National Pork Board
FINAL RESEARCH GRANT REPORT FORMAT

Project Identification Number: NPB 22-074
Project Title: Classical Swine Fever PCR Negative Cohort Study – Partnering to expand testing capacities, support further evaluation and validation of two commercially available CSF PCR assays, and enhance preparedness across the NAHLN.

Principal Investigator
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Institution
Iowa State University Veterinary Diagnostic Laboratory

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Industry Summary (Abstract):

The COVID-19 pandemic clearly illustrated the value of having diversified and scalable supply chains of the testing reagents and consumables used in diagnostic testing; effective private sector, state, and federal partnerships; and fit for purpose diagnostic sample types that are well suited for high throughput testing environments. This study involved leveraging the capabilities and expertise of three swine interest veterinary diagnostic laboratories (VDLs) in the USDA National Animal Health Lab Network (NAHLN) working together with USDA colleagues and industry partners to enhance Classical Swine Fever (CSF) PCR testing capacities and foreign animal disease preparedness across the US. The purpose of this study was to more fully evaluate the specificity of three different CSF PCR assays on two aggregate sample types (oral fluid and processing fluid) obtained from swine herds located throughout the US. Due to the notable implications of dealing with false positive test results in foreign animal disease testing use cases, very clearly understanding assay specificity across a broad range of farms, regions, and sample types is especially important. Each of the three participating VDLs were responsible for conducting CSF PCR testing utilizing the three different assays on 250 swine oral fluid samples and 250 processing fluid samples. Each of the 500 swine samples were tested by the custom-made single-plex CSF PCR assay currently utilized across the USDA NAHLN, as well as by the single-plex CSF PCR assays provided by the two largest suppliers of commercial PCR reagents (i.e., Tetracore® and Thermo Fisher®) to USDA NAHLN labs. Thus, each swine oral fluid and processing fluid sample tested had nine individual PCR assays performed on them, for a total of 4,500 PCR tests conducted. Each laboratory tested each sample using the nucleic acid extraction protocol (first step in the PCR testing process) they routinely use in the PCR testing of oral fluid and processing fluid samples. PCR was run on the extracts, using the current NAHLN CSF PCR assay, the Tetracore® CSF PCR assay, and the Thermo Fisher® CSF PCR assay. All 4,500 tests yielded a valid CSF PCR negative result, suggesting each of the CSF PCR assays evaluated have an estimated diagnostic specificity of 100% on oral fluid and processing fluid samples from US swine. In addition, this study indicated that the extraction techniques used by each of the participating laboratories, which were consistent with what is implemented in each of the laboratories' routine testing, consistently produced valid test results. This supports the suitability of the respective extraction procedures for CSF PCR testing on the sample types tested. Being able to use the same extraction procedure for CSF PCR testing as is being used for the other routine PCR testing on these sample types would allow for streamlined and efficient testing, as this foreign animal disease testing would fit very well into a laboratory's existing PCR workflow and processes. This in turn would enable much higher laboratory testing capacities than if a different extraction process were required. In total, this study made a substantive contribution to the larger set of highly collaborative efforts being made towards

expanding the number of high-quality CSF PCR assays and fit for purpose sample types available for use in the USDA NAHLN laboratories. Such efforts are foundational towards enhancing testing capacities and the overall foreign animal disease preparedness across the network.

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Key Findings:

- Each of the three CSF PCR assays evaluated (i.e., custom-made USDA NAHLN CSF PCR assay, Tetracore® CSF PCR assay, and the Thermo Fisher® CSF PCR assay) were found to have an estimated diagnostic specificity of 100% on oral fluid and processing fluid samples from US swine.

- The nucleic acid extraction techniques routinely used by each of the three participating veterinary diagnostic labs when conducting endemic disease related PCR testing on oral fluid and processing fluid samples consistently yielded valid CSF PCR test results.

- This study made a substantive contribution to the larger set of highly collaborative efforts being made towards expanding the number of high-quality CSF PCR assays and fit for purpose sample types available for use in the USDA NAHLN laboratories. Such efforts are foundational towards enhancing testing capacities and the overall foreign animal disease preparedness across the network.

