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Phylogenetic analysis of canine parvovirus isolates from west Mediterranean region of Türkiye

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Abstract

Canine parvovirus type 2 (CPV-2) causes hemorrhagic enteritis, and is one of the most important and contagious pathogens of dogs. In this study, we aimed to determine the prevalence and antigenic variants of CPV enteritis in dogs. Fecal samples were collected from 35 dogs with mucoid to hemorrhagic diarrhea in the Western Mediterranean region of Türkiye between October 2019 and March 2021. DNA was isolated from the samples and examined using PCR analysis. Twenty-eight out of 35 dogs (80.00%) were detected to be positive for CPV. Of these, three had already been vaccinated. The partial *VP2* genes of 15 CPV positive samples producing strong bands in agarose gels were sequenced. All strains were identified as CPV-2b, and the amino acid changes were identified. Discriminative amino acid changes were detected for different amino acid positions clearly defining new CPV-2b variants. Of the 15 isolates, three had previously unreported synonymous mutations. Phylogenetic analysis indicated that the strains obtained in this study were closely related to isolates from the Mersin province of Türkiye, except for three isolates that had synonymous mutations and were located in a separate branch from the other CPV-2b genetic variants previously detected in Mersin Province and Urfa Province in Türkiye. This study demonstrates the increase in the prevalence rates for CPV-2b circulating in vaccinated and nonvaccinated dogs. Taking into account the data from phylogenetic trees which highlights differences between the vaccine strains and the isolates, re-designing immunization strategies needs necessary.

Introduction

Canine parvovirus type 2 (CPV-2) is one of the most important viral pathogens of dogs causing hemorrhagic gastroenteritis, and myocarditis. The clinical symptoms are fever, leukopenia, hemorrhagic diarrhea, dehydration, and anorexia. The disease is very contagious, and progresses with a mortality of 10.00% in adult dogs and 91.00% in puppies.[1,2,3] The CPV-2 belongs to the family *Parvoviridae*, subfamily *Parvovirinae*, and genus *Protoparvovirus.*[2] It is a non-enveloped, icosahedral, linearized, single-stranded DNA virus. The viral genome is 5.30 kb in length and contains two open reading frames (ORFs).[4,5] The first ORF codes for two non-structural proteins (NS1 and NS2) and the second one codes for two structural proteins, VP1 and VP2. NS1 is responsible for viral replication and the induction of cell apoptosis, while the function of NS 2 is currently unknown.[6] The main capsid protein, VP2, is a key molecule for determining host range, antigenic properties, and receptor binding. Therefore, it is very important to detect certain residues within VP2 to identify variants of CVP-2. During CPV-2 infection, VP3 is derived from the VP2 protein by host proteolytic cleavage, as is presented only on complete (DNA-containing) virions.[7] The virus is genetically related to the feline panleukopenia virus.[8]

Canine parvovirus first emerged in the late 1970s.[9] Three variants were detected, namely CPV-2a (426Asn), CPV-2b (426Asp), and CPV-2c (426Glu) on the basis of amino acid conformation on the capsid protein.[8] These variants have completely replaced the original CPV-2. Currently the original CPV-2 is not found in dog population and is only present in vaccine formulations.[10]

Since its discovery in the 1970s, CPV-2 and its variants have been circulating rapidly in the dog population world-wide. Despite the development of vaccines that included

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its variants and the certain efficacy of the vaccine in preventing CPV-2 disease, high prevalence rates of CPV-2 were reported in previous studies carried out in various countries. Recently, the prevalence rates of CPV-2 in dogs have been detected to be 76.69% (158/206) in Japan,[11] 100% (59/59) in Vietnam,[12] 82.00% (33/40) in Pakistan,[13] 70.42% (50/71) in Colombia,[14] and 55.70% (34/61) in China.[15] In most of these studies, new variants of CPV-2 have been found. Therefore, the prevalence of CPV-2 and surveillance of circulating viruses needs to be re-evaluated. In this study, we aimed to determine the prevalence and antigenic variants of CPV enteritis in dogs from the west Mediterranean region of Türkiye. Using these data, material and moral losses caused by the virus will be prevented, and control programs can be planned and implemented accordingly.

