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Small Animal Diseases

Molecular characterization of carnivore protoparvovirus strains circulating in cats in Turkey[1]

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ABSTRACT.- Hasırcıoglu S., Aslım H.P., Kale M., Bulut O., Koçlu O. & Orta Y.S. 2023. **Molecular characterization of carnivore protoparvovirus strains circulating in cats in Turkey.** *Pesquisa Veterinária Brasileira 43:e07178, 2023.* Veterinary Faculty, Department of Virology, Burdur Mehmet Akif Ersoy *Üniversitesi*, Antalya Burdur Yolu, 15030 Yakaköy/Burdur Merkez/Burdur, Turkey. E-mail: shasircioglu@mehmetakif.edu.tr

Cats are susceptible to feline panleukopenia virus (FPV) and canine parvovirus type 2 (CPV-2). Therefore, coinfection and superinfection with multiple parvovirus strains may occur, resulting in high heterogeneity and recombination. Considering the importance of cats as a potential source of genetic diversity for parvoviruses, we investigated the frequency of parvovirus infection in cats using their blood and fecal samples and performed molecular characterization of parvovirus strains circulating in cat populations. Accordingly, the fecal and blood samples of 60 cats with gastroenteritis symptoms were collected from Turkey's Burdur, Isparta, and Izmit provinces. Of these 15 fecal samples tested as parvovirus-positive by PCR, 14 were confirmed to have been infected with true FPV strains by sequencing analysis. Through the phylogeny analysis, those were located in the FPV cluster, closely related to CPV-2, and one was discriminated in the CPV-2b cluster. Additionally, sequence analysis of the VP2 gene of CPV and FPV revealed that the FPV strains detected in Turkey and the vaccine strains were highly related to each other, with a nucleotide identity of 97.7-100%. Furthermore, 13 variable positions were detected in VP2 of the field and reference FPV strains. Three synonymous mutations were determined in the VP2 gene. Some amino acid mutations in the VP2 protein-affected sites were considered responsible for the virus's biological and antigenic properties. The partial sequence analysis of the VP2 gene revealed that four FPV strains detected in Turkey have a single nucleotide change from T to G at the amino acid position 384 between the nucleotides 3939-3941, which was reported for the first time. Therefore, these four isolates formed a different branch in the phylogenetic tree. The results suggest that both FPV and CPV-2b strains are circulating in domestic cats in Turkey and cats should be considered as potential sources of new parvovirus variants for cats, dogs and other animals.

INDEX TERMS: FPV, CPV, molecular characterization, carnivore protoparvovirus, cats, Turkey.

RESUMO.- [Caracterização molecular de cepas de protoparvovírus carnívoros circulantes em gatos na Turquia.] Os gatos são suscetíveis ao vírus da panleucopenia felina (FPV) e ao parvovírus canino tipo 2 (CPV-2). Portanto, coinfecção e superinfecção com múltiplas cepas de parvovírus podem ocorrer, resultando em alta heterogeneidade e recombinação. Considerando a importância dos gatos como uma fonte potencial de diversidade genética para parvovírus, investigamos a frequência da infecção por parvovírus em gatos usando suas amostras de sangue e fezes e realizamos a caracterização molecular de cepas de parvovírus circulantes nas populações de gatos. Amostras fecais e de sangue de 60 gatos com sinais de gastroenterite foram coletadas nas províncias de Burdur, Isparta e Izmit, na Turquia. Destas, 15 amostras fecais testaram positivas para parvovírus por PCR e 14 foram confirmadas como infectadas com cepas verdadeiras de FPV por análise de sequenciamento. Através da análise filogenética, aqueles foram localizados no agrupamento

