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Discovery of a human parvovirus B19 analog (*Erythroparvovirus*) in cats

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Two feral cats (from the same colony) were presented to the veterinary clinic for weakness, weight loss, and anorexia. The cats were part of a study on feline hepatotropic viruses (collection A, 43 animals). On metaviromic investigation, parvoviral reads were identified in the sera of the two cats. The feline parvovirus genome was 5.3 kb long with an organization similar to other members of the *Erythroparvovirus* genus. In the ORF1 (nonstructural proteins) and ORF2 (VP1/VP2 precursor) the feline virus displayed 43.1% and 49.1% nucleotide identity to human parvovirus B19, and 48.9% and 56.6% to chipmunk parvovirus. Sequence identity to canine/feline protoparvovirus (Protoparvovirus carnivoran 1) was as low as 36.5% % and 29.2% in the ORF1 and ORF2, respectively. Using a quantitative PCR assay, the virus was also identified in an additional ten cats (prevalence 27.6%, 12/43) from collection A and in 15/1150 (1.3%) of archival sera (collection B), revealing a higher infection rate in cats with altered hepatic markers, suggestive of hepatic distress. The findings of our study extend the list of known parvoviruses in the feline host.

Keywords Erythroparvovirus, Hepatitis, Sera, Cat

Erythroparvoviruses (EPVs) have been identified in humans (parvovirus B19), non-human primates, seals, chipmunks, and cows[1–6]. In humans, parvovirus B19 has been associated with a variety of clinical manifestations. In healthy immunocompetent children, parvovirus B19 causes infectious erythema (fifth disease), while in adults it can be associated with acute polyarthropathy[7]. Due to the tropism of parvovirus B19 to erythroid progenitor cells, the virus can cause transient aplastic crisis[8]. In immunocompromised patients, persistent B19 infection is manifested as red cell aplasia and chronic anemia[9]. Due to the immature immune system, in-utero infection can lead to fetal death, hydrops fetalis, or the development of congenital anemia[10]. In macaques, EPVs were identified in animals with anemia[2,11], while bovine parvovirus type 3 and chipmunk parvovirus have been identified in apparently healthy animals[4,5]. Upon experimental inoculation of cynomolgus macaques with simian parvovirus, macaques developed mild clinical signs including transient cessation of erythropoiesis confirming the role of this virus in the observed anemia[12]. In this study, we describe the discovery of a novel EPV in domestic cats. During a survey for hepatotropic viruses of cats, on metaviromic investigation EPV-like sequence reads were identified in the sera of two feral cats living in the same colony, co-infected with feline retroviruses and hepadnavirus. The complete genome sequence and organization of the feline EPVs (FeEPVs) were reconstructed. Using a specific quantitative assay, we searched for FeEPV DNA in collections of feline sera available in our laboratories.

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Materials and methods

Sample collection

Forty-three serum samples were collected from cats presented at the Veterinary Institute of Novara located at Granozzo con Monticello in the prefecture of Novara, Italy between 2021 and 2023 (collection A). The inclusion criteria for the samples were altered liver markers, (i.e. aspartate transaminase (AST), alanine transaminase (ALT) or bilirubin), suggestive of hepatic distress. For each animal, information on gender, age, breed, habitat status (urban or rural), indoor/outdoor lifestyle, cohabitation with other cats, signs of disease and positivity for feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) using a point-of-care test SNAP* FIV/FeLV Combo test (IDEXX Laboratories Inc, Westbrook, Maine, USA) and qPCR assays[13,14] were recorded (Supplementary File 1).

Nucleic acids extraction

Two hundred µL of the serum samples were subjected to nucleic acid extraction using a IndiSpin Pathogen Kit (Indical Bioscience GmbH, Leipzig, Germany), according to the manufacturer's instructions and stored at – 80 °C until use.

Screening for domestic cat hepadnavirus (DCH)

DCH was detected using a qPCR[15]. In detail, 10 µL of the sample DNA or plasmid standard (TOPO XL PCR with 1.4 kb of DCH polymerase) was added to 15 µL of master reaction mix using primers FHBV-for, FHBV- rev and probe FHBV-Pb (Table 1). Thermic file included activation of the iTaq DNA polymerase at 95 °C for 3 min and 42 cycles of denaturation at 95 °C for 10 s and annealing-extension at 60 °C for 30 s[15].

Metaviromic protocol

DCH-positive serum samples were subjected to metagenomic investigation, using sequence-independent single primer amplification (SISPA)-based enrichment protocols and nanopore sequencing as previously described (Table 1)[16]. The preparation of the DNA library for sequencing was performed using the Ligation Sequencing Kit