Materials and Methods

DNA extraction and PCR amplification. Fecal samples were collected from 35 puppies of different breeds and sexes, aged one to twenty months, both vaccinated and nonvaccinated, brought to the Animal Hospital Clinics of Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Burdur. The Animal Care Committee of the University of Mehmet Akif Ersoy, Burdur, Türkiye, approved this study (Approval No. 93773921-555). Fecal samples were collected from dogs with clinical symptoms of gastroenteritis brought to the animal hospital from private veterinary clinics or clinics in the west Mediterranean region of Türkiye and the surrounding provinces: Burdur, Isparta, and Antalya. The samples were stored in a deep freezer at – 80.00 ˚C until DNA extraction. After the fecal samples were mixed and crushed at a ratio of 1:10 in 10x antibiotic phosphate-buffered saline (PBS), they were centrifuged at 3,000 rpm for 20 min. Viral DNA was extracted from the fecal samples using a virus nucleic acid isolation kit (GeneDireX, Taoyuan, Taiwan) following the manufacturer's instructions. The DNA extraction products were stored at – 20.00 ˚C until used in PCR tests. The primer pairs Hfor/Hrev were used to amplify the sequences between 3556 to 4166 nucleotides, including discriminative sequential patterns of the capsid protein genes. For 630 bp fragment amplification PCR, 5.00 µL of Mg free Taq DNA polymerase buffer (Thermo Fisher Scientific, Waltham, USA), 2.00 µL of $MgCl_2$ (25.00 mM) (Thermo Fisher Scientific), 7.00 µL of deoxynucleotide triphosphates (10x; 2.00 mM each) (Thermo Fisher Scientific), 10.00 pmol μL^{-1} of each primer Hfor CAGGTGATGAATTTGCTACA and Hrev CATTTGGATAAACTGGTGGT (Sentebiolab, Ankara, Türkiye) and 1.25 U of Taq DNA polymerase (Thermo Fisher Scientific) were used. The PCR method was as reported by Buonavoglia *et al.*[16] The PCR analysis was performed with following steps; pre-denaturation for 5 min at 95.00 ˚C; 35 denaturation cycles were carried out for 1 min at 95.00 ˚C; annealing was carried out for 1 min at a suitable temperature for each primer pair; extension was carried out for 1 min at 72.00 ˚C; and the final extension was carried out for 10 min at 72.00 ˚C. PCR products were separated by electrophoresis on 1.00% agarose gels. The gels were photographed with a gel documentation system (DNR Bio Imaging Systems, Modi'in-Maccabim-Re'ut, Israel). There were 35 samples, of which the 15 samples with the best PCR results were sequenced (Fig. 1).

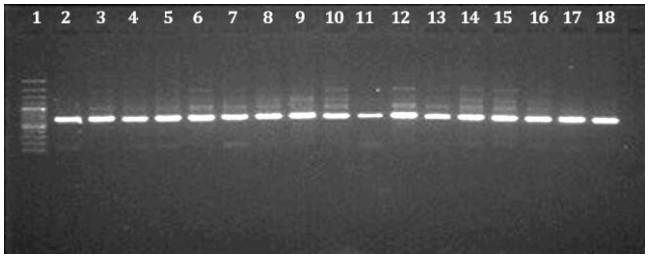

Fig. 1. Amplification products (Hfor and Hrev) of the isolates Lane 1: 1.00 kb DNA Marker; Lane 2: Isolate MZ545656; Lane 3: Isolate MZ545657; Lane 4: Isolate MZ545658; Lane 5: Isolate MZ-545659; Lane 6: Isolate MZ545660; Lane 7: Isolate MZ545661; Lane 8: Isolate MZ545662; Lane 9: Isolate MZ545663; Lane 10: Isolate MZ545664; Lane 11: Isolate MZ545665; Lane 12: Isolate MZ545666; Lane 13: Isolate MZ545667; Lane 14: Isolate MZ-545668; Lane 15: Isolate MZ545669; Lane 16: Isolate MZ545670; Lane 17: Positive control; Lane 18: Positive control.

