Molecular detection and characterization of feline parvovirus in cats in Iran

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Abstract

The feline panleukopenia virus (FPV), a member of the *Parvoviridae* family, 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 even lead to nervous signs. In the present study, during 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 test antigenic detection kit were also collected for cases (if performed) from clinic and hospitals. The presence of FPV was assessed by molecular assay using PCR method. PCR was performed using specific primers to amplify a partial VP2 protein encoding gene, 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 current samples are similar to previously Iranian isolate, IR-

FPV2014.2 which has been obtained from a domestic cat in Tehran in 2014. Homology results also showed high similarity of current isolate with isolate CU4 which used as reference, isolate C14 isolated in Nigeria from domestic cat and isolate "FPV/Raccoon/NJ/RPV-6/90" isolated from *Procyon lotor* (Racoon) in 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. More molecular and epidemiologic studies are recommended to clarify the actual prevalence of FPV in Iran and the infection status among indoor cats and stray cats population.

Key words: Feline panleukopenia virus (FPV), Phylogeny, Molecular diagnosis, Feline, Iran

1. Introduction

Feline Panleukopenia (FPL) which is mainly associated with Feline Parvovirus (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 1900s, the viral nature of disease was discovered in 1928 (2). FPV infection in cats initiate primarily by binding to the feline transferrin receptor (fTfR), expressed on the cell surface and then endocytosis via clathrin-mediated pathway (1). Feline panleukopenia virus, a member of Carnivore protoparvovirus in *Parvoviridae* family, is a small, non-enveloped virus and possess a single-stranded linear DNA as genome which consists of two open reading frames encoding for non-structural proteins (NS1 and NS2) and capsid proteins (VP1 and VP2) (2, 4). In addition to induction of neutralizing antibodies as protective antigen, 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, VP2 encoding gene has been widely characterized in different parvovirus phylogeny studies and different variants can be considered based on variation in VP2 sequence (7).

Feline panleukopenia virus can infect a wide range of Carnivores specially almost all members of Felidae family. In addition, phylogenic and genomic analysis has also revealed intraspecies transmission between different species (4, 8-10). After infection, the disease initiates by showing pyrexia in 3 to 5 days and after that, the virus is extracted in high titer in the feces, which can be transmitted to other susceptible hosts via Oral-Fecal route (11). Infection with FPV in cats can lead to severe leukopenia, gastro-enteritis. The most frequently observed clinical signs are fever, vomiting, diarrhea, anorexia and dehydration and in the kitten 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). 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 weeks after recovery however, shedding of virus is frequently over in 5-7 days (15, 16). Within 18-24 hours after infection, the virus replicates in lymph nodes in oropharynx followed by a viremia in 2-7 days which disseminates the virus through the body (15, 17). Diarrhea and immunosuppression in FPV cases occur as a result of virus tropism to cells which divide fast like intestinal crypt epithelium, lymphoid tissue and bone marrow stem cells (15).

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

Despite its costs and technical difficulty which may make a delay for results from laboratory, polymerase chain reaction (PCR) is a sensitive assay for detection of parvoviruses and considered as reference standard for other FPV diagnostic tests (16). In addition to PCR, in recent years new in-house tests have been introduced to market for using 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 seems to be the origin of canine parvovirus type (CPV-2) which was discovered in 1978. Variation in amino acid sequence of VP2 protein differ FPV from CPV-2 (23). In Iran, Mosallanejad et al, confirmed the presence FPV antigen by using immunochromatography assay test 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 present study, we aimed to perform a molecular study on domestic cats infected with feline panleukopenia virus presenting clinical signs and a phylogenetic analysis using nucleotide sequence of VP2 protein encoding gene in Iran.

2. Material and methods

2.1 Sample collection

During September 2022 to September 2023, 31 rectal and blood samples were collected from cats referred with clinical symptoms of feline panleukopenia. Samples were collected from 4 different veterinary clinics/hospitals in different veterinary clinics/hospitals in Gilan, Mazandaran, and Tehran provinces. After sampling, the swabs were kept into sterile test tubes, which were charged 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 kept in -20°C freezer. Information of collected samples are presented in table 1.