Pathogen	Target gene	Assay	Primer/probes	Sequence 5'–3'	Reference(s)
DCH	pol/surface	qPCR	FHBV- for FHBV- rev FHBV- prob	CGTCATCATGGGTTTAGGAA TCCATATAAGCAAACACCATACAAT [FAM] TCCTCCTAACCATTGAAGCCAGACTACT [BHQ1]	Lanave et al., 2019
-	-	SISPA	FR26RV-N FR40RV-T FR20RV	GCCGGAGCTCTGCAGATATCNNNNNN GCCGGAGCTCTGCAGATATCTTTTTTTTTTTTTTTTTTT GCCGGAGCTCTGCAGATATC	Di Profio et al., 2023
FeEPV	NS1	qPCR	FeEPV 985F FeEPV 1105 R FeEPV 1049 Pb	CGGATCCTGCACAAGACACT GCTCTCCATTGTGGCTCACT [FAM] TACTTTATGTCATTGGCTGGCACA [BHQ1]	This study
FeEPV	Complete genome	First round PCR (fragment 1) Second round PCR (fragment 1) First round PCR (fragment 2) Second round PCR (fragment 2) First round PCR (fragment 3) Second round PCR (fragment 3) First round PCR (fragment 4) Second round PCR (fragment 4) First round PCR (fragment 5) Second round PCR (fragment 5) First round PCR (fragment 6) Second round PCR (fragment 6)	FeEPV 68 F FeEPV 881 R FeEPV 133 F FeEPV 828 R FeEPV 590F FeEPV 440 R FeEPV 672 F FeEPV 440 R FeEPV 1442 F FeEPV 3335R FeEPV 1542 F FeEPV 3297 R FeEPV 3165F FeEPV 4202R FeEPV 3189F FeEPV 4170R FeEPV 3958F FeEPV 5175R FeEPV 3986F FeEPV 5136 R FeEPV 4640 F FeEPV 5159R FeEPV 4708 F FeEPV 5094 R	CTAGCGGAAGTGACGTCAGG GCTCCATGCATACCAACAATCATT GAAGTGACGTGGCGGATGTG TCACAAAGTCCCAATCTGACCG TGTGTTATGTCAGTTAGAACCAGG SAGCCAYTCGCGMACCTCWGT GCGAGGAATTTTAAATGGGGC SAGCCAYTCGCGMACCTCWGT TGTGGAAAGCATTAAAGCTGTT TTGCAGAAACTCGGCATCTG AGTAATGGTGATATGACCGTTGT AAGGATTTCCACCCTGGTGT CACTTACCCACTGACCGGTA AAAGTGTTGACTAGCCCCCA GGTCCTGGGAATCCTTTGGA GGGCTAGCGGTGGAAAACTA AACAAACATTGCCGGGAGAC CAAATGCTTTGTACGTGCTTTTG AGAGGTATACGAACTCCCAAGA GCTGCGTTCATAGCCATTGT GACCGAGAAATGGCAAAACAGTT CTAGCGGAAGTGACGTCAGG ACTGTAACAGAGCAGAGTAGTGC GAAGTGACGTGGCGGATGTG	This study
FeLV	Unique region 3	qPCR	FeLV_U3-exo_f FeLV_U3-exo_r FeLV_U3_probe	AACAGCAGAAGTTTCAAGGCC TTATAGCAGAAAGCGCGCG [FAM] CCAGCAGTCTCCAGGCTCCCCA [TAMRA]	Tandon et al., 2005
FIV	Gag	qPCR	FIV0771f. FIV1081r Probe FIV1010p FIV551f. FIV671r FIV581p	AGAACCTGGTGATATACCAGAGAC TTGGGTCAAGTGCTACATATTG [FAM] TATGCCTGTGGAGGGCCTTCCT [TAMRA] GCCTTCTCTGCAAATTTAACACCT GATCATATTCTGCTGTCAATTGCTTT [FAM] CATGGCCACATTAATAATGGCCGCA [TAMRA]	Leutenegger et al., 1999

Table 1. Primers and probes used in this study. DCH = Domestic cat hepadnavirus; qPCR = quantitative real time PCR; SISPA = sequence-independent single-primer amplification; FeEPV feline erythroparvovirus; FeLV = Feline Leukemia Virus; FIV = feline immunodeficiency virus.

(SQK-LSK110), according to the manufacturer's instructions. Sequencing was carried out on a Flow Cell R9.4.1 using a MinION Mk1c sequencer (Oxford Nanopore Technologies, ONT, Oxford, UK).

Sequence and phylogenetic analyses

Fastq reads obtained were analyzed through the online tool Genome Detective (https://www.genomedetective.com/db/ui/login, accessed on 9 July 2024)[17] using default parameters and a dedicated Nanogalaxy pipeline[18]. The alignment of the obtained sequences was performed using the Multiple Alignment using Fast Fourier Transform (MAFFT) plugin implemented in the Geneious Prime software Version 2022.2.2 (Biomatters Ltd., Auckland, New Zealand). The evolutionary history was inferred in MEGA X[19].

GenBank sequence submission

The nucleotide sequences of FeEPV strains ITA/2021/cat/496.1, ITA/2021/cat/496.2, ITA/2024/cat/4, ITA/2024/cat/24 and ITA/2024/cat/51 used for phylogeny were deposited in the GenBank database under accession nrs. PQ049137- PQ049141, respectively.