Purification of PCR products, DNA sequencing reactions, and phylogenetic analysis. Fifteen PCR products were purified using Exonuclease I (20.00 U μL^{-1}; Thermo Fisher Scientific) and Shrimp Alkaline Phosphatase (Thermo Fisher Scientific). For DNA sequencing reactions, BigDye™ Terminator v3.1 Cycle Sequencing Kits (Thermo Fisher Scientific) were used. Finally, PCR products were analyzed using an ABI 3500 Genetic Analyzer (Applied Biosystems, Carlsbad, USA). Newly obtained sequences were edited by BioEdit version 7.2.5 (https://www.informer.com).[17] Consensus sequences were created and searched in Gen-Bank® using BLAST program, to define the reference sequences included in the phylogenetic analysis. Nucleotide sequences and amino acid sequences of selected references were downloaded from GenBank® in FASTA format and aligned separately using CLUSTAL-W/ BioEdit version 7.2.5.[17] The amino acid sequences of our strains, obtained from ExPASy translate tool (https://web.expasy.org/translate/), were used to examine the effects of mutations arising from amino acid changes. Finally, a phylogenetic tree was constructed was using the Maximum-Likelihood method with 1,000 boot-strap replications, in MEGA-X version 11.0.8 (https:// www.megasoftware.net).[18,19]

Results

Twenty-eight out of 35 dogs (80.00%) were recorded as being PCR positive. Of them, three, aged 1 to 4 months, were vaccinated (Table 1). Based on CLUSTAL-W and ExPASy translate tool analysis, all strains were identified as new CPV-2b (Table 2), a result which was confirmed by a discriminative amino acid changes table, as described by Decaro and Buonavoglia (Table 3).[8] The amino acid changes Val 232 to Ile, T 267 to A, Ser 297 to Ala, Ala 300 to Gly, Asp 305 to Tyr, Asp 323 to Asn, Tyr 324 to Ile, Asn 375 to Asp, Asn 426 to Asp, and Thr 440 to Ala were detected in 15 of the isolates (100%), and are listed in Table 2 for the isolates with the NCBI accession numbers MZ545656, MZ545657, MZ545658, MZ545659, MZ545660, MZ545661, MZ545662, MZ545663, MZ545664, MZ545665, MZ545666, MZ545667, MZ545668, MZ545669, and MZ545670. A synonymous mutation due to a single nucleotide change from A to G at amino acid position 318 between the nucleotides 3740 and 3742 changes the resulting amino acid CAA to CAG which encodes both glutamine or glutamic acid was reported for the first time in three of the isolates (20.00%), with NCBI accession numbers MZ545658, MZ54566, and MZ545670 (Table 2). Finally, a phylogenetic tree was constructed using the Maximum-Likelihood method of with 1,000 bootstrap replications in MEGA-X.[18,19] Phylogenetic analysis showed that the CPV-2b genetic variants sequenced in this study were closely related to Mersin isolates, except for isolates 5 (MZ545658), 10 (MZ54566), and 15 (MZ545670), which had synonymous mutations and were located in a separate branch from those obtained in our study and those from Mersin/Urfa Province completed in Türkiye, previously (Fig. 2).

Table 1. Identification of 15 samples sequenced.

Sample	ID No.	Age (month)	Sex	Vaccination	Genotype
1	MZ545656	2.00	Male	-	2b
2	MZ545657	1.00	Female	-	2b
3	MZ545658	1.50	Male	-	2b
4	MZ545659	4.00	Male	+	2b
5	MZ545660	1.00	Female	-	2b
6	MZ545661	3.00	Male	-	2b
7	MZ545662	2.00	Male	-	2b
8	MZ545663	2.50	Male	-	2b
9	MZ545664	3.00	Female	-	2b
10	MZ545665	4.00	Female	+	2b
11	MZ545666	1.00	Male	+	2b
12	MZ545667	1.00	Female	-	2b
13	MZ545668	2.00	Male	-	2b
14	MZ545669	3.00	Female	-	2b
15	MZ545670	4.00	Male	+	2b

Discussion

Epidemiological studies carried out worldwide have provided information about the distribution of three anti genic variants of CPV in the dog population over the past 20 years. These studies found that the original CPV-2 has disappeared in the dog population, and there is no difference among the antigenic variants in terms of pathogenicity.[20] There have been a limited number of molecular studies conducted into the distribution of CPV-2 antigenic variants in dogs during the last 20 years in Türkiye.

