

Torque teno sus virus (TTSuV) infection at different stages of pig production cycle¹

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ABSTRACT.- Leme R.A., Alfieri A.F. & Alfieri A.A. 2013. ***Torque teno sus virus (TTSuV) infection at different stages of pig production cycle.*** *Pesquisa Veterinária Brasileira* 33(7):840-846. Laboratório de Virologia Animal, Departamento de Medicina Veterinária Preventiva, Universidade Estadual de Londrina, Rodovia Celso Garcia Cid, Campus Universitário, Cx. Postal 10011, Londrina, PR 86057-970, Brazil E-mail: alfieri@uel.br

Torque teno sus virus (TTSuV) infection is present in pig herds worldwide. It has been demonstrated that TTSuV might increase the severity of other important viral diseases with economic and public health impacts. At present, there is no information on the age distribution of pigs infected with TTSuV in Brazilian herds. This study evaluated the frequency of TTSuV infection in pigs at different stages of production. Fecal samples ($n=190$) from pigs at 1 to 24 weeks of age and from breeders at 6 farrow-to-weaning (up to 8 weeks of age) and 9 grower-to-finish (9 weeks of age onwards) farms in the western region of Paraná state, Brazil, were evaluated by PCR. Fragments of the 5' UTRs of TTSuV1 and/or TTSuV2 DNAs were identified in 126 (66.3%) of the fecal samples. Significant differences were found with the percentages of positive samples for TTSuV1, TTSuV2, and mixed infections by both genera between and within the different pig production stages. Fecal samples from the grower-to-finish farms had TTSuV detection rates (90.1%; 64/71) that were significantly ($p<0.05$) higher than those from the farrow-to-weaning farms (52.1%; 62/119). TTSuV detection was significantly ($p<0.05$) more frequent in finisher pigs than in the animals from the other stages. The UTR nucleotide sequences in this study presented higher similarities to strains from Norway (96%, TTSuV1), and Argentina and China (97.1%, TTSuV2). These results suggest that TTSuV infection has spread to pigs of all production stages and that the viral infection rate increases with the age of the animals. In the western region of Paraná state, Brazil, TTSuV1 and TTSuV2-induced infections were more frequently observed in suckling piglets and finisher pigs, respectively. Phylogenetic analysis pointed out the possibility of different strains of TTSuV1 and TTSuV2 circulating in pig herds of Brazil.

INDEX TERMS: *Torque teno sus virus*, *Iotatorquevirus*, *Kappatorquevirus*, TTSuV1 and TTSuV2, age distribution, PCR, swine.

RESUMO.- [Infecção pelo *Torque teno sus virus* (TTSuV) em diferentes categorias do ciclo de produção de suínos.] A infecção pelo *Torque teno sus virus* (TTSuV) está presente em rebanhos suínos em todo o mundo. Tem sido demonstrado que a infecção pelo TTSuV pode aumentar a gravidade de outras importantes doenças virais com impactos econômicos e na saúde pública. Atualmente não

há informações sobre a distribuição da infecção pelo TTSuV, de acordo com a faixa etária, em rebanhos suínos brasileiros. Este estudo avaliou a frequência da infecção pelo TTSuV nas diferentes categorias de produção de suínos. Amostras fecais ($n=190$) de suínos com 1 a 24 semanas de idade e de reprodutores provenientes de 6 unidades produtoras de leitão (até 8 semanas de idade) e 9 unidades de terminação (9 semanas de idade em diante) da região oeste do Paraná, Brasil, foram avaliadas pela técnica de PCR. Fragmentos da região 5' UTR do DNA do TTSuV1 e/ou TTSuV2 foram identificados em 126 (66,3%) amostras fecais. Diferenças significativas foram encontradas em relação às porcentagens de amostras positivas para o TTSuV1,