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FPV que está intimamente relacionado com o CPV-2, e um foi discriminado no agrupamento CPV-2b. Além disso, a análise da sequência do gene VP2 de CPV e FPV revelou que as cepas de FPV detectadas na Turquia e as cepas vacinais eram altamente relacionadas entre si, com uma identidade de nucleotídeos de 97,7-100%. Além disso, 13 posições variáveis foram detectadas em VP2 das cepas de campo e FPV de referência. Três mutações sinônimas foram determinadas no gene VP2. Algumas mutações de aminoácidos nos locais afetados pela proteína VP2 foram consideradas responsáveis pelas propriedades biológicas e antigênicas do vírus. A análise da sequência parcial do gene VP2 revelou que quatro cepas de FPV detectadas na Turquia têm uma única mudança de nucleotídeo de T para G na posição do aminoácido 384 entre os nucleotídeos 3939-3941, o que foi relatado pela primeira vez. Portanto, esses quatro isolados formaram um ramo diferente na árvore filogenética. Os resultados sugerem que ambas as cepas FPV e CPV-2b estão circulando em gatos domésticos na Turquia e os gatos devem ser considerados como fontes potenciais de novas variantes de parvovírus para gatos, cães e outros animais.

TERMOS DE INDEXAÇÃO: Vírus da panleucopenia felina, parvovírus canino, caracterização molecular, gatos, felinos, Turquia.

INTRODUCTION

Cats are among the most common pets and play an important role in providing emotional support to humans. According to estimates, 80-400 million domestic cats are cared for by humans worldwide (Ng et al. 2014). Consequently, people pay significant attention to cat health. Viral diarrhea is common among cats, especially in kittens, and poses a significant threat to their health. Studies conducted in recent years have detected different viruses associated with diarrhea in cats, such as feline astrovirus (FeAstV) (Soma et al. 2020), feline bocavirus (FBoV) (Wang et al. 2020), and feline kobuvirus (FeKoV) (Niu et al. 2019). However, the feline panleukopenia virus (FPV) is the major viral agent that causes diarrhea in cats (Stuetzer & Hartmann 2014).

FPV belongs to the Parvoviridae family (genus *Protoparvovirus*, species Carnivore Protoparvovirus 1) and is a highly contagious viral agent that affects domestic and wild cats (Diakoudi et al. 2019). Its genome is 4.5-5.5kb in length and consists of single-stranded DNA containing two main open reading frames. ORF1 encodes two structural proteins, VP1 (80-85kDa) and VP2 (60-64kDa), and ORF2 encodes two nonstructural proteins (NS1 and NS2). Another protein, VP3, is formed by the cleavage of 15-20 amino acids from the N-terminus of VP2.

FPV is transmitted through the fecal and oral route and primarily infects young kittens aged 3–6 months (Mosallanejad et al. 2014). This oronasal virus targets rapidly dividing cells, such as those in the epithelial crypt, lymphoid tissue, and bone marrow of the small intestine (Awad et al. 2018a). FPV is responsible for causing severe enteric and immunosuppressive diseases characterized by depression, fever, anorexia, acute diarrhea, leukopenia, and hemorrhagic enteritis (Squires 2003). FPV is commonly observed among cats worldwide, causing mortality rates as high as 25-90%, depending on factors such as age, breed, vaccination status, and housing conditions. Moreover, infections with canine parvovirus types-2a, 2b, and 2c (CPV-2a, -2b, and -2c), which belong to the same family as FPV, have been described in domestic cats in several countries (Squires 2003). Although there are slight genetic and amino acid differences between FPV and CPVs, there are differences in important antigens of VP2, which is the major capsid protein of viruses (Hafenstein et al. 2009). Amino acid changes in the capsid protein of CPVs are important molecular determinants in the host range. Although the VP2 capsid protein contains the major antigenic determinant, it plays an important role in detecting viral pathogenicity. Furthermore, the VP2 protein can self-assemble virus-like particles to achieve immune competence. Hence, it serves as a candidate antigen for designing new-generation vaccines. Some point mutations in the VP2 gene region induce changes in the antigenic structure of the virus. Residues 297, 300, 305, and 323 of VP2 effectively determine the cat-dog host (Truyen 2006). Moreover, 426 amino acid residues are the characteristics of the antigenic segregation of the three variants 426Asn-2a, 426Asp-2b, and 426Glu-2c (Decaro et al. 2005).