The CPV-2a was reported to have a significantly higher prevalence than CPV-2b in Türkiye.[21-23] This is the third CPV molecular characterization study published from Turkiye. The isolates in this study were collected from the west Mediterranean region of Türkiye, and the sequencing results were compared to those of strains isolated from Türkiye and worldwide. In order to estimate the viral phylogenetic relationships, a phylogenetic tree was constructed using partial CVP-2 gene sequences. All 15 isolates sequenced in this study were new CPV-2b while the most common variant was reported as type 2a in previous studies from Türkiye.[21-23] The CPV-2b variant has lower prevalence than CPV-2a and CPV-2c in Europe.[24] However, studies from Italy and Australia indicated that CPV-2b has emerged again in recent years, after a long hiatus.[24,25] Battilani *et al.* found that CPV-2b was more genetically stable than CPV-2a, as its sequence analysis showed the highest fraction of non-synonymous mutations, highlighting the significant pheno-typic effects of the accumulated mutations over time.[24] An increased prevalence of CPV-2b was also reported in our study from the west Mediterranean region.[26] These findings indicate that CPV-2b is evolving rapidly. The CPV-2 antigenic variants should therefore be regularly monitored using molecular surveillance to prevent new CPV-2b-induced outbreaks in the dog population in Türkiye.

All of the 15 isolates subjected to sequencing analysis were identical, with V232I, T267A, S297A, A300G, D305Y, D323N, Y324I, N375D, N426D, and Y440A amino acid mutations. Only three of the isolates, MZ545658, MZ54566, and MZ545670, had previously unreported synonymous mutations due to single nucleotide changes from A to G at amino acid position 318 between nucleotides 3740 and 3742, resulting in an amino acid change from CAA to CAG, which encodes both glutamine and glutamic acid. This amino acid change detected in this study may have induced subgroup formation (Fig. 2) and the emergence of the new CPV-2b variants. Additional studies should be carried out to understand whether this amino acid change affects the pathogenicity of this virus.

Amino acid substitutions located in the greatest variable GH loop comprising aa 267-498 of the VP2 protein have been reported.[27-29] Similarly, amino acid changes at residues T267A, S297A, A300G, D305Y, D323N, Y324I, N375D, N426D, and Y440A were also detected in our study. It has been suggested in previous studies that amino acid changes in residue 267 are important for the transmission and infectivity of the virus, and changes in amino acid residue 323 are responsible for binding to

Table 2. Effects of amino acid changes in the isolates.

