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Molecular Diagnosis, Risk factors and Phylogenetic Analysis of Feline Panleukopenia Virus in Cats in Duhok Province, Iraq

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Abstract

THE STUDY aimed to determine the prevalence of feline panleukopenia virus (FPV) in cats in Duhok province-Iraq, using the conventional polymerase chain reaction (c-PCR) technique, evaluate some of the risk factors linked with the prevalence of FPV, and investigate the phylogenetic analysis of FPV detected in the study. Different samples (blood samples, fecal, ocular, and oral swabs) were randomly obtained from 100 cats with different lifestyles, genders, ages, breeds, immune status, health status, pregnancy state, regions, and seasons. Results showed that the overall prevalence of FPV in cats in Duhok province was 70/100 (70%), with no significant difference in the prevalence of virus in various types of samples. The significant risk factors associated with a higher prevalence of FPV recording in stray cats, less than and equal to 1year olds, short hair, non-vaccinated, pregnant cats, the Duhok region, and the autumn and winter seasons. The phylogenetic analysis for 15 local sequences of the VP2 gene that were deposited in the NCBI GenBank under the accession numbers (OQ102246.1-OQ102255.1, and OQ708628.1-OQ708632.1), with highly related (99.29%–100% identity) to other sequences that registered in the GenBank from different countries, including China, Turkey, Thailand, and South Korea. In conclusion; FPV is widespread in Duhok province-Iraq, certain risk factors associated with the higher prevalence of FPV, and the phylogenetic analysis of FPV sequences is important for strategic control of the infection in the study regions.

Keywords: Feline panleukopenia virus, Cats, Conventional PCR, Risk factors, Phylogenetic analysis.

Introduction

The term "domestic cat" or "house cat" is used to differentiate domestic cats (*Felis catus*) from their wild counterparts, as they are the only domesticated species in the Felidae family [1]. Domestic cats are typically kept as home pets, while, feral cats are wild cats that roam free and stay away from people [2].

Feline panleukopenia virus (FPLV) disease is a highly contagious and probably fatal disease of domestic and wild felids, and known by a variety of synonyms, such as feline distemper, feline infectious enteritis, feline parvoviral enteritis, pseudomembranous enteritis, laryngoenteritis, and feline agranulocytosis [3]. The disease clinically manifested by fever, lethargy, depression, anorexia, vomiting, dehydration, and diarrhea. Furthermore, a noticeable decrease in the number of circulating white blood cells (WBC) [4,5].The FPLV disease is caused by the Carnivore protoparvovirus1 of the genus Protoparvovirus. It is a small, non-enveloped, single-stranded DNA virus that belongs to the Parvoviridae family [6,7,8]. Cats are also susceptible to canine parvovirus type 2 (CPV-2a, 2b, and 2c), and co-infection may occur, which infects and causes diseases that are indistinguishable from FPV [9,10]. The virus can be transmitted by direct contact with infected cats or their secretions [11]; placental transmission from pregnant infected cats to the embryo [12]; flies and other insects may also play a role in the spreading of the virus [13].The disease in cats is widely distributed in various continents in Asia, Europe, Africa, Australia, and America [14-18]. Moreover, in Iraq, the FPLV disease has been reported in Baghdad province [19,20], and in Wasit province [21].

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There are many risk factors associated with the prevalence of the FPLV disease in cats, such as lifestyle, gender, age, breed, health status, pregnancy, vaccination status, regions, and the seasons [18,22,23]. The mortality rates of FPV in the acute stage may go up to 25-90% [24], and cause serious effects on many vital organs such as the intestine, heart, kidney, lymph nodes, spleen, lung, liver, and brain [25,26].Several laboratory techniques have been developed to detect FPV in cats, such as virus isolation and elector microscopy [27], Serological tests such as indirect ELISA, hemagglutination inhibition test, and immunofluorescence antibody test [17,20,27], and polymerase chain reaction (PCR) technique [28]. As well as the immunochromatography assay (ICA) is a quick and easy diagnostic technique that owners and veterinarians employ in clinical practice [29].

Material and Methods

Ethical approval

The University of Duhok, College of Veterinary Medicine, Animal Ethics Committee approved the study on January 6, 2021 (DR.199611CV).

Samples collection and animals information

During the period from December 2021 to November 2022, different samples, including fecal swabs (n=100), blood (n=100), ocular swabs (n=20), and oral swabs (n=20), were obtained from one hundred cats with the various lifestyles (stray cats and household cats), gender (males and females), ages (≤ 1 year and > 1 year), breeds (angora, persian, and short hair), immune status (vaccinated and non-vaccinated), health status (clinically healthy and clinically infected), pregnancy state (pregnant and non-pregnant), regions (Zakho, Sumel, and Duhok), and seasons (winter, autumn, summer, and spring).

Extraction of the feline panleukopenia virus DNA

The DNA of FPV was extracted from fecal swabs (n=100), blood (n=100), ocular swabs (n=20), and oral swabs (n=20), using the commercial AddPrep viral DNA extraction kit (Addbio Inc. Korea). That was performed according to the manufacturer's instructions. Using the Nanodrop (BioDrop, Germany), the concentration of extracted DNA was estimated at wavelength 260nm, while the purity of extracted DNA was estimated by calculating the ratio of (A260 nm to A280 nm) as described by Older *et al*. [30].

Amplification of the feline panleukopenia virus DNA

The c-PCR technique was used to amplify the VP2 gene of FPV from fecal swabs (n=100), blood (n=100), ocular swabs (n=20), and oral swabs (n=20). Two oligonucleotides specific primers were used to amplify the VP2 gene; the first primers (VP2A-F and VP2A-R) were designed by Awad *et al*. [31] and the second primers (VP2B-F and VP2B-R) were designed by Aydin and Timurkan, [32], which were provided by Macrogen Inc., South Korean (Table 1). A clinically and laboratory-positive cat's DNA was used as a positive control. Additionally, a healthy and laboratory-negative cat's DNA was used as a negative control. A total volume of 20µl of the PCR reaction was used, comprising 10µl of the master mix (2X), 1µl (10 pmol) of each primer (VP2A-F and VP2A-R), 3µl of template DNA, and 5µl of nuclease-free water. Same-mentioned total volume of the PCR reaction and procedure for the second primers (VP2B-F and VP2B-R). Furthermore, the thermocycler was set with some modifications in steps according to Awad *et al*. [31] and Aydin and Timurkan, [32] (Table 2). The Safe-Red^{TM} dye-stained and 1.5% agarose gel were used to electrophorese the PCR products. Further, to visualize the resultant bands, UV transillumination (BIO-RAD/USA) was utilized.

