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ORIGINAL ARTICLE

First detection of feline bocaparvovirus 2 and feline chaphamaparvovirus in healthy cats in Turkey

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Abstract

The pet cat's population and the number of viruses that infect them are increasing worldwide. Recently, feline chaphamaparvovirus (FeChPV, also called fechavirus) and feline bocaparvovirus (FBoV) infections, which are novel parvovirus species, have been reported in cats from different geographic regions. Here, we investigated FBoV 1–3 and FeChPVs in healthy cats in Turkey using PCR, where nuclear phosphoprotein 1 (NP1) is targeted for FBoV and NP for FeChPV. For this purpose, oropharygeal swabs were obtained from 70 healthy cats with different housing status from June 15 to December 1, 2020. After PCR screening tests, six out of 70 cats (5/47 shelter cats; 1/23 domestic cats) were found to be positive for FBOV, while two were positive for FeChPV (1/47 shelter cats; 1/23 domestic cats). No cat was found in which both viruses were detected. The nucleotide (nt) sequence comparison in the 310 base pair (bp) NP gene of the two FeChPVs identified in this study shared a high identity with each other (95.0% nt and 99% aa identities) and with previously reported FeChPVs (92.4–97.1% nt and 98.1–99.0% aa identities), including 313R/2019/ITA, 49E/2019/ITA, VRI_849, 284R/2019/ITA, and IDEXX-1. Here, the near-full length (1489 nt, 495 amino acids-aa) of the VP2 gene of the FechaV/Tur-2020/68 isolate obtained from the study was also sequenced. The nt and aa identity ratio of this isolate with other FeChPVs was 98.0–98.5%-96–96.5%, respectively. Sequences of the 465 bp NP1 gene of the six Turkish FBoV strains shared high identities with each other (99.6–100% nt and 99.3–100% aa identities) and with those of FBoV-2 strains (97.8–99.1% nt and 98.0–100% aa identities), including 16SY0701, 17CC0505-BoV2, HFXA-6, and POR1. All FBoVs detected in this study were classified as genotype 2, similar to the study conducted in Japan and Portugal. Here, the NS1 (partial), NP1, VP1 and VP2 gene of the FBoV-2/TUR/2020–14 strain obtained from the study were also sequenced and the nt and aa sequences showed high identities to the above-mentioned FBoV-2 strain/isolates (>96%, except for the aa ratio of strain 16SY0701). In conclusion, this study shows that FBoV and FeChPV are present in healthy cats in Turkey, and these viruses can be detected from oropharyngeal swabs. Our findings contribute to further investigation of the prevalence, genotype distribution, and genetic diversity of Turkish FBoVs and FeChPVs, adding to the molecular epidemiology of FBoV and FeChPVs worldwide.

Keywords Cats · FBoV–2 · FeChPV · PCR · Turkey

Introduction

There are approximately 370 million pet cats worldwide, and more than 15 virus families infect these cats (https://www.statista.com/statistics/1044386/dog-and-cat-pet-population-worldwide/). Feline panleukopenia virus (Parrish 1995; Stuetzer and Hartmann 2014), feline coronavirus (Addie and Jarrett 1992), feline calicivirus (Di Martino et al. 2020), and feline astroviruses (Sabshin et al. 2012; Soma et al. 2020) are among the most commonly known enteric pathogens. Recently, the increase in the use of metagenomics-based approaches (virome projects) and consensus primer-based PCR approaches have led to the discovery of many new enteroviruses' candidates, such as feline bocavirus (Lau et al. 2012), feline cyclovirus (Zhang et al. 2014), feline bufavirus (Diakoudi et al. 2019), feline chaphamaparvovirus (Li et al. 2020), feline norovirus (Di Martino et al. 2016), and feline sakobuvirus (Ng et al. 2014). These viruses are still not definitely associated with feline diseases.

Parvoviruses belonging to the *Parvoviridae* family are small (~25 nm diameter) and non-enveloped viruses,

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carrying single-strand negative DNA of 4–6 kilobases (kb) as genetic material. The *Parvoviridae* family comprises three subfamilies: *Hamaparvovirinae*, *Parvovirinae*, and *Densovirinae* (Cotmore et al. 2019; Pénzes et al. 2020).

The Chaphamaparvovirus genus in the *Hamaparvovirinae* subfamily includes Chaphamaparvoviruses (ChPV) that infect vertebrates (such as dogs, cats, bats, rats, mice, simians, pigs, birds, turkeys, peafowl, chickens and Tasmanian devils) (Baker et al. 2013; Reuter et al. 2014; Palinski et al. 2016; Yang et al. 2016; Lima et al. 2019; Kapusinszky et al. 2017; Williams et al. 2018; Chong et al. 2019; Fahsbender et al. 2019; Wang et al. 2019; Liu et al. 2020).

Recently, Cachavirus-1 A and Cachavirus 1 B belonging to Carnivore chaphamaparvovirus species 1 were identified in dogs in the United States in 2019 (Fahsbender et al. 2019). In 2020, another ChPV, called fechavirus or feline chaphamaparvovirus (FeChPV), was detected and analyzed in cats with vomiting and diarrhea. FeChPV DNA encodes three proteins, non-structural protein 1 (NS1), virion protein 1 (VP1), and nuclear phosphoprotein (NP), as in the cachaviruses belonging to Carnivore chaphamaparvovirus spescies 1 (Li et al. 2020).

The subfamily *Parvovirinae* is subdivided into ten genera: Amdoparvovirus, Artiparvovirus, Aveparvovirus, Bocaparvovirus, Dependoparvovirus, Erythroparvovirus, Loriparvovirus, Protoparvovirus, Tetraparvovirus, and Copiparvovirus (Pénzes et al. 2020). Protoparvovirus genus includes viruses such as feline panleukopenia virus and canine parvoviruses, which are highly pathogenic for cats and dogs, respectively (Truyen et al. 2009; Miranda and Thompson 2016).

Bocaparvoviruses in the genus Bocaparvovirus frequently infect young animals and humans through the fecal–oral tract, causing respiratory (Priestnall et al. 2014; Verbeke et al. 2019) and gastrointestinal symptoms (Bodewes et al. 2014; Lin et al. 2020), and have a broad host spectrum that infect animals such as cows, pigs, rats, cats, and dogs (Qiu et al. 2007; Bodewes et al. 2014; Lau et al. 2017; Pfankuche et al. 2016; Piewbang et al. 2019). According to the latest report of the International Committee on Taxonomy of Viruses (ICTV), the genus Bocaparvovirus is divided into 25 species, most of which are novel bocavirus: Carnivore bocaparvovirus 1–6, Chiropteran bocaparvovirus 1–4, Lagomorph bocaparvovirus 1, Pinniped bocaparvovirus 1–2, Primate bocaparvovirus 1–2, Rodent bocaparvovirus 1–2, and Ungulate bocaparvovirus 1–8. Nowadays, Feline bocaparvovirus 1 (FBoV 1), Feline bocaparvovirus 2 (FBoV 2), and Feline bocaparvovirus 3 (FBoV 3) species that infect cats are recognized as members of Carnivore bocaparvovirus 3, 4, and 5, based on NS1 amino acid (aa) sequences (Pénzes et al. 2020).