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TTSuVk2 e infecção mista por ambos os gêneros inter e intra categorias. Amostras fecais provenientes de unidades de terminação apresentaram taxas de detecção de TTSuV (90.1%; 64/71) significativamente ($p < 0.05$) mais altas do que aquelas provenientes de unidades produtoras de leitão (52.1%; 62/119). A detecção do TTSuV em animais de terminação foi significativamente ($p < 0.05$) mais frequente do que nos suínos de outras categorias. As sequências de nucleotídeos da UTR deste estudo apresentaram maior similaridade com cepas da Noruega (96%, TTSuV1) e Argentina e China (97.1%, TTSuVk2). Estes resultados sugerem que a infecção pelo TTSuV encontra-se disseminada em suínos de todas as categorias de produção e que a taxa da infecção viral aumenta de acordo com a idade dos animais. Na região oeste do estado do Paraná, infecções induzidas pelo TTSuV1 e TTSuVk2 foram mais frequentemente observadas em leitões de maternidade e suínos de terminação, respectivamente. A análise filogenética indicou a possibilidade de diferentes cepas de TTSuV1 e TTSuVk2 circulando em rebanhos suinícolas brasileiros.

TERMOS DE INDEXAÇÃO: *Torque teno sus virus*, *Iotatorquevirus*, *Kappatorquevirus*, TTSuV1 e TTSuVk2, distribuição por idade, PCR, suínos.

INTRODUCTION

Torque teno virus (TTV) is a non-enveloped virus with a circular negative-sense single-stranded DNA (ssDNA) genome. This virus was first isolated from a Japanese man with non A-E post-transfusional hepatitis (Nishizawa et al. 1997). Since then, TTV has been shown to infect humans, non-human primates, and farm animals (Leary et al. 1999, Okamoto et al. 2000, 2001, 2002, Brassard et al. 2008).

Torque teno sus virus (TTSuV) infection in pigs was first described by Leary et al. (1999). The first full genome analysis of TTSuV was completed by Okamoto et al. (2002), and the differentiation of TTSuV into separate species was performed by Niel et al. (2005). TTSuV belongs to the family *Anelloviridae* and is classified into the species TTSuV1a (represented by Sd-TTV31 strain) and TTSuV1b (represented by Sd-TTV1p strain) in the genus *Iotatorquevirus*, and TTSuVk2 (represented by Sd-TTV2p strain) in the genus *Kappatorquevirus* (ICTV 2012).

TTSuV is widely distributed in pig herds in a number of countries in Asia, Europe, and America (McKeown et al. 2004, Niel et al. 2005, Gallei et al. 2010, Savic et al. 2010, Pérez et al. 2011, Leme et al. 2012). TTSuV infection is found in both healthy and diseased pigs, and a number of studies have been performed to determine the importance of the virus and the role it plays in infectious diseases (Meng 2012).

The detection of TTSuV infection is currently based on conventional and real-time PCR assays. Recently, the first Western blot and indirect ELISA assays to detect TTSuV2-specific IgG antibodies in pig serum were developed (Huang et al. 2011). The shortage of TTSuV infection studies based on histopathological technique has been discussed (Mei et al. 2011). However, serological assays, immunohistochemical and *in situ* hybridization techniques, and viral culture

systems are specific research tools that are not well established for this virus (Kekarainen & Segalés 2009, Leme et al. 2012).

Studies have demonstrated the presence of TTSuV in the serum and organs of pigs of different ages (Kekarainen & Segalés 2012) and have shown that the prevalence increases with the age of the animals (Sibila et al. 2009b, Aramouni et al. 2010). In Brazil, TTSuV infection has been identified in suckling piglets (Leme et al. 2012) and slaughter-age pigs (Leme et al. 2013), and in the reproductive tracts of boars and sows (Ritterbusch et al. 2012). Despite these studies, no information is available relative to the age distribution of TTSuV infection throughout the Brazilian pig production system.

The aims of this study were to evaluate natural infection by TTSuV in 1 to 24 week-old pigs and in breeders, and to evaluate the frequency of TTSuV infection at different stages of pig production.

