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Research Article

DETECTION OF TORQUE TENO SUS VIRUS (TTSUV) IN CLINICAL SPECIMENS OF PIGS WITH CLASSICAL SWINE FEVER, PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME, AND PORCINE CIRCOVIRUS 2 INFECTIONS

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ABSTRACT: *Torque teno sus viruses* (TTSuVs) have never been implicated in direct causation of any disease; however, their role as cofactors in precipitation of certain disease conditions is gaining some support. Considering the spurt in the number of outbreaks of porcine reproductive and respiratory syndrome (PRRS), porcine circovirus 2 (PCV2) infections and classical swine fever (CSF) in India, we have investigated the extent of association of TTSuVs with the above said viruses by PCR. The TTsuVs were detected in 53% and 26.22% of CSFV-PRRSV-PCV-positive and apparently healthy negative tissue samples respectively. In serum, these were detected respectively in 29.60% and 21.42 % of CSFV-PRRSV-infected and apparently samples. The results obtained for the tissue samples is in concurrence with the observations of previous studies which reported higher prevalence of TTSuVs in CSFV-PRRSV-PCV-positive clinical specimens as compared to the healthy ones. This is the first report of co-infection of TTSuVs with CSFV, PRRSV and PCV from India. Future works are needed to establish pathogenic role of TTSuVs through experimental studies.

Key words: Torque teno sus virus, PCV2, CSFV, PRRSV, India, Pig.

INTRODUCTION

Torque teno sus viruses (TTSuVs) are non-enveloped, circular single stranded DNA viruses with a genome size of approximately 2.8kb (Okamoto *et al.* 2002). The genome contains 3 major open reading frames (ORFs) and an untranslated region (UTR) of promoter and enhancer elements important for replication and transcription activities (Kamada *et al.* 2004, Suzuki *et al.* 2004). Presently, TTSuVs have been classified within the family *Anelloviridae*, which includes the genus *Iota torque virus* (TTSuV1) and *Kappa torque virus* (TTSuVk2). The species *Torque teno sus virus 1a* (TTSuV1a) and *Torque teno sus virus 1b* (TTSuV1b) constitute the genus *Iota torque virus*, while the species *Torque teno sus virus k2a* and *Torque teno sus virus k2b* constitute the genus *Kappa torque virus* (Lefkowitz *et al.* 2018).

TTSuVs have been detected in the pig populations worldwide with variable frequencies of infection (Mckeown *et al.* 2004, Segales *et al.* 2009, Jarosova *et al.* 2011, Subramanyam *et al.* 2019), although they have never been implicated in direct causation of any disease with any observable clinical signs (Ellis *et al.* 2008, Krakowka and Ellis 2008, Vargas-Ruiz *et al.* 2018).

Nevertheless, recent studies have reported a high rate of coinfection between TTSuVs and porcine circovirus (PCV)-associated disease (PCVAD) including in porcine circovirus 3 (PCV3) infections (de Castro *et al.* 2015, Vargas-Ruiz *et al.* 2018) and also with porcine epidemic diarrhea virus (PEDV, Su*et al.* 2019). Among the other diseases, association of TTSuV has been described for porcine circovirus type 2 (PCV2), classical swine fever (CSF), porcine parvovirus (PPV) and porcine reproductive and respiratory syndrome virus (PRRSV)

