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RAPID COMMUNICATION

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Genetic characterization of parvoviruses in domestic cats in Henan province, China

Xiangdong Li[1,*] | Hongchao Wu[1,*] | Lingxiao Wang[1] | Norman Spibey[1] | Caihong Liu[1] | Hangtian Ding[1] | Wujie Liu[1] | Yuxiu Liu[1,2] | Kegong Tian[1,2]

[1]National Research Center for Veterinary Medicine, Luoyang, China

[2]College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou, China

Correspondence
Yuxiu Liu and Kegong Tian, National Research Center for Veterinary Medicine, Luoyang, China.
Emails: qingqingyuxiu1@163.com (YL); tiankg@263.net (KT)

Abstract

Feline panleukopenia virus (FPV) and canine parvovirus (CPV) infections are highly contagious and cause serious enteric diseases, with high fatality rates of cats and dogs. Given the importance of cats as a potential source of genetic diversity for parvoviruses, parvovirus strains detected in cats with gastroenteritis signs were analysed, and molecular characterisation, sequence analysis and phylogeny were evaluated on the VP2 gene. The results showed that FPV, new CPV-2a, and CPV-2 are co-circulating in the cat population in Henan province of China. Moreover, CPV-2 strains (F2016020, and F2016021) with Ser297Ala substitution in VP2 protein was for the first time detected in cats with clinical gastroenteritis. This study provided new important findings about the evolutionary of parvovirus infection in domestic cats.

KEYWORDS
canine parvovirus, domestic cats, feline panleukopenia virus, VP2 gene

1 | INTRODUCTION

Parvoviruses are small, non-enveloped single-stranded DNA viruses which infect a wide range of animals. Canine parvovirus (CPV), feline panleukopenia virus (FPV), and mink enteritis virus (MEV) are members of the genus *Protoparvovirus* and the family *Parvoviridae* (Kelly, 2010). These viruses cause a variety of serious diseases, especially in young animals, since they have a distinct preference for replication in rapidly dividing cells, such as bone marrow and enteric epithelium. Feline panleukopenia virus and CPV infections are highly contagious, can result in high mortality and serious enteric diseases of cats and dogs (Battilani et al., 2011). Except the original CPV-2, the variants of CPV, CPV-2a, 2b and 2c have acquired the feline host-range, and they are able to infect and replicate efficiently in cats, causing diseases indistinguishable from feline panleukopenia induced by FPV in naturally and experimentally infected cats (Battilani, Bassani, Forti, & Morganti, 2006; Hoelzer, Shackelton, Parrish, & Holmes, 2008).

Feline panleukopenia virus and CPV infection of vaccinated cats and dogs have been reported and the viruses have been isolated from diarrhoeic animals (Decaro et al., 2009; Mukhopadhyay et al., 2016). To gain a better understanding of the importance of cats as a potential source of genetic diversity for parvoviruses, parvovirus strains detected in cats with gastroenteritis signs were analysed. Molecular characterisation, sequence analysis, and phylogeny of these strains were evaluated on the VP2 gene.

2 | MATERIALS AND METHODS

A total of 17 faecal samples or rectal swabs from domestic cats with gastroenteritis signs (diarrhoea and/or vomiting) were collected from veterinary clinics in 2016. The cats in the study were unvaccinated. The samples (faecal and rectal swabs samples) were sent to the laboratory for diagnostic purposes and stored at −80°C until analysis. The study was approved by the Animal Care and Ethics Committee of National Research Center for Veterinary Medicine

*These authors contributed equally to this work.

and conventional animal welfare regulations and standards were taken into account.

Viral DNA from the samples was extracted using a viral nucleic acid extraction kit II (Geneaid Biotech Ltd, Taiwan, China) according to the manufacturer's instructions. The extracted template DNA was screened for the presence of CPV/FPV by conventional PCR assay using the universal primers pair (F: 5′-GCACATCAAGATACAGGAAG-3′ and R: 5′-CCTTAACATATTCTAAGGGCAA-3′), which amplifies an about 800-base pair (bp) fragment of VP2 gene encoding capsid protein. All faecal and rectal swabs samples that gave a positive result for CPV/FPV in the preliminary screen were used to further amplify the complete VP2 gene using the primers: VP2F: 5′-AGAGACAATCTTGCACCAAT-3′ and VP2R: 5′-ATGTTAATATAATTTTCTAGGTGCT-3′ (Wu, Li, Wang, Liu, & Tian, 2018). Polymerase chain reaction products were cloned into the pEASY blunt vector (TransGen Biotech Co., Ltd, Beijing, China) and sequenced at least three times (GENEWIZ, Inc., Beijing, China).

