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RESEARCH ARTICLE

Detection and Molecular Characterization of Canine and Feline Bocaparvoviruses in Türkiye

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A B S T R A C T

The genus Bocaparvovirus (BoV) in the subfamily *Parvovirinae* shows a wide host spectrum and causes infection with different clinical findings in various animals. In recent years, canine and feline Bocaparvovirus infections, which are novel parvovirus species, have been reported in different geographical regions. The aim of this study is to investigate the presence of canine and feline Bocaparvovirus genotypes in dog and cat fecal samples and to perform their molecular characterization. The presence of canine and feline bocaparvovirus nucleic acid was investigated in stool samples taken from 74 dogs and cats. After PCR tests, it was found that five out of 46 dogs were positive for CBoV-1 and five out of 46 dogs were positive for CBoV-2, while two out of 28 cats were positive for FBoV-2. The sequence comparison of the capsid protein gene (VP2) of the five CBoV-1 identified in this study shared a high identity with each other (96.4-99.2% nt and 94.6–99.2% aa identities) and with previously reported CBoV-1 strains (93.4-98.9% nt and 93.9–100% aa identities). It was determined that five CBoV-1 strains were located in Lineage 2 in the phylogenetic map. The nucleotide and amino acid comparison showed that the non-structural protein (NS1) gene sequences of the five CBoV-2 strains identified in this study shared a high identity with each other (93.5-97.0% nt and 93.6–100% aa identities) and with previously reported CBoV-2s (92.2–98.6 % nt and 92.8–100% aa identities). The phylogenetic map showed three strains (OR690113, OR690112, OR690117) in Lineage 1 and two strains (OR690114, OR690115) in Lineage 2. The sequence comparison of the nuclear phosphoprotein-1 (NP1) gene sequences of the two FeBoV strains identified in this study shared identity with each other (100% nt and aa identities) and with previously reported FeBoV strains (63.8-99.5% nt and 59.4–100% aa identities). As a result of the sequence analysis, it was determined that the two FBoV-2 strains identified in the study were included in Lineage 1. Findings of the present study compile current genetic data and contribute to the molecular epidemiology of these infections. It is recommended to evaluate Bocaparvoviruses, use molecular methods and conduct further studies on the diagnosis of infections in dogs and cats.

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INTRODUCTION

Viruses in the family *Parvoviridae* have a wide host spectrum and are classified into three sub-families: *Parvovirinae* in vertebrates, *Densovirinae* in insects and *Hamaparvovirinae* in vertebrates and insects (Cotmore *et al.*, 2019; Pénzes *et al.*, 2020; Karapınar *et al.,* 2023). Parvoviruses are small ss DNA (approximately 25 nm in diameter), non-enveloped, icosahedral symmetrical in structure, and have a negative polarity. While other Parvovirus genera contain two Open Reading Frame (ORF) regions, the Bocaparvovirus genome contains three ORF regions (an additional one between NS1 and VP1). The ss DNA genome coding region has hairpin-like foldable terminal repeats that may be different (heterotelomeric) or identical (homotelomeric) (Cotmore *et al.*, 2019). ORF1 encodes the nonstructural protein (NS1) involved in the replication of viral genome. ORF2 encodes two capsid proteins (VP1 and VP2) in the capsid structure. ORF3 in the Bocaparvovirus genome encodes a highly phosphorylated non-structural protein (NP1). The capsid structure is very durable and is resistant to environmental

conditions, high heat, alkaline or acidic pH values, and disinfectants (Pénzes *et al.*, 2020; Capozza *et al.*, 2023).

Bocaparvoviruses, which infect many different species (such as canine, porcine, human, bovine) are divided into 2 genotypes (Canine Minute Virus CBoV-1 and CBoV-2) in dogs and 3 genotypes (FBoV 1-3) in cats (Cotmore *et al.*, 2019; Pénzes *et al.*, 2020). In addition to systemic infection, including digestive and respiratory system diseases, Bocaparvoviruses also cause fetal and neonatal deaths as a result of transplacental transmission and their course is usually subclinical in cats and dogs (Kapoor *et al.*, 2012; Lau *et al.*, 2012).

Since CBoV-1 was first detected in 1967, viral genome positivity has been detected as 7.5, 7.5, 6.3, and 1.2% in Chile, China, Canada, and Japan, respectively (Ohshima *et al.*, 2010; Guo *et al.*, 2016; González-Hein *et al.,* 2020; Canuti *et al.*, 2022a). In Türkiye, CBoV-1 was first reported serologically as 18.0% by IF test in dogs with gastroenteritis (Torun *et al.,* 2005). CBoV-2 was first identified in 2012, and some previous studies reported that viral genome positivity was 10.4, 4.1, 9.6, and 22.78% in Canada, China, North Korea, and the USA, respectively (Kapoor *et al.*, 2012; Lau *et al.*, 2012; Choi *et al.*, 2015; Canuti *et al.*, 2022a). In a study investigating canine bocaparvoviruses in Türkiye, it was determined that CBoV-1 genome positivity was 2.6 and 26.09%, and CBoV-2 genome positivity was 3.94 and 34.78 in adults and puppies, respectively (Isidan and Turan, 2021).

