



Research Paper

Molecular Detection and Characterization of Feline Parvovirus in Cats in Iran



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ABSTRACT

Introduction: The feline parvovirus virus (FPV), a member of the family Parvoviridae, is a major pathogen in cats and is associated with a high risk of mortality and morbidity. Infection in cats can cause severe leukopenia and gastroenteritis, and may also lead to neurological signs.

Materials & Methods: In the present study, from September 2022 to September 2023, 31 blood and fecal samples were collected from cats with clinical symptoms of feline panleukopenia from four different veterinary clinics/hospitals in Gilan, Mazandaran, and Tehran provinces. Reports of rapid antigen detection test kits were also collected for cases (if performed) from clinics and hospitals. The presence of FPV was assessed by molecular assay using the polymerase chain reaction (PCR) method. PCR was performed using specific primers to amplify a partial VP2 gene encoding the viral protein, and the positive samples were subsequently submitted for sequencing. According to the phylogenetic tree generated by MEGA 7 software using the Maximum Likelihood method, samples in this study were clustered with previously submitted FPV isolates, and based on homology analysis.

Results: The current samples are similar to previously reported Iranian isolate IR-FPV2014.2, which has been obtained from a domestic cat in Tehran in 2014. Homology results also showed high similarity of the current isolates with isolate CU4, which was used as a reference, isolate C14, isolated in Nigeria from a domestic cat, and isolate "FPV/Raccoon/NJ/RPV-6/90", isolated from *Procyon lotor* (Raccoon) in the United States. According to the rapid test kit results, 78.57% of indoor cats and 42.86% of outdoor cats were positive for FPV infection. PCR results also revealed that 61.11% of indoor cats and 30.77% of outdoor cats were infected with FPV.

Conclusion: More molecular and epidemiologic studies are recommended to clarify the actual prevalence of FPV in Iran and the infection status among indoor cats and the stray cat population.

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1. Introduction

Feline panleukopenia (FPL), which is mainly associated with feline parvovirus virus (FPV) infection, is a highly contagious and potentially fatal clinical disease in cats [1-3]. Although several cases of an infectious enteritis in cats were reported in the first ten years of the 1900s, the viral nature of the disease was discovered in 1928 [2]. FPV infection in cats is initiated primarily by binding to the feline transferrin receptor (TfR), expressed on the cell surface, and then undergoing endocytosis via a clathrin-mediated pathway [1]. Feline panleukopenia virus, a member of the Carnivore protoparvovirus in the family Parvoviridae, is a small, non-enveloped virus and possesses a single-stranded linear DNA genome that consists of two open reading frames encoding non-structural proteins (NS1 and NS2) and capsid proteins (VP1 and VP2) [2, 4]. In addition to the induction of neutralizing antibodies as a protective response, the main capsid protein, VP2, plays a crucial role in defining the virus antigenicity, cellular tropism, and host range [5, 6]. Due to its importance, the VP2-encoding gene has been widely characterized in different parvovirus phylogeny studies, and different variants can be considered based on variations in the VP2 sequence [7].

FPV can infect a wide range of Carnivores, especially almost all members of the Felidae family. In addition, phylogenetic and genomic analyses have also revealed intraspecies transmission between different species [4, 8-10]. After infection, the disease is initiated with pyrexia within 3 to 5 days, after which, the virus is excreted at high titers in the feces, which can be transmitted to other susceptible hosts via the oral-fecal route [11]. Infection with FPV in cats can lead to severe leukopenia and gastroenteritis. The most frequently observed clinical signs are fever, vomiting, diarrhea, anorexia, and dehydration, and in kittens, the disease can be peracute and may lead to death [12, 13]. Feline panleukopenia virus is also considered one of the viral agents that can cause neurological signs in cats [14]. The virus can be detected in feces, blood, saliva, and vomit of infected cats, with high titers excreted in feces during the 24-48-hours incubation period and even for weeks after recovery; however, viral shedding is frequently over within 5-7 days [15, 16]. Within 18-24 hours after infection, the virus replicates in lymph nodes in the oropharynx, followed by viremia within 2-7 days, which disseminates the virus throughout the body [15, 17]. Diarrhea and immunosuppression in FPV cases occur as a result of the virus's tropism for

rapidly dividing cells, such as intestinal crypt epithelium, lymphoid tissue, and bone marrow stem cells [15].

