Shiraz University

IJVR

ISSN: 1728-1997 (Print)
ISSN: 2252-0589 (Online)

Vol. 21 No. 3 Ser. No. 72 2020

IRANIAN JOURNAL OF VETERINARY RESEARCH

Original Article

Molecular characterization of canine astrovirus, vesivirus and circovirus, isolated from diarrheic dogs in Turkey

Turan, T. and Işıdan, H.*

Virology Department, Faculty of Veterinary Medicine, Sivas Cumhuriyet University, 58140, Sivas, Turkey

****Correspondence:** *H. Işıdan, Virology Department, Faculty of Veterinary Medicine, Sivas Cumhuriyet University, 58140, Sivas, Turkey. E-mail: hisidan@cumhuriyet.edu.tr*

(Received 14 Nov 2019; revised version 2 Mar 2020; accepted 24 Jun 2020)

Abstract

Background: Canine astrovirus (CAstV) has been considered the primary cause of gastroenteritis in young animals worldwide, while canine vesivirus (CVeV) and canine circovirus (CCiV) are occasionally reported. **Aims:** This study aimed to investigate the existence and molecular characteristics for these three viruses in Turkey. **Methods:** Faecal samples from 150 shelter dogs with gastrointestinal problems (127 adults and 23 puppies) were collected and examined by reverse transcription-polymerase chain reaction (RT-PCR) analysis based on the partial sequence of *RdRp* gene (ORF1b) for CAstV, *ORF2* gene of CVeV and capsid protein (Cap) and replication associated protein (*Rep*) gene of CCiV. Randomly selected positive samples were submitted to sequencing and molecular analyses were conducted based on partial sequences. **Results:** It was found that 66% (99/150) of diarrhoeic dogs were positive for CAstV, 3.33% (5/150) for CVeV, and 6% (9/150) for CCiV. Four sub-genotypes for CAstV and two sub-genotypes for CVeV were suggested according to molecular analyses. The phylogenetic relationship of CCiV with other strains obtained from various areas was further demonstrated. **Conclusion:** This study emphasizes the importance of emerging viruses for canids, classification of them and their proportional contribution in gastroenteritis cases. We concluded that astrovirus infection must be considered as the major cause of diarrhea in dogs; However, the prevalences of vesivirus and circovirus were relatively low in cases makes them less important in Turkey.

Key words: Canine astrovirus, canine circovirus, canine vesivirus, Diarrhea, Turkey

Introduction

The *Astroviridae* family consists of approximately 35 nm diameter icosahedral capsids, which comprise a positive, linear, single stranded RNA genome about 6.4-7.4 kb in length. Under the microscope, negative stained preparations show approximately 10% of the viral particles to be as astroviruses (Mendez and Arias, 2013; MacLachlan *et al*., 2017; Zhang *et al*., 2020). These viruses are classified under two genera as *Mamastrovirus* and *Avastrovirus*, whose genomes have three open reading frames (ORFs) (ORF1a, ORF1b, and ORF2) (Zhu *et al*., 2011; Caddy *et al*., 2015; Mihalov-Kovacs *et al*., 2017). Infectious viral RNA acts as both genomic and viral mRNA (Mendez and Arias, 2013; MacLachlan *et al*., 2017). Astroviruses were first identified in the faeces of children with diarrhea in 1975 (Appleton and Higgins, 1975; Madeley and Cosgrove, 1975) and are currently estimated to cause 10% of the gastroenteritis cases in children worldwide (Moser and Schultz-Cherry, 2005). Canine astroviruses (CAstV) have been identified in many countries of the world, and were first investigated in 1980 in faeces of diarrheic puppies in the USA (Williams, 1980; Martelle *et al*., 2011; Zhu *et al*., 2011; Grellet *et al*., 2012; Castro *et al*., 2013; Choi *et al*., 2014).

The host spectrum of caliciviruses comprise both wild and domestic animals, including dogs, cattle, pigs, felines, minks, monkeys, and humans. The family *Caliciviridae* consists of 11 genera, including *Vesivirus*, *Lagovirus*, *Norovirus*, *Sapovirus*, and *Nebovirus*; all of which share specifications such as having 27-40 nm diameter capsids and 7.4-8.3 kb single-stranded, positive-polarity RNA genome (Martella *et al*., 2015; Gutierrez-Escolano, 2017; Renshaw *et al*., 2018; Vinjé *et al*., 2019). The 5´ end of the viral genome is linked to a VPg protein, while the 3´ end has a poly (A) tail. The genome contains 2-4 ORFs depending on the genus. ORF1, located at the 5´ end, encodes a large polyprotein processed only by the viral protease to produce non-structural (NS) proteins. There are two additional ORFs (ORF2 and ORF3) in noroviruses and vesiviruses encoding major (VP1) and minor (VP2) capsid proteins, respectively (Martin-Alonso *et al*., 2005; Gutierrez-Escolano, 2017; Desselberger, 2019).

Vesiviruses were first detected in 1932, in domestic pigs with vesicular disease in the USA. Since then, they have been associated with abortion, hepatitis, respiratory disease, diarrhea, myocarditis, mucosal ulceration and vesicular lesions in human and various animal species (Smith *et al*., 1998; Smith *et al*., 2002; Radford *et al*., 2007). In the early 80s, canine vesivirus (CVeV) was isolated from a dog with glossitis for the first time (Evermann *et al*., 1981). In the following years gastroenteritis cases associated with this virus were reported worldwide (Evermann *et al*., 1985; Shaffer *et*

al., 1985; Gabriel *et al*., 1996; Pratelli *et al*., 2000; Martella *et al*., 2002; Di Martino *et al*., 2009; Binn *et al*., 2018; Renshaw *et al*., 2018).

