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Prevalence of Torque teno viruses among pigs and cattle in Korea

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Abstract : Torque teno virus (TTV), a species of Anellovirus, is a non-enveloped single stranded DNA virus with a wide range of animal hosts. The incidence of TTV is quite ubiquitous throughout the world. A total of 235 serum samples obtained from 137 pigs and 98 cattle at slaughterhouses in Korea during April 2005 to May 2005 were tested by TTV-specific PCR as to monitor prevalence of TTV among swine and cattle. As a result, the prevalent rates of TTVs in pigs and cattle were 43.1% and 4.1%, respectively. It seems that TTV infection is quite prevalent in swine population.

Keywords : cattle, prevalence, swine, TTV

Torque teno virus (TTV) belongs to the floating genus Anellovirus, which includes small, non-enveloped viruses containing a single-stranded, negative-stranded, negative-sense, circular DNA genome [7]. Although the first isolate was detected in a human patient, but more recently, TTV has been identified in domestic animals, including swine, chicken, cow, sheep, cat and dog [4]. Porcine TTV is considered widespread as approximately 33 to 100% of sera examined were positive by TTV genogroup-1 specific PCR [5]. As compared to porcine TTV, less attention was paid to bovine TTV with regards to prevalence survey.

Recently, Kekarainen *et al.* [2] have been reported that TTV was more frequently detected in post-weaning multisystemic wasting syndrome (PMWS)-affected pigs than non-PMWS affected counterparts. In the light of the fact that PMWS incidence is widespread in Korea, being 8.1% of whole herds infected according to a retrospective study [3], TTV should be prevalent among pig herds. It is thus our aim to monitor prevalence of porcine TTV in Korea. In addition, an attempt was done to monitor bovine TTV prevalence in Korea as it has been detected in cattle as reported earlier [4].

To monitor the prevalence rate of TTVs in pigs and cattle, a total of 235 serum samples were obtained from 137 pigs and 98 cattle at slaughterhouses in Korea during April 2005 to May 2005. Sera from pigs and cattle were sampled from 7 and 17 different abattoirs of Korea, respectively. Viral blood DNA was extracted from the serum samples using the QIAmp DNA mini kit according to the manufacturer's recommendation (Qiagen, Germany). Primer pair 1 forward (5′- RMR SWK MCH AAT GGC TGA GTT T - 3′) and reverse-common (5′- YYS CKW GCC CGA AWK GCC CCT - 3′) were designed to amplify conserved sequence of the untranslated region (UTR) of TTVs as indicated previously [6, 7]. In addition, primer pair 2 forward 2 (5′- TAC ACT TCC GGG TTC AGG AGG -3′) and reverse-common (5′- YYS CKW GCC CGA AWK GCC CCT - 3′) were also designed to amplify the conserved sequence among TTVs in this study. All sera samples were subjected to two primer pairs simultaneously. The PCR reaction was conducted using Hot-start Taq premix (Bioneer, Korea); 2 μL of each 25 pmol of primer, 3 μL of extracted DNA, and 15 μL of distilled water. The PCR amplification cycle was done by initial activation at 94°C for 15 min, and 35 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, and extension at 72°C for 30 sec, followed by a final extension at 72°C for 2 min. The amplified products were visualized on a 2% agarose gel. It was considered TTV positive when the samples were detected by either primer pair. However, it is worthy to note that porcine samples were amplified with primer pair 2, whereas none of bovine samples was

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Table 1. Prevalence of Torque teno virus in swine and cattle population in Korea

Species	No. sera tested	No. sera positive	Prevalence, %	95% confidence interval
Swine	137	59	43.1	34.8 – 51.4
Cattle	98	4	4.1	0.2 – 8.0

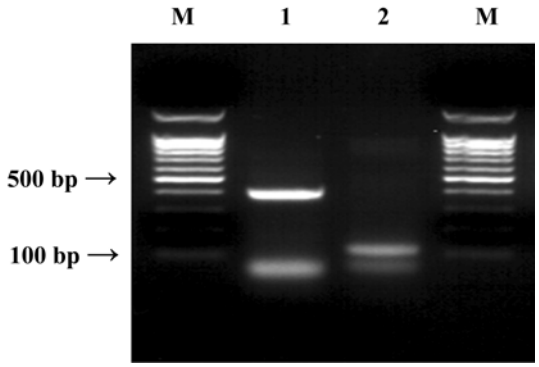

Fig. 1. Torque teno virus (TTV) detection from porcine and bovine blood samples. Each PCR assay was preformed with a primer pair-1 (swine) and pair-2 (bovine), respectively (refer Materials and methods for further information). The amplified products were visualized on a 2% agarose gel. M: Molecular weight markers, lane 1: 410 bp PCR product from swine blood sample, and lane 2: 111 bp PCR product from bovine blood sample.

positive by both primer pairs used in this study (Fig. 1). Among them, 4 amplicons were selected and then they were gel-purified with GeneClean Turbo kit (Qbiogene, USA). Purified products were ligated into pGEM-T Easy vector kit as the manufacturer's protocol (Promega, USA), and both strands were sequenced at commercial compahy (Macrogen, Korea). The nucleotide sequence analysis was performed on four PCR products, which were all confirmed to be TTV DNAs from pigs and cattle using DNASIS (Hitachi, USA).