MATERIALS AND METHODS

One hundred and ninety fecal samples of pigs from the western region of Paraná state, Brazil, were included in this study. The samples were selected independent of their consistency (diarrheic or not), collected between 2008 and 2011, and stored at 4°C. Six farrow-to-weaning farms, which include breeder sows and boars, suckling piglets, and weaned pigs up to 8 weeks of age, and nine grower-to-finish farms, where 9-week-old pigs are housed and fed until they reach 24 weeks of age, were evaluated. A total of 119 fecal samples from farrow-to-weaning farms and 71 samples from grower-to-finish farms were selected.

Fecal samples for each of the pig production stages were analyzed: suckling piglets (1 to 3 weeks old, $n=35$), weaned piglets (4 to 8 weeks old, $n=43$), finisher pigs (9 to 24 weeks old, $n=71$), and breeders ($n=41$).

Fecal suspensions were prepared at 10 to 20% (w/v) in 0.01 M phosphate-buffered saline (PBS), pH 7.2, and centrifuged at 5,000 $\times g$ for 3 min. The supernatants were used for DNA extraction.

To determine the frequency of TTSuV infection, viral ssDNA was extracted by using a combination of the phenol/chloroform/isoamyl alcohol (25:24:1) and silica/guanidinium isothiocyanate nucleic acid extraction methods (Alfieri et al. 2006) and was immediately submitted to a polymerase chain reaction (PCR) assay.

Specific PCR assays were performed using primers for TTSuV1 (*Iotatorquevirus*) and TTSuVk2 (*Kappatorquevirus*) targeting the non-coding region of the viral genome, and the technique was performed as described (Segalés et al. 2009), with modifications (Leme et al. 2012). The amplification reaction was performed in a thermocycler (Swift™ MaxPro Thermal Cycler, Esco Healthcare Pte, Singapore) at 94°C for 5 min for denaturation followed by 40 cycles of 94°C/1 min, 54°C/1 min, and 72°C/1 min and a final extension at 72°C for 5 min. The expected sizes of the amplified products were 305 and 252 bp for TTSuV1 and k2, respectively.

Five positive samples for TTSuV1 and TTSuVk2 were randomly selected for sequence analysis to confirm the specificity of the amplicons obtained in this study. The amplicons were purified by using a QIAquick PCR purification Kit (Qiagen, Valencia, CA, USA), quantified with a Qubit™ Fluorometer (Invitrogen™ Life Technologies, Eugene, OR, USA), and analyzed by electrophoresis on a 2% agarose gel. An ABI3500 Genetic Analyzer and the Big-Dye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) were used for sequencing, with forward and

Table 1. Detection rates of TTSuV1 and TTSuVk2 in single and mixed-infections by PCR assay in pig fecal samples according to stage of pig production cycle

TTSuV detection	Suckling piglets (1 to 3 weeks) <i>n</i> =35 (%)	Weaned piglets (4 to 8 weeks) <i>n</i> =43 (%)	Finisher pigs (9 to 24 weeks) <i>n</i> =71 (%)	Breeders (♂=10/♀=31) <i>n</i> =41 (%)	Total <i>n</i> =190
TTSuV1	10 (28.6) ^{A,a}	10 (23.3) ^{A,a}	4 (5.6) ^{B,a}	8 (19.5) ^{A,a}	32 (16.8)
TTSuVk2	3 (8.6) ^{A,b}	12 (27.9) ^{C,a}	39 (54.9) ^{B,b}	5 (12.2) ^{A,C,a}	59 (31)
TTSuV1 + k2	3 (8.6) ^{A,b}	8 (18.6) ^{A,B,a}	21 (29.5) ^{B,c}	3 (7.3) ^{A,a}	35 (18.4)
Total Positive	16 (45.7)	30 (69.8)	64 (90.1)	16 (39)	126 (66.3)
Total Negative	19 (54.3)	13 (30.2)	7 (9.9)	25 (61)	64 (33.7)

Capital letters (A, B and C) refer to comparisons between stages and lower case letters (a, b, and c) refer to comparisons within each of the stages. Different letters indicate statistically significant differences ($p < 0.05$).

