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Genetic analysis of feline parvovirus reveals predominance of FPV-G1 group among cats in China

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Running title: GENETIC CHARACTERISTIC OF FELINE PARVOVIRUS

ABSTRACT

Feline parvovirus (FPV) or feline panleukopenia virus is a highly contagious, life-threatening infectious virus in cats. Although FPV vaccination is routinely practiced in China, clinical diseases continue to occur. The investigation of genotypes and viral evolution can contribute to the prevention, diagnosis, and treatment of FPV. Therefore, this study aimed to provide an up-to-date understanding of the epidemiological, genotypic, and phylogenetic characteristics of FPV. In total, 152 rectal swabs were collected from diseased cats. All swab samples were tested for FPV using molecular methods. Amplification of the complete viral protein 2 (VP2) gene was performed for further analysis and to infer the genotypic and evolutionary characteristics of FPV. Of the 152 samples, FPV DNA was detected in 17 (17/152, 11.18%). Cats with FPV showed variable clinical signs such as dehydration, anorexia, fever, vomiting, and blood-stained diarrhea. Furthermore, VP2 sequences were identified in 17 PCR-positive cats, confirming the presence of FPV. Phylogenetic and nucleotide pairwise identity analyses revealed high genetic similarity among FPV sequences (99.6%–100%) and clustered them into the FPV-G1 group. Amino acid analysis indicated a novel mutation (Ala91Ser) in all VP2 gene sequences amplified in this study. Our study provides baseline epidemiological data for the better prevention of FPV with respect to vaccination strategies. Genotypic and phylogenetic analyses confirm that FPV-G1 was the predominant FPV group in infected cats in Kunshan. Therefore, a rigorous countrywide investigation of the genotypic and evolutionary characteristics of FPV is warranted.

Keywords: Feline parvovirus, genotyping, evolution, China

Introduction

Feline parvovirus infection, also known as feline panleukopenia, is a highly contagious, life-threatening virus infecting cats which spreads quickly from one infected cat to another [5, 13, 31]. Kittens and non-vaccinated cats are usually affected by feline panleukopenia, leading to high morbidity and mortality rates, especially in young cats or kittens. Although vaccination against feline panleukopenia is routinely practiced in China, disease cases are still observed. Feline panleukopenia can spread directly or indirectly through infected cats, contaminated objects, and bedding materials [22]. Diarrhea, vomiting, fever, and significant decrease in white blood cells are the main characteristics of Feline panleukopenia in cats [5]. The etiology of feline panleukopenia is a small single-stranded DNA virus known as feline parvovirus (FPV). Notably, FPV was first identified in 1928 and later isolated from tissue culture in 1964 [9]. Researchers believe that

canine parvovirus (CPV-2) that infects canines originated from FPV and acquired two key substitution mutations (Lys93Asn and Asp323Asn) in the capsid protein (VP2) [8, 27]. Although the original CPV-2 does not infect cats, new variants of CPV-2 (CPV-2a, CPV-2b, and CPV-2c) can. Amino acid substitution mutations at six key positions (87, 297,300, 305,426, 555) in the VP2 protein have been associated with infection in cats. The pathogenicity of CPV-2 variants (CPV-2a, CPV-2b, and CPV-2c) is apparently mild; however, they can induce severe disease in immunocompromised or co-infected cats. Primarily, FPV targets rapidly dividing cells such as those lining the intestinal mucosa, bone marrow, and lymphoid tissues, leading to a significant reduction in white blood cells (leukopenia) and subsequent immunosuppression [26]. This makes infected cats prone to secondary infections.

Carnivore protoparvovirus 1 is a unique species of viruses in the family *Parvoviridae* which also includes FPV, CPV-2, mink enteritis virus, and raccoon parvovirus [7]. This icosahedral virion has a size of approximately 25 nm and a genome length of approximately 5000 bases, and mainly encodes two non-structural proteins, NS1 and NS2, as well as two structural proteins, VP1 and VP2 [17]. Notably, VP2 is a key and predominant capsid protein and a major target of neutralizing antibodies against FPV. The VP2 gene plays key roles in viral replication and pathogenesis. It encodes the viral capsid protein that facilitates viral entry into host cells and is a major target of host immunity [17, 18]. The host ranges of FPV and CPV-2 are determined by specific amino acids in the VP2 protein, including 80 (L to R), 93 (L to N), 103 (V to A), 232 (V to I), 323 (E to N), 564 (N to S), and 568 (A to G). The antigenicity of FVP or CPV-2 depends on the VP2 protein. Random genetic drift is the main driver of FPV evolution. In addition, genetic recombination has played a crucial role in FPV evolution. Alterations in VP2 amino acids can lead to viral evolution and the generation of different antigenic variants, making it difficult to prevent and treat feline panleukopenia [12, 15, 28].

