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Research article

First molecular report of Feline panleukopenia virus infection in diarrheic cats at Can Tho City, Vietnam

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Abstract

Feline panleukopenia virus (FPV) belongs to the family Parvoviridae and causes an acute viral infection in cats worldwide. Information on the circulation of FPV among cats is currently limited in Vietnam. Herein, the full–length VP2 gene and molecular characteristics of FPV isolated in diarrhea cats in Can Tho City were first exhibited. Phylogenetic analysis based on seven obtained nucleotide sequences revealed that the isolated sequences were clustered into a narrow group with FPV sequences in the neighboring countries such as China, Thailand, and Japan, and distantly grouped with the vaccine strains. Regarding nucleotide and amino acid sequence analysis, the nucleotide and amino acid homology of 99.98–100% and 99.99–100% among obtained sequences, and showed high homology with reference sequences, accounting for 97.38–98.51% and 98.96–99.27%, respectively. Besides, the nucleotide and amino acid sequences were a homology of 98.51% and 99.26% with two vaccine strains in GenBank. Regarding amino acid translation, seven obtained sequences were closely related to FPV strains, meanwhile, they were different from CPV–2 strains in GenBank at amino acid substitutions of K80, K93 and V103. Overall, this is the first detection of FPV in diarrhea cats and illustrated the molecular characteristics of FPV in the cat population in Can Tho City of Vietnam.

Keywords: Cat, Diarrhea, Feline Panleukopenia, Phylogenetic analysis, Vietnam

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INTRODUCTION

Feline panleukopenia (FP) is a highly contagious infectious disease in the cat population, caused by the feline panleukopenia virus (FPV) (Parrish, 1995; Truyen et al., 2009; Miranda et al., 2014; Miranda et al., 2017; Awad et al., 2018; Chowdhury et al., 2021). FPV is a single–stranded DNA virus belonging to the genus of Protoparvovirus, a family of Parvoviridae. The viral genome of 4.5–5.5 kb length, contains open reading frames (ORFs) encoding two structural proteins which are VP1 (83–86 kDa) and VP2 (64–66 kDa), and two non–structural proteins which are NS1 and NS2 (Agbandje et al., 1993; Chowdhury et al., 2021). The VP2 gene encodes the VP2 protein which is a major viral capsid protein and plays an important role in determining the host range (Hueffer et al., 2003; An et al., 2011).

FPV was first discovered in 1928 and was first identified in tissue culture in 1964 (Verge and Cristoforoni, 1928; Johnson, 1965). It is well known that FPV has shared the origination with canine parvovirus (CPV), mink enteritis virus, and raccoon parvovirus, belonging to Carnivore protoparvovirus 1 species. Although these viruses have the same origin, they could be distinguished via differences in host cell specificity (Agbandje et al., 1993; Parrish, 1995; Truyen et al., 2009; Truyen and Parrish, 2013). Prior in vitro and in vivo studies indicated that FPV replicates in feline cells, whereas CPV–2 could replicate in both canine and feline cells (Truyen and Parrish, 1992). Furthermore, the molecular analysis demonstrated that FPV and CPV could be determined via a difference in the amino acid sequences of the VP2 gene at positions 80, 93, 101, 103, 267, 323, and 324 (Agbandje et al., 1993; Chowdhury et al., 2021). Interestingly, recent studies revealed that FPV and CPV could infect both canines and felines (Miranda et al., 2014; Miranda et al., 2017; Hoang et al., 2020). This evidence requires further studies on the interaction between FPV, CPV, and their hosts.

Currently, FPV could be isolated from diarrhea cats among several geographical regions. FPV–suspected cats showed clinical signs associated with enteric diseases such as fever, vomiting, diarrhea, dehydration, abdominal pain, and depression (Decaro et al., 2008; Truyen et al., 2009; An et al., 2011; Awad et al., 2018; Leal et al., 2020; Chowdhury et al., 2021). Although cats of all ages could be infected with FPV, kittens are more susceptible with a high rate of morbidity and mortality (approximately 90%) (Parrish, 1995; Truyen et al., 2009; Truyen and Parrish, 2013). In Vietnam, the information on FPV circulating in canines and felines is limited. To the best knowledge of the authors, only one study reported the genetic characterization of FPV detected among dogs in the North (Hoang et al., 2020) and no investigation analyzed the genetic characterization of FPV isolated among cats in Vietnam. Therefore, the aim of this study was to analyze the molecular characteristic of FPV in cats in Can Tho City of Vietnam. Our findings expand the basic knowledge regarding the circulation of FPV and may contribute to improving the control strategy of this disease in cats in the Mekong Delta region and the whole country in the future.