reverse primers. Sequence quality analysis was performed using Phred and CAP3 software (<http://asparagin.cenargen.embrapa.br/phph/>). Similarity searches were done with similar sequences deposited in GenBank by using the Basic Local Alignment Search Tool - BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). One amplicon of each TTSuV genus was randomly selected for phylogenetic analyses, which was performed by using MEGA package (version 5.10). Two phylogenetic trees based on nucleotide (nt) were obtained using maximum likelihood statistical method based on kimura two-parameter (K2+G) and tamura-nei models, which provided statistical support via bootstrapping with 1,000 replicates. Sequence identity matrix was performed using the BioEdit software version 7.1.11 (<http://www.mbio.ncsu.edu/bioedit/bioedit.html>).

Statistical analysis was performed with Epi Info™, using Chi-square (χ^2) or Fisher exact tests to compare the proportions of TTSuV-positive samples between and within the pig production stages and to compare the detection rates of TTSuV of the different farm types (farrow-to-weaning vs. grower-to-finish). The confidence limit for the statistical tests was set at 95% ($p < 0.05$).

RESULTS

Of the 190 pig fecal samples evaluated, 126 (66.3%) were positive for TTSuV infection. The TTSuV presence was detected in pigs of all stages evaluated. The TTSuV detection results for each stage of pig producing cycle are presented in Table 1.

Among the pig production stages, single infections of TTSuV1 were significantly ($p < 0.05$) more frequent in suckling piglets than finisher pigs, and in breeders relative to finisher pigs. The rate of single infections of TTSuVk2 was significantly ($p < 0.05$) higher in finisher pigs relative to suckling piglets and breeders. A significantly ($p < 0.05$) higher frequency of mixed infections due to TTSuV1 and TTSuVk2 was found in finisher pigs relative to suckling piglets and breeders.

When the proportions of TTSuV1- and TTSuVk2-positive samples within each stage were compared, no significant ($p > 0.05$) differences were found for weaned piglets or breeders. However, the detection of TTSuV1 in suckling piglets was significantly ($p < 0.05$) more frequent than the detection of TTSuVk2 or mixed infections of both genera. When the results of finisher pigs were evaluated, TTSuVk2 detection was significantly ($p < 0.05$) more frequent than the detection of TTSuV1 or mixed infections by both genera. Mixed infections by TTSuV1 and TTSuVk2 were detected

at significantly ($p < 0.05$) higher rates than single infections of TTSuV1.

All farms evaluated had positive results for both TTSuV genera. The rates of TTSuV detection were 52.1% (62/119) and 90.1% (64/71) for samples from farrow-to-weaning and grower-to-finish farms, respectively, and the difference between these rates was significant ($p < 0.05$). TTSuV1 and TTSuVk2 were detected in both single and mixed infections at all farrow-to-weaning farms ($n=6$); for the single infections of TTSuV1 were detected at 4 of grower-to-finish farms ($n=9$), whereas single infections of TTSuVk2 and mixed infections of TTSuV1+TTSuVk2 were observed in all farms evaluated (Table 2).

Positive results for TTSuV1, TTSuVk2, and mixed infections with both genera were observed throughout the period evaluated (2008-2011).

The specificity of the amplicons obtained for each TTSuV genus was confirmed during the sequence analyses. The UTR nt sequences of the TTSuV1 (TTSuV1_BRA16/09) and TTSuVk2 (TTSuV2_BRA26/10) obtained herein contain 311 and 252 bp, respectively. Comparisons of the nt sequence of the TTSuV1 strain in this study to those available in GenBank revealed 96% and 95.6% of nt similarity to strains from Norway (TTV1_NOR02) and Brazil (TTSuV1_BRA11/07), respectively. Slightly smaller similarities (91.3% to 93%) were observed between the TTSuV1 nt sequence in this study and the others from Brazil.