Early and rapid diagnosis is crucial for disease management and prevents the spread of FPV. Conventional tests like ELISA, hemagglutination, and immunochromatography (IC), widely applied in diagnosing FPV, possess low sensitivity and specificity (Liu et al. 2020). With the advancement of molecular technology, conventional and real-time PCR (Awad et al. 2018b, Sun et al. 2018, Temuujin et al. 2019) has been applied to detect CPV-2 with varying specificity and sensitivity. Treatments for the disease should control secondary bacterial infections, recovery dehydration, and electrolyte balance (Awad et al. 2018a). There are commercially available modified live and inactivated vaccines for FPV (Truyen et al. 2009). Cats that have not been vaccinated or received a booster dose of FPV vaccines belong to the high-risk group (Esfandiari & Klingeborn 2000).

Molecular epidemiological studies related to the emergence of new parvovirus variants are important in monitoring its global spread and vaccine development studies (Jakel et al. 2012, Truyen & Parrish 2013). This study was conducted to determine the molecular characterization of FPV and CPV infections through partial sequence analyses of VP2 among domestic cats in Turkey.

MATERIALS AND METHODS

Ethical approval. All procedures and animal care complied with the guidelines of the Burdur Mehmet Akif Ersoy Üniversitesi Veterinary Faculty Ethics Committee (Ethical approval number 877).

DNA extraction and PCR. From December 2021 to January 2022, fecal and blood samples were collected from 60 cats with clinical symptoms of gastroenteritis. Each cat was brought to the animal hospital and veterinary clinics in Burdur and Isparta provinces of the Western Mediterranean region of Turkey and Izmit province in the Marmara region. The collected samples were stored in a deep freezer at -80°C until DNA extraction.

The fecal samples were mixed, crushed in a ratio of 1:10 in 10 × antibiotic PBS, and then centrifuged at 3000rpm for 20 min.

The blood samples were collected from 60 animals in EDTA tubes and centrifuged, and the buffy coat was separated and stored at -20°C until analysis. According to the manufacturer's instructions, viral DNA was extracted from the fecal and blood samples using a virus Nucleic Acid Isolation Kit (GeneDireX, Taoyuan, Taiwan). The resulting DNA extraction products were stored at -20°C until subsequent PCR tests.

Hfor/Hrev primer pairs were used to amplify sequences between 3556 and 4166 nucleotides, including discriminative sequential patterns of the capsid protein genes (Buonavoglia et al. 2001). For 630-bp fragment amplification PCR, 5µl Mg-free Taq DNA polymerase buffer, 2.00µL of $MgCl_2$ (25mM), 7µl deoxynucleotide triphosphate (10×) (2mM each), 10pmol of each primer Hfor CAGGTGATGAATTTGCTACA and HrevCATTTGGATAAACTGGTGGT (Sentebiolab, Ankara, Turkey) and 1.25-U Taq DNA polymerase (Thermo Fisher Scientific, Waltham/MA, USA) enzyme were used. PCR was performed as reported by Buonavoglia et al. (2001) with the following steps: predenaturation at 95°C for 5 min once, 1 min at 95°C for denaturation, annealing for 1 min at 55°C for each primer pair, extension at 72°C for 1 min, this cycling method was repeated 35 times and a final extension at 72°C for 10 min. The PCR products were separated by electrophoresis on a 1% agarose gel. Gel images were photographed on a gel documentation system (DNR Bio-Imaging Systems, Modi'in-Maccabim-Re'ut, Israel). Of the total 60 fecal and 60 blood samples assessed, only 15 were positive fecal samples, and the PCR results were further sequenced.

