



NOTE

Virology

Detection and partial molecular characterization of *Picobirnavirus* in swine from the state of Minas Gerais, Brazil

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ABSTRACT. *Picobirnavirus* (PBV) is a small two-segmented double-stranded RNA (dsRNA) virus that has been identified in diarrheic feces of a large range of animal hosts, including humans. For this reason, PBV has been recognized as an opportunistic agent of gastrointestinal disease. Even under these circumstances, there is a lack of studies regarding this pathogen. Not outstanding, in Brazil, the single description of the PBV occurrence in pigs was provided in the 1980s. Hence, this study aimed to verify the PBV occurrence in Brazilian swine farms and to perform molecular characterization of the identified strains. High genetic variability was found in the analyzed sequences. Further studies comprehending the infection of swine by PBV in Brazilian herds should be performed to provide more accurate information on its epidemiology and to discuss the role of the virus in gastrointestinal diseases.

KEY WORDS: diarrhea, genetic variability, *Picobirnavirus*

J. Vet. Med. Sci.

82(12): 1798–1801, 2020

doi: 10.1292/jvms.19-0565

Received: 17 October 2019

Accepted: 1 October 2020

Advanced Epub:

19 October 2020

Picobirnaviruses (PBVs) are two-segmented double-stranded RNA (dsRNA) viral pathogens [7] belonging to the family *Picobirnaviridae* and the genus *Picobirnavirus*. Although PBVs are classified only into two species, *Human picobirnavirus* and *Rabbit picobirnavirus*, both have been described by infecting several animal hosts from the classes of birds and mammals, in which humans have also been affected. Accordingly, PBVs have been treated as opportunistic enteric pathogens [2], considering that recent studies indicate that PBVs may be viruses of prokaryotes or fungi [8], and a part of the mammalian gut microbiome [14]. The lack of studies regarding PBV infection results in limited knowledge of the viral mechanisms of multiplication in the hosts and its association with gastrointestinal diseases [3].

In 1989, Gatti *et al.* [6] conducted a single study of PBV detection in Brazilian swine. By identifying the virus in the feces of the animals by using polyacrylamide gel electrophoresis (PAGE), the authors found an infection rate of 11.6%. Considering that and other previous reports of PBV infection in swine, along with the association with diarrheic disease, the present study investigated the PBV occurrence in three swine farms in Minas Gerais State, Brazil. Additionally, a part of the isolates was submitted to partial molecular characterization.

The PBV occurrence in swine was assayed in three farms, herein named farm A, farm B, and farm C. The feces samples were collected from three animal categories: sows in gestation, sows in the farrowing period, and suckling piglets. In farm A, 138 animals were sampled: 64 sows in gestation, 25 sows in the farrowing period, and 49 suckling piglets. In farm B, the total number of sampled animals was 46: 11 sows in gestation, 20 sows in the farrowing period, and 15 suckling piglets. Outbreaks of diarrheic disease occurred in the category of suckling piglets in farms A and B, which resulted in high mortality in this population. In farm C, 44 animals were sampled: 18 sows in gestation, 9 sows in the farrowing period, and 17 suckling piglets. The suckling piglets of farm C did not present any diarrheic disease. The sample size for each farm was calculated using the OpenEpi software [10].

For viral detection, all fecal samples were submitted to RT-PCR assays, amplifying a partial sequence (201 bp) of the PBV