Therefore, genetic analysis of the VP2 gene can help identify, characterize different FPV strains and provide valuable insights into the evolution, epidemiology, and possible virulence factors associated with the virus [30]. Several studies have analyzed the full-length VP2 gene of FPV and identified several genetic variations that can be used to track the evolution and spread of the virus. Genetic variations in the VP2 gene can be used to track the origin and spread of the virus, as well as to develop effective vaccines and treatments for the disease [30].

Several epidemiological studies on FPVs have been conducted in China. Between 2021 and 2022, a study conducted in Yanji, China isolated and examined the FPV strain and found that 27 of 80 samples tested positive for the virus [31]. Furthermore, a study performed in Beijing, China, found that cats were predominantly infected with FPV strains, with CPV-2b and CPV-2c variants circulating in the population [24]. The CPV-2c strain has been identified as the dominant antigenic variant prevalent among cats in

Beijing. Other studies have identified FPV-like viruses in dogs in Southwest China, with 28/113 (24.8%) strains identified based on amino acid residues in VP2 [29]. The treatment of feline panleukopenia typically involves supportive care such as fluid therapy and antibiotics to prevent secondary infections. Vaccination is the most effective way to prevent this disease, and all cats should be vaccinated against FPV. Furthermore, isolating infected cats and disinfecting any areas they have visited is important to prevent the spread of the virus to other cats.

As FPV has evolved over time, multiple strains have been identified. Genotypic and viral evolution analyses have contributed to the prevention, diagnosis, and treatment of viral infections. Data on FPV in cats in China is limited. To the best of our knowledge, no published data regarding FPV epidemiology and genotypic and phylogenetic characteristics are available from Kunshan City, indicating the need for epidemiological studies focused on the molecular evolution and genetic characteristics of FPV. Therefore, this study aimed to provide an up-to-date understanding of the epidemiology, genotype, and phylogenetic characteristics of FPV in Kunshan City, Jiangsu Province, China. By investigating the genetic variations and phylogenetic relationships among FPV strains circulating in this specific region and period, we aimed to contribute to the understanding of the molecular epidemiology, evolution, and potential effects on feline health. This information is essential for the development of effective preventive measures, including vaccines and antiviral therapies, and for the implementation of appropriate control strategies to mitigate the spread of FPV in domestic cat populations.

MATERIALS AND METHODS

Ethical approval

The Institutional Animal Care and Use Committee (IACUC) was not required for this non-invasive study; however, the international ethical standards for animal handling and sample collection were strictly followed.

Cat population and sample collection

During the feline disease surveillance program, 152 rectal swabs were collected from cats with diarrhea and other health problems at animal clinics in Kunshan City, China, between January 2022 and September 2023 (Figure 1). Clinical staff recorded data on the breed, age, sex, clinical symptoms, residence, and vaccination status of all cats sampled. Sterile anal swabs (Copan Diagnostics Inc, Italy) were used to collect samples from the rectum of the infected cats. Subsequently, the anal swabs were immersed in a vial containing sterile saline solution. The sample tubes were centrifuged for 10 minutes to separate undigested feed particles from the swab samples. The supernatant was collected and kept at -80 °C for DNA extraction.

DNA extraction and molecular detection of FPV

All samples were processed for DNA extraction and tested for FPV identification using molecular methods. DNA was extracted from all samples using a TaKaRa MiniBEST Viral RNA/DNA Extraction Kit (Cat#9766, Takara, Dalian, China). Molecular screening was conducted by PCR amplification of a 428 bp fragment of the VP2 gene using the primers and thermocycling conditions given in Supplementary Table 1 [1]. PCR was performed using Rapid Taq master mix (Vazyme, Nanjing, China) on a thermocycler (LifeEco TC 96, Bioer, Hangzhou, China*)*. PCR amplicons of approximately 428 bp in length were analyzed by 1.5% agarose gel electrophoresis using a gel imaging analysis system (GenoSens 1880*,* Clinx, Shanghai, China).

Complete VP2 gene amplification and sequencing

The complete VP2 gene was amplified using PCR for genotypic and evolutionary analyses. The complete VP2 gene was amplified with a predicted length of 1932 bp using previously reported primers and conditions [31]. The sequencing primers and conditions are listed in Table 1. Positive and negative controls were added to each reaction to optimize and validate the assays. All PCR products were sequenced by GeneWiz (Suzhou, China).

Genetic and evolutionary analysis

For comparative genetic analysis, we retrieved the reference VP2 gene sequences of FPV and CPV-2 from the National Center for Biotechnology Information (NCBI) GenBank nucleotide database (http://www.ncbi.nlm.nih.gov). A total of 110 global reference sequences were downloaded for comparative analysis. The VP2 sequences from this study were aligned using the ClustalW algorithm with 118 reference sequences, followed by manual editing in BioEdit (Ibis Biosciences, Carlsbad, CA, USA). Phylogenetic relationships were calculated by the neighbor-joining method (500 bootstrap replicates), and evolutionary distances were computed using the maximum composite likelihood method in the MEGA11 software package [23]. The sequences obtained in the present study were compared with the reference sequences of FPV using phylogenetic and nucleotide pairwise identity analyses. The FPV sequences found in the present study were deposited in the NCBI GenBank database (accession numbers OR399559-OR399575).

