Detection of Torque Teno Sus Virus Types 1 and 2 by Nested Polymerase Chain Reaction in Sera of Sows at Parturition and of Their Newborn Piglets Immediately after Birth Without Suckling Colostrum and at 24 hr after Suckling Colostrum

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ABSTRACT. This study was performed to clarify the sow-to-fetus transmission pathway of Torque teno sus virus (TTSuV) types 1 (TTSuV1) and 2 (TTSuV2). For this purpose, detection of TTSuV1 and TTSuV2 (TTSuVs) in sera of 6 sows (Sows 1–6) at parturition and in sera of their newborn piglets immediately after birth without suckling colostrum was performed by nested polymerase chain reaction (nPCR). These sows were bred using semen that had tested negative for TTSuVs. In a TTSuV1- and TTSuV2-positive sow (Sow 1), TTSuV1 and TTSuV2 were detected in 4 and 5 of 12 newborn littersmates, respectively. In a TTSuV1-positive sow (Sow 2), TTSuV1 was detected in 1 of 8 newborn littersmates. In 4 TTSuV1- and TTSuV2-negative sows (Sows 3–6), TTSuV1 was detected in 6 out of the 25 newborn piglets of 3 sows (Sows 3–5), while TTSuVs were not detected in all 13 piglets of 1 sow (Sow 6). In addition, to investigate the possibility of a sow-to-piglet transmission pathway of TTSuV via colostrum, TTSuV1 and TTSuV2 in sera of 12 newborn piglets from Sows 1–3 were examined by nPCR. Immediately after birth without suckling colostrum, TTSuV1 and TTSuV2 were not detected in 10 and 8 of 12 newborn piglets, respectively; however, at 24 hr after suckling colostrum, TTSuV1 was detected in 6 piglets, while TTSuV2 was not detected in any piglets. These results confirmed the existence of a sow-to-fetus transmission pathway of TTSuV during normal pregnancy and suggested a possibility of sow-to-piglet transmission of TTSuV via colostrum.

KEY WORDS: Colostrum, Semen, Sera, Sow-to-fetus and -piglet transmission pathways, Torque teno sus virus (TTSuV) types 1 and 2.

MATERIALS AND METHODS

Experiment 1

Sow-to-fetus TTSuV transmission: This experiment was carried out on a commercial pig farm with 1,500 sows in Kagoshima Prefecture, Japan. Six healthy sows (Sows 1–6) were used. All sows were bred by artificial insemination using pooled semen of 8–10 boars obtained from a total of 13 boars. A portion of each individual semen sample was used to detect the presence of TTSuVs. The blood samples were collected from the sows via the anterior vena cava (about 8 ml) at parturition and from their 58 (7–13 piglets/sow) newborn piglets (about 4 ml) immediately after birth without sucking colostrum, and they were centrifuged at 1,800 × g for 20 min, and the collected sera were stored at –28°C until nPCR was performed.

Experiment 2

Sow-to-piglet transmission of TTSuV via colostrum: Immediately after birth, 12 newborn piglets from 3 sows (4 littersmates/sow; Sows 1–3) in Experiment 1 were used, and each piglet was numbered for identification. These piglets were allowed to suckle sow’s colostrum ad libitum after collection of the blood samples. Then, at 24 hr after sucking colostrum, blood samples were obtained from all piglets via the anterior vena cava, and the collected sera were stored at –28°C until nPCR was performed. The concentration of serum total protein (TP) for all the piglets immediately after birth without sucking colostrum and at 24 hr after sucking colostrum was determined to assess the colostrum uptake by the piglets.

