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Isolation and identification of tiger parvovirus in captive siberian tigers and phylogenetic analysis of VP2 gene

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ABSTRACT

To better understand the prevalence and molecular epidemiology of parvovirus, this study reports the isolation and characterization of a tiger parvovirus (TPV) named CHJL-Siberian Tiger-01/2017 from a captive Siberian tiger in Jilin Province, China. A phylogenetic tree based on the full-length VP2 nucleotide sequence was constructed using the isolated strain in this study and 56 reference strains. The results showed that all the parvoviruses can be grouped into two large branches: the canine parvovirus (CPV) branch and the feline parvovirus (FPV) branch. FPV strains comprised TPVs, FPVs, blue fox parvoviruses (BFPVs), mink enteritis viruses (MEVs), and raccoon feline parvoviruses (RFPVs), and CPV strains comprised CPVs and raccoon dog parvoviruses (RDPVs). RFPVs are also often very closely related to those sampled from other carnivorous species, and raccoons may represent conduits for parvovirus transmission to other hosts. The results of amino acid changes in the VP2 protein of the isolated strain showed that amino acid Ile 101 was mutated to Thr (I 101T). Taken together, a field TPV strain CHJL-Siberian Tiger-01/2017 was isolated, which may be suitable for future studies on FPV infection, replication and vaccine development. This study provided new important findings about the evolution of parvovirus infection in tigers.

1. Introduction

The Siberian tiger (*P. tigris altaica*), also known as the Amur tiger, originated in northeast Asia and is now mainly distributed in the Russian Far East, northeast of China and north of Korea. Today, approximately 500 wild Siberian tigers remain, while only a small amount remain in China with their main activities in the eastern mountain areas of Heilongjiang and Jilin Provinces (Liu et al., 2010). The Siberian tiger is listed as an endangered species by the international union for the conservation of nature and is included in the CITES Appendix 1 (Guo et al., 2014). To increase the population numbers, many of them are captive in zoos or wildlife rescue stations. Under these circumstances, they are considered special animals (e.g., having high ornamental value) with enough food and without natural enemies. However, some serious plagues, such as feline panleukopenia, are currently threatening their lives.

Feline panleukopenia is caused by feline panleukopenia virus (FPV), a single-stranded DNA virus. Little difference in the genome has been detected among viral isolates from the same and different hosts (Battilani et al., 2006b; Decaro et al., 2008b; Hoelzer et al., 2008). Genetically, structurally, and antigenically, it is closely related to blue fox parvovirus (BFPV), mink enteritis virus (MEV) and canine parvovirus (CPV). FPV can cause disease in all members of the family Felidae, and numerous reports of FPV infection or exposure in nondomestic cats exist (Mochizuki et al., 1993). Some Viverridae, Procyonidae and Mustelidae animals, including binturongs, raccoons, coatimundis, ringtailed cats, and minks, are also susceptible. FPV has been reported to threaten the survival of endangered *Panthera tigris* as a severely damaging disease. For example, wild Amur tigers (*Panthera tigris altaica*, $n = 44$) from the Russian Far East were tested for the antibodies to FPV, with an antibody-positive rate of 68% (Goodrich et al., 2012). Furthermore, FPV strains were isolated from the diarrheic faeces of a monkey of the *Macaca* spp. in China (Yang et al., 2008). In this study, a FPV strain was isolated and identified from a captive Siberian tiger in a wildlife park in Jilin Province, China. To study the range and distribution of FPV in wild/captive carnivores, the role of different carnivores in FPV transmission, and the relation between FPV mutations and host ranges, phylogenetic analysis was conducted with the VP2

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gene obtained in this study and all FPV VP2 genes from wild/captive carnivores in GenBank. This study helps to understand the genetic diversity and distribution of wild/captive carnivore FPV.

2. Materials and methods

2.1. Cell strain and ethics statement

F81 cells are from the academy of the Military Veterinary Research Institute of the Academy of Military Medical Sciences. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Jilin Agricultural University, China.

2.2. Clinical samples and preparation

Samples were collected from the intestinal organs of dead Siberian tigers from a wildlife park in Jilin Province, China. Samples were tested by a PCR assay (Niu et al., 2018) as described previously. FPV-positive samples were dissolved in phosphate-buffered saline (PBS) and then homogenized with a vortex. The samples were then centrifuged at 5000*g* for 5 min at 4 °C. Next, the supernatants were filtered using a 0.22 μm microporous filtering film. The filtered supernatants were stored at −80 °C until use.