Amino acid positions	232	267	297	300	305	323	318	324	375	426	440
Nucleotide	3480-3482	3584-3586	3675-3677	3684-3686	3699-3701	3753-3755	3740	3756-3757	3909-3911	4062-4064	4105-4106
Amino acid changes	GTA (Val)-ATA (Ile)	TTT - TAT	TCT (Ser)-GCT (Ala)	GCT (Ala) - GGT (Gly)	GAT (Asp) - TAT (Tyr)	GAC (Asp)-AAC (Asn)	CAA - CAG	TAT (Tyr) - ATT (Ile)	AAT (Asn)-GAT (Asp)	AAT (Asn) - GAT (Asp) /GAA (Glu)	ACA (Thr) - GCA (Ala)
MZ545656	ATA	TAT	GCT (Ala)	GGT(Gly)	TAT (Tyr)	AAC (Asn)	CAA	ATT (Ile)	GAT (Asp)	GAT (Asp)	GCA (Ala)
MZ545657	ATA	TAT	GCT (Ala)	GGT(Gly)	TAT (Tyr)	AAC (Asn)	CAA	ATT (Ile)	GAT (Asp)	GAT (Asp)	GCA (Ala)
MZ545658	ATA	TAT	GCT (Ala)	GGT(Gly)	TAT (Tyr)	AAC (Asn)	CAG	ATT (Ile)	GAT (Asp)	GAT (Asp)	GCA (Ala)
MZ545659	ATA	TAT	GCT (Ala)	GGT(Gly)	TAT (Tyr)	AAC (Asn)	CAA	ATT (Ile)	GAT (Asp)	GAT (Asp)	GCA (Ala)
MZ545660	ATA	TAT	GCT (Ala)	GGT(Gly)	TAT (Tyr)	AAC (Asn)	CAA	ATT (Ile)	GAT (Asp)	GAT (Asp)	GCA (Ala)
MZ545661	ATA	TAT	GCT (Ala)	GGT(Gly)	TAT (Tyr)	AAC (Asn)	CAG	ATT (Ile)	GAT (Asp)	GAT (Asp)	GCA (Ala)
MZ545662	ATA	TAT	GCT (Ala)	GGT(Gly)	TAT (Tyr)	AAC (Asn)	CAA	ATT (Ile)	GAT (Asp)	GAT (Asp)	GCA (Ala)
MZ545663	ATA	TAT	GCT (Ala)	GGT(Gly)	TAT (Tyr)	AAC (Asn)	CAA	ATT (Ile)	GAT (Asp)	GAT (Asp)	GCA (Ala)
MZ545664	ATA	TAT	GCT (Ala)	GGT(Gly)	TAT (Tyr)	AAC (Asn)	CAA	ATT (Ile)	GAT (Asp)	GAT (Asp)	GCA (Ala)
MZ545665	ATA	TAT	GCT (Ala)	GGT(Gly)	TAT (Tyr)	AAC (Asn)	CAA	ATT (Ile)	GAT (Asp)	GAT (Asp)	GCA (Ala)
MZ545666	ATA	TAT	GCT (Ala)	GGT(Gly)	TAT (Tyr)	AAC (Asn)	CAA	ATT (Ile)	GAT (Asp)	GAT (Asp)	GCA (Ala)
MZ545667	ATA	TAT	GCT (Ala)	GGT(Gly)	TAT (Tyr)	AAC (Asn)	CAA	ATT (Ile)	GAT (Asp)	GAT (Asp)	GCA (Ala)
MZ545668	ATA	TAT	GCT (Ala)	GGT(Gly)	TAT (Tyr)	AAC (Asn)	CAA	ATT (Ile)	GAT (Asp)	GAT (Asp)	GCA (Ala)
MZ545669	ATA	TAT	GCT (Ala)	GGT(Gly)	TAT (Tyr)	AAC (Asn)	CAA	ATT (Ile)	GAT (Asp)	GAT (Asp)	GCA (Ala)
MZ545670	ATA	TAT	GCT (Ala)	GGT(Gly)	TAT (Tyr)	AAC (Asn)	CAG	ATT (Ile)	GAT (Asp)	GAT (Asp)	GCA (Ala)
MZ545671	ATA	TTT	TCT (Ser)	GCT (Ala)	GAT (Asp)	GAC	AAT (Asn)	TAT (Tyr)	GAT (Asp)	AAT (Asn)	ACA (Thr)

Table 3. Amino acid changes in FPV and CPV variants[a].[8]

Aminoacid residue	80	87	93	101[b]	103	232	297	300	305	323	375	426[c]	555	564	568
Nucleotide position	3024-3026	3045-3047	3063-3065	3087-3089	3093-3095	3480-3482	3675-3677	3684-3686	3699-3701	3753-3755	3909-3911	4062-4064	4449-4451	4476-4478	4488-4490
Aminoacid changes	AAA(Lys) AGA(Arg)	ATG(met) TTG(Leu)	AAA(Lys) AAC(Asn) AAT(Asn)	ATT(Ile) ACT(Thr)	GUA(Val) GCA(Ala)	GTA(Val) ATA(Ile)	TCT(Ser) GCT(Ala)	GCT(Ala) GGT(Gly)	GAT(Asp) TAT(Tyr)	GAC(Asp) AAC(Asn)	AAT(Asn) GAT(Asp)	AAT(Asn) GAT(Asp) GAA(Glu)	GTA(Val) ATA(Ile)	AAT(Asn) AGT(Ser)	GCT(Ala) GGT(Gly)
FPV	Lys	Met	Lys	Ile	Val	Val	Ser	Ala	Asp	Asp	Asp	Asn	Val	Asn	Ala
CPV-2	Arg	Met	Asn	Ile	Ala	Ile	Ser	Ala	Asp	Asn	Asn	Asn	Val	Ser	Gly
CPV-2a	Arg	Leu	Asn	Thr	Ala	Ile	Ser	Gly	Tyr	Asn	Asp	Asn	Ile	Ser	Gly
CPV-2B	Arg	Leu	Asn	Thr	Ala	Ile	Ser	Gly	Tyr	Asn	Asp	Asp	Val	Ser	Gly
New CPV-2a	Arg	Leu	Asn	Thr	Ala	Ile	Ala	Gly	Tyr	Asn	Asp	Asn	Val	Ser	Gly
New CPV-2b	Arg	Leu	Asn	Thr	Ala	Ile	Ala	Gly	Tyr	Asn	Asp	Asp	Val	Ser	Gly
Asp-300 (2a/2b)	Arg	Leu	Asn	Thr	Ala	Ile	Ala	Asp	Tyr	Asn	Asp	Asn(2a) Asp(2b)	Val	Ser	Gly
CPV-2c	Arg	Leu	Asn	Thr	Ala	Ile	Ala	Gly	Tyr	Asn	Asp	Glu	Val	Ser	Gly