Sequencing of the feline panleukopenia virus DNA

The positive PCR amplicons (n=15) comprising different samples; fecal (n= 6), blood (n= 5), oral (n=2), and eye (n= 2), extracted from cats, were sent to a commercial company for purification and sequencing (Macrogen Inc., South Korea). The retrieved VP2 gene sequences were analyzed using Bio-Edit program version 7.2.5 and using NCBI BLAST (BLAST n) from NCBI (accessible at http://www.Ncbi.nlm.nih.gov), they were compared to other published FPV sequences from the GenBank. Multiple sequence alignments with the online tool (CLUSTALW) Genome Net were used to determine the alignment scores (within and between) obtained sequences. Moreover, the sequences of the VP2 gene for FPV were deposited in the NCBI GenBank using BankIt tool (https://www.ncbi.nlm.nih.gov/WebSub). The Bootstrap analysis with 1000 re-samplings and the Likelihood method on the Tamura-Nei model in MEGA11 software [33], the sequence (ON595914-feline bocavirus, Australia) was used as an outgroup in the created phylogenetic tree [34].

TABLE 1. The primers used in this study

Primers	Sequences 5'-3'	Target gene	Expected size (bp)	References
VP2A-F	TGC CTC AAT C TGAAG GAG CT	VP2	250	Awad *et al*. [31]
VP2A-R	TTT CAT CTG TTT GCG CTC CC			
VP2B-F	CAGGTGATGAATTTGCTACA		630	Aydin and Timurkan, [32]
VP2B-R	CATTTGGATAAACTGGTGGT			

TABLE 2. The PCR program for samples subjected to conventional PCR technique

The steps	Temperature	Time	Cycle
Initial denaturation of DNA	95 ºC	5 min	1
Denaturation of DNA	95 ºC	30 s	
Annealing of primers VP2A (250 bp.)	58 ºC	1min	35
Annealing of primers VP2B (630 bp.)	54 ºC		
Extension	72 ºC	1min	
Final extension	72 ºC	10 min	1
Storage until removal	4 ºC	Variable	

Statistical analysis

The IBM-SPSS Statistics (Version 22) program's two-sided Chi-square and Fischer's exact tests were used to determine the difference in the prevalence of the individual risk variables. Using factors with P values <0.05, which are deemed significant, is advised by Fisher exact test P value results if the anticipated cell value in the Chi-square test is less than 5. Additionally, using 2 by 2 tables in the Epi-InfoTM 7 programme (Version 7), the odds ratio (OR) for the relationship between risk factors for FPV and its 95% confidence interval was calculated.

Results

In the present study, utilizing the Nanophotometer, the extracted DNA concentration at 260 nm wavelength varied from 50.8 to 362.5 ng/µl, while the extracted DNA purity, determined by dividing the absorbance at 260 nm by the absorbance at 280 nm, it was between 1.7 and 1.9. Furthermore, the viral protein 2 (VP2) gene of FPV was amplified using the c-PCR technique, and the positive cats for FPV using the specific primers (VP2AF and VP2AR) and (VP2BF and VP2BR) were detected in approximately band size 250 bp (Figure 1) and 630 bp (Figure 2).

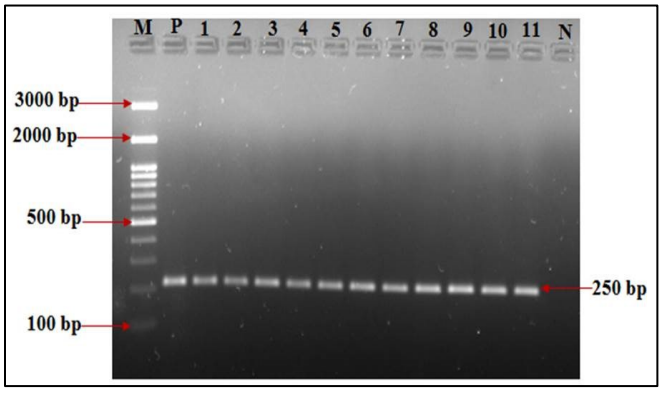

Fig. 1. Conventional PCR technique detected VP2 of the FPV in approximately band size 250bp; (M) representing DNA ladder; (P) representing positive control for FPV; (N) representing negative ol.

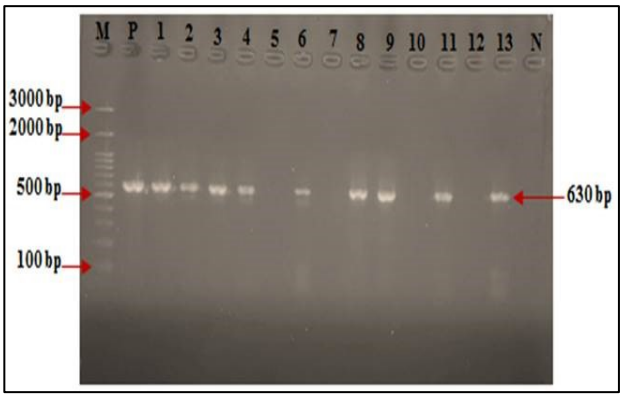

Fig. 2. Conventional PCR technique detected VP2 of the FPV in approximately band size 630bp; (M) representing DNA ladder; (P) representing positive control for FPV; (N) representing negative control.

