

Diarrhea outbreaks in suckling piglets due to rotavirus group C single and mixed (rotavirus groups A and B) infections¹

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ABSTRACT. Lorenzetti E., Stipp D.T., Possatti F., Campanha J.E.T., Alfieri A.F. & Alfieri A.A. 2014. **Diarrhea outbreaks in suckling piglets due to rotavirus group C single and mixed (rotavirus groups A and B) infections.** *Pesquisa Veterinária Brasileira* 34(5):391-397. Laboratory of Animal Virology, Department of Veterinary Preventive Medicine, Universidade Estadual de Londrina, Rodovia Celso Garcia Cid, Campus Universitário, Cx. Postal 10011, Londrina, PR 86057-970, Brazil. E-mail: alfieri@uel.br

Porcine group A rotavirus (PoRVA) is a major cause of neonatal diarrhea in suckling and recently weaned piglets worldwide. The involvement of non-group A rotavirus in cases of neonatal diarrhea in piglets are sporadic. In Brazil there are no reports of the porcine rotavirus group C (PoRVC) as etiologic agent of the diarrhea outbreaks in piglets. The aim of this study was to describe the identification of rotavirus group C in single and in mixed infection with rotavirus groups A and B in three neonatal diarrhea outbreaks in suckling (≤ 21 -day-old) piglets, with 70% to 80% and 20% to 25% of morbidity and lethality rates, respectively, in three pig herds located in the state of Santa Catarina, Brazil. The diagnosis of PoRV in the diarrheic fecal samples was performed using polyacrylamide gel electrophoresis (PAGE) to identify the presence of porcine rotavirus groups A, B (PoRVB), and C, and by RT-PCR (PoRVA and PoRVC) and semi-nested (SN)-PCR (PoRVB) to partially amplify the VP4 (VP8*)-VP7, NSP2, and VP6 genes of PoRVA, PoRVB, and PoRVC, respectively. One RT-PCR (PoRVA and PoRVC) and SN-PCR (PoRVB) product of each group of rotavirus of each diarrhea outbreak was submitted to nucleotide (nt) sequence analysis. Based on the PAGE technique, 4 (25%) and 1 (6.25%) of the 16 diarrheic fecal samples evaluated in the first outbreak presented PoRVA and PoRVC electropherotype, respectively, and 11 (68.75%) were negative. In the second outbreak, 3 (42.85%) of the 7 fecal samples evaluated presented PoRVA electropherotype, and in 3 (42.85%) and in 1 (14.3%) fecal samples were detected inconclusive and negative results, respectively. Three (30%) of the 10 fecal samples of the third outbreak presented PoRVC electropherotype; 5 (50%) and 2 (20%) samples showed negative and inconclusive results, respectively. Based on the RT-PCR and SN-PCR assays in the first neonatal diarrhea outbreak, PoRVC was detected in 13 (81.2%) of the 16 diarrheic fecal samples evaluated. PoRVC single infection was identified in 4 (25%) of these samples and mixed infections with PoRVA and PoRVB in 9 (56.2%) fecal samples. All of the seven diarrheic fecal samples evaluated from the second neonatal diarrhea outbreak were positive for PoRVC, whereas its mixed infection with other PoRV groups was detected in 4 (57.2%) samples. In the third outbreak, PoRVC in single infection was detected in all of the 10 diarrheic fecal samples analyzed. In the nt sequence analysis, the PoRVA strains of the first and second outbreaks demonstrated higher nt identity with G4P[6] and G9P[23] genotypes, respectively. The PoRVB strains (first and second outbreaks) and the PoRVC

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strains (first, second, and third outbreaks) showed higher nt identity and clustered in the phylogenetic tree with PoRVB and PoRVC strains that belong to the N4 and I1 genotypes, respectively. This is the first description in Brazil of the involvement of PoRVC in the etiology of diarrhea outbreaks in suckling piglets. The results of this study demonstrated that PoRVC, in both single and mixed infections, is an important enteropathogen involved in neonatal diarrhea outbreaks in piglets and that the use of more sensitive diagnostic techniques allows the identification of mixed infections involving two or even three groups of PoRV, which may be more common than previously reported.

INDEX TERMS: Diarrhea, rotaviruses, RT-PCR, mixed infection, swine.

