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Short communication

Molecular detection and genomic characterization of Torque teno canis virus in domestic dogs in Guangxi Province, China

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ABSTRACT

The Torque teno canis virus (TTCaV) is a small virus with circular single-stranded DNA that has been reported to cause infections in dogs. The present study aimed to identify the presence of TTCaV in blood samples obtained from domestic dogs, and examine its diversity and evolution of the genomes. Five strains of TTCaV were detected, and the overall prevalence was found to be 7% (28/400). Phylogenetic analysis showed that the five genomes were closely clustered with the previously known Cf-TTV10 and LDL strains and formed a Thetatorque virus. Homology analysis of the whole genome showed a sequence identity of 94.6%–96.8% among the five genomes. The percent sequence similarity among the five complete genomes ranged from 95.3% to 97.4% and from 95.1% to 97% compared to the Cf-TTV10 and LDL strains respectively. The ORF1-encoded amino acid sequences showed 94.4%–97.2% identity among the five isolates. Our findings suggest that the TTCaV has a large genetic diversity and showed that TTCaV and canine parvovirus (CPV) co-infection exists in China. Further studies on the pathogenicity of TTCaV are required.

1. Introduction

The Torque teno virus (TTVs) was first discovered in a Japanese patient with post-transfusion non-A-E hepatitis in 1997 (Nishizawa et al., 1997). A lot of TTVs have been found in the last decade and highly diverse in the genome. TTV infects not only humans but also various animal species including non-human primates, tupaias, pigs, cattle, cats, dogs, and sea lions(Leary et al., 1999; Odemis et al., 2004; Okamoto et al., 2001; Okamoto et al., 2000; Okamoto et al., 2002).

TTCaV was first identified in Japan in 2002 (Okamoto et al., 2002). TTCaV is a small, unenveloped, spherical virus that has a circular, negative single-stranded DNA genome of approximately 2.8 kb in length (Kakkola et al., 2008; Peng et al., 2015). The genome contains three major open reading frames (ORF1, ORF2, and ORF3) and a short stretch of untranslated region (UTR) with high GC content (up to 90%) (Martinez et al., 2006; Matsubara et al., 2001). Although TTV was first identified in a patient with cryptogenic hepatitis, subsequent studies were unable to obtain evidence of a significant role of TTV in the pathogenesis of hepatitis or other diseases (Okamoto, 2009; Zuckerman, 1999). Although human TTV is not considered to be directly associated with a disease, porcine TTV co-infection porcine reproductive and respiratory syndrome virus was recently shown to partially contribute to the experimental induction of porcine dermatitis and nephropathy syndrome (Zhang et al., 2012). However, TTCaV pathogenicity in dogs has not been studied in depth.

In China, specifically, the presence of TTCaV has been poorly investigated (Lan et al., 2011). Furthermore, the pathogenic role of TTCaV is not clear, and its role in co-infection with other pathogens has not been investigated thus far. Therefore, to further explore the association between naturally occurring TTCaV infections and others diseases, in this study, we examined the TTCaV prevalence and the genetic relationships between different TTCaV strains in dogs in the Guangxi Province of China.

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2. Materials and methods

2.1. Sample collection

A total of 400 serum samples were collected from 400 healthy domestic dogs older than 1 year old submissions to the Guangxi Animal Disease Control Center in 2016. All samples were stored in a freezer at −80 °C for further DNA extraction.

2.2. DNA extraction

Viral DNA was extracted from the supernatants using the TaKaRa MinBEST Viral DNA Extraction Kit Ver. 4.0 (Takara Co. Dalian, China) according to the manufacturer's instructions. The isolated DNA was eluted in 80 μL elution buffer and stored in −20 °C for further polymerase chain reaction (PCR) analysis.

2.3. Specific PCR for TTCaV DNA detection

PCR was performed for detection of TTCaV conserved areas using two primer sets (TTCaVD-P1/TTCaVD-P2 and TTCaVD-P3/TTCaVD-P4) with the previously Lan described (Lan et al., 2011) (Table 1). The nested PCR product was run on a 1% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (AxyGene, Hangzhou, China), cloned into a pMD-18T vector (Takara Co. Dalian) and sequenced (KuMei, Changchun, China).

2.4. PCR amplification of TTCaV full-length genomes

PCR-positive blood samples detected were selected for amplification of complete TTCaV genomes. We initially attempted to utilize two primer sets(TTVQ-1-S^b/TTVQ-1-A and TTVQ-2-S^b/TTVQ-2-A) of an inverse PCR (Lan et al., 2011) to amplify the virus genomic DNA. However, no PCR product was obtained after several trials. We subsequently designed three new pairs of primers to amplify the complete genomes: TTCaVD-1F/TTCaVD-1R, TTCaVD-2F/TTCaVD-2F, and TTCaVD-3F/TTCaVD-3R (Table 1).

