

Molecular detection and genomic characterisation of Torque teno canis virus in Turkey

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ABSTRACT

Torque teno virus (TTV) was first detected in humans, and since then it has been reported in many host species, such as monkeys, cats, pigs, seagulls and dogs. The aim of this study was to investigate the presence of Torque teno canis virus (TTCaV) in shelter dogs housed in Sivas Municipal Animal Shelter, Turkey. Faecal specimens, including diarrheic and non-diarrheic (n=202), were collected from dogs of various age groups. In total, 32.18% (65/202) of samples were found positive for TTCaV. Out of the 65 positive samples, 34.64% (44/127) samples were from adult diarrheic dogs and 26.09% (6/23) belonged to diarrheic puppies. On the other hand, 28.84% (15/52) positive samples were detected from clinically healthy dogs. Eight sequences showed close homology among themselves, however, the sequences of two samples (CANEL130 and CANEL140) were genetically distinct from other published sequences. This is the first report on the detection of TTCaV in adult dogs and puppies in Turkey, and provides evidence that TTCaV cannot be considered as the sole cause of diarrhea.

Key words: shelter dogs; Torque teno canis virus; Turkey

Introduction

Torque teno virus (TTV) was first isolated from a human patient, who clinically developed hepatitis after a blood transfusion (NISHIZAWA et al., 1997). Human torque teno virus is a prototype of the *Anelloviridae* family. Anelloviruses contain circular, single stranded DNA, which is 2.1 - 3.8 kb in length, and morphologically appear as small, non-enveloped particles of approximately 30-32 nm in size. The Anelloviridae family is divided into 14 genera, each containing a large number of species, due to high-level genetic heterogeneity.

Anelloviruses have been reported to be widespread all over the world (ICTV 2018 <https://talk.ictvonline.org/taxonomy/>; MANZIN et al., 2015).

Torque teno canis virus (TTCaV) is a member of the genus *Thetatorquevirus*. The presence of TTCaV was first discovered by OKAMOTO et al. (2002). Recently, a metagenomic study on *Rhipicephalus* ticks revealed a novel virus, the Nayun tick torquivirus, showing 42.79% nucleotide identity with TTCaV and it was also classified in the *Thetatorquevirus* genus (XIA et al., 2015).

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A typical TTCaV genome contains three major open-reading frames (ORF1, ORF2 and ORF3), a guanine-cytosine (GC)-rich region (up to 90%) and a short untranslated region (UTR) (MARTÍNEZ et al., 2006). Despite the fact that TTV was first identified in a patient with cryptogenic hepatitis, subsequent studies have not provided evidence that TTV plays an important role in the pathogenesis of hepatitis or other diseases (OKAMOTO, 2009). However, it has been experimentally demonstrated that TTV co-infection with porcine reproductive and respiratory syndrome virus partially contributes to the stimulation of swine dermatitis and nephropathy syndrome (ZHANG et al., 2012).

The pathogenic role of TTCaV in causing infection is unclear due to the very limited reports about its mechanism of pathogenicity, and its association in co-infection with other pathogens has not yet been investigated (SUN et al., 2017). In Turkey, the presence of TTV in human individuals with or without chronic hepatitis B, has been previously reported (KASIRGA et al., 2005; ODEMIS et al., 2004). However, there have been no reports about the detection of TTV in animals in the country. This is the first report about the prevalence of TTCaV in canine species in Turkey.

Materials and methods

Samples and DNA isolation. In the present study, a total of 202 rectal swab specimens were collected from 1100 dogs in Sivas Municipality Animal Shelter. A total of 150 dogs manifested symptoms of gastrointestinal infection, including adult dogs of 1 year old (n=127) and puppies 2-4 months old (n=23); whereas 52 adult dogs were clinically healthy. Samples were transported in cold chain to the laboratory immediately after sampling, and stored at -80 °C until further processing.

