RESEARCH PROGRESS REPORT SUMMARY

Grant 03137: Targeted Next Generation Sequencing Panel for Comprehensive Testing for Reproductive and Neurologic Pathogens of Dogs

Principal Investigator: Rebecca Wilkes, DVM, PhD, DACVM

Research Institution: Purdue University

Grant Amount: $107,510.00

Start Date: 3/1/2023 **End Date:** 2/28/2025

Progress Report: FINAL

Report Due: 2/28/2025

(The content of this report is not confidential and may be used in communications with your organization.)

Original Project Description:

The large number of pathogens that contribute to infectious reproductive or neurologic disease and overlap of clinical signs makes using tests for multi-pathogen detection useful. Routinely, several individual or multiplex PCR assays are performed to identify active infection with these agents. Determining which tests to use for any given case can be difficult. Therefore, investigators propose to validate a comprehensive targeted next generation sequencing (NGS) panel for detection of all known neurologic and reproductive pathogens that infect dogs, as well as other infectious pathogens of dogs, including vector-borne agents previously validated through our work funded by AKC CHF. This takes the guesswork out of proper test selection for infectious disease diagnostics. The amount of data that can be generated with NGS allows for comprehensive testing, and use of a targeted method (adding primers to the protocol for amplification of targets of interest) reduces the cost to the point that this testing can be effectively used in a veterinary diagnostic lab. More and more veterinary laboratories are purchasing sequencing equipment, so this method has merit to be expanded to additional labs. With the comprehensive nature of the panel, epidemiological evaluation of infectious diseases in the dog population can also be improved.

Publications:

None at this time- in preparation

Presentations:

Kattoor J and Wilkes RP. Development of an Ion Torrent Targeted NGS panel for canine infectious neurological and reproductive pathogen detection. Conference of Research Workers in Animal Diseases, Jan. 22, 2024, Chicago, IL.

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Report to Grant Sponsor from Investigator:

Study Overview:
In this study, we aimed to develop a comprehensive targeted next-generation sequencing (tNGS) panel to identify common infectious agents related to neurologic and reproductive disease in dogs, while also incorporating less common zoonotic agents that can spread to humans into a single test. This type of test would allow for the identification of a broader range of infectious agents than the routine tests that are normally performed.

How We Did It:

We developed two pools of primers (short DNA sequences) to detect 34 different infectious agents associated with neurologic/reproductive disease in dogs. We combined the amplification of the agents provided by the use of these primers together with a sequencing assay to both detect, and where possible, characterize the infectious agents if present in the dog's sample.

Testing the Test:

Feasibility Study: We tested the primers with known positive clinical samples and synthetic DNA to determine if all the infectious agents could be detected with the test. We successfully detected 33 out of 34 pathogens included in the panel.
Specificity: We checked if the primers only detected the intended infectious agents, to avoid calling a sample positive if it is not. Some primers were not specific, but we could identify these cases using a database search of the data obtained, thus avoiding false positive results.
Sensitivity: We tested how well the test could detect small amounts of the agents to avoid false negative results. The test was able to detect the infectious agents at low levels, like real-time PCR Ct values from 33-38, and approximately 100-1000 organisms per reaction, depending on the agent.

While detection was not as sensitive as real-time PCR assays for some of the agents, we expect detection at this level will be useful for clinical disease testing.

We tested 76 diagnostic samples with known positive and negative results based on routine testing. The new test correctly identified 89% of the positive and 98% of the negative samples, showing 93% accuracy. The main contributing factor to the discrepancy was reduced sensitivity of the tNGS assay compared to real-time PCR, which was expected. However, unlike with real-time PCR assays, this test could also identify specific strains of certain viruses in positive samples.

We additionally evaluated the reproducibility of the test by having another lab use the same protocol. There was excellent agreement between the two labs. Out of 18 samples tested (that included different combinations of 8 infectious agents), the labs disagreed on two samples. One of the discrepancies was likely due to very low amounts of the infectious agent in the sample. The reason for the discrepancy with the other sample is currently unknown. This was considered a strong agreement between the two labs. Both labs were successful in detecting co-infections and detecting infectious agents that were originally missed by PCR testing because the specific tests needed for these undetected organisms were not performed.