DNA extraction and nPCR for the detection of TTSuV1 and TTSuV2: DNA was extracted from 250 µl of boar semen and sera of sows and newborn piglets by a sodium iodide method using a kit (DNA Extractor WB Kit, Wako Pure Chemical Industries, Osaka, Japan). The nPCR for the detection of TTSuV1 and TTSuV2 was performed by using a method described previously [7, 17]. The first-round 50-µl PCR for TTSuV1 contained 5 µl of DNA, 20 pmole of primer pair forward (5'-TACACTTCCGGGTTCAGGAG-3', reverse), 2.5 mM dNTPs, 2 mM MgCl2 and 0.5 U rTag DNA polymerase (rTag). The amplification conditions were as following: 94°C for 5 min, 35 cycles of 94°C for 30 sec, 52°C for 20 sec and 72°C for 30 sec followed by 72°C for 5 min. Five microliters of amplified product was used as template for nPCR. The primers set and PCR reagents for nPCR of TTSuV1 were 5'-CAATTTGGCTCGCTG-3' (forward) and 5'-ACTCAGCCATTCGGAACCT-3' (reverse), 2.5 mM dNTPs, 2 mM MgCl2 and 0.5 U rTag. The nPCR of TTSuV1 was performed under the same conditions as the first round PCR. The nPCR of TTSuV1 products was run on 2% agarose gel electrophoresis gel, and a band of 260 bp was observed using ethidium bromide under ultra violet (UV) fluorescence. TTSuV2 amplification was carried out under the same procedure as described for TTSuV1.

Briefly, the primer pair set for the first round PCR was 5'-AGTTACATAACCACCAAACC-3' (forward) and 5'-ATTACCGCCCTGCCGATAGGC-3' (reverse), and for the nPCR, the primer set was 5'-CCAAACCCACAGAACTGTGC-3' (forward) and 5'-CTTGACTCCGCTTCAGGAG-3' (reverse), respectively. After electrophoresis, the amplified product of a band of 230 bp was observed under UV fluorescence.

Sensitivity assay: In order to determine the PCR sensitivity, amplicons of TTSuV1 (260 bp) and TTSuV2 (230 bp) from 2% agarose gel after electrophoresis were purified using a commercially available DNA purification kit (QIAquick® Gel Extraction Kit, QIAGEN, Japan) and cloned into a plasmid using a cloning strategy (pJET1.2/blunt cloning vector, Fermentas, Japan) at Hokkaido System Science Co., Ltd., Japan. The sensitivity of the PCR was determined by amplification of tenfold dilutions of known amounts of each plasmid DNA in a PCR. To reconfirm the sensitivity result, the experiment was repeated once. The same plasmids and pure distilled water were used as positive and negative controls, in every PCR reaction.

Statistical analysis: The significance of difference in the concentration of TP between the piglets immediately after birth and 24 hr after sucking colostrum within the same litter was analyzed using the Student’s t-test.

RESULTS

Sensitivity of PCR: The minimum number of TTSuV1 and TTSuV2 copies that could be detected with the current PCR assay was 550 molecules of TTSuV1 and 14 molecules of TTSuV2 per reaction.

Detection of TTSuV1 and TTSuV2 in semen and sera of pigs: Once the sensitivity of the PCR was determined, the detections of TTSuV1 and TTSuV2 in semen of boars and in sera of sows and their piglets were carried out.

The results of detection of TTSuVs in semen of boars are shown in Table 1. TTSuV1 and TTSuV2 were not detected in any semen of boars.

<table>
<thead>
<tr>
<th>Boar No.</th>
<th>TTSuV1</th>
<th>TTSuV2</th>
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<td>13</td>
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</tbody>
</table>

–: Not detected.

Table 1. Detection of TTSuV1 and TTSuV2 by nested PCR in semen of boars
The results of detection of TTSuVs in sera of sows at farrowing and of their newborn piglets immediately after birth without suckling colostrum are shown in Table 2. Both TTSuV1 and TTSuV2 were detected in Sow 1, TTSuV1 but not TTSuV2 was detected in Sow 2 and neither TTSuV1 nor TTSuV2 was detected in Sows 3–6. In Sow 1, TTSuV1 and TTSuV2 were detected in 4 and 5 out of 12 newborn piglets, respectively, and the 6 remaining newborn piglets were negative for TTSuVs. In Sow 2, 1 out of 8 newborn piglets was positive for TTSuV1. For Sows 3–6, TTSuV1 was detected in 6 out of 25 newborn piglets (Sows 3–5), while neither TTSuV1 nor TTSuV2 was detected in any newborn piglet of Sow 6.