Due to its resistance to physical conditions and chemical agents, the virus can survive in the environment for months and even years, increasing the risk of transmission through contaminated individuals and equipment [13]. To reduce this risk, it is recommended to implement population management strategies, including rapid detection of infected cats, appropriate cleaning and disinfection protocols, and quarantine measures [15]. In addition to environmental management, vaccination is strongly recommended for all cats since the presence of antibodies in adult cats correlates with protection against infection [18]. Through vaccination, kittens can develop immunity to acute clinical disease after 1-3 vaccine doses in the absence of maternally derived antibodies (MDA). The presence of MDA can interfere with vaccination and can decrease the immunity response to the vaccine, so kittens with MDA may fail to develop protective immunity [19]. Immunization against FPV can be obtained by the administration of subcutaneous modified live virus (MLV) vaccines. Vaccination in pet cats is not recommended to be initiated before 6 weeks of age; after that, they should be vaccinated at intervals of 3-4 weeks until 16-20 weeks of age [20, 21].

Despite its cost and technical difficulty, which may cause delays in obtaining laboratory results, polymerase chain reaction (PCR) is a sensitive assay for the detection of parvoviruses and is considered the reference standard for other FPV diagnostic tests [16]. In addition to PCR, in recent years, new in-house tests have been introduced to the market for use in veterinary practice, which are mainly based on enzyme-linked immunosorbent assay (ELISA) or immunochromatography technology [22]. Feline panleukopenia virus was first identified in 1928 and is thought to be the origin of canine parvovirus type 2 (CPV-2), which was discovered in 1978. Variations in the amino acid sequence of the VP2 protein differentiate FPV from CPV-2 [23]. In Iran, Mosallanejad et al, confirmed the presence of FPV antigen using an immunochromatography assay among cats in samples collected during 2005-2007 [24]. Later in 2016, Mirzakhani et al. reported feline panleukopenia in a wild cat (*Felis silvestris*), confirmed by PCR assay [25]. In 2017, Nikbakht et al. highlighted the high evolutionary potential of canine parvovirus (CPV) [26]. Given that both FPV and CPV belong to the same viral family, this phenomenon may also apply to FPV. Therefore, continuous monitoring of circulating strains is crucial for understanding the molecular epidemiology and for evaluating the efficacy of commonly used vaccines.

In the present study, we aimed to perform a molecular study on domestic cats infected with feline panleukopenia virus presenting clinical signs and to conduct a phylogenetic analysis using the nucleotide sequence of the VP2-protein encoding gene in Iran.

2. Materials and Methods

2.1 Sample collection

From September 2022 to September 2023, 31 rectal and blood samples were collected from cats presenting clinical symptoms of feline panleukopenia. Samples were collected from four different veterinary clinics/hospitals in Gilan, Mazandaran, and Tehran provinces. After sampling, the swabs were placed into sterile test tubes, which were filled with 2 mL of phosphate-buffered saline (PBS). Subsequently, the swabs were transferred to the Virology Laboratory, Faculty of Veterinary Medicine, University of Tehran, and stored in a -20 °C freezer. Information on the collected samples is presented in Table 1.

2.2 DNA extraction and PCR

Viral DNA was extracted using the viral nucleic acid extraction mini kit (Takapozist, Iran) according to the manufacturer's protocol. Extracted DNA was collected and stored at -20 °C. A PCR assay targeting a 698 bp fragment of the VP2 gene, specific to FPV, was performed using the following primers: forward primer 5' -GCTTTAGATGATACTCATGTA- 3' and reverse primer 5' -GTAGCTTCAGTAATATAGTC- 3' [27]. PCR was conducted with a total volume of 25 µL, containing 10 µL of premix (Ampliqon; Denmark), 1 µL of each forward and reverse primer, 8 µL of nuclease-free water, and 2.5 µL of DNA template. Then, hot-start PCR was carried out; the initial denaturation was done for 3 minutes at 95 °C, followed by 40 cycles of 15 seconds at 94 °C for denaturation, 15 seconds at 55 °C for annealing, and 30 seconds at 68 °C for extension. The final extension was carried out for 7 minutes at 72 °C. PCR products were then loaded in a 1.5% agarose gel and electrophoresed. Agarose gel was stained with ethidium bromide and visualized using a UV transilluminator. The extracted nucleic acid of the commercial trivalent feline vaccine (NOBIVAC, Cambridge, UK), which includes feline viral Rhinotracheitis virus, feline calicivirus, and feline panleukopenia virus, was used as a positive control.