MATERIALS AND METHODS

Sample collection

Over a period from July to October 2022, 357 cats were admitted to the Practical Veterinary Clinic of Can Tho University for treatment of diseases or health checks. 51/357 cats presented the clinical signs of FPV such as vomiting, fever, lethargy, hypothermia, diarrhea with loose or bloody stools, loss of appetite, drooling, and pale mucous membranes (Truyen et al., 2009). The rectal swabs were collected from 51 FPV–suspected cats. Subsequent to collection, the samples were kept at –20oC until used for viral extraction (Chowdhury et al., 2021). The animal procedures of this study followed Vietnam's Animal Husbandry Law (32/2018/QH14). Rectal swabs were collected as per a routine diagnostic process and received the client owner's permission by signing the consent form.

Viral DNA extraction and polymerase chain reaction (PCR)

Total DNA was extracted from 51 rectal swabs using the commercial kit (ABT Equipment Co., Ltd, Vietnam) based on the manufacturer's guidelines. Briefly, the samples were manually 4 homogenized in 0.5% phosphate buffered saline (PBS) and collected the supernatant. A total of 350 µL SRD buffer and 20 µL Proteinase K were then added and incubated at 72°C for 15 min. Following, the supernatant was washed twice using WB1 and WB2 buffer prior to being incubated with 50 µL EB buffer at room temperature for 1 min. Total DNA was collected and stored at –20°C until used.

The full–length VP2 gene fragment 1,710 bp in length was amplified using forward primer (5'–GGTCAACCTGCTGTCAGAAA–3') (at the position from 2,816 to 2,835 in the genome) and reverse primer (5'–AGGTGCTAGTTGAGATTTTTCAT–3') (at the position from 4,525 to 4,503 in the genome) (Kaur et al., 2015). The PCR mixture was performed in 25 µL volume containing 12 µL of GoTaq G2 Green Master Mix (Promega, USA), 1 µL of each forward and reverse primers (10 pmol/µL), 2 µL of template, and 9 µL of nuclease–free water. The thermal cycling conditions were started at 95oC for 5 min, followed by 35 cycles at 95°C for 30 sec, 58°C for 30 sec, 72°C for 2 min, and final extension at 72°C for 5 min. Negative control was performed with non–template controls. The positive products were sequenced via the Sanger method in a commercial company (Macrogen, South Korea).

Phylogenetic analysis

The obtained nucleotide sequences were assembled and aligned using the BioEdit software package version 7.2.5 (Hall, 1999). The sequences were subjected to the BLAST tool of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/blast) to determine their similarity with the GenBank database. Seven sequences derived from the current study were presented in Table 1. A phylogenetic tree was constructed based on the VP2 sequences using the maximum likelihood method in the MEGA software package version 7.0.26 (Kumar et al., 2016) with 1,000 bootstrap replications.

Table 1 Distribution of general signalments and clinical signs of seven FPV–positive cats in the current study

Case No.	Age	Breed	Sex	Vaccination status	Clinical sign
FPV–01	3 years	British Shorthair	Male	No	DLB, F
FPV–02	1 year	Domestic	Male	No	DLB, V
FPV–03	5 months	Mixed	Female	No	DLB, P, V
FPV–04	2 months	Domestic	Female	No	DLB, H, L, P
FPV–05	6 months	British Longhair	Female	No	D, DLB, LA, V
FPV–06	3 months	Domestic	Male	No	H, DLB, P, V
FPV–07	11 months	Domestic	Male	No	DLB, LA, P, V

Drooling=D; diarrhea with loose or bloody stools=DLB; fever=F; hypothermia=H; lethargy=L; loss of appetite=LA; pale mucous membranes=P; Vomiting=V

RESULTS

PCR amplification and phylogenetic analysis

To determine FPV in cats based on molecular analysis, DNA extracted from the rectal swabs of 51 cats was utilized to amplify the full–length VP2 gene by a specific primer pair. The result showed that the full–length VP2 gene was exhibited in clear bands with a 1,710 bp product size (Figure 1) of 7 cats (13,73%).