Recombination analysis in VP2 gene

The complete VP2 gene sequences of FPV found in this study were aligned using MEGA 11 software. Screening was performed to detect any possible recombination among sequences using the RDP4 recombination detection software containing various recombination detection methods (RDP,

GENECONV, Bootscan, MaxChi, Chimaera, SiScan, and 3Seq). The highest acceptable P value was set at 0.05 [24, 27].

RESULTS

Preliminary detection of FPV in samples

Of the 152 samples, we detected FPV DNA in 17 (17/152, 11.18%) using PCR assays. A 428 bp DNA segment of the VP2 gene was targeted for the preliminary detection of FPV in samples. Our results were verified using a positive control by amplifying PCR products of similar size in all reactions. Cats with FPV exhibited various clinical signs (Table 1). No clinical signs were observed in one FPV-positive cat. Among the 17 positive cats, anorexia (10/17, 58.8%), vomiting (7/17, 41.17%), fever (7/10, 41.17%), and diarrhea (5/17, 29.4%) were the prominent clinical signs. Of the 152 cats sampled, nine showed clinical signs of diarrhea. Among these, only four cats with diarrhea tested positive for FPV, whereas five cats with diarrhea tested negative for FPV. All samples from cats with diarrhea were retested for confirmation. Four fully vaccinated cats tested positive for FPV. Three of the vaccinated cats showed mild clinical signs (anorexia, lethargy, diarrhea, dehydration, and fever), whereas one cat was asymptomatic (Table 1). The samples were not tested for other enteric pathogens.

Nucleotide homology analysis and phylogeny

Genotypic and phylogenetic analyses were performed using the complete VP2 gene sequences to infer the genotyping and evolutionary characteristics of FPV in Kunshan. Reference sequences were categorized into three main groups (FPV-G1, FPV-G2, and FPV-G3) and eight subgroups (FPV-G3A, FPV–G3B, FPV–G3C, FPV–G3D, FPV–G3E, FPV–G3F, FPV–G3G, and FPV–G3H) as previously described [30]. Seventeen complete VP2 gene sequences confirmed the presence of FPV. Phylogenetic and nucleotide pairwise identity analyses revealed higher genetic similarity among FPV sequences (99.6%–100%) and showed a close relationship with the FPV-G1 group (Figure 2). None of the sequences from the present study clustered with FPV-G2 or FPV-G3. The VP2 sequences were genetically similar and revealed a close evolutionary association with sequences reported from China (Beijing, Nanjing, Yanji, Yangzhou, Chengdu, Changchun, Jilin, and Nanyang), South Korea, and Argentina. Although the VP2 sequences were more similar to each other, they showed distant relationships with the reference sequences of FPV reported from other countries, including the US, Japan, Turkey, France, Germany, Vietnam, Italy, and Brazil, and clustered separately in the phylogenetic tree.

Deduced amino acid analysis

The key amino acid positions in the VP2 protein (positions 5, 80, 87,91,101, 103,297, 300, 305, 323, 426, 564, and 568) were analyzed by deduced amino acid analysis. The VP2 protein sequences in this study were typed as FPV after analysis of key amino acids. Amino acid analysis indicated a novel mutation (Ala91Ser) in all VP2 sequences in this study. Since 2019, mutations at amino acid position 91(A91S) have become predominant among the Chinese FPV strains. Only OR399569 showed an amino acid substitution (glycine instead of aspartic acid) at position 305 compared to the FPV reference strains (M24004 and M38246). Marked amino acid variations were observed among CPV-2 and FPV reference strains and FPV strains observed in this study. Table 2 shows the variations in amino acids among the FPV sequences observed in this study and the reference FPV strains.

Recombination analysis of VP2 gene

Based on the RDP4 results, no potential recombination events were found within the 17 complete VP2 gene sequences of FPV. This finding suggests that the FPV sequences in this study were not the products of recombination events in other parvoviruses.

DISCUSSION

Feline panleukopenia is a highly contagious disease, characterized by fever, vomiting, diarrhea, severe leukopenia, and abdominal pain. The feline panleukopenia virus is commonly responsible for acute gastroenteritis and leukemia in cats [5]. In addition to domestic cats, FPV has been reported in racoons, minks, and a few wild cat species, including tigers, lions, leopard cats, civets, and wild fishing cats [11]. FPV causes immunosuppression in cats by damaging immune cells, bone marrow, and the gut epithelium, providing an opportunity for coinfection with other enteric viruses. Genetic variations in FPV and differences in host immune responses can alter the severity of disease and clinical signs. Despite the regular practice of vaccination against FPV in China, new cases continue to occur. The genetic and evolutionary characteristics of FPV in Kunshan City, China, have not yet been studied. Therefore, this study aimed to investigate the prevalence, molecular and phylogenetic characteristicsof circulating FPV strains. This is the first report of the genetic and evolutionary analyses of FPV strains in Kunshan, China.

