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Brief communication

Detection of Torque teno sus virus in diarrheic piglet fecal samples positive or negative for porcine group A rotavirus

Raquel de Arruda Leme, DVM, MSc; Elis Lorenzetti, DVM, PhD; Alice F. Alfieri, DVM, PhD; Amauri A. Alfieri, DVM, PhD

Summary

Association of Torque teno sus virus (TTSuV) and porcine group A rotavirus (PoRVA) was evaluated in PoRVA-positive or PoRVA-negative diarrheic piglet fecal samples. Molecular TTSuV detection was 40.4% (21/52) and 53.3% (49/92) in PoRVA-positive and -negative fecal samples, respectively. No association ($P = .19$) was observed between TTSuV and PoRVA diarrhea.

Keywords: swine, intestinal health, diarrhea, porcine enteric viruses, Torque teno sus virus

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Resumen - Detección del Torque teno sus virus en muestras fecales de lechones diarreicos positivas o negativas al rotavirus porcino grupo A

Se evaluó la asociación del Torque teno sus virus (TTSuV) y el rotavirus porcino grupo A (PoRVA por sus siglas en inglés) en muestras fecales de lechones diarreicos negativos o positivos al PoRVA. La detección molecular del TTSuV fue 40.4% (21/52) y 53.3% (49/92) en muestras fecales positivas y negativas al PoRVA, respectivamente. No se observó asociación ($P = .19$) entre TTSuV y PoRVA en las diarreas.

Résumé - Détection du Torque teno sus virus à partir d'échantillons fécaux provenant de porcs diarrhéiques positifs ou négatifs pour le rotavirus porcin du groupe A

L'association du Torque teno sus virus (TTSuV) et du rotavirus porcin de groupe A (PoRVA) fut évaluée dans des échantillons fécaux provenant de porcs diarrhéiques PoRVA-positifs ou PoRVA-négatifs. La détection moléculaire de TTSuV était de 40,4% (21/52) et 53,3% (49/92) dans les échantillons PoRVA-positifs et PoRVA-négatifs, respectivement. Aucune association ($P = 0,19$) ne fut notée entre TTSuV et PoRVA dans les diarrées.

Torque teno virus (TTV), a member of the family *Anelloviridae*, is a non-enveloped virus with a single-stranded, negative-sense, circular DNA genome. Infection has been demonstrated in multiple species, including humans and swine.[1] The virus genome can be detected in various organs, secretions, and excretions from both humans and animals.[1,2]

In pigs, the virus is named *Torque teno sus virus* (TTSuV) and is categorized into two genera. Genus *Iotatorquevirus* includes the species *Torque teno sus virus 1a* and *Torque teno sus virus 1b* (TTSuV1), and the genus *Kappatorquevirus* includes the species *Torque teno sus virus k2* (TTSuV2).[3]

Torque teno sus virus has not been associated with specific clinical pathology or gross or histological lesions, and infection is common in both healthy and diseased pigs.[1] Studies have evaluated TTSuV infection as a contributor to the emergence or worsening of other important viral diseases of economic and public health impact. It is believed that TTSuV may contribute to these clinical syndromes as a co-infection associated with porcine circovirus 2 (PCV2) and hepatitis E virus (HEV) infections.[1,4] Studies have evaluated TTSuV infection in association with porcine reproductive and respiratory syndrome virus (PRRSV) and classical swine fever virus (CSFV); however, no correlation between TTSuV, PRRSV, and CSFV clinical signs or diseases has been identified.[1,5] In contrast, when loads of TTSuV DNA were evaluated by means of a real-time quantitative polymerase chain reaction (PCR) assay in pigs experimentally infected with CSFV, the TTSuV2 serum load was significantly larger in pigs with clinical signs of disease than in the healthy controls.[6]

Torque teno sus virus has been reported in bone marrow and peripheral blood mononuclear cells from clinically healthy pigs and in various fetal tissue samples.[7] The presence of TTSuV DNA in intestinal samples[8-10] and the high rates of TTSuV detection in fecal samples[11-13] suggests that enterocytes might be targets for virus replication.

Maintenance of intestinal health is essential to ensure pig productivity. Neonatal diarrhea is one of the most economically important syndromes affecting piglets worldwide.[14] Occurrence of diarrhea depends on several factors, including host immunity, management procedures, and infectious agents (bacteria, protozoa, and viruses). Microorganisms in single or mixed infections may be the determining factor for occurrence of neonatal diarrhea. The health or immunological status of individual animals, environmental conditions, and management procedures associated with concurrent infections may enhance the severity of clinical disease.[14-16]

Laboratory of Animal Virology, Department of Veterinary Preventive Medicine, Universidade Estadual de Londrina, Londrina, Paraná, Brazil.

Corresponding author: Dr Amauri A. Alfieri, Laboratory of Animal Virology, Department of Veterinary Preventive Medicine, Universidade Estadual de Londrina, Campus Universitário, PO Box 10011, 86057-970, Londrina, Paraná, Brazil; Tel: +55 43 3371 5876; Fax: +55 43 33714485; E-mail: alfieri@uel.br.