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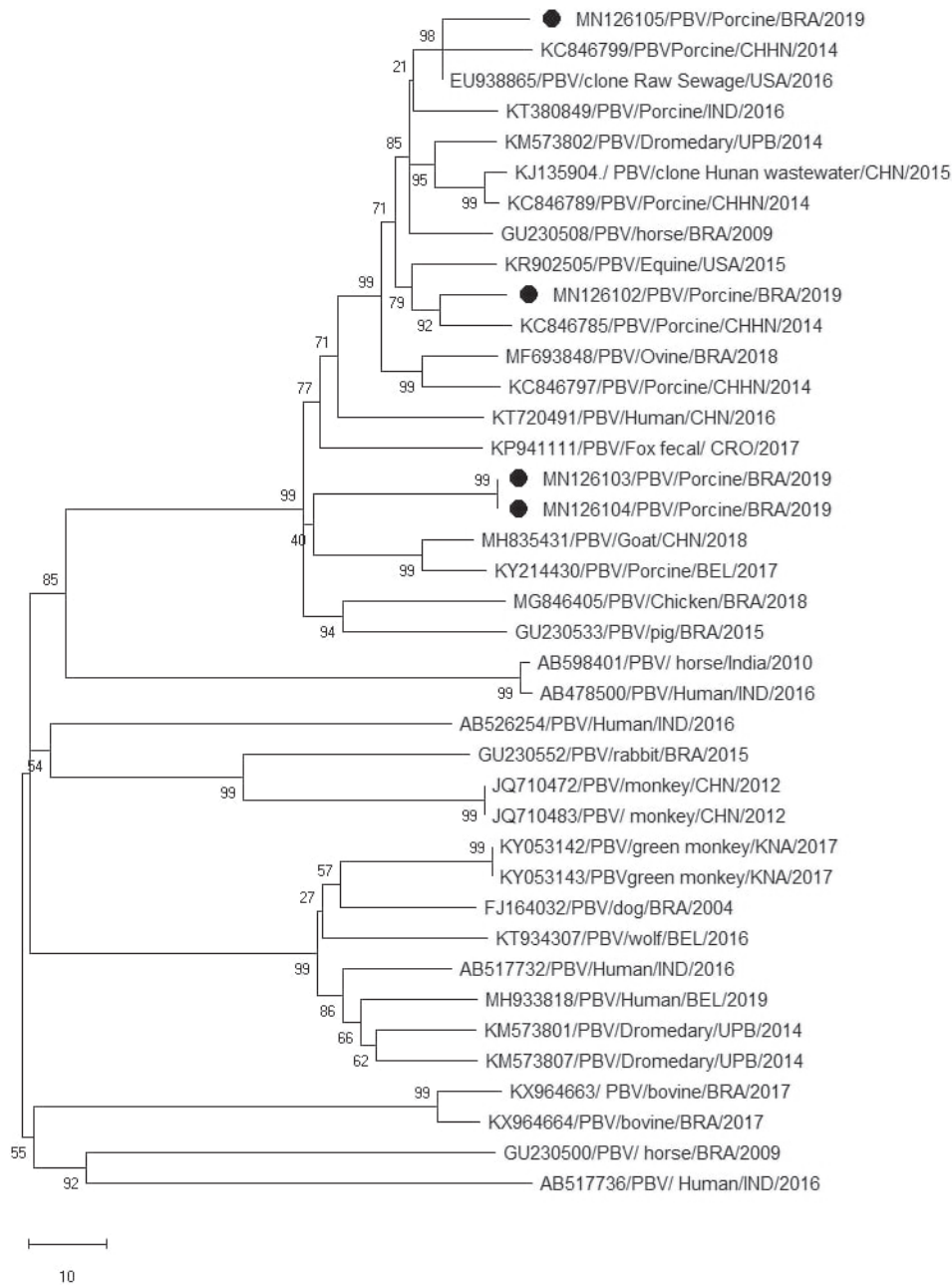


Fig. 1. Phylogenetic analysis of *Picobirnavirus* based on the RdRp gene of 210 bp. The phylogenetic tree was constructed based on the Neighbor-Joining method. For the Kimura evolutionary model, we applied two parameters with 1,000 bootstrap repetitions.

RdRp gene. Gene amplification was performed using the primer pairs for PBVs from genogroup I, previously described by Rosen *et al.* (2000) [11]. As an internal control, a region (107 bp) of swine 18S ribosomal RNA was amplified using the primer pairs designed by Assao *et al.* (2019) [1].

The RT-PCR products of the fourteen positive samples obtained in this study were cloned into *Escherichia coli* DH5 α , using the CloneJET PCR cloning kit (Thermo Fischer Scientific, Waltham, MA, USA), and submitted to nucleotide sequencing. BLAST analysis (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) confirmed that the RT-PCR products belonged to PBVs; however, the sequences were not sufficient quality for phylogenetic analysis, except for four strains. The obtained sequences were compared with the PBV sequences from swine available in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) and aligned with sequences of PBV isolated from diverse hosts.

Farm A was the only property where animals tested positive for PBV, with a prevalence rate of 10.14% (14/138). In this farm, the categories of sows in gestation and sows in the farrowing period presented a PBV prevalence rate of 4% (1/25) and 10.9% (7/64), respectively. No animals belonging to these two categories had diarrhea. In the category of suckling piglets, the virus prevalence rate was 12.2% (6/49), and 8.16% (4/49) of the animals presented diarrheic disease. Asymptomatic suckling piglets represented 4.08% (2/46). As reported by Ganesh *et al.* [4] and Fregolente *et al.* [2], PBV prevalence in different mammalian hosts ranges from 12.5 to 14.3%. Particularly in pigs, a recent study conducted by Kylla *et al.* [9] found 11.5% of PBV prevalence as the most significant rate in diarrheal animals.

Table 1. Percentage of *Picobirnaviruses* (PBVs) identity between sequences obtained from the same farm from Minas Gerais State, Brazil

	MN126102	MN126103	MN126104	MN126105
MN126102	-	69.03	69.19	81.31
MN126103	69.03	-	100	69.50
MN126104	69.19	100	-	67.00
MN126105	81.31	69.50	67.00	-

MN126102/PBV/Porcine/BRA/2019; MN126103/PBV/Porcine/BRA/2019; MN126104/PBV/Porcine/BRA/2019; MN126105/PBV/Porcine/BRA/2019. The identity of sequenced PBVs was calculated using Geneious software (www.geneious.com).