In this study, 152 rectal swabs were collected from cats in Kunshan and analyzed for the presence of FPV DNA. Overall, the FPV detection rate was 11.18%, which was higher than that reported in previous studies conducted in China [24]. In contrast to our findings, higher detection rates have been previously reported [1, 3, 30]. Recently, Wang et al. (2024) reported a prevalence of 41.2% in cats in Shandong Province, China. Similarly, Awad et al. (2018) and Abdel Baky et al. (2023) reported prevalence rates of 45% and 43%,

respectively, among cats in Egypt. A better vaccination program could explain the lower prevalence of FPV in Kunshan. Interestingly, one FPV-positive cat in this study did not show any clinical signs. Among the 17 FPV-positive cats, anorexia (10/17;58.8%), vomiting (7/17;41.17%), fever (7/10;41.17%), and diarrhea (5/17;29.4%) were prominent clinical signs. These clinical signs are similar to those reported in previous studies [1, 18, 19]. However, in this study, four vaccinated cats were also found to be positive for FPV, which could be the result of vaccine failure or insufficient protection provided by current FPV vaccines. Most of the infected cats in the present study were below six months of age (58.8%), whereas 76.47% (13/17) of the infected cats were non-vaccinated, indicating that kittens and non-vaccinated cats can be easily infected with FPV from the environment [16, 18]. Notably, FPV mostly infects cats below 6 months of age, whereas older cats often exhibit mild or no clinical signs [2]. Vaccine failure may be due to improper administration or neutralization of vaccines by maternal antibodies [25]. Viral evolution and crucial mutations in the capsid gene of FPV can also contribute to vaccine insufficiency [16, 18].

Notably, FPV has a conserved genome relative to other feline viruses, which explains the absence of genotypic subgrouping [6, 14, 18, 30]. However, some studies have used different groups for genetic analysis of FPV [24, 30]. For the genotypic and phylogenetic analyses, 110 reference sequences were retrieved from the NCBI GenBank sequence database. A phylogenetic tree was constructed to study the evolutionary relationship between the FVP reference sequences and the FPV sequences obtained in this study. Reference sequences were categorized into three main groups (FPV-G1, FPV-G2, and FPV-G3) and eight subgroups (FPV-G3A, FPV–G3B, FPV–G3C, FPV–G3D, FPV–G3E, FPV–G3F, FPV–G3G, and FPV–G3H). Seventeen complete VP2 gene sequences confirmed the presence of FPV. Phylogenetic and nucleotide pairwise identity analyses revealed higher genetic similarity among FPV sequences (99.6%–100%) and showed a close relationship with the FPV-G1 group (Figure 2). None of the sequences from the present study clustered into FPV-G2 or FPV-G3 and diverged from internationally published sequences. These findings are consistent with the findings of Wang et al. (Wang et al., 2024) and Chen et al. (Chen et al.,2022) who reported the predominance of FPV-G1 in China. In contrast to other countries, only one inactivated vaccine (FPV-Cu4) is used in China. Inoculation with this inactivated vaccine could be one of the reasons for the absence of FPV-G2 in China [30].

The VP2 gene sequences obtained in this study were genetically similar and revealed close evolutionary associations with sequences reported from China, including Beijing, Nanjing, Yanji, Yangzhou, Chengdu, Changchun, Jilin, and Nanyang. Amino acid analysis indicated a novel mutation (Ala91Ser) in all VP2 gene sequences detected in this study. This finding is consistent with previous studies from China [6, 30]. Since 2019, mutations at amino acid position 91(A91S) have become predominant among the Chinese FPV strains. A mutation at position 91 has been reported to alter the biological activities of FPV and its receptor binding

[6, 30]. Interestingly, we observed an amino acid substitution (glycine instead of aspartic acid) in OR399569 compared to other sequences, including the FPV reference sequences. Similar findings have been reported for the FPV sequence (OR194125) from Nanjing, China (unpublished data, sequences published in NCBI GenBank).

