RESEARCH PROGRESS REPORT SUMMARY

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

Principal Investigator: Pedro Diniz, DVM, PhD

Research Institution: Western University of Health Sciences

Grant Amount: $60,717.00

Start Date: 9/1/2016 **End Date:** 6/30/2018

Progress Report: FINAL

Report Due: 6/30/2018 **Report Received:** 6/29/2018

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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.J., 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.J., Billeter SA, Jett LA, Barr MC, Diniz PPVP, Oakley, B. Assessing cat flea microbiomes in northern and southern California by 16S rRNA Next Generation Sequencing. Vector-Borne and Zoonotic Diseases, Published Online:12 Jun 2018. https://doi.org/10.1089/vbz.2018.2282

Presentations:

● Persico E., Qurollo B., Thomas B., Hegarty B., Breitschwerdt E., Diniz P.P. Molecular prevalence of selected canine vector-borne pathogens in the United States (2008-2015). College of Veterinary Medicine Research Day, Western University of Health Sciences, March 20th, 2017.

● Vasconcelos E.J., Oakley B., Diniz, P.P. Building a Computational Workflow for Metagenomics in Veterinary Medicine. College of Veterinary Medicine Research Day, Western University of Health Sciences, March 20th, 2017.

● Geiger J.A., Persico E., Vasconcelos E., Mirrashed H., Qurollo B., Oakley B., Diniz, P.P.V.P. Improving and expanding the broad-range detection of canine tick-borne disease diagnostics using next-generation sequencing. Graduate College of Biomedical Sciences Poster Session. Western University of Health Sciences, May 12th, 2017.

• Vasconcelos E.J., Geiger J.A., Oakley B.B., Diniz P.P. Next-Generation Sequencing Diagnostic Platform for Vector-Borne Diseases. College of Veterinary Medicine Research Day, Western University of Health Sciences, March 19th, 2018.

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. 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. 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 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 millions 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. We confirmed that large-scale DNA sequencing can simultaneously detect one or more microbes in dog blood and can identify which microbe is present based on its unique DNA sequence. We confirmed this in both simulated infections as well as in samples from naturally infected dogs. We also detected potentially new organisms from sick dogs. With the crucial support from the AKC Canine Health Foundation, we were able to expand our expertise in using large-scale DNA sequencing technology for the detection of tick-borne pathogens in dogs and generate a large body of knowledge to support the further development of this technology. Ultimately, the results of this study will support early diagnosis and better medical care to dogs worldwide.