

Article

Detection and Characterization of *Protoparvovirus carnivoran 1* in Blood Samples from Domestic Cats in Southern Brazil

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Abstract

Feline panleukopenia is a viral disease of domestic cats caused by *Protoparvovirus carnivoran 1*. This virus species is generally classified into feline parvovirus (FPV) and canine parvovirus type 2 (CPV-2) according to phylogenetic characteristics and association with diseases in the animal hosts. Despite the availability of vaccines, feline panleukopenia has been reported in cats from Brazil. This study aimed to detect and characterize *Protoparvovirus carnivoran 1* in blood samples from 368 domestic cats in southern Brazil. Total DNA was extracted from these samples, and FPV/CPV-2 detection was performed using a specific real-time polymerase chain reaction (qPCR) assay. The viral protein 2 (VP2) gene was further sequenced to identify the FPV/CPV-2 genotype. The results showed nine blood samples (2.45%) testing positive for *Protoparvovirus carnivoran 1*. Partial VP2 gene was successfully sequenced in six of these positive samples. A phylogenetic analysis with a large dataset classified all protoparvoviruses into three main clades: A, B, and CPV. Four of the viruses sequenced in this study (IDs 9917, 9919, 9927, and 9936) were classified in clade A, one (ID 9944) in clade B, and one (ID 9930) in clade CPV. Furthermore, three FPVs from clade A showed total nucleotide identity among themselves and also with field FPV sequences, while two of them (IDs 9936 and 9944) showed high nucleotide identity with two different modified live virus vaccines (from Clades A and B, respectively). These findings suggest that domestic cats are infected by field-like and/or vaccine-related FPV strains, as well as CPV-2 in southern Brazil.

Keywords: canine parvovirus; feline parvovirus; molecular epidemiology

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1. Introduction

Feline panleukopenia is a severe systemic viral disease in domestic cats caused by feline parvovirus (FPV), a small, non-enveloped, single-stranded DNA virus approximately 25 nm in diameter [1,2]. FPV is very closely related to canine parvovirus type 2 (CPV-2), which causes a highly contagious and often fatal disease in dogs. These viruses are resistant to environmental conditions and can remain infective for up to one year

under favorable conditions, contributing to their widespread dissemination [1,3–5]. FPV is highly infectious and pathogenic to domestic cats, especially kittens, and unvaccinated or immunocompromised individuals, with mortality rates reaching up to 90% in untreated cases [3–7]. Transmission occurs primarily via the fecal–oral route, either through direct contact with infected animals or, more commonly, indirectly via contaminated fomites, given the virus’s environmental resistance [5,6].

Taxonomically, FPV and CPV-2 belong to the family *Parvoviridae* and species *Protoparvovirus carnivoran 1* [8]. The viral genomes have approximately 5100 bases in length, including genes for two non-structural proteins (*NS1* and *NS2*) and two structural capsid proteins (*VP1* and *VP2*) [1]. *VP2* gene encodes the major capsid component, constituting about 90% of its structure, and serves as the main determinant of the virus’s antigenicity, pathogenicity, and host range [1,2].

FPV was first described in the late 1920s [9], nearly one century ago, whereas CPV-2 was identified as a dog pathogen later, in the 1970s. This canine-adapted virus likely originated from an FPV-like ancestor infecting wild carnivores. With species spillover, there was a major global spread of CPV-2 to canine hosts in the 1980s and 1990s [10,11].

Despite their close genetic relationship, FPV and CPV-2 differ by a few critical amino acid substitutions in the *VP2*. The most relevant changes include residues at positions 80 (Lys→Arg), 93 (Lys→Asn), 103 (Val→Ala), 323 (Asp→Asn), 564 (Asn→Ser), and 568 (Ala→Gly), which have been shown to play important roles in receptor recognition and host adaptation [10–13]. In addition, phylogenetic analyses of these viruses have provided valuable insights into viral evolution, including mutation rates, recombination patterns, and geographic dispersal dynamics [12,14,15].

FPV and CPV-2 have tropism for cells with high mitotic activity, such as those in the intestinal epithelium, bone marrow, and lymphoid tissues [1,4,5]. In cats, this viral characteristic results in the destruction of leukocytes and severe immunosuppression [1,4,5]. Clinical signs appear 2 to 10 days post-infection and include fever, lethargy, anorexia, vomiting, and diarrhea, often accompanied by dehydration and pronounced leukopenia [5]. Feline panleukopenia control and prevention are usually assessed with proper immunization of the kittens with modified live vaccines. However, this disease remains endemic worldwide, with recurrent outbreaks, mainly in unvaccinated cat populations or in high-density, stressful environments such as shelters and colonies [5,16].

