RESEARCH ABSTRACT

SWINE HEALTH

Title: Use of Mobile Nanopore Sequencing to Detect and Genotype Porcine Reproductive and Respiratory Syndrome Virus: NPB # 16-205

Investigator: Kevin Lahmers

Institution: Virginia Tech

Date: Submitted: 1/1/18, resubmitted 5/9/18

Scientific Abstract:
Porcine Reproductive and Respiratory Syndrome Virus continues to be a leading cause of disease and decreased production in the swine industry. Detection of virus is not sufficient to determine if this is modified live vaccine, continuing low level infection, or introduction of new strains of the virus that have entered a herd or facility. Therefore, this project looked to combine detection and genotyping using nanopore-based shotgun sequencing. This project was able to successfully detect and genotype virus from experimentally and naturally infected pigs using serum. Using reverse transcription and nanopore sequencing, we were able to detect PRRSV and correctly genotype the virus used for inoculation by using a custom database and the Centrifuge metagenomic classifier. This method is able to detect mixed infections when two samples were mixed. We have demonstrated that this is repeatable with one read per 1600 total reads in multiple library preparations of the same sample. In addition, we have developed a method of data analysis that allows us to identify PRRS virus sequences from among the millions of sequences obtained and to classify the virus within 90 seconds. We are refining this method so that it is automated and better classifies viruses that do not match those sequences found in published databases. This sequencing technology has the ability to selectively sequence and reject unwanted genetic material. Attempts to use this approach were of limited success. Due to the high speed of sequencing reactions and relatively short read lengths, the enrichment was not sufficient to warrant the approach. Alternate enrichment approaches are funded and underway with the intent of making this as fast or faster than current PCR results and at a similar cost. In addition, to increase throughput and efficiency while providing value-added results, we are sequencing ORF5 amplicons at a materials cost similar to that of traditional Sanger sequencing but with the added benefit of being able to detect multiple viruses or modified live vaccine and wildtype virus in the same sample. PCR amplification and sequencing (amp-seq) allows for detection of PRRSV in lower titer samples (preliminary results) as well as detection of multiple isolates within a sample, which is an advantage to sequencing. The amp-seq approach is capable of distinguishing isolates with 99.33% sequence identity over the 604 bp product. The results of this funding will result in 2 manuscripts in preparation, 3 presentations at international meetings, and over $500,000 in additional grant funding to further this line of research in pig disease. We will make protocols and this testing available to clients in the next 2 months.