Feline bocaparvovirus was first detected in the feces, urine, nasal, kidney, and urine of stray cats in Hong Kong from 2009 to 2011 (Lau et al. 2012). Then, FBoV 2 and FBoV 1–3 were detected in the fecal samples of healthy Portuguese and American cats, respectively (Ng et al. 2014; Zhang et al. 2014). FBoV encodes four major ORFs: NS1, NP1, and VP1/VP2 (Ng et al. 2014).

Parvoviral capsid proteins are determinants of host cell tropism and immunogenicity. Minor changes in these proteins can alter host range and pathogenicity (Hoelzer et al 2008; Parrish and Kawaoka 2005). Some parvoviruses, such as FeChPV (Di Profio et al. 2021), FBoV (Lau et al. 2012) and feline bufavirus (FBuV) (Diakoudi et al. 2019), have recently attracted attention with their extraintestinal or possible systemic infections as well as enteric infections. Therefore, in this study, we preferred the oropharynx, which is an important sampling site for respiratory diseases. We also collected samples only from healthy cats in order to obtain data in a short time and eventually contribute to the pathogenicity, pathogenesis and epidemiology of these viruses.

Here, we investigated FBoV 1–3 and FeChPV from the oropharyngeal swabs of 70 healthy cats from Turkey in 2020 using PCR.

Materials and methods

Origin of samples and risk factors

In this study, a total of 70 healthy cats were sampled from three pet clinics and two shelters in two provinces (Kayseri and Elazig) in the Anatolian region of Turkey between 15 June and 1 December 2020. Oropharyngeal swab was preferred as the sampling method. Sampling was randomly conducted with shelter (47/70) and domestic cats (23/70), regardless of age, gender, and race. The survey questionnaire comprising the basic information (breed, age, gender, etc.) and behaviors of the cats was filled out at the time of sampling. The sampled cats were examined for conjunctivitis, diarrhea, fever, respiratory distress, and stomatitis, and they were evaluated as having healthy or no clinical symptoms. Of the 70 cats sampled during this study, 37 were female, 33 were male, 8 were purebreed, 62 were mixed breed, 41 were ≤ 1 year of age (from one months to one year), and 29 were older than 1 year (from 18 months to five years).

In addition, risk factors such as age, gender and housing status for each virus in the cats included in the study were statistically evaluated with the chi-square test using SPSS 22.0 software.

Samples processing and viral nucleic acid extraction

Oropharyngeal swabs were diluted 1:10 in sterile PBS and centrifuged at 4500 rpm for 5 min to remove particles. Nucleic acids were extracted from 200 µl of the supernatant with a spin column (QIAamp MinElute Virus Spin Kit,

Qiagen, Hilden, Germany), according to the manufacturer's instructions. The extracted nucleic acids were eluted in 100 µL elution buffer and were determined to the quality and quantity by absorbance of A260/280 ratio using a NanoDrop2000 spectrophotometer (Thermo Fisher, MA, USA) and stored at – 20 °C until use.

Screening of FBoV and FeChPV by PCR

PCR conditions for FBoV 1–3 and FeChPV detection were performed as follows: 2 × concentrated PCR master mix solution (Thermo Fisher, MA, USA), 5 µl DNA template (50–100 ng), 4 µl primer mix (forward and reverse primers), in a final 50 µl reaction. The nucleotide sequences of the primers used in the PCR step and the target size to be amplified are given in Table 1. Thermal cycling conditions for FBoV detection were 35 cycles of 94 °C for 40 s, 51 °C for 40 s, 72 °C for 45 s, and a final extension at 72 °C for 2 min. Thermal cycling conditions for FeChPV detection in both rounds were 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 35 s, and a final extension at 72 °C for 2 min. As a negative control, PCR master mix with specific primers (no template) was used in parallel with each PCR step. Tests of clinical samples found to be positive in the PCR were repeated once more to confirm positivity.

Amplification of the VP gene for FeChPV and the NS1, NP1, VP1 and VP2 gene for FBoV-2

In this study, in order to obtain more genetic data, one DNA positive in PCR screening was randomly selected and PCR amplifications were performed according to the protocol specified in the reference, using the primer sets in the Table 1.

The amplification products were analyzed in 1.5% agarose gel electrophoresis with ethidium bromide (Sigma-Aldrich, MO, USA) using a 100 bp DNA ladder (NEB, MA, USA). Electrophoresis was performed in TAE buffer (40 mM Tris–acetate, 0.5 M EDTA) for 40 min at 120 V, and the PCR products were visualized under UV light.

Sequence analysis and phylogeny

The PCR products were gel-purified using the QIAquick gel extraction kit (Qiagen, Hilden, Germany) and sequenced in a commercial sequencing service (Macrogen Europe, Amsterdam, Netherlands). This stage was performed in an ABI Prism 3130 genetic analyzer (Applied Biosystems, CA, USA) using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, CA, USA) and the PCR primers.

The resulting bidirectional nucleotide sequences (nt) were aligned, edited, verified with BLASTN (BLAST: Basic Local Alignment Search Tool (nih.gov), and submitted to the GenBank database. Then, nucleotide and amino acid sequences were aligned and compared with strains selected from GenBank using Clustal W software (Thompson et al. 1994) Sequence data have been submitted to the GenBank Nucleotide Sequence Database under accession numbers MZ042258-65 and MZ712099-100. Phylogenetic tree from the aligned sequences was constructed by the Molecular Evolutionary Genetics Analysis (MEGA; version X) software and the Maximum Likelihood method based on the Tamura Nei model with 1000 bootstrap replicates (Tamura and Nei 1993; Kumar et al. 2018).

Table 1 Nucleotide sequences of primers used for screening and sequencing in this study

Primer name	Nucleotide sequences (5′–3′)	Target gene	Amplicon size (bp)	Application	References
FBoV-F	AGAACCRCCRATCACARTCCACT	NP1	465	Screening and sequencing	Zhang et al. 2019
FBoV-R	TGGCRACCGCYAGCATTTCA				
FBoV-2384F	ACAGACGGCGGCAGTGCTCCAGAC	NS1/NP1	1315	Sequencing	Yi et al. 2018
FBOV-3698R	CCATGCCCGCCACCCCCAGAG				
FBoV-3653F	GTGGGGGTGGCAGCGGTGTTGG	VP1/VP2	1014	Sequencing	Yi et al. 2018
FBoV-4666R					
FBoV-4219F	CTAAACGCAGATAATGTGGAAAAA	VP1/VP2	1176	Sequencing	Yi et al. 2018
FBoV-5394R	CAGTGTGGGTGATGTTGAGAAG				
FeChPV-F1	GGTGCGACGACGGAAGATAT		332	Screening and sequencing	Li et al. 2020
FeChPV-R1	CAACACCACCATCTCCTGCT	NP			
FeChPV-F2	GCTGCAGTTCAGGTAGCTCA		310		
FeChPV-2656F	TCTATATCATATTCAAATACTT	VP	702	Sequencing	Di Profio et al. 2021
FeChPV-3358R	TGAACCATTTATTGTCATCTG				
FeChPV-3269	ACCCATCAAGTATAATGGAATT	VP	913	Sequencing	Di Profio et al. 2021
FeChPV-4182R	AATTGAAGTATATCAGGTCATA				

The identity ratio of nt and aa sequences of partial NP and VP genes of FeChPV isolates obtained from this study was compared with that of FeChPV strains/isolates selected from GenBank and calculated using the SIAS software (http://imed.med.ucm.es/Tools/sias.html). The same method was used to calculate the identity ratio between NS1, NP1, VP1, and VP2 genes nt and aa sequences of the FBoV strains obtained from this study and the others.