Faecal samples were diluted 1:10 with 1 M phosphate buffered saline, and centrifuged for 10 min at 3,500 rpm to remove large cellular debris. The collected supernatants were used for nucleic acid extraction using a GF-1 Viral Nucleic Acid Extraction Kit, according to the manufacturer's instructions (Vivantis Technologies, Malaysia). Eluted nucleic acids were stored at -80 °C until further use.

Specific PCR for TTCaV DNA detection. In our study, a set of primers were designed for nested PCR from the ORF3/ORF1 region: TTCaV 2158F (5'-ACCAGCACCCACATTGACAA-3') and TTCaV 2567R (5'-TTTAGAAGGGCCAGCAGTCC-3') for the first round of amplification. In the second round of amplification, TTCaV 2165Fn (5'-CCCA CATTGACAACCCWTGC-3') and TTCaV 2460Rn (5'-GTGGTCCTCCTCCTCCTCCT-3') were used for detection of the virus. The PCR mixture was prepared as a 50 µL final volume containing 5 µL template, 5 µL of 10× PCR buffer, 10 mM of dNTP, 10 pmol/µL of each set of sense/antisense primers, and 5 U of Taq DNA polymerase (Vivantis, Germany). For both rounds of the PCR, the thermal cycling conditions consisted of pre-denaturation at 95 °C for 2 minutes, followed by 40 cycles of denaturation at 94 °C for 45 sec, annealing at 53 °C (the first round) and 55°C (second round) for 45 sec, Extension at 72 °C for 1 min. Final extension was kept at 72 °C for 10 min. A 1 µL aliquot from the first round of PCR was used in the second reaction. PCR products were separated by electrophoresis in 1.5% agarose gels stained with ethidium bromide. The 296 bp PCR amplicons from second round reactions were confirmed under the gel documentation system (BioRad, USA).

Sequencing and phylogenetic analysis. The PCR amplicons were purified with the Wizard SV Gel and PCR Clean-Up System (Promega, USA) and sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, USA) on an ABI 3100 automated sequencer (Applied Biosystems, USA). Nucleotide sequences successfully amplified for this study were deposited in the GenBank database and accession numbers were given between MK496298 and MK496307.

All sequenced products were used to construct phylogenetic data, and for deduction of amino acid sequences. Partial sequences of the ORF3/ORF1 gene were compared with other torque teno virus sequence data available in the National Center for Biotechnology Information (NCBI: <https://www.ncbi.nlm.nih.gov/>) database. Sequence alignment and phylogenetic analysis were drawn with GENEIOUS PRIME software version 2019.2.1 (<https://www.geneious.com>). The neighbor-joining

method and the Tamura-Nei genetic distance model (TAMURA and NEI, 1993) were used to build the tree. The tree was drawn to scale, with branch lengths measured as the number of substitutions per site, and it was bootstrapped with 1000 replicates.

Results

TTCaV DNA prevalence in dog feces. A total of 65 (32.18%) out of 202 samples were found to be positive for TTCaV by nested PCR. Out of 150 diarrheic dogs, 50 samples were positive for TTCaV (33.33%). This included 44 out of 127 adult dogs (34.64%) and 6 out of 23 puppies (26.09%). Out of the 52 healthy adult dogs, 15 (28.84%) were also found positive. However, Chisquare analysis revealed non-significant differences ($p > 0.05$) in the distribution of TTCaV cases between healthy and diarrheic dogs.

Assessment of genetic diversity. The 296 bp nucleotide and 98 amino acid (aa) sequences of the ORF3/ORF1 gene obtained from ten samples were compared among themselves and with nine genomic sequences retrieved from the NCBI GenBank database. Analysis of multiple sequence alignment revealed that eight of ten partial sequences of our study showed 97.31% to 100% nucleotide and 92.86% to 100% amino acid similarity with other available GenBank sequences. The sample CANEL112 sequence in our study showed 100% nucleotide and aa sequence homology to two Chinese isolates GX206 (KX827767) and GX249 (KX827768). However, two isolates in our study (CANEL130 and CANEL140) displayed close identity between each other (97.97% nucleotide and 96.94% aa), but relatively low sequence homology with other samples (88.18% to 91.55% nucleotide and 79.59% to 83.67% aa).

