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Diagnostic Communication

Identification and genetic characterization of a type 2 North American strain of porcine reproductive and respiratory syndrome virus (PRRSV-NA) in sera of sows in Uruguay

Report from the University of Minnesota Veterinary Diagnostic Laboratory (UMVDL) and Center for Animal Health and Food Safety (CAHFS), and the Uruguayan Direccion General de Servicios Ganaderos del Ministerio de Ganadería, Agricultura y Pesca (DGSG/MGAP)

Introduction

In South America, PRRSv has been historically reported in Chile, Colombia, Peru, Bolivia and Venezuela (http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statuslist). In July 2017, positive animals were detected by indirect ELISA and RT-PCR in five premises of Uruguay, located in the departments of Salto and Canelones (https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?reportid=24372). This report describes the identification and genetic characterization of a type 2 North American strain of porcine reproductive and respiratory syndrome virus (PRRSV-NA) in sera of sows in Uruguay.

Diagnostic investigation

In August 2017, the Division of the Veterinary Laboratories (DILAVE), Ministry of Livestock, Agriculture and Fisheries (MGAP) of Uruguay contacted the University of Minnesota Veterinary Diagnostic Laboratory (UMNVDL) and Center for Animal Health and Food Safety (CAHFS) to initiate a serological investigation for confirmatory diagnosis of PRRSV. Prior to shipment, 114 serum samples were heated at temperature of 72^{o}C for 30 minutes, following the USDA protocol for serum inactivation (import permit #53664).

Sera antibodies were investigated using a commercial indirect ELISA (IDEXX PRRS X3®). Positive samples were confirmed by immunofluorescence assay (IFA) using a MN field strain and a reference strain (US VR2332).

Direct detection of PRRSv in the serum was examined through RT-PCR using a commercial kit (VetMAX™ NA and EU PRRSV) targeting European (type 1) and North American (type 2) PRRSV. Positive samples were selected for genetic characterization through restriction fragment length polymorphism (RFLP) analysis and sequencing of the ORF5 gene.

Results

From a total of 24 samples positive for the presence of PRRSv antibodies by indirect ELISA, 15 and 19 sera were also positive by IFA using the US MN field strain and the reference strain US VR2332, respectively. ELISA S/P ratio ranged between 0.413 and 2.197 (mean 1.13, SD 0.66, median 1.01). IFA titters ranged from 1:16 through 1:64.

	IFA			
	US MN strain (n=24)		US VR2332 (n=24)	
Indirect ELISA	+	-	+	-
Positive (n=24)	15	9	19	5

The RT-PCR revealed 13 positive sera for the type 2 North American type (PRRSV-NA) with Ct values ranged from 19.7 through 38.8. All samples were negative for the type 1 European PRRSv (PRRSV-EU).

The three samples with lowest Ct values (19.7; 22.2 and 22.9) were selected for genetic characterization. These samples showed the same RLFP profile (1-4-4) and identical ORF5 nucleotide sequences (Fig 1, blue). Based on the phylogenic analysis of the ORF5 sequences available in the GenBank and in the UMNVDL database, the sequences obtained from Uruguayan sera share common ancestors (95.1-96.2% identities) with North American strains described between 2004 and 2015. These closest ancestors are closest related to the 1-7-4 RFLP types that have been circulating in North America for more than a decade. In addition, the sequences identified in Uruguay showed to be genetically distant from other strains reported in South America (Chilean sequences; Fig 1, red) and from commercial vaccines (Fig 1, green).

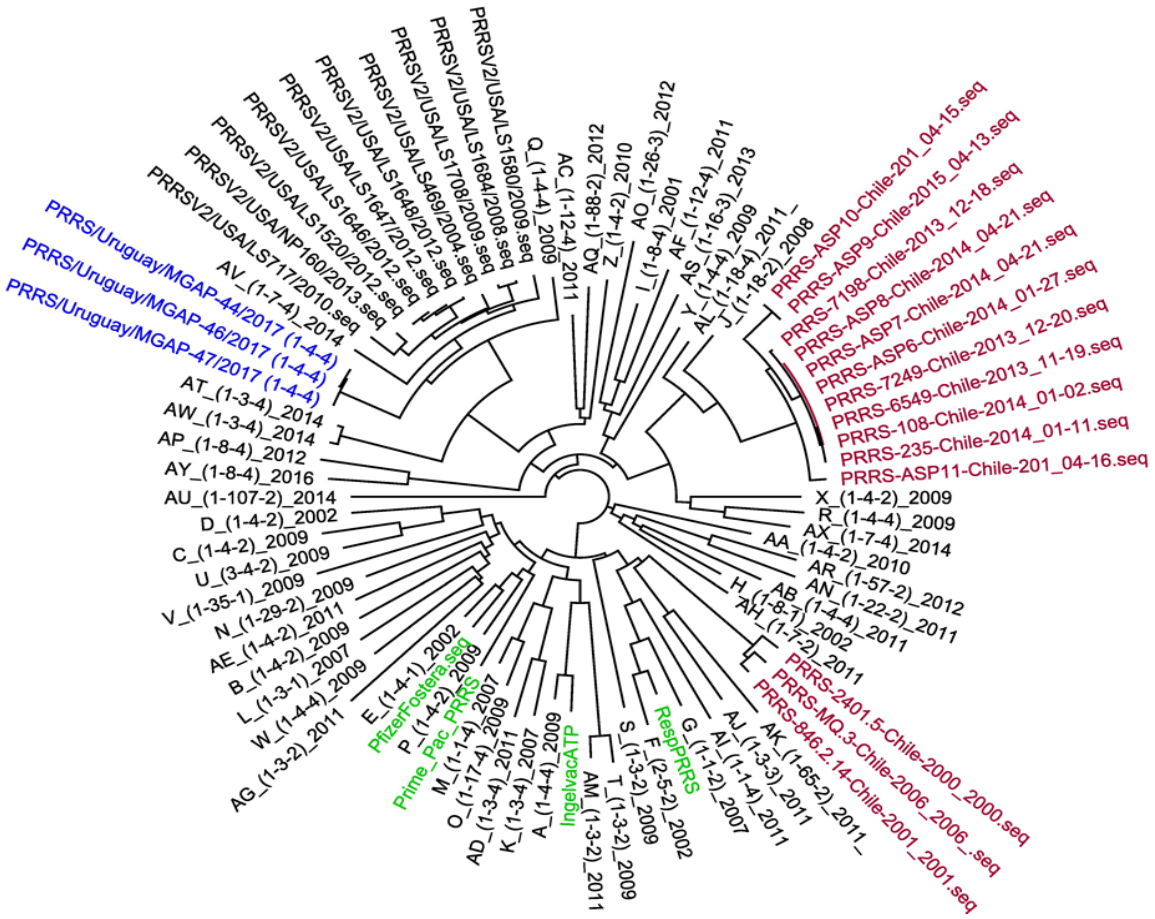

Figure 1. Phylogenetic tree of the PRRSV-NA ORF5 sequences. Strains colored blue are from Uruguay, strains colored black are from USA, strains colored green are commercial vaccines and strains colored red are from South America.

Conclusions

- Evidence of a wild type PRRSV-NA strain circulating in sera of sows in Uruguay.
- Uruguayan ORF5 sequences identical among them but distinct from commercial vaccines strains (Fig 1, green) and other strains reported in South America (Fig 1, red). Although this finding confirms the circulation of wild strains, use of vaccines cannot be ruled out with the data and samples currently available and will be further investigated.
- Sequences from Uruguay share ancestors with strains reported in the USA since 2004 (Fig 1, black).

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