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(Perez *et al.* 2011, Kekarainen and Segalés 2012). In India and elsewhere CSFV, PRRSV, and PCV2 infections are considered economically important and the cases/ outbreaks of CSF are reported every year; while PCV2 infection and PRRS are considered as emerging diseases. The causative agents of CSFV, PRRSV belong to RNA virus families of *Flaviviridae* and *Arteriviridae* respectively, while PCV2 belongs to a DNA virus family *Circoviridae*. CSFV and PRRSV have been known to cause severe to chronic infections resulting in heavy mortality and abortions, while PCV2 is known to cause systemic or subclinical infection resulting in weight loss. Additionally, it has been observed that TTSuV1 infection stimulates B cell hyperplasia, providing more target cells for porcine circo virus 2 replications, while both TTSuV2 and PCV2 infections have been shown to cause positive impact on macrophage infiltration (Lee *et al.* 2014). World over, coinfection of TTSuV with the above said viruses exist, while no such studies are available from India. Thus, considering emergence of PRRS and PCV2 infections in the country, the study on TTSuV was inevitable. Due to the non-availability of suitable cell culture system for isolation of the virus, the diagnosis of TTSuV infection is usually done by the detection of viral DNA using several PCR-based assays including quantitative PCR (qPCR). The present study was aimed to investigate the occurrence of co-infection of TTSuV with important viral pathogens such as PRRSV, CSFV, and PCV2 in India.

MATERIALS AND METHODS

Clinical samples

Ninety-five (95) blood and tissue samples (kidney, lungs, spleen and lymph node) of pigs collected from the confirmed (through PCR in some other lab) cases of PRRSV, CSFV, PCV-2 infection from different states during the period 2016-2018 were designated as 'positive samples' for the purpose of study. Similarly, another 122 samples collected from apparently healthy pigs from abattoir were also studied and designated as 'healthy samples'. Further, a total of 445 purposive serum samples *viz.*, 179 samples from positive cases of CSFV (N=108) and PRRSV (N=71), and 266 samples collected from abattoir or from apparently healthy pigs from different regions of India were also included in the study.

Nucleic acid extraction and Reverse Transcription-Polymerase Chain Reaction

The DNA and RNA of tissue samples were extracted using either DNeasy Blood and Tissue Kit, or RNeasy Mini Kit (Qiagen, Germany) depending on the nature of the virus for which the sample was screened. Reverse transcription PCR (RT-PCR) of the RNA templates of CSFV and PRRSV was performed using previously published primer pairs (Patil *et al.* 2010, Phanikashyap *et al.* 2019) and one step RT-PCR kit (Qiagen, Germany) following the protocols described elsewhere (Patil *et al.* 2010, Phanikashyap *et al.* 2019). The PCR amplification of TTSuV DNA was performed as per the protocol described earlier (Subramnyam *et al.* 2019). The details of the primer sequences and target gene are presented in Table 1. For amplification of ORF2 gene of PCV2, each reaction consisted of 3µl DNA as template, 1x PCR buffer, 2.5 mM MgCl2, 1.0 mM dNTP, 10pmol forward primer and reverse primer each, 0.25 U Taq DNA polymerase (New England Biolabs, USA) and nuclease free water. The thermal profile was as follows: a cycle of 10 min of initial denaturation at 95 ˚C, followed by 35 cycles of 95 ˚C for 30 s, 58 ˚C for 30 s and 72 ˚C for 30 s, and an

Table 1. List of Primers used in the study.

Primer	Sequence 5'-3'	Amplicon Size (bp)	Target gene	Reference
CSFV-UP1	CTAGCCATGCCCWYAGTAGG	421	5' UTR	Paton *et al.* (2000)
CSFV-UP2	CAGCTTCARYGTTGATTGT			
PRRSV-F	TCGTGTTGGGTGGCAGAAAAGC	484	ORF6 & ORF7	Chang *et al.* (2002)
PRRSV-R	GCCATTCACCACACATTCTTCC			
TTSuV1-F	TACACTTCCGGGTTCAGGAGGCT	300	5'UTR	Ozawa *et al.* (2015)
TTSuV1-R	ACTCAGCCATTCGGAACTGCA			
TTSuVk2-F	TCATGACAGGGTTCACCGGA	230	5 UTR	Kekarainen *et al.* (2006)
TTSuVk2-R	CGTCTGCGCACTTACTATATACTCTA			
PCV2-F	AAGGGCTGGGTTATGGTATG	353	ORF-2	Zeng *et al.* (2014)
PCV2-R	CGCTGGAGAAGGAAAAATGG			

Table 2. State-wise details of the tissue samples screened. Values under the virus header indicate number positive*.

