Virus Research 156 (2011) 13–16

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Molecular detection and sequence analysis of feline Torque teno virus (TTV) in China

C.X. Zhu[a,1], T.L. Shan[a,1], L. Cui[a], X.N. Luo[b], Z.J. Liu[b], S.D. Tang[c], Z.W. Liu[a], C.L. Yuan[a], D.L. Lan[a], W. Zhao[a], X.G. Hua[a,*]

[a] *Shanghai Key Laboratory of Veterinary Biotechnology, School of Agriculture and Biology, Shanghai JiaoTong University, Shanghai 200240, China*
[b] *Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences (CAAS), Lanzhou 730046, China*
[c] *Shanghai Minhang Central of Animal Disease Control, 256 Xinsong Road, Shanghai 200240, China*

ARTICLE INFO

Article history:
Received 18 October 2010
Received in revised form 5 December 2010
Accepted 10 December 2010
Available online 24 December 2010

Keywords:
TTV
Cats
China
Sequence analysis

ABSTRACT

In the present study, two isolates (SH-F1 and SH-F2) of Torque teno felis virus (feline TTV) were detected in 16 (12.5%) serum samples collected from cats in China. Their full length genomes were cloned and sequenced. The results showed that they were 2063 bp in length and contained three open reading frames (ORFs) (ORF1: nt438–1748, ORF2: nt268–585 and ORF3: nt268–581, 1461–1842). Phylogenetic analysis showed that they were clustered with the strain of Japan (Fc-TTV4, AB076003) and the strain of France (PRA4, EF538878). Sequence analysis indicated that SH-F1 had high (97.5% and 93.3%) identity with the strain of Japan and the strain of France, and SH-F2 shared 94.5% and 92.1% homology with them, respectively. In conclusion, we demonstrated that feline TTV is present in China.

Crown Copyright © 2010 Published by Elsevier B.V. All rights reserved.

1. Introduction

Torque teno virus (TTV) is a small non-enveloped single-stranded circular DNA virus, which was first discovered in 1997 in Japan from the serum of a patient with acute post-transfusion hepatitis of unknown etiology (Nishizawa et al., 1997). The genome of TTV has a range of 2.1–3.9 kb in length and contains three or four overlapping open reading frames (ORFs) as well as a short stretch of untranslated region with high GC content. In 2009, TTV was classified by the International Committee on Taxonomy of Viruses (ICTV) into the family Anelloviridae, which contains nine genus: *Alphatorquevirus*, *Betatorquevirus*, *Deltatorquevirus*, *Epsilontorquevirus*, *Etatorquevirus* (*Torque teno felis virus*), *Gammatorquevirus*, *Iotatorquevirus*, *Thetatorquevirus* and *Zetatorquevirus*.

TTV is widespread worldwide, and it can transmit not only parenterally but also nonparenterally through fecal–oral transmission (Okamoto et al., 1998) and mother-to-infant transmission (Sugiyama et al., 2001; Indolfi et al., 2007). Due to the lack of a culture system or an animal model to support the viral multiplication, the infection and replication mechanisms and the pathogenicity of TTV are still unknown. Some reports have indicated that several different genotypes of TTV are considered responsible for human diseases (Takahashi et al., 1998). However, TTV has been suggested to be a commensal in normal conditions which should benefit the host, this is an intriguing aspect hitherto unexplored with TTV (Griffiths, 1999).

The infection of TTV is not only restricted to human but also to various animal species including non-human primates (Inami et al., 2000; Okamoto et al., 2001; Ninomiya et al., 2009), domestic animals (porcine, avian, bovine and ovine) (Leary et al., 1999; Brassard et al., 2008), companion animals (feline and canine) (Okamoto et al., 2002), wild animals (wild boar and camels) (Martinez et al., 2006; Al-Moslih et al., 2007) and marine animals (sea lions, sea turtle) (Ng et al., 2009a,b). Among the animals, TTVs of human are best characterized. However, compared with human TTV, the genomic information of feline TTV is limited. Currently, only one full-length feline TTV genomic sequence and two full-length feline Anellovirus genomic sequences have been reported from cats in Japan and France, respectively (Okamoto et al., 2002; Biagini et al., 2007), and only three complete genome sequences from GenBank were available: AB076003 (Fc-TTV4), EF538878 (PRA4) and EF538877 (PRA1). Thus, in our study, pairs of nest primers were designed based on the strain Fc-TTV4 of Japan, which is geographically near China, to detect feline TTV from fresh stool samples and serum samples in Chinese cats.

* Corresponding author at: Zoonosis and Comparative Medicine Group, Shanghai Jiao Tong University, Shanghai, China. Tel.: +86 21 34206149; fax: +86 21 34206263.
E-mail address: hxg@sjtu.edu.cn (X.G. Hua).
[1] Joint first authors.

0168-1702/$ – see front matter. Crown Copyright © 2010 Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2010.12.008

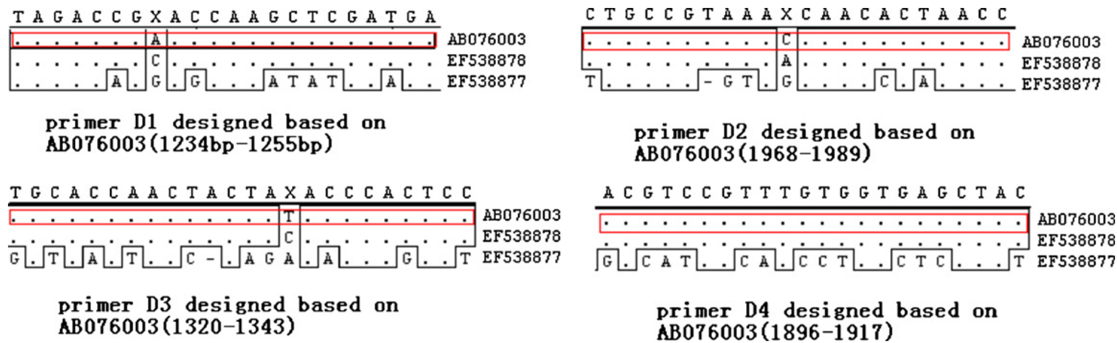

Fig. 1. Aligned of the genomic regions used to design the detected primers.