[a] Positions are referred to the amino acid and nucleotide sequences of strain CPV-b (accession no. M38245).

[b] Codon affected by SNPs used to design type-specific probes differentiating CPV-2 from CPV-2a/2b/2c.

[c] Codon affected by SNPs used to design type-specific probes differentiating CPV-2a from CPV-2b and CPV-2b from CPV-2c.

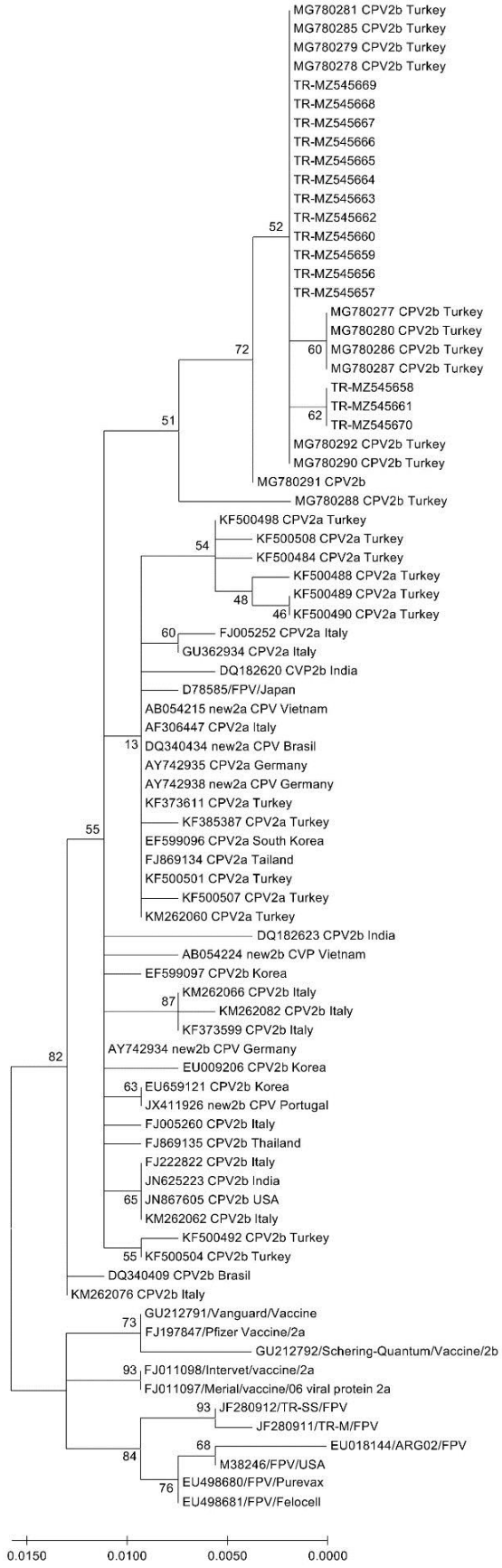

Fig. 2. Phylogenetic tree of the 15 canine parvovirus (CPV2) strains in our study based on 615-bp-long portion of the CPV2 sequence. MEGA-X 11.0.8 was used to construct a Maximum-Likelihood tree and the reliability of the tree was assessed by 1,000 bootstrap replications. The sequences were from our isolates (MZ545656, MZ545657, MZ545658, MZ545659, MZ545660, MZ545661, MZ545662, MZ545663, MZ545664, MZ545665, MZ545666, MZ545667, MZ545668, MZ545669, and MZ545670), reference sequences from Türkiye (KF373611, KF385387, KF500484, KF500488, KF500489, KF500490, KF500498, KF500501, KF500504, KF500506, KF500507, KF500508, KM262060, KM267070, MG780278, MG780279, MG780280, MG780281, MG780283, MG780285, KF500492, MG780275, MG780286, MG780287, MG780290, MG780291, MG780292, MG780277, and MG780288) and reference sequences from various other parts of the world (DQ182623, AY742934, AB054224, KF373599, KM262066, KM262082, EU659121, JX411926, FJ005260, EF599097, FJ869135, EU009206, FJ222822, JN625223, JN867605, KM262062, AF306447, FJ869134, FJ005252, GU362934, EF599096, AY742935, DQ340434, AB054215, AY742938, DQ340409, KM262076, M38246, D78585, JF280912, JF280911, EU018144, GU212791, FJ197847, GU212792, FJ011098, FJ011097, EU498680, and EU498681).

the canine transferrin receptor, affecting the circulation of the virus among different hosts.[30-33] In this study, as in previous studies from China, India, Korea, Japan, and Türkiye, a common mutation in residue Y324I was also detected, and this residue may play a role in CPV host range.[26,34-36] As also reported by Mittal *et al.*, these mutations may lead to the emergence of new CPV-2b variants, and reduce the efficacy of vaccines used in west Mediterranean provinces.[37] Due to the continuing evolution of CPV- 2, RFLP is needed for the detection of shorter mutations on the capsid protein of CPV-2 in samples isolated recently.[21,27,38,39] To detect the antigenic differences in vaccine strain isolates, the three isolates obtained from vaccinated dogs in this study will be subjected to RFLP analysis in future studies. The presence of parvovirus infection in vaccinated dogs has been reported in many of previous studies.