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RAPID COMMUNICATION

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Presence of Torque teno sus virus 1 and 2 in porcine circovirus 3-positive pigs

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Summary

In this study, the co-infection of Torque teno sus virus (TTSuV) and porcine circovirus type 3 (PCV3) was reported. One hundred and ten of 132 (83.3%) PCV3-positive samples were co-infected with Torque teno sus virus 1 (TTSuV1). Ninety-four of 132 (71.2%) PCV3-positive samples were co-infected with Torque teno sus virus 2 (TTSuV2). Sixty-six of 132 (50.0%) of PCV3-positive samples were co-infected with both TTSuV1 and TTSuV2. There were no clinical signs of infection in pigs that were both PCV3-positive and PCV2-negative, in either multiparous sows or live-born infants. The high co-infection rate provides valuable information for the further study of the pathological correlation between PCV3 and TTSuVs.

KEYWORDS
co-infection, porcine circovirus type 3 (PCV3), torque teno sus virus (TTSuV)

1 | INTRODUCTION

Porcine circovirus type 3 (PCV3) is a newly emerging virus, first reported in pigs with unexplained cardiac and multi-organ inflammation in the USA (Phan et al., 2016). At the same time, PCV3 was identified in sows that died acutely with porcine dermatitis and nephropathy syndrome (PDNS)-like clinical signs and reproductive failure. The genomics aspect reveals the PCV3 genome was 2,000 bases in length with two major inversely arranged open reading frames (ORFs) encoding replicase (Rep) and capsid (Cap) proteins (Palinski et al., 2016). The epidemiological aspect shows PCV3 has been reported in the USA, China, Poland and South Korea (Ku et al., 2017; Kwon, Yoo, Park, & Lyoo, 2017; Stadejek, Woźniak, Miłek, & Biernacka, 2017). In China, the detection of porcine circovirus 3 had

been reported in South China (Chen et al., 2017; Shen et al., 2017; Wen et al., 2017) and Central China (Fan et al., 2017; Ku et al., 2017). Our laboratory first reported the occurrence of PCV3 in pigs without clinical infection signs in Shandong Province, eastern China (Zheng et al., 2017).

Torque teno sus virus (TTSuV) is a non-enveloped virus with a circular single-stranded DNA genome just like porcine circovirus (PCV). Two TTSuV species have been described: Torque teno sus virus 1 (TTSuV1) and Torque teno sus virus 2 (TTSuV2). TTSuV is ubiquitous in pig farms worldwide. TTSuV was, until recently, considered non-pathogenic and commonly detected in healthy pigs, but studies have shown that it can serve as a "trigger" or cofactor for porcine circovirus type 2 (PCV2) in the pathogenesis of post-weaning multisystemic wasting syndrome (PMWS) (Novosel, Lipej, Cubric-Curik, & Jungic, 2012). Former researchers have detected TTSuVs in PCV2-positive samples. The sows with PDNS were infected with both PCV3 and TTSuV1 in the USA (Palinski et al., 2016). The importance of TTSuVs infection in the PCV3-positive pigs needs to be further investigated.

2 | MATERIALS AND METHODS

2.1 | Sample preparation

One hundred and thirty-two PCV3-positive but PCV2-negative tissue samples (including livers, lungs, kidneys, spleens and umbilical cords) were collected from 28 multiparous sows and live-born infants from seven large pig farms (Weihai, Yantai, Linyi, Binzhou, Weifang, Laiwu and Liaocheng) in Shandong Province as previously reported (Zheng et al., 2017). Tissue homogenates were prepared for DNA extraction.

2.2 | DNA extraction

500 μl of tissue homogenates in an Eppendorf tube that had been frozen and thawed three times was digested by 50 μl of 10% SDS and 10 μl of proteinase K (20 mg/ml) at 55°C for 2 hr. DNA was extracted with TRIS saturated phenol, chloroform and absolute ethanol according to prior steps. DNA was washed with 75% frozen ethanol and then was dissolved in 20 μl of sterile water and stored at −20°C.

2.3 | TTSuV nested-PCR

DNA was analysed using TTSuV nested-PCRs. TTSuV nested-PCRs were performed in a 50 μl final volume, containing 3 μl DNA, 1 μl each of four primers, 1 μl (10 mmol/L) dNTP (Invitrogen, Shanghai, China), 2 mM $MgCl_2$, 5 μl PCR buffer and 1 U Taq DNA polymerase (Invitrogen).

2.4 | Phylogenetic analysis

Phylogenetic analyses of TTSuV1 and TTSuV2 were performed using MEGA 6.0.