FBoV was first detected in Hong Kong in 2012 (Lau *et al.*, 2012). In previous studies, viral genome positivity was determined as 2.5, 6, 8, 9.9, 25.89, and 47.8% in Hong Kong, Portugal, USA, Japan, China, and Australia, respectively (Lau *et al.*, 2012; Ng *et al.*, 2014; Zhang *et al.*, 2014b; Takano *et al.*, 2016; Zhang *et al.*, 2019; Van Brussel *et al.*, 2022). A Turkish study reported that the positivity rate was 8.5% (Abayli and Can-Sahna, 2022).

The aim of this study is to investigate the presence of Bocaparvoviruses in dogs and cats of various ages that are housed together using molecular methods, to reveal the circulating virus genotypes, and to perform their genetic characterization in the light of the current data.

MATERIALS AND METHODS

The material of the study consisted of stool samples collected from 28 cats and 46 dogs showing signs of gastroenteritis in a shelter in the province of Balıkesir located in western Türkiye between February-April 2022. The cats and dogs were crossbreed and were aged between 6 months and 2 years.

Viral DNA extraction, polymerase chain reaction, and sequence analysis: The stool samples were diluted (1/10) with Phosphate Buffer Saline and centrifuged at 3000 rpm for 10 minutes. Then, the supernatant was taken into stock tubes and stored at –20 ºC until it was tested. For the extraction of viral RNA, a commercial viral RNA/DNA isolation kit (Jena Bioscience, Viral RNA+DNA Preparation Kit, Germany) was used. Table 1 lists primers used in PCR tests. PCR conditions applied in the thermal cycler included 95 °C for 2 min for bocaparvovirus carnivoran 1 and 2, 94 °C for 45 sec at 40 cycles following the first denaturation, annealing at 51 °C (CBoV-1) 55 °C (CBoV-2) for 45 sec and 72 °C for 1 min extension stages. The reaction was completed at 72°C with a 10-min final extension. The PCR conditions applied in the thermal cycler were 95°C for 2 min for FBoV strains, followed by 35 cycles of 94 °C for 40 sec, 51°C for 40 sec, 72 °C for 45 sec. The reaction was completed at 72 °C with a 2-min final extension. Amplified PCR products were evaluated using 2% agarose gel.

PCR products identified as positive were subjected to sequence analysis using the Sanger method (BMLabosis, Ankara, Türkiye). Raw data obtained after the sequence were aligned using the Clustal W algorithm and BioEdit version 7.0.5 (Hall, 1999). The obtained sequences were compared with reference strains obtained from GenBank using the BLAST software available in the NCBI database. Phylogenetic map was created with MEGA v11.0 program (Tamura *et al.,* 2021). Maximum likelihood was used for this purpose and bootstrap value was determined as 1000 repetitions.

RESULTS

Of the 46 dog feces, five were positive for CBoV-1 and five were positive for CBoV-2; whereas, two of the 28 cat feces were positive for FBoV. All samples used in the study were tested with all primers. Accordingly, feline Bocavirus types were not detected in dog stool samples and canine Bocavirus types were not detected in cat stool samples. There were no samples in which CBoV and FBoV strains were co-detected. The samples identified as positive were subjected to sequence analysis. GenBank accession numbers of the strains used in the phylogenetic tree were obtained and presented in Table 2.

For CBoV-1, different similarity rates were obtained when the sequences obtained from the partial VP2 gene study were compared with the sequences obtained from GenBank. Nucleotide sequences of the VP2 gene of the five CBoV-1 strains shared high nt identities with each other (96.4-99.2%) and with those of CBoV-1 strains, including MK503186 (96.7–97.9%), MK503189 (97.4–98.7%), MN947834 (97.2-98.9%), and MN947835 (97.4–98.7%). In addition, nt identities were determined as 93.4-98.9% with other strains. Five strains obtained from the study were included in Lineage 2 in the phylogenetic map (Fig. 1). Amino acid changes were determined between the obtained strains and the strains compared from GenBank (Fig. 2). The amino acid sequence comparison in partial VP2 of the five Turkish CBoV-1 strains showed 93.9–100% identities to the other CBoV-1 strains, and 94.6–99.2% identities to each other.