The complete VP2 nucleotide sequences and deduced amino acid sequences were aligned with 18 reference FPV and 27 reference CPV strains from GenBank database (Supporting Information Table S1) using DNASTAR software. The genotype of the strains in this study was classified based on key amino acid residues of VP2 protein, new CPV-2a was defined as 426Asn and 297Ala, new CPV-2b was defined as 426Asp and 297Ala, CPV-2c was defined as 426Glu and 297Ala, FPV was defined as 80Lys, 93Lys, 103Val, 323Asp, and 568Ala (Martella, Decaro, Elia, & Buonavoglia, 2005). Simplot software was used to calculate and plot the percentage identity of a query sequence against a panel of reference sequences in sliding windows. The full-length of VP2 of two feline CPV-2 in this study was used as query sequences and the previously reported two vaccine strains and field CPV-2 strain (CPV-b) were used as reference sequences. The phylogenetic analyses based on the complete VP2 nucleotide sequences were conducted by the Maximum Likelihood method (Kimura 2 parameter model) in Mega 6.0 (bootstrap replicates = 1000).

3 | RESULTS AND DISCUSSION

Six of a total of 17 suspected samples were tested positive for FPV/CPV by conventional PCR assay (Supporting Information Table S2). The full-length sequence of VP2 gene was successfully amplified from six FPV/CPV positive samples of cats.

The genotype of parvovirus strains in this study was determined based on the key amino acid residues of VP2 protein (Table 1). Sequencing results of six positive samples revealed that one FPV strain (F2016019), three new CPV-2a strains (F2016009, F2016010 and F2016015), and two CPV-2 strains (F2016020 and F2016021) were identified in this study. No mutation was found in F2016019 compared with the reference FPV strains (Table 1), indicating a high degree of conservation in the FPV VP2 protein since its emergence which was consistent with previous report (Miranda et al., 2017).

Muz, Oğuzoğlu, Timurkan, & Akın (2012) found that CPV-2a, CPV-2c, and FPV were circulating in vaccinated and unvaccinated cats living in Turkey between 2006 and 2010. Three new CPV-2a strains (F2016009, F2016010, and F2016015) from domestic cats were identified in this study which possess the Phe267Tyr, Ala300Gly, Tyr324Ile, and Thr440Ala mutations compared with the reference CPV-2 strains (M38245, M19296, EU659116, M23255, FJ197846 and EU914139). These mutations have previously been found in VP2 protein of CPVs isolated from dogs and cats (Pereira, Leal, & Durigon, 2007; Wu et al., 2015; Yi, Tong, Cheng, Song, & Cheng, 2016; Zhou, Zeng, Zhang, & Li, 2017).

Canine parvovirus emerged as a new virus (termed CPV-2) of dogs in the late 1970s and is closely related to FPV (Allison et al., 2014). CPV-2 was named to differentiate it from an unrelated canine minute virus (CPV-1) (Binn, Lazar, Eddy, & Kajima, 1970; Zhou et al., 2017). The original CPV-2 did not replicate in cats (Truyen, Evermann, Vieler, & Parrish, 1996; Truyen & Parrish, 1992). By contrast, the new variants of CPVs such as CPV-2a, 2b, and 2c had acquired the feline host-range and replicated efficiently in cats which led to indistinguishable diseases from feline panleukopenia in naturally and experimentally infected situations (Battilani et al., 2006; Hoelzer et al., 2008). In this study, two CPV-2 (F2016020 and F2016021) were for the first time identified from domestic cats with clinical gastroenteritis. Compared with the vaccine strains (original CPV-2), there were Ser297Ala mutation and Val562Leu mutation in VP2 protein of two CPV-2 strains in this study. Val562Leu mutation was only present in two FPV vaccine strains (EU498680 and EU498681). Ser297Ala mutation was present in new CPV-2a, new CPV-2b, and CPV-2c (Wu et al., 2015).