RESUMO.- [Surto de diarréia em leitões lactentes por rotavírus grupo C em infecções singulares e mistas (rotavírus grupos A e B).] O rotavírus suíno grupo A (PoRVA) é uma das principais causas de diarréia neonatal em leitões lactentes e recém-desmamados em todo o mundo. As descrições do envolvimento de rotavírus não-grupo A em quadros de diarréia neonatal em leitões são esporádicas. No Brasil não há relatos do envolvimento do rotavírus suíno grupo C (PoRVC) na etiologia dos surtos de diarréia em leitões. O objetivo deste estudo foi descrever a identificação de rotavírus grupo C em infecções singulares e mistas com os rotavírus grupos A e B em três surtos de diarréia neonatal em leitões lactentes (≤ 21 dias de idade), com taxas de morbidade de 70% a 80% e de letalidade de 20% a 25%, em três rebanhos suínos localizados no estado de Santa Catarina, Brasil. O diagnóstico de PoRV nas amostras de fezes diarréicas foi realizado por eletroforese em gel de poli-acrilamida (PAGE) para identificar a presença dos grupos A, B (PoRVB), e C de rotavírus suíno e por RT-PCR (PoRVA e PoRVC) e semi-nested (SN)-PCR (PoRVB) com a amplificação parcial dos genes VP4 (VP8*)-VP7, NSP2 e VP6 de PoRVA, PoRVB e PoRVC, respectivamente. Um produto de RT-PCR (PoRVA e PoRVC) e SN-PCR (PoRVB) de cada grupo de rotavírus de cada um dos três surtos de diarréia foi submetido à análise da sequência de nucleotídeos (nt). Com base na técnica de PAGE, 4 (25%) e 1 (6,25%) das 16 amostras de fezes analisadas no primeiro surto apresentaram eletrotipo característico de PoRVA e PoRVC, respectivamente, e 11 (68,75%) amostras fecais foram negativas. No segundo surto, 3 (42,85%) das 7 amostras de fezes analisadas apresentaram perfil eletroforético de PoRVA; em 3 (42,85%) e em 1 (14,3%) amostras de fezes foram detectados resultados inconclusivos e negativos, respectivamente. Três (30%) das 10 amostras de fezes do terceiro surto apresentaram eletrotipo característico de PoRVC; 5 (50%) e 2 (20%) amostras apresentaram resultados negativos e inconclusivos, respectivamente. Com base nos resultados da RT-PCR e SN-PCR, no primeiro surto de diarréia neonatal o PoRVC foi detectado em 13 (81,2%) das 16 amostras de fezes diarréicas analisadas, sendo que em 4 (25%) amostras foi identificada infecção singular e em 9 (56,2%) amostras infecção mista com PoRVA e PoRVB. Todas as sete amostras de fezes diarréicas provenientes do segundo surto de diarréia neonatal foram positivas para o PoRVC, enquanto infecções mistas com outros grupos de PoRV foram detectadas em 4 (57,2%) amostras. No terceiro surto o PoRVC foi detectado em infecção singular em todas as dez amostras de fezes diarréicas analisadas. Na análise da sequência de nt as cepas de PoRVA do primeiro e segundo surtos demons-

traram maior identidade de nt com os genótipos G4P[6] e G9P[23], respectivamente. As cepas de PoRVB (primeiro e segundo surtos) e as cepas de PoRVC (primeiro, segundo e terceiro surtos) mostraram maior identidade de nt com cepas de PoRVB e PoRVC que pertencem aos genótipos N4 e I1, respectivamente. Esta é a primeira descrição realizada no Brasil do envolvimento de PoRVC na etiologia de surtos de diarréia em leitões lactentes. Os resultados deste estudo demonstram que o PoRVC, tanto em infecções singulares quanto em infecções mistas, é um importante enteropatógeno envolvido em surtos de diarréia neonatal em leitões e que o uso de técnicas de diagnóstico mais sensíveis permite caracterizar que infecções mistas, com dois ou até mesmo com três grupos de PoRV, podem ser mais comuns do que anteriormente relatado.

TERMOS DE INDEXAÇÃO: Diarréia, rotavírose, RT-PCR, infecção mista, suínos.