For CBoV-2, different similarity rates were obtained as a result of the phylogenetic analysis run on the partial non-structural protein (NS1) gene sequences obtained from GenBank and the partial sequences obtained from this study (Fig. 3). The phylogenetic map indicated three strains obtained from the study (OR690113, OR690112, OR690117) in Lineage 1 and two strains (OR690114, OR690115) in Lineage 2. Nucleotide sequences of the NS1 gene of the five CBoV-2 strains shared high nt identities with each other (93.5-97.0%) and with those of CBoV-2 strains, including OK546115 (93.5–97%) and other strains (92.2–98.6%). When the amino acid similarity resulting from the sequence analysis with the NS1 gene was

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Table 1: Oligonucleotide primers for the detection and sequencing of the partial VP2, NS1, and NP1 genes of CBoV-1, CBoV-2, and FBoVs used in the study

Primers	Nucleotide sequences (5′–3′)	Target gene	Amplicon Size (bp)	References
CMV4064F	TGTGGGTGGGTCAATAATGA	VP2	500	Isidan and Turan 2021
CMV4563R	TTGTTTGTTCCGTCTTGCAC			
CBoV999F	CCTGACAGAGCAACTCCGTTT	NS1	400	Isidan and Turan 2021
CBoV1398R	TGTGAACCATCTGAAGCAAGGT			
FBoV-F	AGAACCRCCRATCACARTCCACT	NP1	465	Zhang *et al.* 2019
FBoV-R	TGGCRACCGCYAGCATTTCA			

Table 2: Virus Genotype, Strain ID, GenBank accession numbers of Bocaparvoviruses

Virus Genotype	Strain ID	Accession Numbers
	TR/Bal/CanBocaparvo1/cmv1	OR681968
	TR/Bal/CanBocaparvo1/cmv2	OR681969
CBoV-1	TR/Bal/CanBocaparvo1/cmv26	OR681970
	TR/Bal/CanBocaparvo1/cmv29	OR681971
	TR/Bal/CanBocaparvo1/cmv31	OR681972
	TR/Bal/Canine bocaparvovirus 2/cbov8	OR690113
	TR/Bal/ Canine bocaparvovirus 2 /cbov7	OR690112
CBoV-2	TR/Bal/ Canine bocaparvovirus 2 /cbov17	OR690116
	TR/Bal/ Canine bocaparvovirus 2 /cbov10	OR690114
	TR/Bal/ Canine bocaparvovirus 2 /cbov12	OR690115
	TR/Bal/Feline bocaparvo2/fbov50	OR690117
FeBoV-2	TR/Bal/Feline bocaparvo2/fbov51	OR690118

