The Journal of Veterinary Medical Science

NOTE

Wildlife Science

Feline panleukopenia virus as the cause of diarrhea in a banded linsang (*Prionodon linsang*) in Thailand

Natnaree INTHONG[1–3], Kaset SUTACHA[4], Sarawan KAEWMONGKOL[1], Rungthiwa SINSIRI[5], Kriangsak SRIBUAROD[6], Kaitkanoke SIRINARUMITR[3,7] and Theerapol SIRINARUMITR[3,8]*

[1]Department of Veterinary Technology, Faculty of Veterinary Technology, Kasetsart University, Bangkok 10900, Thailand
[2]Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Sean Campus, Nakhon Pathom 73140, Thailand
[3]Center of Excellence on Agricultural Biotechnology (AG-BIO/PERDO-CHE), Bangkok 10900, Thailand
[4]The Veterinary Teaching Hospital, Bang Khaen campus, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand
[5]Molecular Diagnostic Laboratory, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand
[6]Khlong Saeng Wildlife Research Station, Wildlife Research Division, Wildlife Conservation Bureau, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok 10900, Thailand
[7]Department of Small Animal Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand
[8]Department of Pathology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand

J. Vet. Med. Sci.
81(12): 1763–1768, 2019
doi: 10.1292/jvms.19-0238

Received: 13 May 2019
Accepted: 27 August 2019
Advanced Epub:
24 September 2019

ABSTRACT. A banded linsang (*Prionodon linsang*) presented at our hospital with clinical signs of acute diarrhea. Fecal samples were positive for canine parvovirus (CPV) as determined by polymerase chain reaction with primers specific for both CPV and feline panleukopenia virus (FPV). The full-length *VP2* was cloned, sequenced, and compared with sequences of FPV and CPV strains reported in GenBank. The amino acids that determined the host range were similar to those of FPV. Moreover, amino acid analysis of *VP2* revealed over 98% homology to FPV. The FPV isolate was closely related with FPV isolates from Japan, South Korea, and China. To the best of our knowledge, this is the first study to report that banded linsang can be infected with FPV.

KEY WORDS: banded linsang, feline panleukopenia, genetic analysis, *VP2*, wildlife

Feline panleukopenia virus (FPV) is a small, nonenveloped single-stranded DNA virus that usually infects domestic cats and other Felidae, such as those in the families Mustelidae, Procyonidae, and Viverridae, which include raccoons and minks. FPV is highly contagious and is associated with a high mortality and morbidity in young animals. FPV usually causes acute gastroenteritis and leukopenia [4, 17, 18]. The virus is very closely related to canine parvovirus type 2 (CPV-2), with a genomic homology of greater than 98%. These viruses are grouped with other viruses, including mink enteritis virus, raccoon parvovirus (RPV), raccoon dog parvovirus (RDPV), and blue fox parvovirus (BFPV) [16]. Certain amino acids of the *VP2* protein of the parvovirus are very important for determining the host range differences between FPV and CPV, including amino acid positions 80 (Lys to Arg), 93 (Lys to Asn), 103 (Val to Ala), 232 (Val to Ile), 323 (Asp to Asn), 564 (Asn to Ser), and 568 (Ala to Gly) (Table 1) [3, 9, 13, 14, 16, 17, 19].

There have been several reports of FPV infection in wild animals, including an Eurasian lynx (*Lynx lynx*) and European wildcat (*Felis silvestris*) [20], lions (*Panthera leo*) [6, 7], a tiger (*Panthera tigris*) [7] and a monkey [21]. There have been two reports of FPV infection in the Asian palm civet, a member of the family Viverridae [6, 10].

A banded linsang (*Prionodon linsang*) presented at the Veterinary Teaching Hospital, Kasetsart University, Bangkok, Thailand, with clinical signs of acute diarrhea. We wanted to determine whether the banded linsang was infected with parvovirus and to determine whether this parvovirus was CPV or FPV by comparing the complete amino acid sequence of the *VP2* gene of the parvovirus in this case with those of other FPV and CPV strains reported in GenBank.

