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FATAL INFECTION WITH FELINE PANLEUKOPENIA VIRUS IN TWO CAPTIVE WILD CARNIVORES (*PANTHERA TIGRIS* AND *PANTHERA LEO*)

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Abstract: Two cases of fatal infection caused by parvovirus in a white tiger (*Panthera tigris*) and an African lion (*Panthera leo*) at the Lisbon Zoo (Portugal) are described. Gross findings at necropsy were catharral enteritis in the tiger and severe hemorrhagic enteritis in the lion. Histopathologic examination revealed, in both animals, intestinal crypt necrosis and lymphocyte depletion in the germinal centers of the mesenteric lymph nodes. Bacteriologic examination was negative for common bacterial pathogens, including *Salmonella*. Amplification of the parvovirus VP2 complete gene was achieved in both cases and sequencing analysis identified these isolates as feline panleukopenia virus (FPLV). The nucleotide sequences obtained from these two viruses were genetically indistinguishable. The phylogenetic analysis of FPLV strains from domestic cats obtained in the Lisbon area revealed the circulation of FPLV strains highly similar to those isolated from the tiger and lion, which strongly suggests that stray cats may have been the source of infection.

Key words: feline panleukopenia virus, feline parvovirus, lion, *Panthera leo*, tiger, *Panthera tigris*, phylogeny, gene, VP2.

BRIEF COMUNICATION

Feline panleukopenia virus (FPLV) infections in a variety of wild Felidae species, including tigers (*Panthera tigris*) and lions (*Panthera leo*), have been described based on clinical, serologic, virologic, or molecular data.[2,4,8,9,10,11,12] FPLV belongs to the feline parvovirus subgroup within the *Parvovirus* genus along with mink enteritis virus (MEV), blue fox parvovirus (BFPV), raccoon parvovirus (RPV), raccoon dog parvovirus (RDPV), and canine parvovirus type 2 (CPV-2).

Parvoviruses are small (22–26 nm), nonenveloped, single-stranded DNA viruses with icosahedral symmetry. Their genome encodes for two capside (VP1 and VP2) and two nonstructural (NS1 and NS2) proteins.[3] VP2 is a well-characterized gene that has been extensively used for phylogenetic analysis, because it encodes the major protein that determines host range, viral pathogenicity, and immune response.[10]

In young wild and domestic carnivores, the infection usually causes severe gastroenteritis, which is most frequently hemorrhagic. Because of their susceptibility, vaccination of captive carnivores against FPLV is a common practice in the Lisbon Zoo and worldwide.

On 12 May 2006, a 10-mo-old female white tiger was imported from the Zooparc de Beauval (Saint Aignan sur Cher, France), where vaccination against this pathogen was not provided. Blood chemistry values were within normal range. Serologic data concerning the exposure to parvovirus or other feline viral pathogens was not available. In addition, no clinical cases of parvovirus infection were ever reported in this center.

However, 41 days after the arrival at the Lisbon Zoo, the tiger ceased to eat, and 4 days later developed acute, watery diarrhea, dyspnea, ataxia, hypothermia, and pronounced leukopenia. This animal failed to respond to antibiotic and supportive therapy and died less than 24 hr after the onset of symptoms. Sharing the enclosure of the female tiger was a resident male of the same species and age that did not develop clinical signs.

Two weeks after the death of the female tiger, an 11-mo-old male African lion that had been in a neighboring cage of the tiger's enclosure was found dead without exhibiting previous signs of

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Figure 1. Intestinal lesions in the tiger showing catharral enteritis with infiltration of lamina propria by lymphocytes and eosinocytes.

disease. This animal, along with six other cubs also born in 2005, had been vaccinated against FPLV (Fevaxyn, Pentofel, Fort Dodge Animal Health, Southampton SO30 4QH, United Kingdom), about 8 mo before.

Necropsy of the tiger revealed diffuse fibrinocatarrhal enteritis with dilatation of the intestine affecting primarily the ileum. The intestinal lumen contained a dense pinkish liquid with abundant epithelial debris and fibrin. Necropsy of the lion revealed severe hemorrhagic enteritis with marked turgidity of the wall of the ileum. The lumen was filled with blood. Enlarged mesenteric lymph nodes were found in both animals.