	Detection in Infected samples						Detection in abattoir samples		
	PRRSV	CSFV	PCV2	TTSuV1	TTSuVk2	Total screened	TTSuV1	TTSuVk2	Total screened
Assam	0	1	1	1	1	2	0	0	12
Chhattisgarh	0	0	3	1	2	3	1	0	7
Odisha	2	2	0	0	2	2	0	0	0
Goa	2	2	24	4	5	24	2	3	18
Karnataka	27	18	19	12	14	34	1	4	15
Kerala	0	2	2	0	2	2	0	0	0
Madhya Pradesh	0	2	0	0	1	2	0	0	0
Maharashtra	0	0	2	0	1	2	4	10	28
Manipur	0	0	1	0	1	1	0	1	6
Meghalaya	0	0	1	1	1	1	0	0	0
Mizoram	8	8	13	10	13	17	5	9	34
Telangana	0	5	2	4	4	5	0	2	2
Total	39	40	68	33	47	95	13	29	122

*Includes multiply infected samples.

extension cycle of 10 min at 72 ˚C using the primer pair listed in Table 1. The PCR products were visualized on a 1.5% agarose gel. Further confirmation of the specificity of the amplicons was done by nucleotide sequencing of the representative products.

RESULTS AND DISCUSSION

PCR detection of virus genes

Virus gene amplicons of sizes 484bp, 421bp, 353bp, 300bp and 230bp were obtained for PRRSV, CSFV, PCV2, TTSuV1 and TTSuVk2 respectively by using the specific primers (Fig. 1). The nucleotide sequences obtained from the above representative amplicons were subjected to BLAST search at NCBI website to confirm specific amplification of target genes of the different viruses. Considering that nucleotide sequences of the PCR amplicons of PRRSV, CSFV and TTSuVsv did not yield any new information and also that validity/specificity of the primer pairs had been confirmed in our previous studies (Patil *et al*. 2010, Phanikashyap *et al*. 2019, Subramanyam *et al*. 2019), the phylogeny data for the above viruses was not included in the present paper. However, a nucleotide sequence obtained from ORF2 region of PCV2 primer pair was used for phylogenetic analysis, which was done by incorporating all the reported lineages so far (Franzi and Seagles 2018). Nucleotide sequence analysis indicated that strain used in our study belongs to lineage 2d (Fig. 2).

Prevalence of TTSuV in blood and tissue samples

Among the 95 samples positive for CSFV, PRRSV and PCV2, the TTSuV1 and TTSuVk2 were singularly detected in 4 (4.21%) and 18 (18.94%) samples respectively, while 29 (30.52%) samples were positive for both the viruses. Similarly, 17, 8, and 4 samples were singularly positive for PCV2, PRRSV, and CSFV infections respectively. Dual infections of TTSuVk2-TTSuV1, TTSuVk2-PCV2, CSFV-PRRSV and TTSuV1-PCV2 were detected in 10, 8, 4, 2 samples respectively. Additionally, dual infections of CSFV-PCV2, CSFV-TTSuVk2, TTSuV1-PRRSV, and TTSuVk2-PRRSV were detected in one sample each. Triple infections of TTSuV1-TTSuVk2-PCV2, TTSuV1-TTSuVk2-CSFV, TTSuV1-TTSuVk2-PRRSV were observed in 12 (5.94%), 2 (0.99%), and 1 (0.50%) pig samples respectively. Furthermore, 3 samples were infected with all the five pathogens (TTSuV1, TTSuVk2, PCV2, PRRSV and CSFV) studied. The other patterns of co-infection observed in the study are presented in the Figure3. Among the apparently healthy pigs, TTSuV1 was detected in 2.4% (3/122) samples, TTSuVk2 in 15.57% (19/122) samples; another 8.19% (10/122) samples were found to be infected with both the TTSuV1 and TTSuVk2. The state-wise screening results for infected and healthy tissue samples are given in the Table 2.