Design of a qPCR specific for FeEPV

A qPCR assay was designed using specific primers directed to the NS1 genomic region of FeEPV (FeEPV 985F 5′-CGGATCCTGCACAAGACACT-3′, FeEPV 1105 R 5′-GCTCTCCATTGTGGCTCACT-3′, and FeEPV 1049 Pb 5′ Fam TACTTTATGTCATTGGCTGGCACA BHQ1 3′) (Table 1). FeEPV DNA copy numbers were assessed based on standard curves generated by tenfold dilutions of a plasmid standard TOPO XL PCR containing a 500-nt fragment of the NS1 region of strain ITA/2021/cat/496.2. A total of 10 µL of either sample DNA or plasmid standard were added to the 15-µL reaction master mix (IQ Supermix; Bio-Rad Laboratories SRL, Segrate, Italy) containing 0.6 µmol/L of each primer and 0.2 µmol/L of probe. Thermal cycling consisted of activation of iTaq DNA polymerase at 95 °C for 3 min, 45 cycles of denaturation at 95 °C for 15 s, and annealing extension at 60 °C for 30 s.

The specificity of the assay was evaluated with a panel of feline DNA viruses. The qPCR detected as low as 1 $\log_{10}$ genome equivalent (GE)/mL of standard DNA and 0.99 $\log_{10}$GE/mL of DNA template extracted from clinical samples. FeEPV quantification displayed an acceptable level of repeatability over various magnitudes of target DNA concentrations[20],with coefficients of variation of intra and inter-assay ranging from 0.03% to 1.50% and 0.16% to 1.82%, respectively .

The qPCR was used to screen sera of collection A. Also, archival sera (n = 1150) of cats available in our laboratories, mostly obtained from cats for presurgical or routine clinical evaluations between 2018 and 2022 (collection B), were included in the screening.

Hemagglutination (HA) and inhibition of hemagglutination (IHA)

A 1:10 dilution of the serum of cat 496/21–2, which was likely at the peak of viremia (as quantified in the qPCR), was used as virus stock and tested for the ability to induce HA in human group O, porcine and chicken red blood cells (RBCs).

A dilution (8 HA units) of the FeEPV stock was used in IHA assay, and all the sera with low virus load were tested to assess the presence of IHA antibodies. A subset of FeEPV-negative sera was also screened in parallel.

Virus cultivation

Serum samples were centrifuged at 10,000 × g, the supernatant was filtered with 0.22-µm filters, inoculated onto freshly seeded Crandell-Reese Feline Kidney (CRFK) cells and incubated at 37 °C in 5% CO_2. Other cell lines, i.e., Flat Epithelial Atypia (FEA) and Felis catus whole fetus (FCWF-4) were also inoculated. Viral growth was evaluated through 5 serial passages, monitoring the onset of cellular cytopathic effect and testing the cell supernatant by the FeEPV-specific qPCR.

Electron microscopy (EM)

The cat sera sample with a high viral load were examined with negative-staining electron microscopy (nsEM) by using the Airfuge method as previously described[21].

Results

Screening for DCH in collection A

Out of 43, 2 (4.6%) serum samples tested positive for DCH by qPCR with a viral DNA load of 3.19 $\log_{10}$ GE/mL in cat 496/21–1 and of 7.93 $\log_{10}$ GE/mL in cat 496/21–2.

Cat 496/21–1 was a domestic short-air cat, six-year-old, spayed female, and was presented for weakness and weight loss, 15 days after the onset of the clinical signs (Supplementary File 2). Cat 496/21–2 was a 9-year-old domestic short-hair neutered cat living in the same colony as cat 496/21–1 and was presented for weakness, anorexia, and pigmenturia, 5 days after the onset of the signs (Supplementary File 2). Both cats were admitted together at the veterinary clinic for medical care, were not vaccinated, and tested negative for FIV and positive for FeLV (Supplementary File 1). FeLV proviral DNA load was 3.76 $\log_{10}$ GE/mL and 1.68 $\log_{10}$ GE/mL for cat 496/21–1 and cat 496/21–2, respectively.

Reconstruction of the genome sequence of FeEPV

Metagenomic analysis identified EPV-related contigs in the serum of a cat 496/21–2. A primer walking strategy with FeEPV-specific primers was used to close the gaps between non-contiguous sequences (Table 1). The reconstructed complete genome sequence of FeEPV strain ITA/2021/cat/496.2 was about 5.3 kb nucleotide in length with inverted repeats at the 5' and 3' terminations. The open reading frames (ORFs) overlapped by 29