Based on the RDP4 results, no potential recombination breakpoints were found within the 17 complete VP2 gene sequences of FPV observed in this study, suggesting that these FPV sequences were not a result of recombination events from other parvoviruses. These findings are similar to those reported by Liu et al. (Liu et al., 2023) in China, who reported no signs of recombination between FPV sequences. Previously, recombination events were observed within FPV and reference sequences [10, 20, 24]. Tang et al. (Tang et al., 2022) reported recombination events in only one FPV sequence, indicating that recombination events among FPV sequences are rare in China. Random genetic drift is the main driver of FPV evolution. In addition, genetic recombination plays a crucial role in FPV evolution and is recognized as a crucial evolutionary force in several viral families, leading to better replication and survival [4, 15, 21]. To track the spread of FPV and identify potential risk factors for disease outbreaks, epidemiological studies are necessary, in addition to genetic analyses. Furthermore, understanding the transmission routes and reservoir hosts of FPV in China remains crucial to implement effective control measures and prevent future outbreaks. This study has some limitations such as a small sample size and lack of information on the correlation between antibody responses to vaccination and infection. Future studies should include a larger number of FPV isolates from diverse geographical locations in China for genetic analysis. Second, we could not study co-infecting viruses within these samples. Co-infection with enteric viruses could have a serious additive effect on the health of cats with FPV. Finally, the risk factors associated with FPV infection in cats were not studied. Regardless of these limitations, further studies are necessary and should be conducted regularly to better understand FPV evolution.

Conclusion: Our study provides baseline epidemiological data for policymakers to improve the prevention of FPV with respect to vaccination strategies. This study confirms that FPV-G1 carries a novel mutation (A91S) and is the predominant group of FPV infecting cats in Kunshan. Therefore, we urge careful screening of cats for the VP2 gene of FPV during routine diagnosis to determine the genotypes. In addition, a rigorous countrywide investigation of the genotypic and evolutionary characteristics of FPV is warranted.

CONFLICT OF INTEREST. The authors declare no conflicts of interest.

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Legends:

Figure 1. Maps of the study area and sampling locations. Rectal swabs were collected from cats between 2022 and 2023 from veterinary clinics in Kunshan City, Jiangsu Province, China.

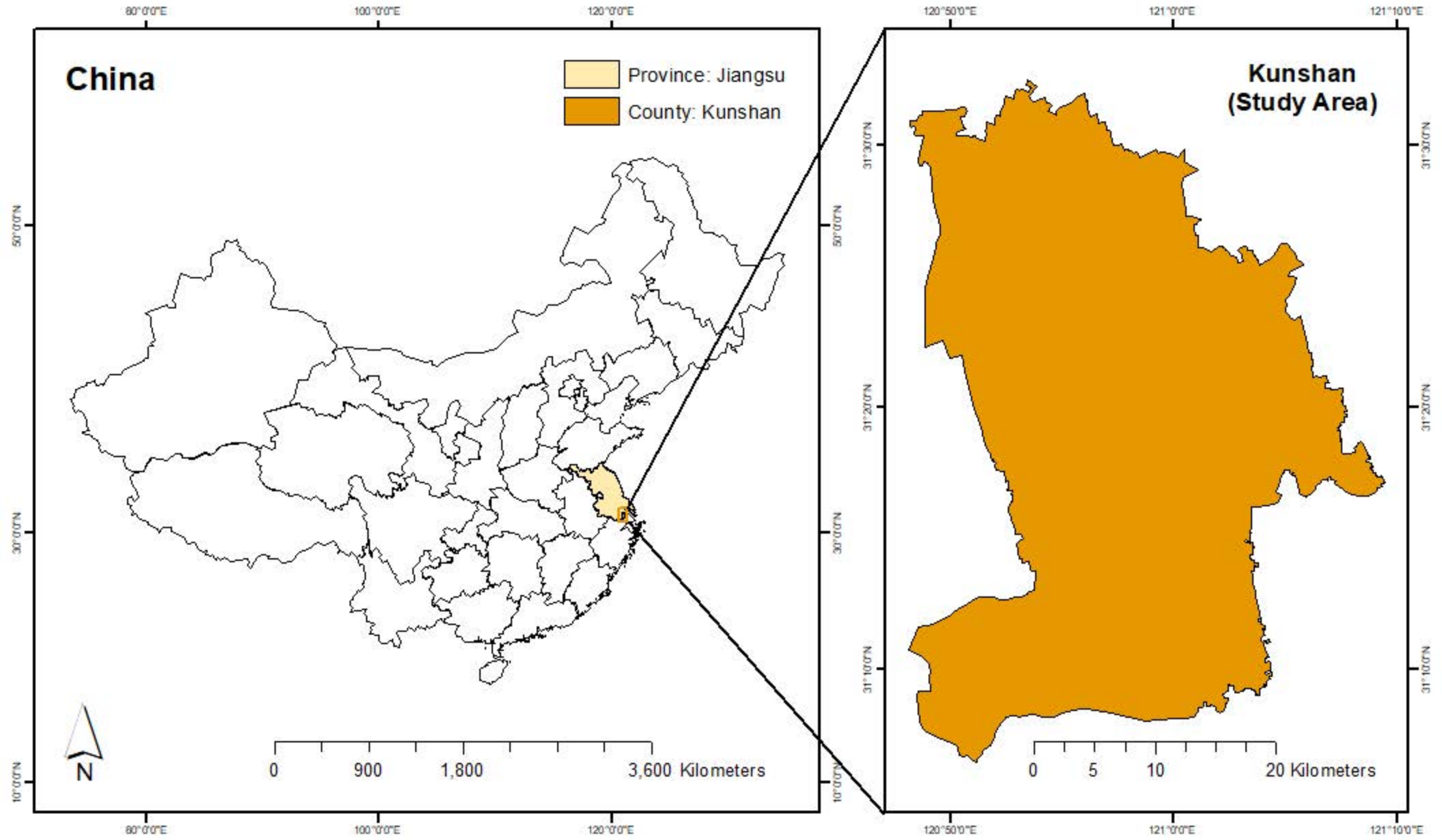
Figure 2.