The overall prevalence of FPV in cats in Duhok province was 70%, with no statistically significant difference in the prevalence of FPV in various types of samples, that examined, including feces, blood, ocular swabs, and oral swabs, which were 70%, 63%, 60%, and 70%, respectively (Table 3).

TABLE 3. Prevalence of FPV in different samples using the c-PCR technique

Type of samples	No. of tested cats	No. of +Ve positive (%)	P value
Feces swab	100	70 (70)[a]	0.368
Blood	100	63 (63)[a]	1.000
Ocular swab	20	12 (60)[a]	0.538
Oral swab	20	14 (70)[a]	1.000

(**+Ve**): Positive. Values that exhibit a significant difference (P < 0.05) are denoted by different letters (a, or b).

Results based on the c-PCR technique, showed that the prevalence of FPV was significantly higher (P<0.0025) among stray cats, 85.4% (OR: 4.64 times, CI: 1.760–12.261), compared to household cats 55.8% (Table 4). Regarding the gender factor, there was no significant difference between male and female cats (Table 4). The study also revealed that the prevalence of FPV was significantly (P<0.0002) higher in cats aged less than and equal to 1 year 82.4% (OR: 6.00 times, CI: 2.352-14.302), compared to cats aged more than 1 year 43.8% (Table 4). The prevalence of FPV was significantly (P< 0.0007) higher in short hair breed cats 85.0% (OR: 8.09 times, CI: 2.443-26.822) compared to angora and Persian breeds 41.2% and 52.2% respectively, but there were no significant (P< 0.0712) differences between the angora breed 41.2% (OR: 1.55 times, CI: 0.439-5.526) compared to Persian breeds 52.2% (Table 4). Furthermore, the prevalence of the FPLV disease was significantly higher (P<0.0025), (P<0.0239) in Duhok regions 76.1% (OR: 8.50 times, CI: 2.012-35.907), and in Sumel regions 72.7% (OR: 7.11 times, CI: 1.400-36.118) respectively, compared to Zakho regions 27.3% (Table 4).

The prevalence was also significantly (P< 0.0008) higher in unvaccinated cats 76.7% (OR: 8.25, CI: 2.333-19.168), compared to vaccinated cats 28.6%. There were no significant (P< 0.0668) differences in the prevalence of FPV between clinically infected cats and clinically healthy cats. While the prevalence was significantly (P< 0.0084) higher in pregnant cats 90.0% (OR: 16.36, CI: 1.825-146.669) than in the non-pregnant cats 35.5% (Table 4).

The current study, revealed that the prevalence of the FPLV disease was significantly (P< 0.0000) higher in the winter 96.7% (OR: 37.70, CI: 4.359-326.00) compared to the spring 43.4% and summer 50.0% seasons, while there were no significant differences between the winter and autumn seasons. The prevalence of the disease was also significantly (P< 0.0145) higher in the autumn 82.6% (OR: 6.17, CI: 1.589-23.994) compared to the spring 43.4% and summer 50.0% seasons, and there were no significant (P< 0.8764) differences between the spring 43.4% (OR: 1.30, CI: 0.412-4.101) and summer 50.0% seasons (Table 5).

TABLE 4. Odds ratio of cat factors linked with prevalence of Feline Panleukopenia virus based on c-PCR technique

Factors	No. of tested cats	Conventional PCR technique			
		No. of +Ve cats (%)	OR	95% CI	P value
Lifestyle					
House hold	52	29 (55.8) [a]	1		
Stray	48	41 (85.4) [b]	4.64	1.760-12.261	0.0025
gender					
Females	41	28 (68.29) [a]	1		
Males	59	42 (71.18) [a]	1.14	0.482-2.727	0.9292
Age					
> 1 year	32	14 (43.8) [a]	1		
≤ 1 year	68	56 (82.4) [b]	6.00	2.352-14.302	0.0002
Breed					
Angora	17	7 (41.2) [a]	1		
Persian	23	12 (52.2) [a]	1.55	0.439-5.526	0.0712
Short Hair	60	51 (85.0) [b]	8.09	2.443-26.822	0.0007
Regions					
Zakho	11	3 (27.3) [a]	1		
Sumel	22	16 (72.7) [b]	7.11	1.400-36.118	0.0239
Duhok	67	51 (76.1) [b]	8.50	2.012-35.907	0.0025
Immune status					
Vaccinated	14	4 (28.6) [a]	1		
Non-vaccinated	86	66 (76.7) [b]	8.25	2.333-19.168	0.0008
Health status					
Clinically healthy	33	19 (57.6) [a]	1		
Clinically infected	67	51 (76.1) [a]	2.34	0.964-5.719	0.0668
Pregnancy					
Non-pregnant	31	11 (35.5) [a]	1		
Pregnant	10	9 (90.0) [b]	16.36	1.825-146.669	0.0084

(+Ve): Positive, (OR): Odds Ratio, (CI): Confidence Intervals, Values that exhibit a significant difference ($P < 0.05$) are denoted by different letters (a, b or c).

TABLE 5. Odds ratio of season's factor associated with prevalence of Feline Panleukopenia virus based on c-PCR technique

Seasons	Conventional PCR technique				
	No. of tested cats	No. of +Ve cats (%)	OR	95% CI	P Value
Spring (March, April & May)	23	10 (43.4) [a]	1		
Summer (June, July & August)	24	12 (50.0) [a]	1.30	0.42-4.101	0.8764
Autumn (September, October & November)	23	19 (82.6) [b]	6.17	1.589-23.994	0.0145
Winter (December, January & February)	30	29 (96.7) [c, b]	37.70	4.359-326.00	0.0000

(**+Ve**): Positive, (OR): Odds Ratio, (CI): Confidence Intervals, Values that exhibit a significant difference ($P < 0.05$) are denoted by different letters (a, b or c).