Regarding the TTSuVk2 UTR nt sequence in this study, higher similarity (97.1%) was obtained to both the strains from Argentina (TTV2_ARG08) and China (SH/China/2010/PTTV2/129). The similarities to the other Brazilian TTSuVk2 nt sequences varied between 93.2% and 94.9%.

Phylogenetic analyses based on two distinct models (kimura two-parameter and tamura-nei) of the same method (maximum likelihood) did not present differences on topology of the trees generated and the bootstrap values presented minimal and not relevant differences among the two phylogenetic trees. Of all the Brazilian TTSuV1 strains available, including that in this study, only one (TTSuV1_BRA15/11) clustered together to the prototypes (Sd-TTV31 and Sd-TTV1p) in the phylogenetic tree (Fig.1), while the others clustered in two distinct branches. For TTSuVk2, all Brazilian strains were distributed in different branches.

Table 2. TTSuV1 and TTSuVk2 single and mixed-infections in pig fecal samples according to farm type

Farms	Pig herds (number of sam- ples evaluated)	TTSuV detection			TOTAL (%)
		TTSuV1	TTSuVk2	TTSuV1 + TTSuVk2	
Farrow-to-weaning (breeder sows and boars, suckling piglets, and weaned pigs until 8 weeks of age) <i>n</i> =6	FW I (<i>n</i> =19)	6	2	3	11 (57.9)
	FW II (<i>n</i> =24)	6	1	3	10 (41.7)
	FW III (<i>n</i> =22)	6	4	1	11 (50)
	FW IV (<i>n</i> =16)	1	8	1	10 (62.5)
	FW V (<i>n</i> =18)	2	3	4	9 (50)
	FW VI (<i>n</i> =20)	7	2	2	11 (55)
Subtotal	<i>n</i> =119	28 (23.5%)	20 (16.8%)	14 (11.8%)	62 (52.1) ^a
Grower-to-finish (9 to 24 weeks of age) <i>n</i> =9	GF I (<i>n</i> =7)	-	4	3	7 (100)
	GF II (<i>n</i> =7)	-	3	3	6 (85.7)
	GF III (<i>n</i> =9)	1	5	1	7 (77.8)
	GF IV (<i>n</i> =5)	1	2	2	5 (100)
	GF V (<i>n</i> =6)	1	1	2	4 (66.7)
	GF VI (<i>n</i> =10)	-	7	3	10 (100)
	GF VII (<i>n</i> =6)	-	3	2	5 (83.3)
	GF VIII (<i>n</i> =4)	1	1	2	4 (100)
	GF IX (<i>n</i> =17)	-	13	3	16 (94.1)
Subtotal	<i>n</i> =71	4 (5.6%)	39 (54.9%)	21 (29.6%)	64 (90.1) ^b
TOTAL	(<i>n</i> =190)	32	59	35	126 (66.3)

Different letters indicate statistically significant differences ($p < 0.05$).