INTRODUCTION

Neonatal diarrhea is the most important health problem in suckling and recently weaned piglets throughout the world (Tubbs et al. 1993). Diarrhea is the result of the combination of several factors, including infectious agents, host immunity, and management procedures (Wittum et al. 1995). Neonatal diarrhea increases the morbidity and mortality rates in the maternity units and nurseries of pig farms worldwide, causing direct and indirect economic losses to the pig industry (Dewey et al. 1995, Wittum et al. 1995).

Newborn piglets are susceptible to infection by several enteric microorganisms, including bacteria (enterotoxigenic *Escherichia coli* and *Clostridium perfringens* type C), protozoans (*Cryptosporidium* spp and *Isospora suis*), and viruses (rotavirus, coronavirus, and calicivirus) (Barry, Alfieri & Alfieri 2008, Zlotowski et al. 2008, Jeong et al. 2009, Linares et al. 2009, Médici et al. 2011, Marthaler et al. 2012).

Rotaviruses are the main viral etiologic agents of diarrhea in children and the young animals of a wide variety of mammalian and avian species throughout the world (Estes & Kapikian 2007). Rotaviruses belong to the *Reoviridae* family and are characterized by double-stranded RNA (dsRNA) containing 11 genomic segments and by capsids composed of three concentric protein layers. Based on the antigenic properties of the capsid protein in the middle layer (VP6), rotaviruses are classified into 5 distinct groups (A to E), and two tentative species (F and G) (ICTV 2013). Recently, a new group (H) was described by Matthijnssens et al. (2012). The A, B, C, and H rotavirus (RV) groups have been found in humans and animals, whereas groups D-G

have been identified only in animals (Estes & Kapikian 2007). However, rotavirus group A (RVA) is the main group of rotaviruses that cause diarrhea in human (infants) and young animal hosts (Estes & Kapikian 2007).

Porcine group A rotavirus (PoRVA) infections are most frequently identified in episodes of diarrhea in piglets worldwide, including in Brazil (Linares et al. 2009, Halaihel et al. 2010, Médici et al. 2011, Lorenzetti et al. 2011); however, the involvement of porcine rotavirus groups B and C (PoRVB and PoRVC) in weaning and post-weaning piglets with diarrhea has also been reported mainly in transversal epidemiological studies (Magar et al. 1991, Zlotowski et al. 2008, Médici et al. 2010a, 2010b, Médici et al. 2011, Marthaler et al. 2012).

RVC has been associated with enteritis in humans, pigs, calves, dogs, and ferrets, and this group of RV is considered an important emerging agent of viral diarrhea in these species (Torres-Medina 1987, Teixeira et al. 1998, Otto et al. 1999, Médici et al. 2011, Park et al. 2011). In Brazil, RVC has been identified as a cause of diarrhea in human and animal hosts (Teixeira et al. 1998, Médici et al. 2010b, 2011).

Most studies have reported the involvement of a single PoRV group with the occurrence of diarrhea in piglets, although some studies showed infection with a mixed of PoRV groups in piglets with clinical signs of diarrhea (Kim et al. 1999, Barreiros et al. 2003, Martella et al. 2007, Linares et al. 2009, Médici et al. 2011). A combination of different groups of PoRVs can intensify the severity of the piglets' diarrhea, although mixed infections have been detected in piglets without these clinical signs (Marthaler et al. 2012).

According to the two outer capsid proteins, VP7 and VP4, the RVA is classified into G (glycoprotein) and P (protease-sensitive) genotypes, respectively (Estes & Kapikian 2007). To date, 27 G and 37 P genotypes of RVA have been described in human and animal hosts (Matthijnssens et al. 2011, Trojnar et al. 2013). Based on the VP6 gene, the RVC strains have been classified into three genotypes, designated I1, I2, and I3. The genotypes I1, I2, and I3 are composed of porcine, human, and bovine strains, respectively (Yamamoto et al. 2011). The NSP2 gene of RVB was recently classified into four genotypes, designated N1, N2, N3, and N4. The genotype N1 is composed by human and murine strains, the genotype N2 by porcine and bovine strains and the genotypes N3 and N4 are formed only by porcine strains (Suzuki et al. 2012).

The aim of this study was to describe the identification of rotavirus group C in single and in mixed infection with rotavirus groups A and B in three neonatal diarrhea outbreaks in suckling piglets in Brazilian pig herds.

