RESEARCH PROGRESS REPORT SUMMARY

Grant 02553: Targeted Next Generation Sequencing Panel for Comprehensive Testing of Vector-borne Pathogens

Principal Investigator: Rebecca Wilkes, DVM, PhD

Research Institution: Purdue University

Grant Amount: $103,245

Start Date: 2/1/2019 **End Date:** 7/31/2021

Progress Report: End-Year 2

Report Due: 1/31/2021 **Report Received:** 1/21/2021

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Original Project Description:

Diagnosing vector-borne disease (VBD) in dogs can be difficult for a number of reasons. First, there are many different disease-causing agents that can be transmitted from ticks/fleas, and the clinical signs caused by these agents in dogs can overlap. Additionally, because ticks/fleas can harbor more than one agent at a time, multiple pathogens may be passed to a dog with a single vector bite, resulting in co-infections. VBD infections can initially present with non-specific signs, such as fever, lethargy, vomiting, diarrhea, and/or respiratory signs. Severe cases can be associated with neurologic signs. These signs can be a diagnostic conundrum. While initial blood work can be helpful and suggest VBD, it does not determine the infecting agent. This study will develop a comprehensive next generation sequencing panel to detect and identify major VBD agents known to cause disease in dogs and to aid in diagnosis of active infections. Additionally, through parallel sequencing with this method, this panel will incorporate testing for additional infectious diseases that may cause GI, respiratory, or neurologic signs in dogs. The comprehensive nature of this sequencing panel should be a useful tool for surveillance of infectious diseases in the canine population for rapid identification of VBD in dogs and protection of pet owners from such zoonotic diseases.

Publications: None at this time.

Presentations: None at this time.

Report to Grant Sponsor from Investigator:

We proposed to develop a comprehensive method for detection of infectious diseases of dogs, taking the guesswork out of determining which tests to use for diagnosis, and potentially improving disease surveillance because of the comprehensive nature of the test. This method takes advantage of the amount of data that can be generated with next-generation sequencing (NGS) but will be performed in a way to keep costs down and maintain adequate turn-around time for diagnostic use. We proposed a combined PCR/NGS method called targeted NGS, and with funding generously provided by AKC CHF, we have developed a comprehensive assay containing 488 primer sets for pathogen detection by NGS. An initial feasibility study was performed with 18 clinical samples (9 pos and 9 neg), previously tested by a collaborating laboratory for tick-borne pathogens by qPCR. This sample set represented multiple pathogens, including *E. canis, Babesia gibsoni*, haemotropic *Mycoplasma*, *Borrelia*, and *Rickettsia* spp. Comparison of the results obtained with the targeted NGS method vs. qPCR resulted in a positive percent agreement between the methods of 86%; negative percent agreement of 64% (NGS was able to detect additional pathogens that were missed by original testing in 4 samples); and Cohen's kappa of 0.46, suggesting a moderate agreement between methods. The ability of the NGS method to detect additional pathogens that were missed by qPCR was expected. The discrepant samples were sent to a third lab for evaluation by real-time PCR +/- sequencing and results are pending. qPCR was not originally performed for some of the pathogens that were detected by the NGS method. This is the benefit of using a comprehensive method, such as the targeted NGS panel.

Initial specificity testing showed no interference of canine DNA with the method. Primers were specific for the tested pathogens, with the exception of cross-detection of *B. gibsoni* with primers for *Cytauxzoon felis*. However, this was easily distinguished with raw sequence evaluation.

Additional studies are required to complete the proposed aims. More characterized clinical samples (by qPCR) will be evaluated with the NGS method, to reach a total of 50 samples. Specificity testing with more pathogens from the panel is needed. Sensitivity testing for relative endpoint detection has yet to be performed but is planned.