Circoviridae family contains 2 genera, *Circovirus* and *Cyclovirus* (Breitbart *et al*., 2017; Rosario *et al*., 2017). The virus has a small (15-25 nm diameter) capsid and circular ssDNA genomes. These genomes range from 1.7 to 2.1 kb and contain two main ORFs encoding replication (Rep) and capsid (Cap) associated proteins (Bexton *et al*., 2015; Breitbart *et al*., 2017). Circoviruses have been described as infectious agents of a group of animals such as birds, pigs, dogs, foxes, and wolves (Kapoor *et al*., 2012; Decaro *et al*., 2014; Hsu *et al*., 2016; Zaccaria *et al*., 2016; Dowgier *et al*., 2017; Rosario *et al*., 2017). Canine circovirus (CCiV) has been found in the blood serum of dogs with hemorrhagic enteritis in the USA (Kapoor *et al*., 2012). Since then, it has been reported in USA, Italy, Germany, Brazil, Taiwan, and Thailand (Li *et al*., 2013; Decaro *et al*., 2014; Hsu *et al*., 2016; Gentil *et al*., 2017; Piewbang *et al*., 2018; Cruz *et al*., 2020). A recent study demonstrated that CCiV was strongly related to the development of canine acute gastroenteritis, especially in case of co-infections with other etiological agents (Dowgier *et al*., 2017).

A large number of pathogens including CAstV, CVeV, and CCiV are responsible for gastroenteritis in dogs. However, none have been reported or investigated before in Turkey. This study, therefore, aimed to study the presence of these viruses in dogs with gastroenteritis and characterize viruses based on genomic data to understand the genetic relationship between strains isolated throughout the world.

Materials and Methods

Primer design

Oligonucleotides for the conventional polymerase chain reaction (PCR) assay were designed based on publicly available data from the database of The National Center for Biotechnology Information (NCBI). Sequences were aligned using MUSCLE alignment (Edgar *et al*., 2004), and the primers were designed using the Geneious Bioinformatics Software Platform (Kearse *et al*., 2012). The program was utilized to generate primer sets from well-conserved areas, which were thereby, capable of recognizing the most sequences in database.

Sampling

A total of 150 rectal swab specimens were collected from Sivas Municipality Animal Shelter, which accepts approximately a thousand dogs per year. All of the sampled dogs were manifesting gastro-intestinal problems, including 127 one-year-old adults and 23 two- to four-month-old puppies. Collected samples were transported to the laboratory immediately and stored at -80°C before being subjected to RNA and DNA isolation.

Virus investigation and phylogenetic analysis

Nucleic acid isolation

Faecal samples were diluted 1:10 with 1 M phosphate buffered saline (PBS) and centrifuged 10 min at 2876 g to remove large cellular debris. After centrifugation, supernatants were submitted to the total nucleic acid extraction procedure using a GF-1 Viral Nucleic Acid Extraction Kit (Vivantis Technologies, Malaysia) according to the manufacturer's instructions. Eluted nucleic acids were stored at -80°C until use.

Reverse transcription (RT)

The cDNA synthesis was carried out in a 20 µL final volume containing 5 µL RNA extract, 10 mM deoxynucleoside triphosphate (dNTP), 2,5 µL 10x RT buffer (50 mM Tris-HCl (pH = 8.3 at 25°C), 75 mM KCl, 3 mM $MgCl_2$ and 10 mM DTT), 50 ng of the random hexamer, 40 U RNasin, and 200 U M-MuLV RT RNase H (Vivantis, Germany). The RT was performed at 37°C for 1 h.

PCR

The PCR was conducted in a 30 µL final volume using 3 µL of the RT reaction mixture or viral nucleic acid extract as template. The PCR mixture contained 3 µL 10x PCR buffer, 10 mM dNTP, 10 pmol/µL of each sense/antisense primer, and 5 U of Taq DNA polymerase (Vivantis, Germany).

Molecular detection of partial *RdRp* gene of CAstV, partial *ORF2* gene of CVeV, partial *ORF1* (*Rep*) gene and *ORF2* (*Cap*) gene of CCiV were conducted by primer sets (Table 1). PCR conditions were adjusted as

Table 1: Primers used in the study. Positions of the forward and reverse primer sets were indicated based on reference sequences (CAstV, NC_026814; CVeV, NC_004542; CCiV, NC_020904.1)

Primer name	Sequence (5´-3´)	Position	Target gene	Amplicon size
CAstV-3484F	GYACTATACCRTCTGATTTAATT	3469-3491	*RdRp* gene (ORF1b) of canine astrovirus	294 bp
CAstV-3777R	AGACYAARGTGTCATAGTTCAG	3741-3762		
CVeV-6193F	ACCGMTGYCTTATGGCTGTGG	6193-6213	*ORF2* gene of canine vesivirus	359 bp
CVeV-6542R	CCAYCCWGTGTACATCTTSGC	6522-6542		
CCiV-1036F	CCCCCTTCGAGGCTGTWTATT	1035-1055	Capsid protein (*Cap*) gene of canine circovirus	250 bp
CCiV-1285R	AGGRGCTAACATGGTMTGGA	1265-1284		
CCiV-241F	GGTGGYCGCGGMCATTTTG	241-259	Replication associated protein (*Rep*) gene of canine circovirus	284 bp
CCiV-524R	ACBTBCACKTCCGTCTTCCA	505-524		

CAstV: Canine astrovirus, CVeV: Canine vesivirus, and CCiV: Canine circovirus

follows: 95°C for 2 min for pre-denaturaton, during 40 cycles, 94°C for 30 s denaturation, 55°C (CAstV) or 56°C (CVeV) or 51°C (both ORF1 and ORF2 of CCiV) for 30 s annealing, 72°C for 45 s for extension and lastly, 10 min for 72°C for the final extension.

Sequencing and phylogenetic analysis

The PCR amplicons were purifed with Wizard SV Gel and a PCR Clean-Up System (Promega, Madison,WI) and were sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) on an automated sequencer (ABI 3100; Applied Biosystems, Foster City, CA). All sequenced products were used to obtain phylogenetic data. Partial sequences were compared with other sequence data provided online by NCBI. Sequence alignment and phylogenetic analysis based on partial nucleotide sequences of 294 bp RdRp gene (canine astrovirus), 359 bp *ORF2* gene (canine vesivirus), 250 bp *ORF1* (*Rep*) and 284 bp *ORF2* (Cap) genes (canine circovirus) were constructed using Geneious software (Kearse *et al*., 2012). The neighbour-joining (NJ) method and the Tamura-Nei genetic distance model (Tamura and Nei, 1993) were used to build the tree. The trees were drawn to scale, where branch lengths were measured based on the number of substitutions per site and bootstrapped with 1000 replicates.

