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: Mid-Year 2

Report Due: 8/31/2024 **Report Received:** 8/8/2024

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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

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.

Report to Grant Sponsor from Investigator:

The goal of this project is to validate respiratory and neurologic disease testing using a targeted next-generation sequencing (tNGS) panel we previously developed. We expect this assay, which includes 34 different pathogens known to be associated with reproductive/neurologic disease in dogs, will perform as well as individual real-time PCR assays designed to detect each of these pathogens individually. This type of comprehensive testing takes the guesswork out of choosing which individual tests or small panel of tests to run to diagnose disease.

We have now completed testing 100% of the pathogens/DNA representing the pathogens that are detectable with this assay, and all but 1 could be detected. The pathogen missed was Campylobacter jejuni. This one was included for its possible role in reproductive disease, but it is more likely to cause diarrhea in dogs. Therefore, we will not redesign the assay at this point.

We performed some additional testing of sensitivity (limit of detection) using synthetic DNA to represent 12 additional pathogens in the assay, for which we could not obtain clinical samples. We determined for 11 of these that we could detect approximately 100-1000 copies of organism in the sample. One of the fungal pathogens had a lower sensitivity, requiring approximately 10,000 copies in the sample to be detected. We can increase the sensitivity of the detection for the fungal pathogen by adding more primers to the panel for this organism.

We performed some additional diagnostic sensitivity and specificity testing using 20 canine abortion cases we received at the Purdue Animal Disease Diagnostic Lab for necropsy. Fetal/neonatal tissues are tested by a real-time PCR panel and an aerobic culture is performed as part of the necropsy, looking for infectious organisms associated with abortion in dogs. Based on comparison between these tests and our tNGS assay, the diagnostic sensitivity and specificity for the tNGS assay were 100%. There were 6 positive and 14 negative samples. To date, we have tested 42/50 samples we planned to test for this aim. We will try to find more positive samples for testing to complete this aim.

We evaluated the data from the blinded panel provided by UC Davis. Unfortunately, they did not have enough positive neurologic samples for us to test. They sent us 7 canine neurologic samples and 11 canine respiratory samples. The point of this testing is to evaluate reproducibility of the tNGS assay between labs. Our collaborator at U Penn has these samples and plans to test them by our tNGS method in September. It will be interesting to see if our collaborator can reproduce our results using our method.

We are on target to complete all the work for the remaining aims by the end of the contract period.