2.3 Sequencing and phylogenetic analysis

Among the positive samples, five were submitted to Codon Genetic Company (Tehran, Iran) for sequencing using the Sanger sequencing method. Sequences were analyzed by BLAST using the NCBI BLAST tool [28] to ensure their accuracy, and the quality of sequences was evaluated using Finch TV software version 1.4.0. For further analysis, sequences were compared with reference FPV strains based on the full lengths of the VP2 region deposited in the GenBank database. The dataset was prepared and trimmed, and two phylogenetic trees were generated using MEGA 7 software. A phylogenetic tree was constructed using the maximum likelihood (ML) method to compare previously submitted FPV isolates and those from the current study with canine parvovirus, based on the VP2 gene. Additionally, a second phylogenetic tree was generated using the Neighbor-Joining method for genotyping [29].

3. Results

Out of 31 collected samples, THE rapid test kit was applied for 21 cases, of which 14 (66.67%) were positive. Among the 31 samples screened by PCR assay, 15 cases (48.39%) were positive. Screening results for both the rapid test kit and PCR assay revealed a higher incidence in indoor cats compared to outdoor or stray cats. Results of the rapid test kit showed that 78.58% of indoor cats and 42.86% of stray cats were positive. PCR assay results also showed positivity in 61.11% of indoor cats and 30.77% of outdoor cats (Table 2 and Figure 1). Four samples tested positive with the rapid test kit but were negative by PCR assay, which may indicate false-positive results from the rapid test. Phylogenetic analysis of sequenced samples clustered them with previously submitted FPV strains (Figure 2). Homology analysis revealed that the current isolates (UT-02590, UT-02589, UT-797, UT-794, and UT-02814) have high similarity with the previously reported Iranian isolate IR-FPV2014.2, which was isolated from a domestic cat in Tehran in 2014 (Table 3). Furthermore, these samples also showed high similarity with isolate CU4, which was used as a reference, isolate C14, isolated in Nigeria, and isolate "FPV/Raccoon/NJ/RPV-6/90", isolated from *Procyon lotor* (Raccoon) in the United States. Sequences are also available in GenBank (NCBI) under the accession numbers: PQ842810, PQ842811, PQ842812, PQ842813, PQ842814.