A fecal sample tested positive for CPV with the screening test kit (Vet-smart Canine CPV/CCV Antigen Duo Test, Pacific Biotech Co., Ltd., Bangkok, Thailand). To confirm the screening test, a multiplex polymerase chain reaction (M-PCR) was

*Correspondence to: Sirinarumitr, T.: fvettps@yahoo.com

Table 1. Important functions of amino acids at different positions in the *VP2* gene of parvoviruses

Amino acid position (*VP2*)	Comparison	Function	References
80	FPV and CPV	Antigenicity and feline host range	[2, 13, 14, 16]
87	CPV-2 and CPV-2a/2b	Antigenicity	[13, 14, 16]
93	FPV and CPV	Antigenicity and feline host range	[2, 7, 13, 14, 16]
103	FPV and CPV	Viability in the presence of changes of residues 93 and 323	[2, 13, 14, 16]
232	FPV and CPV	Antigenicity and feline host range	[16]
300	CPV-2 and CPV-2a/2b	Antigenicity	[10, 11, 13, 14]
305	CPV-2 and CPV-2a/2b	Antigenicity	[10, 11, 13, 14]
323	FPV and CPV	Antigenicity and canine host range	[2, 7, 13, 14]
426	CPV-2 and CPV-2a/2b	Antigenicity	[11, 14, 16]
564	FPV and CPV	Feline host range	[2, 13, 14, 16]
568	FPV and CPV	Feline host range	[2, 13, 14, 16]

FPV, Feline panleukopenia virus; CPV, canine parvovirus; CPV-2, canine parvovirus type 2; CPV-2a/2b, canine parvovirus type 2a/2b.

Table 2. Primers used in this study

Name of set primer	Sequence of primer (5′-3′)	PCR product (bp)	References
F 2a/2b R 2a/2b	GAA GAG TGG TTG TAA ATA ATT CCTATATAACCAAAGTTAGTAC	681	[12]
Fp Rp	TATGGTCCTTTAACTGCATTAAA TTAATATAATTTTCTAGGTGCTAG	404	-
Fw Rw	ATGAGTGATGGAGCAGTTCA TTAATATAATTT TCTAGGTGCTAGTTG	1,755	-

performed using two set of primers (Table 2) for the detection of CPV-2 (Fp and Rp) and CPV-2a/2b (F2a/2b and R2a/2b) [15]. Briefly, the PCR mixture (100 μl) was composed of 10 μl of 10 × buffer (20 mM Tris-HCl (pH 8.4), 50 mM KCl_2), 2 μl of 10 mM dNTPs, 5 μl of 50 mM $MgCl_2$, 1 μl of 100 pmol of each of the forward and reverse primers, 0.5 μl of 5 units/μl of Taq DNA polymerase (Invitrogen, Carlsbad, CA, U.S.A.), 10 μl of DNA template, and distilled water to make the total volume 100 μl. After an initial denaturing at 94°C for 7 min, the amplification was performed using 35 cycles at 94°C for 1 min, annealing at 55°C for 90 sec, extension at 72°C for 90 sec, and a final extension at 72°C for 5 min. The M-PCR products showed a single band of approximately 400 bp in size. This result showed that the banded linsang might be infected with CPV-2.

To determine whether this banded linsang was infected with CPV or FPV, a set of primers was designed for the amplification of the full-length *VP2* genes of both FPV and CPV (Fw and Rw) (Table 2). After an initial denaturing at 94°C for 5 min, the amplification was performed using 35 cycles at 94°C for 40 sec, annealing at 50°C for 40 sec, extension at 72°C for 90 sec, and a final extension at 72°C for 10 min. The PCR products were expectedly 1,755 base pairs in size. The PCR products were purified using an UltraClean®15DNA purification kit (MO BIO Laboratories, Inc., Carlsbad, CA, U.S.A.) and cloned into plasmid pGEM-T easy (Promega Corporation, Madison, WI, U.S.A.). The sequence of the cloned full-length *VP2* was determined at First BASE Laboratories Sdn Bhd, Selangor, Malaysia.