The histopathologic examination of the tiger's ileum revealed necrosis of the crypt epithelium and diffuse lymphocyte infiltration along with some eosinophils present (Fig. 1). More pronounced lesions were found in the lion, which were compatible with an acute course of infection. Extensive necrosis of the crypts as well as of the villi was observed (Fig. 2). Lymphocyte depletion of Peyer's patch follicles and multifocal hemorrhagic lesions in the mucosa were also present. Mesenteric lymph nodes of both animals revealed lymphoid depletion of the germinal centers of the follicles.

Pathogenic bacteria, including *Salmonella*, were not isolated from the clinical samples of the two animals and suggest that bacterial infection was not the primary cause of death. The presence of parvovirus, rotavirus, coronavirus, and canine distemper virus in fecal, intestinal, and blood samples of these two carnivores was evaluated by polymerase chain reaction

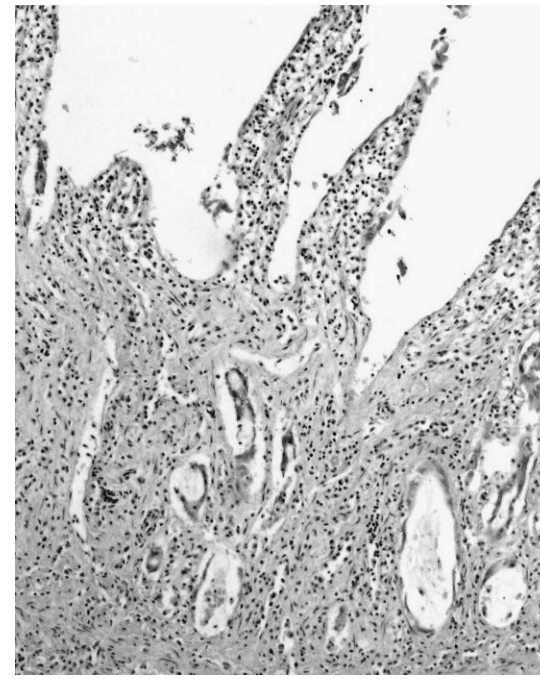

Figure 2. Intestinal lesions in the lion showing necrosis of the villi and ileum glands. Note the diffuse infiltration of lamina propria by lymphocytes.

(PCR), reverse transcription (RT)-PCR, and sequencing methods currently used in the authors' laboratory, and by inoculation of cell cultures (virus isolation). Only parvovirus was detected by PCR. Cytopathogenic effect was observed in Crandell feline kidney (CRFK) cells, 48 hr after inoculation with filtered homogenates of fecal and intestinal samples. The presence of parvovirus in the supernatant of the cell cultures was confirmed by electron transmission microscopy, PCR, and sequencing.

The histopathologic findings, together with the marked leukopenia, virus isolations, PCR results, and sequencing analysis, suggest that parvovirus was the cause of the death in these animals. Moreover, the low parvovirus antibody titer (<20) in the blood sample collected during the onset of symptoms determined by a hemagglutination inhibition (HI) test[1] indicates that the tiger was most probably undergoing an acute parvovirus infection, because there was an absence of seroconversion. The tiger also was tested for the presence of feline leukemia virus (FeLV) p27 antigen (Viracheck FeLV, Synbiotics Corporation, San Diego, California 92127, USA) and feline immunodeficiency virus-specific antibodies via a Western blot with negative results. These

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Table 1. Primer's sequences and size of the amplicon.

Primer[a]	Nucleotide sequence (5′-3′)	Amplicon size (bp)
1F	ACCAGATCATCCATCAACATC	
1R	CAATTAGTTGCCAATCTCCTG	521
2F	AAATTGTAACACCTTGGTCATTG	
2R	AAATGGTGGTAAGCCCAATG	560
3F	ACAGGTGATGAATTTGCTACAG	
3R	TTACAGGAAGGTTAAAGTTAAT	505
4F	CAACAGGAGAAACACCTGAG	
4R	TCTTCTATTTCTTACAGTTATTG	787

[a] F, forward; R, reverse.

results did not suggest that immune depression was caused by the infection with one or both of these two pathogens.