Table 1. Information on collected samples

Case No.	Signs	Gender	Age	Collected Samples	Living Condition	Rapid Kit Result	PCR Result
1	Fever, vomiting, anorexia, diarrhea	Male	2 months	Blood	Outdoor	-	-
2	Fever, anorexia, diarrhea, abdominal pain	Male	4 weeks	Fecal	Indoor	+	+
3	Gastroenteritis, fever, diarrhea	Female	3 weeks	Fecal	Indoor	NP	+
4	Low appetite, diarrhea, abdominal pain	Male	1 month	Fecal	Indoor	-	-
5	Fever, vomiting, anorexia, diarrhea, abdominal pain	Female	4 weeks	Fecal	Indoor	+	+
6	Diarrhea, anorexia, vomiting	Female	1 month and 15 days	Blood	Indoor	+	-
7	Lethargy, fever	Female	4 weeks	Blood	Outdoor	-	-
8	Diarrhea, low appetite, vomiting	Male	2 months	Blood	Outdoor	NP	-
9	Fever, lethargy, abdominal pain	Male	1 month	Fecal	Indoor	+	+
10	Diarrhea, low appetite, vomiting	Male	2 months	Fecal	Indoor	NP	+
11	Diarrhea, low appetite	Female	4 weeks	Fecal	Indoor	-	-
12	Diarrhea, fever, vomiting	Female	3 months	Blood	Indoor	NP	-
13	Diarrhea, lethargy, abdominal pain	Male	3 weeks	Fecal	Indoor	+	+
14	Fever, diarrhea, vomiting, lethargy	Female	2 months and 15 days	Fecal	Indoor	NP	-
15	Abdominal pain, vomiting	Male	1 year	Fecal	Outdoor	NP	+
16	Low appetite, diarrhea, vomiting	Female	3 months	Fecal	Indoor	-	-
17	Low appetite, abdominal pain, vomiting	Female	2 weeks	Blood	Outdoor	+	-
18	Fever, vomiting, lethargy	Female	3 weeks	Fecal	Outdoor	NP	+
19	Low appetite, abdominal pain	Female	1 month and 15 days	Fecal	Outdoor	-	-
20	Low appetite, vomiting, fever, lethargy	Female	4 weeks	Fecal	Indoor	+	+
21	Abdominal pain, fever	Male	2 months	Fecal	Outdoor	NP	-
22	Low appetite, vomiting	Male	4 months	Fecal	Outdoor	+	-
23	Lethargy, diarrhea, lethargy	Female	6 months	Blood	Indoor	+	+
24	Low appetite, abdominal pain, vomiting	Male	5 months	Fecal	Outdoor	+	+
25	Low appetite, vomiting	Male	2 months	Blood	Outdoor	NP	-
26	Vomiting, lethargy, diarrhea	Male	1 year	Fecal	Indoor	+	+
27	Fever, lethargy, diarrhea	Male	4 weeks	Fecal	Outdoor	-	-
28	Abdominal pain, diarrhea	Female	5 weeks	Fecal	Indoor	+	+
29	Vomiting, diarrhea	Male	1 month and 15 days	Fecal	Outdoor	NP	+
30	Fever, vomiting, abdominal pain, diarrhea	Male	4 months	Blood	Indoor	+	-
31	Low appetite, abdominal pain, vomiting	Female	2 months	Blood	Indoor	+	+

NP: Not performed.

Table 2. Results and number of tested samples with both rapid test kit and PCR assay

Test	Cat Population	Number of Tested Samples	No. (%)
			Number of Positive Samples
Rapid test kit	Outdoor cats (stray cats)	7	3(42.86)
	Indoor cats	14	11(78.57)
PCR	Outdoor cats (stray cats)	13	4(30.77)
	Indoor cats	18	11(61.11)

4. Discussion

Feline panleukopenia is a contagious and often fatal disease with a mortality rate ranging from 25-90% for acute cases and reaching up to 100% in peracute infections [30]. The disease can occur in unvaccinated cats or cats who were vaccinated improperly; however, it is most likely to occur in cats younger than 1 year of age. Despite vaccination, there are reports of death in fully vaccinated household kittens, which may occurred due to exposure to large amounts of virus in environment [31]. The presence of antibodies in adult cats, whether acquired by prior vaccination or exposure to the field virus, is associated with immunity against infection; however, it is not currently clear whether cats with pre-existing immunity gain any advantage by revaccination [19]. Studies indicate the importance of the effect of MDA on the efficacy of vaccination. They suggest that although MDA may not completely protect against infection, they can interfere with the immune response induced by vaccination [20, 22, 32]. This phenomenon highlights the

importance of a carefully designed vaccination program, including multiple vaccine doses, to increase the efficacy of vaccine-derived immunity.

Previous studies confirmed the presence and circulation of panleukopenia virus in Iran. In 2009, Mosallanejad et al. reported the presence of FPV antigens in Khuzestan Province, Iran, by an immunochromatographic assay. In this study, out of 67 samples collected from diarrheic cats, 34% were positive [25]. In 2020, Zenad and Radhy investigated the prevalence of FPV among cats in Baghdad, Iraq, a neighboring country to the west of Iran. Out of 180 collected samples, 40 [22.2%] tested positive using a rapid antigen test kit. They also reported higher infection rate in stray cats compared to pet cats [33]. In another study in Iraq published in 2023, among 100 collected samples from stray and household cats, 40% were positive using an immunochromatographic rapid test kit, but PCR results were positive for 70% of samples. They also reported a higher infection rate in stray cats compared to household cats using both immunochromatog-