Purification of PCR products, DNA sequencing reactions, and phylogenetic analysis. A total of 15 PCR products (tested in duplicate), four from Burdur, one from Isparta, and 10 from Izmit, were purified using Exonuclease I (20U/µL) (Thermo Fisher Scientific) and Shrimp Alkaline Phosphatase (Thermo Fisher Scientific). The BigDye™ Terminator v3.1 Loop Sequencing Kits (Thermo Fisher Scientific) were used for DNA sequencing reactions. Finally, the PCR products were analyzed using the ABI 3500 Genetic Analyzer (Applied Biosystems, Carlsbad/CA, USA). The newly obtained sequences were edited using BioEdit Version 7.2.5 (12/11/2013). Consensus sequences were generated and searched in GenBank using the BLAST program to identify reference sequences included in the phylogenetic analysis. The selected references' nucleotide and amino acid sequences were downloaded from GenBank in the FASTA format and aligned separately using CLUSTAL-W/BioEdit Version 7.2.5 (12/11/2013) (Thompson et al. 1994). The amino acid sequences of our strains were obtained from ExPASy Translate. The tool[4] was used to investigate the effects of mutations resulting from amino acid changes. Finally, a phylogenetic tree was constructed using the maximum likelihood method with 1000 bootstrap replications in MEGA-X version 11.0.8. (Rossi 2018, Tamura et al. 2021).

To evaluate the selection pressure driving FPV evolution, we estimated the ratio of synonymous substitutions per nonsynonymous site (dS) to nonsynonymous substitutions per synonymous site (dN) using the Datamonkey Web Interface[5] – a maximum-likelihood-based tool was used for the identification of sites prone to positive or negative selection.

RESULTS

The primer pairs Hfor/Hrev were used to amplify part of the VP2 sequences, including discriminative amino acid residues for FPV and CPV isolates, as shown in bold in Table 1.

All 60 blood samples were negative for FPV, and 15 fecal samples (25%) were positive based on PCR analysis. Moreover, two of these animals were vaccinated (Table 2). Based on the CLUSTAL-W and ExPASy Translate Tool analysis, 14 strains were identified as FPV, and one was identified as CPV-2b. The CPV sequences obtained from this study (OM502014 CPV) and from the same region of Turkey were highly related to each other with a nucleotide identity of 100%, FPV were highly related to each other with a nucleotide identity of 99.5-100%, while the rate of 99-100% compared with vaccine strains Purevax (EU498680) and Felocell (EU498681) and 97.7-99% compared with FPV sequences obtained from the GenBank database. This result was confirmed by a distinctive amino acid substitution table described by Decaro & Buonavoglia (2012). Then, the amino acid changes and NCBI accession numbers for the isolates detected in this study were presented in Table 1. A synonymous mutation due to a single nucleotide change between the nucleotides 3657 and 3959, from C to T (CTA to TTA) at position 291, both of which encode Leu, was reported for our isolates and worldwide (Table 1).

Table 1. Amino acid changes in 15 isolates of this study

Amino acid positions	267	291	297	300	305	323	324	375	384	389	426	440	445	Genotype
Amino acid changes	F-Y	L-L	S-A	A-G	D-Y	D-N	Y-I	N-D	G-G	T-T	N-D/E	T-A	P-L	FPV
MZ545671	F	L	S	A	D	D	Y	D	G	T	N	T	P	FPV
OM502011	F	L	S	A	D	D	Y	D	G	T	N	T	P	FPV
OM502012	F	L	S	A	D	D	Y	D	G	T	N	T	P	FPV
OM502013	F	L	S	A	D	D	Y	D	G	T	N	T	P	FPV
OM502014	Y	L	A	G	Y	N	I	D	G	T	D	A	L	CPV-2b
OM502015	F	L	S	A	D	D	Y	D	G	T	N	T	P	FPV
OM502016	F	L	S	A	D	D	Y	D	G	T	N	T	P	FPV
OM502017	F	L	S	A	D	D	Y	D	G	T	N	T	P	FPV
OM502018	F	L	S	A	D	D	Y	D	G	T	N	T	P	FPV
OM502019	F	L	S	A	D	D	Y	D	G	T	N	T	P	FPV
OM502020	F	L	S	A	D	D	Y	D	G	T	N	T	P	FPV
OM502021	F	L	S	A	D	D	Y	D	G	T	N	T	P	FPV
OM502022	F	L	S	A	D	D	Y	D	G	T	N	T	P	FPV
OM502023	F	L	S	A	D	D	Y	D	G	T	N	T	P	FPV
OM502024	F	L	S	A	D	D	Y	D	G	T	N	T	P	FPV

[4] Available at <https://web.expasy.org/translate/> Accessed on Jan., 2022.