2.3. Viral PCR identification

Total DNA was extracted from the culture supernatant using a DNA Extraction Kit (Takara, Japan) according to the manufacturer's protocol. A PCR assay was performed (Wu et al., 2015) for the preliminary test. Briefly, 25 μL of PCR mixture was prepared with 2.5 μL of 10× Ex-Taq Buffer, 0.5 μL of forward primer, 0.5 μL of reverse primer, 2 μL of dNTPs (10 mM each), 3 μL of DNA template, 0.5 μL of Ex-Taq (5 U/μL) and 16 μL of ddH_2O. The sequences of the primers are FPV-F (5′-GGA TGGGTGGAAATCACAGC-3′) and FPV-R (5′-ATAACCAACCTCAGCTG GTC-3′). The following PCR programs was performed: 5 min hold at 95 °C for denaturation, 35 cycles of 40 s at 94 °C, 1 min at 57 °C and 1 min at 72 °C, and a final extension cycle for 10 min at 72 °C. The amplicons were electrophoresed on a 1% agarose gel. PCR products were cloned into the pEASY blunt vector (TransGen Biotech Co., Ltd., Beijing, China) and sequenced at least 3 times (Genewiz, Inc., Beijing, China).

2.4. Virus isolation

A 500 μL aliquot of the supernatant was placed into a 25 cm^2 flask containing a subconfluent monolayer of F81 cells. The cells were incubated for 1 h, and then Dulbecco's modified Eagle's medium (DMEM) (HyClone, USA) supplemented with antibiotics (without foetal calf serum) was added. Cell cultures were observed every 6 h for a cytopathic effect (CPE). The viruses were stored at −80 °C when 80% CPE was observed. The viruses were passaged to the fifth generation, diluted with DMEM (containing 2% foetal calf serum) and antibiotics to generate 11 serial 10-fold dilutions. Then, F81 cells were grown as a monolayer in 100 μL in 96-well plates. For each dilution, four wells were inoculated with 100 μL of the viral suspension per well. Viral titres were calculated using the Karber statistical formula and expressed in log10.

2.5. Immunofluorescence assay (IFA)

F81 cells were grown in 24-well microtitre plates and infected with FPV, and the multiplicity of infection (MOI) was 0.01. After 24 h, the cells were fixed with 4% paraformaldehyde for 16 min and then washed three times with sodium polyanethole sulfonate (SPS). Cells were permeabilized by Triton X-100 (0.5%) for 20 min at room temperature and incubated with anti-FPV monoclonal antibody (Ingenasa, Spain) at 4 °C overnight and then FITC-conjugated rat anti-mouse secondary antibody (Ingenasa, Spain) for 1 h. Finally, the labelled cells were observed via fluorescence microscopy.

2.6. Electron microscopy observation

The virus isolates were further identified by electron microscopy. F81 cells were infected with field isolates in a 25 cm^2 flask, and the cultures were observed closely for CPE. Approximately 16 h after the infection, the cell culture medium was decanted and the monolayer was overlaid with cold 2.0% paraformaldehyde and 2.5% glutaraldehyde. The flasks were incubated for 1 h and then stored at 4 °C for a few days. Post-fixation processing was performed with 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) at 37 °C for 1–2 h. Grid staining was performed with 2% aqueous uranyl acetate and lead citrate. The samples were observed under an electron microscope.

2.7. Pathogenicity experiments in cats

The experimental cats were screened for FPV, feline calicivirus (FCV), and feline herpes virus (FHV) by RT-PCR and found to be negative. Serum samples were collected and tested before the experiment, and the anti-FPV antibody test was negative. Three-month-old healthy domestic cats ($n = 6$) weighing from 1.2 to 1.5 kg were randomly divided into two groups, and three cats lived in a single animal house (3 m × 3 m). To prevent cross contamination among different rooms, the experimenters were required to wear separate personal protection equipment before entering each room. Cats were challenged with 1.0 mL of 10^7 $TCID_{50}$ of CHJL-Siberian Tiger-01/2017 by intranasal and ocular routes. The control group was mock inoculated with 1.0 mL DMEM. The clinical symptoms and rectal temperatures were monitored daily.