Table 1-Information of collected samples

Case number	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

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 pf the VP2 gene, specific to FPV was performed using following primers: forward primer 5' -GCTTTAGATGATACTCATGTA- 3' and the reverse primer 5' -GTAGCTTCAGTAATATAGTC- 3' (27). Polymerase chain reaction was conducted with a total volume of 25 µL, contained 10 µL of premix (Ampliqon; Denmark), 1µL of each forward and reverse, 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 and then the reaction continued for 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 in 7 minutes at 72°C. PCR products then loaded in 1.5% agarose gel electrophoresed. Agarose gel was stained with ethidium bromide stain and visualized with UV transilluminator. The extracted nucleic acid of the commercial strains of the triple cat vaccine (NOBIVAC, Cambridge, UK), which includes the strain of feline viral Rhinotracheitis, feline Calicivirus, and Panleukopenia (RCPS), was used as a positive control.

2.3 Sequencing and phylogenetic analysis

Among positive samples, five were submitted to Codon Genetic Company (Tehran, Iran), for sequencing using sanger sequencing method. Sequences were analyzed by BLAST using NCBI BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to ensure their accuracy and the quality of sequences evaluated using Finch TV software version 1.4.0. For further analysis, sequences were compared with references FPV strains based on the full lengths of VP2 region that were 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 VP2 gene. Additionally, a second phylogenetic tree was generated using neighbors joining method for genotyping (28).

3. Results

Out of 31 collected samples, rapid test kit was applied for 21 cases and 14 cases (66.67%) were positive. Among 31 samples which were screened by PCR assay, 15 cases (48.39%) were positive. Screening results for both rapid test kit and PCR assay revealed higher incidence in indoor cats compared to outdoor or stray cats. Results of rapid test kit showed that, 78.58% of indoor cats and 42.86% of stray cats are positive. PCR assay results also were positive for 61.11% of indoor cats and 30.77% of outdoor cats (table 2 and figure 1). Four samples tested positive with 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 current isolates, UT-02590, UT-02589, UT-797, UT-794 and UT-02814 have high similarity with previously

Iranian isolate, IR-FPV2014.2 which has been isolated from a domestic cat in 2014 in Tehran (table 3). Furthermore, these samples also showed high similarity with isolate CU4 which used as reference, isolate C14 isolated in Nigeria and isolate "FPV/Raccoon/NJ/RPV-6/90" isolated from Procyon lotor (Racoon) in United States. Sequences also available in GeneBank NCBI, under accession numbers: PQ842810, PQ842811, PQ842812, PQ842813, PQ842814.

Table 2 – results and number of tested samples with both rapid test kit and PCR assay

Test	Cat population	Number of tested samples	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%)

Figure 1 – Comparison of positive samples detection using Rapid test kit and PCR assay.