All the local sequences (n=15) of the VP2 gene, representing 70 positive samples /100 cats, were deposited in the NCBI GenBank under the accession numbers (OQ102246.1-OQ102255.1, and OQ708628.1-OQ708632.1). Furthermore, the alignment score between obtained local sequences (OQ102246.1 to OQ102255.1) was 100%, while it was 97.39%-99.52% between (OQ708628.1 to OQ708632.1), and the alignment score between all obtained local sequences was 83.14% -99.52%, based on the online multiple sequence alignment-CLUSTALW (GenomeNet) programme (Table 6). The individual sequencing using online NCBI Blastn for the local sequences (OQ102246.1, OQ708628.1, OQ708629.1, OQ708630.1, OQ708631.1, OQ708632.1), was revealed a highly related (99.29%–100% identity) with the sequences that registered in the GenBank of different

countries, including China (MW495835.1, MT270543.1, MZ005633.1, MW017628.1, KC814179.1, and MT221234.1), Turkey (MZ391096.1),Thailand (MK425502.1, KP019621.1, MH711908.1, and KT357492. 1), and South Korea (HQ184194.1) (Table 7), (Figure 3).

The phylogenetic tree analysis revealed that the local sequences of the VP2 gene for FPV were closely related (99.29%–100%) to those available sequences of FPV mentioned above, after performing 1000 nucleotide sequence reconstruction using MEGA 11 software and Bootstrap analysis, the tree was rooted with ON595914.1-feline bocavirus-Australia, which was employed as an outgroup (Figure 3).

TABLE 6. Alignment score within and between obtained local sequences of feline panleukopenia virus using multiple sequence alignment- CLUSTALW.

Sequences Accession No.	Alignment score
OQ102246.1 **to** OQ102255.1	100
OQ708628.1 **to** OQ708632.1	97.39%-99.52%
OQ102246.1 **to** OQ708632.1	83.14% -100%

TABLE 7. Using NCBI BLASTn, the similarity between the local sequences of the feline panleukopenia virus (FPV) and the same pathogen sequences in the GenBank

Accession No. of local sequences	Identified Pathogen	Query Cover %	Similarity Number %	Accession Number in the GenBank	Identification Country
OQ102246	**FPV**	100	100	MW495835.1	China
OQ708628		100	99.76	MT270543.1	China
		100	99.53	MZ005633.1	China
		100	99.53	MW017628.1	China
OQ708629		100	99.53	KC814179.1	China
		100	99.53	MZ391096.1	Turkey
		100	99.29	MK425502.1	Thailand
OQ708630		100	99.29	KP019621.1	Thailand
		100	99.29	MH711908.1	Thailand
OQ708631		100	99.29	KT357492.1	Thailand
OQ708632		100	99.29	HQ184194.1	South Korea

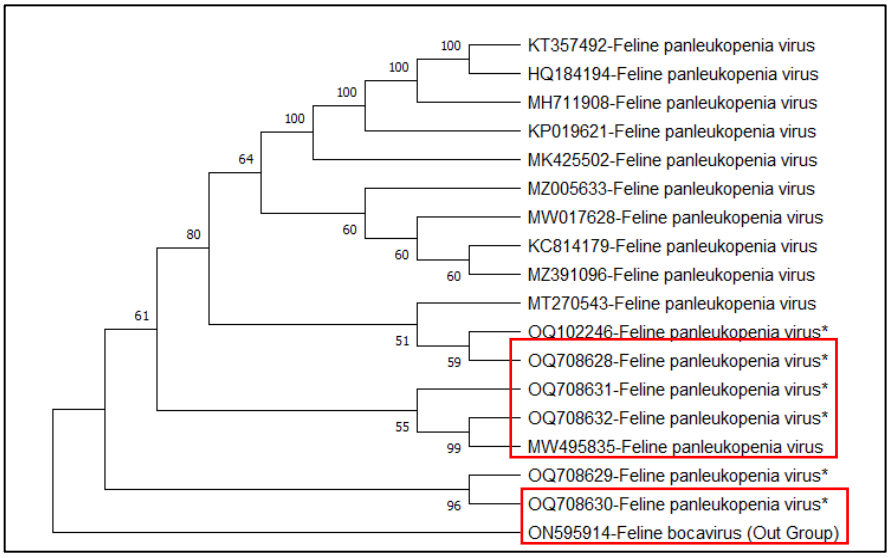

Fig. 3. Phylogenic tree of feline panleukopenia virus from Duhok province, Iraq (*). The phylogenic tree was constructed using the Maximum Likelihood method based on the Tamura-Nei model in MEGA11 software and bootstrap analysis with 1000 re-samplings. Partial DNA sequences of concatenated partial VP2 gene were used as input data. The feline bocavirus (ON595914.1- Australia) was used as outgroup.

Discussion

In this study, the overall prevalence of feline panleukopenia virus (FPLV) disease in cats in Duhok province was 70% using the conventional polymerase chain reaction technique (c-PCR). This result is higher than other studies conducted in Iraq and other countries such as: Baghdad-Iraq was 40%, 21.6% and 13.3% using c-PCR technique, indirect enzyme-linked immunosorbent assay (i-ELISA), and immunochromatography assay (ICA) respectively [19,20], in Ahvaz-Iran was 34.3% using ICA [35], in Burdur, Isparta and Izmit-Turkey was 25% using c-PCR technique [28], in Al Ain-United Arab Emirates was 2.18% using ICA [36], in Suhag, Assiut, and Cairo-Egypt was 35% and 43% using ICA and c-PCR, respectively [18], in Gimcheon-South Korea was 36.4% using quantitative real time polymerase chain reaction (q-PCR) technique [17], in Malang-Indonesia was ICA [37], in Jilin-China was 37.06% using multiplex PCR technique [38], in Bangladesh was 8.16% and 18.37% using ICA and c-PCR, respectively [39], in Bavaria- Germany was 65.8% using virus culture [40], and in Ontario/ Toronto-Canada was 11.72% using q-PCR technique [41]. The prevalence of FPV varied across different regions and countries due to different management approaches, environmental factors, efficient diagnostic techniques used in studies, and the presence or lack of additional parameters such as the host's age, physical condition, and immune status [16,18,20,28].

