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OVERCOMING THE PRACTICAL LIMITATIONS OF PRRS ORF5 SEQUENCING

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BACKGROUND & 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 characterization. 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

Samples: A total of 89 specimens were analyzed. Samples consisted on 28 type-1 (EU) and type-2 (NA) PRRSV isolates worldwide from internal viral culture collection, and 61 ORF7-qPCR positive field samples (pooled sera and tissues) coming from 5 European countries.

Laboratorial procedure: Viral RNA was purified from all samples by using RNeasy Mini kit (Qiagen. Germany) in automatic robot (Qiacube. Qiagen) following manufacturer's instructions. Three different primer-pair RT-PCR protocols (2 EU and 1 NA) previously described[1,2,3] were adapted to SybrGreen methodology and used to amplify complete ORF5 gene of all the samples. The purified PCR products were sequenced using adapted Sanger methodology[4], and nucleotide sequences were analyzed using Geneious Pro software (Biomatters, Ltd. New Zealand). The entire laboratorial procedure was performed in Diagnos-HQ (HIPRA, Spain).

RESULTS

Complete ORF5 gene from 84/89 (94.4%) specimens were successfully amplified after attempting with the tested protocols (Image 2). More than one ORF5 nucleotide sequences were obtained in two clinical samples of pooled sera, so individual sera sequencing was performed on that samples and successful results were reached obtaining separate and different sequences.

Phylogenetic analysis of nucleotide sequences allowed the characterization of all of them. The identity and presumptive genotype characterization of all PRRSV isolates was confirmed after phylogenetic study and all PRRSV detected on clinical samples were classified as type-I strains (Table 1). Results were obtained in 24-72 hours, although the ORF5 gene from 2 viral isolates (from Italy and Iowa) and 3 field samples (from Spain) with high Ct values were not amplified with any of the protocols. Also, different kind of samples such as oral fluids had been tested with successful results (data not shown).

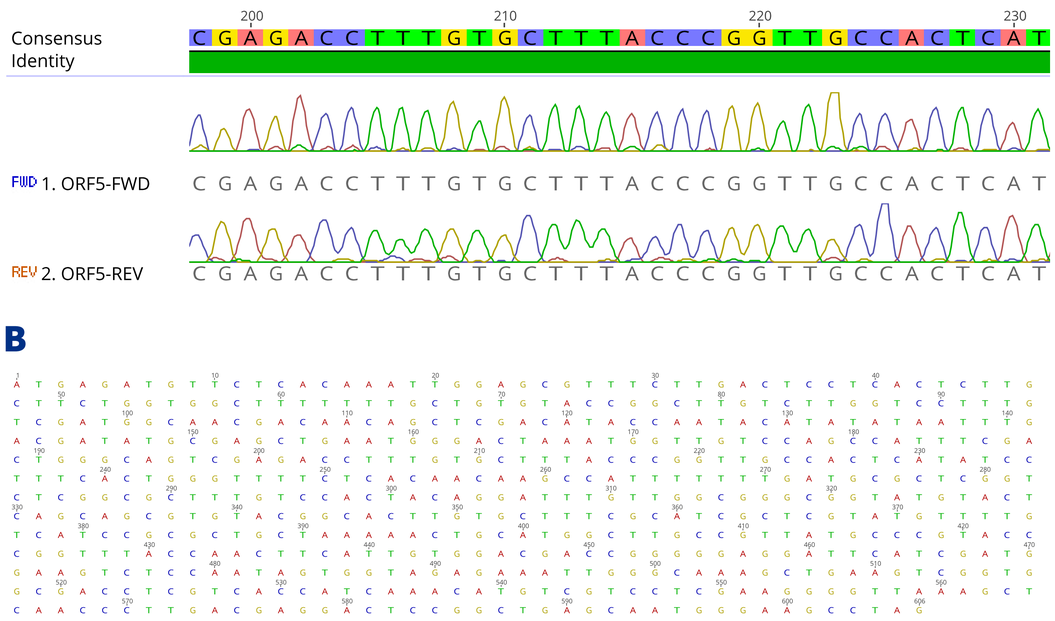
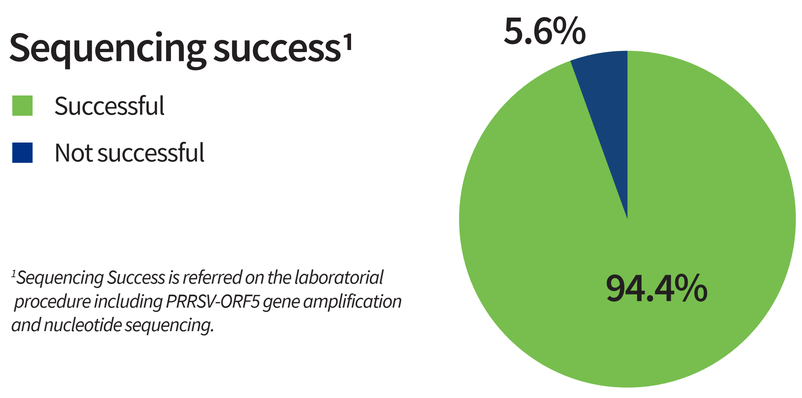


Image 1. Alignment electropherogram of forward and reverse type-I PRRSV-ORF5 nucleotide sequences (A) and consensus nucleotide sequence (B).



[1]Sequencing Success is referred on the laboratorial procedure including PRRSV-ORF5 gene amplification and nucleotide sequencing.

Image 2. PRRSV-ORF5 sequencing results of a total of 89 specimens including clinical samples (n=61) and pure viral isolates (n=28).

Type of sample	Origin	Nº of samples in each genogroup with successful sequencing results	
		Type-I PRRSV	Type-II PRRSV
PRRSV isolate	Spain, Poland, Italy, Slovakia, Italy, Hungary, Czech Republic, Germany, China, Vietnam.	13	13
Field sample[1]	Spain, France, Poland, Germany, Denmark	58	0

[1]Field sample: Pooled sera or tissues (lung, lymph nodes, tonsil, thymus)

Table 1. Origin and genotype characterization of PRRSV with ORF5 successful sequencing results (n=84).

DISCUSSION AND 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.

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