MATERIALS AND METHODS

The neonatal diarrhea outbreaks occurred in June 2007 (first), November 2011 (second), and August 2013 (third) in three different pig farms located in the state of Santa Catarina, south of Brazil, and exhibited 70% to 80% and 20% to 25% of morbidity and lethality rates, respectively. These complete-cycle pig farms of medium size (\pm 400 sows) had a confinement system (all-in/all-out) and good nutritional and health management practices including sows vaccination against etiological agents of neona-

tal diarrhea such as *Escherichia coli* (K88, K99, 987P, and F41), PoRVA (genotypes G4 and G5), and *Clostridium perfringens* type C. Sampling consisted of 33 diarrheic fecal samples with a watery consistency from suckling (\leq 3-week-old) piglets, with 16 fecal samples coming from the first diarrhea outbreak, 7 samples from the second, and 10 samples from the third. All diarrheic fecal samples analyzed in this study were collected from live piglets.

Viral dsRNA was obtained from the fecal samples using a combination of phenol/chloroform/isoamyl alcohol (25:24:1) and silica/guanidinium isothiocyanate nucleic acid extraction methods, modified as described by Alfieri et al. (2006). The OSU strain amplified in MA104 cells was used as the positive control for PoRVA and two porcine fecal samples with electropherotype characteristics of group B or C that were confirmed by nucleotide sequence analysis were used as positive controls for PoRVB and PoRVC, respectively (accession numbers: EF577257 and EU002783) (Médici et al. 2010a,b). Aliquots of Tris-Ca²⁺ buffer were used as a negative control. Positive and negative controls were used for all of the viral RNA extraction, RT-PCR, and semi-nested (SN)-PCR procedures.

All diarrheic fecal samples were tested by 7.5% polyacrylamide gel electrophoresis (PAGE) followed by silver staining as described by Herring et al. (1982) and Pereira et al. (1983), respectively, to verify the presence of porcine rotavirus groups A, B, and C.

The diagnoses of PoRV in the diarrheic fecal samples were also conducted using RT-PCR for RVA, with primers that amplified 876-bp and 1,062-bp products from the VP4 (VP8*) and VP7 genes, respectively (Gouvea et al. 1990, Gentsch et al. 1992). SN-PCR was performed for RVB using primers that amplified a 434-bp product of the NSP2 gene (Gouvea et al. 1991), and RT-PCR was performed for RVC using primers that amplified a 270-bp product of the VP6 gene (Alfieri et al. 1999).

The RT-PCR (PoRVA and PoRVC) and SN-PCR (PoRVB) products were analyzed using 2% agarose gel electrophoresis, staining with ethidium bromide, and visualization under UV light.

One RT-PCR (PoRVA and PoRVC) and SN-PCR (PoRVB) product of each group of rotavirus of each diarrhea outbreak was purified using the GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK) and quantified using a Qubit[®] Fluorometer (Invitrogen Life Technologies, Eugene, OR, USA). The DNA sequences were obtained using a BigDye[®] Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an ABI3500 Genetic Analyzer sequencer (Applied Biosystems, Foster City, CA, USA). Sequence quality analyses were conducted using Phred and CAP3 software (<http://asparagin.cenargen.embrapa.br/phph/>). Sequence similarity searches were performed using the BLAST program (<http://blast.ncbi.nlm.nih.gov/>), and identity matrix analyses were performed using BioEdit version 7.1.3.0. Phylogenetic trees were constructed using the neighbor-joining method and the Kimura two-parameter model in MEGA software version 5.05. The bootstrap probabilities of each node were calculated using 1,000 replicates.

The sequences of PoRVA, PoRVB, and PoRVC described in the present study have been deposited in the GenBank database under the accession numbers: KF991082 to KF991090.

