Infection Dynamics of *Torque Teno Sus Virus* Types 1 and 2 in Serum and Peripheral Blood Mononuclear Cells

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**ABSTRACT.** This study was carried out to investigate the presence of *Torque teno sus virus* types 1 (TTSuV1) and 2 (TTSuV2) in a longitudinally (14 to 150 days of age) collected paired sera (pSE) and peripheral blood mononuclear cells (pPBMCs) using nested polymerase chain reaction. The detection rate of TTSuV1 in pSE increased from 14 to 90 days of age, but a progressive decline was observed from 120 to 150 days of age, while in pPBMC, a high value was maintained till the end of growing-finishing period. On the contrary, except in PBMCs at 30 days of age, high detection rates of TTSuV2 were found in both pSE and pPBMCs in all sampling ages. The detection rate of TTSuVs between pSE and pPBMCs was positively correlated at all sampling ages except for TTSuV1 at 150 days of age. This is the first study showing the presence of TTSuVs in PBMCs from pigs and describing the in vivo infection dynamics of TTSuV in paired sera and PBMCs during the entire growing and finishing periods of pigs reared in conventional farms.

**KEY WORDS:** peripheral blood mononuclear cells, serum, swine, TTSuV.

Torque teno virus (TTV) is a small, nonenveloped, single-stranded and circular DNA virus [21]. TTV was first detected in a human patient with post-transfusion hepatitis of unknown aetiology [19], but later detected in many other vertebrate animals including pigs [3, 7, 10, 16, 21, 22]. In pigs, two distinct species of TTV, *Torque teno sus virus* (TTSuV), types 1 (TTSuV1) and 2 (TTSuV2) have been described [17, 22], and they are currently grouped under the genus *Iotatorquevirus* of family *Anelloviridae* [2]. A definite pathogenic role of TTSuVs is yet to be demonstrated.

TTSuV1 and TTSuV2 (TTSuVs) have been detected from serum, plasma, semen, faeces, colostrum, nasal secretion and various tissues [5, 8, 13, 26, 29, 30] exclusively by Polymerase chain reaction (PCR), and different pig rearing countries have reported prevalence rate ranging from 24 to 100% [4, 8, 14–17, 24–26]. Limited longitudinal studies employing serum [17, 26] and cross-sectional studies employing serum and tissues [1, 8, 9, 27] have shown that TTSuV infection increases with age. However, little is known about primary infection with TTSuV and sites of viral persistence and reactivation after infection in the pigs, and to the best of our knowledge there is no report on the detection of TTSuVs in peripheral blood mononuclear cells (PBMCs) in pigs. Therefore, the purpose of this study was to investigate the presence of TTSuVs in PBMCs and compare its infection dynamics to that of sera. For this purpose, individual serum from 8 sows at 1-week before farrowing and paired pooled sera (pSE) and pooled PBMC (pPBMCs) obtained from their piglets at 14, 30–150 days of age at 30 days interval were examined for the presence of TTSuVs by nested PCR (nPCR).

This study was conducted in a commercial hybrid (Hypor) pig farm in Kagoshima prefecture, southern Japan having a total of 1,500 breeding sows. Eight sows and their 24 pigs (3 piglets/sow) were used for this study. All piglets appeared apparently healthy during the whole study period. Blood samples were collected from the sows at 7 days before farrowing and their piglets at 14, 30, 60, 90, 120 and 150 days of age and centrifuged at 1,800 × g for 20 min to obtained sera. The resultant sera were stored at −28°C until nPCR was performed. For obtaining PBMCs, 2 ml of the freshly collected whole blood per piglet was transferred to a 15 ml heparinized tubes which were pooled for the same sow (3 piglets/sow). The pooled heparinized blood samples were diluted to 3 fold dilutions using sterile PBS and centrifuged on a Ficoll-Conrey gradient solution at 1,400 × g for 20 min as described previously [6]. The PBMCs fraction was washed thrice with sterile PBS and stored below −28°C until use for the analysis. DNA was extracted from 250 µl of the individual serum of sows, pSE (pSE contain sera of 3 pigs from same sow) and pPBMCs of their pigs by a sodium iodide method using a kit (Wako Chemicals, Tokyo, Japan). The nPCR for the detection of TTSuV1 and TTSuV2 was performed by using a method described previously [8, 30]. Specific amplicons of TT-
SuV1 (260 bp) and TTSuV2 (230 bp) were located after performing electrophoresis in 2% agarose gel stained with ethidium bromide. Eighteen paired amplified products of pSE and pPBMCs (10 from TTSuV1 and 8 from TTSuV2) of two sows and their piglets at 30 and 120 days of age were excised from the agarose gel and purified by using QIAquick gel extraction kit (Qiagen, Tokyo, Japan). The obtained sequences according to the general sequencing protocol (Hokkaido University, Sapporo, Japan). The construction of phylogenetic tree was carried out using the neighbor-joining (NJ) method in the MEGA 4 package. The construction of phylogenetic tree was carried out using the neighbor-joining (NJ) method in the MEGA 4 software package [28].