A phylogenetic tree was constructed based on the six full-length VP2 sequences obtained from domestic cats, in addition to 45 sequences of FPV/CPV strains retrieved from GenBank (Figure 1). It revealed that the FPV strain (F2016019) was closely related to the reference FPV strains (DQ099430, DQ474236, HQ184203) and was distinct from the commercial FPV vaccine strains (EU498680 and EU498681). Three new CPV-2a strains (F2016009, F2016010 and F2016015) also closely related to some reported Chinese CPV-2a strains (KF676668, KY386852, KJ438805, KM386822) but had a distant relationship to commercial CPV-2 vaccine strains (FJ197846 and EU914139). Interestingly, two CPV-2 strains (F2016020 and F2016021) clustered together in a single clade and were related to commercial CPV-2 vaccine strains.

In 1978, CPV-2 was first identified from dogs and thought not to be able to replicate in cats. The original CPV-2 type was soon extinct and replaced by CPV-2a and CPV-2b in the 1980s (Zhang et al., 2010). Therefore, the origin of two feline CPV-2 (F2016020 and F2016021) in this study remains unknown. One possibility is that CPV-2 we thought extinct could evolve in cats or some other carnivore species since one previous study provided lines of evidence to suggest FPV and CPV arose independently from an ancestral parvovirus in wildlife (Allison et al., 2014). We cannot exclude the possibility that CPV-2 vaccine or field viruses independently evolving in

TABLE 1 Amino acid mutations in VP2 protein of FPV and CPVs

Strain	GenBank accession no.	Mutations sites: amino acid residue																							Gene types
		46	80	85	87	91	93	101	103	219	232	267	297	300	301	305	323	324	426	440	556	562	564	568	
CPV-b	M38245	N	R	N	M	A	N	I	A	I	I	F	S	A	T	D	N	Y	N	T	D	V	S	G	CPV-2
CPV 2 int (vaccine)	FJ197846	N	R	N	M	A	N	I	A	V	I	F	S	A	T	D	N	Y	N	T	D	V	S	G	CPV-2
CPV 2 Pfizer (vaccine)	EU914139	N	R	N	M	A	N	I	A	K	I	F	S	A	T	D	N	Y	N	T	D	V	S	G	CPV-2
CPV-N	M19296	N	R	N	M	A	N	I	A	I	I	F	S	A	T	D	N	Y	N	T	D	V	S	G	CPV-2