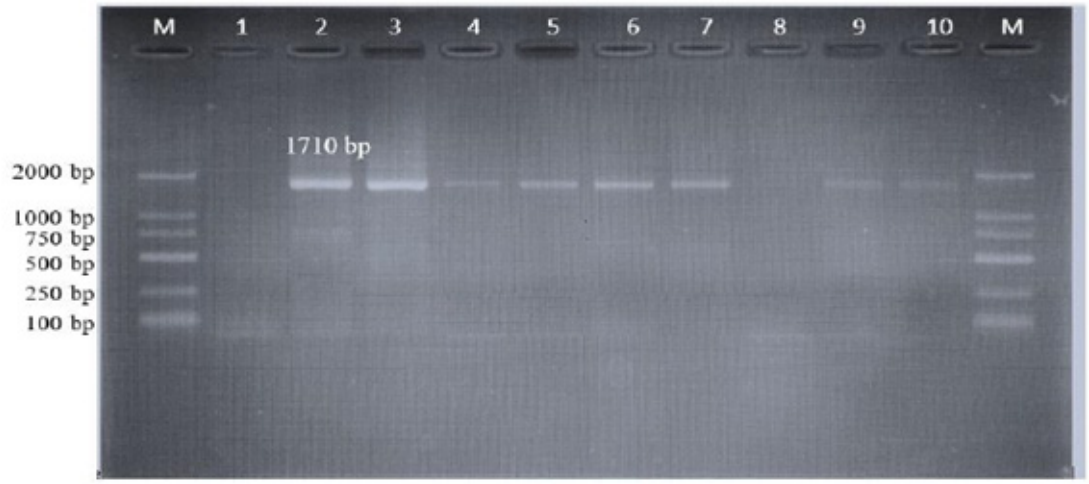


Figure 1 Amplified result of the VP2 gene of FPV by PCR. The positive results were shown at 1,710 bp. Lanes M represented the 2,000 bp DNA marker (Bioline, United Kingdom), lanes 1 and 8 represented the negative control, and lane 2 represented the positive control.

Subsequent to sequencing, seven sequences were harvested and analyzed. The phylogenetic tree was constructed according to prior publications (Garigliany et al., 2016; Charoenkul et al., 2019; Yi et al., 2022). The phylogenetic analysis revealed that the obtained sequences were closely related to FPV sequences detected from neighboring countries such as China, Thailand, and Japan. Interestingly, the obtained sequences were clustered into a narrow branch with FPV sequences isolated from China and Thailand. Notably, the obtained sequences were separated with two vaccine strains and formed a distinct branch with CPV–2 sequences in the GenBank database (Figure 2).

Figure 2 Phylogenetic relationship of FPV based on the full–length VP2 nucleotide sequences. The phylogenetic tree was constructed using the maximum likelihood method with 1,000 bootstrap replications. The FPVs obtained from cats in Can Tho City were presented in red dots and two vaccine strains were presented in green squares.