The nucleotide sequences of the full-length *VP2* were translated, and multiple alignments of the amino acid sequences were identified using the Bioedit biological sequence alignment editor computer package (version 7.1.3; Ibis Biosciences, Carlsbad, CA, U.S.A.). The amino acid sequence of the cloned full-length *VP2* in this study showed more than 98% homology with the *VP2* gene from FPV. The amino acids at positions 80, 93, 103, 232, 323, 564, and 568 were similar to FPVs (Fig. 1).

For performing phylogenetic analysis, the amino acid sequence of the full-length *VP2* in this study was compared with 39 reference FPV and CPV strains available in the GenBank database (Table 3). The amino acid phylogenetic analysis was created using MEGA (version 6.0; The Bio Design Institute, Tempe, AZ, U.S.A.), and a phylogenetic tree was constructed using the neighbor-joining method and by running 1,000 replicates in the bootstrap to test the reliability of the phylogenetic tree for the *VP2* region. The phylogenetic analysis of the full-length *VP2* amino acid sequence was closely related to FPV from cats in Japan (AB000056) and South Korea (HQ184198), mink enteritis virus (KJ186148), and FPV isolated from a tiger (FJ405225) (Fig. 2). Based on these results, the banded linsang in the current study was infected with FPV.

In this study, the fecal sample of the banded linsang was found to be positive for CPV by both screening and PCR analysis. The amino acids of this cloned *VP2* at positions 80, 93, 103, 232, 323, 564, and 568 were similar to FPV but not CPV. These results indicated that the banded linsang in this study was infected with FPV. Currently, it is unclear whether the banded linsang is also susceptible to CPV. It is possible that the banded linsang may be infected with CPV, since this virus can infect both canine and feline cells with similar efficiency by binding to both canine and feline transferrin receptors (TfR) [2]. The *TFCR* gene, which encodes TfR, of carnivore species has up to a 10% difference in DNA sequence, with the changes distributed throughout