This study observed that there was no significant difference in the prevalence of FPV in various types of samples (feces, blood, ocular swabs, and oral swabs) when examined using the c-PCR technique. This finding corresponds to Radhy and Zenad, [19] who stated that there was no significant difference among the types of samples for detecting FPV infection in cats. The explanation for the reasons is that the virus is present in different secretions of infected cats, such as feces, urine, saliva, and nasal secretions [25,41].

Results also showed that the prevalence of FPV was significantly higher in stray cats than household cats. These findings are in agreement with Amoroso *et al*. [16]; Radhy and Zenad, [19] and Bukar-Kolo *et al*. [23]. This may be due to the virus spreading to healthy domestic cats through stray cats that are clinical or subclinical infected, as these cats shed the virus over extended periods of time or when they are on the move and looking for food [21,42]. Additionally, the majority of domestic cat owners maintain their cats' health by vaccinating them and keeping them away from stray cats [22]. Conversely, Jenkins *et al*. [14]; Abdel-Baky *et al*. [18] and Bergmann *et al*. [43] they stated that there was no significant difference between outdoor stray and indoor pet cats.

The present study revealed that there was no significant difference in the prevalence of FPV between male and female cats. This result is in accordance with Jenkins *et al*., (14); Zenad and Radhy, [20] and Awad *et al*. [31]. On the other hand, Al-Bayati, [21] found that the incidence of the infection is greater in females than males, these probably due to the physiological effects of the females during pregnancy and lactation period that are associated with immunological and hormonal changes.

Additionally, the prevalence of FPV was significantly higher in cats aged ≤1 year compared to cats >1 year. This finding agreed with those of Bukar-Kolo *et al*. [23] and Zenad and Radhy, [20] who mentioned that the prevalence of FPV is significantly higher in kittens than in adult cats and may cause fatal infection in kittens, whereas mild immunosuppressive disease occurs in adult cats. Furthermore, the adult cats may constitute reservoirs for the spread of the disease and are considered a potential risk for kittens [44]. Whereas these results were in conflict with Jenkins *et al*. [14] and Al-Bayati, [21] whose findings found no significant difference between cats less than one year and more than one year.

This present study also found that the prevalence of FPV was significantly higher in short hair breed cats compared to angora and Persian breeds, but there was no significant difference between angora and Persian breeds. This finding may be due to the fact that most of the short hair breeds are usually stray cats and live outdoors, while Angora and Persian usually live indoors as pet cats. The frequent exposure of stray cats to the virus in the outdoors makes them more susceptible to infection [42].

The results revealed that the prevalence of FPV in cats was significantly higher in the Duhok and Sumel regions compared to the Zakho regions. This result is comparable to Abdel-Baky *et al*. [18]; Radhy and Zenad, [19] and Al- Bayati, [21] they found that there is a different prevalence of FPV in cats in various regions of the same country. The difference in prevalence may be related to management practices, cat lifestyles, sampling size, and various climatic factors such as temperature, humidity, and rainfall [18,28,45].

In the current study, the prevalence of FPV was significantly higher in unvaccinated cats compared to vaccinated cats. This result agrees with the findings of Bukar-Kolo *et al*. [23] and Bergmann *et al*. [43] they recorded a higher prevalence of FPV in unvaccinated cats. Furthermore, Rehme *et al*. [40] who recorded that high titers of antibody were formed in vaccinated cats that protect cats against FPLV disease, so vaccination is the most important

measure to reduce the risk of disease and virus shedding.

No significant differences were recorded between clinically infected and healthy cats. The same result was recorded by Jenkins *et al*. [14]; Zhang *et al*. [38] and Oğuzoğlu *et al*. [44]. On other hand, Kim *et al*. [22] found that the prevalence of FPV was higher in healthy cats. Moreover, Radhy and Zenad, [19] mentioned that infected cats with diarrhea would be expected to enhance the shedding of viruses from damaged intestinal epithelium.

The prevalence of FPV was significantly higher in pregnant cats compared to non-pregnant cats. This finding may be due to significant reproductive problems caused by the virus when transported through the placenta, and causing infection to the embryo, or hardly weakening pregnant cats without congenital infection [46].

In the present study, according to the seasons, the prevalence of FPV was significantly higher in the winter and autumn seasons compared to the spring and summer seasons. The same findings were conducted by Amoroso *et al*. [16]; Radhy and Zenad, [19] and Al Eissaee *et al*. [36]. One explanation for these results may be that the virus is stable for long periods in organic material, debris, and solid fomites, increasing infection during the winter; however, this may be merely due to an increase in susceptible new born kittens [24]. Conversely, the outbreak of the FPLV disease increases from early summer until autumn [47]. The seasonal difference of FPV outbreaks between countries may be due to different climates within different geographical areas [18].

Various targeting genes have been used for the detection of FPV in cats. However, in this study, the virus protein 2 (VP2) gene was used because it is more commonly used in epidemiology and genetic diversity studies [17,19], the conservation of these gene sequences at the species level exists in various copies in the genome, and the gene sequences are available in molecular databases [48]. Moreover, regarding the sequencing and phylogenic analyses of PCR amplicons (n=15) of the VP2 gene for FPV obtained from cat's blood samples, fecal, oral, and ocular swabs, these genetic sequences were identified for the first time in Duhok province, Iraq. It was found to have phylogenetic characteristics and have a very tight evolutionary relationship with the other FPV sequences included in the NCBI GenBank for various countries, including China [49-52], Turkey [28], Thailand [53,54], and South Korea [55], with a highly related (99.29%–100% identity) after 1000 re-samplings using the Likelihood method on the Tamura-Nei model in MEGA11 software and Bootstrap analysis [33].

Conclusions

The feline panleukopenia virus is widely spread in Duhok province-Iraq. There are several risk factors linked to the higher prevalence of FPV such as stray cats, less than and equal to 1year olds, short hair, non-vaccinated, pregnant cats, the Duhok region, and the autumn and winter seasons. The phylogenetic analysis of FPV local sequences (OQ102246.1 - OQ102255.1, and OQ708628.1 - OQ708632.1) is important for controlling of the FPV in the study regions.