The 296 bp sequence data obtained using nested primer pairs of TTCaV 2158F/2567R and TTCaV 2165Fn /TTCaV 2460Rn were compared among themselves and with the present data in GenBank. Analysis of the multiple sequence alignment of the partial ORF3/ORF1 regions' nucleotide and aa translations revealed that several mutations resulted in amino acid variations at multiple positions (See Figs. 1, 2 and 3.)

Phylogenetic analysis of ORF3/ORF1 nucleotide sequences revealed two clades, with group classification similar to that identified from the multiple sequence alignment analysis, as shown in Fig. 1 and 2. Clades were well supported by bootstrap values for each nodes (See Fig. 4).

Discussion

Torque teno virus (TTV) can infect many host species, including humans (KASIRGA et al., 2005; ODEMIS et al., 2004), and animals such as pigs (LEE et al., 2012; XIA et al., 2015), non-human primates (OKAMOTO et al., 2001), marine mammals (NG et al., 2009), cattle and sheep (BIAGINI, 2009). Torque teno virus (TTV) was first described in dogs in Japan (OKAMOTO et al., 2002), and since then a few cases have been reported from China and Brazil (LAN et al., 2011; SUN et al., 2017; WEBER et al., 2018). In our study, a total of 32.18% samples were positive for TTV. The prevalence of TTV virus in specimens from dogs with abnormal fecal appearance was reported as 13% in China (LAN et al., 2011). Another study from China reported 7% prevalence of TTCaV in serum samples of clinically healthy dogs (SUN et al., 2017). On the other hand, three out of eight clinically healthy dogs (38%) were detected to be positive in Japan (OKAMOTO et al., 2002). Although there are differences in sampling size, the findings of our study are concordant with the study reported from Japan. However, the prevalence of TTCaV in Turkey was relatively higher than the reports from China. Considering the studies reported from China, our study revealed a considerably higher percentage of prevalence in both diarrheic and clinically healthy dogs, including adult dogs and puppies. Epidemiological differences may be associated with sampling and geographical variations

Multiple sequence alignment of ORF3/ORF1 revealed amino acid sequence differences at multiple positions (29/98 substitutions). Despite amino acid chain similarity between the reference sequence (NC_014071.1) and the general tendency of Turkish samples ranging between 93.88 to 96.94%, CANEL130 and CANEL140 presented a relatively lower percentage of similarity, 79.59 and 81.63%, respectively (Fig. 2).