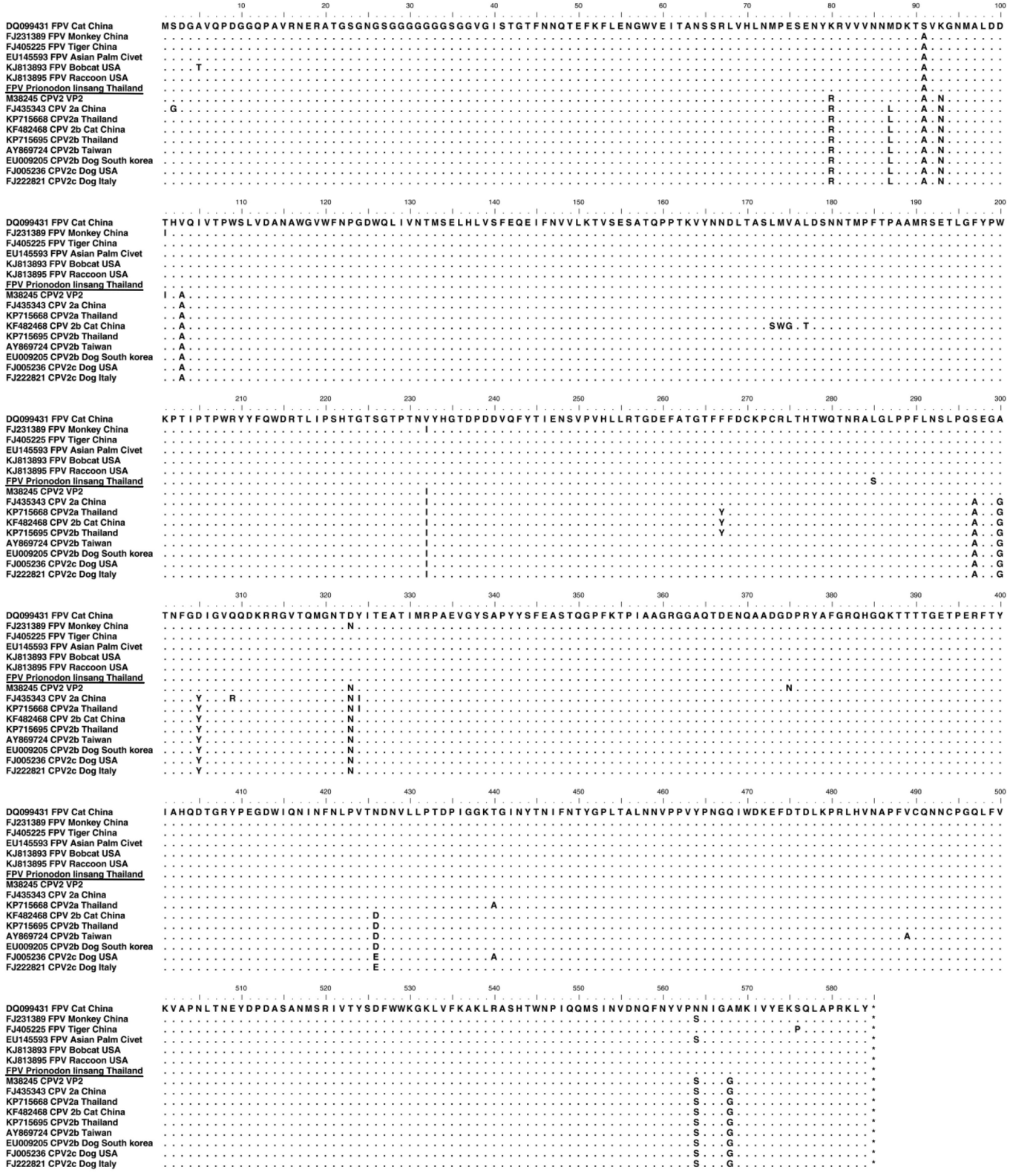

Fig. 1. The full-length *VP2* amino acid sequence of the banded linsang compared with 15 other isolates of feline panleukopenia virus (FPV), canine parvovirus type 2 (CPV-2), canine parvovirus type 2a (CPV-2a), canine parvovirus type 2b (CPV-2b), and canine parvovirus type 2c (CPV-2c).

Table 3. Canine parvovirus and feline panleukopenia virus strains used for construction of the phylogenetic tree

No.	Isolate	Origin	GenBank accession No.
1	FPV/ARG07	Argentina	FJ440713
2	FPV/ARG08	Argentina	FJ440714
3	FPV/Tiger	China	FJ405225
4	FPV 389/07 Asian Palm Civet	Hungary	EU145593
5	FPV strain 42/06-G10/Cat	Italy	EU498705
6	FPV strain 143/04/Cat	Italy	EU498692
7	FPV, Obihiro/Cat	Japan	AB000056
8	FPV/Cat	Japan	AB000061
9	FPV strain:V211/Cat	Japan	AB054227
10	FPV isolate PT271/14/Cat	Portugal	KT240136
11	FPV PT210/13/Cat	Portugal	KT240134
12	FPV strain KS2/Cat	South Korea	HQ184204
13	FPV, KS18/Cat	South Korea	HQ184198
14	FPV Cat	Taiwan	AF015223
15	**FPV Prionodon linsang**	**Thailand**	**MH669800 (present study)**
16	FPV strain 97/06-11/Cat	U.K.	EU498714
17	FPV strain 490/07/Cat	U.K.	EU498719
18	FPV/ND/979/2013/Bobcat	U.S.A.	KJ813893
19	FPV isolate Raccoon	U.S.A.	KJ813895
20	Purevax vaccine	-	EU498680
21	Felocell vaccine	-	EU498681
22	Mink enteritis virus/mink	China	KJ186148
23	CPV 2	U.S.A.	M38245
24	CPV 2a Dog	China	FJ435343
25	CPV 2a Dog	South Korea	FJ197834
26	CPV 2a Dog	Taiwan	U72698
27	CPV 2a Dog	Thailand	KP715668
28	CPV 2a Dog	Thailand	KP715675
29	CPV 2a Dog	Thailand	KP715684
30	CPV 2a Dog	Thailand	GQ379047
31	CPV 2a Dog	Thailand	GQ379048
32	CPV 2a Dog	Thailand	GQ379049
33	CPV 2b Dog	China	KF482468
34	CPV 2b Dog	Thailand	KP715695
35	CPV 2b Dog	Thailand	FJ869122
36	CPV 2b Dog	Thailand	FJ869123
37	CPV 2b Dog	Thailand	FJ869124
38	CPV2c Cat	Italy	HQ025913
39	CPV2c Dog	Italy	FJ222821
40	CPV2c Dog	Germany	FJ005202