Results

Molecular detections were conducted based on 294 bp partial *RdRp* gene (*ORF1b*) of the CAstV genome, 359 bp partial *ORF2* gene of the CVeV genome, 284 bp partial *Rep* gene and 250 bp *Cap* gene of the CCiV. For CCiV, all positive samples gave exact bands for both *Cap* and *Rep* genes. Polymerase chain reaction assay showed that 82 samples for CAstV (64.57%), 4 samples for CVeV (3.15%) and 8 samples for CCiV based on both *Rep* and *Cap* genes (6.30%) were positive within the 127 adult dogs. For the puppies, 17 samples (73,91%) for CAstV, one in each sample (4.35%) for both CVeV and CCiV, were detected from faecal samples (Table 2).

Ten amplicons of the 294 bp partial *RdRp* gene region of CAstV were randomly selected and sequenced from positive samples (GenBank Accession No.: MK507563.1-MK507572.1). Partial sequence data were compared with each other and the available nucleotide sequence data in GenBank. According to phylogenetic analyses based on nucleotide alignment, there were four distinct clades (Fig. 1). All Turkish isolates were part of clade 1, which also included the RefSeq strain (Gillingham/2012/UK). Clades (2, 3 and 4) were named according to the distance of the nucleotide identity from clade 1. The identity of clade 1 strains varies from 91.40 to 100.00%. Likewise, the nucleotide identity of Turkish CAstV strains varied from 94.22% to 100%. In comparison with clade 1 and 2, the identity level dramatically reduced between 73.21% to 80.08%, while clade 2 strains varied from 91.76 to 97.63%. Between clade 1 and 3, the identity level was varied from 68.20% to 73.53%, whereas clade 3 strains were 95.73% within the group. Lastly, clade 4 strains showed an identity between 91.80% to 100.00% within the group, and the nucleotide identity was between 58.04% to 63.16% when compared to clade 1.

From the positive samples, two 359 bp length sequence data were obtained from the partial *ORF2* gene of CVeV and deposited to the GeneBank database (Acc. No.: MK783212.1 and MK783213.1). These data were compared with the other available CVeV sequences according to nucleotide identity level. The results showed that these two strains were segregated into two distinct clades (Fig. 2). The identity percentage between clade 1 and 2 varied between 67.04% to 71.75%. Clade 1 strains showed 84.59% to 100.00% identity within the group, whereas clade 2 strains were between 87.74% to 99.16%.

Phylogenetic analysis demonstrated that all Turkish CCiV strains (GenBank Acc. No.: MK783214.1-MK783223.1) were clustered with a Chinese CD17/2016 (MG266899.1), three USA 214 (JQ821392.1), UCD2-32162 (KC241984.1), and OH19098-1 (MF457592.1), two German Ha3 (KF887949.1) and FUBerlin-JRS (KT283604.1) strains which were isolated from dogs and 18 Italian strains, isolated from dogs, wolves and badgers, based on 250 bp partial *cap* gene of CCiV. Similar results were observed in the phylogenetic tree based on the replication associated protein (*Rep*) gene of CCiV with the contribution of five more Chinese strains including COS8/2016, COS2/2016, CDX2/2017, COM7/2016, and CDX8/2017 (MG266900-MG266904) in the same branch (Figs. 3A and B).

Moreover, the percentage of nucleotide identity of the novel strains ranged between 97.54% to 99.65% within the group, while the rest of the members in the clade were between 94.01% to 97.54%. On the other hand, the similarity between Turkish strains and the strains being drawn in the separate branches were 79.93% to 91.20%. While, similarity levels based on the *Cap* gene of CCiV seemed to be a little confusing, the tree substitution looked like a counterpart of the *Rep* gene. Nucleotide

Table 2: Overall PCR results for detecting CAstV, CveV, and CCiV from adults and puppies

Age	Total Samples	CAstV	CVeV	CciV	Dual infection with CAstV and CVeV	Dual infection with CAstV and CCiV	Dual infection with CVeV and CCiV	Triple infection
Adults	127	64.57% (82/127)	3.15% (4/127)	6.30% (8/127)	1.58% (2/127)	5.51% (7/127)	0.79% (1/127)	0.79% (1/127)
Puppies	23	73.91% (17/23)	4.35% (1/23)	4.35% (1/23)	4.35% (1/23)	4.35% (1/23)	4.35% (1/23)	4.35% (1/23)
Overal	150	66.00% (99/150)	3.33% (5/150)	6.00% (9/150)	2% (3/150)	5.33% (8/150)	2% (3/150)	2% (3/150)

PCR: Polymrase chain reaction, CAstV: Canine astrovirus, CVeV: Canine vesivirus, and CCiV: Canine circovirus, x/y: Positive/total sample ratio