The result of detection of TTSuV1 and TTSuV2 from serum and PBMCs samples in sows and their pigs is shown in Table 1. From the individual serum samples of 8 sows analyzed at 7 days before farrowing, TTSuV1 was detected in 1 sow (Sow 8) and TTSuV2 in all 8 sows.

In their growing pigs, the detection rate of TTSuV1 in serum increased with the age, being lowest at 14 days of age (25%, 2/8), and highest at 60 and 90 days of age (75%, 6/8), and then gradually declining at 120 (62.5%, 5/8) and 150 days of age. The virus was detected in all pSE (100%, 8/8) at 60, 90, 120 and 150 days of age; however, the virus was detected in all pSE (100%, 8/8) again at 60, 90, 120 and 150 days of age. The co-infection rates of the two virus types were 25.0% (2/8), 37.5% (3/8), 75.0% (7/8), 75.0% (7/8), 62.5% and 12.5% (1/8) at 14, 30, 60, 90, 120 and 150 days of age, respectively. From the 8 pSE analyzed, 12.5% (1 out of 8) each was TTSuV1 PCR positive (No. 8) and negative (No.2) at all 6 sampling ages. Six out of eight (75%) pSE analyzed were TTSuV1 positive (No. 3–8) in more than one sampling times and showed consecutive PCR positivity in at least 2 sampling ages. On the contrary, 62.5% (5 out of 8) of pSE were TTSuV2 PCR positive (No. 3–6, 8) at all sampling ages while none of the pSE was TTSuV2 PCR negative throughout the study period. All pSE (100%, 8/8) were TTSuV2 PCR positive in more than one sampling age and showed PCR positive in at least 2 consecutive sampling ages.

Regard the TTSuV1 and TTSuV2 detection in pPBMCs of growing pigs, similar to the detection trend in sera, the detection rate of TTSuV1 and TTSuV2 increased over time; the lowest value for TTSuV1 (12.5%, 1/8) and TTSuV2 (50%, 4/8) was detected at 30 days of age (TTSuVs detection was not analyzed at 14 days of age), while the highest value for TTSuV1 (75%, 6/8) and TTSuV2 (100%, 8/8) was detected at 90 and 60 days of age, respectively. Thereafter, the highest value for TTSuV1 was maintained until the last sampling age, while for TTSuV2, a decrease detection rate from 100.0% to 87.5% (7/8) was observed at 150 days of age. Co-infection of TTSuV1 and TTSuV2 increased progressively over time with the lowest detection rate (12.5%, 1/8) observed at 30 days of age and the highest (87.5%, 7/8) observed at 120 days of age. One out of 8 pPBMCs (12.5%) each was TTSuV1 PCR positive (No. 4) and negative (No. 5) at all 6 sampling ages. Seven out of 8 (87.5%) pPBMCs were TTSuV1 positive (No. 1–3, 5–8) in more than one sampling ages and 6 out of 8 (75%) showed consecutive PCR positivity (No. 2–4, 6–8) in at least 2 consecutive sampling ages.

### Table 1. Detection of TTSuV1 and TTSuV2 in individual sera of sows at 7 days before farrowing, and in paired pooled sera and peripheral blood mononuclear cells (PBMCs) of their growing and finishing pigs

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**Detection rate (%)**

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†: Age of piglets (days). + : Detected, – : Not detected, NE : Not examined.
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sampling ages. On the contrary, 3 out of 8 (37.5%) pPBMCs were TTSuV2 PCR positive (No. 4, 6, 8) and none were PCR negative at all sampling ages. All pPBMCs (100%, 8/8) were TTSuV2 PCR positive in more than one sampling age and showed consecutive PCR positive in at least 2 sampling ages.