2.8. Histopathologic examination

Tissue samples were collected and set in 10% buffered formalin solution, processed and embedded in paraffin for histopathological analysis. Three millimetre-thick sections were stained with haematoxylin and eosin (H&E). Histopathologic changes were evaluated in 5 sections from each sample. Three samples from each group were analysed.

2.9. Phylogenetic analysis

The full-length VP2 gene was amplified according to a published report (Niu et al., 2018). Fifty-six complete VP2 nucleotide sequences (FPV, CPV, MEV, BFDV, and RDPV) were aligned from the GenBank database (Supplemental Fig. 4) using DNAStar software. Phylogenetic analyses were performed using Molecular Evolutionary Genetics Analysis software MEGA 6.06. The maximum likelihood method was used for the construction of the phylogenetic tree.

3. Results

3.1. Virus isolation and identification

One FPV strain, designated FPV CHJL-Siberian Tiger-01/2017, was successfully isolated from a sample from Jilin Province, China. Typical CPE was observed within 24 h in F81 cell cultures exposed to the swab supernatant. Compared to the normal F81 cells (Fig. 1A), F81 cells infected with FPV exhibited cytogamy, rounding, karyopyknosis and cluster-like forms; they eventually shed completely and remained suspended, and the border cells appeared long and thin with large cell margins (Fig. 1B). By applying an immuno fluorescence assay (IFA), the specificity of the virus was confirmed. Bright green fluorescence was found in infected F81 cells probed with anti-FPV monoclonal antibody

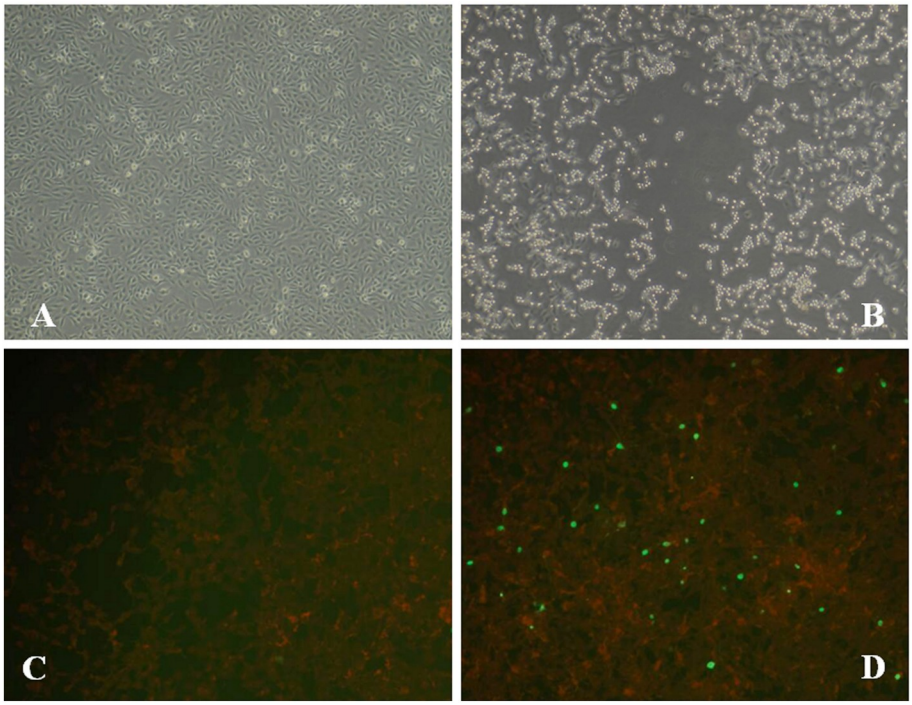

Fig. 1. Detection of FPV through virus isolation and IFA. (A) Normal F81 cells. (B) Cells infected with FPV, cell rounding, karyopyknosis and cluster-like forms. (C) Negative control. (D) Cells infected with FPV are shown. Green fluorescence represents the FPV antigen within infected cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 1C), in contrast, there was no fluorescence for normal F81 cells (Fig. 1D).

3.2. Electron microscopy observation

After infection with FPV CHJL-Siberian Tiger-01/2017 for 16 h ultracryotomy and transmission electron microscopy of F81 cells were used. The virus particle was round or oval, and the diameter was approximately 20 nm, which was consistent with the structural characteristics of panleukopenia virus (Fig. 2).