the gene, including the apical domain region that is associated with parvovirus binding [11]. A single amino acid change or new glycosylation site in the apical domain of TfR, especially at amino acid position 384, may reduce or eliminate both parvovirus binding and infection [8, 11, 12]. If such a change in the TfR of the banded linsang is present, CPV infection may not occur. Whether the absence of CPV infection in the banded linsang is due to a lack of clinical cases or natural resistance to infection remains to be determined. There have been reports of FPV infection in members of the family Viverridae, such as the Asian palm civet *(Paradoxurus hermaphrodites)* [5] and Formosan gem-faced civets (*Paguma larvata taivana*) [10]. However, there has been no report of FPV infection in the banded linsang (*Prionodon linsang*), a member of the family Prionodontidae, which is closely related to the family Viverridae [2]. According to molecular phylogenetics, Prionodontidae has a sister relationship with Viverridae [1]. Thus, the *TFCR* gene of Prionodontidae might have minimal or no variation from that of the family Viverridae. This might be the reason why the banded linsang can be infected with FPV, as other feliform species have also been infected with FPV. Thus, to the best of our knowledge, this is the first report of FPV infection in the banded linsang (*Prionodon linsang*), demonstrating that parvoviruses have been continuously expanding their host range. Based on the findings of this study, it is important to undertake effective biosecurity measures and vaccination to prevent interspecies transmission of FPV in the zoo.