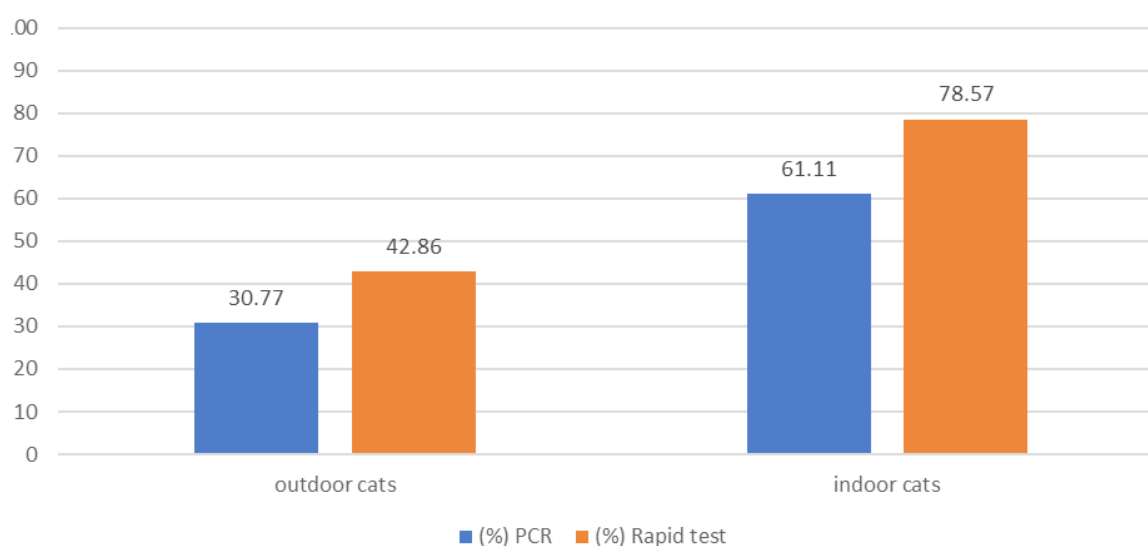
**Figure 1.** Comparison of positive sample detection using rapid test kit and PCR assay

Table 3. The similarity of nucleotide sequences of the FPV partial VP2-encoding gene of current isolates compared with other isolates submitted to GenBank (NCBI)

	Isolate Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	19	20	21
1	UT-02590																				
2	UT-02589	100																			
3	UT-797	99.7	99.7																		
4	UT-794	99.7	99.7	99.7																	
5	UT-02814	99.5	99.5	99.7	99.5																
6	KP081409.1 IR-FPV2014.2	100	100	99.7	99.7	99.5															
7	M24004.1 CU4	99.8	99.8	99.5	99.5	99.4	99.8														
8	OP985513.1 C14	99.7	99.7	99.3	99.4	99.2	99.7	99.8													
9	JN867594.1 FPV/Raccoon/NJ/RPV-6/90	99.7	99.7	99.3	99.4	99.2	99.7	99.8	100												
10	KX900570.1 HH-1/86	99.5	99.5	99.2	99.2	99.1	99.5	99.7	99.8	99.8											
11	MF069447.1 FPV/Raccoon/RC18/BC_2016	99.4	99.4	99.2	99.1	98.9	99.4	99.5	99.7	99.7	99.5										
12	EU498704.1 42/06-G8	99.4	99.4	99	99.1	98.9	99.4	99.2	99.4	99.4	99.5	99.1									
13	EU145593.1 389/07	99.2	99.2	98.8	98.9	98.7	99.2	99.4	99.5	99.5	99.7	99.2	99.2								
14	HQ184200.1 KS42	99.2	99.2	98.8	98.9	98.7	99.2	99.1	99.2	99.2	99.4	98.9	99.5	99.1							
15	MW650831.1 FPV-SH2001	99.2	99.2	98.8	98.9	98.7	99.2	99.4	99.5	99.5	99.7	99.2	99.2	99.7	99.1						
16	KX685354.1 HN-ZZ1	99.2	99.2	98.8	98.9	98.7	99.2	99.4	99.5	99.5	99.7	99.2	99.5	99.4	99.4	99.4					
17	DQ099431.1 JF-3	99.1	99.1	98.6	98.8	98.6	99.1	99.2	99.4	99.4	99.5	99.1	99.1	99.5	98.9	99.5	99.2				
18	MN862748.1 FPV/River otter/OTVI-16/BC_2019	99.1	99.1	98.6	98.8	98.6	99.1	99.2	99.4	99.4	99.5	99.1	99.1	99.2	98.9	99.2	99.2	99.1			
19	MN862744.1 FPV/American pine marten/MAVI-36/BC_2016	98.8	98.8	98.3	98.4	98.3	98.8	98.9	99.1	99.1	99.2	98.8	98.8	98.9	98.6	98.9	98.9	98.8	99.7		
20	MT250783.1 JL (MEV)	98.9	98.9	98.5	98.6	98.4	98.9	99.1	99.2	99.2	99.4	98.9	99.2	99.1	99.1	99.1	99.4	98.9	98.6		
21	KJ674819.1 si (CPV)	97.7	97.7	97.2	97.4	97.2	97.7	97.9	97.7	97.7	97.9	97.4	97.7	97.6	97.6	97.6	97.9	97.4	97.4	97.1	97.7