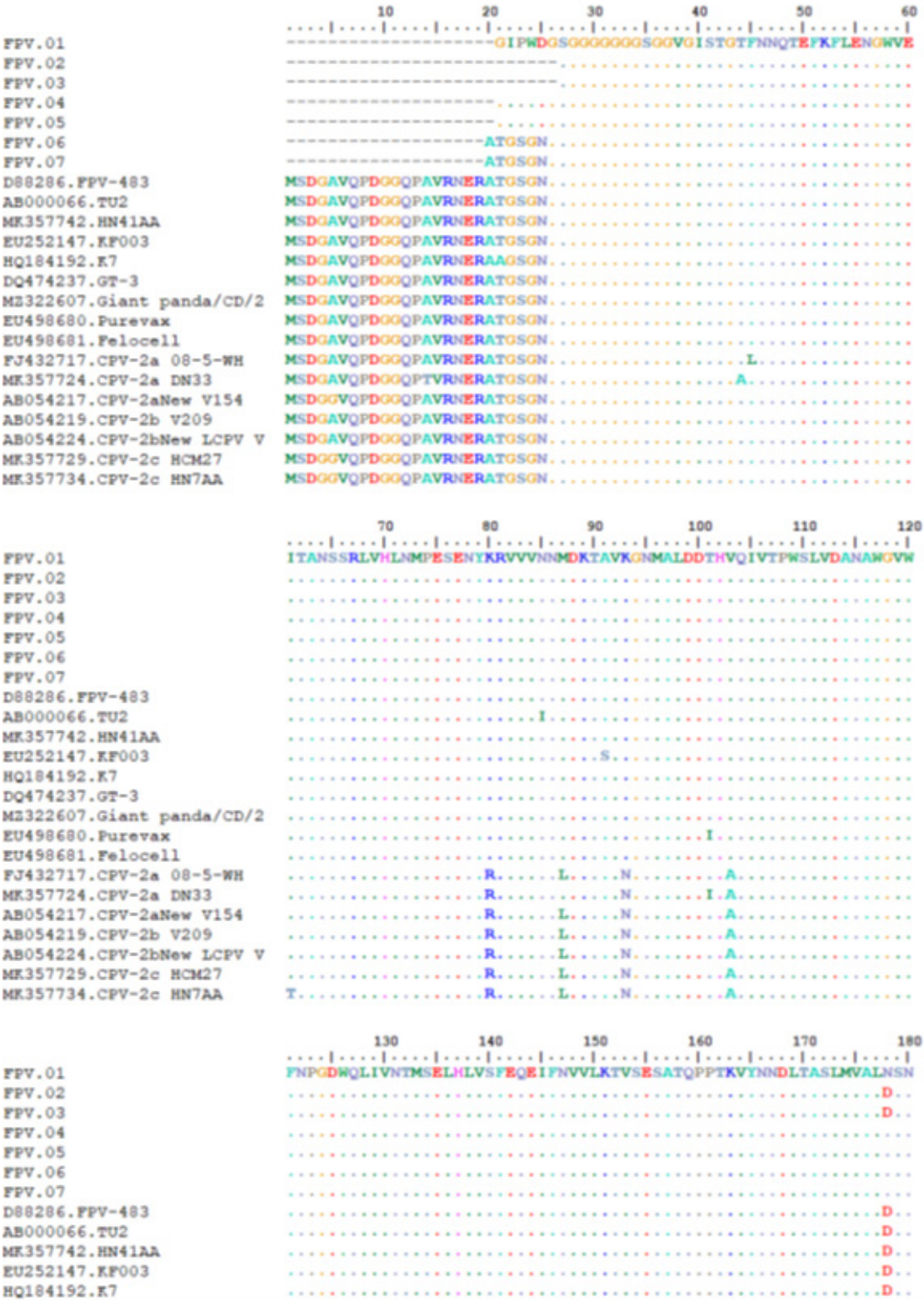
Nucleotide and amino acid sequence analysis

The nucleotide and amino acid sequences of the full–length VP2 gene obtained in this study were compared together and with reference sequences in GenBank. The results indicated that nucleotide homology of 99.98–100% among seven isolated FPVs in Can Tho City. However, the homology decreased to 97.38–98.51% when compared with reference sequences. Also, the seven Vietnamese sequences displayed a nucleotide homology of 98.51% in comparison with two vaccine strains and low homology with CPV–2 strains (95.02–97.76%). Likewise, the amino acid sequence of FPV isolated from cats at Can Tho City showed a homology of 99.99–100%. Meanwhile, the obtained amino acid sequences displayed a high homology with reference sequences in GenBank, accounting for 98.96–99.27%. Moreover, the Vietnamese amino acid sequences were a homology of 99.26% with two vaccine strains and revealed a homology of 98.49–99.05% compared with CPV–2 strains in GenBank (Table 2).