As a result, apparent prevalence of swine and bovine TTV was 43.1% and 4.1%, respectively (Fig. 1). The prevalence rate of bovine TTV observed in this study was much lower than that of previous report [4, 5]. Leary *et al.* [4] reported that TTV prevalence in farm animals was ranged from 19 to 30%. In addition, the prevalence of bovine TTV was higher than that of swine TTV (25% *vs.* 20%). The discordant result in prevalence of bovine TTV between our study and that by Leary *et al.* [4] may in part be raised by limited numbers of samples evaluated. Furthermore, as shown by McKeown *et al.* [5] who observed unequal prevalence in swine TTV among countries, the regional variation in TTV prevalence may exist. In any events, TTV prevalence could be different according to the type of herds, namely the swine TTV infection was more frequent in finishing herds than in farrow-to-finish herds (40.1% *vs.* 11.0%). In addition, an interesting study has been recently reported by Kekarainen *et al.* [2] who observed that TTV was more frequently detected in PMWS-affected pigs than non-PMWS affected counterparts. At this stage, implication of TTV in the development of PMWS needs to be further assessed.

The amplified target sequences were aligned with that of previously reported sequence (Fig. 2). The nucleotide sequences of the suspected TTV isolates in this study were all confirmed to be porcine TTV DNAs. Sequence comparison of the porcine TTV DNAs revealed that nucleotide similarity shared 92% identical between Japanese TTV strain (GenBank accession No.: AB076001) and TTV isolates. And the nucleotide sequences of the bovine TTV isolates (Fig. 3) reported in this study revealed that nucleotide similarity shared 95% identical each other and 89-91% with the porcine TTV isolate (GenBank accession No.: AB076001). In this regard, our study is well in line with the study by McKeown *et al.* [5], who reported that sequence analyses of porcine TTV isolates shared 86-100% nucleotide sequence identity each other. However, it is worthy to note that in contrast to porcine TTV, amplified signals were not detected from bovine samples using primer pair 2 forward 2 (5′- TAC ACT TCC GGG TTC AGG AGG -3′) and reverse-common (5′- YYS CKW GCC CGA AWK GCC CCT - 3′).

It is known that TTV could be isolated from various tissues, being lung the most frequently positive organ [1]. Moreover, TTV DNAs were detected in all examined tissues, with the viral load being equal to or up to 300 times higher than that in the corresponding serum [6]. Therefore, further investigation is necessary to monitor porcine TTV infection and the possible interaction with other infectious agents in the field. The result of this study clearly indicates that the TTV was

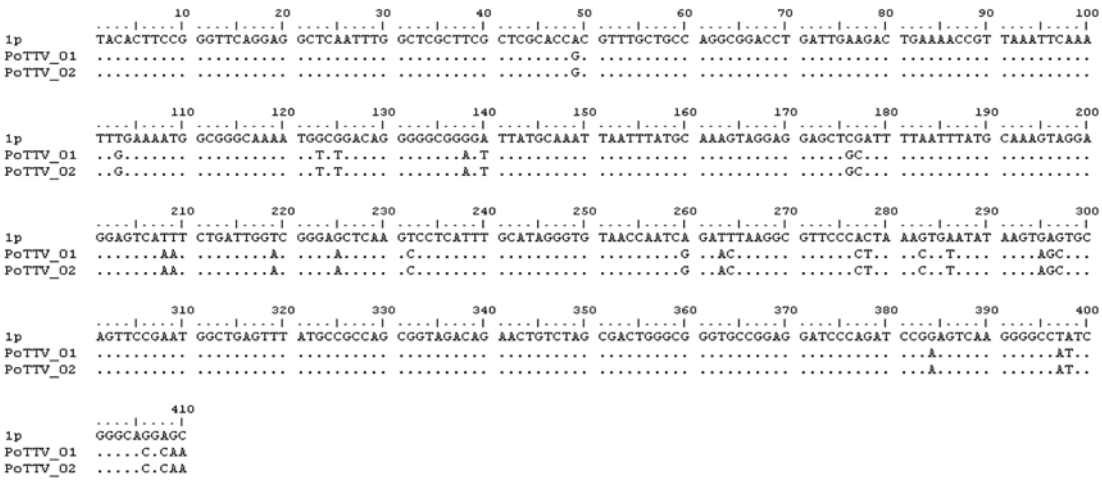

Fig. 2. Multi-alignment of the nucleotide sequences of porcine TTVs. The amplified target sequences were aligned with previously reported sequence, Japanese TTV strain (GenBank accession No. AB076001). Porcine TTV isolates used in this study have been submitted to GenBank (PoTTV_01, Bankit 879035; PoTTV_02, Bankit 879036).

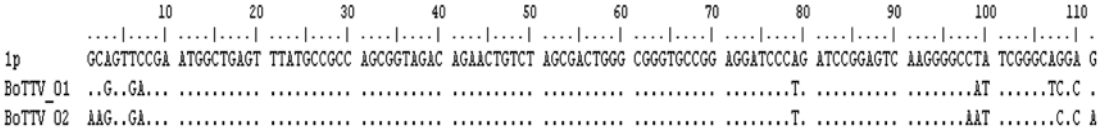

Fig. 3. Multi-alignment of the nucleotide sequences of bovine TTVs. The amplified target sequences were aligned with previously reported sequence, Japanese TTV strain (GenBank accession No. AB076001). Bovine TTV isolates have been submitted to GenBank (BoTTV_01, Bankit 879041; BoTTV_02, Bankit 879042).

more significantly prevalent among pig herds whereas less frequent in cattle herds in Korea.

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