CPV-5.us.79	EU659116	N	R	N	M	A	N	I	A	I	I	F	S	A	T	D	N	Y	N	T	D	V	S	G	CPV-2
CPV-d Cornell 320	M23255	N	R	N	M	A	N	I	A	I	I	F	S	A	T	D	N	Y	N	T	D	V	S	G	CPV-2
CPV-15	M24003	N	R	N	L	A	N	T	A	I	I	F	S	G	T	Y	N	Y	N	T	D	V	S	G	CPV-2a
CPV-39	M74849	N	R	N	L	A	N	T	A	I	I	F	S	G	T	Y	N	Y	D	T	D	V	S	G	CPV-2b
CPV-2b W42	AF306444	N	R	N	L	A	N	T	A	I	I	F	S	G	T	Y	N	Y	D	T	D	V	S	G	CPV-2b
CPV-339	AY742933	N	R	N	L	A	N	T	A	I	I	F	A	G	T	Y	N	Y	N	T	D	V	S	G	New CPV-2a
Henan42	KJ438805	N	R	N	L	A	N	T	A	I	I	Y	A	G	T	Y	N	I	N	A	D	V	S	G	New CPV-2a
GY-3	KY386852	N	R	N	L	A	N	T	A	I	I	Y	A	G	T	Y	N	I	N	A	D	V	S	G	New CPV-2a
HLJ-02	KM386822	N	R	N	L	A	N	T	A	I	I	Y	A	G	T	Y	N	I	N	A	D	V	S	G	New CPV-2a
CPV-JS2	KF676668	N	R	N	L	A	N	T	A	I	I	Y	A	G	T	Y	N	I	N	A	D	V	S	G	New CPV-2a
CPV2a	AJ564427	N	R	N	L	A	N	T	A	I	I	F	A	G	T	Y	N	Y	N	A	D	V	S	G	New CPV-2a
02B9	DQ025950	N	R	N	L	A	N	T	A	I	I	F	A	G	T	Y	N	Y	N	A	D	V	S	G	New CPV-2a
K022	EU009203	N	R	N	L	A	N	T	A	I	I	F	A	G	T	Y	N	Y	N	A	D	V	S	G	New CPV-2a
LZ2	JQ268284	N	R	N	L	A	N	T	A	I	I	Y	A	G	T	Y	N	I	D	A	D	V	S	G	new CPV-2b
BM-11	JQ743894	N	R	N	L	A	N	T	A	I	I	Y	A	G	T	Y	N	I	D	A	D	V	S	G	new CPV-2b
Wuhan2	KC881278	N	R	N	L	A	N	T	A	I	I	Y	A	G	T	Y	N	I	D	A	D	V	S	G	new CPV-2b
LCPV V204	AB054221	N	R	N	L	A	N	T	A	I	I	F	A	G	T	Y	N	Y	D	A	D	V	S	G	new CPV-2b
DH326	EF599097	N	R	N	L	A	N	T	A	I	I	F	A	G	T	Y	N	Y	D	T	D	V	S	G	new CPV-2b
HRB-A6	KT156832	N	R	N	L	A	N	T	A	I	I	Y	A	G	T	Y	N	I	E	T	D	V	S	G	CPV-2c
06-09	GU380303	N	R	N	L	A	N	T	A	I	I	Y	A	G	T	Y	N	I	E	T	D	V	S	G	CPV-2c
G367_97	FJ005202	N	R	N	L	A	N	T	A	I	I	F	A	G	T	Y	N	Y	E	T	D	V	S	G	CPV-2c
ME10	KF149963	N	R	N	L	A	N	T	A	I	I	F	A	G	T	Y	N	Y	E	T	D	V	S	G	CPV-2c
ME28	KF149984	N	R	N	L	A	N	T	A	I	I	F	A	G	T	Y	N	Y	E	T	D	V	S	G	CPV-2c
G333_99	FJ005204	N	R	N	L	A	N	T	A	I	I	F	A	G	T	Y	N	Y	E	T	D	V	S	G	CPV-2c
M124	KC196108	N	R	N	L	A	N	T	A	I	I	F	A	G	T	Y	N	Y	E	T	D	V	S	G	CPV-2c
ME1	KF149962	N	R	N	L	A	N	T	A	I	I	F	A	G	T	Y	N	Y	E	T	D	V	S	G	CPV-2c
F2016009	MH329283	N	R	N	L	A	N	T	A	I	I	Y	A	G	T	Y	N	I	N	A	D	V	S	G	New CPV-2a
F2016010	MH329284	N	R	N	L	A	N	T	A	I	I	Y	A	G	T	Y	N	I	N	A	D	V	S	G	New CPV-2a