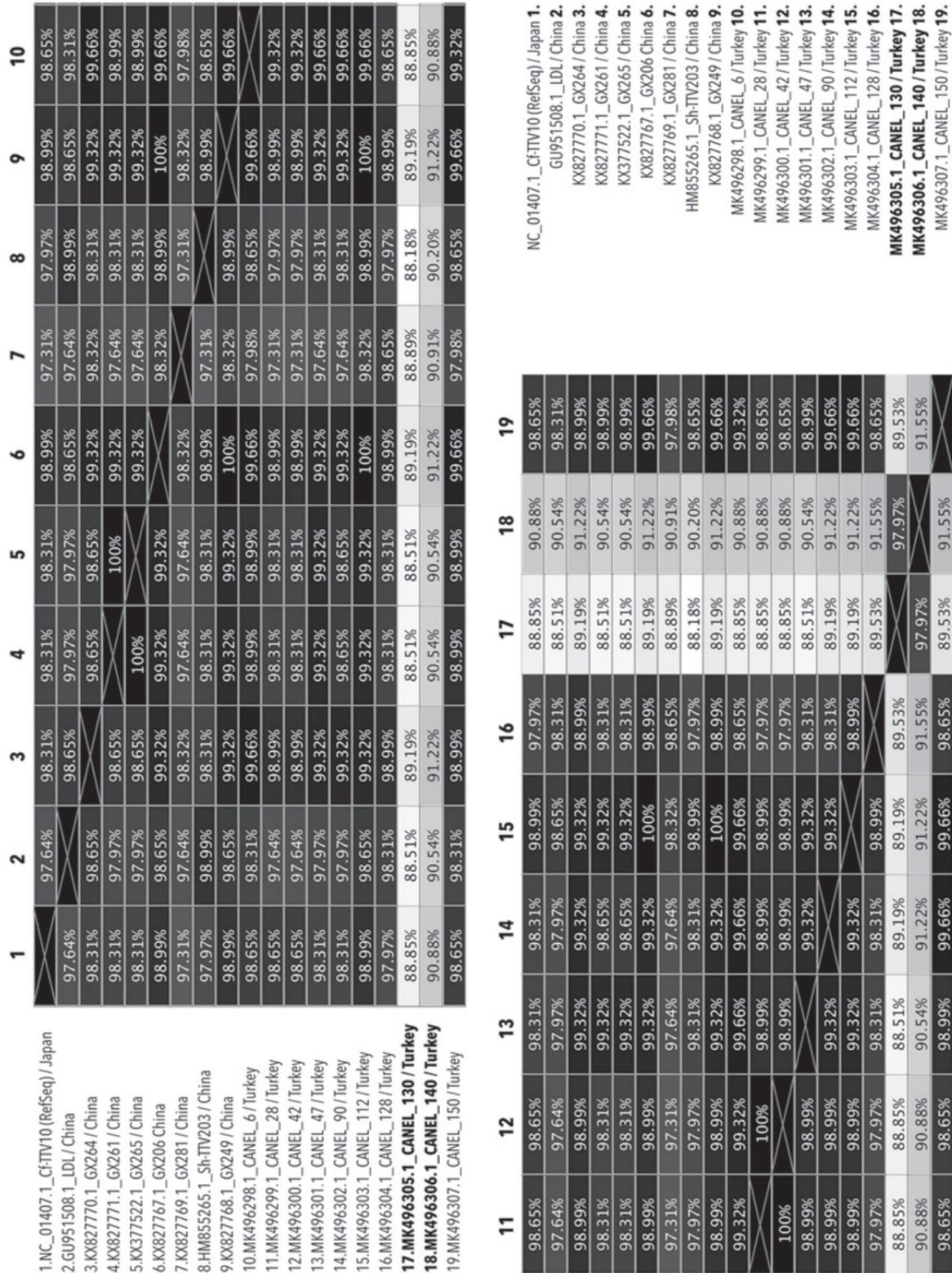


Fig. 1. Nucleotide % Identity matrix of TTCaV isolates based on 296 bp partial sequences of ORF1/ORF3.