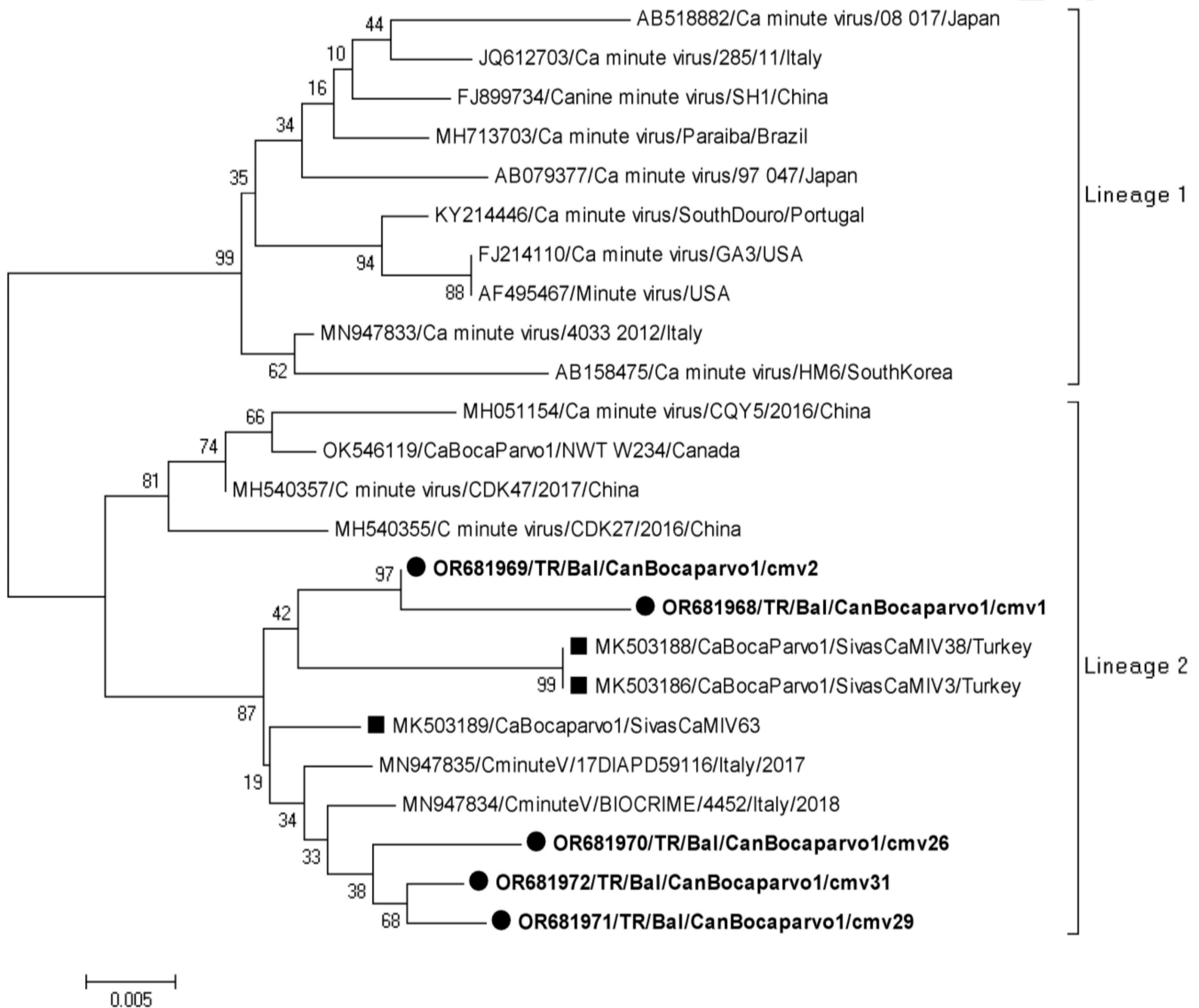

Fig. 1: Phylogenetic tree based on the partial nucleotide sequences of the VP2 gene of CBoV-1. The novel consensus sequences obtained in this study are indicated by a solid black circle

investigated, it was determined that there were changes between the strains obtained and the strains compared from GenBank (Fig. 4). The amino acid sequence comparison in partial NS1 of the five Turkish CBoV-2 strains showed 92.8–100% identities to other CBoV-1 strains, and 93.6–100% identities to each other.

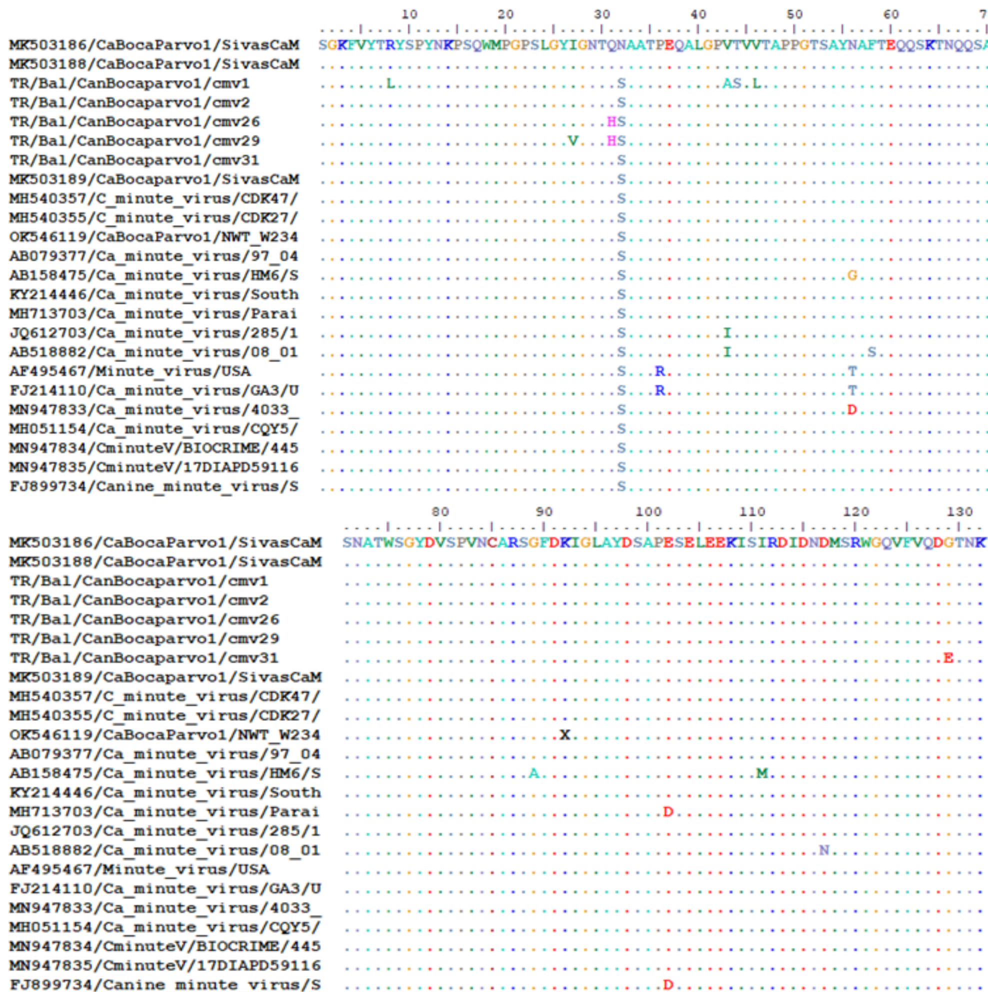

Fig. 2: Non-synonymous amino acid substitutions identified in the partial sequences of the VP2 proteins of CBoV-1 determined in this study. The figure shows the changes between the 1371st aa and the 1502nd aa of the genome.