Complete VP2 gene-based phylogenetic tree of Feline parvovirus (FPV)strains circulating in Kunshan. Phylogenetic relationships were calculated by the neighbor-joining method (500 bootstrap replicates). The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. Evolutionary analyses were conducted in MEGA11. FPV isolates collected in the present study have been marked with “red” circles.

Table 1. Correlation between clinical signs and feline panleukopenia virus-positive samples in this study

Table 2. Variation of amino acids among Feline parvovirus (FPV) sequences observed in the present study and reference FPV strains.

Supplementary Table 1. Primers used PCR to amplify the VP2 gene of FPV for detection and sequencing.


China
Province: Jiangsu
County: Kunshan
N
0
900
1,800
3,600 Kilometers
Kunshan
(Study Area)
0
5
10
20 Kilometers
80°0'0"E
100°0'0"E
120°0'0"E
120°50'0"E
121°0'0"E
121°10'0"E
50°0'0"N
30°0'0"N
10°0'0"N
31°30'0"N
31°20'0"N
31°10'0"N

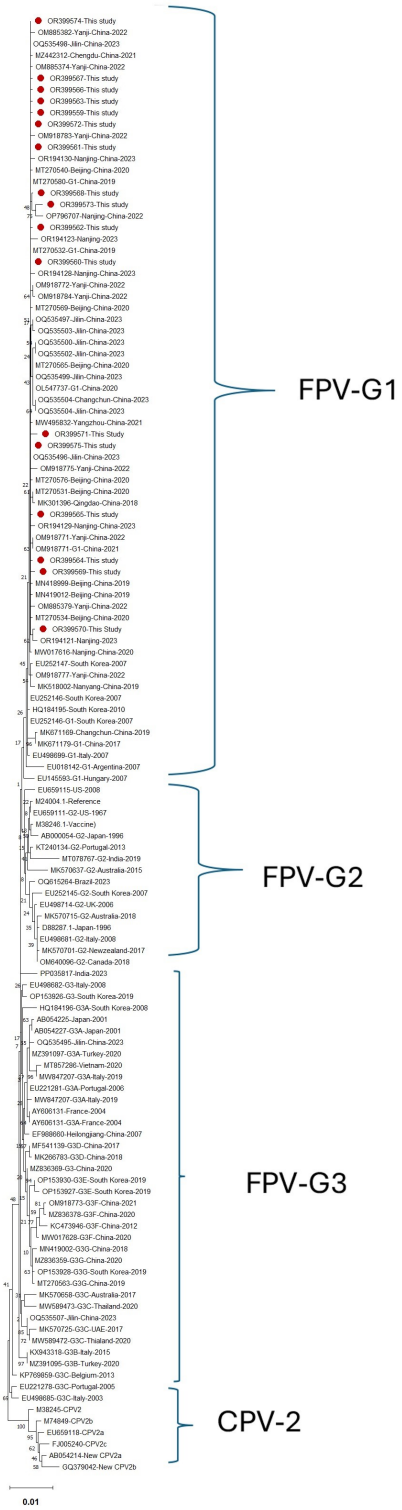

OR399574-This study
OM885382-Yanji-China-2022
OQ535498-Jilin-China-2023
MZ442312-Chengdu-China-2021
OM885374-Yanji-China-2022
OR399567-This study
OR399566-This study
OR399563-This study
OR399559-This study
OR399572-This study
OM918783-Yanji-China-2022
OR399561-This study
OR194130-Nanjing-China-2023
MT270540-Beijing-China-2020
MT270580-G1-China-2019
OR399568-This study
OR399573-This study
OP796707-Nanjing-China-2022
OR399562-This study
OR194123-Nanjing-2023
MT270532-G1-China-2019
OR399560-This study
OR194128-Nanjing-China-2023
OM918772-Yanji-China-2022
OM918784-Yanji-China-2022
MT270569-Beijing-China-2020
OQ535497-Jilin-China-2023
OQ535503-Jilin-China-2023
OQ535500-Jilin-China-2023
OQ535502-Jilin-China-2023
MT270565-Beijing-China-2020
OQ535499-Jilin-China-2023
OL547737-G1-China-2020
OQ535504-Changchun-China-2023
OQ535504-Jilin-China-2023
MW495832-Yangzhou-China-2021
OR399571-This Study
OR399575-This study
OQ535496-Jilin-China-2023
OM918775-Yanji-China-2022
MT270576-Beijing-China-2020
MT270531-Beijing-China-2020
MK301396-Qingdao-China-2018
OR399565-This study
OR194129-Nanjing-China-2023
OM918771-Yanji-China-2022
OM918771-G1-China-2021
OR399564-This study
OR399569-This study
MN418999-Beijing-China-2019
MN419012-Beijing-China-2019
OM885379-Yanji-China-2022
MT270534-Beijing-China-2020
OR399570-This Study
OR194121-Nanjing-2023
MW017616-Nanjing-China-2020
EU252147-South Korea-2007
OM916777-Yanji-China-2022
MK518002-Nanyang-China-2019
EU252146-South Korea-2007
HQ184195-South Korea-2010
EU252146-G1-South Korea-2007