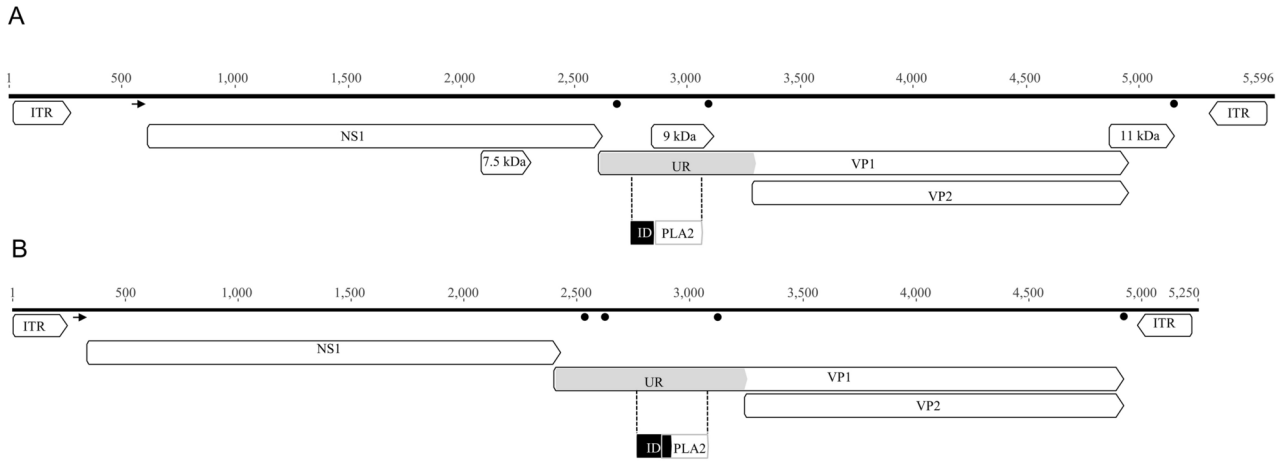

Fig. 1. Genome structure of human parvovirus B19 (panel **A**) and feline erythroparvovirus (panel **B**). Black arrow indicates p6 promoter. Black circles indicate the polyadenylation sites. Intrinsically disordered (ID) and phospholipase A2 regions, in the unique region (UR) of the VP1 of human parvovirus B19 and feline erythroparvovirus are indicated.

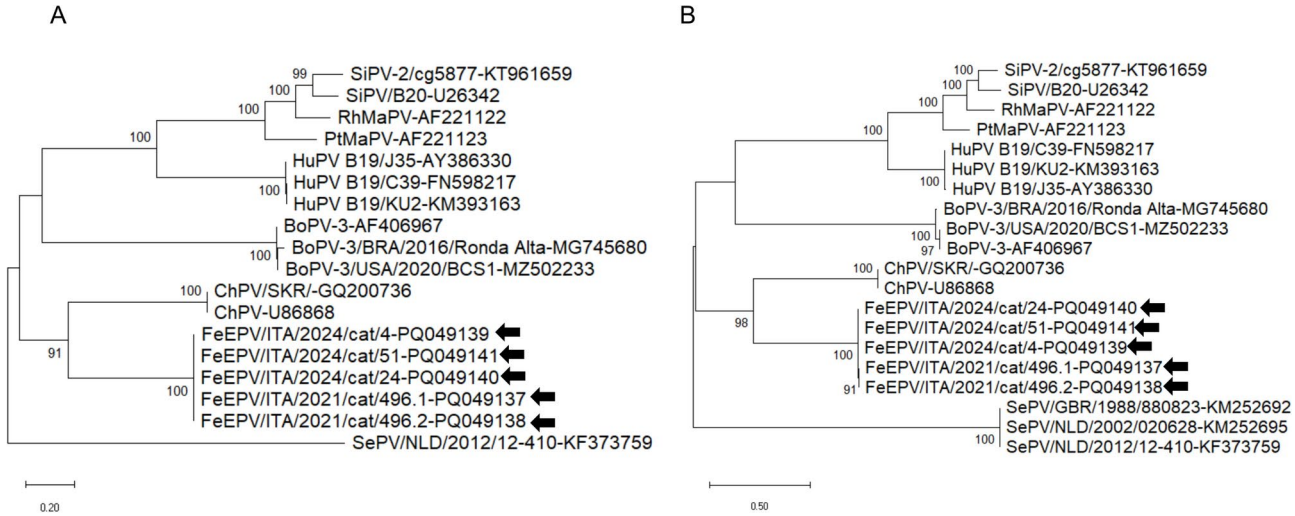

Fig. 2. Maximum likelihood phylogenetic trees of the feline erythroparvovirus identified in this study and reference strains of *Erythroparvovirus* genus recovered in the GenBank database. Complete NS (2041 nt) (panel **A**) and VP (1795 nt) (panel **B**) sequence-based phylogenetic trees were reconstructed using Tamura-Nei model (four parameters) with a gamma distribution. A total of 1,000 bootstrap replicates were used to estimate the robustness of the individual nodes on the phylogenetic tree. Bootstrap values greater than 75% were indicated. Black arrows indicate the feline erythroparvovirus strain detected in this study. The numbers of nucleotide substitutions are indicated by the scale bar. FeEPV: feline erythroparvovirus, BoPV-3: bovine parvovirus 3, ChPV: chipmunk parvovirus, HuPV B19: human parvovirus B19, PtMaPV: pig-tailed macaque parvovirus, RhMaPV: rhesus macaque parvovirus, SePV: seal parvovirus, SiPV: simian parvovirus.

nucleotides (nt) and encoded a nonstructural protein (NSP1) of 698 amino acids (aa) and a capsid protein (VP1/VP2) of 781/558 aa (Fig. 1). Regulatory signals were identified in the genome, including a consensus promoter sequence upstream of the NSP1 starting codon and polyadenylation sites after the termination of ORF1 and ORF2.

