RESEARCH PROGRESS REPORT SUMMARY

Grant 02292: Broad-Range Detection of Canine Tick-Borne Disease and Improved Diagnostics Using Next-Generation Sequencing

Principal Investigator: Dr. Pedro Paul Diniz, DVM, PhD

Research Institution: Western University of Health Sciences

Grant Amount: $60,717.00

Start Date: 9/1/2016 **End Date:** 2/28/2018

Progress Report: End-Year 1

Report Due: 8/31/2017 **Report Received:** 8/30/2017

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Original Project Description:

Diagnostic tests based on the detection of DNA of infectious organisms from clinical samples have revolutionized veterinary medicine in the last decades. Currently, diagnostic panels for several tick-borne organisms are available through universities and private laboratories in the USA and abroad. However, the vast majority of results from clinically ill dogs are negative for tick-borne diseases, which frustrates veterinarians and dog owners trying to reach a definitive diagnosis and improve treatment options. These panels are based on the detection of previously known DNA sequences of each pathogen, with little room for detecting new organisms. Consequently, the current assays may suffer from "myopia": a self-fulfilling effect that prevents the detection of new or emerging organisms. Using an innovative approach, the investigators will employ next-generation sequencing (NGS) to overcome the limitations of current diagnostic technology. With NGS, the investigators can generate millions of individual gene sequencing reads from each clinical sample, allowing for the identification and characterization of multiple organisms from a single sample. Testing samples from dogs naturally exposed to tick-borne diseases, NGS will detect not only new organisms but also characterize genetic differences among known organisms. The resulting dataset of a large number of DNA sequences of known tick-borne organisms and previously undetected organisms in naturally-infected dogs will support the development of diagnostic tools to simultaneously advance canine and human health.

Publications:

Manuscript under preparation:

- 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). Journal of American Veterinary Medical Association.

- Vasconcelos E., Oakley B., Diniz, P.P.V.P. Strategies for assessing vector-borne diseases 16S rRNA next generation sequencing data in veterinary clinical samples.
BMC Microbiology or BMC Veterinary Research.

- Vasconcelos E., Oakley, B., Billeter SA, Jett LA, Wournell AL, Kjemtrup AM, Padgett KA, Yoshimizu MH, Metzger ME, Barr MC, Diniz PPVP. Epidemiology of flea-borne microbiomes detected in northern and southern California by universal 16S rRNA generation sequencing. Veterinary Microbiology.

Report to Grant Sponsor from Investigator:

Dogs from any breed, age or gender can be infected with microbes transmitted by ticks or fleas. These diseases can cause devastating effects and even death not only to dogs but also to humans. Ticks are present everywhere in the US, and they bring the risk of transmitting the microbes to dogs and humans. In fact, dogs infected with these microbes have been reported at every US state including Hawaii, and more recently, Alaska. It is still very difficult to diagnose these conditions, and approximately 95% of suspected dog cases are negative when we use current diagnostic technology. One of the biggest limiting factors for the development of better diagnostic tools is the insufficient funding opportunities for large-scale projects. CHF has implemented a new funding mechanism to address such limitation, which supports this study. The long-term goal of our research team is to expand the current diagnostic tools to include a larger spectrum of potentially hazardous microorganisms. Our innovative approach is based on four pillars: (1) large-scale DNA sequencing to identify known and potentially new organisms present in blood of 500 dogs naturally exposed to vector-borne diseases; (2) increase in sensitivity and specificity of large-scale sequencing by targeting major families of potentially hazardous organisms, (3) advanced bioinformatic analysis of billions of DNA sequences from a large number of dogs suspected of infection; and (4) comprehensive quality-control measures in order to support and validate the impact of our results. In the last six months, expanded the sample size to 537, to account for missing samples. We have completed the preparation of the samples from all canine samples, and have submitted them for deep DNA sequencing. We also design novel family-level primers for the Anaplasmataceae, Bartonellaceae and Mycoplasmataceae families using a unique computational approach and confirmed their sensitivity and specificity based on rigorous benchtop experiments. We sought further training to update and expand our computational protocols to remain at the cutting-edge workflow of analysis of large-scale DNA sequences in metagenomics. Our bioinformatics pipeline is ready to quickly analyze the raw data coming from our large-scale DNA sequencing effort. Finally, we already identified key elements to

improve the use of this new technology in veterinary diagnostics. Our results will probably become a paradigm shift in the detection and identification of the cause of vector-borne diseases in dogs, by unveiling thousands of novel potentially hazardous microorganisms. Our data will serve as the new foundation for the implementation and expansion of novel diagnostic methods, and will ultimately support early diagnosis and better medical care to dogs worldwide. By sponsoring this CHF research initiatives, breed clubs and other sponsors will have a positive impact in animal health at a global level.