The present study investigated the molecular occurrence of FPV in blood samples from an urban population of domestic cats in southern Brazil. Additionally, phylogenetic analyses of partial *VP2* sequences from FPV and CPV-2 strains were performed to characterize viral diversity and identify the main circulating lineages.

2. Materials and Methods

2.1. Sampling Population

This study was based on a previously sampled domestic-cat population [17,18], including 368 domestic cats treated in nine different veterinary clinics located in Caxias do Sul (Rio Grande do Sul, southern Brazil), between March 2021 and March 2023. The study was approved by the Animal Use Ethics Committee (CEUA) of the University of Caxias do Sul (protocol no. 006/2022).

2.2. Sample Collection and Processing

Owners provided written informed consent and completed a questionnaire collecting information on age, sex, reproductive status, vaccination history, and clinical signs. Some additional clinical data were obtained from veterinary record forms. The main recorded diseases in the animals included lymphoma, leukemia, non-hematopoietic ne-

oplasms, anemia, respiratory diseases, dermatological conditions, and feline lower urinary tract disease (FLUTD). Based on all this information, the veterinarians who treated the cats performed a general assessment of each animal, classifying them as apparently healthy or sick, and also as lethargic or alert.

Blood samples were collected via venipuncture (cephalic or jugular vein), obtaining 1 mL per animal, which was divided into two 0.5 mL microtubes containing EDTA and maintained refrigerated until processing as previously described [17]. Briefly, all samples were centrifuged (12,000 rpm for 3 min) to separate the main layers of the blood components: red blood cells at the bottom, buffy coat (containing the various white blood cells and platelets) in the middle, and the blood plasma at the top of the tube. These different blood layers were removed with a micropipette to be stored in separate cryotubes in a freezer (−20 °C) for all laboratory analyses.

2.3. DNA Extraction and qPCR

Viral DNA was extracted using the commercial NewGene® Prep and PreAmp kits, following the manufacturer's instructions (Simbios Biotecnologia, Cachoeirinha, Brazil). The qPCR assay was performed using the NewGene® FPVamp master mix, also according to the manufacturer's instructions (Simbios Biotecnologia, Cachoeirinha, Brazil). Briefly, all assays were conducted with an initial denaturation at 95 °C for 3 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s (fluorescence was collected at the end of each amplification cycle), and carried out on the Step One Plus™ Real-Time PCR System (Life Technologies Corporation, Carlsbad, CA, USA). FPV detection was considered positive for samples exhibiting a sigmoidal amplification curve and a cycle threshold (Ct) value of 38 or lower, according to the manufacturer's validation criteria. Borderline samples were retested to confirm the results. Positive and negative controls were included in all reactions.

2.4. DNA Sequencing

Partial VP2 gene amplification was performed by PCR prior to nucleotide sequencing, as previously described [19]. Sequencing was carried out using two pairs of PCR primers: Hfor (5'-CAGGTGATGAATTTGCTACA-3') and Hrev (5'-CATTTGGATAAACTGGTGGT-3'), corresponding to positions 3556–4183 of the CPV genome, and 555for (5'-CAGGAAGATATCCAGAAGGA-3') and 555rev (5'-GGTGCTAGTTGATATGTAATAAACA-3'), corresponding to positions 4003–4585. These primer pairs amplify fragments of approximately 629 bp and 583 bp, respectively, including the primer sequences. Sample amplification for sequencing was conducted using the following cycling program: initial denaturation at 95 °C for 3 min, followed by 40 cycles of 95 °C for 20 s, annealing at 50 °C for 40 s, and extension at 72 °C for 60 s. PCR-amplified products were purified using the NewGene® PCR Purification Kit according to the manufacturer's instructions (Simbios Biotecnologia, Cachoeirinha, Brazil).

Sanger sequencing was performed using the same forward/reverse PCR primers above and the BigDye Terminator v.3.1 cycle sequencing kit (Applied Biosystems, Waltham, MA, USA). Sequencing reactions were carried out on the Veriti 96 thermocycler under the same cycling conditions as above (Applied Biosystems, Waltham, MA, USA). All samples were purified by ethanol-EDTA-sodium acetate precipitation, and the DNA pellets were resuspended in Hi-Di formamide, denatured at 95 °C for 2 min, and loaded onto the automated ABI 3500 XL Genetic Analyzer (Applied Biosystems, Waltham, MA, USA). Nucleotide sequences were obtained using Data Collection v.1.0.1, and electropherograms were analyzed with Sequencing Analysis v.5.3.1 (Applied Biosystems, Waltham, MA, USA). Sequence quality was assessed by visual inspection of chromatograms, and low-quality bases at the 5' and 3' ends were trimmed prior to assembly.