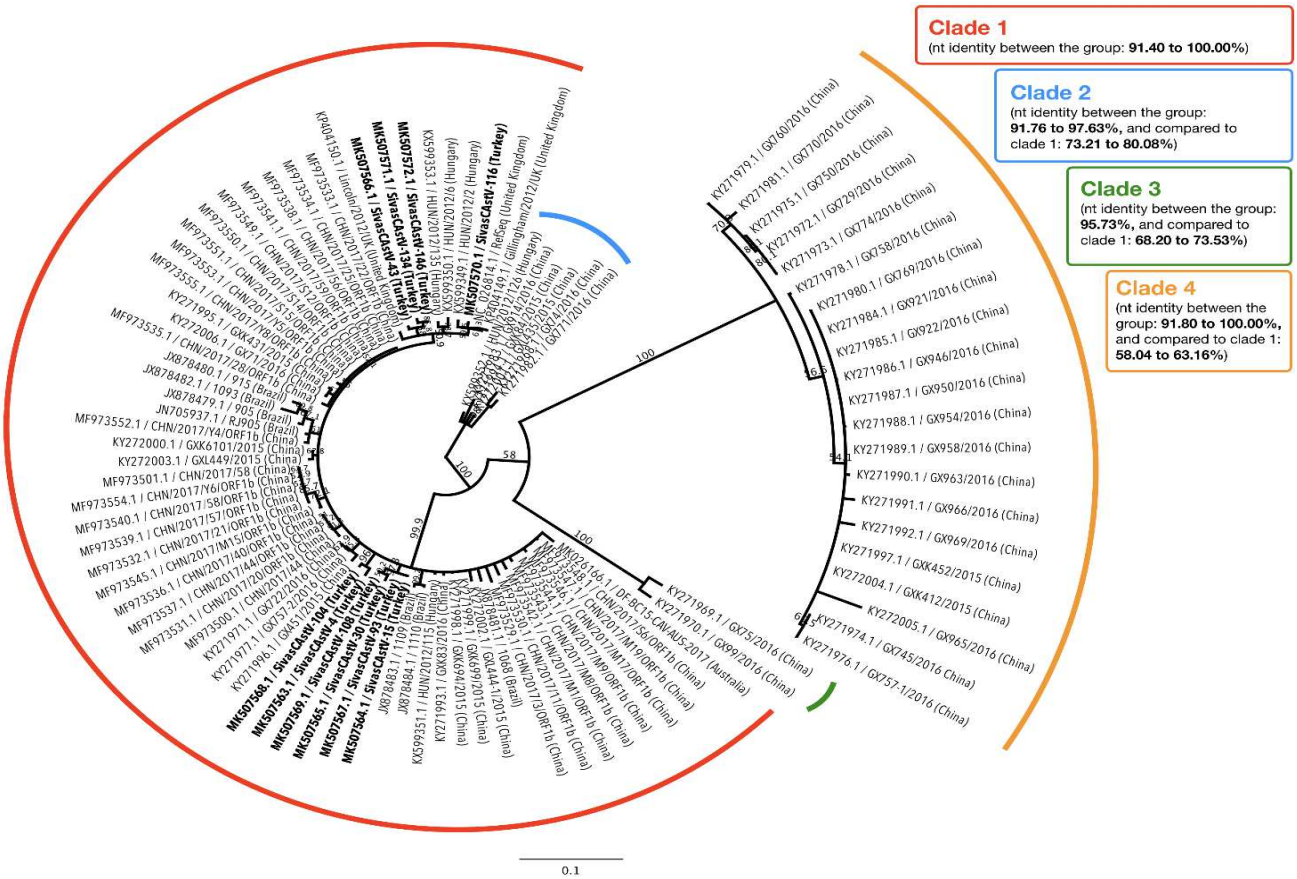

Fig. 1: Cladogram representing the consensus (1000 replicates) neighbour-joining phylogenic tree of canine astroviruses based on 294 bp partial *RdRp* gene sequences. Tree construction was built following the Tamura-Nei (1993) genetic distances model using Geneious Prime software version 2019.2.3 (available at https://www.geneious.com, Accessed Oct.11.2019). Novel strains are illustrated in bold text

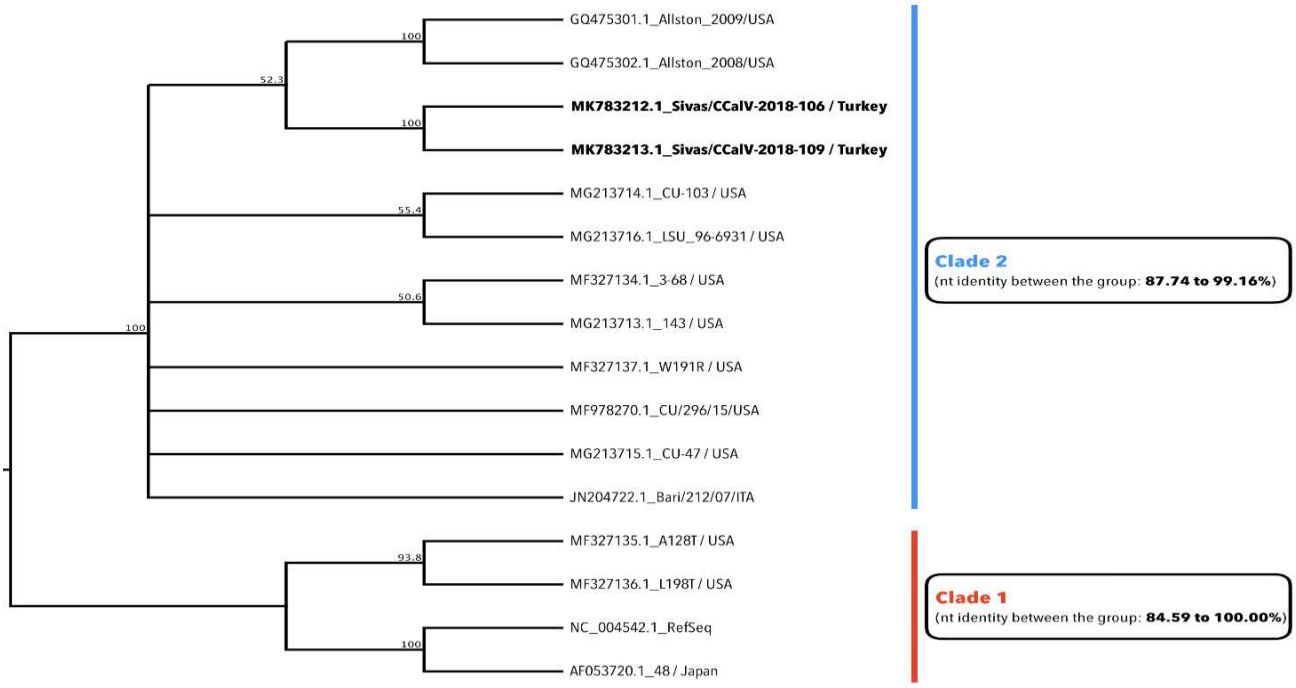

Fig. 2: Cladogram representing the consensus (1000 replicates) neighbour-joining phylogenic tree of canine vesiviruses based on 359 bp partial *ORF2* gene sequences. Tree construction was built following the Tamura-Nei (1993) genetic distances model using Geneious Prime software version 2019.2.3 (available at https://www.geneious.com, Accessed Oct.11.2019). Novel strains are illustrated in bold text