To analyze the complete VP2 gene, total DNA was extracted from biologic samples, and viral DNA was obtained from clarified and filtered supernatants of CRFK-infected cultures, in a BioSprint 96 nucleic acid extractor (QIAGEN GmbH, 40724 Hilden, Germany), accordingly with the manufacturer's recommendation. Amplification was conducted with primers designed from conserved regions (Table 1) by using the High Fidelity PCR Master Mix (Roche Diagnostics GmbH, 68305 Mannheim, Germany), by performing 40 cycles consisting of denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, and extension at 72°C for 30 sec, followed by a final extension step of 10 min at 72°C. The resulting overlapping amplicons were cloned into pCR2.1 vector (Invitrogen, Carlsbad, California 92008, USA) and sequenced using a 3130 Genetic Analyser (Applied Biosystems, Foster City, California 94404, USA). Amplification and sequencing analysis generated the same data obtained directly from tissue and fecal samples.

Because stray cats can easily enter the zoo premises and therefore be a possible source of parvovirus infection, the VP2 gene nucleotide sequences of four FPLV isolates obtained from domestic cats of the Lisbon area during 2005 and 2006 also were analyzed, by using the same PCR system. The nucleotide sequences of the VP2 gene of the isolates studied were submitted to GenBank under the accession numbers EF418568 (FPLV/Tiger/PT06), EF418569 (FPLV/Lion/PT06), EU221278 (FPLV/cat/52171/PT05), EU221279 (FPLV/cat/46912/PT05), EU221280 (FPLV/cat/39897/PT06), and EU221281 (FPLV/cat/31609/PT06). Multiple alignments of nucleotide sequences were generated by CLUSTALW program, version 1.6, and phylogenetic analysis was carried out by maximum likelihood analysis with the TREE-PUZZLE program, version 5.1, by using a quartet-puzzling algorithm to generate the tree. The analysis was run with Hasegawa–Kishino–Yano (HKY-85) model of substitution[6] and quartet-puzzling support values based on 1,000 puzzling steps were calculated. Several VP2 nucleotide sequences obtained from wild carnivores (leopards, tiger, and cheetah) that are available in GenBank also were included as well as other members of the feline parvovirus group, namely, CPV-2, CPV-2a, CPV-2b, MEV, RPV, RDPV, and BFPV.

The phylogenetic analysis showed that FPLV/Tiger/PT06 and FPLV/Lion/PT06 isolates grouped together along with three other strains obtained from Portuguese domestic cats in an independent branch within the FPLV cluster apart from the CPV-2 group (Fig. 3). The fourth Portuguese cat strain (FPLV/cat/31609/PT06) was found the most divergent among the domestic strains analyzed, grouping closer to the Chinese tiger isolate GT-2.

The comparison of the FPLV/Tiger/PT06 and FPLV/Lion/PT06 VP2 gene with those from domestic cats demonstrated a low level of variability that ranged from one (FPLV/cat/52171/PT05 and FPLV/cat/46912/PT05) to 10 (FPLV/cat/31609/PT06) silenced nucleotide substitutions. All the Portuguese cat FPLV isolates analyzed so far, including some collected during 2007 (Fevereiro, unpubl. data) differ from the two wild isolates in nucleotide 871, the first in codon 291. The majority of the FPLV isolates currently available in the database as well as the Portuguese isolates from domestic cats use a CTA for Leu-291, whereas the lion and tiger isolates and some other FPLV isolates from Asia (South Korea [EU252147, EU252146], China [DQ003301, strain GT-2]), and Japan [AB000070, AB000068, and AB000064]) and Europe (Germany (AY742937) use a TTA for Leu-291. More data on VP2 sequences obtained