Table 3. State-wise details of the serum samples screened. Values under the virus header indicate number positive*.

State	Detection in infected samples				Detection in abattoir/healthy samples			
	PRRSV	CSFV	TTSuV1	TTSuVk2	Total	TTSuV1	TTSuVk2	Total
Arunachal Pradesh	0	0	0	0	0	1	6	24
Chhattisgarh	0	10	2	2	10	0	0	0
Goa	0	9	0	0	9	0	0	0
Karnataka	0	10	1	1	10	0	0	5
Kerala	0	0	0	0	0	0	0	22
Madhya Pradesh	0	0	0	0	0	0	0	0
Maharashtra	0	21	3	14	21	0	0	21
Manipur	0	0	0	0	0	0	0	14
Meghalaya	0	0	0	0	0	0	0	5
Mizoram	71	58	16	28	129	17	43	171
Punjab	0	0	0	0	0	0	0	4
Total	71	108	22	45	179	18	49	266

*Includes multiply infected samples.

Prevalence of TTSuV in serum samples

Of the 445 sera examined in the study, 179 samples were from pigs infected with CSFV and/or PRRSV, while the remaining 266 samples were from apparently healthy pigs. Among the samples from infected animals TTSuV1 and TTSuVk2 were detected in 4.47% (8/179) and 17.32% (31/179) samples respectively, besides presence of both the viruses in 7.82% (14/179) of the samples. Among the samples from apparently healthy pigs TTSuV1 and TTSuVk2 were detected in 2.63% (7/266) and 15.04% (40/266) samples respectively, besides 3.76% (10/266) samples being positive for both TTSuV1 and TTSuVk2. The state-wise screening results are given in Table 3.

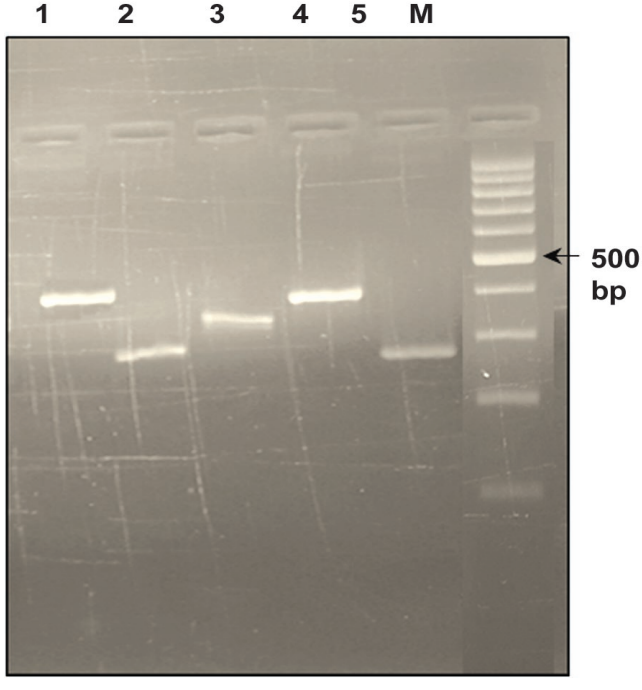

Fig. 1. Agarose gel electrophoresis of PCR amplicons generated from different regions of various viruses.
[Lane 1: PRRSV, Lane 2: TTSuV1, Lane 3: PCV2, Lane 4: CSFV, Lane 5: TTSuVk2, Lane M: 100 bp Mol. Wt. marker (500 bp product is shown by arrow)].