Keywords: include at least 5 keywords
Diagnostic
Oral fluid
Processing fluid
CSF
PCR
Cohort
NAHLN
Specificity

Scientific Abstract:

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USDA NAHLN labs. Thus, each swine oral fluid and processing fluid sample tested had nine individual PCR assays performed on them, for a total of 4,500 PCR tests conducted. Each laboratory tested each sample using the nucleic acid extraction protocol (first step in the PCR testing process) they routinely use in the PCR testing of oral fluid and processing fluid samples. PCR was run on the extracts, using the current NAHLN CSF PCR assay, the Tetracore® CSF PCR assay, and the Thermo Fisher® CSF PCR assay. All 4,500 tests yielded a valid CSF PCR negative result, suggesting each of the CSF PCR assays evaluated have an estimated diagnostic specificity of 100% on oral fluid and processing fluid samples from US swine. In addition, this study indicated that the extraction techniques used by each of the participating laboratories, which were consistent with what is implemented in each of the laboratories' routine testing, consistently produced valid test results. This supports the suitability of the respective extraction procedures for CSF PCR testing on the sample types tested. Being able to use the same extraction procedure for CSF PCR testing as is being used for the other routine PCR testing on these sample types would allow for streamlined and efficient testing, as this foreign animal disease testing would fit very well into a laboratory's existing PCR workflow and processes. This in turn would enable much higher laboratory testing capacities than if a different extraction process were required. In total, this study made a substantive contribution to the larger set of highly collaborative efforts being made towards expanding the number of high-quality CSF PCR assays and fit for purpose sample types available for use in the USDA NAHLN laboratories. Such efforts are foundational towards enhancing testing capacities and the overall foreign animal disease preparedness across the network.

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Introduction:

Foreign animal diseases such as Classical Swine Fever (CSF) could have devastating impacts on the US swine industry. Surveillance strategies for enhancing early detection and supporting evidence of freedom of disease and the ongoing movement of animals and products in the event of an outbreak are imperative to a speedy recovery from an outbreak situation. The validation of diagnostic tools for aggregate testing are needed to make efficient use of diagnostic and field resources in the midst of an outbreak. More specifically, the specificity of the assay, which is the proportion of true negative samples that will actually yield a negative test result, reflects how well an assay performs in a disease-free population. A highly specific test will minimize the fraction of false positive test results and consequently reduce the risk of having to investigate, trace back, and re-test any positive sample, which could have negative economic impacts for animal health programs and even impact trade. The validation of the specificity of the RT-PCRs that will be used for detection of CSF in an outbreak situation will provide information on number of false positives test results that should be expected when testing a disease-free population. In an outbreak situation, this becomes particularly important when the disease is in the eradication phase and/or the surveillance goal is to support evidence of freedom of disease. Having multiple high quality PCR assays available that includes assays manufactured by commercial PCR reagent providers with substantive production facilities located within the US is desirable. Also, the ability of a testing lab to use workflows already established in their laboratories would assist in streamlining and scaling up testing numbers. Generally, the most laborious or cumbersome step of the PCR process is extracting nucleic acid from the clinical specimen. Not being required to utilize a different extraction process for CSF (foreign animal disease) testing would be extremely beneficial.

Objectives:

Objective 1: To validate the diagnostic specificity of the currently approved USDA NAHLN PCR and the Tetracore® and Thermo Fisher® commercial RT-PCR kits for detection of CSF in oral fluids and processing fluids of US swine.

Objective 2: To assess the suitability of the three participating (swine interest) veterinary diagnostic laboratories to utilize their routine validated nucleic-acid extraction protocols for use with the above mentioned CSF PCR assays when testing oral fluids and processing fluids of US swine.

Materials & Methods:

The Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) collected, aliquoted, and froze back 250 oral fluid samples and 250 processing fluids, with efforts made to have representation from diverse geographical regions across the US.