RESULTS

In PAGE analysis, 11 (68.75%), 4 (25%), and 1 (6.25%) of diarrheic fecal samples evaluated in the first outbreak presented negative results, PoRVA, and PoRVC electropherotype, respectively (Table 1). In the second outbreak, 3 (42.85%), 3 (42.85%), and 1 (14.3%) of the fecal samples presented PoRVA electropherotype, inconclusive, and negative results, respectively (Table 1). Five (50%) fecal samples of the third outbreak were negative in PAGE, 3 (30%)

Table 1. Porcine rotavirus (PoRV) groups identified by PAGE technique, RT-PCR and SN-PCR assays in three diarrhea outbreaks in suckling pigs

Infection	PoRV group	Outbreaks / Number of positive samples (%)							
		PAGE				RT-PCR/SN-PCR			
		First	Second	Third	Total (%)	First	Second	Third	Total (%)
Single	A	4 (25)	3 (42.85)	0	7 (21.2)	1 (6.25)	0	0	1 (3)
	B	0	0	0	0	0	0	0	0
	C	1 (6.25)	0	3 (30)	4 (12.1)	4 (25)	3 (42.8)	10 (100)	17 (52)
Mixed	A + B	0	0	0	0	0	0	0	0
	A + C	0	0	0	0	6 (37.5)	2 (28.6)	0	8 (24)
	B + C	0	0	0	0	1 (6.25)	0	0	1 (3)
	A + B + C	0	0	0	0	2 (12.5)	2 (28.6)	0	4 (12)
Subtotal		5 (31.25)	3 (42.85)	3 (30)	11 (33.3)	14 (87.5)	7 (100)	10 (100)	31
Inconclusive#		0	3 (42.85)	2 (20)	5 (15.2)	0	0	0	0
Negative*		11 (68.75)	1 (14.3)	5 (50)	17 (51.5)	2 (12.5)	0	0	2 (6)
Total		16	7	10	33	16	7	10	33

PAGE = polyacrylamide gel electrophoresis; RT-PCR = reverse transcriptase-polymerase chain reaction; SN-PCR = semi-nested PCR; # inconclusive samples in PAGE; * negative samples in PAGE, RT-PCR, and SN-PCR.

exhibited PoRVC electropherotype, and 2 (20%) fecal samples presented inconclusive results (Table 1).

Based on the RT-PCR and SN-PCR assays, 14 (87.5%) of the 16 diarrheic fecal samples evaluated in the first neonatal diarrhea outbreak were PoRV-positive. PoRVC was the most prevalent group, being identified in 13 (81.2%) of these fecal samples. Single infections of PoRVC were identified in 4 (25%) of the fecal samples and mixed infections with other PoRV groups were identified in 9 (56.2%) of the samples (Table 1). In the second outbreak, PoRV was identified in all ($n=7$) of the fecal samples analyzed. PoRVC was identified in all of fecal samples from this diarrhea outbreak, as a single infection in 3 (42.8%) samples and as a mixed infection with other PoRV groups in 4 (57.2%) of the fecal samples (Table 1). All of the 10 diarrheic fecal samples evaluated from the third outbreak were positive for PoRVC in single infection (Table 1).

Sequence analysis of the VP7 gene of RVA and comparison with the 27 known G genotypes revealed that the BRA900/2007-Po (first outbreak) PoRVA strain displayed 83% and 84.2% nucleotide (nt) identity with the human (ST3 strain) and porcine (Gottfried strain) prototypes of the G4 genotype, respectively, and the BRA82/2011-Po (second outbreak) PoRVA strain displayed 88.4% and 91% nt identity with the human (WI61 strain) and porcine (JP29-6 strain) prototypes of the G9 genotype, respectively (Fig.1).

Comparison of the VP4 gene (VP8* subunit) of RVA with the 37 known P genotypes revealed that the BRA900/2007-Po (first outbreak) PoRVA strain exhibited 88.4% and 79.6% nt identity with the human (ST3 strain) and porcine (Gottfried strain) prototypes of the P[6] genotype, respectively, and the BRA82/2011-Po (second outbreak) PoRVA strain exhibited 91.5% nt identity with the porcine (A34 strain) prototype of the P[23] genotype (Fig.2).

The NSP2 sequence of the two Brazilian wild-type PoRVB strains of the first and second outbreaks (BRA900/2007-Po and BRA82/2011-Po) displayed 79.3 to 90.5 nt identity with the PoRVB strains that belong to the N4 genotype and showed 100% nt identity each other. These sequences exhibited high nt identity with the Brazilian PoRVB strains (89% to 90.5%) (Fig.3).