Sequence identity in the NSP1 was 48.9% nt and 42.8% aa to chipmunk parvovirus and 43.1% nt and 33.1% aa to human parvovirus B19, whilst in the VP2 it was 56.6% nt and 54.6% aa to chipmunk parvovirus and 49.1% nt and 38.6% aa to human parvovirus B19. The identity to carnivore protoparvovirus 1 was as low as 36.5% nt and 19.9% aa in the NSP1 and 37.9% nt and 19.5% aa in the VP2. The full genome of FeEPV strain ITA/2021/cat/496.1 was also obtained. On genome sequencing, the FeEPV strains ITA/2021/cat/496.1 and ITA/2021/cat/496.2 were highly related (> 99.9% nt identity) to each other. On phylogenetic analysis based on the NSP1 and VP2 nt and aa sequences, the FeEPVs segregated in a branch with chipmunk parvovirus, in a position basal to primate EPVs (Fig. 2).

In the unique region of the VP1 (VP1uR), the phospholipase A2 (PLA_2) motifs[22] were conserved, i.e. the HDXXY motif, found in the catalytic site of PLA_2[23] and the YXGXG motif found on the Ca2 + binding loop of PLA_2[23]. Upstream the PLA_2region, an intrinsically disordered (ID) region was mapped with MobiDB-lite 3.0[24]via InterProScan[25]. Interestingly, an analogous ID region was also mapped in the VP1uR of parvovirus B19 and other EPVs in or around the same VP1 portion (Fig. 1), although the mapped ID regions markedly varied.

Structural homology modeling using parvovirus B19 VP2 as a template (data not shown) and sequence alignment with other EPVs allowed for the identification in FeEPV of the conserved regions mapped in primate EPVs (at aa 160–177, 182–194, 206–216, 246–257, 511–524 and 534–543), mostly located in the canyon structure laying between the fivefold axis and the 5/twofold wall of the asymmetric unit of the viral capsid[26].

Screening for FeEPV by qPCR

Using the qPCR assay, the viral load of FeEPV in the sera of cats 496/21–1 and 496/21–2 was 4.22 $\log_{10}$ GE/mL and 10.69 $\log_{10}$ GE/mL respectively. FeEPV DNA was also detected, at lower viral load in an additional 10 cats of collection A. The overall infection rate of collection A was 27.6% (12/43) with a viral load ranging from 1.24 $\log_{10}$ GE/mL and 10.69 $\log_{10}$ GE/mL (mean = 2.94 $\log_{10}$ GE/mL, median = 2.31 $\log_{10}$ GE/mL) (Fig. 3). No statistically significant differences ($p > 0.05$). were observed according to the metadata (i.e. gender, age, breed, habitat, lifestyle, clinical signs, co-infections) available for the 12 positive animals of collection A (Table 2).

A total of 1150 sera from collection B were also screened for FeEPV. The overall infection rate observed was 1.3% (15/1150) with a viral load ranging from 1.90 $\log_{10}$ GE/mL and 8.48 $\log_{10}$ GE/mL (mean = 3.74 $\log_{10}$ GE/mL, median = 3.11 $\log_{10}$ GE/mL) (Fig. 3). The 21-fold increase in FeEPV infection rate in the subset of sera from collection A vs collection B was statistically significant ($p < 0.000001$). Viral load of the two collections displayed differences in terms of the averages of the data sets, although without statistical significance ($p > 0.05$) (Fig. 3). Only in three animals (cat 4/24, 24/24 and 51/24) the viral load was higher than 4 $\log_{10}$ GE/mL, possibly still in the peaks of viral acute phase. Cat 24/24 tested positive for FeEPV (4.96 $\log_{10}$ GE/mL) and DCH (6.97 $\log_{10}$ GE/mL). The animal was a free-roaming cat visited for a leg injury and displayed gastrointestinal signs during the recovery. The FeEPV-positive cat 51/24 (7.94 $\log_{10}$ GE/mL), was a stray animal and was co-infected with FeLV (7.28 $\log_{10}$ GE/mL). At clinical examination, cat 51/24 showed depression with respiratory signs (nasal and ocular discharge, respiratory distress, and pulmonary rattle) and acute regenerative anemia (microhematocrit:16%, range 25–45%, with anisocytosis). Mucosal swabs of cat 51/24 were also available for testing and FeEPV was detected in the ocular-conjunctival (OC) (3.39 $\log_{10}$ GE/mL) and oro-pharyngeal (OF) (2.78 $\log_{10}$ GE/mL) swabs, suggesting active virus shedding. The swabs of this cat also tested positive for feline calicivirus (FCV) (3.88 $\log_{10}$ GE/mL in the OF swab and 2.54 $\log_{10}$ GE/mL in the OC swab), feline herpesvirus (FHV) (1.45 $\log_{10}$ GE/mL in OC swab) and FeLV (4.41 $\log_{10}$ GE/mL in the OF swab and 4.54 $\log_{10}$ GE/mL in the OC swab). The FeEPV-positive animal 4/24 (8.48 $\log_{10}$ GE/mL) was a stray cat that did not display apparent signs at clinical evaluation.