Table 2. Percentage of identity between the sequences obtained in this study and *Picobirnavirus* sequences deposited in GenBank from Brazil and other countries

	KJ650569 India	KY214430 Belgium	KC846785 China	KC846799 China	KT380849 India	KC846797 China	KC846786 China	GU230533 Brazil	KC846789 China
MN126102	70.20	71.21	88.88	80.80	86.86	81.81	72.22	68.18	81.81
MN126103	72.08	72.58	66.49	65.99	69.54	69.03	72.08	68.02	67.51
MN126104	72.36	72.86	66.36	65.82	69.84	68.84	71.85	68.34	67.83
MN126105	67.67	67.67	80.30	85.85	83.33	79.29	75.25	67.67	84.34

The identity of the isolates was calculated using Geneious software (www.geneious.com).

Because PBV can infect humans, employees from farm A were sampled to investigate the presence of the virus in their feces. Fifteen feces samples were collected and analyzed by RT-PCR. Although the employees had been in contact with PBV-infected animals, all tested negative.

The category of suckling piglets belonging to farm A—the single farm positive for PBV—had a mortality rate of 8.32% due to the diarrhea outbreak. Both the diarrheic disease outbreak and the collection of samples occurred in February 2017. In this period, infection by *E. coli* was detected in the piglets presenting diarrhea; thus, these animals were treated with neomycin. After the antibiotic therapy, the piglets recovered from the diarrheic clinical status. To verify the possibility of PBV association with the diarrheic disease in the piglets, a second collection of feces was performed after the outbreak, in October 2017, only in farm A: 73 animals from the three categories were sampled. None of these samples tested positive for PBV in the RT-PCR assay. Under these circumstances, it is possible to infer the association between *E. coli* and PBV infections in the diarrheic outbreak in farm A. The positive results for PBV may be related to a co-infection that allowed the viral circulation on the farm.

Four of the 14 samples positive for PBV were submitted to nucleotide sequencing of a ~200-bp fragment. The sequences were deposited in GenBank under accession numbers MN126102 to MN126105. Analyzed samples were distantly positioned in the phylogenetic tree, presenting a heterogeneous clustering profile. Interestingly, MN126102 is positioned close to an equine PBV (KC846785), while MN126103 and MN126104 are in a close clade of a PBV identified in goat (MH83543). The MN126105 is positioned close to a PBV identified in sewage sludge containing human waste (Fig. 1). Gatti *et al.* [6] report that PBV can infect several hosts. Herein, we identified swine PBVs related to goats, equines, and humans. Our results differ from previous findings described by Wilburn *et al.* [13], in which sequenced samples from farms located in the same region in Thailand were positioned in the same clade in the phylogenetic tree.

Nucleotide alignment detected a considerable identity variability between PBV-sequenced samples. When compared with each other, the sequences obtained in this study presented identity profiles ranging from 69.03 to 81.31% for MN126102, 69.03 to 100% for MN126103, 67.00 to 100% for MN126104, and 67.00 to 81.31% for MN126105 (Table 1). These data show that PBV demonstrated a high genetic diversity, although the samples were taken from the same pig farm.

Considering that swine is a target host for reassortment and recombination by RNA segmented viruses, as in the case of the Influenza A virus [12], the similarity of the isolates obtained in this study was compared with all PBV sequences available in GenBank. When compared with the isolate GU230533 from Brazil, MN126102, MN126103, MN126104, and MN126105 presented similarities of 68.18, 68.03, 68.34, and 67.67%, respectively. The sequences were also compared with other PBV sequences from Asia and Europe. The identity of the isolates ranged from 65.82 to 88.88% with PBV sequences identified in China (KC846785, KC846786, KC846789, KC846797, and KC846799). In comparison with PBV sequences from India, an identity variability ranging from 69.54 to 86.86% was observed, while when compared with a PBV from Belgium, the identity profile ranged from 67.67 to 72.86% (Table 2). These results show that PBV possesses a high genetic diversity in swine hosts from diverse countries. This feature can lead to viral evolution, as previously described by Ganesh *et al.* [5].

Gatti *et al.* [6] published the singular description of PBV in Brazilian pigs. Thus, in our study, we describe the PBV occurrence in Brazil after 30 years. Besides virus detection, the molecular characterization of the samples evidenced a high genetic diversity of PBV in pigs. Complementary studies regarding PBV detection in Brazilian swine herds should be developed to estimate the virus distribution and its relation to gastrointestinal disease in piglets.

COMPETING INTERESTS. This work was financially supported by FAPEMIG, CAPES, and CNPq. The authors declare that they have no competing interests.

ACKNOWLEDGMENT. We thank Professor Ricardo Seiti Yamatogi for contributing to the bioinformatic analyses.

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