[24,25,37,40-43] The main cause of vaccination failure has been demonstrated to be due to the interfering role of maternal antibodies, especially in puppies.[44] However, due to the ongoing evolution of the virus and the detection of the new variants currently circulating in the canine population all around the world, the efficacy of vaccines used against to CPV infection is questioned,.[11,45,46] It has also been found that the pre-exposure to CPV-2 prior to vaccination could be a factor contributing to the occurrence of parvovirus infection in puppies.[24] In the present study, CPV infection was detected in three vaccinated puppies of one to four months of age, with the new 2b variant with synonymous mutations (Table 1). The strains of canine parvovirus obtained from dogs in present study constituted a completely different branch in the phylogenetic tree than the vaccine strains (Fig. 2). Additional studies are needed to completely understand the antigenic differences between vaccine and field strains.

Different strategies have been proposed to overcome vaccination failure caused by maternal derived antibody interference such as high-titer vaccines[47] and intranasal vaccination.[48] It is also necessary to conduct further vaccine development studies on this topic.

In the present study, sequence comparison showed 100% nucleotide identities among our CVP positive isolates, which had V232I, T267A, S297A, A300G, D305Y, D323N, Y324I, N375D, N426D, and Y440A amino acid mutations. These results indicated that a new CPV-2b variant is prevalent in the west Mediterranean region of Türkiye. Three of the isolates had previously unreported synonymous mutations, resulting in subgroup formation in a phylogenetic tree for CPV-2b, constructed using data from our study and reference strains selected from Türkiye. These results suggest that further studies are needed to understand the possible effects of these specific mutations on the pathogenicity of CPV-2. In order to prevent CPV-2b outbreaks in the west Mediterranean region of Türkiye, vaccines should be updated in response to the new variants currently circulating in the canine population, and molecular surveillance studies should be performed regularly to monitor the emergence and spread of the new CPV-2 variants.

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Conflict of interest

There is no conflict of interest.

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