The complete genome sequences (about 5.3 kb) of the FeEPV strains ITA/2024/cat/4, ITA/2024/cat/24 and ITA/2024/cat/51 were also determined, revealing a high identity (> 99.2% nt) to the FeEPV strains ITA/2021/cat/496.1 and ITA/2021/cat/496.2.

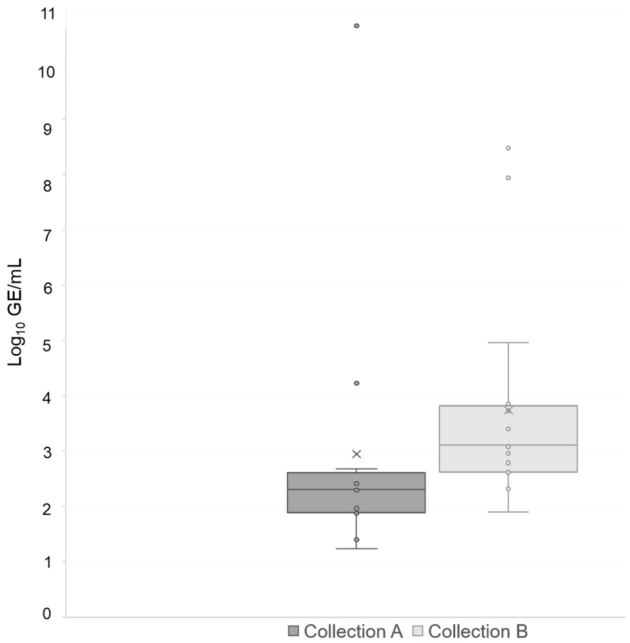

Fig. 3. Distribution of the viral load of feline erythroparvovirus positive samples of the collection A and collection B.

Variable	Category	FeEPV Pos Nr (%)	FeEPV Neg Nr (%)	df	*p*-value
Gender	M	1 (8.3)	1(3.2)	3	0.67
	F	0 (0.0)	2 (6.5)		
	MN	5 (41.7)	17 (54.8)		
	FN	6 (50.0)	11 (35.5)		
	Total	12 (100)	31 (100)		
Age	Junior (1–2 yo)	1 (8.3)	0 (0.0)	4	0.35
	Adult (3–6 yo)	5 (41.7)	9 (29.0)		
	Mature (7–10 yo)	4 (33.3)	9 (29.0)		
	Senior (11–14 yo)	0 (0.0)	13 (42.0)		
	Geriatric (≥ 15 yo)	2 (16.7)	0 (0.0)		
	Total	12 (100)	31 (100)		
Breed	DSH	11 (91.7)	23 (74.2)	6	0.21
	sacred birman	0 (0.0)	1 (3.2)		
	Abyssian	0 (0.0)	1 (3.2)		
	British SH	0 (0.0)	1 (3.2)		
	siamese	1 (8.3)	1 (3.2)		
	ragdoll	0 (0.0)	1 (3.2)		
	Maine coon	0 (0.0)	3 (9.7)		
	total	12 (100)	31 (100)		
Habitat status	urban	8 (66.7)	19 (61.3)	1	0.74
	rural	4 (33.3)	12 (38.7)		
	total	12 (100)	31 (100)		
Lifestyle	indoor	6 (50.0)	16 (51.6)	2	0.91
	outdoor	0 (0.0)	3 (9.7)		
	outdoor/indoor	6 (50.0)	12 (38.7)		
	total	12 (100)	31 (100)		
Cohabiting cats	cattery	2 (16.7)	4 (12.9)	2	0.86
	owned cats	4 (33.3)	13 (41.9)		
	none	6 (50.0)	14 (45.2)		
	Total	12 (100)	31 (100)		
Clincal signs	diarrhea	1 (8.3)	2 (6.5)	1	0.82
	jaundice	1 (8.3)	2 (6.5)	1	0.82
	lethargy	6 (50.0)	9 (29.0)	1	0.19
	strangury	0 (0.0)	1 (3.2)	1	0.84
	vomit	6 (50.0)	14 (45.2)	1	0.77
	acute kidney injury	0 (0.0)	1 (3.2)	1	0.84
	anemia	1 (8.3)	1 (3.2)	1	0.80
	anorexia	0 (0.0)	3 (9.7)	1	0.88
	ascites	1 (8.3)	1 (3.2)	1	0.49
	constipation	1 (8.3)	0 (0.0)	1	0.84
	depression	0 (0.0)	3 (9.7)	1	0.88
	diabetes	0 (0.0)	2 (6.5)	1	0.86
	head injury	0 (0.0)	1 (3.2)	1	0.84
	hyperthermia	0 (0.0)	2 (6.5)	1	0.86
	inappetence	6 (50.0)	20 (64.5)	1	0.38
	injured tail	1 (8.3)	0 (0.0)	1	0.84
	pigmenturia	1 (8.3)	0 (0.0)	1	0.84
	polydipsia	1 (8.3)	2 (6.5)	1	0.82
	polyphagia	0 (0.0)	1 (3.2)	1	0.84
	polyuria	0 (0.0)	2 (6.5)	1	0.86
	urethral obstruction	0 (0.0)	1 (3.2)	1	0.84
	weightloss	0 (0.0)	2 (6.5)	1	0.86
Time of the onset of clinical signs	< 5 days	3 (25.0)	11 (35.5)	1	0.51
	≥ 5 days	9 (75.0)	20 (64.5)		
	Total	12 (100)	31 (100)		