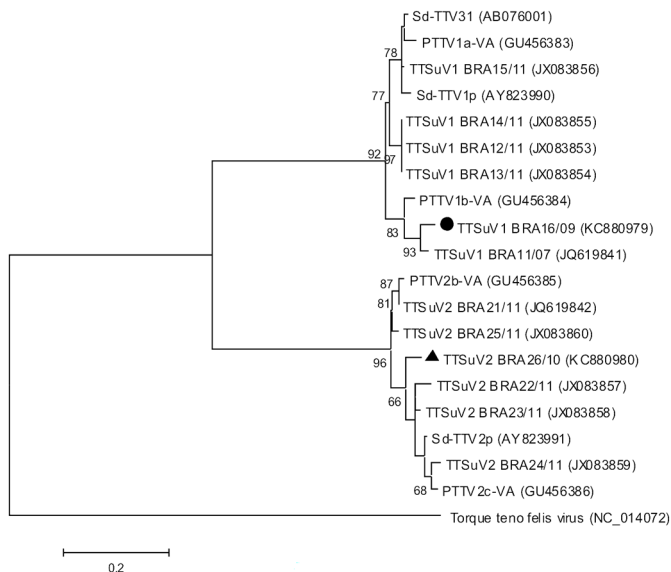


Fig.1. Maximum likelihood phylogenetic tree construction using the Tamura-Nei model based on partial (261 nt: 53-314 bp) untranslated region. Bootstrap values (1,000 replicates) higher than 65% are shown. GenBank accession number of prototypes (Sd-TTV31, Sd-TTV1p, Sd-TTV2p), Brazilian (TTSuV1_BRA11/07, TTSuV1_BRA12/11, TTSuV1_BRA13/11, TTSuV1_BRA14/11, TTSuV1_BRA15/11, TTSuV2_BRA21/11, TTSuV2_BRA22/11, TTSuV2_BRA23/11, TTSuV2_BRA24/11, and TTSuV2_BRA25/11), other reference sequences (PTTV1a-VA, PTTV1b-VA, PTTV2b-VA, PTTV2c-VA), and this study TTSuV strains (● TTSuV1_BRA16/09, ▲ TTSuV2_BRA26/10) are indicated between parentheses. Torque teno felis virus was used as out group.

DISCUSSION

As far as the authors are aware, this is first study that investigated the rates of TTSuV detection at different stages

of pig production in Brazil. These results indicate that TTSuV infection has spread to pigs of all production stages. Finisher pigs had significantly higher rates of TTSuV detection than animals from the other stages, in agreement with previous studies that have reported that TTSuV infection increases with the age of the animals (Sibila et al. 2009b, Aramouni et al. 2010).

In our study, the overall percentage of TTSuV-positive pigs (66.3%) was higher than that described in a study performed with rectal swabs (Sibila et al. 2009b), which reported a low percentage (<20%) of TTSuV detection. Previous studies reported higher rates for TTSuV in fecal samples, suggesting that the fecal-oral route is an important route of TTSuV transmission (Brassard et al. 2008, Leme et al. 2012).

An inversion in the frequencies of infection with the two TTSuV genera relative to the age of pigs within the production cycle was observed during this study. TTSuV1 detection was higher ($p < 0.05$) in suckling piglets and progressively less frequent with the increasing age up to finisher pigs, whereas the detection rates of single infections of TTSuVk2 and mixed infections increased proportionally with the age of the animals; with finisher pigs presenting higher ($p < 0.05$) rates of TTSuVk2 infection than the animals of the other stages. These findings are corroborated by the results at the farm level; 5 of the 9 grower-to-finish farms did not present positive results for single infections of TTSuV1. A similar result was obtained in a longitudinal study performed with 1 to 15 week-old animals, which evaluated the presence of TTSuV in different biological samples (Sibila et al. 2009b). Specifically, in that study TTSuV1 was detected more frequently than TTSuV2 from the nasal swabs of animals up to 7 weeks of age, but the rate of TTSuV2 detection was higher ($p < 0.05$) than the rate of TTSuV1 in pigs

that were at 11 and 15 weeks of age. Moreover, analysis of the rectal swabs demonstrated that the percentage of TT-SuV2-positive pigs increased progressively from 7 weeks of age onwards, whereas the detection of TT-SuV1-positive pigs was fairly constant throughout the study (Sibila et al. 2009b). Although the data from both studies show similar trends, there is no clear explanation for the inversion in the rates of infection by TT-SuV species.

A study performed within Czech pig population demonstrated the increasing frequency of TT-SuV infection with ageing, although has revealed no differences between the TT-SuV1 and 2 detection rates for piglets, weaned pigs, and adult animals (Jarosova et al. 2011). The prevalence of TT-SuV infection in pigs of different ages also has been evaluated in the United States and it was demonstrated that both TT-SuV1 and TT-SuV2 infections increased with the age of the animals, with the prevalence of TT-SuV1 DNA being higher ($p < 0.05$) than that of TT-SuV2 in each of the age groups analyzed (Xiao et al. 2012). Although a serodiagnostic technique was used in the American study, the different geographical regions used in these studies might explain the divergence in the results obtained, suggesting that the behavior of the virus may vary between distinct geographical locations.