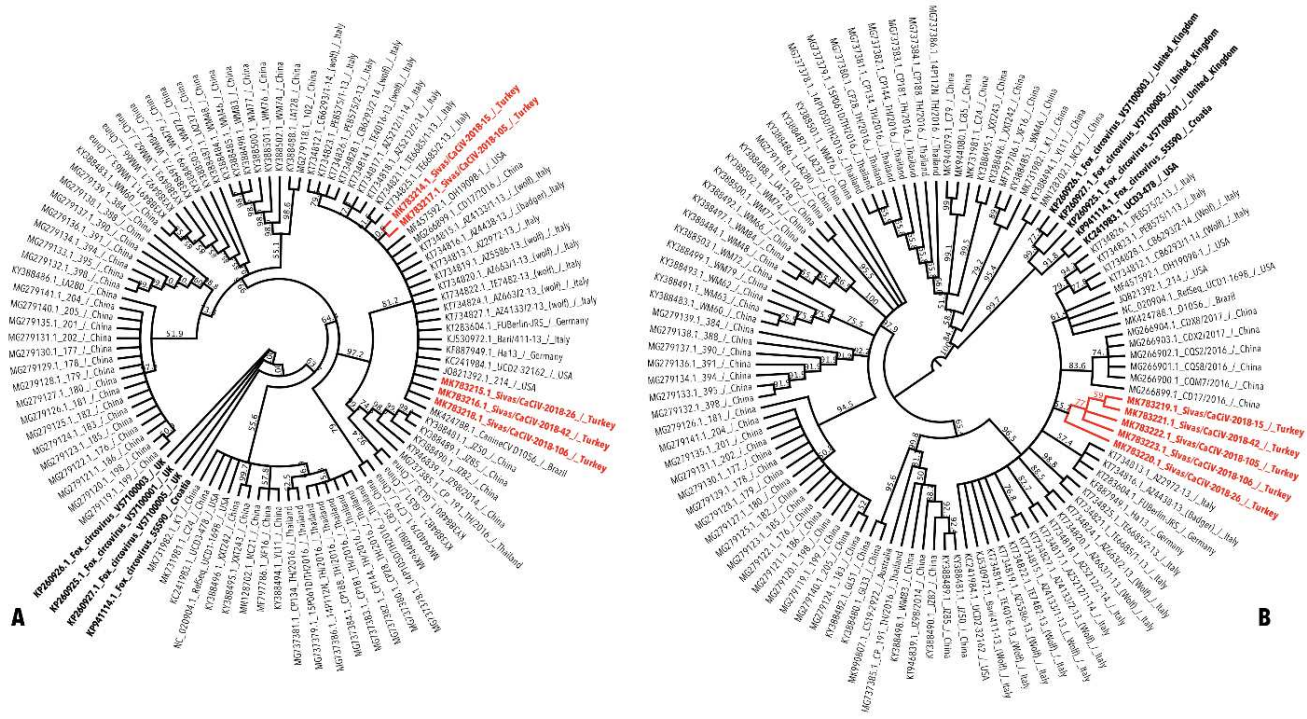

Fig. 3: Trees representing the consensus (1000 replicates) neighbour-joining phylogenic tree of canine circoviruses based on (**A**) 250 bp partial capsid protein (*Cap*) gene and (**B**) 284 bp replication associated protein (*Rep*) gene of CCiV strains. Tree construction was built following the Tamura-Nei (1993) genetic distances model using Geneious Prime software version 2019.2.3 (available at https://www.geneious.com, Accessed Oct.11.2019). Novel strains are illustrated in red color

similarity of the novel strains varied from 97.20% to 100% based on the *Cap* gene comparison in the branches from 95.60% to 100%. On the other hand, similarity of Turkish strains were 83.60% to 92.40% compared to other branches. The most distant strains to our isolates were the Chinese group (MG279119 to MG279140) with similarity levels downs to 83.60%.

Discussion

The main goals of this study were to show the common circulation of CAstV, CVeV, and CCiV between dog populations in Turkey, and reveal molecular characterizations of the identified sequences. In our study, CAstV was found to be 66% (99/150). This ratio was 64.57% (82/127) in adults and 73.91% (17/23) in puppies. The presence of CAstV has been reported in countries such as China, Korea, England, Italy, the USA and Hungary. In these studies, the positivity rates of CAstV in diarrheic dogs ranged from 2.2% to 26.9% (Williams, 1980; Martella *et al*., 2011; Zhu *et al*., 2011; Choi *et al*., 2014; Caddy *et al*., 2015; Mihalov-Kovacs *et al*., 2017). Although there was no statistically significant difference in the positivity rates between adults and puppies (P>0.05), the positivity rates of both (73.91%) were slightly higher than those reported in previous studies. The main role of CAstV in viral enteric disorder still remains unknown (Mihalov-Kovacs *et al*., 2017), however, it is known to be a significant viral cause of gastroenteritis in children (Olortegui *et al*., 2018). The higher incidence of infection implies that CAstV might be considered as a factor causing diarrhoea in dogs. However, a comprehensive study of other geographic areas is needed to support this argument so that CAstV could be included as a component of future multivalent vaccines, similar to the work published by Xia *et al*. (2016) on the trivalent, recombinant subunit vaccine against human astrovirus infections, along with norovirus and hepatitis E (Xia *et al*., 2016). It must be noted that we designed a universal primer set for CAstV for this study. The higher incidence may also validate the accuracy of the designed primer sets, since cumulative data in the GenBank database had been utilized to create unique, degenerated oligonucleotides from the most conserved regions.

Sequence comparison in the ORF1b (RdRp) was calculated using a 294 nt (98 aa) long fragment located at 3484 to 3777 nt position (based on the RefSeq) from the 5′ UTR of CAstV genome which makes the last quarter of the *ORF1b* gene. Mihalov-Kovács *et al*. (2017) conducted a comprehensive study about CAstV and suggested that three distinct genetic sub-lineages existed based on the partial *ORF1b* gene sequences. We thus included all the sequence data deposited in the GenBank including novel strains (94 sequences in total) to evaluate the overall results precisely. The phylogenetic tree and similarity heat map showed that four distinct sub-lineages were available, and as shown in Supplementary Material 1 (SM1), the identity percentages gradually differed from >90% to <60%.