	1	2	3	4	5	6	7	8	9	10
1.NC_01407.1_CFTV10 (RefSeq) / Japan	93.88%	93.88%	95.92%	94.90%	94.90%	96.94%	91.84%	94.90%	96.94%	96.94%
2.GU951508.1_LDL / China	95.92%	97.96%	97.96%	94.90%	94.90%	96.94%	93.88%	98.98%	96.94%	96.94%
3.KX827770.1_GX264 / China	94.90%	94.90%	96.94%	96.94%	96.94%	98.98%	95.92%	96.94%	98.98%	98.98%
4.KX827771.1_GX261 / China	94.90%	94.90%	96.94%	100%	100%	97.96%	92.86%	95.92%	97.96%	97.96%
5.KX377522.1_GX265 / China	94.90%	94.90%	96.94%	100%	97.96%	97.96%	92.86%	95.92%	97.96%	97.96%
6.KX827767.1_GX206 China	96.94%	96.94%	98.98%	97.96%	97.96%	97.96%	94.90%	97.96%	100%	100%
7.KX827769.1_GX281 / China	91.84%	93.88%	95.92%	92.86%	92.86%	94.90%	92.86%	92.86%	94.90%	94.90%
8.HM855265.1_Sh-TTV203 / China	94.90%	98.98%	96.94%	95.92%	95.92%	97.96%	92.86%	97.96%	97.96%	97.96%
9.KX827768.1_GX249 / China	96.94%	96.94%	98.98%	97.96%	97.96%	100%	94.90%	97.96%	100%	100%
10.MK496298.1_CANEL_6 / Turkey	96.94%	96.94%	98.98%	97.96%	97.96%	100%	94.90%	97.96%	100%	100%
11.MK496299.1_CANEL_28 / Turkey	96.94%	94.90%	96.94%	95.92%	95.92%	97.96%	92.86%	95.92%	97.96%	97.96%
12.MK496300.1_CANEL_42 / Turkey	96.94%	94.90%	96.94%	95.92%	95.92%	97.96%	92.86%	95.92%	97.96%	97.96%
13.MK496301.1_CANEL_47 / Turkey	95.92%	95.92%	97.96%	98.98%	98.98%	98.98%	93.88%	96.94%	98.98%	98.98%
14.MK496302.1_CANEL_90 / Turkey	95.92%	95.92%	97.96%	96.94%	96.94%	98.98%	93.88%	96.94%	98.98%	98.98%
15.MK496303.1_CANEL_112 / Turkey	96.94%	96.94%	98.98%	97.96%	97.96%	100%	94.90%	97.96%	100%	100%
16.MK496304.1_CANEL_128 / Turkey	93.88%	95.92%	97.96%	94.90%	94.90%	96.94%	95.92%	94.90%	96.94%	96.94%
17.MK496305.1_CANEL_130 / Turkey	79.59%	79.59%	81.63%	78.57%	78.57%	80.61%	79.59%	78.57%	80.61%	80.61%
18.MK496306.1_CANEL_140 / Turkey	81.63%	81.63%	83.67%	80.61%	80.61%	82.65%	81.63%	80.61%	82.65%	82.65%
19.MK496307.1_CANEL_150 / Turkey	95.92%	95.92%	97.96%	96.94%	96.94%	98.98%	93.88%	96.94%	98.98%	98.98%

	11	12	13	14	15	16	17	18	19
11	96.94%	96.94%	95.92%	95.92%	96.94%	93.88%	79.59%	81.63%	95.92%
12	94.90%	94.90%	95.92%	95.92%	96.94%	95.92%	79.59%	81.63%	95.92%
13	96.94%	97.96%	97.96%	97.96%	98.98%	97.96%	81.63%	83.67%	97.96%
14	95.92%	95.92%	98.98%	96.94%	97.96%	94.90%	78.57%	80.61%	96.94%
15	95.92%	95.92%	98.98%	96.94%	97.96%	94.90%	78.57%	80.61%	96.94%
16	97.96%	97.96%	98.98%	98.98%	100%	96.94%	80.61%	82.65%	98.98%
17	92.86%	92.86%	93.88%	93.88%	94.90%	95.92%	79.59%	81.63%	93.88%
18	95.92%	95.92%	96.94%	96.94%	97.96%	94.90%	78.57%	80.61%	96.94%
19	97.96%	97.96%	98.98%	98.98%	100%	96.94%	80.61%	82.65%	98.98%
20	100%	100%	96.94%	96.94%	97.96%	94.90%	80.61%	82.65%	96.94%
21	96.94%	96.94%	96.94%	97.96%	98.98%	95.92%	79.59%	81.63%	97.96%
22	96.94%	96.94%	97.96%	97.96%	98.98%	95.92%	81.63%	83.67%	100%
23	97.96%	97.96%	98.98%	98.98%	95.92%	96.94%	80.61%	82.65%	98.98%
24	94.90%	94.90%	95.92%	95.92%	96.94%	83.67%	81.63%	83.67%	95.92%
25	80.61%	80.61%	79.59%	81.63%	80.61%	81.63%	96.94%	81.63%	83.67%
26	82.65%	82.65%	81.63%	83.67%	82.65%	83.67%	96.94%	81.63%	83.67%
27	96.94%	96.94%	97.96%	100%	98.98%	95.92%	81.63%	83.67%	83.67%