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Although TTSuV is excreted in diarrheic feces, to the knowledge of the authors, no studies have sought association of TTSuV with important enteric virus infections.

Porcine group A rotavirus (PoRVA) is implicated in enteric diseases of pigs. It causes a common health problem and is the most frequent viral etiological agent involved in the pig neonatal diarrhea complex throughout the world.[16] Most studies on TTSuV infection in association with other viruses have focused on hepatic, respiratory, reproductive, or multisystemic diseases. The aim of this study was to determine the frequency of TTSuV DNA detection in feces of diarrheic piglets previously identified as PoRVA-positive or PoRVA-negative by polyacrylamide gel electrophoresis (PAGE).

Materials and methods

This study is in agreement with the ethical principles determined by the Brazilian College of Animal Experimentation (COBEA) and was approved by the Ethics Committee on Animal Experimentation of the Universidade Estadual de Londrina.

In total, 144 piglet diarrheic fecal samples were included in this study. The samples were derived from a collection of feces (2004 to 2012) that had been stored at 4°C. Fecal samples were selected on the basis of the Brazilian state of origin (specifically, South, Midwest, and Southeast areas of Brazil where commercial swine production is concentrated), age of the animals, fecal consistency, and previous conclusive results for PoRVA diarrhea by the PAGE technique. Samples with doubtful PAGE results (eg, polyacrylamide gel bands of low intensity or in anomalous positions, extra bands or undefined electropherotype or both) were not selected for analysis. Fecal samples previously evaluated for TTSuV[12] (n = 97) and 47 other samples meeting the terms of the inclusion criteria were selected.

Fecal samples from diarrheic piglets originated from a total of 43 pig herds located in the South (n = 61), Midwest (n = 38), and Southeast (n = 45) Brazilian regions. Fifty-two PoRVA-positive and 92 PoRVA-negative diarrheic fecal samples were included from piglets in their first week of life (0 to 7 days of age, n = 43), second week of life (8 to 14 days of age, n = 48), and third week of life (15 to 21 days of age, n = 53). The distribution of samples by their date of collection was 16 for 2004, 10 for 2005, 13 for 2006, 20 for 2007, six for 2008, three for 2009, four for 2010, 46 for 2011, and 26 for 2012.

Fecal suspensions were prepared and the supernatants were used for nucleic acid extraction.[17] Polymerase chain reaction assays were performed using specific primers for TTSuV1 and TTSuV2 in a previously described technique.[12,18] Two positive samples for each TTSuV genus were randomly selected by drawing lots for sequence analysis to confirm the specificity of the amplicons obtained.

Statistical analysis was performed with Epi Info (`http://wwwn.cdc.gov/epiinfo/`) using chi-square (χ^2) analysis to compare the percentages of positive samples for each TTSuV genus between and within both groups of fecal samples analyzed (PoRVA-positive and PoRVA-negative), and to determine whether detection of TTSuV was associated with PoRVA diarrhea. The confidence limit for the statistical tests was set at 95% ($P < .05$).

Results

Of the 144 diarrheic suckling piglet fecal samples included in this study, 48.6% (70) were positive for TTSuV. The specificity of the amplicons obtained for each TTSuV genus was confirmed during sequence analysis. The detection rate for TTSuV1 was higher ($P < .05$) than that for either TTSuV2 or a combination of both genera in both groups evaluated (PoRVA-positive and PoRVA-negative samples). However, TTSuV1, TTSuV2, or co-infection detection rates did not differ between the PoRVA-positive and PoRVA-negative groups ($P > .05$). The TTSuV was most frequently detected in samples from piglets during their first week of life (55.8%; 24 of 43), followed by animals at the second (47.9%; 23 of 48) and third weeks of life (43.4%; 23 of 53) (Table 1).

Of the PoRVA-positive diarrheic piglets, 40.4% (21 of 52) tested positive for TTSuV and 59.6% (31 of 52) tested negative. Of the PoRVA-negative diarrheic piglets, 53.3% (49 of 92) tested positive for TTSuV and 46.7% (43 of 92) tested negative. Overall TTSuV detection did not differ between PoRVA-positive and PoRVA-negative samples ($P = .19$).

Discussion

This study was drafted to evaluate TTSuV infection in association with an enteric viral pathogen. Porcine group A rotavirus was used as the model enteropathogen because it is the most common viral agent involved in piglet neonatal diarrhea. While all fecal samples included in this analysis were diarrheic, the study did not intend to evaluate TTSuV as a causative agent of diarrhea. For this, the presence of other enteric pathogens (bacteria, protozoa, and various viruses) should be investigated.

Results based on each TTSuV genus in piglets aged 1 to 3 weeks are in agreement with a Brazilian study[13] that evaluated TTSuV infection at various stages of the pig production cycle. The TTSuV1 genus was detected in fecal samples from suckling piglets more frequently ($P < .05$) than the TTSuV2 genus or mixed infections of both genera.