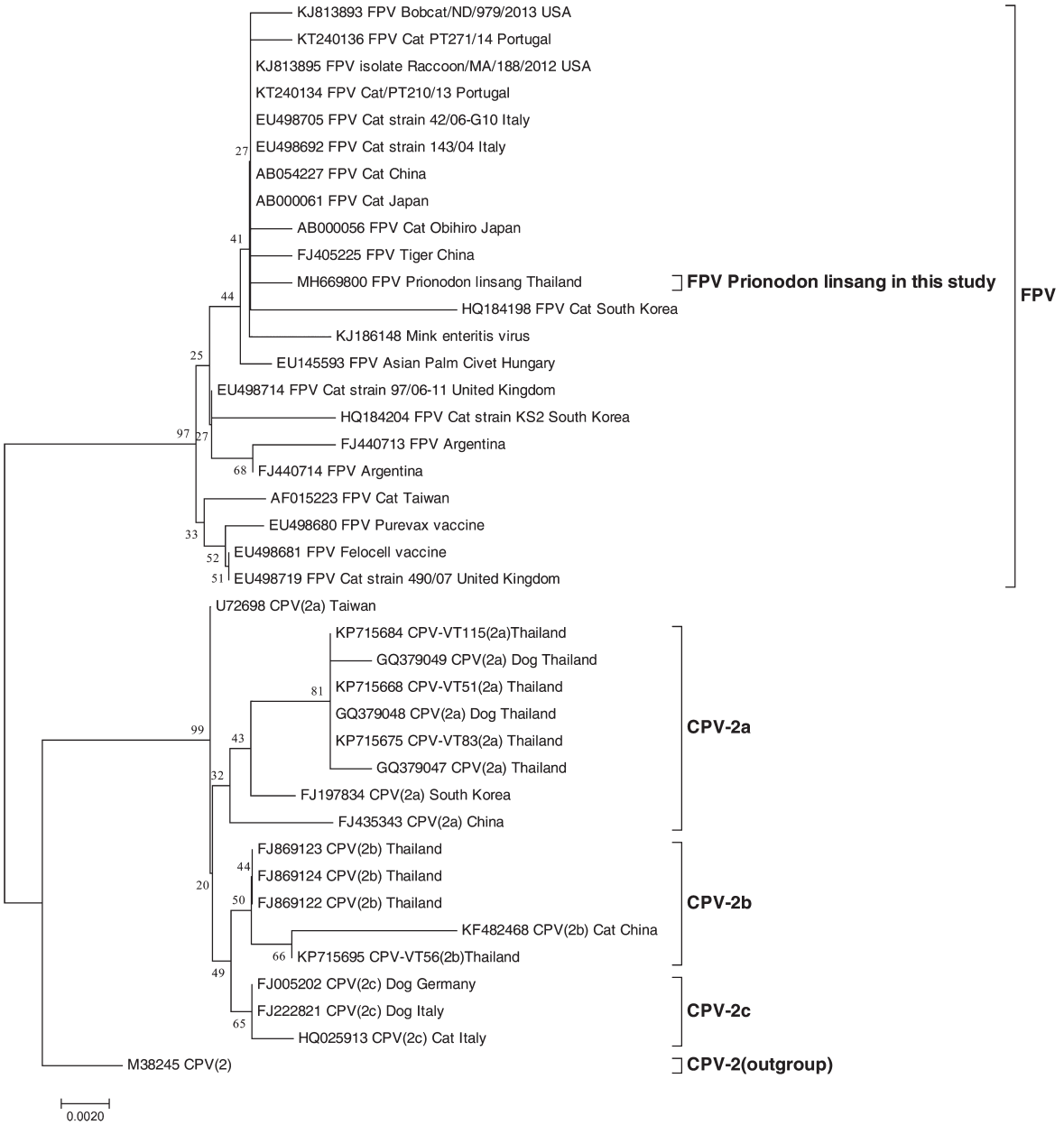

Fig. 2. Phylogenetic analysis based on the entire *VP2* gene amino acid sequence of feline panleukopenia virus (FPV) isolated in this study compared with canine parvovirus and feline panleukopenia virus strains obtained from the GenBank database. A phylogenetic tree was constructed using the MEGA6 program with the neighbor-joining method.

ACKNOWLEDGMENTS. This research was supported by a grant from the Center of Excellence on Agricultural Biotechnology, Science and Technology Postgraduate Education and Research Development Office, Office of Higher Education Commission, Ministry of Education, Science, Research and Innovation (AG-BIO/PERDO-CHE), Bangkok, Thailand.

REFERENCES

1. Agnarsson, I., Kuntner, M. and May-Collado, L. J. 2010. Dogs, cats, and kin: a molecular species-level phylogeny of Carnivora. *Mol. Phylogenet. Evol.* **54**: 726–745. [Medline] [CrossRef]
2. Allison, A. B. and Parrish, C. R. 2014. Chapter 3−parvoviruses of carnivores: their transmission and the variation of viral host range. pp. 39–61. *In*: The Role of Animals in Emerging Viral Diseases. Academic Press, Boston.
3. An, D. J., Jeong, W., Jeoung, H. Y., Yoon, S. H., Kim, H. J., Park, J. Y. and Park, B. K. 2011. Phylogenetic analysis of feline panleukopenia virus (FPLV) strains in Korean cats. *Res. Vet. Sci.* **90**: 163–167. [Medline] [CrossRef]
4. Barker, I. K., Povey, R. C. and Voigt, D. R. 1983. Response of mink, skunk, red fox and raccoon to inoculation with mink virus enteritis, feline panleukopenia and canine parvovirus and prevalence of antibody to parvovirus in wild carnivores in Ontario. *Can. J. Comp. Med.* **47**: 188–197.

[Medline]