	NC_01407.1_CFTV10 (RefSeq) / Japan	GU951508.1_LDL / China	KX827770.1_GX264 / China	KX827771.1_GX261 / China	KX377522.1_GX265 / China	KX827767.1_GX206 China	KX827769.1_GX281 / China	KX827768.1_GX249 / China	MK496298.1_CANEL_6 / Turkey	MK496299.1_CANEL_28 / Turkey	MK496300.1_CANEL_42 / Turkey	MK496301.1_CANEL_47 / Turkey	MK496302.1_CANEL_90 / Turkey	MK496303.1_CANEL_112 / Turkey	MK496304.1_CANEL_128 / Turkey	MK496305.1_CANEL_130 / Turkey	MK496306.1_CANEL_140 / Turkey	MK496307.1_CANEL_150 / Turkey
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Fig. 2. Amino acid % Identity matrix of TTcCaV isolates based on 98 aa partial sequences of ORF1/ORF3.

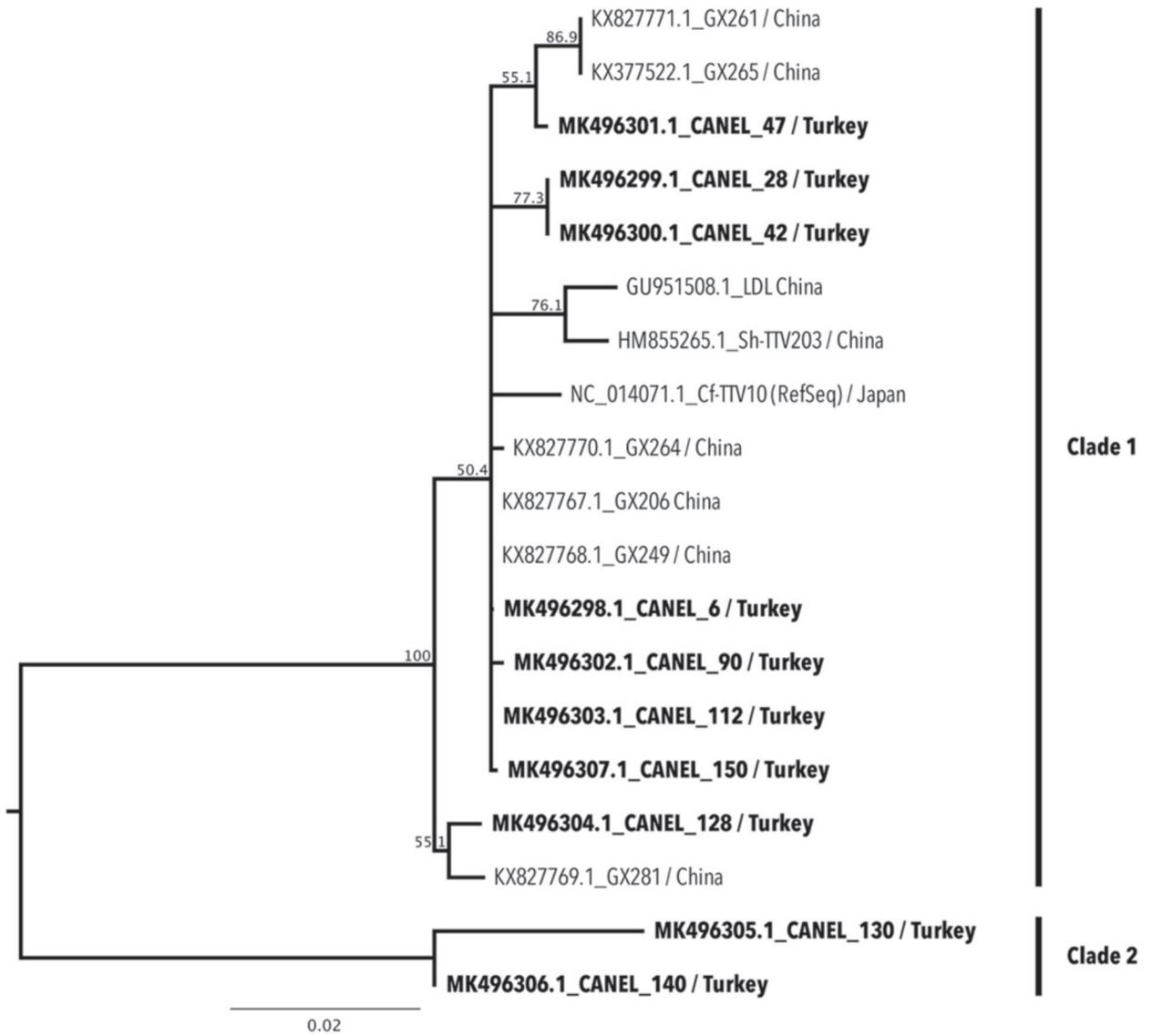


Fig. 3. Multiple alignment of 98 aa partial sequences of the ORF1/ORF3 region of TTCaV isolates.

Indeed, further investigations must be undertaken to determine the reasons of divergence in the two sequences obtained in this study. However, analysis of partial sequences, both nucleotide and amino acids, clearly indicates two distinct sub-genotypes of TTCaV, even if they are a small part of the genome (Figs 2, 3, 4).

In conclusion, our study provides the first information on the prevalence of TTCaV in both clinically healthy and diarrheic dogs in Turkey, and reveals the partial genomic sequence data from canine faecal samples. We found no correlations in the presence of TTCaV in terms of age or health condition. Therefore, we are of the opinion that TTCaV cannot be considered as a cause of diarrhea. On the basis of the phylogenetic analysis, we propose that the two