MK671169-Changchun-China-2019
MK671179-G1-China-2017
EU498699-G1-Italy-2007
EU018142-G1-Argentina-2007
EU145593-G1-Hungary-2007
EU659115-US-2008
M24004.1-Reference
EU659111-G2-US-1967
M38246.1-Vaccine)
AB000054-G2-Japan-1996
KT240134-G2-Portugal-2013
MT078767-G2-India-2019
MK579637-G2-Australia-2015
OQ615264-Brazil-2023
EU252145-G2-South Korea-2007
EU498714-G2-UK-2006
MK570715-G2-Australia-2018
D88287.1-Japan-1996
EU498681-G2-Italy-2008
MK570701-G2-Newzealand-2017
OM640096-G2-Canada-2018
PP035817-India-2023
EU498682-G3-Italy-2008
OP153926-G3-South Korea-2019
HQ184196-G3A-South Korea-2008
AB054225-Japan-2001
AB054227-G3A-Japan-2001
OQ535495-Jilin-China-2023
MZ391097-G3A-Turkey-2020
MT857296-Vietnam-2020
MW847207-G3A-Italy-2019
EU221281-G3A-Portugal-2006
MW847207-G3A-Italy-2019
AY606131-France-2004
AY606131-G3A-France-2004
EF988660-Heilongjiang-China-2007
MF541139-G3D-China-2017
MK266783-G3D-China-2018
MZ836369-G3-China-2020
OP153930-G3E-South Korea-2019
OP153927-G3E-South Korea-2019
OM918773-G3F-China-2021
MZ836378-G3F-China-2020
KC473946-G3F-China-2012
MW017628-G3F-China-2020
MH419002-G3G-China-2018
MZ836359-G3G-China-2020
OP153928-G3G-South Korea-2019
MT270563-G3G-China-2019
MK570658-G3C-Australia-2017
MW589473-G3C-Thailand-2020
OQ535507-Jilin-China-2023
MK570725-G3C-UAE-2017
MW589472-G3C-Thailand-2020
KX943318-G3B-Italy-2015
MZ391095-G3B-Turkey-2020
KP769859-G3C-Belgium-2013
EU221278-G3C-Portugal-2005
EU498685-G3C-Italy-2003
M38245-CPV2
M74849-CPV2b
EU659118-CPV2a
FJ005240-CPV2c
AB054214-New CPV2a
GQ379042-New CPV2b
0.01
FPV-G1
FPV-G2
FPV-G3
CPV-2

Table 1. Correlation of clinical signs and feline panleukopenia virus-positive specimens detected in this study

Specimen ID	Age (months)	Clinical sign	Vaccine history	GenBank ID
13	4	Vomiting, diarrhea	-	OR399559
19	9	Anorexia, lethargy	+	OR399560
34	7	Fever, anorexia	-	OR399561
41	12	Vomiting, fever, anorexia	-	OR399562
42	6	Anorexia, vomiting	+-	OR399563
56	5	Dehydration, fever, diarrhea	-	OR399564
59	9	Diarrhea, anorexia	+	OR399565
73	13	Fever, diarrhea, anorexia	-	OR399566
77	16	No clinical sign	+	OR399567
89	8	Vomiting, sticky feces	-	OR399568
109	2	Vomiting, fever	-	OR399569
111	5	Vomiting, anorexia	-	OR399570
122	3	Dehydration, fever	+	OR399571
133	9	Sticky feces, anorexia	-	OR399572
141	4	Vomiting, anorexia	-	OR399573
143	2	Anorexia, lethargy	-	OR399574
149	5	Dehydration, fever, diarrhea	-	OR399575

+= Vaccinated; -= non vaccinated; +-= incomplete vaccination

Table 2: Variation of amino acids among FPV sequences of present study and reference FPV strains.