Canine calicivirus infections belonging to *Norovirus* (Ntafis *et al*., 2010; Martella *et al*., 2019) *Sapovirus* (Li

et al., 2011) and *Vesivirus* (Roerink *et al*., 1999; Mochizuki *et al*., 2002; Castro *et al*., 2013; Martella *et al*., 2015; Renshaw *et al*., 2018) genera have been reported throughout the world. Canine vesiviruses were first included in the *Vesivirus* genus by Matsuura *et al*. (2002) and the incidence of CVeV literature reports variations between 1.1% and 64.8% (Mochizuki *et al*., 2002; Martella *et al*., 2015). In our study, this ratio was 3.33% (5/150). The positivity rates of adults (3.15%) and puppies (4.35%) were found not to be statistically significant (P>0.05). Nonetheless, this result is important, as it is the first investigation of CVeV in Turkey.

According to the phylogenetic analysis, two distinct sub-lineages of CVeV (Fig. 2) were demonstrated. As shown in Supplementary Material 2 (SM2), in comparison between clade 1 and 2, the nucleotide similarity level decreased from 67.04% to 71.75%, while clade 1 strains were from 84.59% to 100.00% within the group and clade 2 strains were from 87.74% to 99.16%.

Canine circovirus infections have been detected by various researchers in Europe (Matsuura *et al*., 2002; Kapoor *et al*., 2012; Decaro *et al*., 2014), the USA (Zaccaria *et al*., 2016; Anderson *et al*., 2017), Brazil (Weber *et al*., 2018; Kostias *et al*., 2019), Australia (Neef *et al*., unpublished; Bhatta *et al*., 2019) and the Far East (Hsu *et al*., 2016; Sun *et al*., 2019), and the incidence has been reported to range from 3.64% to 32.42%. Infection percentage was found to be 6% (9/150) in this study, and the positivity rates of both adults (6.30%) and puppies (4.35%) were found to be statistically insignificant (P>0.05). According to the phylogenetic analysis based on both *Rep* and *Cap* genes, our isolates substituted each other with other European and American strains, apart from fox circoviruses clustered together in a distinct branch. Despite the fact that Turkish strains included some of the far eastern strains, other branches did not include any European, American and Turkish strains.

In conclusion, we reported the presence of CCiV, CVeV, and CAstV from diarrheic dogs and provided initial genomic data for Turkey. We also suggested four sub-genotypes of CAstV and two sub-genotypes of CVeV. In addition, we also conducted the phylogenetic analysis of CCiV based on two gene regions according to molecular analyses of partial genomes. This is, to our knowledge, the first report showing proof of existence for these viruses in Turkey.

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

Anderson, A; Hartmann, K; Leutenegger, CM; Proksch, AL; Mueller, RS and Unterer, S (2017). Role of canine circovirus in dogs with acute haemorrhagic diarrhoea. Vet. Rec., 180: 542.

Appleton, H and Higgins, PG (1975). Viruses and gastro-enteritis in infants. Lancet. 1: 1297.

Bexton, S; Wiersma, LC; Getu, S; van Run, PR; Verjans, GMGM; Schipper, D; Schapendonk, CME; Bodewes, R; Oldroyd, L; Haagmans, BL; Koopmans, MMP and Smits, SL (2015). Detection of circovirus in foxes with Meningoencephalitis, United Kingdom, 2009-2013. Emerg. Infect. Dis., 21: 1205-1208.

Bhatta, TR; Chamings, A; Vibin, J and Alexandersen, S (2019). Detection and characterisation of canine astrovirus, canine parvovirus and canine papillomavirus in puppies using next generation sequencing. Sci. Rep., 9: 4602.

Binn, LN; Norby, EA; Marchwicki, RH; Jarman, RG; Keiser, PB and Hang, J (2018). Canine caliciviruses of four serotypes from military and research dogs recovered in 1963-1978 belong to two phylogenetic clades in the *Vesivirus* genus. Virol. J., 15: 39.

Breitbart, M; Delwart, E; Rosario, K; Segales, J and Varsani, A (2017). ICTV virus taxonomy profile: *Circoviridae*. J. Gen. Virol., 98: 1997-1998.

Caddy, SL and Goodfellow, I (2015). Complete genome sequence of canine astrovirus with molecular and epidemiological characterisation of UK strains. Vet. Microbiol., 177: 206-213.

Castro, TX; Cubel Garcia, RCN; Costa, EM; Leal, RM; Xavier, MDPT and Leite, JPG (2013). Molecular characterisation of calicivirus and astrovirus in puppies with enteritis. Vet. Rec., 172: 557.

Choi, S; Lim, S; Kim, Y; Cho, Y; Song, J and An, D (2014). Phylogenetic analysis of astrovirus and kobuvirus in Korean dogs. J. Vet. Med. Sci., 78: 1141-1145.

Cruz, TF; Batista, TN; Vieira, EM; Portela, LMF; Baccarin, AM; Gradiz, JJ and Junior JPA (2020). Genomic characterization of canine circovirus detected in a dog with intermittent hemorrhagic gastroenteritis in Brazil. Ciência Rural. 50: e20190909.

Decaro, N; Martella, V; Desario, C; Lanave, G; Circella, E; Cavalli, A; Elia, G; Camero, M and Buonavoglia, C (2014). Genomic characterization of a circovirus associated with fatal hemorrhagic enteritis in dog, Italy. PLoS One. 9: e105909.

Desselberger, U (2019). *Caliciviridae* other than noroviruses. Viruses. 11: 286.

Di Martino, B; Di Rocco, C; Ceci, C and Marsilio, F (2009). Characterization of a strain of feline calicivirus isolated from a dog faecal sample. Vet. Microbiol., 139: 52-57.