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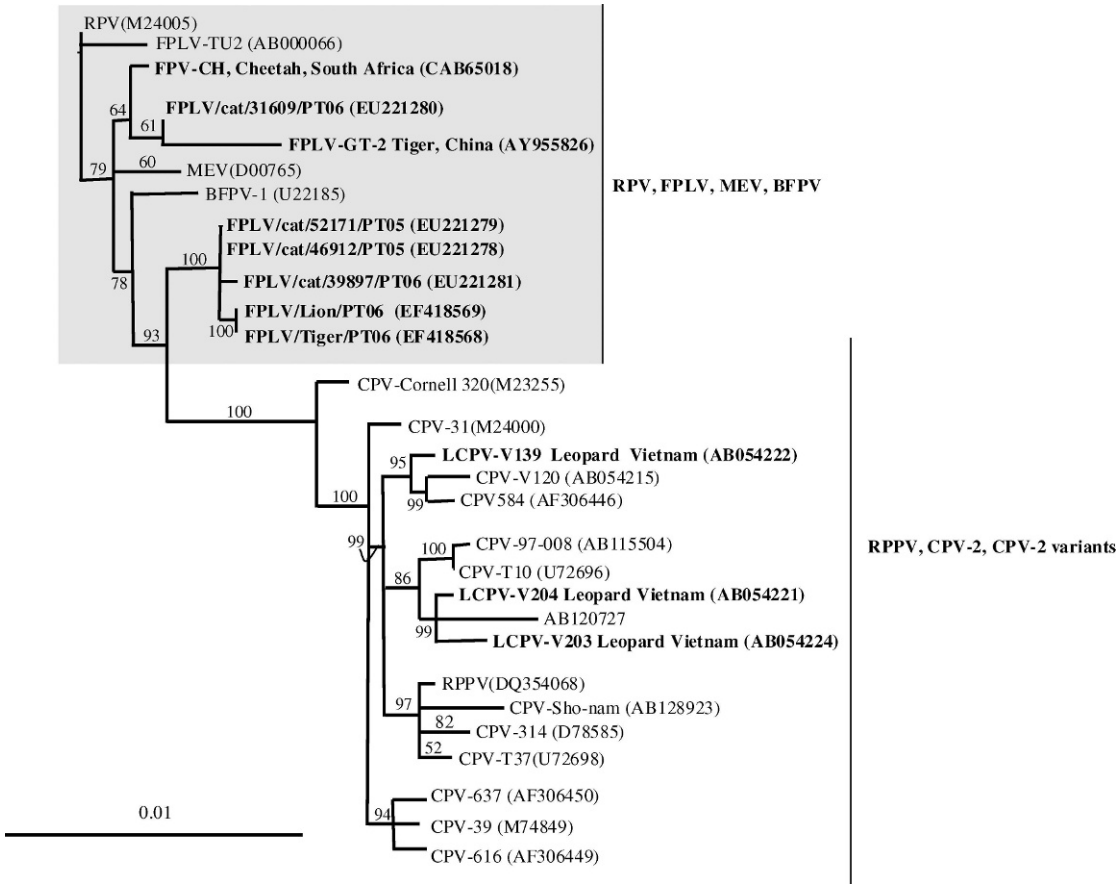

Figure 3. Phylogenetic analysis based on the VP2 gene nucleotide sequence of different parvovirus. Maximun likelihood tree performed by PUZZLE, version 5.1, with the HKY-85 model generated by using a transition/transversion of 3.84, a nucleotide frequency of A = 0.352; C = 0.157; G = 0.197; T = 0.294. Log likelihood = −3103.90 branch lengths represent genetic distances between sequences. The branch values represent the support based on quartet-puzzling steps of 1,000 replicates. Isolates from wild carnivores are in bold. GenBank accession numbers are shown in brackets.

from domestic and stray cats in the Lisbon area are needed to ascertain whether the two codons are used by the FPLV circulating in Portugal. Whether this nucleotide variation can be used as a molecular marker has yet to be determined.

The analysis of the deduced VP2 amino acid sequences demonstrated that the two wild carnivore isolates encode all the critical amino acids that define the FPLV group (Lys-80, Met-87, Lys-93, Ile-101, Val-103, Ser-297, Ala-300, Asp-305, Asp-23, Asn-426, Val-555, Asn-564, and Ala-568) (Table 2).

The 100% identity between the VP2 nucleotide sequences of the tiger and lion isolates strongly suggests that both viruses share the same origin. Although the young lion did not have direct contact with the sick tiger, the water used to clean the area where this tiger was accommodated may have contaminated the lion's contiguous premises by carrying the infection from the tiger to the lion. Two distinct viral infections between the putative stray cat and the tiger and lion also could have occurred.

The small amount and the poor quality of the lion blood sample collected at necropsy hampered the evaluation of the animal immune status to parvovirus by the titration of HI antibodies. Of the seven putatively exposed lions, only this one lion died.

