DETECTION OF SAPOVIRUS IN BRAZILIAN PIG FARMS

ABSTRACT

Sapoviruses (Caliciviridae) are considered important gastroenteritis worldwide affecting animals and humans. In pig farming, the epidemiology has not completely understood, because can affect all stages of production, with symptomatic (diarrhea) or asymptomatic pigs. The aim of our study was investigated Sapovirus occurrence in Brazilian pig farms. A total of 166 fecal samples of pigs, with different ages, from Minas Gerais, São Paulo and Mato Grosso States were submitted to RT-PCR reactions and confirmed with nucleotide sequencing of Sapovirus RdRp gene. In total, six (3.61%) samples were positive and four of them had partial RdRp gene sequenced, putatively belonging to GVII.1 genogroup, also previously reported in swine herds in Brazil.

Key words: Sapoviruses; Calicivirus; swine; RdRp gene; genogroup.

RESUMO

Sapovírus (Caliciviridae) são considerados importantes gastroenterites em todo o mundo, afetando animais e humanos. Na suinocultura, sua epidemiologia ainda não foi totalmente esclarecida, pois pode afetar todas as fases da produção, com suínos sintomáticos (diarréia) ou assintomáticos. O objetivo do nosso estudo foi investigar a ocorrência de Sapovírus em granjas de suínos brasileiras. Um total de 166 amostras fecais de suínos, com diferentes idades, dos estados de Minas Gerais, São Paulo e Mato Grosso foram submetidas a reações de RT-PCR e confirmadas com sequenciamento de nucleotídeos do gene RdRp do Sapovírus. No total, seis (3,61%) amostras foram positivas e quatro delas tinham sequenciamento parcial do gene RdRp, supostamente pertencente ao genogrupo GVII.1, previamente relatado em rebanhos suínos no Brasil.

Palavras-chave: Sapoviroses; Calicivirus; suínos; RdRp gene; genogrupo.

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INTRODUCTION

Sapovirus (SaV) is a non-enveloped linear RNA (+) virus, belonging to Caliciviridae family, presenting *Sapporo virus* as unique viral species, presenting nineteen genogroups (GI-GXIX), additionally subdivided into different genotypes (ICTV, 2020). Besides, these viruses can be found in a wide variety of hosts: humans (GI, GII, GIV, GV), pigs (GIII, GV-GXI), sea lion (GV), mink (GXII), dogs (GXIII), bats (GXIV, GXVI-GXIX), rodents (GXV) (Yinda et al., 2017; Diez-Valcarce et al., 2018).

Although SaV are considered important childhood gastroenteritis worldwide (fecal-oral route), behind only Rotaviruses (Reoviridae) and Noroviruses (Caliciviridae) in terms of occurrence (Gutiérrez et al., 2011; Becker-Dreps et al.,2020), in pig farming, the virus epidemiology has not completely understood (Lauristen et al., 2015; Kuroda et al., 2017; Desselberger, 2019). SaV can cause economic losses in all stages of production, especially in newly weaned piglets, which some animals presenting diarrhea with viral elimination and small intestine lesions (Guo et al., 2001; Martella et al., 2008; Lu et al., 2016). Meanwhile, it was reported the SaV detection in asymptomatic swine (Chao et al., 2012; Valente et al., 2016).

The disease is considered endemic in Brazilian pig herds and described in the States of Mato Grosso do Sul (Barry et al., 2008a), Minas Gerais, Paraná, Santa Catarina, Rio Grande do Sul (Barry et al., 2008b), Rio de Janeiro (Cunha et al., 2010) and the Amazon region (Hernandez et al., 2014), with higher prevalence of the GIII, GVII and GVIII genogroups. In addition, in Paraná, was the circulation of the GIX? group also evident (Valente et al., 2016).

Due the scarcity of data available on SaV in Brazilian pig herds, the aim of our study was investigated SaV occurrence among pig farms from 3 different states, providing a better understanding on virus circulation.

MATERIALS AND METHODS

Here, 166 fecal samples of pigs with different ages (maternity to adult) in multiple Brazilian commercial farms in Minas Gerais, São Paulo and Mato Grosso States were collected between 2017/2018 and tested for SaV.

This study was authorized by the Ethics Committee on the Use of Animals of the Faculty of Veterinary Medicine and Zootechnics under the protocol 4416230217.

Viral RNA was extracted from fecal samples diluted in ultrapure water, proportion of 50% (w/v) and clarified by centrifugation at 2,000 g/15 minutes, using the TRIzol reagent (Invitrogen™, Carlsbad, CA, US) according to manufacturer's instructions. Primers (upCavF 5' TACTCCARGTGGGAYTCCAC 3' and upCavR 5' TGACAATGTAATCATCMCCRT 3') were designed both for reverse transcription (RT-PCR) and polymerase chain reaction (PCR) targeting a 328 nt-long fragment of the Sapovirus RNA-dependent RNA polymerase (RdRp) gene.