3.3. Infection with FPV contributes to severe intestinal damage

In the challenge group, all cats displayed anorexia, weight loss, and fever, and two of them died on the 5th day after onset. There were petechial and ecchymotic haemorrhages on the serosal surfaces (Fig. 3A), and the intestinal wall was thin (Fig. 3B). Compared to normal intestinal tissue (Fig. 3C), histologic abnormalities in the intestine included dilated crypts, with sloughing of epithelial cells and necrotic debris into the lumen. Crypt-lining cells may slough completely so that only the basement membrane remains (Fig. 3D).

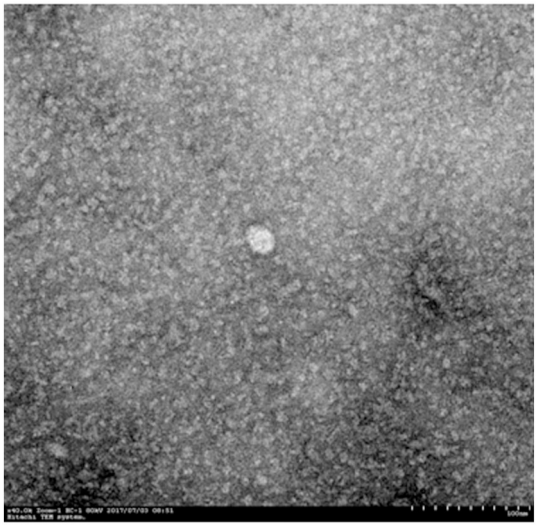

Fig. 2. Transmission electron microscopy technique of negative staining (80,000×). The virus particle was round, the diameter was around 20 nm, which was consistent with the structural characteristics of panleukopenia virus.

3.4. Comparison of the CHJL-Siberian Tiger-01/2017 VP2 gene sequence with those of other FPVs

The full-length VP2 gene was successfully amplified. Sequence analysis showed that the nucleotide sequence was highly related to those from other tigers, with nucleotide sequence identity ranging from 97.44%–99.49%. To further analyse mutations in the isolated strain, amino acid changes were determined in the VP2 protein of the isolated strain. The results showed that the amino acid Ile 101 was mutated to Thr (I 101T) (Table 1).

A phylogenetic tree based on the full-length VP2 nucleotide sequence was constructed using the isolated strain in this study and 56 reference strains (13 TPVs, 1 civet parvovirus, 18 raccoon parvoviruses (RFPVs), 7 mink enteritis viruses (MEVs), 1 mongoose parvovirus (MPV), 1 lion parvovirus, 4 BFPVs, 3 wild cat FPVs, 3 raccoon dog parvoviruses (RDPVs), 3 CPVs and 2 vaccine strains) from Europe, America and Asia (Fig. 4). The phylogenetic analysis indicated that all the parvoviruses can be grouped into two large branches: the CPV branch and the FPV branch. FPV strains comprised of TPVs, FPVs, BFPVs, MEVs, and RFPVs and consisted of two strains from Japan, one strain from Korea, one strain from India, three strains from Portugal, nine strains from the USA, one strain from the UK and sixteen strains from China, while two FPV vaccine strains (EU498680 and EU498681) were classified in the FPV branch. Fourteen TPV sequences (EU697387, EU697383, FJ405225, EU697386, EU697384, AB054227, CHJL- Siberian Tiger-01/2017, DQ099430, KX685354, AY955826, EF988660, EU252146, EU145593, EU498692) formed a cluster, and two sequences (EF418568 and EF418569) from Portugal formed different clades. CHJL- Siberian Tiger-01/2017 was closely related to the Japan-isolated strain (AB054227) and was distant from the Portugal-isolated TPV (EU221278, ef418568). All the CPV strains consisted comprised CPVs and RFPVs.

