RESEARCH REPORT
SWINE HEALTH

Title: Project: Rapid detection of porcine reproductive and respiratory syndrome virus using real-time MinION nanopore sequencing - **NPB #18-176**

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Scientific Abstract:
The global spread and constant evolution are challenges to the control of the Porcine reproductive and respiratory syndrome virus (PRRSV), one of the most important viruses affecting the swine industry. PRRSV is divided into two major phylogenetic clades, PRRSV Type 1 (more prevalent in Europe) and Type 2 (more prevalent in North America). Effective control of PRRSV benefits from genotyping, which currently relies on Sanger sequencing. A sensitive and specific protocol was developed with the aim to sequence a ~1600 bp region of the genome covering the complete ORF5 (envelope protein) and ORF6 (membrane protein) using MinION amplicon sequencing. Six 10-fold sequential dilutions of four different PRRSV isolates including type I and II were sequenced using the PCR barcoding amplicons protocol (Oxford nanopore Technologies, ONT) in two independent MinION runs. Aliquots of the same RNA used for the sequencing experiments were tested by RT qPCR. After 20 minutes of sequencing it was possible to detect PRRSV reads in all dilutions, showing the sensitivity of the protocol. Viral loads resulting in qPCR Cts as high as 35 and 37 for type I and II respectively, were still possible to be sequenced and retrieved 25 and 18 reads respectively, corresponding to the full-length amplicons. From RNA extraction to start the sequencing run, the average time to process 24 samples was approximately 17 hours. Clinical swine serum samples with Cts ranging from 15 to 35 were also tested. The sequencing resulted in reads for all the samples, enabling the classification into type I or II after phylogenetic analysis. Besides amplicon sequencing, the whole genome of seven different PRRSV isolates was obtained by random sequencing using an adaptation of the PCR Barcoding Genomic DNA protocol (ONT) and were compared to the results of a sequencing using the Miseq platform (Illumina). The average genome coverage obtained from MinION random sequencing was 99.88% and the identity between platforms was 99.61% on average. A fast turnover time, portability, repeatability and an accuracy high enough for genotyping suggests that this is a useful platform with the potential to improve the understanding of virus evolution and provide important informations for the control of PRRSV. Constant optimization of protocols together with the use of fast and accurate bioinformatic pipelines will potentially decrease the turnaround time, improving an already cost-effective platform for PRRSV genotyping.