The results of detection of TTSuVs in sera of 12 newborn piglets immediately after birth without suckling colostrum are shown in Table 3. TTSuV1 and TTSuV2 were detected in 4 and 5 of 8 newborn piglets, respectively, and the remaining newborn piglets were negative for TTSuVs. The concentration of TP (g/dl) was 2.5–3 times higher in all individual piglets at 24 hr after suckling colostrums (6.3 ± 0.7, 7.4 ± 0.4 and 6.5 ± 0.5) compared with those at immediately after birth without suckling colostrums (2.2 ± 0.1, 2.5 ± 0.3 and 2.2 ± 0.1), and this difference was significant between the two groups of pigs (P<0.001) in Sows 1–3, respectively (result not shown).

DISCUSSION

In swine, it has been assumed that sow-to-fetus transmission of TTSuVs occurs during pregnancy because TTSuVs were detected in the sera of stillborn and nonstillborn gnotobiotic piglets derived by caesarean section and in the tissues of aborted and nonaborted fetuses [1, 14, 15, 24]. Further, intrauterine infection/transmission of TTSuV in pregnant sows has also been reported as a possibility based on the detection of TTSuVs in the semen of boars by PCR [5, 8, 13]. However, in these studies, detection of TTSuVs in the semen used for breeding the sows was not performed. Therefore, in this study, to exclude the effect of TTSuV infection/transmission to the fetus in the uterus by semen, sows were bred using semen in which TTSuV1 and

<table>
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<th>Sows</th>
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<td>No.</td>
<td>TTSuV1</td>
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<td>6 – –</td>
<td>1 – –</td>
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</table>

+: Detected; - : Not detected.

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TTSuV2 were not detected by nPCR.

TTSuV1 and/or TTSuV2 were detected in the sera of newborn piglets immediately after birth prior to suckling colostrum. This result indicated the existence of a TTSuV transmission pathway from sow to fetus during pregnancy. Moreover, TTSuV1 was detected in the piglets of sows without detectable levels of TTSuVs in sera at parturition, indicating that the sow-to-fetus TTSuV transmission pathway exists even in sows with undetectable levels of TTSuV in sera of sows at parturition. TTSuV movement within and between tissues has previously been suggested because TTSuVs have been detected in different tissues and not simply in serum [1, 10, 24]. Therefore, the detection of TTSuVs in sera of newborn piglets of TTSuV-negative sows in this study may indicate the possible movement of the virus from serum to other tissues after the occurrence of sow-to-fetus transmission.

It has been assumed that suckling colostrum may be one of the routes of TTSuV transmission in swine because TTSuVs were detected in colostrum of sow [14]. In this experiment, immediately after birth without suckling colostrums, TTSuV1 and TTSuV2 were not detected in 10 and 8 out of 12 newborn piglets from 3 sows; however, at 24 hr after suckling colostrum, TTSuV1 was detected in 6 of 10 piglets. The TTSuV1 detected in these piglets at 24 hr after birth following suckling colostrum most likely originated from the colostrum since the concentrations of TP indicated that all the piglets had adequately consumed colostrum during this postnatal period. However, a possibility of piglet-to-piglet or sow-to-piglet transmission of TTSuV via feces, urine and nasal secretions cannot be ignored, since TTSuV dissemination by these routes has been reported by some authors [4, 26–28]. On the other hand, TTSuV2 was not detected in any piglet at 24 hr after suckling colostrum indicating that TTSuV2 might be less transmissible via colostrum compared with TTSuV1.

In conclusion, it was confirmed that a sow-to-fetus transmission pathway of TTSuV exists during normal pregnancy and that there might be a sow-to-piglet transmission pathway of TTSuV via colostrum.

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