India has sizeable pig population which are reared under different biosecurity and management practices. Various viral diseases are major threats to the pig population in India and outside. The coinfection of multiple viruses not only increases the severity of the disease, it may also have negative influence on vaccine efficacy (Opriessnig *et al.* 2013, Genzow *et al.* 2009). Further, several studies have suggested that coinfection of TTSuVs particularly *Iotatorquevirus* species with another virus increases the severity of disease as a result of synergism (Gallei *et al*. 2010, Huang *et al*. 2011, Perez *et al*. 2011). Therefore, it is important to monitor coinfections for better prevention and control of diseases. Additionally, we believe that the data generated on the extent of coinfections of TTSuVs with other important pig pathogens would help to define the role of the former viruses on the clinical outcome.

In the present investigation, TTSuVs were detected in 53% (51/95) and 26.22% (32/122) of CSFV-PRRSV-PCV-positive and apparently healthy tissue samples respectively. Rammohan *et al.* (2012) also reported that

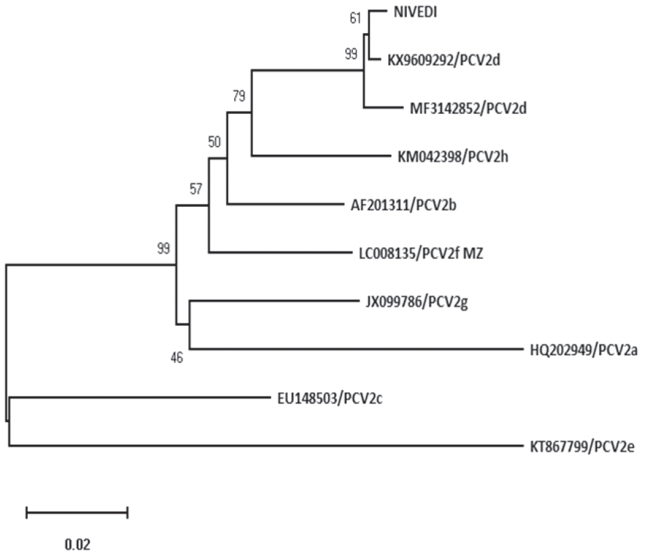

Fig. 2. Neighbor joining tree showing the grouping of Indian strain (NIVEDI) of PCV 2 with others in the ORF-2 region.
[The optimal tree with the sum of branch length = 0.41868803 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site. All ambiguous positions were removed for each sequence pair. There was a total of 353 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.* 2018). Lineage classifications are as per Franzo and Seagles (2018)].

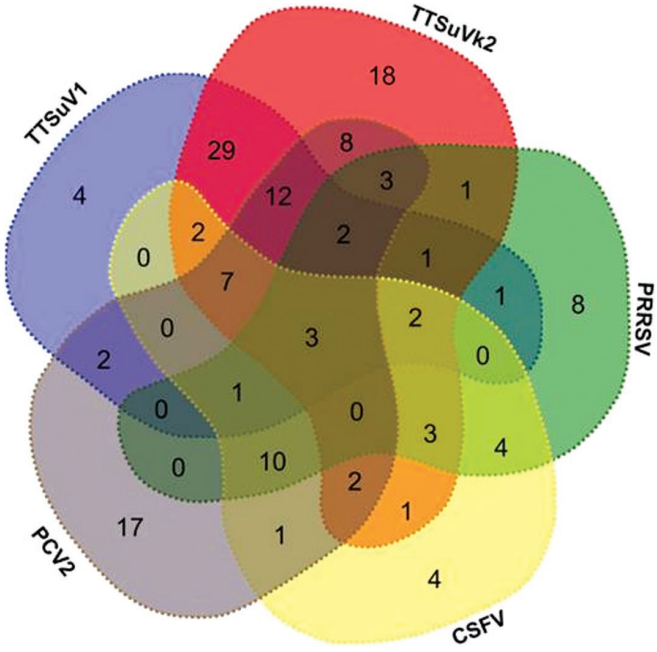

Fig. 3. Venn diagram showing the different patterns of coinfections observed in the study.
[Each colored shape represents a virus infection. The cells of the intersecting regions represent various combinations of coinfections. Values within each cell indicate number positive. The Venn diagram was created using the URL_http://bioinformatics.psb.ugent.be/webtools/Venn/].