(Continues)

TABLE 1 (Continued)

Strain	GenBank accession no.	Mutations sites: amino acid residue																							Gene types
		46	80	85	87	91	93	101	103	219	232	267	297	300	301	305	323	324	426	440	556	562	564	568	
F2016015	MH329285	N	R	N	L	A	N	T	A	I	I	Y	A	G	T	Y	N	I	N	A	D	V	S	G	New CPV-2a
F2016020	MH329287	N	R	N	M	A	N	I	A	I	I	F	A	A	T	D	N	Y	N	T	D	L	S	G	CPV-2
F2016021	MH329288	N	R	N	M	A	N	I	A	I	I	F	A	A	T	D	N	Y	N	T	D	L	S	G	CPV-2
ZF-5	DQ099430	N	K	N	M	A	K	T	V	I	V	F	S	A	T	D	D	Y	N	T	D	V	N	A	FPV
JF-3	DQ099431	N	K	N	M	S	K	T	V	I	V	F	S	A	T	D	D	Y	N	T	D	V	N	A	FPV
JF-1	DQ474236	N	K	N	M	A	K	T	V	I	V	F	S	A	T	D	D	Y	N	T	D	V	N	A	FPV
389/07	EU145593	N	K	N	M	A	K	T	V	I	V	F	S	A	T	D	D	Y	N	T	D	V	S	A	FPV
933/07	EU360958	N	K	N	M	S	K	T	V	I	V	F	S	A	T	D	D	Y	N	T	D	V	N	A	FPV
Purevax RCP Merial	EU498680	N	K	N	M	A	K	I	V	I	I	F	S	A	T	D	D	Y	N	T	D	L	N	A	FPV
Felocell CVR Pfizer	EU498681	N	K	N	M	A	K	T	V	I	I	F	S	A	T	D	D	Y	N	T	D	L	N	A	FPV
198/01	EU498682	N	K	N	M	A	K	T	V	I	V	F	S	A	T	D	D	Y	N	T	D	V	N	A	FPV
189/03	EU498686	N	K	N	M	A	K	T	V	I	V	F	S	A	T	D	D	Y	N	T	D	V	N	A	FPV
42/06-G8	EU498704	N	K	N	M	A	K	T	V	I	V	F	S	A	T	D	D	Y	N	T	D	V	N	A	FPV
42/06-G11	EU498706	N	K	N	M	S	K	T	V	I	V	F	S	A	T	D	D	Y	N	T	D	V	N	A	FPV
443/07	EU498718	N	K	N	M	A	K	T	V	I	V	F	S	A	T	D	D	Y	N	T	D	V	N	A	FPV
KS42	HQ184200	N	K	N	M	A	K	T	V	I	V	F	S	A	T	D	D	Y	N	T	D	V	N	A	FPV
KS58	HQ184203	N	K	N	M	A	K	T	V	I	V	F	S	A	T	D	D	Y	N	T	D	V	N	A	FPV
50_07-1	EU498716	N	K	N	M	A	K	T	V	I	I	F	S	A	T	D	D	Y	N	T	D	V	N	A	FPV
CO_952_10	JX475245	N	K	N	M	A	K	T	V	I	V	F	S	A	T	D	D	Y	N	T	D	V	N	A	FPV
FPV-b CU4	M24004	N	K	N	M	A	K	T	V	I	I	F	S	A	T	D	D	Y	N	T	D	V	N	A	FPV
CU-4	M38246	N	K	N	M	A	K	I	V	I	V	F	S	A	T	D	D	Y	N	T	D	V	N	A	FPV
F2016019	MH329286	N	K	N	M	A	K	T	V	I	V	F	S	A	T	D	D	Y	N	T	D	V	N	A	FPV

FPV, Feline panleukopenia virus; CPV, canine parvovirus.

FPV and CPV isolates in this study were indicated by italics. The shaded colour showed the point mutations of amino acids among different virus strains.