Table 2 Percentage of nucleotide and amino acid identity (%) between obtained sequences and 300 reference sequences in GenBank

No.	Accession number	Original country	Strain %	Nucleotide identity%	Animo acid identity
1	FPV–01	Vietnam	FPV	100	100
2	FPV–02	Vietnam	FPV	99.98	99.99
3	FPV–03	Vietnam	FPV	99.98	99.99
4	FPV–04	Vietnam	FPV	100	100
5	FPV–05	Vietnam	FPV	100	100
6	FPV–06	Vietnam	FPV	99.98	99.99
7	FPV–07	Vietnam	FPV	99.98	99.99
8	DQ474237	China	FPV	98.51	99.26
9	AF015223	Taiwan	FPV	97.75	99.05
10	EU221279	Korea	FPV	98.13	99.26
11	AB054225	Japan	FPV	98.14	99.16
12	MH711909	Thailand	FPV	98.51	99.25
13	EU360959	Hungary	FPV	98.14	99.16
14	EU498682	Italy	FPV	98.14	99.16
15	EU498681	Italy	Vaccine	98.51	99.26
16	EU498680	Italy	Vaccine	98.51	99.26
17	AY665655	Russia	FPV	98.51	99.12
18	KU248463	Portugal	FPV	98.14	99.16
19	JX475270	USA	FPV	98.51	99.27
20	EU018145	Argentina	FPV	97.38	98.96
21	M38245	USA	CPV	97.76	99.05
22	JN867615	USA	CPV	96.60	98.80
23	FJ197847	Korea	CPV	97.76	99.05
24	AB054222	Vietnam	CPV	96.60	98.80
25	AB054224	Vietnam	CPV	96.98	98.90
26	MK357728	Vietnam	CPV	95.02	98.49
27	MK357734	Vietnam	CPV	95.41	98.56
28	KP715700	Thailand	CPV	95.80	98.66
29	JN625223	India	CPV	96.20	98.70
30	KP071956	India	CPV	96.60	98.80
31	FJ222823	Italy	CPV	96.60	98.80
21	M38245	USA	CPV	97.76	99.05

Regarding amino acid translation, two amino acid substitutions (N178 and V562) were distinguished among seven Vietnamese sequences. Besides, the field sequences isolated in Can Tho City were closely correlated to other FPV strains in GenBank. However, the obtained sequences were different from CPV–2 strains in GenBank at positions K80, K93, and V103 (Figure 3).


10 20 30 40 50 60
FPV.01 ----------GIPWDGSGGGGGGGSGGVGISTGTFNNQTEFKFLENGWVE
FPV.02 ----------
FPV.03 ----------
FPV.04 ----------
FPV.05 ----------
FPV.06 ----------ATGSGN
FPV.07 ----------ATGSGN
D88286.FPV-483 MSDGAVQPDGGQPAVRNERATGSGN
AB000066.TU2 MSDGAVQPDGGQPAVRNERATGSGN
MK357742.HN41AA MSDGAVQPDGGQPAVRNERATGSGN
EU252147.KF003 MSDGAVQPDGGQPAVRNERATGSGN
HQ184192.K7 MSDGAVQPDGGQPAVRNERAAGSGN
DQ474237.GT-3 MSDGAVQPDGGQPAVRNERATGSGN
MZ322607.Giant panda/CD/2 MSDGAVQPDGGQPAVRNERATGSGN
EU498680.Purevax MSDGAVQPDGGQPAVRNERATGSGN
EU498681.Felocell MSDGAVQPDGGQPAVRNERATGSGN
FJ432717.CPV-2a 08-5-WH MSDGAVQPDGGQPAVRNERATGSGN.....L.....
MK357724.CPV-2a DN33 MSDGAVQPDGGQPTVRNERATGSGN.....A.....
AB054217.CPV-2aNew V154 MSDGGVQPDGGQPAVRNERATGSGN
AB054219.CPV-2b V209 MSDGAVQPDGGQPAVRNERATGSGN
AB054224.CPV-2bNew LCPV V MSDGAVQPDGGQPAVRNERATGSGN
MK357729.CPV-2c HCM27 MSDGGVQPDGGQPAVRNERATGSGN
MK357734.CPV-2c HN7AA MSDGGVQPDGGQPAVRNERATGSGN
70 80 90 100 110 120
FPV.01 ITANSSRLVHLNMPESENYKRVVVNNMDKTAVKGNMALDDTHVQIVTPWSLVDANAWGVW
FPV.02 .....
FPV.03 .....
FPV.04 .....
FPV.05 .....
FPV.06 .....
FPV.07 .....
D88286.FPV-483 .....
AB000066.TU2 .....I.....
MK357742.HN41AA .....
EU252147.KF003 .....S.....
HQ184192.K7 .....
DQ474237.GT-3 .....
MZ322607.Giant panda/CD/2 .....
EU498680.Purevax .....I.....
EU498681.Felocell .....
FJ432717.CPV-2a 08-5-WH .....R.....L.....N.....A.....
MK357724.CPV-2a DN33 .....R.....N.....I.A.....
AB054217.CPV-2aNew V154 .....R.....L.....N.....A.....
AB054219.CPV-2b V209 .....R.....L.....N.....A.....
AB054224.CPV-2bNew LCPV V .....R.....L.....N.....A.....
MK357729.CPV-2c HCM27 .....R.....L.....N.....A.....
MK357734.CPV-2c HN7AA T.....R.....L.....N.....A.....
130 140 150 160 170 180
FPV.01 FNPGDWQLIVNTMSELMLVSFEQEIFNVVLKTVSESATQPPTKVYNNDLTASLMVALNSN
FPV.02 .....D..
FPV.03 .....D..
FPV.04 .....
FPV.05 .....
FPV.06 .....
FPV.07 .....
D88286.FPV-483 .....D..
AB000066.TU2 .....D..
MK357742.HN41AA .....D..
EU252147.KF003 .....D..
HQ184192.K7 .....D..

