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TITLE
OVERCOMING THE PRACTICAL LIMITATIONS OF PRRS ORF5 SEQUENCING

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CONTENT
Background and Objectives

Porcine Respiratory and Reproductive Syndrome Virus (PRRSV) genomic variation determined by sequencing is used to understand virus epidemiology and to drive control strategies, with nucleotide sequence analysis of the ORF5 gene as the cornerstone of virus characterisation. The main limitations are primer design due to high genetic variability, cost and waiting time for results. The aim of this study is to report on the way to overcome some of the practical limitations of PRRSV ORF5 sequencing in a diagnostic laboratory.

Material & Methods

A total of 28 type-1 (EU) and type-2 (NA) PRRSV isolates and 61 field samples (serum and tissues ORF7-qPCR positive coming from 5 European countries) were analyzed. Three different primer-pair RT-PCR protocols (2 EU and 1 NA) previously described were adapted to SybrGreen methodology and used to amplify complete ORF5 gene of all the samples. The purified PCR products were sequenced using Sanger methodology, and nucleotide sequences were analyzed.

Results

Complete ORF5 gene from 84/89 (94.4%) specimens were successfully amplified after attempting with the tested protocols. Phylogenetic analysis of obtained nucleotide sequences allowed the characterization of all of them. Results were obtained in 24-72 hours, although the ORF5 gene from 2 viral isolates and 3 field samples (5.6%) were not amplified with any of the protocols. Also, different kind of samples such as oral fluids had been tested with successful results.

Discussion & Conclusion

Despite the high genetic variation of ORF5 in PRRSV, sequencing can be performed with high success rate in a short time, and at an affordable cost. However, several primer pairs are needed to get valid sequences from most samples. These results encourage continuing testing more samples, and new technologies such as Next Generation Sequencing are being tested as an alternative to characterize strains that would otherwise remain unknown.