rate of detection of TTSuVs in pigs with clinical signs of the coinfecting pathogens is roughly double that of clinically healthy pigs. Interestingly, among the 179 serum samples from CSFV-PRRSV infected animals, TTSuVs were detected in 29.60% (53/179) of samples, while the detection rate was 21.42 % (57/266) in apparently healthy serum samples, thus indicating insignificantly higher rate of detection among diseased animals. The lower prevalence of virus in serum samples of clinically infected animals, as compared to the other tissues described above, might arise from the fact that high load of virus is generally detected in blood during the initial viraemia stage and the load decreases when the virus localizes in different organs resulting in damage to internal organs and frank clinical onset of the disease. Aramouni *et al.* (2013) observed significantly higher TTSuV load in serum during incubation period following experimental infection of the animals with CSFV. This is substantiated by the fact that TTSuVs were detected at a higher percentage in tissues than in serum, as all the tissues in our study were collected during the necropsy. In view of the above, our findings support the earlier observations that TTSuVs may have a role in increased pathogenesis caused by above mentioned viruses in affected animals or, alternatively, immunosuppression associated with post weaning multisystemic wasting syndrome (PMWS) may up-regulate TTSuV replication (Aramouni *et al.* 2011, Nieto *et al.* 2011).

It has been reported (Lee *et al.* 2014) that TTSuVs, TTSuV1 in particular, may act as an immune stimulation inducer. Immuno-stimulation has been regarded as a pivotal event in the induction of wasting disease in PCV2-infected pigs (Krakowka *et al.* 2001, Opriessnig *et al.* 2007). Lymphoid hyperplasia, an indicator of immune-stimulation, is usually present in sub-clinically PCV2-infected pigs with a low virus load (Krakowka *et al.* 2002) and lymphoid hyperplasia occurring in the early stage of TTSuV infection may have a significant impact on PCV2 propagation via the provision of more target cells for PCV2 replication (Opriessnig and Halbur 2012), which may lead certain PCV2-infected pigs to subsequently transform into PMWS (Ellis *et al.* 2008). Similar to

PCV2, TTSuVk2a, but not TTSuV1, has a positive impact on macrophage infiltration with a significant correlation between TTSuVk2a and the above-mentioned lymphoid lesions (Lee *et al.* 2014). In our study, we found, 40 of the 68 (58.82%) of PCV2 infected tissues to harbor TTSuVs. Out of 40 PCV2 infected samples, 12 were infected by both the genera, while 13 were co-infected with TTSuVk2 and three were with TTSuV1. Though our study cannot provide any experimental proof about definitive role of TTSuVs in PMWS or PCVAD, the results obtained nevertheless support earlier observation of their increased detection (Nieto *et al.* 2011, Lee *et al.* 2014).

In conclusion, our study supports increased detection of TTSuVs in CSFV-PRRSV-PCV-positive clinical samples as compared to healthy ones. Additionally, this is the first report of co-infection of TTSuVs with CSFV, PRRSV and PCV from India. Future focus is required to establish pathogenic role of TTSuV through experimental studies.

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REFERENCES

Aramouni M, Kekarainen T, Ganges L, Tarradas J, Segales J (2013) Increased viral load and prevalence of Torque teno sus virus 2 (TTSuV2) in pigs experimentally infected with classical swine fever virus (CSFV). Virus Res 172: 81-84.

Aramouni M, Segales, J, Sibila M, Martin-Valls GE, Nieto D *et al.* (2011) Torque teno sus virus 1 and 2 viral loads in postweaning multisystemic wasting syndrome (PMWS) and porcine dermatitis and nephropathy syndrome (PDNS) affected pigs. Vet Microbiol 153: 377-381.