[5] Available at <http://www.datamonkey.org> Accessed on Jan., 2022.

A synonymous mutation due to a single nucleotide change from T to G at the amino acid position 384 between the nucleotides 3939 and 3941 that changes the codon GGT to GGG, both of which encode glycine, was reported for the first time and was shown in Figure 1.

A synonymous mutation due to a single nucleotide change from C to T at the amino acid position 389 between the nucleotides 3951 and 3953 changes the codon ACC to ACT, both of which encode Thr, which can be seen in Figure 1.

Finally, a phylogenetic tree was generated with 15 isolates from this study and sequences obtained from the GenBank database using MEGA-X version 11.0.8 by applying the maximum likelihood method with 1000 bootstrap replications were presented in Figure 2. Two clusters were formed by partial VP2 nucleotide sequences (630 bp) analysis: the CPV and FPV branches. The CPV strain with the accession number OM502014 obtained in this study formed a group along with the other CPV Turkey strains (Fig.2). FPV isolates in this study formed two clusters:

Table 2. Identification of 15 samples sequenced

Sample No.	ID No.	Age (month)	Sex	Vaccination status	Genotype
1	OM502011	1.5	F	-	FPV
2	OM502012	2	F	-	FPV
3	OM502013	6	F	-	FPV
4	OM502024	1	M	+	FPV
5	MZ545671	1.5	F	+	FPV
6	OM502015	3	M	-	FPV
7	OM502019	3.5	F	-	FPV
8	OM502017	1	M	-	FPV
9	OM502018	2	M	-	FPV
10	OM502020	2	F	-	FPV
11	OM502016	3.5	M	-	FPV
12	OM502021	2.5	F	-	FPV
13	OM502022	4	F	-	FPV
14	OM502023	1	M	-	FPV
15	OM502014	3	F	-	CPV-2b

a small cluster consisting of the nine isolates and Purevax and Felocell due to synonymous mutation located in amino acid position 291 and large cluster formed five FPV strains in this study and nine strains from Italy, five strains from China, one strain from Portugal, one strain from Belgium and two strains from Argentina and two strains from Turkey. The four FPV isolates (OM502015, OM502017, OM502018, OM502020) in a large cluster formed a separate sub-cluster due to a single nucleotide mutation at position 384 (Fig.1).

DISCUSSION AND CONCLUSION

The probable diagnosis of parvovirus infection is generally based on clinical manifestations, and an IC test is performed for confirmation in veterinary clinics. However, the IC test can yield false-negative results (Desario et al. 2005). The virus isolation can be used for diagnostic purposes. However, it requires enormous effort, time, and specialized personnel, and additional tests such as immunofluorescence and hemagglutination must be performed to detect the viral antigens (Raheena et al. 2017). Due to its high sensitivity and specificity, the PCR technique is widely applied to diagnose several diseases (Chowdhury et al. 2021).

There have been limited studies conducted on the molecular characterization of parvovirus in cats in the last 20 years in Turkey. This is the third parvovirus molecular characterization study published on domestic cats from Turkey. In this study, we investigated the presence of parvovirus DNA by PCR by targeting the VP2 region in the fecal and blood samples of 60 cats with gastroenteritis symptoms during 2021-2022. Only 15 fecal samples (25%) were positive for parvovirus. The partial VP2 gene of the parvovirus samples was sequenced. Of them, one was identified as CPV-2b, and 14 as FPV. These findings show both CPV-2b and FPV circulating in cats in Turkey. Previous studies from Turkey have reported that CPV-2a/2c and FPV were circulating in domestic cats (Muz et al. 2012, Aydin & Timurkan 2018). The CPV-2b variant has a lower prevalence than CPV-2a, and CPV-2c in Europe (Battilani et al. 2019), an increased prevalence of CPV-2b was also reported in another study carried out in dogs from the same region of

