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SHORT COMMUNICATION

Simultaneous infection with distinct strains of *Torque teno sus virus* (TTSuV) in healthy slaughter-age pigs

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Abstract The aims of this study were to evaluate *torque teno sus virus* (TTSuV) infection in healthy slaughter-age pigs and to compare the similarities of the untranslated region (UTR) nucleotide (nt) sequences obtained from different biological samples of the same animals. Fifty-eight pigs were evaluated by PCR assay for the presence of TTSuV in paired samples of liver and serum (Group 1, n=27) and lung and serum (Group 2, n=31). All the pigs were positive for TTSuV infection in the organs sampled and 94.8 % (n=55) presented with viraemia. The nt sequence similarities between the Groups 1 and 2 varied from 91.7 % to 96.6 % (TTSuV1) and 91 % to 95 % (TTSuV2). In Group 1, the nt sequence similarities were 93 % (TTSuV1) and 95.4 % (TTSuV2). In Group 2, the nt sequence similarities were 95 % (TTSuV1) and 91 % (TTSuV2). These results revealed the simultaneous infection with distinct strains of TTSuV1 and 2 in healthy pigs at slaughter age.

Keywords Liver · Lung · Serum · TTSuV1 and 2 · Multiple infection · PCR

Introduction

Torque teno virus (TTV) was first isolated in a Japanese patient as the causative agent of an acute post-transfusion hepatitis of unknown aetiology (Nishizawa et al. 1997). TTV has since been identified infecting humans and animals (Kekarainen and Segalés 2012).

Porcine TTV belongs to the family *Anelloviridae*, which is divided into the genus *Iotatorquevirus*, which includes the species *torque teno sus virus 1a* (TTSuV1a) and *torque teno sus virus 1b* (TTSuV1b), and the genus *Kappatorquevirus*, which includes the specie *torque teno sus virus k2* (TTSuVk2) (ICVT 2012).

TTSuV is a non-enveloped virus with a circular, negative-sense, single-stranded DNA (ssDNA) genome (Okamoto et al. 2002). The TTSuV DNA genome is 2.8 kb long and includes four open reading frames (ORFs) and an untranslated region (UTR), which is considered a useful molecular marker in polymerase chain reaction (PCR) assays for TTSuV species differentiation (Segalés et al. 2009; Meng 2012).

TTSuV has not been linked to any specific disease. However, studies have associated important pig pathologies of viral aetiology, like porcine circovirosis due to porcine circovirus-2 (PCV-2), hepatitis E, porcine reproductive and respiratory syndrome, and classical swine fever with TTSuV infection (Kekarainen et al. 2006; Ellis et al. 2008; Savic et al. 2010; Zhu et al. 2012).

TTSuV is known to infect pigs from different geographical regions worldwide, including Asia, Europe, and the Americas (McKeown et al. 2004; Gallei et al. 2010; Savic et al. 2010; Pérez et al. 2011; de Arruda Leme et al. 2012). Marked genetic variability is present among TTSuV species (Cortey et al. 2011), and multiple infections by distinct types and subtypes of TTSuV species in a single pig may occur (Gallei et al. 2010; Huang et al. 2010).

The aims of this study were to evaluate the presences of TTSuV1 and 2 in organs and concomitant viraemia in healthy slaughter-age pigs, and to evaluate the occurrence of simultaneous infection by distinct strains of the same TTSuV species in these same pigs.

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Materials and methods

Samples were derived from a collection of paired tissue and serum samples collected in two separate pig slaughterhouses under the control of the Inspection Service of Parana state (SIP), Brazil, in July, 2011, for other research purposes and were stored at −80 °C. Pigs were from different herds in this same state; the animals were aged 25 weeks or older and clinically healthy.

A total of 116 samples were included in this study. Paired liver and serum samples of 27 pigs and paired lung and serum samples of 31 pigs from the southern and western regions of Parana state, respectively, were evaluated.

Organs homogenates (10–20 %w/v) were prepared in 0.01 M phosphate-buffered saline (PBS), pH 7.2, and the nucleic acids were extracted using a combination of the phenol/chloroform/isoamyl alcohol (25:24:1) and silica/guanidinium isothiocyanate nucleic acid extraction methods (Alfieri et al. 2006). Nucleic acid extraction was performed by the silica/guanidinium isothiocyanate nucleic acid extraction method on 200 μl aliquots of the serum samples (Boom et al. 1990).