isolates of our study are new genotypes of Torque teno canis virus. The present study is also one of the pioneer studies revealing the existence of TTCaV, after the reports from Japan, China and Brazil. The findings of our study may be used as an indication to compare with future studies for the presence of canine TTV worldwide.

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TURAN, T., H. İŞİDAN, M. O. ATASOY: Molekularno otkrivanje i genomska svojstva psećeg virusa Torque teno u Turskoj. *Vet. arhiv* 90, 467-475, 2020.

SAŽETAK

Virus Torque teno (TTV) otkriven je najprije u ljudi, no otada je zabilježen i u mnogim drugim domaćinima kao što su majmuni, mačke, svinje, galebovi i psi. Cilj ovoga rada bio je istražiti prisutnost psećeg virusa Torque teno (TTCaV) u pasa u azilu Sivas Municipal Animal Shelter u Turskoj. Uzorci izmeta (n = 202), uključujući one s proljevom i bez proljeva, prikupljeni su od pasa različitih dobnih skupina. Ukupno je 32,18 % (65/202) uzoraka bilo pozitivno na TTCaV. Od 65 pozitivnih uzoraka njih 34,64 % (44/127) potjecalo je od odraslih pasa s proljevom, a 26,09 % (6/23) od štenadi s proljevom. S druge strane, 28,84 % (15/52) pozitivnih uzoraka utvrđeno je u klinički zdravih pasa. Osam je sekvencija pokazalo međusobnu blisku srodnost, no sekvencije dvaju uzoraka (CANEL130 i CANEL140) genetski su se razlikovale od drugih sekvencija. Ovo je prvi nalaz TTCaV u odraslih pasa i štenadi u Turskoj i dokazuje da se TTCaV ne može smatrati jedinim uzročnikom proljeva.

Ključne riječi: psi iz azila; virus Torque teno canis; Turska