The comparison of detection rates of the two virus types in pSE and pPBMCs showed no significant differences in the detection rate of either TTSuV1 or TTSuV2 between pSE and pPBMCs at any sampling age. The detection rate of TTSuV1 between pSE and pPBMCs was positively correlated from 30 to 120 days of age and was negatively correlated at 150 days of age. On the contrary, the detection rate of TTSuV2 between pSE and pPBMCs showed a positive correlation at all sampling ages.

The phylogenetic tree (Fig. 1) shows the grouping of TTSuVs in two main types, TTSuV1 and TTSuV2. The sequence similarity among the respective TTSuV types (AB679090-AB679107) and to that of their respective references (TTSuV1:GU570208, TTSuV2:AB076001) ranged from 89 to 98%. It can also be seen that except for one paired sample from piglets at 30 days of age of Sow 4, the phylogenetic distances were different between the DNA sequences obtained from the paired pSE and pPBMCs.

In the present study, the presence of TTSuV nucleic acids in paired sera and PBMCs during the entire growing-finishing period was investigated. To date the prevalence of TTSuVs in PBMC has not been studied, although its homologous counterpart has been detected in PBMCs from humans [20, 23]. The detection rate of TTSuV1 increased over time starting from 14 to 90 days of age and declining thereafter, while for TTSuV2 an increased in the detection rate was evident from 30 to 60 days (but a high value detected at 14 days of age) of age and a constant value was maintained until the last sampling age. Our result is in partial agreement with the increasing prevalence of both TTSuVs in sera in a longitudinal study carried out with pigs aged from 7 to 105 days of age [26]. The reason for such minor difference in prevalence rate, particularly for TTSuV2 is open to speculation. For example, it may be attributed to
the high percentage of TTSuV2 positive sows as observed in this study, since it has been suggested that TTSuVs can be transmitted by both vertical and horizontal routes [5, 13, 24, 30], and as a result of these events, it is likely that TTSuV2 is more widespread than TTSuV1 in this farm. Furthermore, it is likely that the slightly higher PCR sensitivity of TTSuV2 compared to that of TTSuV1 (550 versus 14 molecules per reaction) reported in our previous study [30] might have contributed to the higher detection rate of TTSuV2 observed in this study. Other plausible explanation could be due to different infection dynamics of the two virus types under different epidemiological settings. Further studies are required to elucidate these hypotheses.

The detection rate of TTSuVs in pPBMCs was similar to that in pSE. In fact, they were positively correlated at all sampling ages except for the detection rate of TTSuV1 at 150 days of age which was negatively correlated. In humans, PBMCs and bone marrow hematopoitic cells have been suggested as the two possible sites of TTV replication [11, 12, 31]. However, it is unknown whether the detection of TTSuVs in PBMC in our study corresponds to the virus tropism to PBMC. Apart from replication, TTSuVs could simply be engulfed without replication. Nevertheless, the detection of TTSuV in PBMCs opens room for further investigations, particularly to find out whether TTSuV can replicate in the PBMCs, and if so, which cell subset may be involved in the viral replication.

The duration of TTSuV1 and TTSuV2 in pigs has been reported to last up to 56–77 and 105 days, respectively [18, 26], but the viruses have not been tested thereafter in these longitudinal studies. Few other cross-sectional studies [1, 27] including our recent publication [29] have detected the two virus types in sera of pigs from the last stage of their growing-finishing period. In the present study, TTSuV1 and TTSuV2 nucleic acids were detected from 14 days to 150 days of age in both sera and PBMCs samples, further confirming the lifelong presence of the two virus types in pigs. Although, TTSuVs were consecutively detected in most of the pSE, it was unclear whether it was due to virus persistence or re-infection. It is possible that TTSuVs in PBMC being secluded from the circulating antibodies might be acting as a source of long lasting viremia and playing an important role in the transmission of the viruses in some clinical and epidemiological conditions.

In conclusion, this is the first study showing the presence of TTSuVs in PBMCs from pigs and describing the in vivo infection dynamics of TTSuV in paired sera and PBMCs during the entire growing-finishing period. The obtained results indicate the lifelong presence of TTSuVs in both sera and PBMCs of pigs reared in a conventional pig farm.

REFERENCES