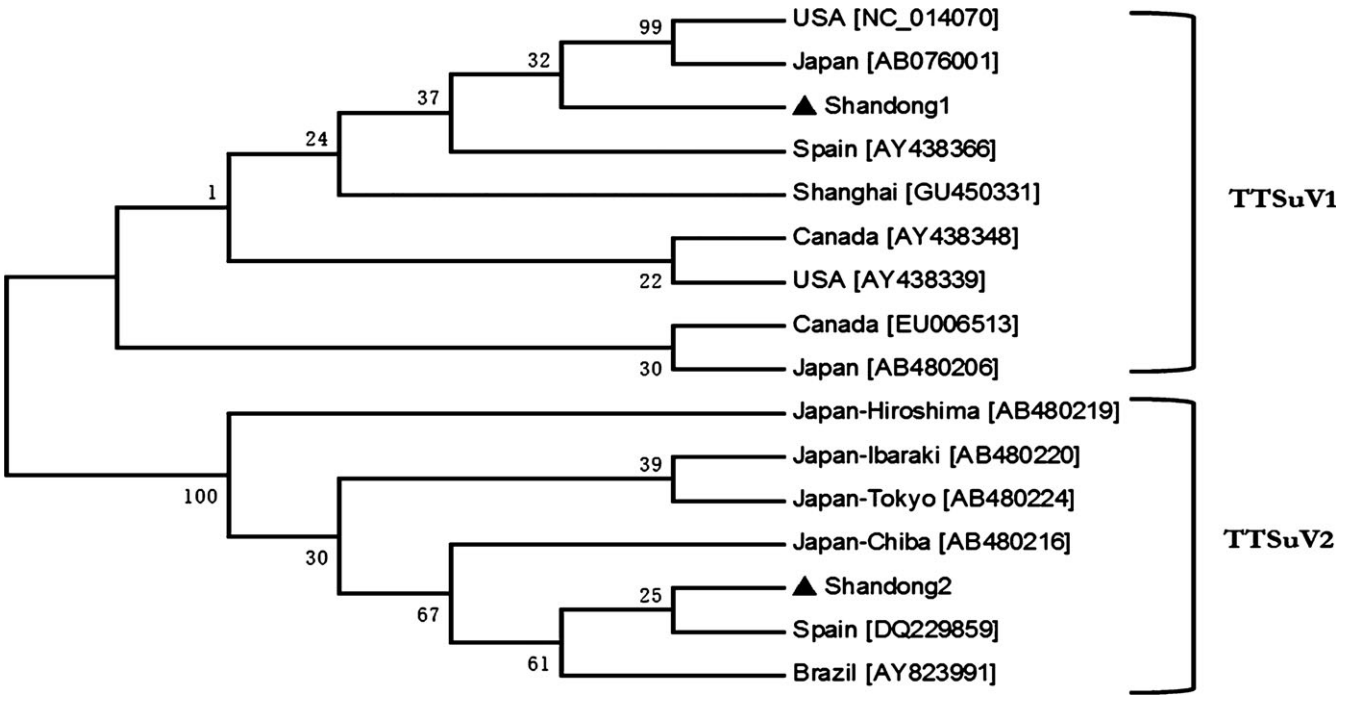

FIGURE 1 Phylogenetic tree of TTSuV1 and TTSuV2. Phylogenetic tree was constructed using the maximum-likelihood algorithm of MEGA 6.0 with 1,000 bootstrap trials

FIGURE 2 Geographical distribution of porcine circovirus type 3 (PCV3) and the co-infection with Torque teno sus virus (TTSuV) in Shandong Province, eastern China. Red stars represent the PCV3-positive pig farms. Yellow stars represent the pig farms which were co-infected with TTSuV

3 | RESULTS AND DISCUSSION

TTSuV1 nested-PCR was performed using primers A1/A2 and B1/B2 (A1: 5′-TACACTTCCGGGTTCAGGAGGCT-3′, A2: 5′-ACTCAGC CATTCGGAACCTCAC-3′, B1: 5′-AGTTACACATAACCACCAAACC-3′, B2: 5′-ATTACCGCCTGCCCGATAGGC-3′). TTSuV2 nested-PCR was performed using primers A3/A4 and B3/B4 (A3: 5′-CAATTTGGCT CGCTTCGCTCGC-3′, A4: 5′-TACTTATATTCGCTTTCGTGGGAAC-3′, B3: 5′-CCAAACCACAGGAAACTGTGC-3′, B4: 5′- CTTGACTCCGC TCTCAGGAG-3′). Nested-PCR amplification was carried out using 35 cycles at 94°C for 30 s, primers annealing at 52°C for 20 s, extension at 72°C for 30 s and a final extension for 10 min at 72°C. The amplified products (260 bp for TTV1 and 230 bp for TTV2) were run in a 2% agarose gel with 5 μg/ml of ethidium bromide. The phylogenetic analyses of TTSuV1 and TTSuV2 are shown in Figure 1.

This is the first report evaluating the occurrence of TTSuV1 and TTSuV2 in PCV3-positive pigs with no signs of clinical infection. One hundred and thirty-two PCV3-positive but PCV2-negative samples were examined for TTSuV1 and TTSuV2 using TTSuV nested-PCR. Geographical distribution of porcine circovirus type 3 (PCV3) and the co-infection with Torque teno sus virus (TTSuV) in Shandong Province showed three (Binzhou, Linyi and Yantai) of the seven PCV3-positive pig farms were co-infected with both TTSuV1 and TTSuV2 (Figure 2). The co-infection of Torque teno sus virus (TTSuV) and porcine circovirus type 3 (PCV3) was reported: 110 of 132 (83.3%) PCV3-positive samples were co-infected with Torque teno sus virus 1 (TTSuV1). Ninety-four of 132 (71.2%) PCV3-positive samples were co-infected with Torque teno sus virus 2 (TTSuV2). Sixty-six of 132 (50.0%) PCV3-positive samples were co-infected with both TTSuV1 and TTSuV2. The high prevalence of TTSuV1 and TTSuV2 in PCV3-positive but PCV2-negative pigs suggests that variation could exist among the TTSuVs strains in China. In the first report of PCV3, sows with PDNS were infected with both PCV3 and TTSuV1 (Palinski et al., 2016). However, in this study, the co-infection of TTSuVs with PCV3 was detected among clinically healthy multiparous sows and live-born infants. The high co-infection rate provides valuable information for the further study of the pathological correlation between PCV3 and TTSuVs.

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CONFLICT OF INTEREST

None.

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