Dowgier, G; Lorusso, E; Decaro, N; Desario, C; Mari, V; Lucente, MS; Lanave, G; Buonavoglia, C and Elia, G (2017). A molecular survey for selected viral enteropathogens revealed a limited role of canine circovirus in the development of canine acute gastroenteritis. Vet. Microbiol., 204: 54-58.

Edgar, RC (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res., 32: 1792-1797.

Evermann, JF; Bryan, GM and Mckeirnan, A (1981). Isolation of a calicivirus from a case of canine glossitis. Canine Pract., 8: 36-39.

Evermann, JF; Mckeirnan, AJ; Smith, AW; Skilling, DE and Ott, RL (1985). Isolation and identification of caliciviruses from dogs with enteric infections. Am. J. Vet. Res., 46: 218-220.

Gabriel, S; Tohya, Y and Mochizuki, M (1996). Isolation of a calicivirus antigenically related to feline caliciviruses from feces of a dog with diarrhoea. J. Vet. Med. Sci., 58:

1041-1043.

Gentil, M; Gruber, AD and Müller, E (2017). Nachweishäufigkeit von dog circovirus bei gesunden und an Durchfall erkrankten Hunden. Tierarztl Prax Ausg K Kleintiere Heimtiere. 45: 89-94.

Grellet, A; De Battisti, C; Feugier, A; Pantile, M; Marciano, S; Grandjean, D and Cattoli, G (2012). Prevalence and risk factors of astrovirus infection in puppies from French breeding kennels. Vet. Microbiol., 157: 214-219.

Gutierrez-Escolano, AL (2017). Calicivirus biology. In: Ludert, JE; Pujol, FH and Arbiza, J (Eds.), *Human virology in Latin America from biology to control*. (1st Edn.), Switzerland, Springer. PP: 43-54.

Hsu, HS; Lin, TH; Wu, HY; Lin, LS; Chung, CS; Chiou, MT and Lin, CN (2016). High detection rate of dog circovirus in diarrheal dogs. BMC Vet. Res., 12: 116.

Kapoor, A; Dubovi, EJ; Henriquez-Rivera, JA and Lipkin, WI (2012). Complete genome sequence of the first canine circovirus. J. Virol., 86: 7018.

Kearse, M; Moir, R; Wilson, A; Stones-Havas, S; Cheung, M; Sturrock, S; Buxton, S; Cooper, A; Markowitz, S; Duran, C; Thierer, T; Ashton, B; Meintjes, P and Drummond, A (2012). Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 28: 1647-1649.

Kotsias, F; Bucafusco, D; Nuñez, DA; Lago Borisovsky, LA; Rodriguez, M and Bratanich, AC (2019). Genomic characterization of canine circovirus associated with fatal disease in dogs in South America. PLoS One., 14: e0218735.

Li, L; McGraw, S; Zhu, K; Leutenegger, CM; Marks, SL; Kubiski, S; Gaffney, P; Dela Cruz Jr, FN; Wang, C; Delwart, E and Pesavento, PA (2013). Circovirus in tissues of dogs with Vasculitis and Hemorrhage. Emerg. Infect. Dis., 19: 534-541.

Li, L; Pesavento, PA; Shan, T; Leutenegger, CM; Wang, C and Delwart, E (2011). Viruses in diarrhoeic dogs include novel kobuviruses and sapoviruses. J. Gen. Virol., 92: 2534-2541.

MacLachlan, NJ; Dubovi, EJ; Barthold, SW; Swayne, DE and Winton, JR (2017). *Caliciviridae* and *Astroviridae*. In: *Fenner's veterinary virology*. (5th Edn.), London, Elsevier. PP: 497-510.

Madeley, CR and Cosgrove, BP (1975). 28 nm particles in faeces in infantile gastroenteritis. Lancet. 2: 451-452.

Martella, V; Decaro, N; Lorusso, E; Radogna, A; Moschidou, P; Amorisco, F; Lucente, MS; Desario, C; Mari, V; Elia, G; Banyai, K; Carmichael, LE and Buonavoglia, C (2019). Genetic heterogeneity and recombination in canine noroviruses. J. Virol., 83: 11391-11396.

Martella, V; Moschidou, P; Lorusso, E; Mari, V; Camero, M; Bellacicco, A; Losurdo, M; Pinto, P; Desario, C; Banyai, K; Elia, G; Decaro, N and Buonavoglia, C (2011). Detection and characterization of canine astroviruses. J. Gen. Virol., 92: 1880-1887.

Martella, V; Pinto, P; Lorusso, E; Di Martino, B; Wang, Q; Larocca, V; Cavalli, A; Camero, M; Decaro, N; Bányai, K; Saif, LJ and Buonavoglia, C (2015). Detection and full-length genome characterization of novel canine vesiviruses. Emerg. Infect. Dis., 21: 1433-1436.

Martella, V; Pratelli, A; Gentile, M; Buonavoglia, D; Decaro, N; Fiorante, P and Buonavoglia, C (2002). Analysis of the capsid protein gene of a feline-like calicivirus isolated from a dog. Vet. Microbiol., 85: 315-322.

Martin-Alonso, JM; Skilling, DE; Gonzalez-Molleda, L; del Barrio, G; Machin, A; Keefer, NK; Matson, DO; Iversen, PL; Smith, AW and Parra, F (2005). Isolation and characterization of a new vesivirus from rabbits. Virology. 337: 373-383.

Matsuura, Y; Tohya, Y; Nakamura, K; Shimojima, M; Roerink, F; Mochizuki, M; Takase, K; Akashi, H and Sugimura, T (2002). Complete nucleotide sequence, genome organisation and phylogenic analysis of the canine calicivirus. Virus Genes. 25: 67-73.

Mendez, E and Arias, C (2013). Astroviruses. In: Knipe, DM and Howley, P (Eds.), *Field's virology*. (6th Edn.), Philadelphia, Lippincott Willliams & Wilkins. PP: 609-628.

Mihalov-Kovács, E; Martella, V; Lanave, G; Bodnar, L; Fehér, E; Marton, S; Kemenesi, G; Jakab, F and Bányai, K (2017). Genome analysis of canine astroviruses reveals genetic heterogeneity and suggests possible inter-species transmission. Virus Res., 232: 162-170.