Chang CC, Yoon KJ, Zimmerman JJ, Harmon KM, Dixon PM *et al*. (2002) Evolution of porcine reproductive and respiratory syndrome virus during sequential passages in pigs. J Virol 76: 4750-4763.

de Castro AMM, Baldin CM, Favero CM, Gerber PF, Cipullo RI *et al.* (2015) Torque teno sus virus 1 and 2 viral loads in faeces of porcine circovirus 2-positive pigs. Acta Vet Brno 84: 91-95.

Ellis J, Allan G, Krakowka S (2008) Effect of coinfection with genogroup 1 porcine torque teno virus on porcine circovirus type 2-associated postweaning multisystemic wasting syndrome in gnotobiotic pigs. Am J Vet Med Res 69: 1608 - 1614.

Franzo G, Segalés J (2018) Porcine circovirus 2 (PCV-2) genotype update and proposal of a new genotyping methodology. PLoS One. 13: e0208585.

Gallei A, Pesch,S, Esking WS, Keller C, Ohlinger VF (2010) Porcine Torque teno virus: determination of viral genomic loads by genogroup-specifics multiplex rt-PCR, detection of frequent multiple infections with genogroups 1 or 2, and establishment of viral fulllength sequences. Vet Microbiol 143: 202–212.

Genzow M, Schwartz K, Gonzalez G, Anderson G, Chittick W (2009) The effect of vaccination against Porcine reproductive and respiratory syndrome virus (PRRSV) on the Porcine circovirus-2 (PCV-2) load in porcine circovirus associated disease (PCVAD) affected pigs. Can J Vet Res 73: 87–90.

Huang YW, Dryman BA, Harrall KK, Vaughn EM, Roof MB *et al.* (2010) Development of SYBR green-based real time PCR and duplex nested PCR assay for quantitation and differential detection of species or type specific porcine Torque Teno viruses. J Virol Methods 170: 140–148.

Jarosova V, Pogranichniy R, Celer V (2011) Prevalence and age distribution of porcine torque teno sus virus (TTSuV) in the Czech Republic. Folia Microbiol 56: 90-94.

Kamada K, Kamahora T, Kabat P, Hino S (2004) Transcriptional regulation of TT virus: promoter and enhancer regions in the 1.2-kb noncoding region. Virology 321: 341-348.

Kekarainen T (2006) Prevalence of swine Torque teno virus in post-weaning multisystemic wasting syndrome (PMWS)-affected and non-PMWS-affected pigs in Spain. J Gen Virol 87: 833-837.

Kekarainen T, Segales J (2012) Torque teno sus virus in pigs: an emerging pathogen? Transbound Emerg Dis 59: 103-108.

Krakowka S, Ellis J (2001) Evaluation of the effects of porcine genogroup 1 torque teno virus in gnotobiotic swine. Am J Vet Med Res 69: 1623-1629.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35: 1547-1549.

Lefkowitz EJ, Dempsey DM, Hendrickson RC, Orton RJ, Siddell SG *et al.* (2018) Virus taxonomy: the database of the international committee on taxonomy of viruses (ICTV). Nucleic Acids Res Spec Publ 46(D1): D708-D717.

Lee Y, Lin CM, Jeng CR, Pang VF (2014) Detection of torque teno sus virus 1 and 2 in porcine tissues by *in situ* hybridization using multi-strained pooled probes. Vet Microbiol 172: 390-399.

McKeown NE, Fenaux M, Halbur PG, Meng XJ (2004) Molecular characterization of porcine TT virus, an orphan virus, in pigs from six different countries. Vet Microbiol 104: 113-117.

Nieto D, Aramouni M, Grau-Roma L, Segales J, Kekarainen T (2011) Dynamics of Torque teno sus virus 1 (TTSuV1) and 2 (TTSuV2) DNA loads in serum of healthy and postweaning multisystemic wasting syndrome (PMWS) affected pigs. Vet Microbiol 152: 284-290.