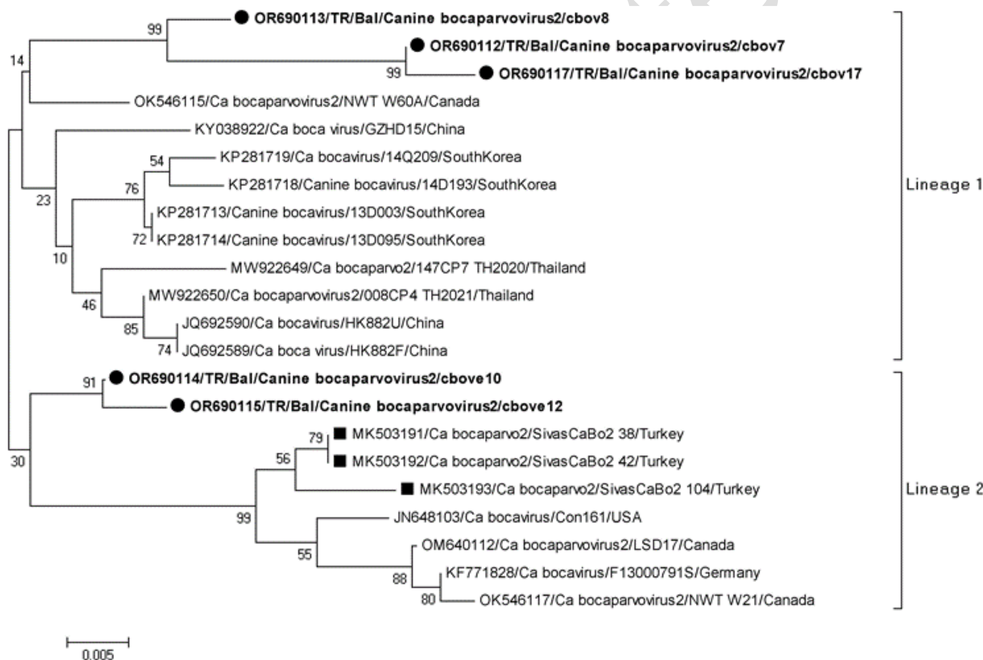

Fig. 3: Phylogenetic tree based on the partial nucleotide sequences of the NS1 gene of CBoV-2. The novel consensus sequences obtained in this study are indicated by a solid black circle

The phylogenetic analysis run on the partial nuclear phosphoprotein-1 (NP1) gene sequences obtained from GenBank for FBoV strains and the partial sequences obtained from this study revealed different similarity rates (Fig. 5). By the nucleotide sequence comparison, NP1 gene of the two FeBoV strains identified in this study shared a high identity with each other (100 %) and with previously reported FBoV strains (63.8-99.5%). As a result of the sequence analysis, it was determined that two FBoV-2 strains obtained from the study were included in Lineage 1. The amino acid sequence comparison in partial NP1 of the two Turkish FBoV-2 strains showed 59.4–100% identities to other FBoV-1 strains, and 100% to each other.

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MK503191/Ca_bocaparvo2/SivasCa   HFACSGKTYAATFVNGKWVLPQVRKQWLNYLRDSVCQKADPVFSGDMFENLPKVPRATWEADVSSNKSKI
MK503192/Ca_bocaparvo2/SivasCa   ......................................................................
MK503193/Ca_bocaparvo2/SivasCa   ....................................H.................................
TR/Bal/Ca_bocaparvo2/cbov7       ...............................P...........................S........T.
TR/Bal/Ca_bocaparvo2/cbov8       ...........................................................S....A.....
TR/Bal/Ca_bocaparvo2/cbove10     ...........................................................S..........
TR/Bal/Ca_bocaparvo2/cbove12     ...........................................................S..........
TR/Bal/Ca_bocaparvo2/cbov17      .....E.........................P...........................S........T.
JQ692589/Ca_boca_virus/HK882F/   ...........................................................S..........
KY038922/Ca_boca_virus/GZHD15/   ...........................................................S..........
KP281714/Canine_bocavirus/13D0   ...........................................................S..........
KP281718/Canine_bocavirus/14D1   ...........................................................S..........
JQ692590/Ca_bocavirus/HK882U/C   ...........................................................S..........
KF771828/Ca_bocavirus/F1300079   .............................................................E........
OK546117/Ca_bocaparvovirus2/NW   .............................................................E........
OM640112/Ca_bocaparvovirus2/LS   ...........................................................A.E........
MW922649/Ca_bocaparvo2/147CP7_   ............Y..............................................S..........
KP281719/Ca_bocavirus/14Q209/S   ...........................................................S..........
JN648103/Ca_bocavirus/Con161/U   ...........................................................SVE........
OK546115/Ca_bocaparvovirus2/NW   ....T......................................................S..........
MW922650/Ca_bocaparvovirus2/00   ...........................................................S..........
KP281713/Canine_bocavirus/13D0   ...........................................................S..........