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Conflict of interest

The authors claim no conflicts of interest

References

1. Clutton-Brock, J. Camels and llamas. A Natural History of Domesticated Mammals. 2nd ed. Cambridge, England: The Press Syndicate of The University of Cambridge, pp.151-159 (1999).
2. Cove, M. V., Gardner, B., Simons, T. R., Kays, R. and O'Connell, A. F. Free-ranging domestic cats (Felis catus) on public lands: estimating density, activity, and diet in the Florida Keys. *Biological Invasions*, **20**, 333-344 (2018).
3. Tuzio, H. Feline panleukopenia. Infectious disease management in animal shelters, 2nd ed.; Miller, L.., Janeczko, J., Hurley, K., Eds.; John Wiley & Sons, USA, pp. 337-366 (2021).
4. Raj, V. P. R. P. and Haryanto, A. Clinical Study and Rapid Detection of Feline Parvovirus in Suspected Cats by Polymerase Chain Reaction Method. *Indonesian Journal of Veterinary Sciences*, **1**(1), 15-23 (2020).
5. Riya, B., Rathish, R. L., Deepa, P. M., John, L., Janus, A. and Vijaykumar, K. Clinical manifestations in cats with feline panleukopenia. *Journal of Veterinary and Animal Sciences,* **51**(1), 97-100 (2020).
6. Cotmore, S. F., Agbandje-McKenna, M., Canuti, M., Chiorini, J. A., Eis-Hubinger, A. M., Hughes, J., Mietzsch, M., Modha, S., Ogliastro, M., Pénzes, J.J. and Pintel, D.J. ICTV virus taxonomy profile: Parvoviridae. *Journal of General Virology*, **100**(3), 367-368 (2019).
7. Mietzsch, M., Pénzes, J. J. and Agbandje-McKenna, M. Twenty-five years of structural parvovirology. *Viruses*, **11**(4), 362-396 (2019).
8. Gallinella, G. Parvoviridae; In Encyclopedia of Infection and Immunity; Rezaei, N., Ed.; Elsevier: Oxford, UK, pp. 259-277 (2022).
9. Radhy, A. M., Mahmoud, M. H. S. and Khalaf, J. M. Novel detection of Canine Parvovirus 2c in domestic cats at Baghdad city, Iraq. *Systematic Reviews in Pharmacy*, **12**(3), 213-219 (2021).
10. Pan, S., Jiao, R., Xu, X., Ji, J., Guo, G., Yao, L., Kan, Y., Xie, Q. and Bi, Y. Molecular characterization and genetic diversity of parvoviruses prevalent in cats in Central and Eastern China from 2018 to 2022. *Frontiers in Veterinary Science*, **10**,1-8 (2023).
11. Raheena, K. P., Priya, P. M., Mani, B. K., Mini, M. and Pillai, U. N. Comparison of different diagnostic test to detect feline panleukopenia virus among cats in Kerala, India. *Indian Journal of Animal Research*, **51**(2), 347-349 (2017).
12. Stuetzer, B. and Hartmann, K. Feline parvovirus infection and associated diseases. *The Veterinary Journal*, **201**(2), 150-155 (2014).
13. Aiello, S. E., Moses, M. A. and Allen, D. G. (2016). The Merck veterinary manual. White Station, NJ, USA: Merck & Company, Incorporated, pp. 796-798 (2016).
14. Jenkins, E., Davis, C., Carrai, M., Ward, M. P., O'Keeffe, S., van Boeijen, M., Beveridge, L., Desario, C., Buonavoglia, C., Beatty, J.A. and Decaro, N. and Barrs, V. R. Feline parvovirus seroprevalence is high in domestic cats from disease outbreak and non-outbreak regions in Australia. *Viruses*, **12**(3), 320-332 (2020).
15. Jacobson, L. S., Janke, K. J., Giacinti, J. and Weese, J. S. Diagnostic testing for feline panleukopenia in a shelter setting: a prospective, observational study. *Journal of Feline Medicine and Surgery*, **23**(12), 1192-1199 (2021).
16. Amoroso, M. G., Serra, F., Miletti, G., Cardillo, L., de Martinis, C., Marati, L., Alfano, F., Ferrara, G., Pagnini, U., De Carlo, E., Fusco, G. and Montagnaro, S. A. Retrospective Study of Viral Molecular Prevalences in Cats in Southern Italy (Campania Region). *Viruses*, **14**(11), 2583-2596 (2022).
17. Yang, D. K., Park, Y. R., Park, Y., An, S., Choi, S. S., Park, J. and Hyun, B. H. Isolation and molecular characterization of feline panleukopenia viruses from Korean cats. *Korean Journal of Veterinary Research*, **62**(1), 10-1 (2022).
18. Abdel-Baky, M. M., El-Khabaz, K. A., Abdelbaset, A. E. and Hamed, M. I. Clinico-epidemiological survey of feline parvovirus circulating in three Egyptian provinces from 2020 to 2021. *Archives of Virology*, **168**(4), 126-133 (2023).
19. Radhy, A. M. and Zenad, M. M. Molecular and epidemiological study of pan-leukopenia in cats. *Online Journal of Veterinary Research,* **23**(10), 1032-1037 (2019).
20. Zenad, M. M. and Radhy, A. M. Clinical, serological and antigenic study of feline panleukopenia virus in cats in Baghdad, Iraq. *Iraqi Journal of Veterinary Sciences*, **34**(2), 435-439 (2020).
21. Al-Bayati, H.A.M. Detection of feline parvovirus (FPV) from cats infected with Enteritis using rapid test and Polymerase chain reaction in Iraq. *Kufa Journal for Veterinary Medical Sciences*, **7**(2),61-70 (2016).
22. Kim, S. G., Lee, K. I., Kim, H. J. and Park, H. M. Prevalence of feline panleukopenia virus in stray and household cats in Seoul, Korea. *Journal of Veterinary Clinics*, **30**(5), 333-338 (2013).
23. Bukar-Kolo, Y. M., Buba, E., Igbokwe, I. O. and Egwu, G. O. Prevalence of feline panleukopenia virus in pet and stray cats and associated risk factors in Maiduguri, Nigeria. *Alexandria Journal for Veterinary Sciences*, **59**(1), 92-96 (2018).
24. Greene, C.E. Feline parvovirus infection. Infectious Diseases of the Dog and Cat, 4th ed.; Greene, C.E., Ed.; Saunders Elsevier: St. Louis, MO, USA, pp. 80-90 (2012).
25. Sykes, J. E. Feline panleukopenia virus infection and other viral enteritides. Canine and Feline Infectious Diseases. 1st ed.; United States, California. Elsevier Saunders: St. Louis, MO, USA, pp. 141-194 (2014).
26. Al-Bayati, H. A. A. and Akaby, S. R. A. Study of histopathological changes associated with Feline panleukopenia virus infection in naturally infected

cats. *Journal of Education College Wasit University*, **1**(26), 511-520 (2017).

