RESEARCH ABSTRACT

SWINE HEALTH

Title: Rapid, Pen-side Molecular Diagnostic Tool for Foreign Animal Disease **NPB# 18-040**

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Scientific Abstract: Foreign animal diseases present a major threat to the global swine industry. FMDV is particularly challenging since any vesicular disease case must be addressed and FMDV ruled out so that animal transport is permitted. In pigs, several other viral vesicular diseases, including swine vesicular disease (SVD), Senecavirus A (SVA), and vesicular exanthema virus infection, cannot be distinguished from FMDV on the basis of clinical findings. The economic implications of the prohibition of pig movement and export due to vesicular disease outbreaks can range from severe for endemic viruses, to catastrophic for FAD. The proposed project thus directly addresses the swine health research priority for rapid, differential diagnostics of vesicular diseases, including FMDV, which is an important FAD. The primary goal is to benchmark rapid sequencing methods for RNA virus detection and evaluate their sensitivities in biological samples that could be collect on the farm or at the slaughterhouse. Rapid, direct sequencing will provide new insights for FMDV detection and differentiation from other pathogens that cause vesicular diseases, such as SVA. Benchmarks will establish key strengths and limitations that must be improved. Our research is directly applicable to all disease pathogens with RNA as the genetic material.

Using SVA as a model, which has been observed as a cause of vesicular disease in different countries throughout the world since 2015, we demonstrated that Oxford Nanopore MinION sequencing could be used as a robust tool for investigation of vesicular diseases. Our results identified the presence of a pathogen from a clinical sample, allowing for identification at the species and strain level. SVA whole genome sequences were generated using both direct RNA sequencing and cDNA-PCR sequencing, with a consensus accuracy of 94% and 99% respectively. The advantages of direct RNA sequencing lie in its simplicity of library preparation and direct RNA strand information which can indicate potential nucleic acid modifications, while cDNA-PCR sequencing excelled at generating highly accurate sequences. This study developed whole genome sequencing methods to facilitate the diagnosis of SVA and provide a reference for investigations of FMD. Next, we used PRRSV, which is the most economically devastating pathogen in the US swine industry, to test the method we established above and to aid in developing more precise detection of endemic diseases, such as examination of co-infection. A nearly full length PRRSV genome was successfully generated from raw sequence reads, achieving an accuracy of 96% after consensus genome generation. Direct RNA sequencing reliably detected the PRRSV strain present with an accuracy of 99.9% using as few as 5 raw sequencing reads and successfully differentiated multiple co-infecting strains present in a sample. In addition, PRRSV strain information was quickly obtained from clinical samples containing 10^4 to 10^6 viral copies or more within 6 hours of sequencing.

Overall, our study not only accelerated the development of robust, rapid, on-site, real-time disease diagnosis but also created a disease diagnostic model for FMD and other FAD outbreaks. Sequencing followed by bioinformatic analysis proves to be a promising approach for investigation of infectious diseases, allowing for more precise prevention and control strategies during outbreaks.