MK503191/Ca_bocaparvo2/SivasCa   TKKETLMIDCIDRCEKNHLLTYEDLVNECSDLVIMLGSQPGGTKLIETLLQMVHX
MK503192/Ca_bocaparvo2/SivasCa   .......................................................
MK503193/Ca_bocaparvo2/SivasCa   .......................................................
TR/Bal/Ca_bocaparvo2/cbov7       ............S.......A.....R........................S...
TR/Bal/Ca_bocaparvo2/cbov8       ...................................................W...
TR/Bal/Ca_bocaparvo2/cbove10     .......................................................
TR/Bal/Ca_bocaparvo2/cbove12     .......................................................
TR/Bal/Ca_bocaparvo2/cbov17      ............S.......A.....R........................S...
JQ692589/Ca_boca_virus/HK882F/   .......................................................
KY038922/Ca_boca_virus/GZHD15/   .......................................................
KP281714/Canine_bocavirus/13D0   .......................................................
KP281718/Canine_bocavirus/14D1   .......................................................
JQ692590/Ca_bocavirus/HK882U/C   .......................................................
KF771828/Ca_bocavirus/F1300079   .......................................................
OK546117/Ca_bocaparvovirus2/NW   .......................................................
OM640112/Ca_bocaparvovirus2/LS   .......................................................
MW922649/Ca_bocaparvo2/147CP7_   .......................................T...............
KP281719/Ca_bocavirus/14Q209/S   .......................................................
JN648103/Ca_bocavirus/Con161/U   .......................................................
OK546115/Ca_bocaparvovirus2/NW   .......................................................
MW922650/Ca_bocaparvovirus2/00   .......................................................
KP281713/Canine_bocavirus/13D0   .......................................................
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Fig. 4: Non-synonymous amino acid substitutions identified in the partial sequences of the NS1 proteins of CBoV-2 determined in this study. The figure shows the changes between the 296th and 420th aa of the genome.

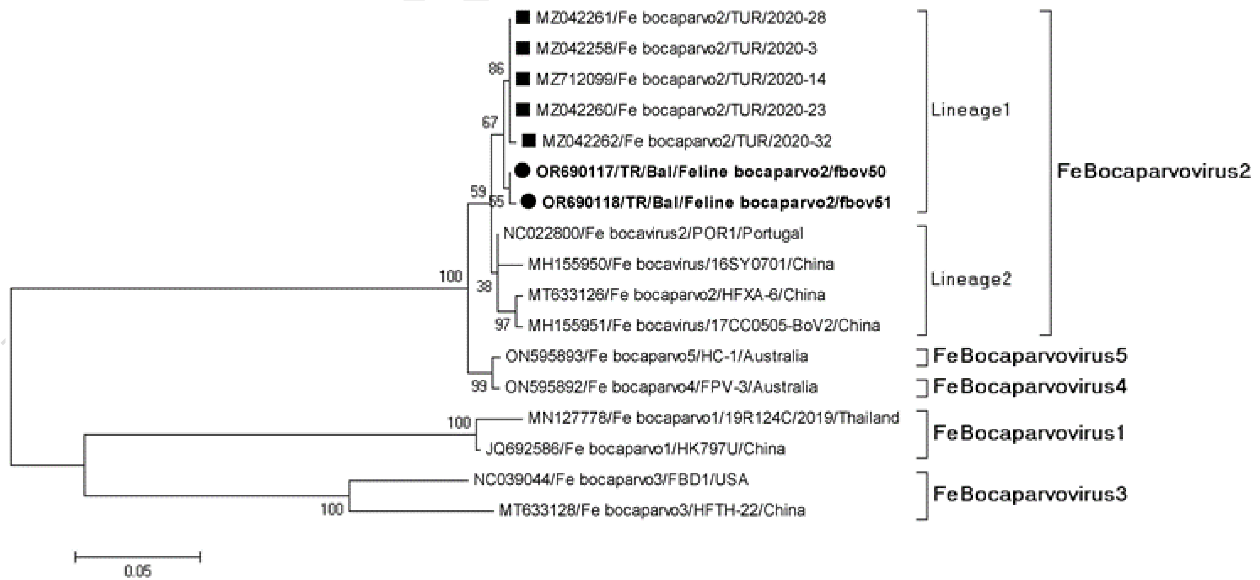

Fig. 5: Phylogenetic tree based on the partial nucleotide sequences of the NP1 gene of FBoV-2. The novel consensus sequences obtained in this study are indicated by a solid black circle

DISCUSSION

In recent years, new bocaviruses identified in animals and humans have begun to attract worldwide attention and be investigated. The aim of the study was to detect the infectious agents in cats and dogs infected with canine and feline Bocaparvovirus infections and housed together to determine their strains by molecular characterization of the viruses in positive samples.

