Torque teno sus virus 1 (TTSuV1) and 2 (TTSuV2) viral loads in serum of postweaning multisystemic wasting syndrome (PMWS)-affected and healthy pigs in Brazil

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Abstract

Associations between Torque teno sus viruses (TTSuVs) and the occurrence of postweaning multisystemic wasting syndrome (PMWS) have been reported with controversial results. Currently, no studies have been performed comparing simultaneously viral loads of TTSuVs and PCV2. To examine the role for TTSuV1 in PMWS-affected animals, a SYBR Green-based quantitative PCR (qPCR) was designed to detect and quantify TTSuV1, TTSuV2 and PCV2 genomes in swine sera. TTSuV1 genome loads were significantly higher in healthy adults than in young and SPF animals (p < 0.05) suggesting that the prevalence of TTSuV1 infection increases with age and bears no association with PMWS. Regarding TTSuV2, no significant variation was detected in viral loads within any of the groups. As expected, PCV2 genome loads were higher in PMWS-affected swine than in healthy or SPF animals (p < 0.001). These findings provide clear evidence to indicate that neither TTSuV1 nor TTSuV2 viral loads have any correlation with the occurrence of PMWS.

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TTSuV2 and PCV2 DNA viral loads and to determine genome prevalence in sera of PMWS-affected pigs and healthy animals.

Serum samples (181) were sent to laboratory from pig farms in the state of Rio Grande do Sul, Brazil. Forty-nine sera from PMWS-affected pigs (1–4 months old), plus 50 serum samples from healthy young pigs (1–4 months old), as well as from 50 healthy adult pigs (6–19 months old) were used. Additionally, 32 serum samples from SPF pigs (6–19 months old) were examined. PMWS was diagnosed with basis on clinical signs, histopathological lesions and identification of PCV2 in tissues by conventional PCR as reported (Segales et al., 2005). DNA was extracted from 500 μL of serum with a standard phenol–chloroform protocol (Sambrook and Russel, 2001). Primers used for the quantitative TTSuV1, TTSuV2 and PCV2 SYBR Green real-time PCR (qPCR) are shown in Supplemental Table 1. Positive controls used to construct the standard curve are described in Supplemental methods (Dezen et al., 2011). Descriptive statistics and the Kruskall–Wallis test (with Dunn’s as posttest) were performed using the GraphPad Prism5 soft-ware. Differences were considered significant when p ≤ 0.05.

The standard curve obtained with the TTSuV1 plasmid control (pCR2.1 + TTSuV1) showed an efficiency of ~99.4%. The detection limit of the assay was 10 copies of plasmid DNA per reaction (Supplemental Fig. 1). For TTSuV2 qPCR, the standard curve with the TTSuV2 control (pCR2.1 + TTSuV2) showed an efficiency of ~99.8%, with a detection limit of 100 copies of plasmid DNA per reaction (Supplemental Fig. 2). The regression coefficients (R²) were ≥ 0.999 on both curves. The observed temperature of dissociation for TTSuV1 and TTSuV2 qPCR was 78.75 °C and 77.41 °C, respectively. Negative controls did not give rise to any amplification product, as revealed by the absence of spurious peaks on the dissociation curve on both reactions.

Frequencies of detection of TTSuV1, TTSuV2 and PCV2 genomes in the four groups of pigs in this study are summarized in Table 1. Both TTSuV species and PCV2 were detected at high frequencies in sampled animals in all groups, and no significantly difference was observed (p > 0.05). Four serum samples were negative for both TTSuV1 and TTSuV2 (4/181, 2.2%); one of these was from a PMWS-affected pig, two were from healthy young pigs and the remainder from a SPF pig. Regarding PCV2, only 17 sera were negative; five of those from adult pigs and twelve from SPF pigs.

Mean viral loads (MVLs) of TTSuV1 and TTSuV2 genomes were quantified and compared in all groups (Fig. 1). For TTSuV1, the MVL in PMWS-affected pigs was 2.3 × 10⁶ copies/mL (SD ± 9.8 × 10⁵), whereas in healthy young animals (1–4 months old) the MVL was 1.6 × 10⁵ copies/mL (SD ± 5.5 × 10⁴). In adult healthy animals (6–19 months old) the TTSuV1 MVL was 8.0 × 10⁵ copies/mL (SD ± 4.0 × 10⁵). In SPF animals, the MVL was 5.9 × 10⁵ copies/mL (SD ± 1.4 × 10⁵). TTSuV1 loads in healthy adults were significantly higher than those in healthy young and SPF animals (p < 0.05).

For TTSuV2, the MVL in PMWS-affected pigs was 2.84 × 10⁶ copies/mL (SD ± 1.4 × 10⁶) whereas in young healthy animals (1–4 months old) it was 5.2 × 10⁵ copies/mL (SD ± 3.5 × 10⁵). In adult healthy animals (6–19 months old) the MVL was 2.4 × 10⁶ copies/mL (SD ± 1.5 × 10⁵) and in SPF animals the MVL was 7.6 × 10⁵ copies/mL (SD ± 2.6 × 10⁵). These findings reveal no significant differences (p > 0.05) among TTSuV2 viral loads in any of the different groups tested (Fig. 1).