The reference strains were compared with the present strains on the phylogenetic trees.

Virus cultivation

Virus isolation from oropharyngeal swaps was tried on Madin–Darby Canine Kidney (MDCK) cells. Growth medium (DMEM supplemented with 15 mM/L HEPES, 1.5 gr/L sodium bicarbonate, l-glutamine, 100 U mL^{-1} penicillin and 100 µg mL^{-1} streptomycin, and 10% fetal bovine serum) was removed from 70% confluent MDCK cells and these cells were washed with sterile PBS. Then, supernatants of diluted clinical samples were inoculated into MDCK cells. After incubation at 37 °C for 1 h at 5% CO2, washing was repeated twice with sterile PBS and fresh medium was added onto the cells. MDCK cells were examined under the microscope for 5–7 days.

Results

PCR screening and risk factors

After PCR screening tests, six out of 70 cats were found to be positive for FBOV, while two were positive for FeChPV. There were no cats in which FBOV and FeChPVs were co-detected.

In this study involving 70 cats aged one month to five years, the age of one of the FeChPV-positive cats was two months and the other four years, while the age of the FBoV-positive cats was between one and 12 months. Table 2 shows the detailed information of PCR positive cats.

Based on the age-based analysis of virus distribution, the presence of FBoV was more common (6/6) between 0–12 months compared to other age groups (> 12 months) ($p = 0.03$). When examining the relationship between factors such as housing (5/47 shelter cats; 1/23 domestic cats) and gender (4/33 male; 2/37 female) and the frequency of FBoV in cats, the difference was statistically insignificant ($p > 0.05$).

There was no statistically significant difference between the frequency of FeChPVs in cats and factors such as age, housing (1/47 shelter cats; 1/23 domestic cats), and gender (2/33 male; 0/37 female) ($p > 0.05$).

Virus cultivation

After the 1th and 2nd passages, FBoV-2 was not detected in PCR, while FeChPV was detected until the third passage. While no specific cytopathic efffect was observed, a significant increase in the number of cells that rounded and then died was observed.

Sequence analysis and phlogeny

Nucleotide sequences of the 465 bp NP1 gene of the six Turkish FBoV strains shared high nt identities with each other (99.6–100%) and with those of FBoV-2 strains, including 16SY0701 (97.8–98.2%),17CC0505-BoV2 (98.0–98.2%), HFXA-6 (98.0–98.2%), and POR1 (98.9–99.1%). The six identified Turkish FBoV-2 strains were named as followed: FBoV-2/TUR/2020–3, FBoV-2/TUR/2020–14, FBoV-2/TUR/2020–23, FBoV-2/TUR/2020–28, FBoV-2/TUR/2020–32, and FBoV-2/TUR/2020–34. Upon phylogenetic analyses based on the NP1 nt sequences, all FBoVs were divided into three genogroups, and all positive FBoVs reported in this and earlier studies (16SY0701, 17CC0505-BoV2, HFXA-6, and POR1) clustered in the genogroup

Table 2 Demographic information of cats with positive results by PCR

Label	Age (month)	Breed	Gender	Lifestyle	Location	Strain name
2	48	Shelter	M	Mixed	A	FechaV/Tur-2020/2
3	3	Shelter	M	Mixed	A	FBoV-2/TUR/2020–3
14	4	Domestic	F	Angora	B	FBoV-2/TUR/2020–14
23	12	Shelter	M	Mixed	A	FBoV-2/TUR/2020–23
28	1	Shelter	M	Mixed	B	FBoV-2/TUR/2020–28
32	1	Shelter	M	Mixed	B	FBoV-2/TUR/2020–32
34	1	Shelter	F	Mixed	B	FBoV-2/TUR/2020–34
*68	2	Domestic	M	British shothair	B	FechaV/Tur-2020/68

The symbol (*) represents cats vaccinated against FCV, FHV and FPLV. The letters A and B represent the provinces of Elazig and Kayseri, respectively