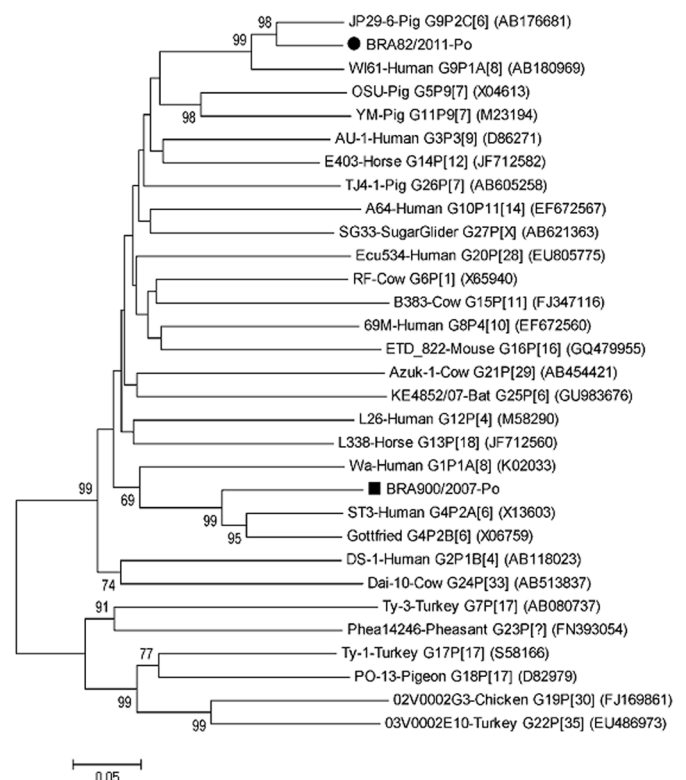


Fig.1. Phylogenetic tree based on the partial nucleotide sequences (nt 103-1020) of the VP7 gene from PoRVA strains described in this study and those of representative strains of each 27 genotypes recognized thus far. The tree was constructed using neighbor-joining and Kimura two-parameter as a nucleotide substitution model. The bootstrap values are shown at the branch nodes (values < 50% not shown). The Brazilian PoRVA strains from the first and second diarrhea outbreaks are marked with a filled square and a filled circle, respectively.

Sequence analysis of the VP6 gene of the three Brazilian wild-type PoRVC strains of the three outbreaks (BRA905/2007-Po, BRA82/2011-Po, and BRA1437/2013-Po) displayed 83.8% to 96.9% nt identity with the PoRVC strains that belong to the I1 genotype. The VP6 sequence of the three PoRVC strains described in this study displayed

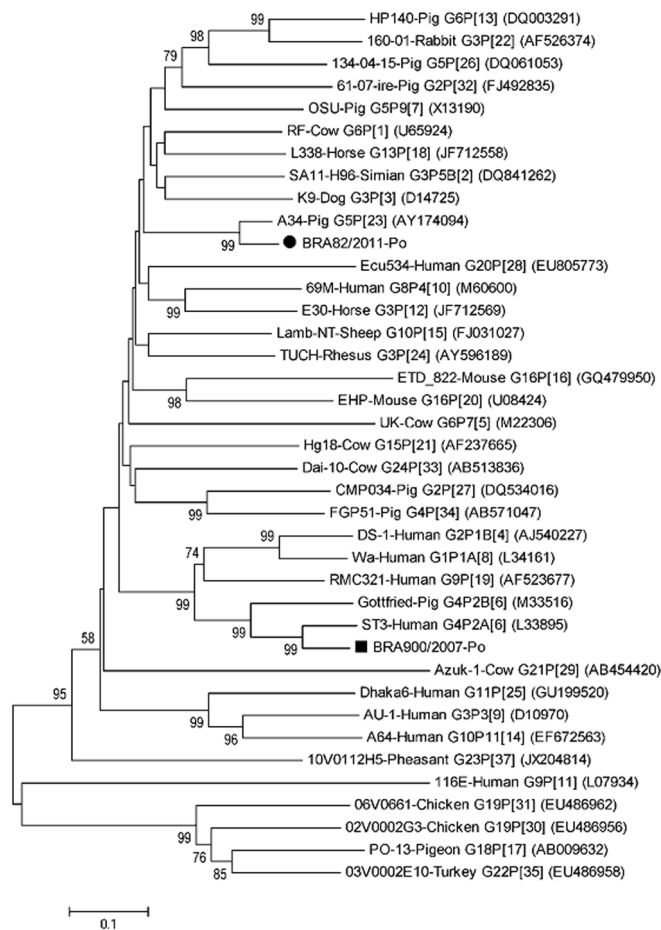


Fig.2. Phylogenetic tree based on the partial nucleotide sequences (nt 60-778) of the VP4 (VP8*) gene from PoRVA strains described in this study and those of representative strains of each 37 genotypes recognized thus far. The tree was constructed using neighbor-joining and Kimura two-parameter as a nucleotide substitution model. The bootstrap values are shown at the branch nodes (values < 50% not shown). The Brazilian PoRVA strains from the first and second diarrhea outbreaks are marked with a filled square and a filled circle, respectively.