Each PCR reaction mixture consisted of 5 μL template DNA and 1 μL primers (25 μM each primer). For the PCR, we used 2.5 U TransStart® FastPfu Fly DNA Polymerase (TransGen Biotech, Beijing, China), 5 μL of 5 × TransStart® FastPfu Fly DNA buffer (TransGen Biotech, Beijing, China), and ddH_2O and made up the volume to 25 μL. The amplification was initiated by preheating for 2 min at 95 °C; followed by 35 cycles of 30 s at 95 °C, 20 s at 65 °C, 30 s at 72 °C; and a final extension for 10 min at 72 °C. The amplified product was purified with an AxyPrep DNA Gel Extraction Kit (AxyGene, Hangzhou, China) and cloned into the pMD-18T Vector System (Takara Co. Dalian, China) according to the manufacturer's instructions. The cloned products were then sequenced (Takara Co. Dalian, China).

In order to determine whether TTCaV co-infection canine parvovirus (CPV), two primers CPV-2b-F/CPV-2b-F to amplify the virus genomic DNA with the previously described (Zhao et al., 2016).

Table 1
Specific primers used in this study.

Primer name	Primer sequence(5′–3′)	Size(bp)
TTCaVD-P1	AACATCACAAATACCCATTAACATTCCC	453 bp
TTCaVD-P2	TGCTGTCGCTGTCTTCGCTCAC	
TTCaVD-P3	CCAAGGGACCAGCACCCACATT	385 bp
TTCaVD-P4	CTGTCGCTGTCTTCGCTCACCC	
CPV-F	GAATCTGCTACTCAGCCACCAAC	560 bp
CPV-R	GTGCACTATAACCAACCTCAGC	
TTCaVD-1F	ACACATAGCCAAAACCAAAAAAAACAAA	1014 bp
TTCaVD-1R	TAGAAATGTATTGTCTTTTGGTG	
TTCaVD-2F	TCGTAGACGACGCGGCTATGCTCGCCG	1710 bp
TTCaVD-2R	CCATTCTTTGTCCTCGGGGTAGG	
TTCaVD-3F	TGGCCAGATTTATGTCAAATGGTGGCCCA	700 bp
TTCaVD-3R	GGGGGGGGAGGCGCCAGGGGGGGTT	

2.5. Sequences and phylogenetic analysis

The genome sequence of TTCaV in this study was analyzed using MegAlign software (DNAStar Inc., Madison, WI, USA). A phylogenetic tree of the full-length genome was generated by the Neighbor-joining (NJ) method using 1000 bootstrap replicates in a heuristic search with MEGA 5 software program (USA). Two representative full-length genomes of TTCaV and other representative full-length genomes of TTVs were obtained from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) (Table 2).

3. Results

3.1. TTCaV DNA prevalence in domestic dog sera

Using specific primers (TTCaVD-P1/TTCaVD-P2) to amplify the target fragments, we found that the overall prevalence of TTCaV was 7% (28/400) among domestic dogs in Guangxi Province. 3 CPV positives were identified from TTCaV positives.

3.2. Sequencing and phylogenetic analysis of TTCaV

Using three pairs of primers, five full-length genomes of TTCaV strains were cloned and designated GX206, GX249, GX281, GX264, and GX261 (GenBank IDs: KX827767, KX827768 KX827769, KX827770, and KX827771, respectively) (Table 2).

The whole-genome length varied from 2791 bp to 2799 bp. Three ORFs were identified in these strains genome: ORF1 (576aa), ORF2 (101aa), and ORF3 (243aa) (Table 3). Homology analysis of the whole genome showed sequence identity of 94.6%–96.8% among the strains. The percent sequence similarity among the five complete genomes ranged from 95.3% to 97.4%, and from 95.1% to 97% in a comparison with the Cf-TTV10 and LDL strains. In addition, we assessed the sequence similarity of the ORF1 protein of the five complete TTCaV genomes. The sequence similarity of the ORF1 protein ranged from 94.4%–97.2% among the five strains, and the percent sequence similarity of the ORF1 protein among the five strains ranged from 94.4% to 95.5% and from 95.3% to 98.8% in a comparison with the Cf-TTV10 strain and LDL strains.

A phylogenetic tree (Fig. 1) was constructed on the basis of the full-length of other TTVs and reference representative strains (Table 2). The NJ tree analysis showed that the five strains were clustered with the Cf-TTV10 and LDL strains (Fig. 1). Another NJ phylogenetic tree of the ORF1 of other TTVs in this study was confirmed to the result of complete genome sequences. These ORF1 sequences confirmed that the five strains formed a special cluster with TTCaV (Fig. 2).

4. Discussion

TTV is known to infect several hosts including humans, cows, and sheep (Biagini et al., 2006; Lefrere et al., 2000; Lyra et al., 2005). TTCaV has been previously also reported in dogs in Japan. However, knowledge about its prevalence in China is still limited. The present study was the first attempt to evaluate the occurrence of TTCaV infection among domestic dogs in Guangxi Province, China, in 2016. The prevalence of TTCaV was found to be 7% (28/400) in the current study. In contrast, the prevalence was 13% (20/158) among dogs aged > 1 year in Shanghai and 38% among dogs in Japan (Okamotoe-tal.,2002). This difference in the prevalence of TTCaV infection in geographically distinct dog herds could be due to the differences in regions and samples.

Recent studies have reported high prevalence rates and viral loads of Torque teno sus virus in pigs affected with porcine circovirus strain