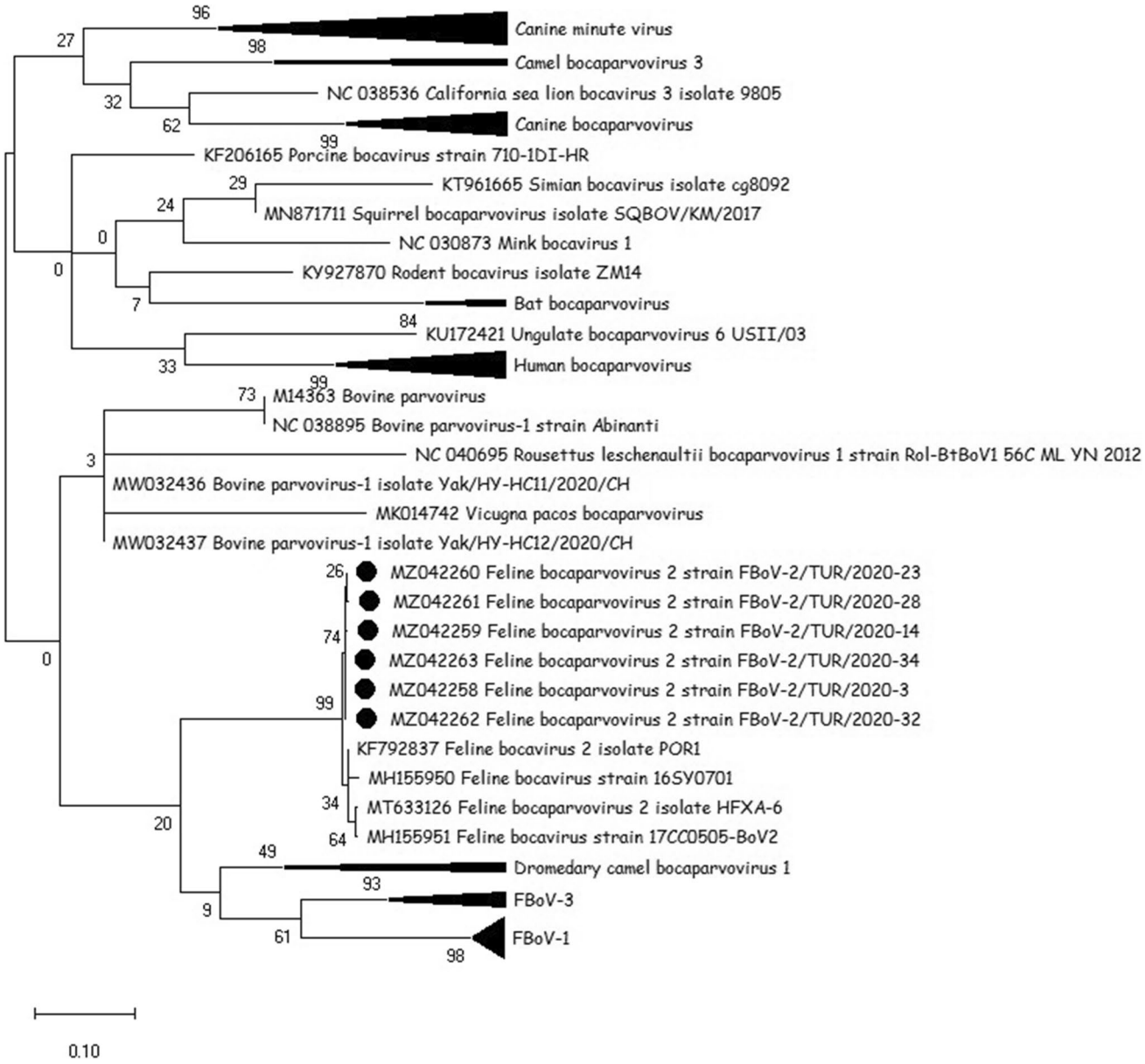

Fig. 1 Phylogeny of FBoV NP1 nt sequences. The tree was constructed using the maximum likelihood method. Bootstrap support values based on 1000 replicates are given at the nodes. Black circles represent the FBoV strains in the present study. Others are BoV strains or isolates from different countries

called genotype 2, and none of them were not included in genotypes 1 and 3 (Fig. 1).

The amino acid sequence comparison in partial NP1 of the six Turkish FBOV-2 s showed 98.0–100% identities to other FBoV-2 s, 48.0–73.91% to other BoVs, and 99.3–100% to each other.

According to the partial NP1 aa alignments of FBoVs based on the aa position of the FBoV-2 POR1 strain, the FBoV-2 specific aa substitutions, also included in Turkish strains, were as follows: M99V, E105S, Q/G106S, P111N, E140S, N/M141Y, S/C142A, K/N143T, L/M155I, L163V, W172Y, M176L, D/H181N, K188Q, A/S191T, L199I, Q202I, E203Q.

Furthermore, strain FBoV-2/TUR/2020–14 was randomly selected and in addition to its partial NS1 gene (287 nt, 94 aa), its full-length encoding NP1 (689 nt, 229 aa), VP1 (2159 nt, 719 aa), and VP2 genes (1736 nt, 578 aa) were sequenced. The nucleotide and aa identity of the FBoV-2/TUR/2020–14 FBOV-2 and strains/isolates selected from the GenBank database were as follows, respectively: 75.3–78.0% -54.0–56.8% for partial NS1; 98.4–99.3%-98.7–100 for NP1; 96.4–97.0%-96.9–97.0% for VP1; 96.5–97.2%-97.7–98.1%

Table 3 Percentage of nucleotide-amino acid identity of strain FBoV-2/TUR/2020–14 (MZ712099) obtained from this study with some others

Gene	FBoV-1	FBoV-2					FBoV-3
	HK797U	KU-61	POR1	HFXA-6	17CC0505-BoV2	16SY0701	FBD1
	(JQ692586)	(LC148407)	(KF792837)	(MT633126)	(MH155951)	(MH155950)	(NC_039044)
Partial NS1	78.0–56.0	99.6–98.9	98.9–97.8	98.9–97.8	98.6–96.8	98.2–96.8	83.2–72.5
NP1	72.4	–	99.3-%100	98.5–99.6	98.5–99.6	98.4–98.7	72.5–67.1
VP1	69.4–69.0	–	97.0–97.1	96.4–96.9	96.5–96.2	96.7–96.7	68.6–67.8
VP2	69.4–70.2	–	97.2–98.1	96.5–98.1	96.9–97.7	96.9–69.3	67.8–69.6

for VP2. Similarly, nt and aa identities compared with different feline bocavirus genotypes were as follows: 62.2–86.2%-54–72.5% for NS1; 71–73.5%-62.4–67.0% for NP1; 68.0–69.0%-67.8–71.5% for VP1; 67.8–70.7%-29.6–72.0% for VP2. Some of the nt and aa comparisons of FBoVs was detailed in Table 3.

By the nucleotide sequence comparison, the 310 base pair (bp) NP gene of the two FeChPVs identified in this study shared a high identity with each other (95.03%) and with previously reported FeChPVs, including 313R/2019/ITA (92.4–95.2%), 49E/2019/ITA (94.3–95.2%), VRI_849 (93.3–96.2%), 284R/2019/ITA (94.3–97.1%, and IDEXX-1 (92.4–97.1%) while sharing a lower identity (16.0–62.5%) with other ChPV's mentioned above. The two identified Turkish FeChPVs were named as followed: FechaV/Tur-2020/2 and FechaV/Tur-2020/68. Upon phylogenetic analyses based on the NP nt sequences, FechaV/Tur-2020/2 and FechaV/Tur-2020/68 were in the same cluster as FeChPVs from other geographic regions (Fig. 2).

The amino acid sequence comparison in partial NP of FechaV/Tur-2020/2 and FechaV/Tur-2020/68 showed 98.1–99.0% identities to other FeChPVs, 64.2–84.4% to other ChPVs, and 99.0% to each other.

According to the partial NP aa alignments of FeChPVs and Carnivore ChPVs based on the aa position of the 49E/2019/ITA strain, the FeChPV-specific aa substitutions, also included in Turkish isolates, were as follows: G65A, R67T, S/G68N, I93Q, F103Y, I119V, E121S, M123I, L125M, A134T, M137I, Q141E, V151T, D152T/A, H155I, Q156L, Q158R. The FechaV/Tur-2020/2 isolate, unlike the other FeChPVs, had a new aa substitution (A to D) at position 73. FechaV/Tur-2020/2, FechaV/Tur-2020/68, and 49E/2019/ITA had a different aa (I to M) not found in the others at position 117.

In addition, the nearly complete VP2 coding region (1489 nt, 495 aa) of the randomly selected FechaV/Tur-2020/68 isolate was sequenced in this study. The VP gene nt and aa sequence of the FechaV/Tur-2020/68 isolate were 98.0–98.5%-96.0–96.5% and 20.5–80.7%-30.7–80.7, respectively, when compared to other FeChPVs and other ChPVs selected from the GenBank database.