Forward and reverse sequences were assembled into consensus sequences using Geneious v.2024.0 (Biomatters, Newark, NJ, USA), and ambiguous nucleotide positions were manually reviewed and corrected when necessary. The 555 and H regions were assembled, generating an amplicon of approximately 950 bp within the VP2 gene (1755 bp). The nucleotide sequences generated in this study were deposited in GenBank under accession numbers PZ338780–PZ338785 (Table S3).

2.5. Phylogenetic Analysis

Complete nucleotide sequences of the VP2 gene of FPV and CPV-2 were obtained from GenBank (<https://ncbi.nlm.nih.gov/genbank/>; accessed on 1 November 2025) to compose two distinct datasets for comparative analysis. The first dataset (Table S1) was constructed for initial FPV and CPV-2 identification. It comprised 515 sequences, including FPV vaccine strains ($n = 7$), other FPVs ($n = 465$), CPV-2 ($n = 1$), CPV-2a ($n = 11$), CPV-2b ($n = 10$), CPV-2c ($n = 4$), and unclassified *Protoparvovirus* ($n = 17$) according to previously published [15]. The second dataset (Table S2) was obtained from a previous study [20] and was used for CPV-2-specific analysis. It included 659 sequences: CPV-2 ($n = 26$), CPV-2a ($n = 332$), CPV-2b ($n = 81$), and CPV-2c ($n = 220$). Selected sequences corresponded to samples obtained from felines, canines, other animal hosts, and vaccine strains, with associated information on country and year of collection. The same sequence datasets previously described [15,20] were used here, and no additional filtering of identical or highly similar sequences was performed prior to phylogenetic analysis.

Additionally, the six partial VP2 gene sequences generated in this study were included in both datasets (Table S3). Reference VP2 data and sequences generated in this study were trimmed to the same length (approximately 950 bp). All datasets were aligned using the MAFFT multiple sequence alignment software version 7 according to previously described [21]. Phylogenetic trees were reconstructed using the maximum likelihood (ML) method implemented on the W-IQ-TREE web server [22]. The optimal nucleotide substitution model was selected using ModelFinder [23], and 1000 ultrafast bootstrap replicates were performed [24]. Final phylogenies were edited in FigTree v.1.4.4 (available at <http://tree.bio.ed.ac.uk/software/figtree/>; accessed on 18 November 2025).

Based on the generated alignment, key VP2 amino acid residues discriminating between viral types FPV and CPV-2 (positions 267, 297, 300, 305, 323, 324, 564, 568) and among CPV types 2a, 2b, and 2c (positions 300, 305, 426) were analyzed according to previously described [10–13].

2.6. Statistical Analysis

Clinical and molecular data were analyzed using IBM SPSS® software, version 23.0 (IBM Corporation, Armonk, NY, USA). Associations between *Protoparvovirus carnivoran 1* detection and categorical epidemiological or clinical variables, including sex, reproductive status, vaccination status, health condition, behavior, anemia, respiratory disease, and feline lower urinary tract disease (FLUTD), were evaluated using Pearson's chi-square test or Fisher's exact test when expected cell counts were lower than 5. Age distributions between positive and negative cats were compared using descriptive statistical analysis. Odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated when applicable. A significance level of 5% ($p < 0.05$) was adopted for all analyses.

3. Results

3.1. FPV Detection

Nine (2.45%) cats tested positive for FPV by qPCR. In an initial analysis of some generic epidemiological data, no significant associations were observed between viral detection and sex, reproductive status, vaccination, health condition, and behavior (Table 1). The overall prevalence was 3.0% in males and 1.8% in females. Among intact cats, 1.8% tested positive compared to 2.4% in neutered animals. Protoparvoviruses were detected in 2.0% of unvaccinated and 2.0% of vaccinated cats. Sick cats showed a prevalence of 2.0%, compared to 2.5% among apparently healthy animals. Likewise, 3.2% of lethargic and 2.3% of alert cats had positive results.

Age distribution of FPV-positive and negative cats was also evaluated (Figure 1). Positive cats were younger, with a median age of 1.5 years and lower variability (estimated standard deviation: 1.2 years). In contrast, negative cats exhibited a wider age range, with a median of 4 years (standard deviation: approximately 4 years), including several outliers up to 18 years of age. Despite this apparent difference in age distribution between groups, the association was not statistically significant ($p = 0.119$).

