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Short Communication

Detection of *Torque teno sus virus 1* and *2* in tissues from stillborn piglets delivered by sows *via* natural farrowing

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We detected *Torque teno sus virus 1* and *2* (TTSuV1 and TTSuV2) in tissue samples from 18 stillborn piglets using nested polymerase chain reaction. The detection rates of TTSuV1 and TTSuV2 were 78% and 50%, respectively, with 83% of the stillborn piglets positive for TTSuV1 or TTSuV2. TTSuV1 was detected highest in the liver (72%) followed by heart (56%), spleen (38%) and tonsils (38%) while TTSuV2 was detected highest in the tonsils (38%) followed by liver (33%), spleen (25%), and heart (17%). These results indicate that TTSuVs are commonly present but not equally distributed among the tissues of stillborn piglets.

Keywords: natural farrowing, sow, stillborn piglet, tissue, TTSuV

Torque teno virus (TTV) is a small, non-enveloped virus with single-stranded, circular DNA grouped into the *Anelloviridae* family (International Committee on Taxonomy of Viruses, USA). TTV was first detected in a human patient with post-transfusion hepatitis of unknown etiology [10]. In pigs, the homologous counterpart of human TTV, *Torque teno sus virus* (TTSuV), was first reported in 1999 [5] although it was shown that this virus had been circulating in pig farms as early as 1985 [12]. Since then, the reported prevalence of TTSuV has varied between 24～100% in pigs from different countries [2,4,8,9,12,14]. However, the impact of TTSuV on the health of both human and animals is still not yet clear. Two species of TTSuVs (TTSuV1 and TTSuV2) belonging to the genus *Iotatorquevirus* have been reported [9,11].

Apart from serum, plasma, semen, feces, colostrum, and nasal secretions, TTSuVs have been also detected by polymerase chain reaction (PCR) in various tissues from pigs including those of fetuses [1,6]. Additionally, these viruses have been detected in sera of stillborn piglets derived by ceasarian section and those derived from sows with occasional stillbirths [7]. However, to the best of our knowledge there are no reports of TTSuVs detected in tissues from stillborn piglets. Moreover, it is still unclear whether TTSuVs have any role in reproductive failure of sows. To provide further insights on the dynamics of TTSuV infection in stillborn piglets, we performed the present study to determine the detection rates of TTSuVs from selected stillborn piglet tissues by performing nested PCR (nPCR).

The tissue samples evaluated in this study were obtained from the frozen stillborn piglets submitted to the Laboratory of Farm Animal Production Medicines, Faculty of Agriculture, Kagoshima University (Japan) in October 2008. A total of 18 stillborn Berkshire piglets delivered by 16 sows were received from a conventional farm housing 2,400 breeding sows. All stillborn piglets were delivered by sows *via* natural farrowing (1～2 stillborn piglets/sow) along with other live piglets. The average litter size of the sows ranged from nine to 11 piglets. All sows on the farm were regularly vaccinated against pathogens associated with major reproductive failure such as Aujeszky's disease virus (ADV), encephalomyocarditis virus (EMV), Getah virus (GV), Japanese encephalitis virus (JEV), porcine enterovirus (PEV), porcine parvovirus (PPV), and porcine reproductive and respiratory syndrome virus (PRRSV). At the time of farrowing, all sows appeared healthy without any clinical signs of reproductive problems. Body weights of the stillborn piglets at birth (1.2～1.4 kg) were comparable to those of the live piglets. Analysis of reproductive data collected for 1 year from the farm revealed an increased number of stillborn piglets to 0.7 per

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litter during the sampling month compared to that observed 6 months prior to (0.25 stillborn piglets/litter/month) and after (0.35 stillborn piglets/litter/month) the sampling month. During necropsy, all stillborn piglets were found to have collapsed lungs and enlarged livers, but the heart and spleen were normal.

Tissues from the heart, liver, spleen, and tonsils were collected from the stillborn piglets, homogenized manually by mortar and pestle in 20% phosphate buffered saline (pH 7.4), and centrifuged at 1,800 × g for 20 min. The supernatant was then collected. DNA was extracted from 250 μL of the tissue supernatant using a commercially available kit (DNA Extractor WB Kit; Wako Chemicals, Japan). Nucleic acids specific for porcine circovirus type 2 (PCV2) and TTSuVs were detected by PCR as previously described [3,4]. For PRRSV detection, RNA was extracted from the same quantity of individual tissue supernatant used for DNA recovery using a commercially available kit (Isogen-LS; Nippon Gene, Japan). The presence of PRRSV RNA was detected by reverse transcription PCR as previously described [13].