The presence of TTSuV was determined using a previously described PCR assay for differential detection of species 1 and 2 (Segalés et al. 2009) with modifications (de Arruda Leme et al. 2012). The expected sizes of the amplified products were 305 bp and 252 bp for TTSuV1 and 2, respectively.

Amplified products from 4 different positive pigs were randomly selected for sequencing analysis to confirm the results. Sequence alignment and identity matrix creation were performed using BioEdit software version 7.0.8.0 (http://www.mbio.ncsu.edu/bioedit/bioedit.html).

Results and discussion

TTSuV was detected in the vast majority of the examined serum, liver, and lung samples. Of all 116 samples analysed, 87.9 % (n=102) showed mixed infection by both TTSuV species. Only 9.5 % (n=11) of the samples showed single infection (1.7 % for TTSuV1 and 7.8 % for TTSuV2). Three samples tested negative for TTSuV infection (Table 1).

A comparison was made between the sequences found in this study, other sequences derived from pigs in Brazil in other study, and the complete genome sequences (prototypes) available in GenBank. The results are shown in Table 2.

The presence of TTSuV infection in organ samples paired with serum samples of the same healthy adult pigs at the same time has never previously been reported. All 58 pigs were positive for TTSuV infection in the organ samples and 55 (94.8 %) of them presented with viraemia.

Aramouni et al. (2010) suggested that when TTSuV infection occurs before the age of immunocompetence, the host potentially becomes tolerant toward the virus and develops a persistent infection throughout its lifetime. Therefore, the persistence of the TTSuV infection might be related to host immunocompetence. Alternatively, the virus could develop mechanisms to avoid immune responses, such as replicating in low amounts to prevent inflammation of the target tissue (Savic et al. 2010). Another study suggested that after overcoming the infection, the individual suffers re-infection (Sibila et al. 2009). These facts might explain the high number of viral-positive organ samples in this study, as well as the high incidence of viraemia.

Table 1 TTSuV1 and 2 PCR assay positive results for serum samples paired with their respective tissue samples

	Paired samples (%)			
	G1 (n=27)		G2 (n=31)	
	Serum (%)	Liver (%)	Serum (%)	Lung (%)
TTSuV1	2 (7.4)	0	0	0
TTSuV2	0	0	8 (25.8)	1 (3.2)
TTSuV1+2	25 (92.6)	27 (100)	20 (64.5)	30 (96.8)
Negative	0	0	3 (9.7)	0
TOTAL	27 (100)	27 (100)	31 (100)	31 (100)

Most of the studies that have been performed have reported the prevalence of TTSuV using serum samples (McKeown et al. 2004; Kekarainen et al. 2006; Segalés et al. 2009; Taira et al. 2009; Huang et al. 2010; Cortey et al. 2011; Tshering et al. 2012). The prevalence of TTSuV1 and 2 in serum samples has been demonstrated to increase with the age of the animals (Sibila et al. 2009; Jarosova et al. 2011).

Based on studies that have demonstrated the presence of TTSuV in organs (Bigarré et al. 2005; Aramouni et al. 2010; Gallei et al. 2010; Savic et al. 2010; Pérez et al. 2011; Zhu et al. 2012) it can be concluded that there is no single target tissue for TTSuV. In addition to the increased prevalence of TTSuV viraemia with age, the prevalence of TTSuV infection in organ samples has also been demonstrated to increase with the age of the animals (Aramouni et al. 2010).

None of the previously mentioned studies evaluated serum and organ samples collected at the same time from the same healthy adult animals. A study performed by Tackás et al. (2008) tested liver and intestine samples paired with serum samples for the presence of TTSuV, and the analysed organ samples were found to be infected at lower rates than the serum samples. However, the samples tested were derived from weaned pigs.

Zhu et al. (2012) evaluated TTSuV infection in organ, serum, and stool samples from the same ill pigs at 1.5 to 5 months of age and found the prevalence of TTSuV in tissues much higher than in the stool and serum samples.