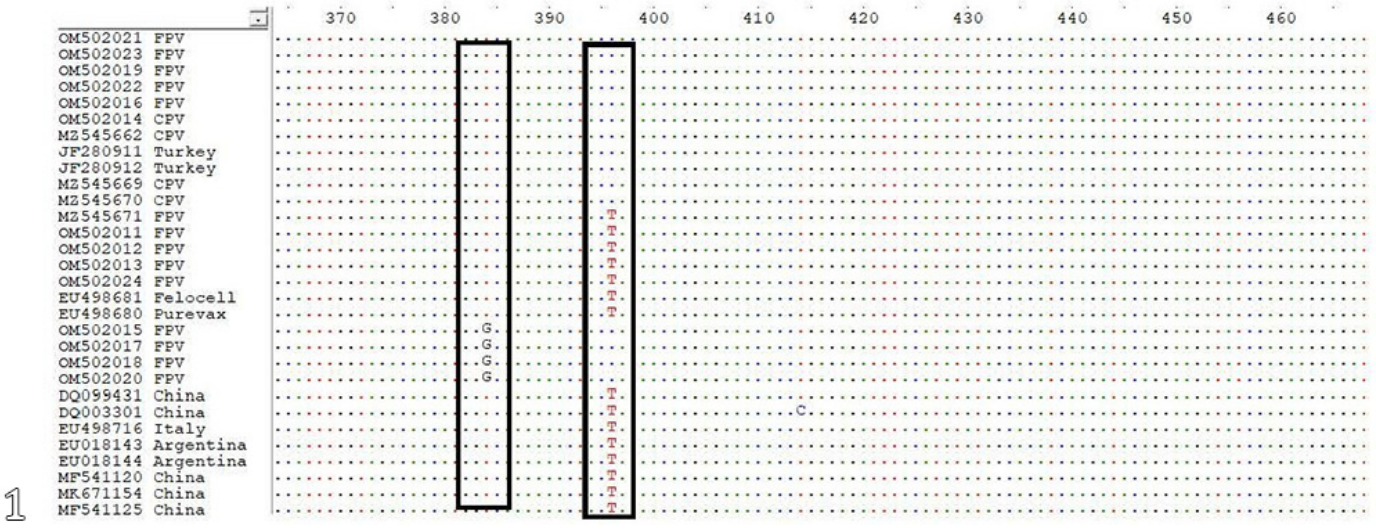

Fig.1. Synonymous mutation determined in positions 384 and 389 obtained in this study and from worldwide.

Turkey (Hasircioğlu 2023). This finding indicates that CPV-2b is evolving rapidly. Therefore, all CPV-2 antigenic variants and FPV should be regularly monitored using molecular surveillance to prevent new CPV-2 variant-induced outbreaks in the cat and dog populations in Turkey. In addition, coinfection of CPV subtypes and FPV has been detected in domestic and wild cats (Wang et al. 2017, Balboni et al. 2018). This mixed infection increases the chance of genetic mutation and recombination of CPV and FPV. It suggests that cats act as carriers of different parvoviruses and as a source of new variants of parvovirus that infect cats, dogs and other animals.