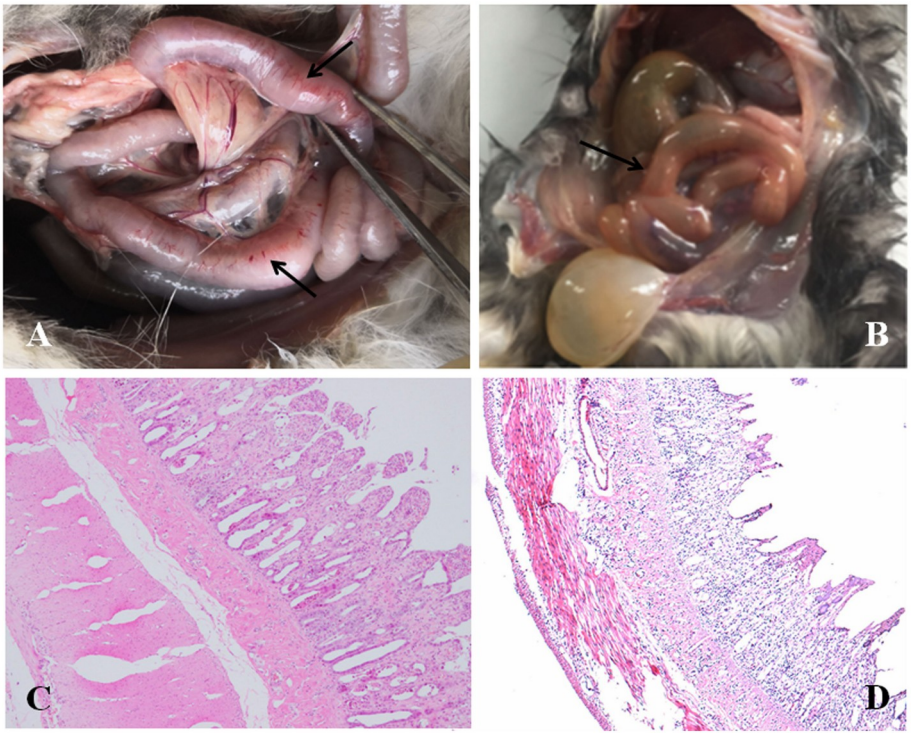

Fig. 3. Segmental hyperaemia of the intestine seen at necropsy of a cat. (A) There were petechial and ecchymotic haemorrhages on the serosal surfaces. (B) The intestinal wall was thin. (C) Normal intestinal villi (H & E stain, ×100). (D) Dilated crypt lumina and collapsed villi are visible in the lower part of the figure; there is sloughing of epithelial cells. Necrotic debris and overlying inflammatory exudate are presented in the intestinal lumen in the upper part of the fig. (H & E stain, ×100).

4. Discussion

Parvoviruses are small, nonenveloped, DNA-containing viruses that infect a wide range of animals. The viruses have been named according to the host from which they were isolate rather than their genetic relatedness. In this study, a TPV was isolated and identified. The results proved that tiger parvovirus disease is one of the most important diseases endangering tiger health. The reason may be that all parvoviruses are extremely stable and resistant to adverse environmental influences.

Currently, eighty-five VP2 nucleotide sequences are available in GenBank since some VP2 sequences from the same country or province are identical to each other, thus, fifty-six VP2 sequences were collected. The result of the evolutionary tree showed that Asia-isolated FPVs form one branch, and the Portugal-isolated FPVs form another branch. The FPV CHJL-Siberian Tiger-01/2017 strain belonged to the Asia-isolated branch and was closely related to the Japan-isolated strain (AB054227) but had a distant relationship to the Portugal-isolated FPV (EU221278, ef418568) and commercial FPV vaccine strains (EU498681, EU498680). This result indicates that TPV transmission and epidemics tend to be consistent with geographical features. All sequences obtained from China, Korea, Japan and India are in a cluster and may share the same ancestral origins. The cause might be the Siberian tiger originated in northeast Asia. All the FPV-like tiger viruses from this study and the GenBank were sampled between 2001 and 2017, such that they represent the current genetic diversity of parvoviruses in tigers. The relationship between isolates and vaccine strains is distant, the captive tigers were vaccinated using a cat vaccine, and the effect will not be ideal. Finally, the FPV-like phylogenetic also contained individual sequences from a virus sampled from a masked palm civet (EU145593) that was closely related to the Korea-isolated strain (EU252146). Because there were only single sequences for some of the vituses, it was difficult to determine whether they simply represent transient spillover infections.

Determining how viruses infect and spread in new host species is central to the study of disease emergence (Holmes, 2009; Parrish et al., 2008). To better determine the natural host range for the CPV- and FPV-relevant viruses, 56 amino acid sequences from wild/captive carnivores were downloaded from GenBank. The phylogeny analysis of the VP2 sequence indicated that the most notable are those viruses sampled from raccoons. A minority of raccoon-derived sequences (3 of 14) fell into the FPV-like group, one of which was sampled more than 50 years ago (from 1967). The majority of raccoon parvoviruses (11 of 14) exist within CPV-like group and occupy diverse positions. In particular, there are seven CPV 2a, one CPV 2b, as well as nine new CPV 2a and two new CPV 2b, that occupy a phylogenetic position in the CPV branch (Fig. 4). That this multihost viral lineage diverged early in the evolutionary history of CPV, yet has persisted to the present day, indicates that it has circulated for an extended time period.