5. Demeter, Z., Gál, J., Palade, E. A. and Rusvai, M. 2009. Feline parvovirus infection in an Asian palm civet (*Paradoxurus hermaphroditus*). *Vet. Rec.* **164**: 213–216. [Medline] [CrossRef]
6. Demeter, Z., Palade, E. A. and Rusvai, M. 2010. Feline panleukopenla virus infection in various species from Hungary. *Lucr. St. Med. Vet. Timisoara* **43**: 73–81.
7. Duarte, M. D., Barros, S. C., Henriques, M., Fernandes, T. L., Bernardino, R., Monteiro, M. and Fevereiro, M. 2009. Fatal infection with feline panleukopenia virus in two captive wild carnivores (*Panthera tigris* and *Panthera leo*). *J. Zoo Wildl. Med.* **40**: 354–359. [Medline] [CrossRef]
8. Goodman, L. B., Lyi, S. M., Johnson, N. C., Cifuente, J. O., Hafenstein, S. L. and Parrish, C. R. 2010. Binding site on the transferrin receptor for the parvovirus capsid and effects of altered affinity on cell uptake and infection. *J. Virol.* **84**: 4969–4978. [Medline] [CrossRef]
9. Hueffer, K., Govindasamy, L., Agbandje-McKenna, M. and Parrish, C. R. 2003. Combinations of two capsid regions controlling canine host range determine canine transferrin receptor binding by canine and feline parvoviruses. *J. Virol.* **77**: 10099–10105. [Medline] [CrossRef]
10. Ikeda, Y., Miyazawa, T., Nakamura, K., Naito, R., Inoshima, Y., Tung, K. C., Lee, W. M., Chen, M. C., Kuo, T. F., Lin, J. A. and Mikami, T. 1999. Serosurvey for selected virus infections of wild carnivores in Taiwan and Vietnam. *J. Wildl. Dis.* **35**: 578–581. [Medline] [CrossRef]
11. Kaelber, J. T., Demogines, A., Harbison, C. E., Allison, A. B., Goodman, L. B., Ortega, A. N., Sawyer, S. L. and Parrish, C. R. 2012. Evolutionary reconstructions of the transferrin receptor of Caniforms supports canine parvovirus being a re-emerged and not a novel pathogen in dogs. *PLoS Pathog.* **8**: e1002666. [Medline] [CrossRef]
12. Palermo, L. M., Hueffer, K. and Parrish, C. R. 2003. Residues in the apical domain of the feline and canine transferrin receptors control host-specific binding and cell infection of canine and feline parvoviruses. *J. Virol.* **77**: 8915–8923. [Medline] [CrossRef]
13. Parrish, C. R., Aquadro, C. F. and Carmichael, L. E. 1988. Canine host range and a specific epitope map along with variant sequences in the capsid protein gene of canine parvovirus and related feline, mink, and raccoon parvoviruses. *Virology* **166**: 293–307. [Medline] [CrossRef]
14. Parrish, C. R., Aquadro, C. F., Strassheim, M. L., Evermann, J. F., Sgro, J. Y. and Mohammed, H. O. 1991. Rapid antigenic-type replacement and DNA sequence evolution of canine parvovirus. *J. Virol.* **65**: 6544–6552. [Medline]
15. Senda, M., Parrish, C. R., Harasawa, R., Gamoh, K., Muramatsu, M., Hirayama, N. and Itoh, O. 1995. Detection by PCR of wild-type canine parvovirus which contaminates dog vaccines. *J. Clin. Microbiol.* **33**: 110–113. [Medline]
16. Steinel, A., Munson, L., van Vuuren, M. and Truyen, U. 2000. Genetic characterization of feline parvovirus sequences from various carnivores. *J. Gen. Virol.* **81**: 345–350. [Medline] [CrossRef]
17. Steinel, A., Parrish, C. R., Bloom, M. E. and Truyen, U. 2001. Parvovirus infections in wild carnivores. *J. Wildl. Dis.* **37**: 594–607. [Medline] [CrossRef]
18. Stuetzer, B. and Hartmann, K. 2014. Feline parvovirus infection and associated diseases. *Vet. J.* **201**: 150–155. [Medline] [CrossRef]
19. Truyen, U., Gruenberg, A., Chang, S. F., Obermaier, B., Veijalainen, P. and Parrish, C. R. 1995. Evolution of the feline-subgroup parvoviruses and the control of canine host range in vivo. *J. Virol.* **69**: 4702–4710. [Medline]
20. Wasieri, J., Schmiedeknecht, G., Förster, C., König, M. and Reinacher, M. 2009. Parvovirus infection in a Eurasian lynx (*Lynx lynx*) and in a European wildcat (*Felis silvestris silvestris*). *J. Comp. Pathol.* **140**: 203–207. [Medline] [CrossRef]
21. Yang, S., Wang, S., Feng, H., Zeng, L., Xia, Z., Zhang, R., Zou, X., Wang, C., Liu, Q. and Xia, X. 2010. Isolation and characterization of feline panleukopenia virus from a diarrheic monkey. *Vet. Microbiol.* **143**: 155–159. [Medline] [CrossRef]