INTRODUCTION

Torque teno viruses (TTVs), a group of non-enveloped, circular single-strand DNA viruses with 3.6- to 3.9-kb genome size, were first discovered in a Japanese patient in 1997 (Nishizawa et al., 1997). According to the International Committee on Taxonomy of viruses (ICTV), TTVs were assigned into Anelloviridae family including 14 genera (Walker et al., 2020). Of which, \textit{Alphatorquevirus}, a genus

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mainly found in human and primates, can be divided into at least seven different genogroups with great genetic diversity (Hsiao et al., 2016; Mi et al., 2014; Ninomiya et al., 2008). Among them, genogroup 3 was the most widely spreading (AbuOdeh et al., 2015; Pinho-Nascimento et al., 2011).

Besides in humans, TTVs were found in a wide range of other hosts such as primates, pigs, cats, and dogs. The TTVs of pig origin can be classified into two major groups: Torque teno sus virus 1 (TTSuV1, genus Iotatorquevirus) and Torque teno sus virus 2 (TTSuV2, genus Kappatorquevirus), consisting of three subtypes (1a to 1c) and seven subtypes (2a to 2 g), respectively (Li et al., 2013). TTSuV1 was considered to cause clinical symptoms in gnotobiotic pigs (Ellis et al., 2008; Krakowka et al., 2008).

It is reported that TTVs common in human infection can be found in pig’s serum (Ssemadaali et al., 2016). The authors also provided evidence about the possible infection of human cells by TTSuV1, which raised the question of transmission of TTVs among humans and animals. Recently, a study based on molecular analysis supported the hypothesis of human to animal transmission event of TTVs (Sarairah et al., 2020).

Up to date, few studies focused on the prevalence of TTSuVs and other TTVs in Korean swine farms. Therefore, the aim of this study is to investigate the appearance of TTSuVs and TTV genogroup 3 in sick pigs from Korean domestic swine farms in South Korea during the 2017–2018 period.

2 | MATERIALS AND METHODS

From January 2017 to December 2018, 470 clinical samples (sera, tissues in lung, kidney, liver and lymph node; samples are pooled in each group) from nine provinces of South Korea were sent to the lab for diagnosis of respiratory viral diseases. The total DNA was extracted using a viral DNA/RNA extraction kit (iNtRON Biotechnology Inc., Gyeonggi, South Korea) and was immediately used for amplification or stored at −20°C.

Methods for detection of TTSuV1 and TTSuV2 were following the previous studies (Li et al., 2013). We further investigated the most widely spread Torque teno virus of genogroup 3 (TTV3) to confirm cross species infection. Detection of TTV3 uses AI-1F and AI-1R as mentioned below (Dencs et al., 2009). The polymerase chain reaction (PCR) was performed using an i-StarMaster mix PCR kit (iNtRON Biotechnology Inc.). For genetic characterization, we followed TTSuVs of the three strains (M117, N86 and N116) completely sequenced by a primer walking method (Biagini et al., 2000).

In this study, pigs showing signs of respiratory problems (n = 470) were collected on the detection rates of TTSuVs in 2017 and 2018; the positive rates of TTSuV1 were 17% (47/280), 15% (28/190) in 2017 and 2018, and for TTSuV2, they were 34% (95/280) and 39% (73/190) in 2017 and 2018, respectively. In total, positive rates of 16% (75/470) and 36% (168/470) were detected for TTSuV1 and TTSuV2. Co-infection of both groups (TTSuV1 and TTSuV2) was 8% (38/470) of the total sample. Among the positive samples, three strains (M117, N86 and N116) were registered in GenBank with accession numbers MK452763–MK452765. Regarding the genetic relationship within the complete genome references in TTSuVs, two strains of M117 and N86 belonged to 1b and 1c of TTSuV1, and the other N116 strain is located in subtype 2b (Figure S1). From the collected samples (n = 470), we further investigated the most widely spread TTV3 genogroup to confirm cross species infection. Interestingly, the results detected only two field strains (M265_Korea_2017, MK452766; and N119_Korea_2018, MK452767) for which the PCR amplicon band site is 350 bps. In the sequencing blast results, the strains of M265 and N119 were shown 96% and 94% homology with TUPB (AF247137) strain. For genetic characterization, the two strains (M265_Korea_2017 and N119_Korea_2018) were completely sequenced by using a primer walking method (Biagini et al., 2000).

