



## 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:** 8/31/2021  
**Progress Report:** FINAL  
**Report Due:** 8/31/2021      **Report Received:** 10/2/2021

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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 AKC CHF-funded study #02292.

### **Publications:**

#### *In preparation:*

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*.

Blood microbiome profile is influenced by infection type, rather than locality, sex or age of the canine. Chayan Roy, Elton J. R. Vasconcelos, Brian B. Oakley, Pedro Diniz. (journal to be defined).



Oney, K., Koo, M., Roy, C., Ren, S., Quorollo, B., Juhasz, N. B., Vasconcelos, E. J. R., Oakley, B., & Diniz, P. P. V. P. (2021). Evaluation of a commercial microbial enrichment kit used prior DNA extraction to improve the molecular detection of vector-borne pathogens from naturally infected dogs. *Journal of Microbiological Methods*, 106163. <https://doi.org/10.1016/j.mimet.2021.106163>

Vasconcelos, E. J. R., Roy, C., Geiger, J. A., Oney, K. M., Koo, M., Ren, S., Oakley, B. B., & Diniz, P. P. V. P. (2021). Data analysis workflow for the detection of canine vector-borne pathogens using 16 S rRNA Next-Generation Sequencing. *BMC Veterinary Research*, 17(1), 262. <https://doi.org/10.1186/s12917-021-02969-9>

#### **Presentations:**

Oney K, Vasconcelos EJR, Quorollo B, Oakley B, Diniz PPVP. Enhancing Microbial Detection of Vector-Borne Pathogens from Clinical Samples. One Health Day, College of Veterinary Medicine, Western University of Health Sciences, Pomona, CA. November 1, 2019. (No proceedings).

Diniz PPVP. Tick-borne diseases and their tricks. 27th Ljudevit Jurak: International Symposium on Comparative Pathology with One Health Session, Zagreb, Croatia, May 31st and June 1st, p.33, 2019.

Koo M, Oney K, Quorollo B, Ren S, Oakley B, Diniz PPVP. Piroplasmiosis Detection Using Next-Generation Sequencing. 2019 Boehringer Ingelheim National Veterinary Scholars Symposium, Tufts University, Grafton, MA. July 25-28, 2019.

#### **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 microorganism, which limits their ability to detect novel organisms, or the presence of co-infection without performing multiple assays. With the AKC-CHF support, we were able to develop a novel diagnostic tool based on high-throughput next-generation sequencing (NGS) capable of testing a large number of canine samples at once for the detection of one or more organisms in one single assay. We were able to expand the level of specificity of the NGS when compared to traditional approaches by targeting a different gene to precisely detect each organism present in clinical specimens. We confirmed in-vitro the assay's ability to detect low levels of infection with one organism even in the presence of high levels of other organisms. We also evaluated a method to decrease the presence of host DNA to improve the detection of vector-borne pathogens and developed a computational pipeline to help facilitate the analysis of the sequencing data. Collectively, our efforts yielded a novel diagnostic platform for the broad detection of tick- and blood-borne organisms in dogs. The AKC-CHF support was instrumental in the development of such diagnostic tools in veterinary medicine.