Accession number	type	Origin	amino-acid positions [A]																			
			5	80	87	91	93	101	103	262	267	297	300	305	323	324	370	375	426	440	555	564	568
M24004	FPV	Reference	A	K	M	A	K	T	V	A	F	S	A	D	D	Y	Q	D	N	T	V	N	A
M38246	FPV	Vaccine	A	K	M	A	K	I	V	A	F	S	A	D	D	Y	Q	D	N	T	V	N	A
M38245	CPV2	Reference	A	R	M	A	N	I	A	A	F	S	A	D	N	Y	Q	D	N	T	V	S	G
EU659118	CPV2a	Reference	A	R	L	A	N	I	A	A	F	S	G	Y	N	Y	Q	D	N	T	V	S	G
M74849	CPV2b	Reference	A	R	L	A	N	I	A	A	F	S	G	Y	N	Y	Q	D	D	T	V	S	G
FJ222821	CPV2c	Reference	A	R	L	A	N	I	A	A	F	A	G	Y	N	Y	Q	D	E	T	V	S	G
AB054214	New CPV2a	Reference	A	R	L	A	N	I	A	A	F	A	G	Y	N	Y	Q	D	N	T	V	S	G
GQ379042	New CPV2b	Reference	A	R	L	A	N	I	A	A	F	A	G	Y	N	I	Q	D	N	T	V	S	G
OR399559	FPV	cat	A	K	M	S	K	I	V	A	F	S	A	D	D	Y	Q	D	N	T	V	N	A
OR399560	FPV	cat	A	K	M	S	K	I	V	A	F	S	A	D	D	Y	Q	D	N	T	V	N	A
OR399561	FPV	cat	A	K	M	S	K	I	V	A	F	S	A	D	D	Y	Q	D	N	T	V	N	A
OR399562	FPV	cat	A	K	M	S	K	I	V	A	F	S	A	D	D	Y	Q	D	N	T	V	N	A
OR399563	FPV	cat	A	K	M	S	K	I	V	A	F	S	A	D	D	Y	Q	D	N	T	V	N	A
OR399564	FPV	cat	A	K	M	S	K	I	V	A	F	S	A	D	D	Y	Q	D	N	T	V	N	A
OR399565	FPV	cat	A	K	M	S	K	I	V	A	F	S	A	D	D	Y	Q	D	N	T	V	N	A
OR399566	FPV	cat	A	K	M	S	K	I	V	A	F	S	A	D	D	Y	Q	D	N	T	V	N	A
OR399567	FPV	cat	A	K	M	S	K	I	V	A	F	S	A	D	D	Y	Q	D	N	T	V	N	A
OR399568	FPV	cat	A	K	M	S	K	I	V	A	F	S	A	D	D	Y	Q	D	N	T	V	N	A
OR399569	FPV	cat	A	K	M	S	K	I	V	A	F	S	A	G	D	Y	Q	D	N	T	V	N	A
OR399570	FPV	cat	A	K	M	S	K	I	V	A	F	S	A	D	D	Y	Q	D	N	T	V	N	A
OR399571	FPV	cat	A	K	M	S	K	I	V	A	F	S	A	D	D	Y	Q	D	N	T	V	N	A
OR399572	FPV	cat	A	K	M	S	K	I	V	A	F	S	A	D	D	Y	Q	D	N	T	V	N	A
OR399573	FPV	cat	A	K	M	S	K	I	V	A	F	S	A	D	D	Y	Q	D	N	T	V	N	A
OR399574	FPV	cat	A	K	M	S	K	I	V	A	F	S	A	D	D	Y	Q	D	N	T	V	N	A
OR399575	FPV	cat	A	K	M	S	K	I	V	A	F	S	A	D	D	Y	Q	D	N	T	V	N	A

[a] amino-acid positions refer to the prototype FPV (M24004). FPV, feline panleukopenia virus; CPV, canine parvovirus; A, Ala; D, Asp; E, Glu; G, Gly; K, Lys; L, Leu; M, Met; N, Asn; R, Arg; S, Ser; V, Val; Y, Tyr.

Supplementary Table 1

Primers used for polymerase chain reaction analysis to amplify VP2 gene for detection and sequencing purposes

Designation	Oligonucleotide (5' → 3')	Target gene	Length (bp)	Remarks	Thermocycling conditions	Reference
FPV-F1	TTG ARG CRT CTA CAC AAG GG	VP2	428	Detection	95°C –5 min 40 cycles 95°C – 15 sec 60°C – 30 sec 72°C – 15sec	(Abdel-Baky et al., 2023)
FPV-R1	TGG TGC ATT TAC ATG AAG TCT TGG					
FPV-F2	ATGAGTGATGGAGCAGTTCAAC	VP2	1932	Sequencing	95°C –5 min 40 cycles 94°C – 30 sec 57°C – 60 sec 72°C – 120sec	(Xue et al., 2023)
FPV-R2	GTATACCATATAACAAACCTTC					