In the analysis of the clinical signs of the nine positive cats, two presented anemia, one had respiratory disease, and another had urinary disease (FLUTD). In addition, four cats were considered apparently healthy at the time of sampling, while the clinical information was missing for the other FPV-positive animal.

Table 1. General variables and FPV positivity in domestic cats.

Variable	FPV Positive <i>n</i>	%	FPV Negative <i>n</i>	%	Crude OR (95% CI)	<i>p</i>
Sex						
Male	6	3.0	195	97.0	1.6 (0.41–6.83)	0.519 [‡]
Female	3	1.8	164	98.2	1.0 Ref.	
Reproductive status *						
Intact	2	1.8	112	98.2	0.7 (0.14–3.68)	0.705 [‡]
Neutered	6	2.4	246	97.6	1.0 Ref.	
Vaccination **						
Unvaccinated	4	2.0	183	98.0	0.9 (0.22–3.75)	>0.999 [‡]
Vaccinated	4	2.0	169	98.0	1.0 Ref.	
Health condition ***						
Apparently sick	4	2.0	199	98.0	0.7 (0.19–3.18)	0.735 [‡]
Apparently healthy	4	2.5	156	97.5	1.0 Ref.	
Behavior ****						
Lethargic	2	3.2	61	96.8	1.3 (0.28–6.83)	0.656 [‡]
Alert	7	2.3	296	97.7	1.0 Ref.	

OR: Odds Ratio; Ref.: reference; 95% CI: 95% Confidence Interval; Test: [‡] = Fisher's Exact Test; * Missing data = 2; ** Missing data = 8; *** Missing data = 5; **** Missing data = 2.

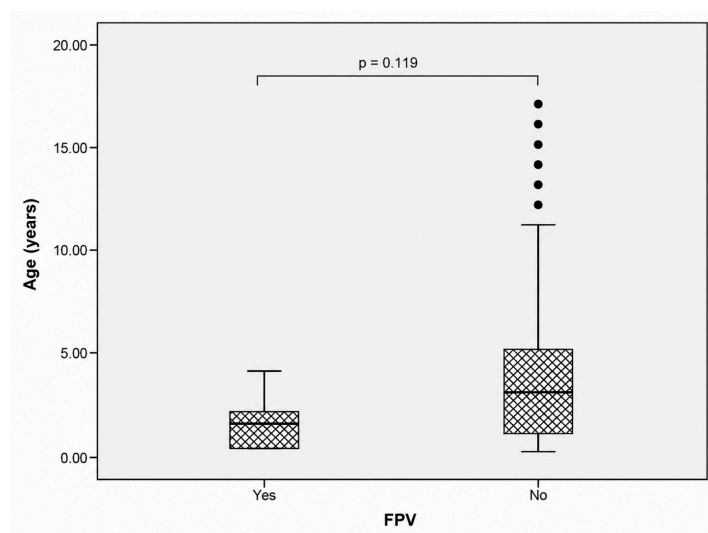


Figure 1. Comparison of age (years) between cats positive (Yes) and negative (No) for FPV. Boxes represent the interquartile range (IQR), the central line indicates the median, and whiskers extend to values within $1.5 \times \text{IQR}$. Black dots represent outliers. Statistical difference assessed using the Mann–Whitney test ($p = 0.119$).

3.2. Phylogenetic Relationships of *Protoparvovirus carnivoran 1*

Protoparvovirus carnivoran 1 VP2 gene could be sequenced in six of the nine positive samples. The other three samples showed low viraemia (less than 1000 DNA copies/reaction), as demonstrated by the high Ct values (>30) in the qPCR (Table S3). Consequently, all three were negative in the PCR assay performed to amplify the larger fragments of the VP2 gene required for sequencing. The phylogenetic analysis of the dataset, comprising 515 *Protoparvovirus* sequences from public databases, together with the six sequences obtained in this study, revealed three well-distinct clades, here referred to as Clades A, B, and CPV (Figure 2).

Clade A comprised 130 publicly available sequences, five FPV vaccine sequences, and four sequences generated in this study (IDs 9917, 9919, 9927, and 9936). The publicly available FPV sequences originating from Asia ($n = 39$), Oceania ($n = 36$), North America ($n = 25$), Europe ($n = 18$), South America ($n = 10$), and Africa ($n = 1$), with one sequence of unknown geographic origin. They were isolated between 1964 and 2021 from both domestic and wild animals (Figure 2 and Table S1). Three FPV samples sequenced here (IDs 9917, 9919, and 9927) showed 100% nucleotide identity among themselves and clustered with eight FPV samples previously sequenced from domestic cats. The other sample (ID 9936) clustered with 15 sequences from Oceania (*Felis catus*), two sequences from North America (*Lynx rufus* and *Procyon lotor*), one from Europe (*Felis catus*), and four vaccine strains (M24002_vaccine_USA_1988, D88287_FPV_vaccine_Japan_1996, EU498681_FPV_vaccine_PfizerFelocell, and EU498680_FPV_vaccine_PurevaxMerial), showing 99.9% identity, suggesting a possible vaccine-derived origin for this strain (Figure 2 and Table S5).