Focusing on the TTV 3 strains, we found that each M265 and N119 strains has a 3,817 full-length genome and the M265 has three ORFs (ORF1, ORF2 and ORF3) while the N119 strain has four ORFs (ORF1, ORF2, ORF 2-2 and ORF 3) (Figure 1a,b). The strains showed 98.4% similarity in nucleotide and 98.4% in amino acid, and ORF3 showed 98% and 98% homology, respectively. As inferred based on the ORF1 alignment with previous phylogenetic tree study within TTVs, the M265 and N119 strains belong to genogroup 3 of subgroup 3c, which are close to the TUPB strain (Figure 1c).

Further analysis indicated that the putative ORF1 of both two TTV genogroup 3 strains contained (1) the Arginine-rich region located at N-terminator and (2) three conserved motifs of

3 | RESULTS

For phylogenetic study, the best nucleotide substitution model and the complete genome sequence model were selected automatically by specifying the ‘-m TEST’ option in IQ-TREE version 1.3.8 (Nguyen et al., 2014). In this study, the best plot model (GTR + G4) was used for phylogenetic analysis. For genotyping, we collected from the TTSuVs reference sequences (Li et al., 2013) and the TTVs reference sequences (Hsiao et al., 2016).
replicate-associated protein including motif 1 (FSL), motif 3 (YxxK) and motif 4 (GxGK: P-loop) (Figure 2a,b). Similarly, the conserved motif (WX₇HX₃CX₅H) of ORF2- TTV was observed in M265 strain while an aa change H→Q in the first histidine of the motif was found in N119 (Figure 2c). Of the putative ORF3, a serine-rich domain was found in the C-terminate of both strains (Figure 2d).

**4 | DISCUSSION**

It was reported that TTSuV infections existed in many countries around the world (McKeown et al., 2004). In our study, the prevalence rate of TTSuVs in Korea during 2017–2018 was 44% (the positive rates of TTSuV1 and TTSuV2 were 16% and 36%, respectively, and the co-infection of both type of TTSuVs was approximately 8%). The prevalence of TTSuVs in Korea in this study was equivalent to the positive rate of one in Thailand (McKeown et al., 2004). However, the detection rate of TTSuV1 and/or TTSuV2 in this study was lower compared with reports in other countries (Blois et al., 2014; Li et al., 2013; Sibila et al., 2009) and even that of Korea before 2004 (McKeown et al., 2004).

In this study, we focused our investigation of sick pigs in the potential appearance of TTVs genogroup 3. As a result, two out of 470 samples were TTV genogroup 3 positive. Sequence comparison and phylogeny analysis indicated that the two strains share 98.4% sequence homology and belonged to the subgroup 3c. Interestingly, genome organization prediction of N119 strain revealed an additional ORF2-2 besides the common ORFs 1-3 found in Anelloviridae family (Biagini et al., 2012).

In this study, the TTVs genogroup 3 detected in pigs were predicted to contain the three common ORFs observed in other TTVs. Of the ORF1, the N-terminated region was featured by the Arginine-rich region (Figure 2a) which is similar to capsid proteins of circoviruses (Mou et al., 2019). Furthermore, three replication-associated motifs were also observed (Figure 2b). The presented motifs in ORF1 in TTVs were previously reported elsewhere (Tanaka et al., 2001).
Previous study suggested that ORF1 might encode a bifunctional structural protein: the N-terminus played a role as capsid while the function of the C-terminus might be a co-response to the replication (Kakkola et al., 2008). Of the remaining ORFs, putative ORF2 of M265 contained the well-observed motifs of Wx7Hx3CxCx5H in TTVs while a cluster of Leucine rich regions followed by Serine rich regions in C-terminus were observed in the present strains. These features are highly conserved in other Anelloviruses (Vibin et al., 2020). As far as our knowledge, this is the first time the strains belonging to Alphaternovirus were detected and studied.

In conclusion, the present study provided information of TTSuVs prevalent in swine farms in Korea. Our results also highlight the presence of TTV genogroup 3 strains in pig.

5 | CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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AUTHOR CONTRIBUTION

Van Giap Nguyen: Formal analysis; Investigation. Cheong Ung Kim: Investigation. Quynh Do Hai: Formal analysis. Yong Ho Park: Conceptualization; Formal analysis. Bong-Kyun Park: Conceptualization. Hee Chun Chung: Conceptualization; Formal analysis; Writing-original draft.

ETHICAL STATEMENT

This article does not contain any studies with live animals performed by any of the authors.

PEER REVIEW

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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