Piglet fecal samples included in this study were tested for PoRVA immediately after collection. In acute infections, high loads of PoRVA are shed in feces ($10^{10\text{-}12}$ virus particles per gram of feces).[9] This facilitates diagnosis by the PAGE technique, which is considered of high specificity for PoRVA detection. The same does not apply, for example, to the atypical rotaviruses, which are eliminated in feces in smaller loads[19] and cannot always be detected by the PAGE technique. For this reason, and to maintain consistency since 2004, the PAGE technique is considered a useful tool to screen fecal samples for PoRVA.

Fecal samples included in this study were stored for diagnostic purposes at 4°C to avoid repeated freezing and thawing, which would accelerate degradation of nucleic acid in the samples.[20] Molecular assays targeting small fragments of the most conserved region of enteric virus genomes have successfully been performed using fecal samples stored at 4°C (data not shown). However, the authors cannot exclude the possibility of some degree of degradation of virus nucleic acid in samples stored at 4°C. Consequently, occurrence of TTSuV may be underestimated due to false-negative findings in both the PoRVA-positive and PoRVA-negative piglet samples.

The role of TTSuV as a triggering factor or an opportunistic pathogen has been extensively investigated, primarily in multifactorial diseases.[1] Porcine group A rotavirus is sufficiently pathogenic to independently cause clinical signs of disease. However, one study[14] reported multiple pathogens involved in 30% of piglet diarrhea cases, with rotavirus the most frequently detected agent, alone or in combination with other agents. Our results revealed that TTSuV shedding in the feces of diarrheic suckling piglets did not differ significantly between

Table 1: Detection of TTSuV1 and TTSuV2 in single or mixed infections using PCR assays in piglet diarrheic fecal samples previously diagnosed as positive or negative for PoRVA by the PAGE technique*

	Piglet age (week)	TTSuV-positive (%)			Total (%)
		TTSuV1	TTSuV2	TTSuV1 + TTSuV2	
PoRVA-positive (n = 52)					
	1st (n = 19)	9 (47.4)	0	2 (10.5)	11 (57.9)
	2nd (n = 16)	7 (43.8)	1 (6.3)	0	8 (50.0)
	3rd (n = 17)	2 (11.8)	0	0	2 (11.8)
Subtotal	NA	18 (34.6)^{A,a}	1 (1.9)^{A,b}	2 (3.8)^{A,b}	21 (40.4)
PoRVA-negative (n = 92)					
	1st (n = 24)	8 (33.3)	1 (4.2)	4 (16.7)	13 (54.2)
	2nd (n = 32)	10 (31.3)	0	5 (15.6)	15 (46.9)
	3rd (n = 36)	16 (44.4)	2 (5.6)	3 (8.3)	21 (58.3)
Subtotal	NA	34 (37)^{A,a}	3 (3.3)^{A,b}	12 (13)^{A,c}	49 (53.3)
Total (n = 144)	**NA**	**52 (36.1)^{A,a}**	**4 (2.8)^{A,b}**	**14 (9.7)^{A,c}**	**70 (48.6)**

* 144 fecal samples from a collection of pig feces (2004 to 2012) were selected according to the Brazilian state of origin, age of piglet, fecal consistency, and previously conclusive results for PoRVA diarrhea by the PAGE technique. Fifty-two PoRVA-positive and 92 PoRVA-negative diarrheic fecal samples from suckling piglets during their first week of life (0 to 7 days of age, n = 43), second week of life (8 to 14 days of age, n = 48), and third week of life (15 to 21 days of age, n = 53) were evaluated for TTSuV. Specific PCR assays were performed to detect and differentiate TTSuV1 (genus *Iotatorquevirus*) and TTSuV2 (genus *Kappatorquevirus*).

A Within a column, values with the superscript "A" do not differ significantly ($P > .05$; chi-square).

a,b,c Within a row, values with different lowercase superscript letters differ significantly ($P < .05$; chi-square).

TTSuV = Torque teno sus virus; PoRVA = porcine group A rotavirus; PCR = polymerase chain reaction; PAGE = polyacrylamide gel electrophoresis; NA = not applicable.

PoRVA-positive and PoRVA-negative animals, and no association between PoRVA and TTSuV infection was identified. It has been suggested that the biological behavior of TTSuV may vary with conditions of co-infection and that variation in the immunological status of the host due to mixed infections may regulate TTSuV replication.[6]

To the best of our knowledge, this is the first study conducted to detect TTSuV in association with a specific enteric viral pathogen (PoRVA). The potential pathogenic role of TTSuV infections has been previously investigated.[1] The neonatal diarrhea complex in pigs depends on many factors. Interaction between viruses may enhance the severity of clinical signs and, consequently, may impact productivity. Further studies seeking associations among emerging and classic enteric viral agents are needed to provide tools that enable prophylactic procedures and strategies to improve pig intestinal health.

Implications

- Under the conditions of this study, fecal shedding of TTSuV is independent of PoRVA infection in diarrheic piglets aged 1 to 3 weeks.
- Considering that porcine enteric viral agents are common throughout the pork industry and that the maintenance of pig intestinal health is essential to ensure productivity, continued surveillance for viral enteric infections and their potential associations cannot be ignored.

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Conflict of interest

None reported.

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