Clade B comprised 334 publicly available sequences, two FPV vaccine sequences, and one sequence generated in this study (ID 9944). The publicly available sequences originated from Asia ($n = 178$), Europe ($n = 79$), Oceania ($n = 70$), North America ($n = 4$), and Africa ($n = 1$), two seven sequences of unknown geographic origin, and were isolated between 1964 and 2020 from both domestic and wild animals. The here sequenced sample (ID 9944) clustered with two sequences from Oceania (*Felis catus*) and one vaccine strain (OQ615264_FPV_vaccine_Nobivac), showing 100% identity within the analyzed partial VP2 fragment.

CPV comprised 45 publicly available sequences and one sequence generated in this study (ID 9930). The public sequences included CPV-2 ($n = 1$), CPV-2a ($n = 11$), CPV-2b ($n = 10$), CPV-2c ($n = 4$), FPV ($n = 2$), and *Protoparvovirus* ($n = 17$) (Figure 2 and Table S1). This finding suggests that the sample ID 9930 shares genetic similarity with CPV lineages. To further explore this result, this sequence was included in a dataset composed exclusively of CPV sequences [20]. This additional phylogenetic analysis revealed that sequence ID9930 clustered within clade W.1, together with CPV-2a sequences ($n = 11$) isolated in Brazil between 2017 and 2020 (Table S2 and Figure S1).

Amino acid comparison of the VP2 protein revealed that the samples IDs 9917, 9919, 9927, 9936, and 9944 showed amino acid patterns consistent with FPV reference strains, characterized by the residues Asp (D), Asp (D), Tyr (Y), Asn (N), and Ala (A) at positions 305, 323, 324, 564, and 568, respectively. Notably, the unique variable amino acid spot among these samples was 562, with Val (V) in three (IDs 9917, 9919, and 9927) and Leu (L) in the other two (IDs 9936 and 9944) samples. Also, these sequences did not display any of the amino acid substitutions typically associated with CPV variants. In contrast, sample ID 9930, which clustered within CPV-2a, exhibited the amino acids Gly (G), Tyr (Y), Ile (I), Ser (S), and Gly (G) at the same positions, as well as Asn (N) at position 426, an important key marker of CPV-2a. However, this same sample presented Asp (D) at position 323, similarly to FPV strains (Table 2).