Continued

Variable	Category	FeEPV Pos Nr (%)	FeEPV Neg Nr (%)	df	*p*-value
Co-infection with FIV	Pos	1 (8.3)	1 (3.2)	2	0.64
	Neg	7 (58.3)	22 (71.0)		
	NA	4 (33.3)	8 (25.8)		
	Total	12 (100)	31 (100)		
Co-infection with FeLV	Pos	2 (16.7)	2 (6.5)	2	0.45
	Neg	6 (50.0)	21 (67.7)		
	NA	4 (33.3)	8 (25.8)		
	Total	12 (100)	31 (100)		
Co-infection with DCH	DCH Pos	2 (16.7)	0 (0.0)	1	0.12
	DCH Neg	10 (83.3)	31 (100)		
	Total	12 (100)	31 (100)		

Table 2. Inferential statistics testing the association between the identification of feline erythroparvovirus and different variables of the samples of the collection A. Df: Degree freedom; M: Male; F: Female; MN: Male neutered; FN: Female neutered; NA not available; SH: Short hair; DSH: Domestic short hair; FIV Feline Immunodeficiency virus, FeLV Feline leukemia virus, DCH domestic cat hepadnavirus, FeEPV feline erythroparvovirus.

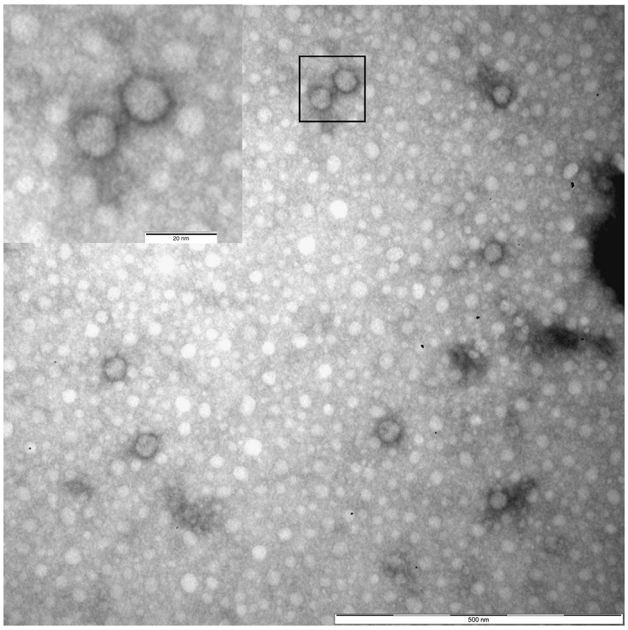

Fig. 4. Electron microscopy observation of parvovirus-like particles from cat serum samples. Negative staining (NaPT, 7.2 pH) microphotograph of some dispersed 25–27 nm rounded particles. Bar = 500 nm.

HA and IHA

FeEPV was found to hemagglutinate human group O RBC (pH 7.0 and T = 4 °C), with a hemagglutination (HA) titer of 1:640, whilst HA activity was not observed with porcine and chicken RBCs.

IHA antibodies were detected in 6/14 (42.8%) low-viremic (less than 4 $\log_{10}$ GE/mL) sera and 3/17 (17.6%) non-viremic sera with the titers reaching 1: 320.

Attempts of in vitro adaptation

Attempts were made to infect feline cells with FeEPV strains monitoring virus load by the specific qPCR. However, despite several attempts in different cell lines (CRFK, FEA, and FCWF-4), the virus did not replicate in these substrates.

Identification of parvovirus-like particle by EM observation

On electron microscope observation, typical parvovirus-like particles were visible by negative.

staining. Since the feline virus was related to members of the *Erythroparvovirus* genus, a human serum with antibodies for parvovirus B19 was used in immuno-electron microscopy. However, the virus was not immuno-precipitated using the control human serum (QED Bioscience Inc, San Diego, California, USA) (Fig. 4).

Discussion

During a study for hepatotropic viruses in cats, a novel parvovirus was identified in two cats living in the same colony in the Alps mountains. On sequence and phylogenetic analysis, the novel parvovirus was more related to members of the genus *Erythroparvovirus* (Fig. 2). The colony's cats were free to roam the mountain areas. To understand if FeEPV was a sporadic spillover from wildlife or if it was also present in other cats, we screened nearly 1200 feline sera of various geographical areas, and FeEPV was detected in 12/43 sera of collection A and 15/1150 animals of collection B.