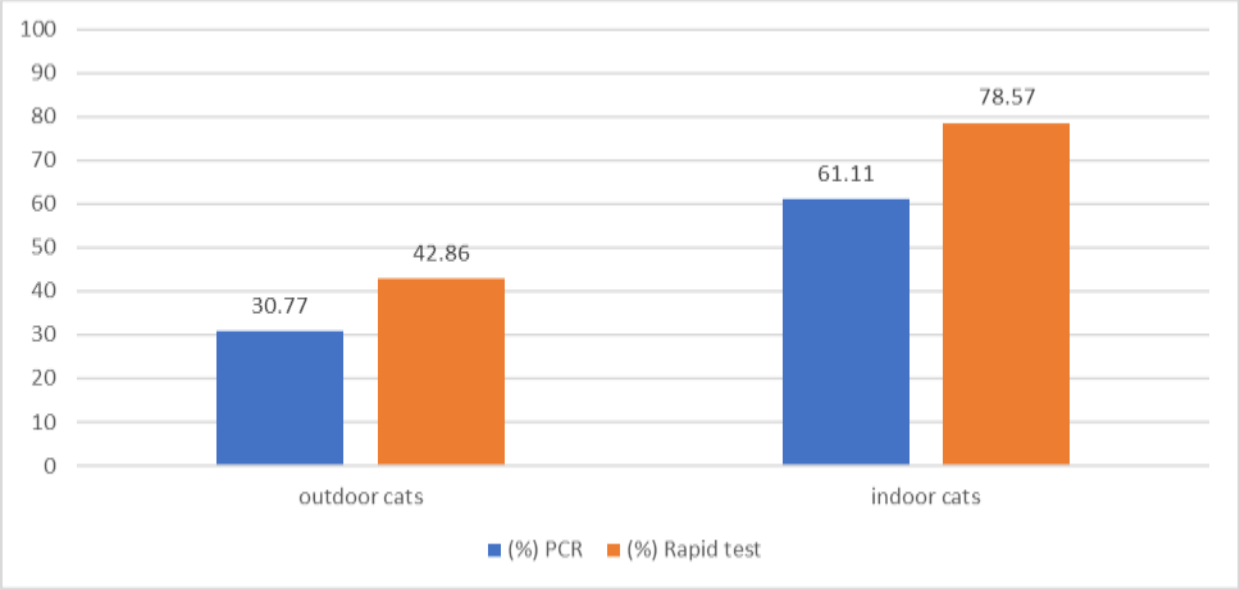

Table 3- The similarity of nucleotide sequence of feline parvovirus partial VP2 protein encoding gene of current isolates compared with other isolates submitted to GeneBank NCBI

		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	98.9	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