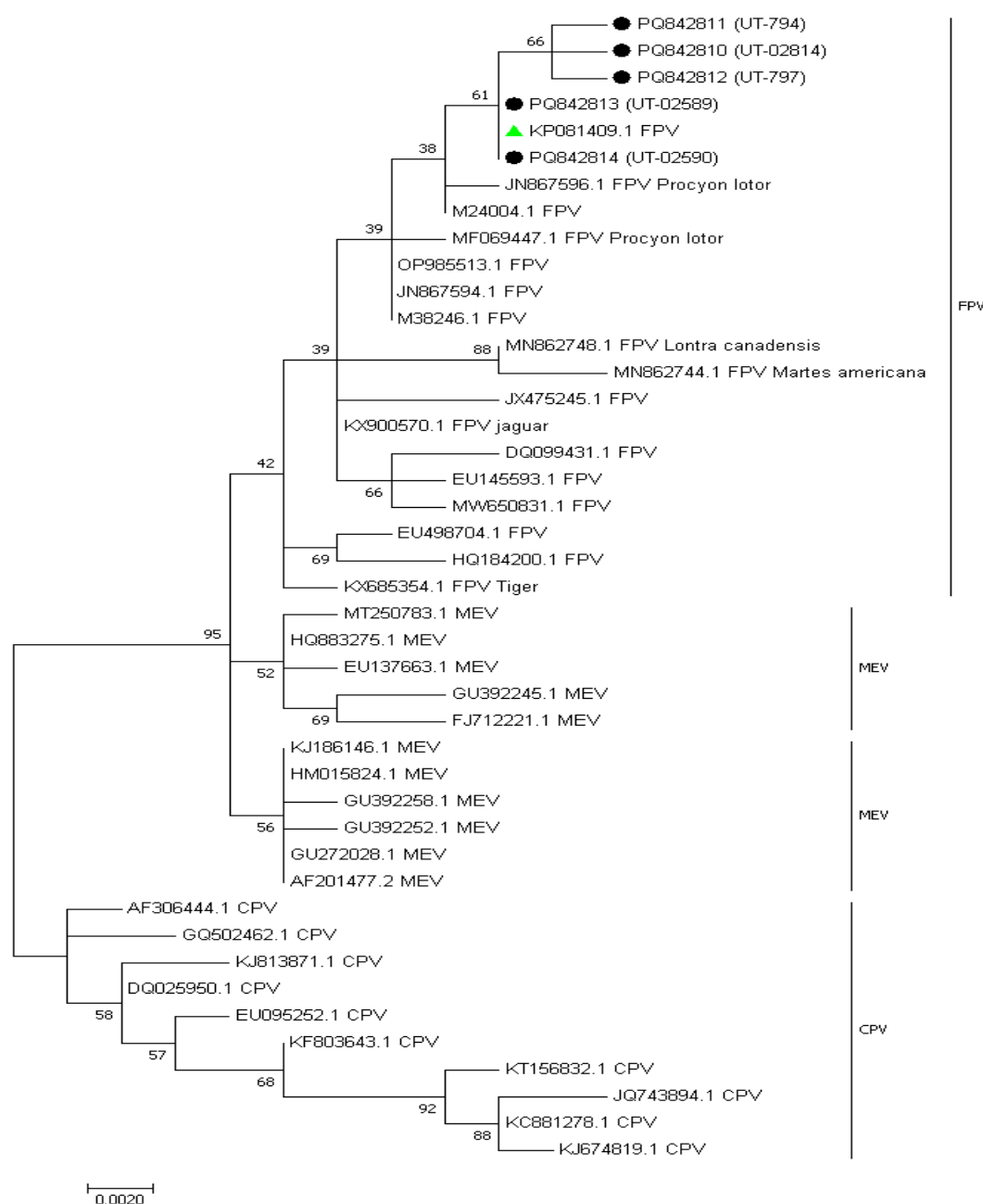


Figure 2. Phylogenetic tree based on the VP2-encoding gene of different FPVs, canine parvoviruses, and mink enteritis viruses submitted to the NCBI database

Note: The phylogenetic tree was generated using MEGA 7 by maximum likelihood method. Numbers in each branch indicate the bootstrap value of each node. According to this tree, the current isolates are clustered with other FPVs. Current isolates are marked with black circle and the previously reported isolate from Iran marked with green triangle.

raphy assay and PCR methods [34]. In another study in Bangladesh, Islam et al reported that among 58 samples collected from pet and stray cats, 22.41% of samples were positive using a rapid antigen detection kit. They also reported a higher prevalence of the disease in stray cats (41.67%) than pet cats (17.39%) [35]. In a study conducted in Bangladesh, Kabir et al, used a PCR assay

for the molecular detection of FPV and reported an overall prevalence of 22.9% among 161 rectal swab samples collected from pet hospitals between July 2021 and December 2022. They also reported the mortality rate and case fatality rate as 10.6% and 45.9%, respectively [30]. In the current study, a rapid detection kit was used on 21 cases, of which 14 cases (66.67%) tested positive and

7 cases (33.33%) tested negative. A higher incidence of FPV was detected using only the rapid detection kit among indoor cats (78.57%) compared to outdoor or stray cats (42.86%). PCR assay was conducted for all 31 collected samples, and according to PCR results, 15 samples (48.39%) were positive. PCR results also indicate a higher prevalence in indoor cats (61.11%) compared to outdoor cats (30.77%).

Phylogenetic analysis of isolates in the present study clustered them with previously submitted FPV isolates. These isolates showed a high similarity with the previously submitted Iranian isolate, IR-FPV2014.2, isolated from *Felis catus* in 2014. In another study by Dishow et al. reported an overall prevalence of 70% in samples collected from 100 cats. Phylogenetic