2. Materials and methods

2.1. Samples

A total of 26 samples (16 sera and 10 stools) were collected from 26 cats (Felis catus) aged 9–24 months in March 2010 from pet clinics in Shanghai. The study protocol was approved by the Animal Care and Use Committee (ACUC) of Shanghai Jiaotong University. Samples were stored at −80 °C until the testing was performed.

2.2. DNA extraction

The fecal samples were diluted in 10× volume of phosphate-buffered saline (PBS, pH 7.4) and centrifuged at 12,000 × g for 10 min at room temperature, and the supernatant was collected. Viral DNA was extracted from 100 μl serum samples and 100 μl stool extracts using a MiniBEST Viral RNA/DNA Extraction Kit Ver.4.0 (TaKaRa, Dalian, China) following the manufacturer's instructions and stored at −80 °C for further PCR analysis.

2.3. PCR detection of TTV DNA

TTV DNA was detected by the amplification of a 598 bp fragment by nested PCR with TTV-specific primers D1–D4 (Table 1) based on the published genomic sequence of feline TTV, Fc-TTV4 (GenBank accession number: AB076003). The primer regions were also aligned to the strains PRA4 and PRA1 (Fig. 1), and the primers D1–D4 shared 18.2–47.6% homology with the divergent strain PRA1. All PCR reactions were carried out as follows: 32 cycles of denaturation at 94 °C for 30 s with an additional 7 min in the first cycle, annealing at 57 °C for 30 s, extension at 72 °C for 50 s, and with an additional 7 min in the last cycle. The amplification products were excised from 1% agarose gels containing ethidium bromide (0.5 μg/ml), purified with the AxyPrep DNA Gel Extraction Kit (Axygene, Silicon Valley, USA), cloned into the pMD-18T vector (TaKaRa, Dalian, China), and sequenced (TaKaRa, Dalian, China).

Table 1
Primers employed in these experiments.

Primer	Polarity	Nt position	Nucleotide sequence
D1	Sense	1234–1255	5′-TAG ACC GAA CCA AGC TCG ATG A-3′
D2	Antisense	1968–1989	5′-CTG CCG TAA ACC AAC ACT AAC C-3′
D3	Sense	1320–1343	5′-TGC ACC AAC TAC TAT ACC CAC TCC-3′
D4	Antisense	1896–1917	5′-ACG TCC GTT TGT GGT GAG CTA C-3′
Q1	Sense	1559–1580	5′-CGA AGA CGA GGC TTA TGA ACG A-3′
Q2	Antisense	1533–1555	5′-ATC CCA TCA CTG TCG AGA TCC CC-3′
Q3	Sense	1900–1920	5′-CTC ACC ACA AAC GGA CGT CGC-3′
Q4	Antisense	1379–1400	5′-CCA AAT GTT TTC TCC GGT GAC C-3′

2.4. PCR amplifications of the full-length feline TTV genomes

Based on the sequence obtained from detected PCR, an inverted nested PCR with TTV specific primers (Q1–Q4) was also performed to obtain an overlapping PCR fragment containing the remainder of the circular genome (including the GC rich region) (Table 1). Amplification was performed using Takara LA Taq polymerase and GC buffer I (LA PCR Kit Ver.2.1, TaKaRa, Dalian, China) and the following cycling conditions: 7 min at 94 °C, followed by 32 cycles of 40 s at 94 °C, 40 s at 60 °C, and 2 min at 72 °C, with a final extension step of 7 min at 72 °C. The remaining protocol was carried out as above.

2.5. Phylogenetic analysis

The genome sequences of feline TTV isolated in this study were analyzed using the MegAlign software (DNAStar Inc., Madison, WI, USA). Phylogenetic trees were constructed by the alignment of the full-length genome of feline TTV isolated in this study and the referenced strains (GenBank number and source of regions are shown in Fig. 2). They were evaluated using the neighbor-joining method with 1000 bootstrap replicates in a heuristic search with the Molecular Evolutionary Genetics Analysis program (MEGA, version 4.0, USA).

3. Results

3.1. Detection of TTV in cats

In the first round of PCR and the subsequent nested PCR, 2 out of the 16 serum samples (12.5%) were found to be positive for TTV DNA, whereas no TTV DNA was detected in the 10 stools. The two positively amplified products were sequenced. The products are 598 bp in length and shared 98.7% and 98.5% nucleotide identities with Fc-TTV4, respectively.

3.2. Full-length nucleotide sequences of feline TTV

The complete genomes of the two isolates were 2063 bp in length and contained three ORFs. ORF1 is 1311 bp (nt438–1748) in length and encodes a 437 aa protein rich in Arg at the N-terminus. ORF2 is 318 bp (nt268–585) in length and encodes a 106 aa protein. ORF3 encodes a putative joint protein of 232 aa. It overlaps with ORF1 (1461–1842 nt), contains a part of ORF2 (268–581 nt), and is rich in Ser in the C terminus. These data are consistent with the composition of TTV strains reported in other studies. They were

Download English Version:

https://daneshyari.com/en/article/6143515

Download Persian Version:

https://daneshyari.com/article/6143515

Daneshyari.com