# RESEARCH PROGRESS REPORT SUMMARY

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

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| **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:** | End-Year 3 |

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*(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:**

Oney, K., Koo, M., Roy, C., Ren, S., Qurollo, 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

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**Page 1 of 3**

Under review:
Comprehensive workflow for Next-Generation Sequencing applied to vector-borne disease diagnostics. Elton J. R. Vasconcelos, Chayan Roy, Joseph Geiger, Brian B. Oakley, Pedro Diniz. *BMC Veterinary Research.*

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)

**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:**

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. Using an innovative approach, our study proposes the adaptation of high-throughput next-

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**Page 2 of 3**

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. However, adaptations and optimizations of the technique are required for adequate efficiency and accuracy in detecting tick-borne bacteria. With support from the AKC Canine Health Foundation, we are investigating methods to concentrate the harmful bacteria in clinical samples, precisely detect them using NGS, and better characterize these bacteria using genetic sequences. Our most recent efforts 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. Since your last report, we have identified three candidates for the best "DNA fingerprints", and are performing extensive benchtop and genetic testing to identify the best candidate. 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.

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Page 3 of 3