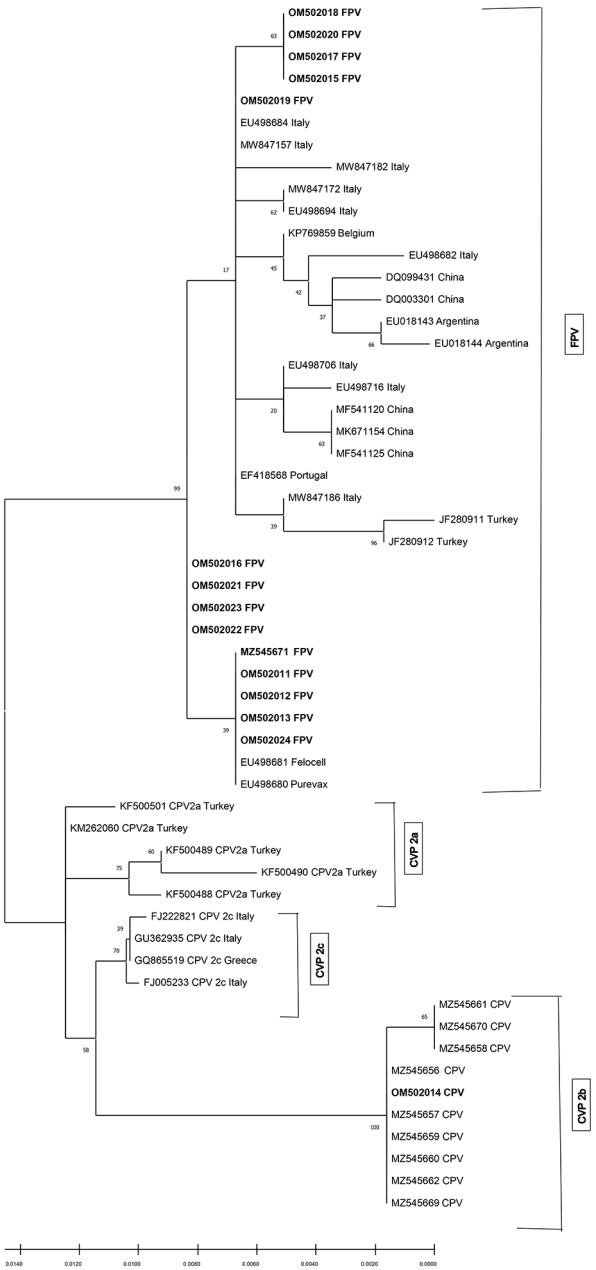

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Fig.2. The phylogenetic tree based on the partial VP2 gene was constructed using the maximum likelihood method (Mega 11.0.8.) and a bootstrap analysis with 1000 replicates. The phylogenetic relationship between 15 Turkish sequences from this study is shown in bold characters, and CPV-2 and FPLV reference sequences were obtained from the GenBank database.

CPV-2a/2b/2c can infect cats with a clinical picture that cannot be distinguished from FPV infection (Miranda et al. 2014, Mukhopadhyay et al. 2017). Although CPV variants demonstrated a high degree of mutations in their nucleotide sequences, the sequences of FPV strains remained relatively genetically stable (Battilani et al. 2019, Wen et al. 2021). Point mutations occurring in the genetic structure can develop antigenic differences between variants and target host differentiation (Hoelzer & Parrish 2010). The amino acid changes of 15 isolates in this study were examined at critical positions 267, 297, 300, 305, 323, 324, 375, 426, 440, and 445 to differentiate between FPV and CPV variants as reported in previous studies (Parrish 1991, Decaro et al. 2008, Takahisa & Mochizuki 2008). Synonymous mutations reported before due to a single nucleotide change were detected at the amino acid positions 291, 384, and 389, which supports the fact that the FPV genome is stable. However, according to variations detected in 297, 300, 305, and 323 amino acid residues of the VP2 protein are of key importance in determining the host range (Martella et al. 2005), and 324, 426, 440, and as for 445 amino acid residues, one of the isolates was CPV-2b according to these amino acid residues (Table 1). The results of previous parvovirus investigations in cats showed that more than 95% of cases were caused by FPV (Muz et al. 2012, Aydin & Timurkan 2018, Niu et al. 2018). Similarly, the findings in this study suggest that FPV strains cause the majority of parvovirus outbreaks in cats in Turkey.

Balboni et al. (2018) detected FPV in the blood of clinically healthy cats. In the present study, FPV was detected only in fecal samples and not in blood samples of cats with symptoms of gastroenteritis. This difference can be attributed to the fact that this disease does not exhibit many clinical signs during the viremia period, and manifestations occur only after the virus has been transferred to the tissues; hence, the infection is noticed late by the patient's owners. Previous studies have demonstrated that parvovirus in cats' blood can be diagnosed without clinical signs or even if the virus is not detected in the feces (Schunck et al. 1995, Muz et al. 2012). Moreover, as the virus is transmitted through the fecal–oral route (Koç et al. 2018), a high titer of the virus is shed in the feces a few days after the infection is transmitted to the susceptible host.