Several parvoviruses belonging to different taxa have been identified in cats[27–32]but carnivore protoparvovirus 1 (including feline parvovirus, FPV, and canine parvovirus, CPV) is the only known parvovirus associated with diseases (enteritis and cerebellar ataxia) in cats[33]. The findings of our study extend the list of known parvoviruses in the feline host. Since EPVs have been associated with a broad range of clinical signs in human and non-human primates, alone or in conjunction with immuno-suppressive pathogens/conditions[7–12,34], it will be important to investigate if FeEPV also has a pathogenic role in cats. For instance, we observed a statistically significant disproportion in terms of infection rates in the collection of sera obtained from cats with altered hepatic markers (collection A). This possible association with liver tropism should be confirmed in larger serological and virological studies in cohorts of animals.

Of interest, the low infection rate of FeEPV observed in feline sera from collection B (archival samples) is similar to the infection rates of B19 parvovirus reported in healthy persons. On screening of blood donors, the prevalence of B19 DNA has been found to range from 0.03–0.6%, while the seroprevalence in different countries varied from 9.78 to 79.1%[35–38]. The low prevalence rates and low viral titers in collection B would be, therefore, consistent with acute infections in healthy cats, detected during either the lingering decline (the tail) of viremia or in the initial replicative phase.

An intriguing feature of our analysis was the identification of a potential ID region in the VP1uR of FeEPV. Although ID regions and proteins fail to acquire distinctive 3D structures, they retain essential functions for the control of cellular signaling networks, regulation of various biological processes, and disease-related pathways[39]. Previous studies have established that ID proteins/regions in viruses play diverse roles, such as adaptation to their host, oncogenicity, binding to host cells, and virus replication and pathogenesis[40]. The N-terminus of the VP1uR in FeEPV was longer and poorly conserved in comparison to the counterpart regions of human and nonhuman primate EPVs, where a receptor binding region has been mapped[41].

By homology modeling, we were able to reconstruct the structure of the VP2 using human B19 virus as a template. The reconstructed structure of FeEPV appeared highly conserved to other EPVs. The sequence conservation in the canyon of the viral capsid has been related to the utilization of similar receptor molecules. Also, the conserved regions aa 511–524 and aa 534–543 flank an insertion unique to EPVs. The amino acid sequence of this unique insertion differs greatly between human parvovirus B19 and the animal counterparts, including FeEPV. Since residues known to be involved in the control of the host range of FPV (amino acids 564 and 568)[42]are at about the same site, this variable region has been hypothesized to modulate the host range of EPVs[26].

As observed for human parvovirus B19, FeEPV was able to hemagglutinate human group O RBCs. The ability to hemagglutinate human group O RBCs has been related to the tropism of parvovirus B19 and of other primate EPVs to the erythroblastoid cell line that seems the main target of parvovirus B19 replication[43]. We developed a IHA assay, using the virus present in the serum of cat 496/21–2 and we found IHA antibodies (with titers up to 1: 320) in low-viremic and non-viremic sera. The rationale of testing sera with low virus load, for the presence of FeEPV-specific IHA antibodies, relies on the parallelism with human parvovirus B19, since the decline of parvovirus B19 titer after acute infection is related to the appearance of serum IgM and IgG[9,44]. IHA screening of the sera had several limitations. Interpreting the results of IHA is challenging since several factors can confound the reaction. For instance, testing human sera with or without parvovirus B19-specific IgG and/or IgM, using parvovirus B19 in IHA, has identified non-specific inhibition (titers > 1:80), non-removable by standard treatments, including receptor destroying enzyme, and a role of non-specific IgMs has been hypothesized[45]. Also, low-viremic sera could be of cats at either the initial or terminal phase of infection, with a different immunological status. Finally, since we could not cultivate the virus, we used as stock virus for the IHA experiments aliquots of the sera of cat 496/21–2 which was likely at the peak of viremia, and we could only screen a limited number of sera.

B19 parvovirus has been cultivated in cells from bone marrow and primary cells from fetal liver in the presence of erythropoietin hormone (EPO)[46]. Likewise, seal EPV has been cultivated on bone marrow cells supplemented with EPO[3]. Overall, cultivation of EPVs is fastidious[47] and this could account for our failure to adapt the virus to growth in vitro.

In conclusion, we identified a novel EPV in domestic cats and obtained evidence that this virus is common in the feline population. Since human parvovirus B19 is associated with several clinical signs/pathologies[2,7–11,34], it will be interesting to understand if FeEPV also has a pathogenic role in cats.

Data availability

Sequence data of the FeEPV strains ITA/2021/cat/496.1, ITA/2021/cat/496.2, ITA/2024/cat/4, ITA/2024/cat/24 and ITA/2024/cat/51 used for phylogeny and supporting the findings of this study have been deposited in the GenBank database under accession nrs. PQ049137- PQ049141. Any additional data or materials related to this study are available from the corresponding author upon reasonable request.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics statement

All applicable international, national and institutional guidelines for the care and use of animals were followed. The study is reported in accordance with ARRIVE guidelines (https://arriveguidelines.org). The study was approved by the Ethics Committee of the Department of Veterinary Medicine, University of Bari (Authorization 23/2018).

Additional information

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