As shown in Table 1, 83% (15/18) of the stillborn piglets were infected with either species of virus. TTSuV1 was detected in 78% (14/18) of the stillborn piglets while TTSuV2 was found in 50% (9/15). Co-infection with both species of virus was observed in 44% (3/18) of the stillborn piglets. In the tissue samples, TTSuV1 was detected more frequently than TTSuV2, particularly in the liver (72%, 13/18 *vs.* 33%, 6/18) followed by heart (56%, 10/18 *vs.* 17%, 3/18) and spleen (38%, 3/8 *vs.* 25%, 2/8). The detection rate of either TTSuV1 or TTSuV2 from a particular tissue was the highest in the liver (78%, 14/18) followed by tonsils (63%, 5/8), spleen (63%, 5/8), and heart (61%, 11/18). PCV2 and PRRSV were not detected in any tissues from the stillborn piglets (results not shown).

TTSuVs have been detected by PCR in various tissues from healthy pigs including those of fetuses [1,6]. However, there are currently no reports of TTSuV detection in tissues from stillborn piglets although the viruses have been found in sera [7]. Therefore, we performed nPCR in the present study to detect the presence of TTSuV1 or TTSuV2 in selected tissues from stillborn piglets delivered by sows *via* natural farrowing. A high percentage of the tissues we evaluated were positive for TTSuVs, indicating that apart from serum these viruses could also reside in various tissues of stillborn piglets. Interestingly, the detection rate for the two TTSuV species was different among the tissues. TTSuV1 was more frequently found in the liver followed by heart, spleen, and tonsils. In contrast, the detection rate of TTSuV2 was highest in the tonsils followed by liver, spleen, and heart. The presence of TTSuV1 and TTSuV2 in tissues we examined may be attributed to passive distribution of the viruses through normal blood circulation. However, it seems more likely that this represents tissue tropism or indicates preferential sites for TTSuV replication and/or accumulation since both virus species were not equally distributed among the tested tissues. However, the exact mechanism and biological implications of our findings require further investigation.

Overall, 83% of the stillborn piglets were infected with either TTSuV1 or TTSuV2, and 70% and 50% were positive for TTSuV1 and TTSuV2, respectively. Interestingly, these detection rates are comparatively higher than those previously reported [7] from sera of stillborn piglets derived by caesarean section and those derived from sows with occasional stillbirths (TTSuV1, 50% and TTSuV2, 7%). It is unknown whether the high TTSuV detection rate observed in this study could be related to type of samples that were assayed (tissue *vs.* serum) or pathological conditions of the stillborn piglets. Our recent report of TTSuVs prevalence for sera taken from newborn piglets prior to consuming colostrum at a different farm showed that 18.9% and 8.6% of the samples were positive for TTSuV1 and TTSuV2, respectively [15]. These figures are comparatively lower than the values for the tissues found in our current study.

The stillborn piglets examined in the present investigation came from apparently healthy sows. However, the number of stillbirths increased at the farm housing these animals during the sampling month. PRRSV and PCV2 seemed to

Table 1. Detection rates of *Torque teno sus virus 1* (TTSuV1) and *2* (TTSuV2) in tissues from stillborn piglets delivered by sows *via* natural farrowing

Tissue	TTSuV1	TTSuV2	TTSuV1 and TTSuV2	TTSuV1 or TTSuV2
Liver	72 (13/18)	33 (6/18)	28 (5/18)	78 (14/18)
Heart	56 (10/18)	17 (3/18)	11 (2/18)	61 (11/18)
Spleen	38 (3/8)	25 (2/8)	0 (0/8)	63 (5/8)
Tonsils	38 (3/8)	38 (3/8)	13 (1/8)	63 (5/8)
Total infected stillborn piglets	78 (14/18)	50 (9/18)	44 (8/18)	83 (15/18)

Values are expressed as the percentage with the number of samples infected with TTSuVs over the total number of samples examined shown in parentheses.

have not promoted stillbirth since they were not detected by PCR in the present study. Moreover, all sows at the farm in our study were regularly vaccinated for ADV, EMV, GV, JEV, PEV, PPV, and PRRSV, thereby minimizing the probability that these viruses were associated with stillbirth. In addition, the farm has had no previous record of reproductive failure related to other infectious and non-infectious agents. Based on our results and observations, we speculate that TTSuV was a potential cause of increased stillbirths at the farm examined in our study. However, since this investigation was not designed to test the causal relationship between TTSuV infection and stillbirth, our hypothesis must be interpreted with caution and deserves further investigations.

In summary, the results of our study indicated that TTSuVs are widely prevalent in the tissues of stillborn piglets delivered by sows *via* natural farrowing. Furthermore, we demonstrated that TTSuV1 and TTSuV2 were not equally or uniformly distributed among the tissues of these piglets suggesting that the two TTSuV virus types may have different predilection sites.

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