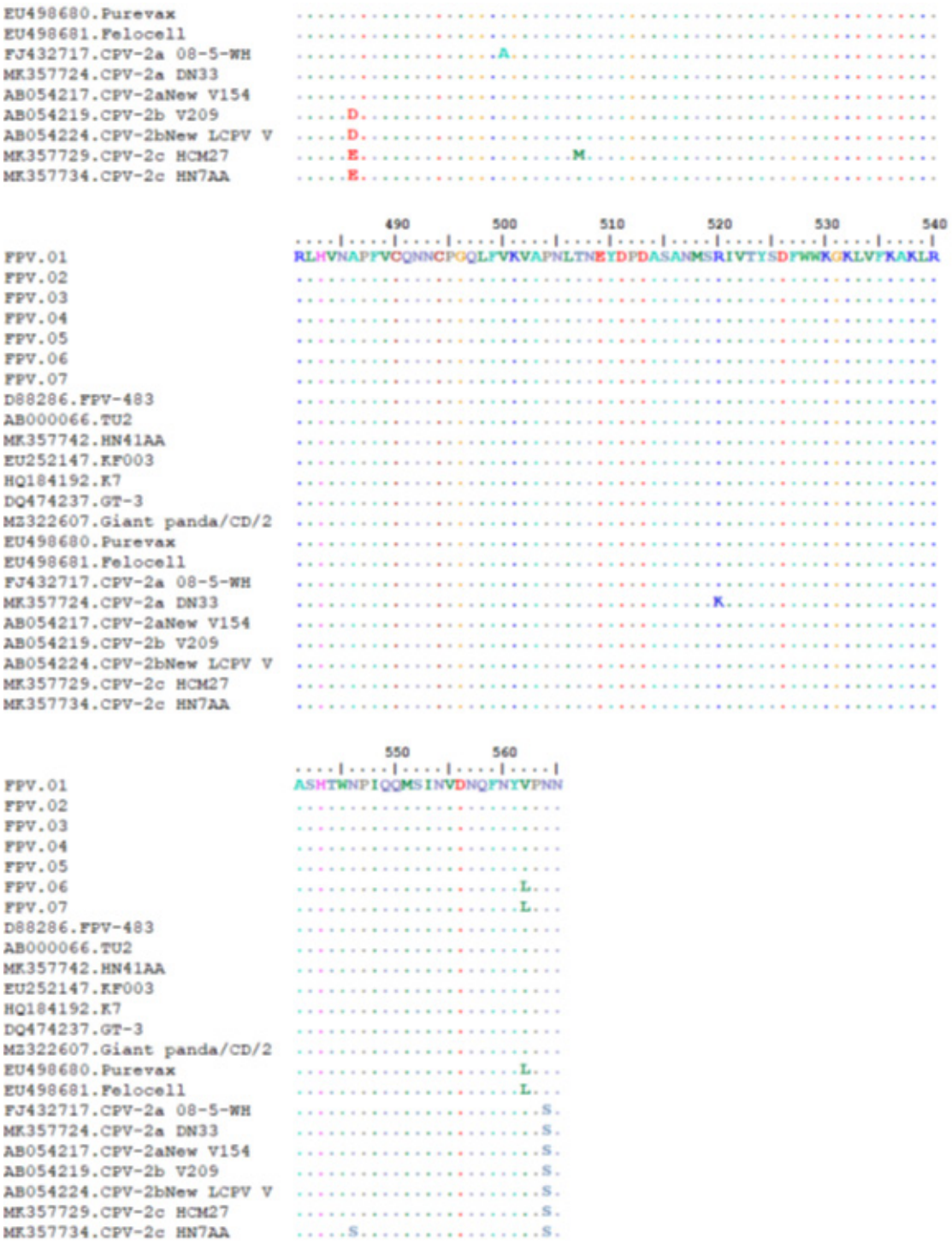


Figure 3 Comparison between the deduced amino acid sequences in the VP2 region of FPV collected in the Mekong Delta of Vietnam and other references of FPV and CPV–2 in GenBank. Dots represent the identical residues meanwhile letters represent the differences among sequences.

DISCUSSION

FP is a highly fatal infectious disease among cats worldwide, caused by FPV. Cats suspected of FPV have shown clinical signs associated with enteric disease (Parrish, 1995; Truyen et al., 2009; Hoang et al., 2020). Even though the presence and molecular characteristics of FPV in diarrheic cats have recently been documented in several geographical regions (Decaro et al., 2008; Truyen et al., 2009; An et al., 2011; Awad et al., 2018; Leal et al., 2020; Chowdhury et al., 2021), the information on the circulation and the molecular characteristics of FPV among cats has not well been studied and remain to be elucidated in Vietnam. Therefore, it is essential to perform the investigation of FPV in Vietnam, especially in Can Tho City which is the central city of the Mekong Delta. Herein, FPV was detected in cats that exhibited clinical signs related to diarrhea. Our results first revealed the molecular characteristics of FPV among diarrheic cats in Can Tho City and the relationship between the obtained sequences and previously available FPV sequences in the GenBank database. The result of this study is the initial report of FPV isolated among diarrheic cats in the investigated area and the whole country as well as expanding the window of investigation of FPV in the world.

In the current study, the full–length VP2 gene of FPV was amplified via a specific primer pair (Kaur et al., 2015). FPV and CPV are two major genogroups of the Carnivore protoparvovirus 1 species and share over 98% homology in DNA sequence. Over the past decades, there have been few changes in genetic variations of FPV, however, their role is obscure (Mochizuki et al., 1996; Decaro et al., 2008). Interestingly, some specific mutations in the VP2 gene of FPV (I101T) could be found in CPV–2 variants (Decaro et al., 2008). In fact, the transmission of FPV and their hosts is an interestingfield, and several studies indicated that FPV and CPV–2 (a, b, and c) could infect both canine and feline (Miranda et al., 2014; Miranda et al., 2017; Hoang et al., 2020). A study focusing on feline parvovirus–314 in cats revealed that the virus was finally decided as CPV due to the lack of a specific antigenic epitope in the genome (Mochizuki et al., 1996).