In relation to PCV2, the MVL in PMWS-affected pigs was 3.2 × 10⁵ copies/mL (SD ± 1.0 × 10⁵) while in young healthy animals (1–4 months old) the MVL recorded was 5.1 × 10⁵ copies/mL (SD ± 2.1 × 10⁵). In adult healthy animals (6–19 months old) the MVL was 2.7 × 10⁵ copies/mL (SD ± 1.5 × 10⁵), whereas in SPF animals the MVL was 1.0 × 10⁵ copies/mL (SD ± 3.1 × 10⁵). These findings reveal that the PCV2 MVL was higher in PMWS-affected swine than in healthy and SPF animals (p < 0.001). In SPF animals, unlike in the other groups of animals, the PCV2 MVL was significantly lower than TTSuV1 and TTSuV2 MVLs (p < 0.001).

The role for TTSuVs as a causative agent of disease in pigs has become a matter of debate since its discovery (Niel et al., 2005; Okamoto et al., 2002). In particular, associations between TTSuV and PMWS have been investigated, since relationships between the latter and other pathogens have been confirmed in a number of occasions (Ellis et al., 2000; Pogranichnyi et al., 2002). Previous studies have searched for TTSuVs without simultaneously monitoring PCV2 viral loads. However, it is mandatory to examine PCV2 viral loads since MVLs > 10⁶/ml is a hallmark in establishing PMWS diagnosis (Brunborg et al., 2004). Thus, to define potential associations with disease, it is of importance to examine not only the viral loads of TTSuVs, but also PCV2 viral loads. Although several studies have reported on TTSuV viral loads (Aramouni et al., 2013; Brassard et al., 2010, 2013; Leblanc et al., 2014; Lee et al., 2012; Nieto et al., 2012; Zheng et al., 2014), the high-light of the present study is, for the first time, to compare simultaneously viral loads of both PCV2 and TTSuVs in healthy and PMWS-affected pigs. In order to address this issue, here, highly sensitive qPCRs were developed to detect and quantify TTSuV1, TTSuV2 and PCV2 viral loads. The results obtained showed no correlation between TTSuV1 and TTSuV2 viral loads and the occurrence of PMWS. Genomes of both TTSuV1 and TTSuV2 were detected in all groups of animals tested. Additionally, a significant finding was that older animals tend to have higher TTSuV1 MVLs, regardless of their PMWS status.

Some studies have detected a significant increase in TTSuV2 viral loads in PMWS-affected pigs (Aramouni et al., 2011). However, the findings reported here are in agreement with other researchers, albeit employing different approaches, no correlation between TTSuVs and occurrence of PMWS could be established (Blomstrom et al., 2010; Lee et al., 2010). In addition, TTSuV1 viral loads in adult animals were significantly higher than those in healthy young and SPF animals; this is in accordance with previous reports (Martinez-Guiño et al., 2009a; Sibila et al., 2009a, 2009b) where TTSuV prevalence in serum was found to increase with ageing.

Regarding the SPF animals, the PCV2 viral load was much lower than those of TTSuV1 and TTSuV2. Clearly, these were not expected to be infected with such viruses; however, to the knowledge of the authors, TTSuV testing is not included in screening test protocols for SPF animals. Therefore, before conducting experiments with SPF pigs, it might be of interest to investigate the animal status in relation to PCV2 and TTSuVs to prevent misleading results.

### Table 1

<table>
<thead>
<tr>
<th>Source of serum</th>
<th>TTSuV1</th>
<th>TTSuV2</th>
<th>TTSuV1/TTSuV2</th>
<th>PCV2</th>
<th>PCV2/TTSuV1</th>
<th>PCV2/TTSuV2</th>
<th>TTSuV1/TTSuV2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy young pigs</td>
<td>89.8 (44/49)</td>
<td>89.8 (44/49)</td>
<td>81.6 (40/49)</td>
<td>89.8 (44/49)</td>
<td>81.6 (40/49)</td>
<td>80.0 (40/50)</td>
<td>80.0 (40/50)</td>
</tr>
<tr>
<td>Healthy adult pigs</td>
<td>90.0 (45/50)</td>
<td>80.0 (40/50)</td>
<td>74.0 (37/50)</td>
<td>90.0 (45/50)</td>
<td>90.0 (45/50)</td>
<td>90.0 (45/50)</td>
<td>74.0 (37/50)</td>
</tr>
<tr>
<td>SPF pigs</td>
<td>100 (50/50)</td>
<td>72.0 (36/50)</td>
<td>72.0 (36/50)</td>
<td>86.0 (43/50)</td>
<td>80.0 (40/50)</td>
<td>62.0 (31/50)</td>
<td>62.0 (31/50)</td>
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</table>

<table>
<thead>
<tr>
<th>Source of serum</th>
<th>TTSuV1</th>
<th>TTSuV2</th>
<th>TTSuV1/TTSuV2</th>
<th>PCV2</th>
<th>PCV2/TTSuV1</th>
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<td>74.0 (37/50)</td>
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<tr>
<td>SPF pigs</td>
<td>100 (50/50)</td>
<td>72.0 (36/50)</td>
<td>72.0 (36/50)</td>
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<td>80.0 (40/50)</td>
<td>62.0 (31/50)</td>
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In conclusion, this study provides quantitative and qualitative data to demonstrate that neither TTSuV1 nor TTSuV2 viral loads have any correlation with the occurrence of PMWS. Furthermore, TTSuV1 viral load was found to increase with age, whereas TTSuV2 viral load is not affected by age.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.rvsc.2015.05.016.

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