Takahashi M, Nishizawa T, Tawara A, Fukai K, Muramatsu U *et al.* (2002) Genomic characterization of TT viruses (TTVs) in pigs, cats and dogs and their relatedness with species-specific TTVs in primates and tupaias. J Gen Virol 83: 1291-1297.

Opriessnig T, Halbur PG (2012) Concurrent infections are important for expression of porcine circovirus associated disease. Virus Res 164: 20-32.

Opriessnig T, Meng XJ, Halbur PG (2007). Porcine circovirus type 2 associated disease: update on current terminology, clinical manifestations, pathogenesis, diagnosis, and intervention strategies. J Vet Diagn Invest 19: 591-615.

Opriessnig T, Xiao CT, Gerber PF, Halbur PG (2013) Emergence of a novel mutant PCV2b variant associated with clinical PCVAD in two vaccinated pig farms in the US concurrently infected with PPV2. Vet Microbiol 163: 177-183.

Ozawa M, Kawabata T, Okuya K, Nagano K, Kanda T *et al.* (2015) Full genome sequences of torque teno sus virus strains that coinfected a pig with postweaning multisystemic wasting syndrome in Japan: Implications for genetic diversity. Arch Virol 160: 3067-3074.

Patil SS, Hemadri D, Shankar BP, Raghavendra AG, Veeresh H *et al.* (2010) Genetic typing of recent classical swine fever isolates from India. Vet Microbiol 141: 367-73.

Paton DJ, McGoldrick A, Greiser-Wilke I, Parchariyanon S, Song JY *et al.* (2000) Genetic typing of classical swine fever virus. Vet Microbiol 73: 137-157.

Phanikashyap S, Hiremath J, Subramanyam V, Patil SS, Roy P *et al.* (2019) PCR-based baseline survey indicates widespread prevalence of porcine reproductive and respiratory syndrome (PRRS) in India. J Comp Microbiol Immunol Infect Dis 40: 99-102.

Perez LJ, de Arce H, Frias MT, Perera CL, Ganges L *et al.* (2011) Molecular detection of Torque teno sus virus in lymphoid tissues in concomitant infections with other porcine viral pathogens. Res Vet Sci 91: 154-157.

Rammohan L, Xue L, Wang C, Chittick W, Ganesan S *et al.* (2012) Increased prevalence of torque teno viruses in porcine respiratory disease complex affected pigs. Vet Microbiol 157: 61-68.

Segales J, Martinez-Guino L, Cortey, M, Navarro N, Huerta E *et al.* (2009) Retrospective study on swine Torque teno virus genogroups 1 and 2 infection from 1985 to 2005 in Spain. Vet Microbiol 134: 199-207.

Su M, Li C, Qi S, Yang D, Jiang N *et al.* (2019) A molecular epidemiological investigation of PEDV in China: characterization of co-infection and genetic diversity of S1-based genes. Transbound Emerg Dis 67(3): 1–12.

Subramanyam V, Hemadri D, Kashyap SP, Hiremath J, Barman NN *et al.* (2019) Detection of torque teno sus virus infection in Indian pigs. Vet World 12: 1467-1471.

Suzuki T, Suzuki R, Li J, Hijikata M, Matsuda M *et al.* (2004) Identification of basal promoter and enhancer elements in an untranslated region of the TT virus genome. J Virol 78: 10820-10824.

Vargas-Ruiz A, Garcia-Camacho LA, Ramirez-Alvarez H, Rangel-Rodriguez I. C, Alonso-Morales RA, *et al.* (2018) Molecular characterization of the ORF2 of Torque teno sus virus 1a and torque teno sus virus 1b detected in cases of postweaning multisystemic wasting syndrome in Mexico. Transbound Emerg Dis 65: 1806-1815.

Zeng Z, Liu Z, Wang W, Tang D, Liang H *et al.* (2014) Establishment and application of a multiplex PCR for rapid and simultaneous detection of six viruses in swine. J Virol Methods 208: 102-106.

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