For cDNA synthesis, the enzyme M-MLV Reverse Transcriptase (Invitrogen™, US) was used according to the manufacturer's recommendations, and the amplification reaction occurred with 2.5 µL cDNA added to the mix of 1 × PCR Buffer™ (Invitrogen™, US), 0.2 mM of each dNTP, 0.5 µM of forward and reverse primer, 2.0 mM $MgCl_2$, and 1.25 U Platinum Taq Polymerase™ (Invitrogen™, US) and nuclease-free water to obtain the final volume of 25 µL. Thermocycling conditions were: initial denaturation at 94° C for 2 min, followed by 40 cycles at 94° C for 30 s, 48° C for 40 s and 72° C for 1 min, followed by final extension at 72° C for 10 minutes.

Amplicons were purified using ExoSAP-IT® PCR Product Cleanup (USB Products Affymetrix, Cleveland, USA) and BigDye Terminator 3.1™ kit (Applied Biosystems, Foster City, USA), according to manufacturer's protocol and a 3500™ Genetic Analyzer system (Applied Biosystems, Foster City, USA).

RESULTS AND DISCUSSION

In total, 3.61% (6/166) samples were positive for Sapovirus, originated from: Minas Gerais - MG (2), Jaguariúna - SP (3) and Itu - SP (1) (Table 1). Out of 6 samples with positive results, 4 had partial RdRp gene sequenced (Genbank accession numbers MW086610, MW086611, MW086612, and MW086613).

In an attempt to characterize the capsid protein (VP1), all positive samples were tested, according to Kojima et al. (2002), but there was no amplification of this gene.

SaV RdRp gene neighbor joining phylogenetic trees were constructed with MEGA®X software (Kumar et al., 2018), considering 236 positions in final dataset, using maximum composite likelihood as substitution model and 1,000 bootstrap replicates, including representatives from genogroups GI to GXI (Fig. 1). There is no public RdRp sequence data available for SaV genogroups GXII-GXIX, which have been found in species such as mink, dogs, bats and rodents, and hence, not present in the tree.

Our samples clustered together with GVII.1 genogroup (Figure 1), considering the RdRp gene. Sapovirus sequences obtained come from animals aged between maternity and daycare (0 to 70 days). However, Kuroda et al. (2017) describes the SaV infection being common in nursery animals (31 to 70 days) going against the highest frequency in maternity animals (0 to 30 days) (Valente et al., 2016; Barry et al., 2008b).

While the prevalence of SaV in the Brazilian swine herd is not known, surveys of this viral disease present the GIII genogroup as prevalent in all regions of the country already addressed in previous studies (Barry et al., 2008a; Barry et al., 2008b; Cunha et al., 2010; Hernandez et al., 2014; Valente et al., 2016). Our results, putatively defined as GVII.1 genogroup, corroborate the findings of Hernandez et al. (2014) and Cunha et al. (2010), who describes this genogroup in the Amazon Region and Rio de Janeiro State, respectively.

In addition, GVII.1 genogoups are commonly demonstrated in pig farming in different countries, as Belgium (Mauroy et al., 2008), Canada (L´Homme et al., 2009), Denmark (Reuter et al., 2010), Japan (Nakamura et al., 2010). Although the viral classification is based on the VP1 gene, Schuffenecker et al. (2001) have been shown that when comparing different SaVs strains in the gene RdRp, the 3′ORF, capsid overlap ORF and 3′UTR (untranslated region) sequences demonstrate the same classification.

Thereby, using as reference the RdRp gene of porcine Sapovirus GVII.1 (KF241961) (Hernandez et al., 2014), our samples presented 5 different substitutions (13I→V; 20V→I; 23D→N; 31S→N; 65T→V), including the cluster of samples from the city from Jaguariúna-SP which were identical to each other and differed (4 aa / 4.65%) from those of Itu-SP, suggesting the circulation of two different viral subpopulations.

The results with the RdRp detection take in consideration the fact that this gene is highly conserved, given its biological role in viral replication, but can suffer genetic drift mechanism, as recombination process, insertion, deletion and mutations contributing to viral fitness (Wang et al., 2020). To overcome the limitations of partial nucleotide sequencing when genotyping these viruses, the complete genome sequencing may be used, providing a more comprehensive data. In fact, Kuroda et al. (2017) analyzed complete genomes and the phylogenetic tree based on the RdRp region and found that it was essentially similar to the trees obtained from the VP1 and VP2 regions.

In conclusion, this study demonstrates the circulation of SaV in Brazilian pig farms (São Paulo and Minas Gerais States), where they could be detected with a relative low frequency of occurrence and they should be considered when studying diarrhea etiology and its impact on animal health.

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Table 1: Distribution and positivity by location of Brazilian swine production and the age of fecal samples used to detect porcine Sapovirus.