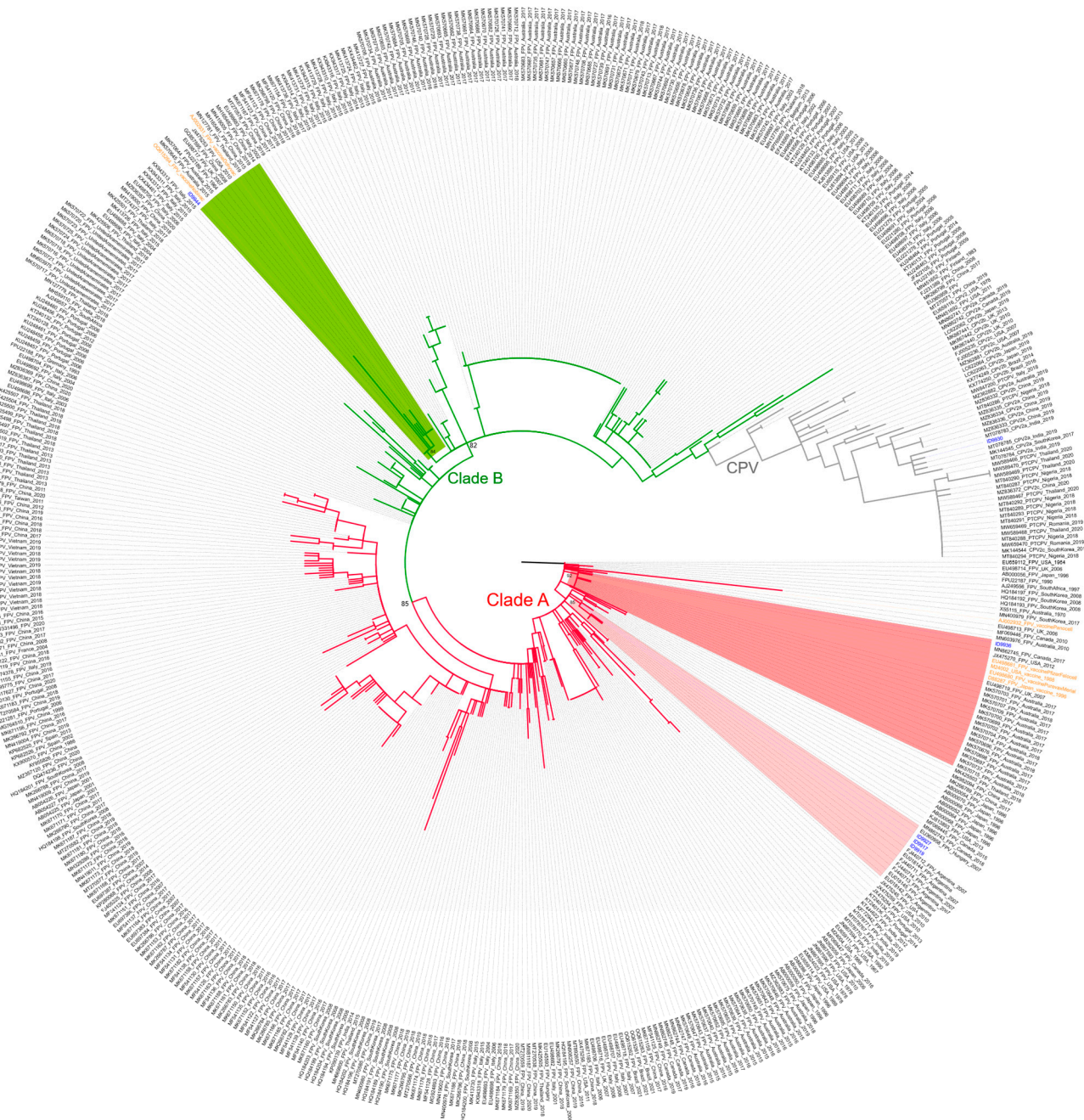


Figure 2. Maximum likelihood phylogeny based on nucleotide partial VP2 gene sequences of FPV and CPV-2. Blue labels show the sequences generated in this study, while orange labels indicate the FPV vaccine strains. The dataset also includes FPV, CPV-2, CPV-2a, CPV-2b, CPV-2c, and other CPV sequences (Table S1). FPV and CPV sequences are classified into clades A (red), B (green), and CPV (gray). The main bootstrap values are indicated at the nodes.

Table 2. Amino acids in the VP2 protein of feline and canine parvovirus strains, as well as vaccine samples, at key positions.

Clade	Subtype	Origin	Sequence	VP2 Amino Acid Position														
				267	297	300	305	323	324	340	411	419	426	434	440	562	564	568
A	-	Animal	AB054225	F	S	A	D	N	Y	A	E	N	N	D	T	V	N	A
A	-	Animal	M24004	F	S	A	D	D	Y	A	E	N	N	D	T	V	N	A
A	-	Vaccine	AJ002932	F	S	A	D	D	Y	A	E	N	N	D	T	V	N	A
A	-	Vaccine	M24002	F	S	A	D	D	Y	A	E	N	N	D	T	L	N	A
A	-	Animal	ID9917	F	S	A	D	D	Y	A	E	N	N	D	T	V	N	A
A	-	Animal	ID9919	F	S	A	D	D	Y	A	E	N	N	D	T	V	N	A
A	-	Animal	ID9927	F	S	A	D	D	Y	A	E	N	N	D	T	V	N	A
A	-	Animal	ID9936	F	S	A	D	D	Y	A	E	N	N	D	T	L	N	A
B	-	Vaccine	OQ615264	F	S	A	D	D	Y	A	E	N	N	D	T	L	N	A
B	-	Vaccine	AJ002931	F	S	A	D	D	Y	A	A	N	N	D	T	L	N	A
B	-	Animal	ID9944	F	S	A	D	D	Y	A	E	N	N	D	T	L	N	A
CPV	CPV-2	Animal	EU659116	F	S	A	D	N	Y	A	E	N	N	D	T	V	S	G
CPV	CPV-2a	Animal	MW648348	Y	S	G	Y	N	I	A	E	N	N	D	T	V	S	G
CPV	CPV-2a	Animal	ID9930	Y	A	G	Y	D	I	A	E	N	N	D	A	V	S	G
CPV	CPV-2b	Animal	KX774249	F	A	G	Y	N	L	A	E	N	D	D	T	V	S	G
CPV	CPV-2c	Animal	MZ836372	Y	A	G	Y	N	I	A	E	N	E	D	T	V	S	G

VP2: viral protein 2; CPV: canine parvovirus; Samples with the ID prefix were sequenced in this study. Amino acid residues are shown using the standard one-letter amino acid nomenclature.