Genome positivity, which was 1.2-7.5% reported by some international studies, was determined as 3.94% in adult dogs in a national study (Ohshima *et al.*, 2010; Guo *et al.*, 2016; González-Hein *et al.,* 2020; Isidan and Turan, 2021; Canuti *et al.*, 2022b). In this study, genome positivity was determined to be 10.8% in samples obtained from dogs older than 6 months.

Recombination is very important for the evolution and persistence of RNA viruses. Despite having a single-stranded DNA genome, parvoviruses have been determined to be capable of rapid evolution to form new genotypes or species and have a mutation rate close to that of RNA virus (Fu *et al.*, 2011). Novel bocaparvovirus variants emerging in different species are the result of rapid evolution and frequent recombination in the virus genome (Brožová *et al.*, 2016). In Bocaparvoviruses, ORF1 encodes the NS1 protein and ORF2 encodes the overlapping capsid proteins VP1/VP2, while ORF3 encodes NP1(Capozza *et al.*, 2023). These gene regions were selected for genetic characterization of circulating strains in the study.

Two distant lineages emerge in the phylogenetic tree constructed based on the partial VP2 gene-based molecular analysis of CBoV-1. All of the strains obtained in this study were involved in Lineage 2, along with the previous strains from Türkiye, China, Canada, and Italy. There are many studies in Türkiye to investigate the existence of CPV-2 and circulating virus strains. However, there is only one study conducted in the eastern Türkiye (Sivas) to investigate canine Bocaparvoviruses. As a result of phylogenetic analysis, it was determined that two of the CBoV-1 strains were highly similar to these strains, while the other three were more similar to the Italian strains than these strains. Considering that Balıkesir province, where the present study was conducted, is located in the west and is far away from Sivas province, it is seen that different strains coming from the west can be found in Türkiye.

Amino acid changes were determined in the partial VP2 gene of the CBoV-1 genome (Fig. 2). In the gene region replicated in the study, amino acid changes were detected at four different points (1378th aa Arg (R) to Leu (L), 1413th aa Val (V) to Ala (A), 1414th aa Thr (T) to Ser (S) and 1416th aa Val (V) to Leu (L)) in the OR681968 strain compared to other compared strains and other strains obtained from the study circulating in the same region. A change was detected at one point each in OR681970 (1401th aa Gln(Q) to His(H)) and OR681972 (1499th aa Gly(G) to Glu (E)) strains, and at two points in OR681971 (1397th aa Ile(I) to Val (V), 1401th aa Gln(Q) to His(H)) strain. Capsid proteins of parvoviruses are responsible for determining cellular tropism and the host's immune response (Nandi and Kumar, 2010). Therefore, the genetic diversity of the detected CBoV-1 strains may change the pathogenesis of the virus. The detected amino acid changes suggest that strains with different pathogenesis and virulence may circulate in the same environment.

When CBoV-2 strains were compared with CBoV-1 (CMV) strains, it was determined that they were similar at 63, 62, and 64% at the levels of NS, NP and VP genes, respectively (Kapoor *et al.*, 2012). Based on the partial NS1 gene-based molecular analysis of CBoV-2, two distant lineages emerge. Three of the strains obtained in the present study (OR690113, OR690112, OR690116), together with strains from Canada, China, South Korea, and Thailand, were included in Lineage 1. The other two strains (OR690114, OR690115) were detected in Lineage 2 together with the strains previously identified in Canada, USA, Germany, and Türkiye. In the phylogenetic analysis performed for CBoV-2, it was determined that two of the strains were found in Lineage 2 together with the previous strains reported from Türkiye (Sivas). However, the other three strains were quite distant from these strains and their similarity to Canadian and far eastern strains such as China and South Korea was revealed as interesting data.

Viruses within the Bocaparvovirus genus are more than 30% monophyletic at the NS1 amino acid level. Amino acid changes were determined in the partial NS1 gene of the CBoV-2 genome. OR690112 and OR690116 strains showed aa changes at six points (327th aa Arg (R) to Pro (P), 364th aa Lys (K) to Thr (T), 378th aa Arg (R) to Ser(S), 386th aa Thr (T) to Ala (A), 392th aa Asn (N) to Arg (R), 417th aa Met (M) to Ser (S)). In addition, strain OR690116 (302th aa Lys (K) to Glu (E)) underwent amino acid change at one point, and strain OR690113 (360th aa Ser (S) to Ala (A), 417th aa Met (M) to Trp (W)) underwent amino acid change at two points.