Host specificity was affected by the regions in the capsid structure located around VP2 residues, which can influence viral binding with specific host cell transferrin receptors and thus the specificity of infectivity of host species (Allison et al., 2012; Cotmore and Tattersall, 2007; Hafenstein et al., 2007; Hueffer et al., 2003a; Palermo et al., 2006; Wang et al., 2017). Mutations in the genes controlling these capsid moieties and selective pressures allow the parvoviruses to evolve and infect new hosts (Franzo et al., 2017; Hueffer et al., 2003b). For example, CPV-2a, CPV-2b and CPV-2c strains predominated in isolates from large exotic cats in southeast Asia (Nakamura et al., 2001; Steinel et al., 2001). In addition, CPV-2a, CPV-2b, and CPV-2c strains have been isolated from healthy cats (Mochizuki et al., 1996) and from those with signs of feline panleukopenia (Battilani et al., 2006a, 2006b; Mochizuki et al., 1999; Truyen and Parrish, 1992). CPV-2a has been reported to cause a naturally occurring fatal disease identical to feline panleukopenia in a kitten (Decaro et al., 2010). A new CPV-2c variant was isolated from leopards in southeast Asia (Ikeda et al., 2000, 2002) and from domestic cats worldwide (Battilani et al., 2006b; Decaro et al., 2008a,b). In contrast to CPV strains infecting cats, FPV has limited replication in dogs after experimental inoculation; it does not infect the gut epithelium, and therefore, it is not shed (Url et al., 2003). In this study, compared with the amino acid sequence of VP2 in vaccine strains (original FPVs), the amino acid Ile 101 of VP2 was mutated to Thr (I 101 T) in the isolated strain. The isolated strain possessing the I 101 T substitution in this study was observed for the first time. In summary, tiger-borne feline parvovirus has been found in most provinces and