Phylogenetic analysis showed that the obtained sequences were closely clustered together and with the FPV sequences isolated from the neighboring countries. The findings of this study were similar to the past investigation, FPV detected in clinically suspected cats in the United Kingdom (UK) exhibited a tight relationship to FPV sequences in Italia and Russia (Decaro et al., 2008). Likewise, FPV detected in domestic cats in Portugal were closely grouped with previous sequences in the whole country and with FPV sequences in neighboring countries in the European region (Miranda et al., 2017). Moreover, FPV isolated from the Korean cats was clustered with FPV in Japan and China (An et al., 2011). Furthermore, FPV isolated in diarrhea cats in Bangladesh is located in the same group of FPV in the Middle East, China, Thailand, and Europe countries (Chowdhury et al., 2021). In China, surveillance of FPV in domestic cat population was performed and the results displayed that FPV isolated in domestic cats were clustered by region and located in the same subgroup as FPV in Australia and the USA (Leal et al., 2020). To date, it is well known that the transmission route of FPV is the faecal–oral route (Truyen et al., 2009). However, the studies on the transboundary movement of FPV

globally are limited. There was evidence that FPV detected in domestic cats in Portugal was introduced from other geographical regions (Miranda et al., 2017). Therefore, the transboundary movement of FPV globally would be an interesting field for future research.

Notably, seven Vietnamese sequences collected from seven unvaccinated cats showed a distant relationship with two FPV vaccine strains. In this study, we recruited two FPV vaccine strains including Felocell CVR (Pfizer) and Purevax RCP (Merial) circulating in the UK (Miranda et al., 2017) due to the limited information on the FPV vaccine in Vietnam. The homology of nucleotide and amino acidbetween obtained sequences and both two vaccine strains were 98.51% and 99.26%, respectively. These results displayed important information for developing vaccine strategies against FPV among cat populations in Vietnam in the future. Especially, there was evidence that new FPV strains isolated in domestic cats in China emerged under selective pressures of FPV immune response (Leal et al., 2020). Therefore, further investigations on the selection of suitable vaccine strains for FPV in Vietnam should be promoted to control this disease in the future.

In addition, all obtained sequences were clustered in the same branch with FPV sequences globally and were separately grouped with CPV–2 sequences in GenBank. The homology of nucleotide and amino acid of obtained sequences was low in comparison with previous CPV–2 sequences (95.02–97.76% and 98.49–99.05%, respectively). Interestingly, the amino acid translation results revealed that three amino acid substitutions (K80, K93, and V103) were identified in the obtained sequences compared to CPV–2 strains in GenBank. Indeed, FPV could be distinguished from CPV at amino acid positions 80, 93, 101, 103, 267, 323, and 324 in the genome (Agbandje et al., 1993; Decaro et al., 2008; Chowdhury et al., 2021). Prior studies showed that amino acid positions N93, A103, and N323 in the VP2 gene of CPV play an important role in the ability to replicate in canine, meanwhile, feline could closely associate with amino acid positions K80, N564, and A568 in the VP2 gene (Agbandje et al., 1993; Decaro et al., 2008). The findings of the current study clearly indicated that the obtained sequences were FPV due to a close relationship with FPV sequences worldwide and separate from previous CPV–2 sequences in GenBank. Although FPV and CPV shared a high homology in the genome (above 98%), FPV created a group with more homologous genetic and antigenic than CPV (Mochizuki et al., 1996). Moreover, prior reports demonstrated that FPV isolated among cats was separated into two major branches with CPV (Decaro et al., 2008; Chowdhury et al., 2021). Altogether, the phylogenetic analysis revealed that seven FPV sequences isolated in Can Tho City were closely clustered together and with the FPV sequences isolated from the neighboring countries, meanwhile, the obtained sequences were distant to vaccine strains and separate from CPV–2 sequences in GenBank.

CONCLUSIONS

In summary, the present study first exhibited the molecular characteristic of FPV associated with feline enteritis in Can Tho City of Vietnam. The findings of this study may contribute to expanding the basic knowledge of FPV among cats in Vietnam, promoting further investigations on the distribution of FPV in the whole country.

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AUTHOR CONTRIBUTIONS

B.T.N. and T.T.T. conceptualized, designed, and supervised the experiment. T.T.M.D. and T.M.V. collected the samples, performed laboratory work, and analyzed the data. T.T.M.D., T.T.T., T.Q.L. and B.T.N. interpreted data and wrote the first draft. T.T.M.D., T.Q.L. and B.T.N. reviewed and edited the manuscript. All the authors read and approved the manuscript.

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

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