Diarrhea caused by viral infections in domestic cats can often be serious enough to be life-threatening. Parvoviruses, which generally infect the gastrointestinal system, are associated with diarrhea. Although Feline Bocavirus (FBoV) is frequently detected in cats with signs of enteritis, they have also been detected in healthy cats (Zhang *et al.*, 2014a). Previous studies reported that the positivity rate in all samples was determined mostly in stool, and the studies, in which infection was detected, reported no significant correlation between infection and clinical symptoms (Takano *et al.*, 2016; Kelman *et al.*, 2020). Genome positivity, which varies between 2.5-47.8% in various countries, was determined to be 7.1% in this study.

ORF3, found in Bocaparvoviruses, encodes NP1, a highly phosphorylated protein involved in RNA processing. NP1 regulates splicing of VP-encoding RNAs and ensures readthrough of proximal polyadenylation. It has been determined that mutations on the NP1 gene have a significant effect on RNA processing, independent of genome replication (Fasina *et al.*, 2016; Capozza *et al.*, 2023). In this study, NP1 gene was used to investigate and detect FBoV. FBoV genotypes are divided into three genotypes. In previous studies, Genotype-1 was detected in Hong Kong, Belgium, and Thailand, Genotype-2 in Türkiye, Portugal, and Japan, Genotype-1 and Genotype-3 in the USA, Genotypes-1 and Genotype-2 in northeastern China (Lau *et al.*, 2012; Ng *et al.*, 2014; Zhang *et al.*, 2014b; Takano *et al.*, 2016; Liu *et al.*, 2018; Yi *et al.*, 2018; Piewbang *et al.*, 2019; Zhang *et al.*, 2019; Abayli and Can-Sahna, 2022). In the phylogenetic tree created based on the

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NP1 gene, two FBoV strains obtained from the study were identified as FBoV-2 genotype and were included in Lineage 1 together with the strains previously obtained from Türkiye.

It has been determined that genetic rearrangement and recombination can occur in human and porcine bocaviruses, as in RNA viruses. In another study, co-infection of two different swine bocaviruses in the same host and significant inter- and intra-host genetic variation were detected (Kapoor *et al.*, 2009; Lau *et al.*, 2011). Cats and dogs often share common living spaces and it is known that since they are closely related, some interspecies viruses (feline and canine parvoviruses, herpesviruses, coronaviruses) are transmitted between these two animals (Sun *et al.*, 2009; Lau *et al.*, 2012). When the differences between parvoviruses are examined, CPV-2 emerged as a result of several amino acid mutations in FPV. Over time, CPV2 has evolved and the CPV2a lineage and its variants have emerged, which can infect both dogs and cats. Since the emergence of CPV in the 1970s, different variants (CPV2a, CPV2b, CPV2c) have emerged, resulting in a wider host range and greater infectivity and epidemics (Truyen, 2006). In this study, all samples were investigated with different selected gene regions, and no mixed infection with different Bocaparvovirus types was detected in the same host. However, the fact that different amino acid changes have been determined between different strains of the same viruses shows that different variants can emerge in cats and dogs in the same environment.

The rapid adaptation of Canine parvovirus 2 (CPV-2), which originates from the feline parvovirus, to dogs is due to the high mutation rate in the major capsid gene and the positive selection of mutations (Truyen, 2006). Studies have reported that feline and canine Bocaparvoviruses are closely related, but the genetic difference between them is much greater than that between FPV and CPV (Lau *et al.*, 2012). In this study, samples were obtained from cats and dogs housed together, and no transmission was detected between them. Based on all this information, it can be concluded that although feline and canine Bocaparvoviruses are closely related and the virus has co-evolved between cats and dogs, it is unlikely that recent interspecies transmission has occurred.The geographical location of Türkiye has an important place in revealing the genetic diversity of a wide variety of viral infections belonging to different animal species. Phylogenetic analysis results obtained from the present study revealed that strains from the East and the West circulated together in Türkiye. Moreover, it should not be forgotten that the detection of these infections, which are mostly subclinical, is important and may pave the way for other infections that may occur together in a mixed manner. Recently, the increase in the use of advanced molecular techniques, metagenomic studies, and consensus primer-based PCR approaches has enabled the identification of different enteroviruses (such as bocaparvovirus, chaphamaparvovirus, and bufavirus). The relationship between these viruses and cat and dog diseases has not been exactly established so far.

Conclusions: In conclusion, comprehensive epidemiological surveillance and genetic characterization of virus strains circulating in different regions should be performed to clarify the genetic diversity of bocaparvovirusların and their relationship with enteric diseases in cats and dogs.

Authors' contributions All authors contributed to the study conception and design. The preparation of the material and molecular analyses were performed by ZK, and the sequence analysis was performed by MOT. The draft of manuscript was written by ZK and was reviewed, developed, and finalized by all authors. ZK and MOT contributed to drawing of figures and editing. All authors read and approved the final manuscript.

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