high (85.3% to 96.9%) nt identity with the Brazilian PoRVC strains (Fig.4).

DISCUSSION

Identification of enteric pathogens as causative agents of diarrhea in piglets has been conducted worldwide. The majority of the surveys evaluated only one pathogen, resulting in insufficient information about the frequency and severity of diarrhea caused by concomitant infections with multiple enteropathogens (Barreiros et al. 2003, Linares et al. 2009); however, more recently some studies have identified several different enteric agents in pig fecal samples (Martella et al. 2007, Jeong et al. 2009, Halaihel et al. 2010, Médici et al. 2011, Marthaler et al. 2012).

In Brazil, the association of different PoRV groups in piglet enteritis has been reported in transversal epidemiological studies carried out in distinct pig farms, not all of which were involved in a diarrhea outbreak (Médici et al. 2011). Considering the variety of enteric pathogens that

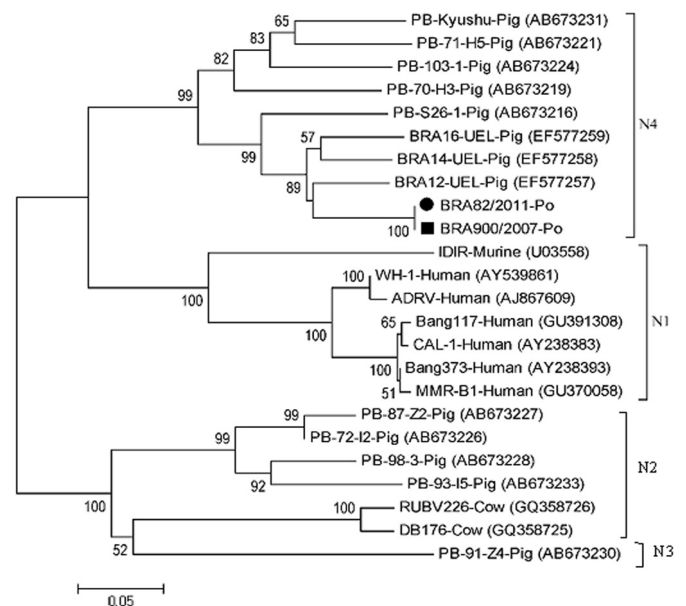


Fig.3. Phylogenetic tree based on the partial nucleotide sequences (nt 58-405) of the NSP2 gene from PoRVB strains described in this study and representative strains of the 4 genotypes recognized thus far. The tree was constructed using neighbor-joining and Kimura two-parameter as a nucleotide substitution model. The bootstrap values are shown at the branch nodes (values < 50% not shown). The Brazilian PoRVB strains from the first and second diarrhea outbreaks are marked with a filled square and a filled circle, respectively.

