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: FINAL

Report Due: 7/31/2021 **Report Received:** 7/30/2021

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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: Abstract submitted to CRWAD (Conference for Research Workers in Animal Diseases) for presentation in Dec. 2021.

Report to Grant Sponsor from Investigator:

We developed a comprehensive method for detecting infectious diseases in 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. The method is a targeted next-generation sequencing (NGS) assay, which takes advantage of the amount of data that can be generated with NGS but also includes a PCR step prior to sequencing. This reduces costs associated with the sequencing and provides adequate turn-around time for diagnostic use. The goal of this project was to validate the assay for detection of vector-borne pathogens. An initial feasibility study was performed to determine if the primer sets included in the assay were able to accurately detect the intended pathogens. The sensitivity of the method (the ability to detect low amounts of pathogen) was compared to qPCR, which is the standard method for diagnosis of active vector-borne infections. The diagnostic capability of the assay was also evaluated through testing 51 clinical samples from dogs that had been determined to be positive or negative by qPCR. Primer sets for 17/23 tick-borne pathogen species included in the panel were evaluated and all successfully amplified the intended pathogens. Validated strains/samples with significant amounts of pathogen could not be obtained for the other six pathogens. The targeted NGS assay is highly specific, able to differentiate between closely related pathogens, and highly sensitive, with detection equivalent to qPCR Ct values of approximately 35 (very low amounts). The targeted NGS assay had an almost perfect agreement with qPCR for detection of pathogens in diagnostic samples. The assay was able to detect multiple pathogens in a single sample with a single test. Additionally, the method was able to detect pathogens that were originally missed because they were not included in original testing. While the method is slightly less sensitive than qPCR for detection of vector-borne pathogens in whole blood samples, it is a viable option for detection of these pathogens for diagnosis of vector-borne diseases in dogs.