results of their study showed a high (99.29-100%) similarity between their isolates and other previously submitted sequences from other countries such as China, Turkey, Thailand, and South Korea [36]. In another study in Bangladesh, Chowdhury et al. conducted the first molecular characterization and phylogenetic analysis of FPV based on the VP2 gene in Bangladesh. They reported that 18.37% of cases were positive among 98 collected samples using a PCR assay. Their newly sequenced Bangladeshi strain showed the highest sequence identity with strains from the United Arab Emirates (UAE) [24].

5. Conclusion

Results of the current study show the presence and circulation of FPV in Iran, and the current isolates seem to belong to the same genogroup and are similar to the previously submitted isolate from Iran in NCBI GenBank (IR-FPV2014.2). This genotype-based differentiation is crucial for understanding the epidemiology of FPV and developing targeted control measures. However, more studies are needed to determine whether additional FTP genotypes are currently present in Iran. Further studies are also recommended to clarify the epidemiological situation of currently circulating FPV genotypes in Iran and to evaluate the efficacy of commonly used vaccines against them.

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Compliance with ethical guidelines

We declare that all ethical standards related to animal health and welfare have been respected in the present study.

Data availability

The data supporting the findings of this study are available upon request from the corresponding author.

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Authors' contributions

Conceptualization, study design, and supervision: Arash Ghalyanchilangeroudi; Data acquisition: Dornaz Mehinarparvar Irani, Arian Abbassioun, and Fahimeh Jamiri; Analysis and data interpretation: Zahra Ziafati Kafi, Naser Sadri, and Soroush Sarmadi; Writing: Naser Sadri, Dornaz Mehinarparvar Irani; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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