4. Discussion

Protoparvovirus carnivoran 1 is an important pathogen for the health of dogs and cats, responsible for systemic diseases with high morbidity and mortality [7,25,26]. In domestic cats, this parvovirus infection primarily manifests as an acute enteric disease characterized by diarrhea, vomiting, and dehydration, often leading to death in young or immunocompromised animals [1]. Viral particles are detected in large quantities in the feces of these acutely infected animals, both symptomatic and asymptomatic [27].

In this study, blood DNA samples from a previously sampled population of domestic cats were tested for the presence of *Protoparvovirus carnivoran 1*, similarly to two other studies [28,29]. Nine cats (2.45%) tested positive in this analysis, including four asymptomatic animals. This result demonstrates that domestic cats can have this virus circulating in their blood, even in the absence of clinical manifestations. However, the frequency of this parvovirus observed here was low. Studies based on serological assays have reported anti-FPV prevalence above 25% and exceeding 90% in certain populations of unvaccinated adult cats [5,30–32]. Since serological studies assess previous exposure rather than active viraemia, higher prevalence estimates are expected. Notably, similarly low frequencies have also been observed in previous studies investigating viral DNA in blood samples [28,29]. More studies with other clinical samples (feces, anal swabs, etc.) and larger populations of domestic cats are needed to determine the prevalence and understand the molecular epidemiology of this parvovirus in southern Brazil.

Another approach was to analyze the occurrence of *Protoparvovirus carnivoran 1* with general information obtained from clinical forms. This virus was detected more frequently in younger cats (1.5 years for positive vs. 4 years for negative), in agreement with previous studies [3–6]. Regarding the other variables analyzed (sex, reproductive status, vaccination, health condition, and behavior), no statistically significant association with parvovirus infection was detected. Again, the type of sample analyzed (blood) and the small number of positive domestic cats limit the scope of these epidemiological interpretations, which should be interpreted with great caution.

In addition, interesting results were obtained from the phylogenetic analysis. Firstly, the phylogenetic tree topology and clade clustering were the same as reported in a recent study, and the samples sequenced here were classified into the three main clades previously reported: A, B, and CPV [13]. Furthermore, five parvoviruses sequenced here clustered into the two main FPV clades, A and B (comprising only FPV strains), while the sixth sequence joined the CPV clade (comprising CPV-2 strains). Clade A has both vaccine and field FPV isolates from North and South America; clade B mainly includes field FPV strains from Europe, Asia, and Oceania; and the CPV clade encompasses all CPV-2 strains worldwide [13].

Among the five FPV samples sequenced in this study, three (IDs 9917, 9919, and 9927) belonged to Clade A and showed 100% nucleotide identity with each other. They also clustered with VP2 sequences of FPV previously reported in domestic cats from Argentina, a country sharing borders with southern Brazil, highlighting the genetic similarity of FPV strains circulating in the Southern region. These findings suggest that these FPVs are highly clonal and belong to lineages currently circulating in the region. Furthermore, two samples sequenced in this study (IDs 9936 and 9944) showed high nucleotide identity (99.9 to 100%) with two strains frequently used as modified live virus (MLV) vaccines, one from clade A and the other from clade B. However, other field FPV sequences from our dataset also showed total or near-total nucleotide identity with vaccine strains from clades A and B, indicating that more complete genome sequencing would be important to distinguish vaccine-derived viruses from circulating field strains. More data with full-length VP2 gene and even a complete genome will be required to further investigate this possibility. It is also necessary to consider vaccine lineages in molecular surveillance studies, particularly when interpreting low-divergence sequences that cluster closely with commercially used vaccine strains [33,34]. This raises the need to conduct further studies with field strains that may arise from virulence reversion of attenuated vaccines, as previously suggested for other parvoviruses [35,36].

It is also important to highlight the detection of a CPV clade strain in domestic cats in the present study. Further analysis, using a dataset composed exclusively of CPV sequences, revealed that the CPV-2 positive sample (ID 9930) clustered with CPV-2a strains previously reported in cats in Brazil [37]. Additionally, the Asn426 amino acid substitution, characteristic of CPV-2a, was detected in this sample (Table 2). This previous study has detected FPV, CPV-2a, and CV-2b in fecal specimens from kittens with diarrhea [37]. This finding also highlights the ability of CPV-2 variants to infect felines and occasionally cause clinical manifestations similar to panleukopenia [4,5,37]. FPV molecular surveillance approaches should be able to accurately differentiate FPV and CPV-2 field and vaccine variants, helping with effective epidemiological monitoring of cats and dogs [12,15].