Mochizuki, M; Hashimoto, M; Roerink, F; Tohya, Y; Matsuura, Y and Sasaki, N (2002). Molecular and seroepidemiological evidence of canine calicivirus infections in Japan. J. Clin. Microbiol., 40: 2629-2631.

Moser, LA and Schultz-Cherry, S (2005). Pathogenesis of astrovirus infection. Viral Immunol., 18: 4-10.

Ntafis, V; Xylouri, E; Radogna, A; Buonavoglia, C and Martella, V (2010). Outbreak of canine norovirus infection in young dogs. J. Clin. Microbiol., 48: 2605-2608.

Olortegui, MP; Rouhani, S; Yori, PP; Salas, MS; Trigoso, DR; Mondal, D; Bodhidatta, L; Platts-Mills, J; Samie, A; Kabir, F; Lima, A; Babji, S; Shrestha, SK; Mason, CJ; Kalam, A; Bessong, P; Ahmed, T; Mduma, E; Bhutta, ZA; Lima, I; Ramdass, R; Moulton, LH; Lang, D; George, A; Zaidi, AKM; Kang, G; Houpt, ER; Kosek, MN and on behalf of the MAL-ED Network (2018). Astrovirus infection and diarrhea in 8 countries pediatrics. 141: e20171326.

Piewbang, C; Jo, WK; Puf, C; van der Vries, E; Kesdangsakonwut, S; Rungsipipat, A; Kruppa, L; Jung, K; Baumgärtner, W; Techangamsuwan, S; Ludlow, M and Osterhau, ADME (2018). Novel canine circovirus strains from Thailand: Evidence for genetic recombination. Sci. Rep., 8: 7524.

Pratelli, A; Greco, G; Camero, M; Normanno, G and Buonavoglia, C (2000). Isolation and identification of a calicivirus from a dog with diarrhea. New Microbiol., 23: 257-260.

Radford, AD; Coyne, KP; Dawson, S; Porter, CJ and Gaskell, RM (2007). Feline calicivirus. Vet. Res., 38: 319-335.

Renshaw, RW; Griffing, J; Weisman, J; Crofton, LM; Laverack, MA; Poston, RP; Duhamel, GE and Dubovi, EJ (2018). Characterization of a vesivirus associated with an outbreak of acute hemorrhagic gastroenteritis in domestic dogs. J. Clin. Microbiol., 56: e01951-17.

Roerink, F; Hashimoto, M; Tohya, Y and Mochizuki, M (1999). Organization of the canine calicivirus genome from the RNA polymerase gene to the poly(A) tail. J. Gen. Virol., 80: 929-935.

Rosario, K; Breitbart, M; Harrach, B; Segales, J; Delwart, E; Biagini, P and Varsani, A (2017). Revisiting the taxonomy of the family *Circoviridae*: establishment of the genus *Cyclovirus* and removal of the genus *Gyrovirus*. Arch. Virol., 162: 1447-1463.

Schaffer, FL; Soergel, ME; Black, JW; Skilling, DE; Smith, AW and Cubitt, WD (1985). Characterization of a new

calicivirus isolated from feces of a dog. Arch. Virol., 84: 181-195.

Smith, AW; Skilling, DE; Cherry, N; Mead, JH and Matson, DO (1998). Calicivirus emergence from ocean reservoirs: zoonotic and interspecies movements. Emerg. Infect. Dis., 4: 13-20.

Smith, AW; Skilling, DE; Matson, DO; Kroeker, AD; Stein, DA; Berke, T and Iverse, PL (2002). Detection of vesicular exanthema of swine-like calicivirus in tissues from a naturally infected spontaneously aborted bovine fetus. J. Am. Vet. Med. Assoc., 220: 455-458.

Sun, W; Zhang, H; Zheng, M; Cao, H; Lu, H; Zhao, G; Xie, C; Cao, L; Wei, X; Bi, J; Yi, C; Yin, G and Jin, N (2019). The detection of canine circovirus in Guangxi, China. Virus Res., 259: 85-89.

Tamura, K and Nei, M (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol., 10: 512-526.

Vinjé, J; Estes, MK; Esteves, P; Green, KY; Katayama, K; Knowles, NJ; L'Homme, Y; Martella, V; Vennema, H; White, PA; ICTV Report Consortium (2019). ICTV virus taxonomy profile: *Caliciviridae*. J. Gen. Virol., 100: 1469-1470.

Weber, MN; Cibulski, SP; Olegario, JC; da Silva, MS; Puhl, DE; Mosena, ACS; Alves, CDBT; Paim, WP; Baumbach, LF; Mayer, FQ; Fernandes, ARF; Azevedo, SS and Canal, CW (2018). Characterization of dog serum virome from Northeastern Brazil. Virology. 525: 192-199.

Williams, F (1980). Astrovirus-like, coronavirus-like, and parvovirus-like particles detected in the diarrheal stools of beagle pup. Arch. Virol., 66: 215-226.

Xia, M; Wei, C; Wang, L; Cao, D; Meng, XJ; Jiang, X and Tan, M (2016). A trivalent vaccine candidate against hepatitis E virus, norovirus, and astrovirus. Vaccine. 34: 905-913.

Zaccaria, G; Malatesta, D; Scipioni, G; Di Felice, E; Campolo, M; Casaccia, C; Savini, G; Di Sabatino, D and Lorusso, A (2016). Circovirus in domestic and wild carnivores: An important opportunistic agent? Virology. 490: 69-74.

Zhang, W; Wang, R; Liang, J; Zhao, N; Li, G; Gao, Q and Su, S (2020). Epidemiology, genetic diversity, and evolution of canine astrovirus. Transbound. Emerg. Dis., 00: 1-10.

Zhu, LA; Zhao, W; Yin, H; Shan, TL; Zhu, CX; Yang, X; Hua, XG and Cui, L (2011). Isolation and characterization of canine astrovirus in China. Arch. Virol., 156: 1671-1675.

Supporting Online Material

Refer to web version on PubMed Central® (PMC) for Supplementary Material.