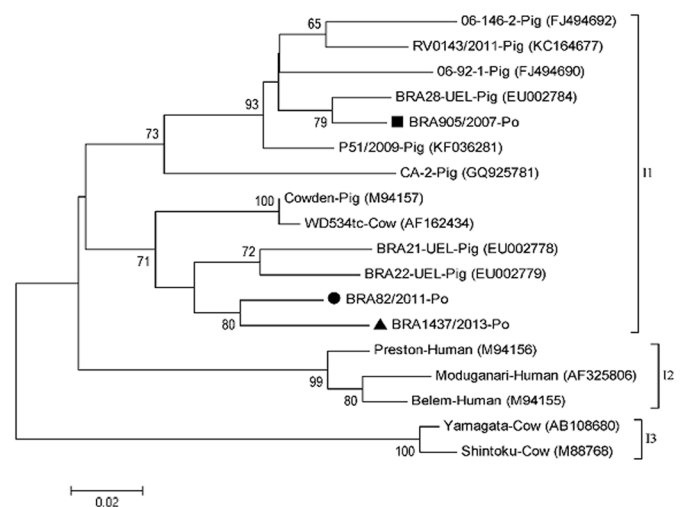


Fig.4. Phylogenetic tree based on the partial nucleotide sequences (nt 53-250) of the VP6 gene from PoRVC strains described in this study and representative strains of 3 the genotypes recognized thus far. The tree was constructed using neighbor-joining and Kimura two-parameter as a nucleotide substitution model. The bootstrap values are shown at the branch nodes (values < 50% not shown). The Brazilian PoRVC strains from the first, second, and third diarrhea outbreaks are marked with a filled square, a filled circle, and a triangle filled, respectively.

can be isolated from the fecal samples of suckling piglets, the lack of recent multiple etiological studies is compromising the success of preventive measures.

PoRVC has been described as a cause of diarrhea outbreaks in weaning and post-weaning pigs, which in some cases leads to substantial morbidity rates (Morin et al. 1990, Kim et al. 1999, Martella et al. 2007). Single infections of PoRVC have been detected in piglets, but this PoRV group was more frequently detected in piglets in association with other PoRV groups or with other enteric viruses (Médici et al. 2011, Marthaler et al. 2012). Martella et al. (2007) reported that in piglets, PoRVC was more frequently detected in association with other viruses, such as PoRVA and calicivirus, than as a single infectious agent.

The high morbidity (70% to 80%) and lethality (20% to 25%) rates observed in the three neonatal diarrhea outbreaks reported in this study might have involved a pathogenic PoRVC strain in the third outbreak and by the simultaneous infections with different groups of PoRV in the other two outbreaks. Dual infections involving bovine RVA and RVC have been reported in neonatal diarrhea episodes in gnotobiotic calves, generally with intestinal lesions, such as villous atrophy and lymphoid hyperplasia, which were more severe than the single-infection lesions (Chang et al. 1999).

The lower frequency of reports of piglet diarrhea caused by PoRVB and PoRVC cannot be a consequence of a low infection rate but rather of the use of less sensitive diagnostic techniques. Polyacrylamide gel electrophoresis is the most practical and low-cost method used for RV diagnosis in animal production; however, this diagnostic method exhibits low sensitivity and thus PoRVB and PoRVC infections are frequently not identified (Xu et al. 1990, Médici et al. 2011). The several enzyme-linked immunosorbent assay (ELISA) kits available (commercial or in-house) are specifically designed to identify only RVA. Some studies have confirmed that the frequency of diagnosis of RVB and RVC as single or mixed infections increased with the use of more sensitive diagnostic techniques, such as RT-PCR assay (Alfieri et al. 1999, Médici et al. 2011).

The G4P[6] and G9P[23] genotypes of RVA detected in the first and second diarrhea outbreaks, respectively, have been detected in Brazilian pig herds (Lorenzetti et al. 2011, Tonietti et al. 2013). Similarly, the genotypes N4 of RVB (NSP2 gene) and I1 of RVC (VP6 gene) have been described in fecal samples of piglets in Brazilian pig herds (Médici et al. 2010a,b).

The immune pressure induced by mass vaccination could promote reassortment, rearrangement, and zoonotic transmission of RVA strains between human and animal hosts (Matthijnsens et al. 2009). Lorenzetti et al. (2011) suggested that the immune pressure induced by commercial vaccine on neonatal diarrhea control might have allowed the selection and emergence of PoRVA strains. In this study, the sows vaccinated with an attenuated commercial vaccine for neonatal diarrhea control that included PoRVA protected most of the piglets for group A rotavirus infection and might have influenced in the selection or emergence of PoRVC strains.

The present study indicated that PoRVC, both in single and mixed infections with PoRVA and PoRVB was the main PoRV group detected in the three neonatal diarrhea

outbreaks and that the use of more sensitive diagnostic techniques allowed the identification of mixed infections of PoRV groups and the elucidation of the etiology of diarrhea. PoRVC can also be considered an important viral etiologic agent in the diarrhea outbreaks in suckling piglets, and therefore more studies of infections involving the atypical PoRV groups are needed. To the best of our knowledge, this is the first study about the involvement of PoRVC in diarrhea outbreaks in suckling piglets in Brazil.

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