Discussion

Parvoviruses are generally known as enteric pathogens, as they commonly infect the gastrointestinal tract, and their transfer to the susceptible host is via fecal contaminated water or any food, i.e., fecal oral route (Gerba 2009). Although FBoVs are often detected in feces (Ng et al. 2014; Zhang et al. 2014; Yi et al. 2018) and even associated with enteric disease (Liu et al. 2018; Yi et al. 2018; Li et al. 2020), we still have limited information about the clinical disorders they cause, as they are also detected in healthy cats (Takano et al. 2016). Other members of bocaviruses cause respiratory diseases in humans (Meriluoto et al. 2012) or animals (Kapoor et al. 2012) alongside enteric diseases, but there are no such reports yet for feline bocaviruses. To date, three genotypes of feline bocavirus have been reported: genotype 1 in Hong Kong, Belgium, and Thailand; genotype 2 in Japan and Portugal; genotypes 1 and 3 in the United States, and genotypes 1 and 2 in northeastern China (Lau et al. 2012; Ng et al. 2014; Zhang et al. 2014; Piewbang et al. 2019; Takano et al. 2016; Liu et al. 2018; Yi et al. 2018). We identified FBoV (6/70) in oropharyngeal swabs of 70 healthy Turkish cats with PCR-based screening tests where the partial genes for NP1 for FBoV were targeted. All FBoVs detected in this study were classified as genotype 2, similar to the study conducted in Japan and Portugal. According to our current knowledge, there has been no report on the detection and identification of feline bocavirus in healthy or unhealthy cats in Turkey, and this study is the first report.

The more widespread detection of FBoV-1 in cats with enteric disease (Yi et al. 2018) may indicate that different genotypes may exhibit different phenotypic characteristics, just like some members of bocaparvoviruses. For example, human bocaparvovirus 1 (HuBoV-1) is often associated with respiratory disease (Koseki et al. 2012; Meriluoto et al. 2012; Ziemele et al. 2019) while HuBoV-2–4 has been associated with enteric diseases (Cheng et al. 2011; Jin et al. 2011). Garigliany et al. (2016) reported the presence of FBoV-1 in the brain of an FPV-infected cat with neurological findings. A recent study reported that FPV coinfection with FBoV-1 infection can exacerbate bloody diarrhea (Piewbank et al. 2019). There are no

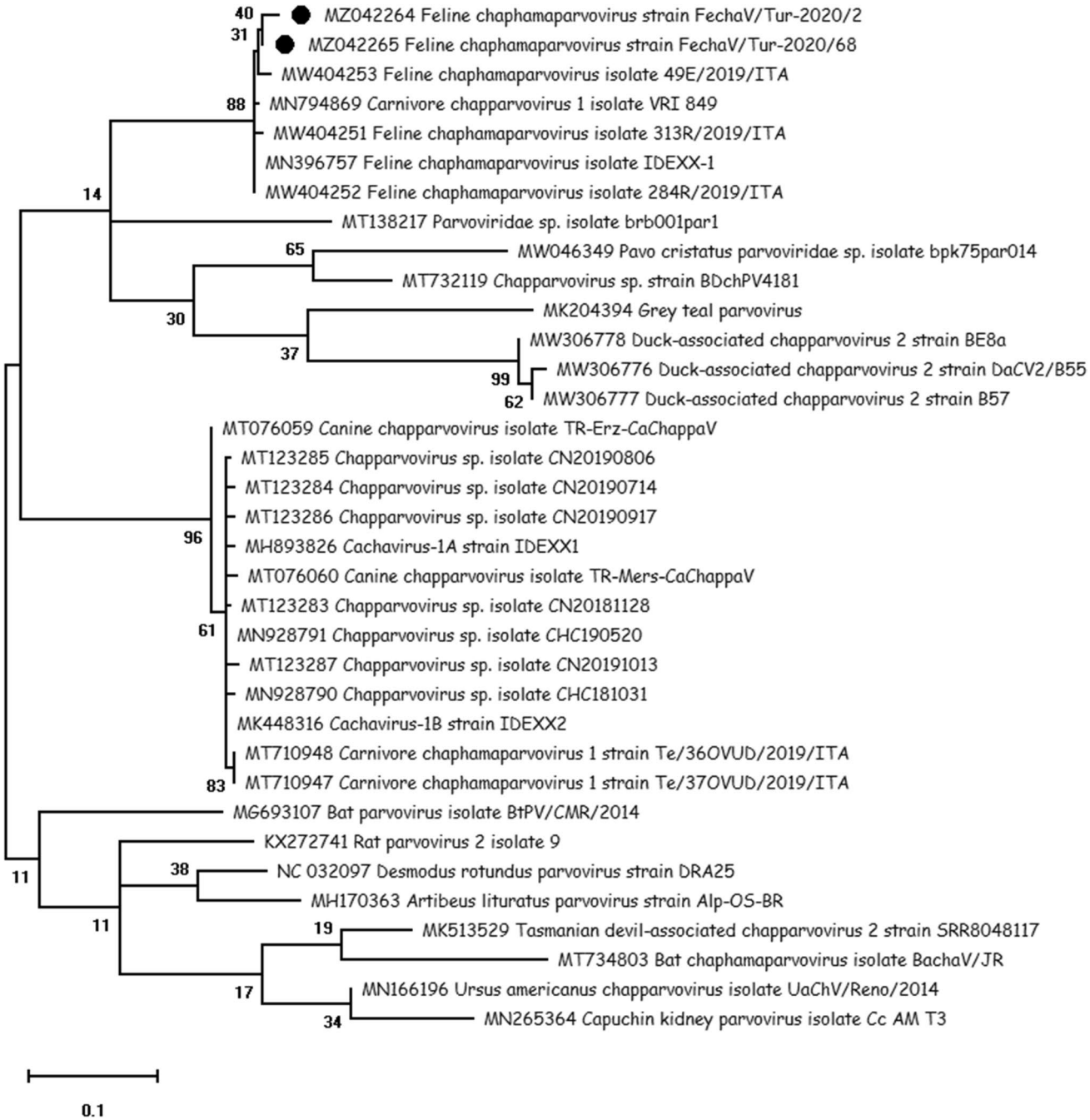

Fig. 2 Phylogeny of FeChPV NP nt sequences. The tree was constructed using the maximum likelihood method. Bootstrap support values based on 1000 replicates are given at the nodes. Black circles represent the FeChPV isolates in the present study. Others are ChPV strains or isolates from different countries

similar reports yet for FBoV 2 and 3, perhaps they may be reported in the future more often in healthy cats or cats with respiratory or other diseases; more studies are needed for this. While demonstrating the phenotypic characteristics of bocaviruses, the age of the infected animal should be considered because these viruses generally cause clinical disease at an early age and subclinical infection at an older age (Manteufel and Truyen 2008; Takano et al. 2016). Conversely, all FBoV-2 positive cats in this study showed no clinical symptoms, although they were between the ages of 1 and 12 months. Interestingly, the nt sequence of the NP1 gene showed that the Portuguese FBoV-2 strain (POR1) detected in the feces of a healthy cat in 2014 was genetically closest to (98.9–99.1% nt and 99.3–100% aa

identities) the six Turkish FBoV-2 strains detected in the oropharyngeal swaps of healthy cats in this study (in 2020). Moreover, the nt and aa acid sequences of some other genes (NS1, NP1, VP1 and VP2) of strain FBoV-2/TUR/2020–14 obtained from this study showed very high identity (> %97) to those of POR1. Although these results suggest that the origin of the virus may be Portugal, it is very difficult to say anything at this stage as there is limited data in the Genbank and this may change with new data from other countries. For example, the partial NS1 sequence of strain FBoV-2/TUR/2020–14 from this study revealed more similarity to Japanese FBOV-2 strain (KU-61), but data for this strain were limited in the database.