Although the detection of viral DNA in blood does not directly indicate active viral shedding or transmission potential, asymptomatic animals have previously been reported as possible sources of viral dissemination and environmental contamination [5,38]. This scenario is particularly concerning in settings with high animal density and turnover, such as shelters and colonies, where such infections may contribute to outbreaks in susceptible individuals [4,37,39]. Further studies, including fecal samples and viral load analyses, are necessary to better understand the role of these animals in the epidemiology and transmission dynamics of *Protoparvovirus carnivoran 1* infections.

Also, the detection of sequences similar to vaccine strains in this study points to an additional issue: tests such as qPCR, although highly sensitive, cannot distinguish whether the detected viral DNA originates from a field virus or from transient excretion of the vaccine virus, particularly when MLV vaccines are used [38–40]. According to previous studies, vaccine-derived DNA can be detected in feces for several days or even

weeks after vaccination, with a peak around the seventh day [39–41]. Although the viral load associated with vaccination is generally lower than that observed in clinical cases, the overlap in PCR results highlights the critical need for careful interpretation [39]. This means that any positive result should always be evaluated in conjunction with the animal's recent vaccination history and the clinical signs presented [38,39]. Furthermore, definitive discrimination between vaccine-derived and field strains would require full-length VP2 gene sequencing, or ideally whole-genome sequencing, to provide higher-resolution genetic information.

It is also necessary to mention some limitations of the study. First, the convenient blood sampling here obtained does not accurately represent the scenario for panleukopenia disease in the domestic cat population in southern Brazil. The small number of positive animals ($n = 9$) also restricts the interpretation of possible associations and risk factors. Therefore, the epidemiological patterns observed here should be interpreted with caution, as previously mentioned. Also, the VP2 gene was partially sequenced, providing a limited view of the virus's overall genomic diversity and restricting the resolution of the phylogenetic analyses. Full-length *Protoparvovirus carnivoran 1* genome sequencing would be necessary to provide a more comprehensive understanding of viral diversity in this population. Furthermore, serological analyses to assess antibody prevalence could provide complementary information on the relationship between viral DNA detection and the host immune response.

Overall, the detection and genetic characterization of *Protoparvovirus carnivoran 1* performed in this study provide additional information on the circulation and genetic diversity of this viral species in domestic cats from southern Brazil. The identification of FPV and CPV-2 viral DNA in some animals highlights the need for further epidemiological investigations covering larger feline populations, additional sample types (such as feces), and more extensive genomic analyses of the parvoviruses present in these animals. The present study demonstrated the active circulation of at least three distinct FPV/CPV-2 lineages among domestic cats, indicating a need for more robust epidemiological studies on feline panleukopenia. These findings also underscore the importance of molecular surveillance in monitoring the circulation and evolution of parvoviruses, aiming to assist in the prevention and control of this significant disease affecting domestic cats.

5. Conclusions

In conclusion, *Protoparvovirus carnivoran1* was detected in blood samples from domestic cats in southern Brazil. Additional phylogenetic analyses revealed the coexistence of field and vaccine FPV lineages as well as CPV-2 in the domestic cat population evaluated, highlighting the evolutionary plasticity of carnivore parvoviruses. These findings underscore the value of genomic surveillance for monitoring viral diversity and cross-species transmission.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pets3030029/s1>, Table S1: Metadata of 515 VP2 sequences analyzed in this study; Table S2: Metadata of 659 VP2 sequences analyzed in this study; Table S3: Metadata of the nine sequences obtained in this study; Table S4: Association between major clinical problems and feline panleukopenia virus (FPV) positivity in domestic cats; Table S5: Matrix percentage of identical bases among 525 sequences (FPV and CPV) and six sequences from this study; Figure S1: Maximum likelihood phylogeny inferred from 660 VP2 gene sequences of CPV. Yellow highlights the single sequence obtained in this study. The dataset comprises 26 CPV-2, 332 CPV-2a, 81 CPV-2b, and 220 CPV-2c sequences. Label colors indicate CPV genotypes: pink = CPV-2, red = CPV-2a, blue = CPV-2b, green = CPV-2c. Clades are classified according to de Oliveira Santana et al.

[20] as follows: red = O clade, orange = W clade, purple = W.1 clade, dark blue = W.2 clade, green = W.3 clade, and light blue = W.4 clade.

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