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

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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
performed using two sets 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 5 min. The PCR products were expectedly 1,755 base pairs in size. The PCR products were purified using an UltraClean® DNA 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 FPVs 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

| Name of set primer | Sequence of primer (5′-3′) | PCR product (bp) | References |
|--------------------|-----------------------------|------------------|------------|
| F 2a/2b            | GAA GAG TGG TTG TAA ATA ATT | 681              | [12]       |
| R 2a/2b            | CCTATATAACCAAGTATGAC         |                  |            |
| Fp                 | TATGGTCTCTTAACTGCTTTTCT     | 404              | -          |
| Rp                 | ATCTAAATAATTITTCAGGTGCTAG    |                  |            |
| Fw                 | ATGAGTGATGAGGACGTTCA         | 1,755            | -          |
| Rw                 | TTAATATAATTTCCTAGGTGCTATT   |                  |            |

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

### 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 NV211/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              |
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