27. Yang, D. K., Park, Y., Park, Y. R., Yoo, J. Y., An, S., Park, J. and Hyun, B. H. Expression of the VP2 protein of feline panleukopenia virus in insect cells and use thereof in a hemagglutination inhibition assay. *Korean Journal of Veterinary Research*, **61**(2), 1-7 (2021).

28. Hasırcıoglu, S., Aslım, H. P., Kale, M., Bulut, O., Koçlu, O. and Orta, Y. S. Molecular characterization of carnivore protoparvovirus strains circulating in cats in Turkey. *Pesquisa Veterinária Brasileira*, **43**, e07178 (2023).

29. Ahmed, N., Riaz, A., Zubair, Z., Saqib, M., Ijaz, S., Nawaz-Ul-Rehman, M. S., Al-Qahtani, A. and Mubin, M. Molecular analysis of partial VP-2 gene amplified from rectal swab samples of diarrheic dogs in Pakistan confirms the circulation of canine parvovirus genetic variant CPV-2a and detects sequences of feline panleukopenia virus (FPV). *Virology Journal*, **15**(1), 1-7 (2018).

30. Older, C. E., Diesel, A., Patterson, A. P., Meason-Smith, C., Johnson, T. J., Mansell, J., Suchodolski, J. S. and Rodrigues Hoffmann, A. The feline skin microbiota: The bacteria inhabiting the skin of healthy and allergic cats. *PLoS One*, **12**(6), e0178555 (2017).

31. Awad, R. A., Khalil, W. K. and Attallah, A. G. Epidemiology and diagnosis of feline panleukopenia virus in Egypt: Clinical and molecular diagnosis in cats. *Veterinary World*, **11** (5), 578-584 (2018).

32. Aydin, H. and Timurkan, M. O. A pilot study on feline astrovirus and feline panleukopenia virus in shelter cats in Erzurum, Turkey. *Revue de Medecine Veterinaire*, **169**(1-3), 52-57 (2018).

33. Tamura, K., Stecher, G., and Kumar, S. MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution***, 38**(7), 3022-3027 (2021).

34. Van Brussel, K., Wang, X., Shi, M., Carrai, M., Feng, S., Li, J., Holmes, E.C., Beatty, J.A. and Barrs, V. R. The enteric virome of cats with feline panleukopenia differs in abundance and diversity from healthy cats. *Transboundary and Emerging Diseases*, **69**(5), e2952-e2966 (2022).

35. Mosallanejad, B., Avizeh, R. and Ghorbanpour, N. M. Antigenic detection of Feline Panleukopenia virus (FPV) in diarrhoeic companion cats in Ahvaz area. *Iranian Journal of Veterinary Research, Shiraz University,* **10** (3) 289-293 (2009).

36. Al Eissaee, S. A., Mohteshamuddin, K., Mahel, Z. and Ameni, G. Retrospective longitudinal study on canine and feline parvovirus infections in Al Ain, United Arab Emirates. *Emirates Journal of Food and Agriculture*, **32**(11), 762-767 (2020).

37. Priambudi, M. Z. D. R., Haskito, A. E. P., Inayah, K. and Adrenalin, S. L. Detection of feline panleukopenia with antigen test kit. *ARSHI Veterinary Letters*, **6**(1), 3-4 (2022).

38. Zhang, Q., Niu, J., Yi, S., Dong, G., Yu, D., Guo, Y., Huang, H. and Hu, G. Development and application of a multiplex PCR method for the simultaneous detection and differentiation of feline panleukopenia virus, feline bocavirus, and feline astrovirus. *Archives of Virology*, **164**, 2761-2768 (2019).

39. Chowdhury, Q. M. K., Alam, S., Chowdhury, M. S. R., Hasan, M., Uddin, M. B., Hossain, M. M., Islam, M.R., Rahman, M. M. and Rahman, M. M. First molecular characterization and phylogenetic analysis of the VP2 gene of feline panleukopenia virus in Bangladesh. *Archives of Virology*, **166**(8), 2273-2278 (2021).

40. Rehme, T., Hartmann, K., Truyen, U., Zablotski, Y. and Bergmann, M. Feline panleukopenia outbreaks and risk factors in cats in animal shelters. *Viruses*, **14**(6), 1248-1261 (2022).

41. Jonathan, R. K., Susan, F C., Marshall E. B., Michael, R. L. and Colin R. P. Parvoviruses, United States, New York, Hodder Arnold, Euston Road London, pp. 323-324 (2006).

42. Clegg, S. R., Coyne, K. P., Dawson, S., Spibey, N., Gaskell, R. M. and Radford, A. D. Canine parvovirus in asymptomatic feline carriers. *Veterinary Microbiology*, **157**(1-2), 78-85 (2012).

43. Bergmann, M., Schwertler, S., Speck, S., Truyen, U., Reese, S. and Hartmann, K. Fecal shedding of parvovirus deoxyribonucleic acid following modified live feline panleucopenia virus vaccination in healthy cats. *Veterinary Record*, **185**(3), 83-83 (2019).