Table 2 Percentage of nucleotide sequence similarities of TTSuV1 and 2 UTR sequences obtained in this study, sequences derived from pigs in Brazil, and the complete genome sequences (prototypes) available in GenBank

TTSuV strains (Genbank access number)		Paired samples			
		G1		G2	
		Serum	Liver	Serum	Lung
		TTSuV1			
G1	Serum (JX083853)	100	93	96.3	92
	Liver (JX083854)		100	96.6	91.7
G2	Serum (JX083855)			100	95
	Lung (JX083856)				100
TTV1_BRA11/07 (JQ619841)		93.9	87.9	91.1	91.8
Sd-TTV31 (AB076001)		91.7	91.4	94.7	96.6
Sd-TTV1p (AY823990)		91.4	91.1	94.3	96.3
PTTV1a-VA (GU456383)		90.7	90.4	93.7	96
PTTV1b-VA (GU456384)		90.4	90.1	93.4	94.7
20N (1c) (GU570199)		90.7	90.4	93.7	95.3
		TTSuV2			
G1	Serum (JX083857)	100	95.4	95	91
	Liver (JX083858)		100	94.6	91.9
G2	Serum (JX083859)			100	91
	Lung (JX083860)				100
TTV2_BRA21/11 (JQ619842)		82.6	83	92.1	83.3
Sd-TTV2p (AY823991)		93.1	95.2	94.7	90.4
PTTV2b-VA (GU456385)		91.1	91.6	90.3	93.6
TTV2_G61(2d) (GU570207)		95.5	92.8	92.7	90.8
TTV2_G64(2e) (GU570208)		92.3	92.4	90.7	92
TTV2_GE1(2f) (GU570209)		90.3	90	89.5	90.4
TTV2_G31(2g) (GU570204)		92.3	92	91.5	91.2

Only three of the evaluated pigs presented TTSuV-positive serum and organ samples.

In Brazil, Niel et al. (2005) detected TTSuV1 and 2 infections in serum samples for the first time. Ritterbusch et al. (2012) in Santa Catarina state, Brazil, found TTSuV2 infection more frequently than TTSuV1, primarily during co-infection with PCV2, in reproductive organs, semen, ovarian follicular fluid and lymph nodes of adult pigs. Another study showed that early infection with TTSuV1 was significantly higher than TTSuV2 in pig herds from different Brazilian regions by using faecal samples of suckling piglets (de Arruda Leme et al. 2012). No studies on systemic infection by TTSuV have been performed in Brazil until now.

The nt sequence similarities between the strains in this study and other Brazilian strains obtained from faecal samples (de Arruda Leme et al. 2012) varied (87.9 % to 93.9 % for TTSuV1 and 82.6 % to 92.1 % for TTSuV2). Based on the most conserved region of the viral genome, these data reveal the genetic variability among Brazilian TTSuV strains.

The UTR sequence in this study showed variation between the sequences of the same TTSuV species from the same animals (93 % and 95 % for TTSuV1; 95.4 % and 91 % for TTSuV2). The similarities between the same UTR sequence portions of the prototypes were analysed and were also found to vary between each other (93.6 % to 96.8 % for TTSuV1; and 93.5 % to 96.9 % for TTSuV2). Based on the variability of the conserved region nt sequences similarities, these results suggest that the TTSuV strains present in the organ samples in this study were not the same strains that were present in the serum samples.

Multiple infections with both TTSuV species and distinct TTSuV strains within the same species commonly occur (Gallei et al. 2010; Huang et al. 2010). The simultaneous presence of several related but distinct TTSuV strains has been suggested to favour immune evasion by the virus, establishment of infection, and, eventually, disease induction (Gallei et al. 2010).

To our knowledge, this study represents the first description of TTSuV infection in organs paired with serum samples at the same time in the same clinically healthy adult pigs. The results are not restricted to viraemia but also provide data on the distribution of infection in organs. This study showed that

slaughter-age pigs were commonly co-infected with both the TTSuV species. Based on the partial sequences of the conserved region, we concluded that the TTSuV strains in this study differ between the animals and, particularly, that nt sequences obtained from the same pigs differ between each other. This result provides evidence of multiple infections with distinct strains of the same TTSuV species. Further studies are necessary to understand the sanitary risks of the TTSuV infection and the mechanisms of survival and evasion of the host immune responses, tissue tropism, and persistence of the infection by this virus.

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