Aliquots of each of the 250 oral fluid and processing fluid samples were distributed to the three participating laboratories (ISU VDL, South Dakota State University Animal Disease Research and Diagnostic Laboratory, and the University of Minnesota Veterinary Diagnostic Laboratory) and were tested in each laboratory by the three different CSF PCR assays being evaluated. Each laboratory utilized their own validated nucleic acid extraction protocols for these specimen types (**Figure 1**).

Table 1. Overview of this CSF PCR negative cohort study.[1]

CSF PCR Assay	Sample Types	
	Oral Fluids	Processing Fluids
USDA NAHLN (Custom)	250 samples	250 samples
Tetracore® (Commercial)	250 samples	250 samples
Thermo Fisher® (Commercial)	250 samples	250 samples

[1]Aliquots of the 250 oral fluid samples and 250 processing fluid samples were tested by the current USDA NAHLN, Tetracore®, and Thermo Fisher® CSF PCR assays at ISU VDL, SDSU ADRDL, and UMN VDL.

Re-testing was conducted on any tests results yielding an “Inconclusive” result due to the observance of an Internal Control Failure (ICF). Internal controls were used in all PCR assays and protocols conducted in efforts to avoid reporting false negative results. All test results were sent to Karen Krueger at the Iowa State University Veterinary Diagnostic Lab for compilation of results.

Results:

Each PCR test that was run produced a valid negative CSF PCR test result.

Table 2. Results of CSF PCR testing

	ISU VDL	SDSU ADRDL	UMN VDL	Total
Processing fluids				
NAHLN, # Positive	0	0	0	0
Tetracore®, # Positive	0	0	0	0
Thermo Fisher®, # Positive	0	0	0	0
Total, # Positive				0
NAHLN, # Negative	250	250	250	750
Tetracore®, # Negative	250	250	250	750
Thermo Fisher®, # Negative	1500	1500	1500	750
Total, # Negative				2,250
Oral fluids				
NAHLN, # Positive	0	0	0	0
Tetracore®, # Positive	0	0	0	0
Thermo Fisher®, # Positive	0	0	0	0
Total, # Positive				0
NAHLN, # Negative	250	250	250	750
Tetracore®, # Negative	250	250	250	750
Thermo Fisher®, # Negative	250	250	250	750
Total, # Negative				2,250

Discussion:

No false positive PCR test results were observed across the three CSF PCR assays being evaluated on the 4,500 individual PCR assays conducted on swine oral fluid and processing fluid samples at the ISU VDL, SDSU ADRDL and UMN VDL. These results suggest the three CSF PCR assays evaluated have an estimated diagnostic specificity of 100% on oral fluid and processing fluid samples from US swine. In addition, this study supports that lab validated processes for nucleic acid extraction of oral fluids and processing fluids are suitable for testing of CSF by PCR. The expansion of the number of approved CSF PCR assays to include domestically produced commercial PCR assays would improve the number of options available to the USDA NAHLN labs and greatly increase the CSF PCR test reagent manufacturing capacities in the US. Such improvements and increasing the number of CSF PCR assay options available for use would all but ensure CSF PCR test reagents would not be a limiting factor in supporting the response to a CSF outbreak in the US. Additionally, the ability of laboratories to utilize their routine extraction processes would aid in streamlining testing and allowing laboratories to dramatically scale up the volume of testing for surveillance or in the event of an outbreak.

In total, the findings from this study represent a substantive contribution to the larger set of highly collaborative efforts being made towards expanding the number of high-quality CSF PCR assays and fit for purpose sample types available for use in the USDA NAHLN laboratories. All such efforts are

foundational towards enhancing testing capacities and the overall foreign animal disease preparedness across the network.

Special Note:

This study was made possible and done in cooperation and synergy with a companion African Swine Fever (ASF) PCR Negative Cohort Study funded ($140,128) by the 2021 USDA NAHLN Farm Bill Request for Proposals. The National Pork Board's financial support ($20,000) for this project also leveraged USDA's provision of an additional $92,000 to complete this CSF PCR Negative Cohort study in total. A highly collaborative and successful effort amongst industry, federal, and land-grant university partners.