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

Research Institution: Purdue University

Grant Amount: $107,510.00

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

Progress Report: End-Year 1

Report Due: 2/29/2024 **Report Received:** 2/26/2024

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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 approximately 70% (23/34) of 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 also began testing the sensitivity of the tNGS assay, compared to real-time PCR testing. Based on the results from 13 pathogens tested, the approximate limit of detection, based on real-time PCR Ct values is in the mid-30s. One of the pathogens, bluetongue virus, had a detection limit of approximately 25. This suggests additional primers for this pathogen should be added to the assay to increase the sensitivity for this organism. We started testing the diagnostic sensitivity and specificity of the assay by evaluating 22 CSF samples submitted from dogs with neurologic disease for canine distemper virus (CDV) real-time PCR. Only one of these samples was positive for CDV by real-time PCR. We were also able to detect this virus in this sample by the tNGS method. Additionally, we were able to identify the variant as an America-5 genotype with the new assay. All the other real-time PCR negative samples were also negative by the tNGS assay for CDV, and for all other neurologic pathogens in the tNGS assay as well. We have also begun testing the blinded samples for Aim 4 of the study. We are currently evaluating the data and will share the samples and the primer pools with our collaborator at the University of Pennsylvania. We are currently ahead of schedule, based on the timeline submitted for the project.