44. Oğuzoğlu, T. Ç., Muz, D. İ. L. E. K., Timurkan, M. Ö., Maral, N. and Gurcan, I. S. Prevalences of feline coronavirus (FCoV), feline leukaemia virus (FeLV), feline immunodeficiency virus (FIV) and feline parvovirus (FPV) among domestic cats in Ankara, Turkey. *Revue de Medecine Veterinaire*, **164**(11), 511-516 (2013).

45. Barrs, V. R. Feline panleukopenia: a re-emergent disease. *Veterinary Clinics: Small Animal Practice*, **49**(4), 651-670 (2019).

46. Decaro, N., Carmichael, L. E. and Buonavoglia, C. Viral reproductive pathogens of dogs and cats. *Veterinary Clinics: Small Animal Practice*, **42**(3), 583-598 (2012).

47. Litster, A. and Benjanirut, C. Case series of feline panleukopenia virus in an animal shelter. *Journal of Feline Medicine and Surgery*, **16**(4), 346-353 (2014).

48. Chang, D., Liu, Y., Chen, Y., Hu, X., Burov, A., Puzyr, A., Bondar V. and Yao, L. Study of the immunogenicity of the VP2 protein of canine parvovirus produced using an improved Baculovirus expression system. *BMC Veterinary Research*, **16**, 1-9 (2020).

49. Niu, J. T., Yi, S. S., Hu, G. X., Guo, Y. B., Zhang, S., Dong, H., Zhao, Y.L. and Wang, K. Prevalence and molecular characterization of parvovirus in domestic kittens from Northeast China during 2016-2017. *Japanese Journal of Veterinary Research*, **66**(3), 145-155 (2018).

50. Guo, Z., Deng, M., Liu, Z., Xin, S., Wang, T., Ding, C., Xu, X., Li, Y., Chen, L. and Yang, Y. Feline panleukopenia virus associated Hypogranular cerebellar hypoplasia in young captive lions. *Authorea Preprints*, 1-17 (2022).

51. Tang, Y., Tang, N., Zhu, J., Wang, M., Liu, Y. and Lyu, Y. Molecular characteristics and genetic evolutionary analyses of circulating parvoviruses derived from cats in Beijing. *BMC Veterinary Research*, **18**(1), 1-11 (2022).

52. Xue, H., Hu, C., Ma, H., Song, Y., Zhu, K., Fu, J., Mu, B. and Gao, X. Isolation of feline panleukopenia virus from Yanji of China and molecular epidemiology from 2021 to 2022. *Journal of Veterinary Science*s, **24**(2), 1-12 (2023).

53. Charoenkul, K., Tangwangvivat, R., Janetanakit, T., Boonyapisitsopa, S., Bunpapong, N., Chaiyawong, S. and Amonsin, A. Emergence of canine parvovirus type 2c in domestic dogs and cats from Thailand. *Transboundary and Emerging Diseases*, **66**(4), 1518-1528 (2019).

54. Chaiyasak, S., Piewbang, C., Banlunara, W. and Techangamsuwan, S. Carnivore protoparvovirus-1 associated with an outbreak of hemorrhagic gastroenteritis in small Indian civets. *Veterinary Pathology*, **57**(5), 706-713 (2020).

55. An, D. J., Jeong, W., Jeoung, H. Y., Yoon, S. H., Kim, H. J., Park, J. Y. and Park, B. K. Phylogenetic analysis of feline panleukopenia virus (FPLV) strains in Korean cats. *Research in Veterinary Science*, **90**(1), 163-167 (2011).

التشخيص الجزيئي وعوامل الخطورة وتحليل الشجرة الجينية لفيروس طاعون القطط في القطط في محافظة دهوك، العراق

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الخلاصة

هدفت الدراسة إلى تحديد مدى انتشار فيروس طاعون القطط في القطط في محافظة دهوك-العراق، باستخدام تقنية تفاعل البلمرة المتسلسل التقليدي، وتقييم بعض عوامل الخطورة المرتبطة بانتشار فيروس طاعون القطط، والتحقيق من تحليل النشوء الجيني لفيروس طاعون القطط المشخص في هذه الدراسة. تم الحصول على عينات مختلفة (عينات دم ومسحات براز، ومسحات من العيين، والفم) من 100 قطة مختلفة في أنماط الحياة، والجنس، والاعمار، وسلالات، والحالة المناعية، والحالة الصحية، والحمل، والمناطق، والمواسم. تم استخدام جين البروتين الفيروسي 2 (VP2) للكشف وتحليل النشوء الجيني لفيروس طاعون القطط. أظهرت النتائج أن نسبة انتشار فيروس طاعون القطط في القطط في محافظة دهوك بلغت 100/70 (70٪)، مع عدم وجود اختلاف معنوي في نسبة انتشار الفيروس في الانواع المختلفة للعينات. شملت عوامل الخطورة المرتبطة بارتفاع نسبة انتشار فيروس طاعون القطط، القطط السائبة والقطط التي عمرها سنة واحدة واقل من سنة، والقطط قصيرة الشعر، وغير المحصنة، والقطط الحوامل، ومنطقة دهوك، وفصلي الخريف والشتاء. اظهر التحليل النشوئي للتسلسلات الجينية محليًة (15 تسلسل) للجين VP2 التي تم تسجيلها في بنك الجينات المركزي الوطني لمعلومات التكنولوجيا بأرقام تسلسلية (OQ102246.1 - OQ102255.1)، و (OQ708632.1 - OQ708628.1)، أن هذه التسلسلات المحلية كانت عالية التشابه (99.29% - 100%) تماثل) بالتسلسلات الأخرى المسجلة في بنك الجينات من بلدان مختلفة كالصين وتركيا وتايلاند وكوريا الجنوبية. أستنتج من هذه الدراسة، أن فيروس طاعون القطط واسع الانتشار في محافظة دهوك-العراق. وهناك العديد من عوامل الخطر المرتبطة بارتفاع نسبة انتشار الفيروس، ويعد تحليل النشوء الجيني للتسلسلات الجينية المحلية للفيروس مهمًا لاستراتيجية السيطرة على المرض في مناطق الدراسة.