Until now, the FeChPV has been reported from Canada (Li et al. 2020) and Italy (Di Profio et al. 2021) there is no previous report of this virus in Turkey.

In Italy, FeChPV was detected in 36.8% (14/38) of the enteric samples of cats with acute gastroenteritis between 2019 and 2020, while this ratio was 2% (1/50) in healthy cats. In the same study, FeChPV was investigated by PCR in oropharyngeal clinical samples of 183 cats with URTD (57%) and 140 healthy cats (43.3%) in 2014–2017, and FeChPV DNA was detected in 12 clinical samples, six of which belonged to healthy animals. It was reported that there was no statistically significant relationship between FeChPV and URTD (Di Profio et al. 2021). Our findings of the prevalence of FeChPV in the oropharyngeal swabs of healthy cats supported theirs, which was 1.42% (2/70) and 4.28% (6/140), respectively. The NP nt and aa sequence of the FechaV/Tur-2020/2 and FechaV/Tur-2020/68 isolates obtained from this study shared 92.38–97.14% and 98.08–99.03% identity, respectively, with FeChPVs (313R/2019/ITA, 49E/2019/ITA, and 284R/2019/ITA) identified in Italy by Di Profio et al. (2021). The results were also similar for the VP gene sequence of the FechaV/Tur-2020/68 strain (> 98% nt and > 96% aa identities).

We still have limited information about the clinical disorders caused by FeChPVs. It is difficult to associate new virus strains emerging with the disease, as the effects of many microbiological agents must be eliminated. The most reliable way to prove this is to inoculate viruses in pure form into susceptible animals. Clinical disorders can only be associated if they produce a specific disease in the host inoculated with the virus. PCR screening tests from oropharyngeal swabs may be useful in the early diagnosis of viral infection and preventing further viral transmission. Even if these viruses are not directly associated with feline diseases, it will be important to identify and monitor them, as they can have synergistic effects with others (Piewbang et al. 2019).

Currently, emerging parvovirus members as pathogen candidates are attracting great attention by researchers worldwide; there are few studies about FeBoV and FeChPVs obtained from different geographic regions, thereby making the data obtained from this study useful.

Using a single sampling method in sampled cats, excluding different sampling methods such as blood collection and anal swab, and not comparing our results with these methods limited this study. In addition, the diversity of the sampling region and the number of cats could be increased.

Here we report the detection of FeBoV-2 and FeChPV in the oropharynx of healthy cats. A larger-scale study is planned in the future, in which other upper respiratory tract infectious agents, including feline bufavirus, will be investigated. We think that the pharynx is one of the important junctions on the respiratory and digestive systems and if this region is included in studies with enteric viruses that may cause extraintestinal infections, some new parvoviruses may not be missed.

There is also the possibility that viral DNA detected from enteric samples may have been obtained incidentally from digested meat or any of them. We think that virus isolation attempts and/or calculation of viral load should be performed in epidemiological studies involving the association of new viruses with disease.

In conclusion, this study shows that FBoV and FeChPV are present in healthy cats in Turkey, and these viruses can be detected from oropharyngeal swabs. Our findings contribute to the further investigation of the prevalence, genotype distribution, and genetic diversity of Turkish FBoVs and FeChPVs, adding to the molecular epidemiology of FBoVs and FeChPVs worldwide.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by HA, and KCS. The first draft of the manuscript was written by HA and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Code availability Not applicable.

Declarations

Ethical approval No attempt was made to adversely affect animal health or disrupt the tissue integrity of the cats selected in this study. All procedures performed in animal studies were in compliance with the institution (Fırat University Animal Experiments Local Ethics Committee), and international ethical standards.

Consent to participate All the authors consented to participate in this study.

Consent for publication All the authors consent to publication of this article.

Informed consent All the authors consent to publish. The human participants and their personal data are not included in this article. The consent of animal owners was obtained at the time of sampling.

Conflict of interest The authors declare that they have no conflict of interest.

References

Addie DD, Jarrett O (1992) A study of naturally occurring feline coronavirus infections in kittens. Vet Rec 130:133–137. https://doi.org/10.1136/vr.130.7.133

Baker KS, Leggett RM, Bexfield NH, Alston M, Daly G, Todd S, Tachedjian M, Holmes CE, Crameri S, Wang LF, Heeney JL, Suu-Ire R, Kellam P, Cunningham AA, Wood JL, Caccamo M, Murcia PR (2013) Metagenomic study of the viruses of African straw-coloured fruit bats: detection of a chiropteran poxvirus and isolation of a novel adenovirus. Virology 441:95–106. https://doi.org/10.1016/j.virol.2013.03.014

Bodewes R, Lapp S, Hahn K, Habierski A, Förster C, König M, Wohlsein P, Osterhaus AD, Baumgärtner W (2014) Novel canine bocavirus strain associated with severe enteritis in a dog litter. Vet Microbiol 174:1–8. https://doi.org/10.1016/j.vetmic.2014.08.025

Cheng W, Chen J, Xu Z, Yu J, Huang C, Jin M, Li H, Zhang M, Jin Y, Duan ZJ (2011) Phylogenetic and recombination analysis of human bocavirus 2. BMC Infect Dis 11:50. https://doi.org/10.1186/1471-2334-11-50

Chong R, Shi M, Grueber CE, Holmes EC, Hogg CJ, Belov K, Barrs VR (2019) Fecal viral diversity of captive and wild tasmanian devils characterized using virion-enriched metagenomics and metatranscriptomics. J Virol 93:e00205-e219. https://doi.org/10.1128/JVI.00205-19

Cotmore SF, Agbandje-McKenna M, Canuti M, Chiorini JA, Eis-Hubinger AM, Hughes J, Mietzsch M, Modha S, Ogliastro M, Pénzes JJ, Pintel DJ, Qiu J, Soderlund-Venermo M, Tattersall P, Tijssen P (2019) Ictv Report Consortium. ICTV virus taxonomy profile: *Parvoviridae*. J Gen Virol 100:367–368. https://doi.org/10.1099/jgv.0.001212