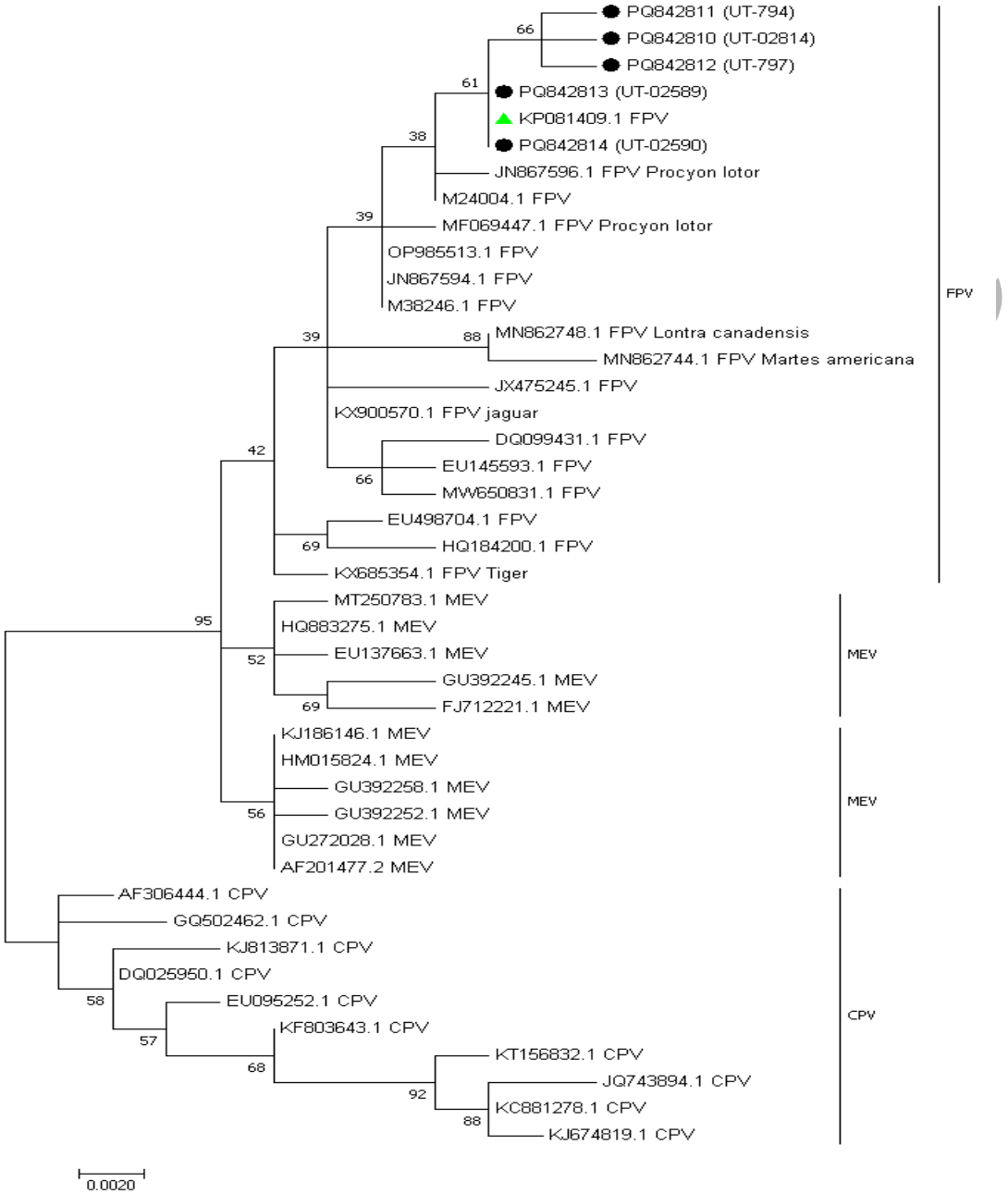

Figure 2 – Phylogenetic tree based on VP2 protein encoding gene of different feline parvoviruses, canine parvoviruses and mink enteritis viruses submitted in NCBI database. Phylogenetic tree was generated using MEGA 7, by Maximum Likelihood method. Numbers in each branch indicates the Bootstrap of each node. According to this tree, current isolate is clustered with other feline parvoviruses. Current isolates are marked with black circle. Previously reported isolate from Iran marked with green triangle.

4. Discussion

Feline panleukopenia is a contagious and often fatal disease with a mortality rate ranges from 25-90% for acute cases and can reach up to 100% in per acute infections (29). The disease can occur in unvaccinated cats or cats who were vaccinated improperly, however its most likely to occur in cats younger than 1 year of age. Despite vaccination, there are reports of death in household fully vaccinated kittens which may occurred due to exposure to large amounts of virus in environment (30). 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's not currently clear that cats with pre-existing immunity gain any advantage by revaccination (18). Studies indicate the importance of MDA effect on efficacy of vaccination. They suggest that although maternally derived antibodies may not completely protect against infection, they can interfere with immunity response provoked by vaccination (19, 21, 31). This phenomenon highlights the importance of carefully designed vaccination program and even multiple doses of vaccines to increase the efficacy of vaccine derived immunity.

Previous studies confirmed presence and circulation of panleukopenia virus in Iran. in 2009, Mosallanejad et al reported the presence of FPV antigens in Khouzestan province of Iran by immunochromatography assay. In this study, out of 67 samples collected from diarrheic cats, 34% were positive (24). In 2020, Zenad and Radhy investigated the prevalence of FPV among cats in Baghdad, Iraq, a neighbor country to the west of Iran. Out of 180 collected samples, 40 (22.2%) tested positive using rapid antigen test kit. They also reported higher infection rate in stray cat compared to pet cats (32). In another study in Iraq published in 2023, among 100 collected samples from stray and household cats, 40% were positive using immune chromatography rapid test kit but PCR result were positive for 70% of samples. They also reported higher infection rate in stray cat compared to household cats using both immunochromatography assay and PCR methods (33). 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 rapid antigen detection kit. They also reported more prevalence of disease in stray cats (41.67%) than pet cats (17.39%) (34). In a study conducted in Bangladesh, Kabir et al, used 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 of 10.6% and 45.9% respectively (29). 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. Higher incidence of FPV was detected using only rapid detection kit among indoor cats (78.57%) compared to outdoor or stray cats (42.86%). PCR assay conducted for all 31 collected samples and according to PCR results, 15 samples (48.39%) were positive. PCR results also indicate higher prevalence in indoor cats (61.11%) compared to outdoor cats (30.77%).

Phylogenetic analysis of isolates in present study, clustered them with previously submitted FPV isolates. These isolates showed a high similarity with 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 represent high (99.29%-100%) similarity between their isolates and other previously submitted sequences from other countries such as China, Turkey, Thailand and South Korea (35). In

another study in Bangladesh, Chowdhury et al, conducted the first molecular characterization and phylogenetic analysis of FPV based on VP2 gene in Bangladesh. They reported that 18.37% of cases were positive among 98 collected samples, using PCR assay. Their newly sequenced Bangladeshi strain, showed the highest sequence identity with strains from United Arab Emirates (UAE) (23).

Results of current study show presence and circulation of FPV in Iran and current isolates seems to belong to same genogroup and similar to previous isolate submitted in NCBI GenBank from Iran (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 show if there are more genotypes of the FPV virus currently present in Iran. More studies are recommended for clarify the epidemiological situation of current circulating FPV genotypes in Iran and the efficacy of commonly used vaccines against them.

5. Conflict of interests

The authors declare no conflict of interests.

6. Authors Contributions

Acquisition of data: DMI, AA. FJ

Analysis and interpretation of data: ZZK, NS, SS

Drafting of the manuscript: NS, DMI

Study concept and design: AGL

Study supervision: AGL

All authors reviewed the manuscript.

7. Ethics committee Approval

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

8. Data availability

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

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