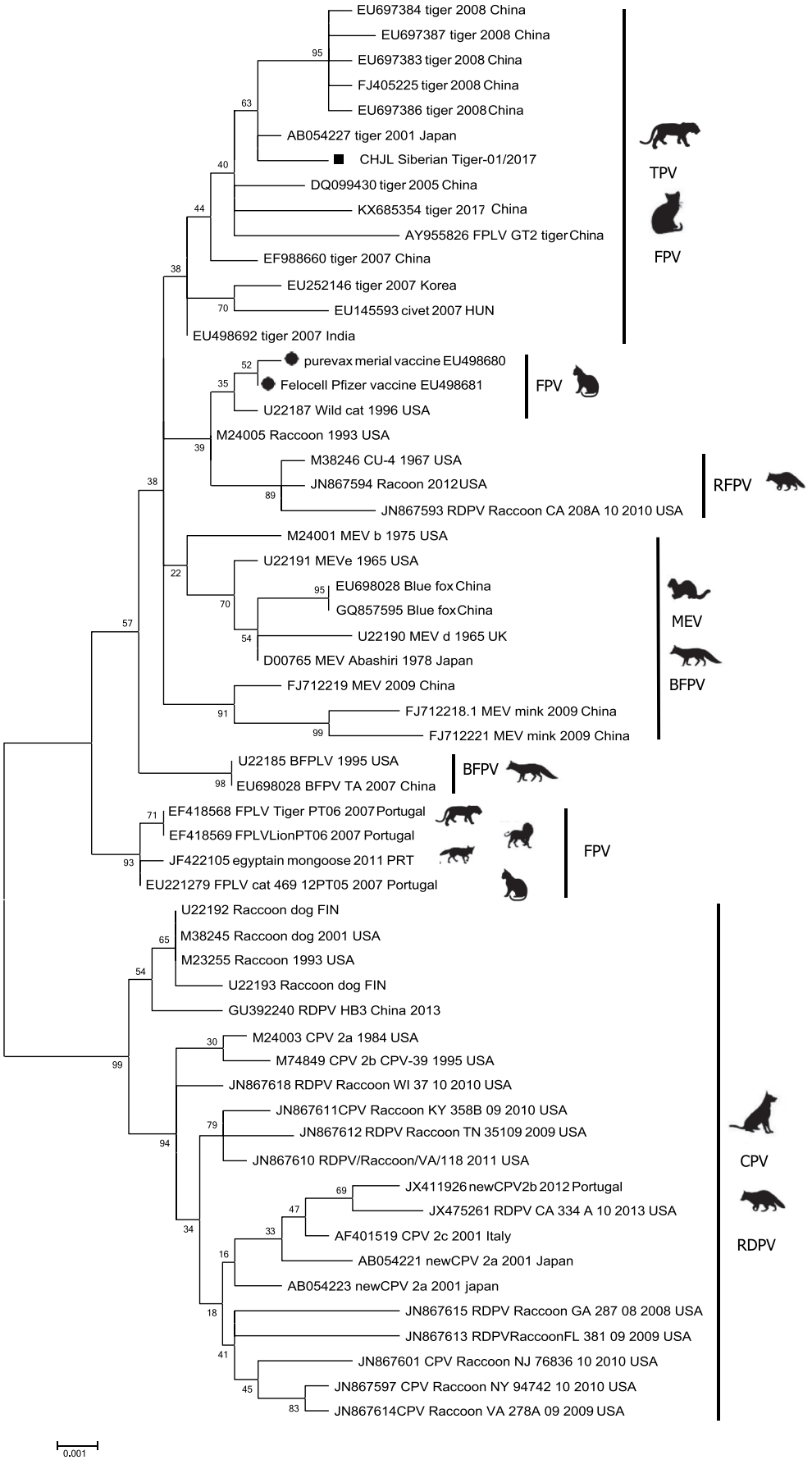

Fig. 4. Phylogenetic analysis based on VP2 gene sequences of different panleukopenia viruses. Note: CHJL-Siberian Tiger-01/2017 from this study is marked with "■", vaccine strains are marked with "●".

Table 1
Amino acid mutations in VP2 protein of FPV and CPV.