Di Martino B, Di Profio F, Melegari I, Sarchese V, Cafiero MA, Robetto S, Aste G, Lanave G, Marsilio F, Martella V (2016) A novel feline norovirus in diarrheic cats. Infect Genet Evol 38:132–137. https://doi.org/10.1016/j.meegid.2015.12.019

Di Martino B, Lanave G, Di Profio F, Melegari I, Marsilio F, Camero M, Catella C, Capozza P, Bányai K, Barrs VR, Buonavoglia C, Martella V (2020) Identification of feline calicivirus in cats with enteritis. Transbound Emerg Dis 67:2579–2588. https://doi.org/10.1111/tbed.13605

Di Profio F, Sarchese V, Palombieri A, Fruci P, Massirio I, Martella V, Fulvio M, Di Martino B (2021) Feline chaphamaparvovirus in cats with enteritis and upper respiratory tract disease. Transbound Emerg Dis. https://doi.org/10.1111/tbed.14032

Diakoudi G, Lanave G, Capozza P, Di Profio F, Melegari I, Di Martino B, Pennisi MG, Elia G, Cavalli A, Tempesta M, Camero M, Buonavoglia C, Bányai K, Martella V (2019) Identification of a novel parvovirus in domestic cats. Vet Microbiol 228:246–251. https://doi.org/10.1016/j.vetmic.2018.12.006

Fahsbender E, Altan E, Seguin MA, Young P, Estrada M, Leutenegger C, Delwart E (2019) Chapparvovirus DNA found in 4% of dogs with diarrhea. Viruses 11:398. https://doi.org/10.3390/v11050398

Garigliany M, Gilliaux G, Jolly S, Casanova T, Bayrou C, Gommeren K, Fett T, Mauroy A, Lévy E, Cassart D, Peeters D, Poncelet L, Desmecht D (2016) Feline panleukopenia virus in cerebral neurons of young and adult cats. BMC Vet Res 12:28. https://doi.org/10.1186/s12917-016-0657-0

Gerba CP (2009) Environmental Microbiology. In: Maier RN, Pepper IL, Gerba CP (eds) Environmentally transmitted pathogens, 2nd edn. Academic press, Massachusetts, pp 445–484. https://doi.org/10.1016/B978-0-12-370519-8.00022-5

Hoelzer K, Shackelton LA, Holmes EC, Parrish CR (2008) Within-host genetic diversity of endemic and emerging parvoviruses of dogs and cats. J Virol 82:11096–11105. https://doi.org/10.1128/JVI.01003-08

Jin Y, Cheng WX, Xu ZQ, Liu N, Yu JM, Li HY, Jin M, Li DD, Zhang Q, Duan ZJ (2011) High prevalence of human bocavirus 2 and its role in childhood acute gastroenteritis in China. J Clin Virol 52:251–253. https://doi.org/10.1016/j.jcv.2011.07.012

Kapoor A, Mehta N, Dubovi EJ, Simmonds P, Govindasamy L, Medina JL, Street C, Shields S, Lipkin WI (2012) Characterization of novel canine bocaviruses and their association with respiratory disease. J Gen Virol 93:341–346. https://doi.org/10.1099/vir.0.036624-0

Kapusinszky B, Ardeshir A, Mulvaney U, Deng X, Delwart E (2017) Case-control comparison of enteric viromes in captive rhesus macaques with acute or idiopathic chronic diarrhea. J Virol 91:e00952-e1017. https://doi.org/10.1128/JVI.00952-17

Koseki N, Teramoto S, Kaiho M, Gomi-Endo R, Yoshioka M, Takahashi Y, Nakayama T, Sawada H, Konno M, Ushijima H, Kikuta H, Ariga T, Ishiguro N (2012) Detection of human bocaviruses 1 to 4 from nasopharyngeal swab samples collected from patients with respiratory tract infections. J Clin Microbiol 50:2118–2121. https://doi.org/10.1128/JCM.00098-12

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549. https://doi.org/10.1093/molbev/msy096

Lau SK, Yeung HC, Li KS, Lam CS, Cai JP, Yuen MC, Wang M, Zheng BJ, Woo PC, Yuen KY (2017) Identification and genomic characterization of a novel rat bocavirus from brown rats in China. Infect Genet Evol 47:68–76. https://doi.org/10.1016/j.meegid.2016.11.014

Lau SKP, Woo PCY, Yeung HC, Teng JLL, Wu Y, Bai R, Fan RYY, Chan KH, Yuen KY (2012) Identification and characterization of bocaviruses in cats and dogs reveals a novel feline bocavirus and a novel genetic group of canine bocavirus. J Gen Virol 93:1573–1582. https://doi.org/10.1099/vir.0.042531-0

Li Y, Gordon E, Idle A, Altan E, Seguin MA, Estrada M, Deng X, Delwart E (2020) Virome of a feline outbreak of diarrhea and vomiting includes bocaviruses and a novel chapparvovirus. Viruses 12:506. https://doi.org/10.3390/v12050506

Lima DA, Cibulski SP, Tochetto C, Varela APM, Finkler F, Teixeira TF, Loiko MR, Cerva C, Junqueira DM, Mayer FQ, Roehe PM (2019) The intestinal virome of malabsorption syndrome-affected and unaffected broilers through shotgun metagenomics. Virus Res 261:9–20. https://doi.org/10.1016/j.virusres.2018.12.005

Lin MY, Chan HC, Chi H, Chiu SC, Nora-Krukle Z, Rasa-Dzelzkaleja S, Vilmane A, Murovska M, Lin JH, Liu HF (2020) Genetic diversity and phylogenetic analysis of human bocavirus 2 in pediatric patients with acute gastroenteritis in Taiwan. Int J Environ Res Public Health 17:1086. https://doi.org/10.3390/ijerph17031086

Liu C, Liu F, Li Z, Qu L, Liu D (2018) First report of feline bocavirus associated with severe enteritis of cat in Northeast China, 2015. J Vet Med Sci 80:731–735. https://doi.org/10.1292/jvms.17-0444

Liu X, Wang H, Liu X, Li Y, Chen J, Zhang J, Wang X, Shen S, Wang H, Deng F, Wang M, Guan W, Hu Z (2020) Genomic and transcriptional analyses of novel parvoviruses identified from dead peafowl. Virology 539:80–91. https://doi.org/10.1016/j.virol.2019.10.013

Manteufel J, Truyen U (2008) Animal bocaviruses: a brief review. Intervirology 51:328–334. https://doi.org/10.1159/000173734

Meriluoto M, Hedman L, Tanner L, Simell V, Mäkinen M, Simell S, Mykkänen J, Korpelainen J, Ruuskanen O, Ilonen J, Knip M, Simell O, Hedman K, Söderlund-Venermo M (2012) Association of human bocavirus 1 infection with respiratory disease in childhood follow-up study, Finland. Emerg Infect Dis 18:264–271. https://doi.org/10.3201/eid1802.111293

Miranda C, Thompson G (2016) Canine parvovirus: the worldwide occurrence of antigenic variants. J Gen Virol 97:2043–2057. https://doi.org/10.1099/jgv.0.000540