It has been demonstrated that a single pig can be infected with more than one strain of the TT-SuV genera (Huang et al. 2010, Gallei et al. 2010, Leme et al. 2013). This is probably because molecular variation in the TT-SuV genomic sequences could lead to antigenic differences as a mechanism to avoid the host immune response.

An increasing level of anti-TT-SuV2-ORF1 antibodies has been shown to be associated with a decrease in the viral DNA load within the serum (Huang et al. 2011). Although the result refers to TT-SuV2, and considering the previous results that TT-SuV1 species more frequently infects suckling piglets from Brazil (Leme et al. 2012), the progressive production of anti-TT-SuV1 antibodies in response to early exposure is likely. Such antibody production might be at least partially responsible for decreases in virus excretion via feces and in the frequency of positive results for this species of the virus in older animals, since piglets are typically exposed to TT-SuV1 earlier in life. However, the immunological response could not lead to the clearance of TT-SuV1 infection, and the increasing incidence of infection by TT-SuV2 with ageing might also explain the increase in co-infection rates.

Age has been suggested to be an important factor affecting the profile of TT-SuV2 infection because older pigs have been found to have higher prevalences of viremia and antibodies (Huang et al. 2011). The slow development of immunity as the animals become exposed and matures, suggested by Xiao et al. (2012), and the progressive increase in the frequency of TT-SuV2 infection with age could explain the high frequency of TT-SuV2-positive animals of this study.

Jarosova et al. (2011) reported higher frequencies of both TT-SuV1 and TT-SuV2 viremia in weaned and adult pigs of Czech Republic. The lower rates of TT-SuV detection in serum samples from newborn piglets in that study suggest that young animals developed their immunological respon-

se to TT-SuV infection later. The occurrence of immunological tolerance to the virus due to the infection before the immunocompetence age may be responsible for persistent TT-SuV infection throughout the pig productive life (Aramouni et al. 2010).

TT-SuV2-induced infections have been demonstrated to be more frequent in porcine circovirus associated disease (PCVAD)-affected pigs than in non-affected pigs (Kekalainen et al. 2006, Blomström et al. 2010, Aramouni et al. 2011), although no association between TT-SuV species infection and PCVAD has been identified (Lee et al. 2010). In Brazil, Ritterbusch et al. (2012) found TT-SuV2 infection more frequently than TT-SuV1 infection in the reproductive organs, semen, ovarian follicular fluid and lymph nodes of adult pigs, primarily in the context of co-infection with porcine circovirus 2 (PCV2).

In the present study, 18.4% (35/190) of the fecal samples were positive for both genera of TT-SuV. A study performed by our group evaluated paired organs and serum samples from clinically healthy slaughter-age pigs for TT-SuV infection, where it was demonstrated co-infection due to both TT-SuV genera in 87.9% (102/116) of the samples, whereas single infections of TT-SuV1 and TT-SuV2 were found in only 2 and 9 of the 116 samples analyzed, respectively (Leme et al. 2013). The difference in the detection rate of TT-SuV-induced mixed infections between our two studies may be due to the type of samples evaluated (fecal vs. serum vs. organs). Based on the results obtained from the fecal samples, it can be suggested that mixed infections due to TT-SuV genera may interfere with viral shedding, leading to lower rates of detection in feces. In the study based on the systemic infection (Leme et al. 2013) it was not possible to determine whether action of the two genera of TT-SuV is competitive or synergetic. Thus, it is clear that TT-SuV genera co-infection is not an unusual event, but understanding the implications of this result has not been elucidated since it is not known whether the presence of one of the genus limits or favors infection by the other or affects the level of fecal viral shedding (Leme et al. 2012).