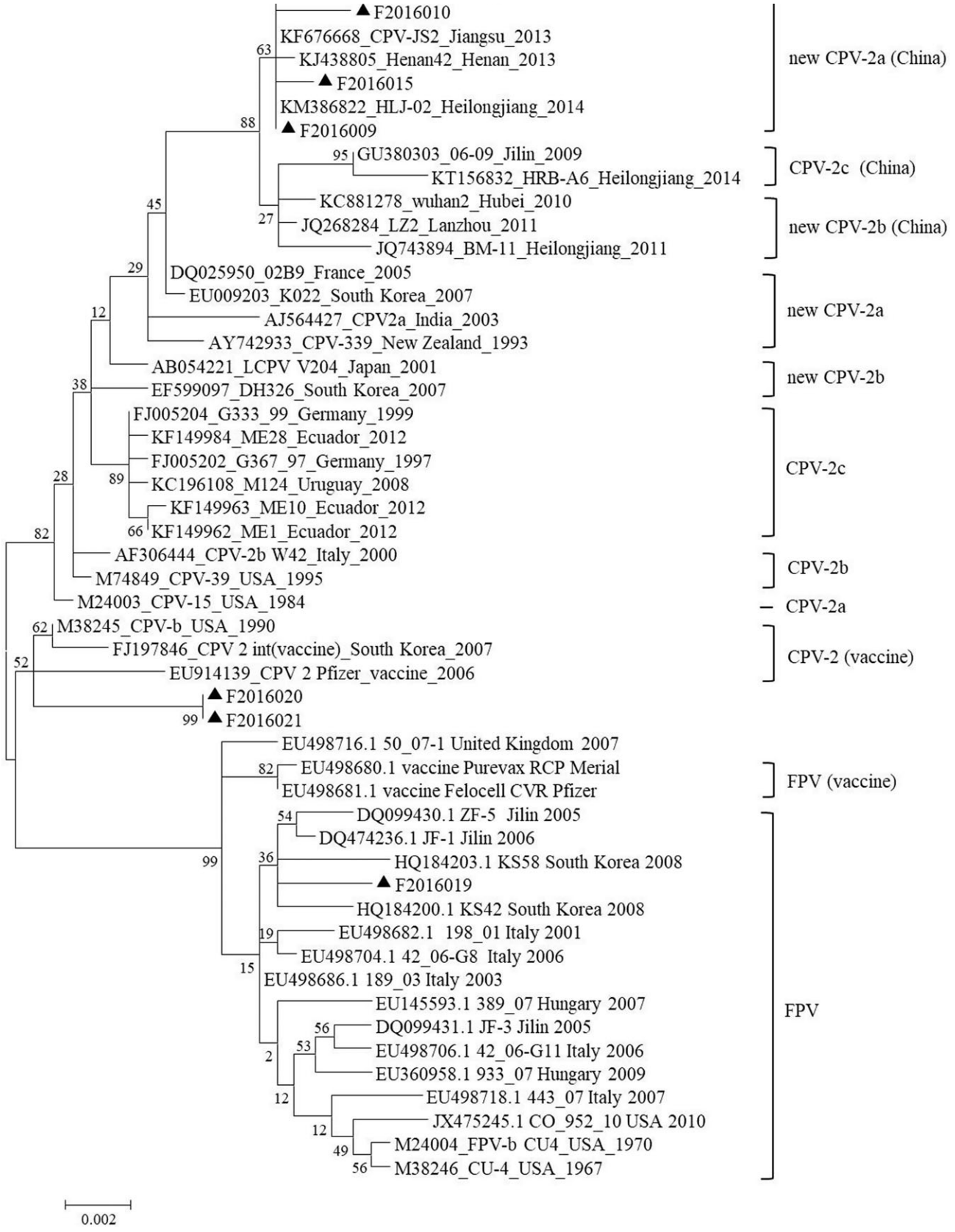

FIGURE 1 A Phylogenetic tree based on six VP2 gene sequences from domestic cats and 45 reference feline panleukopenia virus/canine parvovirus (FPV/CPV) strains was constructed by Maximum Likelihood method using the MEGA software, version 6.0 (http://www.megasoftware.net/). ▲ indicates the FPV/CPV strains in this study. Horizontal branch lengths are proportional to genetic distances. Scale bars indicate nucleotide substitutions per site. Bootstrap values were calculated based on 1,000 replicates

cats but the chance of contracting with other wild carnivore species was minimum in these domestic cats in China.

Another more reasonable explanation is that two feline CPV-2 in this study came from vaccine strain since they clustered together in a single clade and were more related to commercial CPV-2 vaccine strains. The original CPV-2 were previously identified from sick and vaccinated dogs (Zhang et al., 2010). In Zhang's report, three canine CPV-2 collected in 2008 were found having Lys93 Asn mutation in dogs (Zhang et al., 2010). By contrast, Ser297Ala and Val562Leu mutations in VP2 protein were found in these two feline CPV-2 strains in our study. The Ser297Ala mutation was previously reported to be responsible for changes in the antigenicity of CPV variants and may have a marked influence on the process of continuing host adaptation (Pereira et al., 2007; Truyen, 2006; Yi et al., 2016). Therefore, two feline CPV-2 strains in this study may originate from vaccinated dogs and undergo key point mutations to gain the feline tropism.

The co-infection of CPV vaccine strain with field strain was previously reported in dogs with enteritis (Decaro et al., 2007). There is a possibility that two feline CPV-2 in this study were recombination strains between field and vaccine strains. To answer this question, the VP2 genes of F2016020 and F2016021 strains as query sequences compared with CPV-2 Pfizer and Invervet vaccine strains and field isolate (CPV-b). No recombination was found between two CPV-2 strains in this study and CPV-b or vaccine strains (Supporting Information Figure S1).

In conclusion, our analysis of the parvoviruses identified from domestic cats revealed that FPV, new CPV-2a, and CPV-2 were co-circulating in Henan province of China. The CPV-2 strains (F2016020 and F2016021) possess Ser297Ala substitution in this study were the first time observed. These results demonstrate the need for continuing surveillance of parvovirus infections in cats within China.

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CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

ORCID

Kegong Tian http://orcid.org/0000-0001-5362-1415

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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