GenBank number	Mutation sites: amino acid residue													
	Animal	80	87	93	101	103	297	300	305	323	426	556	564	568	Vitus types
M38246	Cat	K	M	K	I	V	S	A	D	D	N	D	N	A	FPV
EU498680	Cat	K	M	K	I	V	S	A	D	D	N	D	N	A	FPV
EU221279	Wild cat	K	M	K	T	V	S	A	D	D	N	D	N	A	FPV
U22187	Wild cat	K	M	K	T	V	S	A	D	D	N	D	N	A	FPV
EU498681	Wild cat	K	M	K	T	V	S	A	D	D	N	D	N	A	FPV
EU252146	Tiger	K	M	K	**T**	V	S	A	D	D	N	D	N	A	**TPV**
AB054227	Tiger	K	M	K	**T**	V	S	A	D	D	N	D	N	A	**TPV**
EF418568	Tiger	K	M	K	**T**	V	S	A	D	D	N	D	N	A	**TPV**
EF988660	Tiger	K	M	K	**T**	V	S	A	D	D	N	D	N	A	**TPV**
EU498692	Tiger	K	M	K	**T**	V	S	A	D	D	N	D	N	A	**TPV**
EU697384	Tiger	K	M	K	**T**	V	S	A	D	D	N	D	N	A	**TPV**
DQ099430	Tiger	K	M	K	**T**	V	S	A	D	D	N	D	N	A	**TPV**
CHJL	Tiger	K	M	K	**T**	V	S	A	D	D	N	D	N	A	**TPV**
FJ405225	Tiger	K	M	K	**T**	V	S	A	D	D	N	D	N	A	**TPV**
EU697386	Tiger	K	M	K	**T**	V	S	A	D	D	N	D	N	A	**TPV**
EU697387	Tiger	K	M	K	**T**	V	S	A	D	D	N	D	N	A	**TPV**
KX685354	Tiger	K	M	K	**T**	V	S	A	D	D	N	D	N	A	**TPV**
AY955826	Tiger	K	M	K	**T**	V	S	A	D	D	N	D	N	A	**TPV**
EU697383	Tiger	K	M	K	**T**	V	S	A	D	D	N	D	N	A	**TPV**
EU145593	Palm Civet	K	M	K	T	V	S	A	D	D	N	D	S	A	FPV
EF418569	Lion	K	M	K	T	V	S	A	D	D	N	D	N	A	FPV
EU698028	blue fox	K	M	K	I	V	S	P	D	D	N	D	N	A	BFPV
GQ857595	blue fox	K	M	K	I	V	S	P	D	D	N	D	N	A	BFPV
U22185	blue fox	K	M	K	T	V	S	V	D	D	N	D	N	A	BFPV
EU698028	blue fox	K	M	K	T	V	S	V	D	D	N	D	N	A	BFPV
JF422105	Mongoose	K	M	K	T	V	S	V	D	D	N	D	N	A	BFPV
FJ712218	Mink	K	M	K	T	V	S	V	D	D	N	D	N	A	MEV
FJ712219	Mink	K	M	K	T	V	S	V	D	D	N	D	N	A	MEV
U22191	Mink	K	M	K	T	V	S	A	D	D	N	D	N	A	MEV
U22190	Mink	K	M	K	T	V	S	A	D	D	N	D	N	A	MEV
D00765	Mink	K	M	K	T	V	S	A	D	D	N	D	N	A	MEV
M24001	Mink	K	M	K	T	V	S	V	D	D	N	D	N	A	MEV
FJ712221	Mink	K	M	K	T	V	S	V	D	D	N	D	N	A	MEV
U22192	Raccoon dog	R	M	N	I	A	S	A	D	N	N	D	S	G	RDPV
U22193	Raccoon dog	R	M	N	I	A	S	A	D	N	N	D	S	G	RDPV
M38245	Raccoon dog	K	M	N	I	A	S	A	D	N	N	D	S	G	RDPV
GU392240	Raccoon	R	M	N	I	A	S	A	D	N	N	D	S	G	CPV 2a
JN867610	Raccoon	R	M	N	T	A	A	D	D	N	N	D	S	G	CPV 2a
JX475261	Raccoon	R	M	N	T	A	A	D	H	N	D	D	S	G	CPV 2a
M24003	Canine	R	M	N	T	A	S	G	N	D	N	D	S	G	CPV 2a
M74849	Canine	R	M	N	T	A	S	G	N	D	N	D	S	G	CPV 2b
AF401519	Canine	R	M	N	T	A	A	G	N	D	N	D	S	G	CPV 2c
M24005	Raccoon	K	M	K	T	V	S	A	D	D	N	D	N	A	CPV 2a
M23255	Raccoon	R	M	N	I	A	S	A	N	D	N	D	S	G	CPV 2a
JX411926	Raccoon	K	M	N	T	A	A	G	Y	N	D	D	S	G	New CPV 2b
AB054221	Raccoon	R	M	N	T	A	A	G	Y	N	D	D	S	G	New CPV 2b
AB054223	Raccoon	R	M	N	T	A	A	D	Y	N	N	D	S	G	New CPV 2a
JN867594	Raccoon	K	M	K	T	V	S	A	D	D	N	D	N	A	CPV 2a
JN867611	Raccoon	R	M	N	T	A	A	D	D	N	N	D	S	G	New CPV 2a
JN867618	Raccoon	R	M	N	T	A	S	D	D	N	N	D	S	G	CPV 2a
JN867597	Raccoon	R	M	N	T	A	A	D	H	N	N	D	S	G	New CPV 2a
JN867601	Raccoon	R	M	N	T	A	A	D	H	N	N	D	S	G	New CPV 2a
JN867593	Raccoon	K	M	K	T	V	A	A	D	D	N	D	N	A	New CPV 2a
JN867615	Raccoon	R	M	N	T	A	A	D	H	N	N	D	S	G	New CPV 2a
JN867612	Raccoon	R	M	N	T	A	A	D	D	N	N	D	S	G	New CPV 2a
JN867613	Raccoon	R	M	N	T	A	A	D	H	N	N	D	S	G	New CPV 2a
JN867614	Raccoon	R	M	N	T	A	A	D	H	N	N	D	S	G	New CPV 2a

cities in China (Wang et al., 2017). The high incidence of FPV indicates that FPV is a serious threat to the health of tigers. The gene mutation of the isolated strain was found to be composed of sequences from CPV and FPV. Our study not only provides new gene sequences for the global study of FPV-infected tigers, but also helps to further study the incidence and genetic diversity of FPV in China.

Declaration of Competing Interests

The authors of this work do not have any personal or financial conflicts or biases that can inappropriately influence the contents of the document.

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