Similar to the reports of Dall'Ara et al. (2019) and Islam et al. (2010), the frequency of FPV infection was higher in female cats (60%) than in male cats (40%). Although female animals may have a more effective immune response due to their gonadotropic hormones, testosterone may exert an immunosuppressive effect in male animals (Klein 2000, Bilbo & Nelson 2001). Furthermore, male cats are more likely to be FIV-positive due to their fighting and biting behavior and are, therefore, less immunoreactive (Dall'Ara et al. 2019).

Jakel et al. (2012) reported that two animals, aged one and 1.5 months, respectively, exhibited similar behavior to FPV infection despite being vaccinated. They observed that 36.7% of kittens (aged 8-12 weeks) showed no seroconversion despite administering the third vaccine dose. They argued that even extremely low maternal antibody titers could inhibit the development of active immunity. Bergmann et al. (2018) reported that only 47.3% (53/112) of cats responded adequately

to vaccination with FPV. Nakamura et al. (2001) reported low cross-protection against CPV strains in cats experimentally infected with FPV or vaccinated with an inactivated FPV vaccine. In the present study, CPV infection was detected in two vaccinated kittens aged 1-1.5 months (Table 2). Therefore, we concluded that, despite being vaccinated, the animals were infected with FPV, suggesting that they did not complete the vaccination program, were exposed to maternal antibody intervention, or faced a problem such as an application error or the vaccine did not work. Furthermore, the FPV subgroup in the vaccine may not have provided adequate protection against the subgroup present in the region. Vaccine-related infections may develop after administering FPV vaccines (Freisl et al. 2017, Bergmann et al. 2019). It supports this possibility that two FPV strains (OM502024 and MZ545671) isolated from vaccinated kittens in this study and FPV vaccine strains (Felocell, Purevax) were placed into the same branch, the highest similarity (100 %) was found between these two isolates and the vaccine strain and these kittens have been vaccinated with a modified live FPV vaccine recently (Fig.2). Hence, further studies on the FPV vaccine may be necessary.

A different substitution (SAA) at position 297 (due to the first-base change T889G) was recently described for the antigenic variants of CPV-2 currently circulating throughout the world (Nakamura et al. 2004, Wang et al. 2005, Meers et al. 2007). Residue 297 is located in the antigenic region close to epitope B, and substitutions at this position may be responsible for changes in the antigenicity of CPV-2 variants (Truyen 2006). Analogously, studies have described a change at position 440 (TAA) for Italian, Indian, Korean, and American CPV-2 isolates (Battilani et al. 2002, Chinchkar et al. 2006, Kapil et al. 2007, Kang et al. 2008) as well as for a clone from a mixed CPV-2 population detected in a domestic cat from Italy (Battilani et al. 2006). Both residues 297 and 440 are present in the GH loop region of the VP2 protein, where the greatest variability among parvoviruses has been observed (Battilani et al. 2002). The results of our research also support these findings. An isolate (OM502014) was identified as CPV2 because of the amino acid differences detected in these residues.

To our knowledge, this is the first study to confirm the presence of CPV-2b in cats in Turkey and to determine the incidence of FPV infection in cats with gastroenteritis diarrhea. These data will facilitate the diagnosis and management of FPV and CPV and enhance our understanding of the molecular and genetic evolution of FPV in Turkey. These results indicate that both FPV and CPV-2b are circulating in domestic cats in Turkey and suggest that cats may be carriers of different parvoviruses and new variants of parvovirus that can infect cats, dogs, and other animals. However, FPV strains (OM502015, OM502017, OM502018, OM502019, OM5020) isolated in Turkey and FPV vaccine strains were placed into different branches in this study. Therefore, available vaccines may provide inadequate protection against these FPV field strains. According to the investigation results, additional studies are required to fully understand the protection potential of vaccines containing only FPV strains used to provide protection against infections induced by these FPV and CPV field strains obtained from domestic cats in Turkey.

Conflict of interest statement.- The authors declare that they have no competing interests.

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