Understanding PRRSv diversity at the pig and litter level using whole genome sequencing

Mariana Kikuti; Carles Vilalta; Juan Sanhuezza; Nakarin Pamormchainavakul; Jessica L. Kevill; Igor A.D. Paploski; Ross Kiehne; Kimberly VanderWaal; Declan Schroeder; Cesar A. Corzo

1Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, MN; 2Departamento de Ciencias Veterinarias y Salud Pública, Universidad Católica de Temuco; 3Swine Vet Center, St. Peter, MN

Key points
- Viral diversity within the piglet population is generally small, but higher diversity was found in ORFs 4 and 5a
- Within animal consensus changes were observed in a period as short as 2 weeks, which means piglets might be going to the GF sites with different viruses than the ones identified closer to farrowing

Introduction
Although genetic variability has been estimated to be around 3% at both the within herd and individual level using ORF5 sequences, whole genome variability within a naturally infected herd and within individuals is expected to be higher, more informative, and has yet to be documented. Here, we describe preliminary results of overall, within litter, and within piglet PRRSv genetic diversity on a farm undergoing an outbreak. A PRRSv naive 2500-sow farrow-to-wean farm was selected for this study. The farm broke with PRRSv type 2 after maintaining a naive status for over three years. As part of the PRRS outbreak management protocol, live-virus inoculation was performed in the entire herd nine days after identifying the newly introduced PRRSv. Two weeks after inoculation, all piglets 3-5 days of age (DOA) were individually ear tagged and bled. Surviving piglets were bled again at 17-19 DOA (pre-weaning). Samples were individually tested for PRRSv by RT-PCR, and all positive samples were submitted for MINION sequencing. Consensus whole genome sequences were generated from reads by mapped to the reference MH651739. ORFs 2-7 were analyzed. Samples that were only partially sequenced in any of the ORFs 2-7 were excluded from further analysis.

Results
A total of 127 piglets from 21 litters were sampled at 3-5 DOA and all animals tested PRRSv RT-PCR positive. All 63 surviving piglets at 17-19 DOA were still PRRSv RT-PCR positive. Complete consensus reads were obtained for 101 samples (54 from 3-5 DOA and 47 from 17-19 DOA). Percent identity amongst samples was overall high for most comparisons (>99.0%). The lowest overall and within litter percent nucleotide identity was 95.8% found in ORF4, particularly in litters 10 and 12 at 17-19 DOA. The second lowest percent nucleotide identity was the overall 97.7% identity in ORF5a at 3-5 DOA, and overall and within litter 98.5% in ORF5a at 17-19 DOA. Within animal percent identity between both samplings was also assessed, with lower identity also found in ORFs 4 and 5a at 97.2% and 98.5%, respectively. While most sequences differed less than 1% from the overall ORFs 2-7 consensus, only 30.7% (31/101) of all the sequences were identical to the consensus (Figure 1).

Discussion
Although viral diversity within the piglet population is generally small, higher diversity was found in ORFs 4 and 5a. ORF5a overlaps with the beginning of ORF5, where sites under selective pressure were previously described. PRRSv mutation rate has been estimated at the order of 10-2/site/year, so the high nucleotide difference between the two samples found might represent turnover of a co-circulating virus that was not detected in the first sampling. These animals were also followed at 40-41, 65-66, and 109-110 DOA and will have viral diversity assessed at these stages as well.

Figure 1. Distribution of ORFs 2-7 percent nucleotide identity of each sequence to the consensus by litter. Litters are identified by the numbers in the horizontal axis, separated by each sampling point.

References