Ng TF, Mesquita JR, Nascimento MS, Kondov NO, Wong W, Reuter G, Knowles NJ, Vega E, Esona MD, Deng X, Vinjé J, Delwart E (2014) Feline fecal virome reveals novel and prevalent enteric viruses. Vet Microbiol 171:102–111. https://doi.org/10.1016/j.vetmic.2014.04.005

Palinski RM, Mitra N, Hause BM (2016) Discovery of a novel *Parvovirinae* virus, porcine parvovirus 7, by metagenomic sequencing of porcine rectal swabs. Virus Genes 52:564–567. https://doi.org/10.1007/s11262-016-1322-1

Parrish CR (1995) Pathogenesis of feline panleukopenia virus and canine parvovirus. Baillieres Clin Haematol 8:57–71. https://doi.org/10.1016/s0950-3536(05)80232-x

Parrish CR, Kawaoka Y (2005) The origins of new pandemic viruses: the acquisition of new host ranges by canine parvovirus and influenza A viruses. Annu Rev Microbiol 59:553–586. https://doi.org/10.1146/annurev.micro.59.030804.121059

Pénzes JJ, Söderlund-Venermo M, Canuti M, Eis-Hübinger AM, Hughes J, Cotmore SF, Harrach B (2020) Reorganizing the family *Parvoviridae*: a revised taxonomy independent of the canonical approach based on host association. Arch Virol 165:2133–2146. https://doi.org/10.1007/s00705-020-04632-4

Pfankuche VM, Bodewes R, Hahn K, Puff C, Beineke A, Habierski A, Osterhaus AD, Baumgärtner W (2016) Porcine bocavirus infection associated with encephalomyelitis in a pig, Germany. Emerg Infect Dis 22:1310–1312. https://doi.org/10.3201/eid2207.152049

Piewbang C, Kasantikul T, Pringproa K, Techangamsuwan S (2019) Feline bocavirus-1 associated with outbreaks of hemorrhagic enteritis in household cats: potential first evidence of a pathological role, viral tropism and natural genetic recombination. Sci Rep 9:16367. https://doi.org/10.1038/s41598-019-52902-2

Priestnall SL, Mitchell JA, Walker CA, Erles K, Brownlie J (2014) New and emerging pathogens in canine infectious respiratory disease. Vet Pathol 51:492–504. https://doi.org/10.1177/0300985813511130

Qiu J, Cheng F, Johnson FB, Pintel D (2007) The transcription profile of the bocavirus bovine parvovirus is unlike those of previously characterized parvoviruses. J Virol 81:12080–12085. https://doi.org/10.1128/JVI.00815-07

Reuter G, Boros Á, Delwart E, Pankovics P (2014) Novel circular single-stranded DNA virus from turkey faeces. Arch Virol 159:2161–2164. https://doi.org/10.1007/s00705-014-2025-3

Sabshin SJ, Levy JK, Tupler T, Tucker SJ, Greiner EC, Leutenegger CM (2012) Enteropathogens identified in cats entering a Florida animal shelter with normal feces or diarrhea. J Am Vet Med Assoc 241:331–337. https://doi.org/10.2460/javma.241.3.331

Soma T, Ogata M, Ohta K, Yamashita R, Sasai K (2020) Prevalence of astrovirus and parvovirus in Japanese domestic cats. J Vet Med Sci 82:1243–1246. https://doi.org/10.1292/jvms.20-0205

Stuetzer B, Hartmann K (2014) Feline parvovirus infection and associated diseases. Vet J 201:150–155. https://doi.org/10.1016/j.tvjl.2014.05.027

Takano T, Takadate Y, Doki T, Hohdatsu T (2016) Genetic characterization of feline bocavirus detected in cats in Japan. Arch Virol 161:2825–2825. https://doi.org/10.1007/s00705-016-2972-y

Tamura K, 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. https://doi.org/10.1093/oxfordjournals.molbev.a040023

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680. https://doi.org/10.1093/nar/22.22.4673

Truyen U, Addie D, Belák S, Boucraut-Baralon C, Egberink H, Frymus T, Gruffydd-Jones T, Hartmann K, Hosie MJ, Lloret A, Lutz H, Marsilio F, Pennisi MG, Radford AD, Thiry E, Horzinek MC (2009) ABCD guidelines on prevention and management. J Feline Med Surg 11:538–546. https://doi.org/10.1016/j.jfms.2009.05.002

Verbeke V, Reynders M, Floré K, Vandewal W, Debulpaep S, Sauer K, Cardoen F, Padalko E (2019) Human bocavirus infection in Belgian children with respiratory tract disease. Arch Virol 164:2919–2930. https://doi.org/10.1007/s00705-019-04396-6

Wang Y, Yang S, Liu D, Zhou C, Li W, Lin Y, Wang X, Shen Q, Wang H, Li C, Zong M, Ding Y, Song Q, Deng X, Qi D, Zhang W, Delwart E (2019) The fecal virome of red-crowned cranes. Arch Virol 164:3–16. https://doi.org/10.1007/s00705-018-4037-x

Williams SH, Che X, Garcia JA, Klena JD, Lee B, Muller D, Ulrich W, Corrigan RM, Nichol S, Jain K, Lipkin WI (2018) Viral diversity of house mice in New York City. Mbio 9:e01354-e1417. https://doi.org/10.1128/mBio.01354-17

Yang S, Liu Z, Wang Y, Li W, Fu X, Lin Y, Shen Q, Wang X, Wang H, Zhang W (2016) A novel rodent Chapparvovirus in feces of wild rats. Virol J 13:133. https://doi.org/10.1186/s12985-016-0589-0

Yi S, Niu J, Wang H, Dong G, Zhao Y, Dong H, Guo Y, Wang K, Hu G (2018) Detection and genetic characterization of feline bocavirus in Northeast China. Virol J 15:125. https://doi.org/10.1186/s12985-018-1034-3

Zhang W, Li L, Deng X, Kapusinszky B, Pesavento PA, Delwart E (2014) Faecal virome of cats in an animal shelter. J Gen Virol 95:2553–2564. https://doi.org/10.1099/vir.0.069674-0

Zhang Q, Niu J, Yi S, Dong G, Yu D, Guo Y, Huang H, Hu G (2019) Development and application of a multiplex PCR method for the simultaneous detection and differentiation of feline panleukopenia virus, feline bocavirus, and feline astrovirus. Arch Virol 164:2761–2768. https://doi.org/10.1007/s00705-019-04394-8

Ziemele I, Xu M, Vilmane A, Rasa-Dzelzkaleja S, Hedman L, Hedman K, Söderlund-Venermo M, Nora-Krukle Z, Murovska M, Gardovska D (2019) Acute human bocavirus 1 infection in child with life-threatening bilateral bronchiolitis and right-sided pneumonia: a case report. J Med Case Reports 13:290. https://doi.org/10.1186/s13256-019-2222-5