In this study, the frequency of TT-SuV infection for breeders was not significantly different for that of suckling piglets ($p > 0.05$). The results are in agreement with the detection of TT-SuV in sows in a study performed in Spain (Sibila et al. 2009a). However, our results differ from the reported results of a study in Czech Republic, which detected TT-SuV infection in higher frequencies in gilts and sows than in piglets, with no significant differences according to each TT-SuV genus (Jarosova et al. 2011). The results presented also differ from the results obtained from wild boar serum samples, which showed that TT-SuV2 infection was more common ($p < 0.05$) than TT-SuV1 (Martínez et al. 2006).

Two studies evaluated the TT-SuV infection in female breeders and in both the frequencies of detection were higher in young parity relative to old parity sows (Sibila et al. 2009a, Jarosova et al. 2011). In the present study such evaluation could not be done, since we have no information about the age of the female breeders analyzed. Anyway, parity number has not been associated with TT-SuV infection in sows (Sibila et al. 2009a).

The breeders included in this study had a higher rate of TTSuV1 detection. Although this rate was not different ($p > 0.05$) from the detection rates of TTSuV2 or mixed infections, this result reinforces the hypothesis that breeders may be a source of TTSuV infection for piglets. Studies reported piglets at first week of age as positive for TTSuV infection and suggested that sow-to-piglet transmission is most likely to occur (Sibila et al. 2009a, Jarosova et al. 2011).

There are few Brazilian TTSuV nt sequences available for phylogenetic studies. The UTR nt sequences similarities of the TTSuV strains in this study and other Brazilian strains revealed discrete genetic variability within each TTSuV genus. However, both the TTSuV amplicons presented higher similarities with foreign TTSuV strains (Argentina, China, and Norway), indicating the absence of barriers for viral spread.

Recently, a new classification for this virus was established based on the complete genome sequences of the known prototypes (Sd-TTV31, Sd-TTV1p, and Sd-TTV2p). The UTR is the most conserved region among anelloviruses and, even belonging to different virus species in the same genus, the TTSuV1 UTR nt sequences of the prototypes are similar and they both grouped at same branch of the phylogenetic tree presented herein. Two of the Brazilian nt sequences available were identified from piglet fecal samples of distinct states of Brazil, TTSuV1_BRA11/07 from Santa Catarina state and TTSuV2_BRA21/11 from Minas Gerais state (Leme et al. 2012). The other TTSuV strains were originated from Paraná state (Leme et al. 2013). Despite the low variability among the Brazilian TTSuV1 and TTSuV2 strains, the phylogenetic tree shows that they did not group in same branches of the prototypes, as well as not always clustered with each other. Moreover, TTSuV1 nt sequence in this study clustered together to the TTSuV1 strain of Santa Catarina state. It is strongly possible that distinct strains of the same TTSuV genus have circulating in Brazilian pig herds, as suggested previously (Leme et al. 2013).

Emerging viral infections of pigs have been recently reported in Brazil; these include infections due to rotaviruses B and C (Médici et al. 2011), caliciviruses (Barry et al. 2008, Cunha et al. 2010), kobuvirus (Barry et al. 2011), and hepatitis E virus (Gardinali et al. 2012). The associations of these emerging viruses and of TTSuV with disease in pigs have not been elucidated (Meng 2012). However, the zoonotic potential of these viruses is a major concern, and the surveillance of epidemiological data is important. Moreover, porcine enteric viral infections, emerging or not, require continuous attention in pig herds since intestinal health is directly related to productivity.

CONCLUSIONS

To the best of our knowledge, this is the first study on the age distribution of TTSuV infection to be performed in South America.

TTSuV infection was widespread in all stages of pigs maintained within the production cycle during the period evaluated (2008-2011).

Infections due to TTSuV1 and TTSuV2 were found more frequently in suckling piglets and finisher pigs, respectively, in Brazil.

The observation of inversion of TTSuV genera infection with the age of infected pigs, and the variation in the number of TTSuV genera-induced mixed infections detected might be related to the different biological samples used, and highlights the biological properties of this virus.

Distinct strains of TTSuV1 and TTSuV2 are present in Brazilian pig herds.

Studies based on the immune response to TTSuV in hosts may help to elucidate the dynamics of TTSuV genera infection and the pathogenicity of these viruses.

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