	Maternity	**Nursery**	**Maternity/ Nursery**	**Adults**	**No record**	**Total**
Minas Gerais (MG)	11	30 (2)	-	-	8	**49**
São Paulo (SP)						
Jaguariúna	-	-	38 (3)*	-	-	**38**
Itu	19 (1)*	-	-	-	-	**19**
Pereiras	4	-	-	-	-	**4**
Holambra	8	-	-	-	-	**8**
Cunha	3	1	-	-	-	**4**
Fartura	2	-	-	-	-	**2**
Bragança Paulista	11	-	-	-	-	**11**
Jundiaí		2		3	-	**5**
São Roque			2	10	4	**16**
Mato Grosso (MT)						
Sorriso	3	-	-	-	-	**3**
Castanheira	3	-	-	-	1	**4**
No record[4]	2	1				**3**
TOTAL	**66**	**34**	**40**	**13**	**13**	**166**

[1]: Maternity, pig up to 30 days;
[2]: Nursery, pig between 31 to 70 days;
[3]: Adults, pig over 70 days;
[4]: No record in our database about the location of the sample.
( ): number of positive animal for Sapovirus;
*: sequenced positive

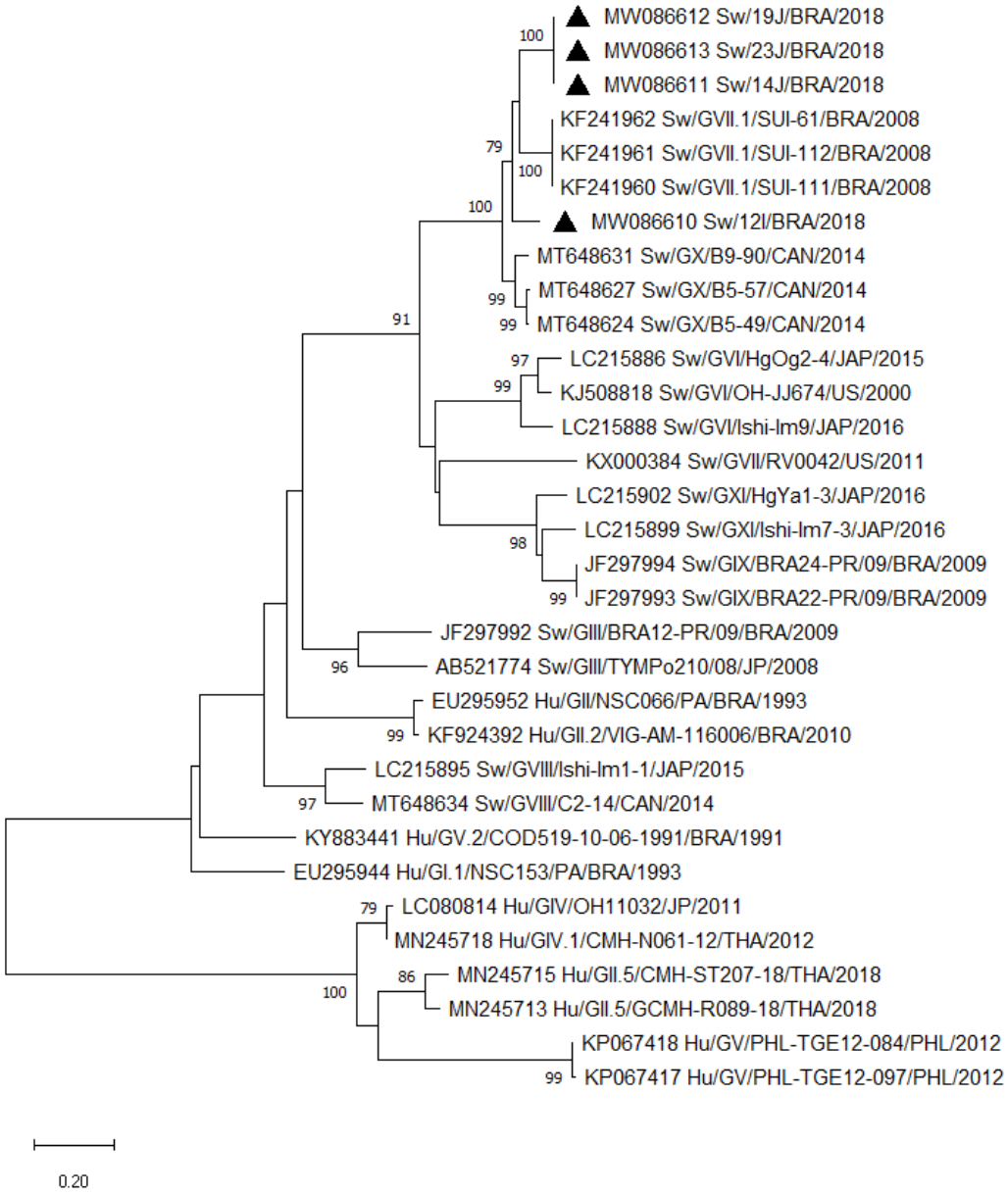

Fig. 1. Nucleotide neighbor-joining distance tree (maximum composite likelihood substitution model) for the partial RdRp Sapovirus gene showing the known genotypes. Strains detected in the present study are preceded by black triangles. The numbers at each node are bootstrap values greater than 75% from 1000 replicates. The bar represents the number of substitutions per site.