The white tigers had been temporarily accommodated in facilities occupied previously by bears (*Ursus thibetanus* and *Ursus arctus*). Antibodies against parvovirus were described in Florida black bears (*Ursus americanus*), and Marsican

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Table 2. Amino acid residues at key positions in the VP2 protein of Parvovirus from wild carnivore species.

Virus	VP2 amino acids													Species	Source[a]
	80	87	93	103	232	297	300	305	323	426	555	564	568		
CPV-2b	—[b]	—	—	—	—	—	Gly	Tyr	Asp	Asp	—	Ser	Gly	*Acinonyx jubatus*	(a)
CPV-2b V204	Arg	Leu	Asn	Ala	Ile	Ala	Gly	Tyr	Asn	Asp	Val	Ser	Gly	*Leo pardus*	(b)
CPV-2a	—	—	—	—	—	—	Gly	Tyr	Asp	Asn	—	Ser	Gly	*Panthera tigris altaica*	(c)
CPV-2c(a) V139[c]	Arg	Leu	Asn	Ala	Ile	Ala	Asp	Tyr	Asn	Asn	Val	Ser	Gly	*L. pardus*	(d)
CPV-2c(b) V203[c]	Arg	Leu	Asn	Ala	Ile	Ala	Asp	Tyr	Asn	Asp	Val	Ser	Gly	*L. pardus*	(e)
FPV	Lys	Met	Lys	Val	—	—	Ala	Asp	Asn	Asn	—	Asn	Ala	*Felis sylvestris*	(f)
FPV	—	—	—	—	—	—	—	—	—	Asn	—	Asn	Ala	*A. jubatus*	(g)
FPLV GT-2	Lys	Met	Lys	Val	Ile	Ser	Ala	Asp	Asp	Asn	Val	Asn	Ala	*P. tigris*	(h)
FPLV-CH1	Lys	Met	Lys	Val	Val	Ser	Ala	Asp	Asp	Asn	Val	Asn	Ala	*A. jubatus*	(i)
FPV	Lys	Met	Lys	Val	Val	Ser	Ala	Asp	Asp	Asn	Val	Asn	Ala	*Panthera uncia*	(j)
FPLV/Tiger/PT06	Lys	Met	Lys	Val	Val	Ser	Ala	Asp	Asp	Asn	Val	Asn	Ala	*P. tigris*	(k)
FPLV/Lion/PT06	Lys	Met	Lys	Val	Val	Ser	Ala	Asp	Asp	Asn	Val	Asn	Ala	*Panthera leo*	(l)

[a] Source of the amino acid residues from scientific publications (a, c, f, and g) and GenBank accession numbers of the sequences (b, d, e, h, i, and j). (a) Steinel et al. (2000); (b) AB054221; (c) Steinel et al. (2000); (d) AB054222; (e) AB054224; (f) Steinel et al. (2000); (g) Steinel et al. (2000); (h) AY955826; (i) CAB65018; (j) BAE96024; (k) EF418568, present study; (l) EF418569, present study.

[b] —, data not available.

[c] According to the classification based on residue 426, LCPV139 and LCPV203 belong, respectively, to the CPV-2a and CPV-2b subtypes.

brown bears (*Ursus arctos marsacanus*).[5,7] To investigate whether a subclinical parvovirus infection of these bears could have been the origin of infection, feces were collected and tested for the presence of parvovirus. However, no specific amplification was obtained from any of the bear samples, and virus particles were not observed by transmission electron microscopy.

In addition, blood and fecal samples from the asymptomatic male white tiger, collected when the female became sick, tested negative for parvovirus DNA by PCR. Several fecal samples, collected for successive months after the outbreak, also tested negative, suggesting that this animal was neither infected nor the source of infection of the cohabitant female. The VP2 sequence analysis suggests that infected stray cats that are known to access the zoo premises could have been the source of the virus.

In domestic cats and dogs, parvovirus infection has a very brief incubation period, usually shorter than 1 wk. However, little is known about the duration of this period in wild carnivores. The possibility that the female tiger had been infected before being accommodated in the Lisbon Zoo is unlikely, given the absence of anti-parvovirus antibodies determined by the serologic examination.

Clinical and serologic reports have showed for decades that large Felidae are susceptible to this virus;[10] however, the number of strains from wild species that have been characterized by molecular methods is very limited as can be observed by the scarce complete VP2 sequences available in GenBank. Therefore, the sequences reported here contribute to enrich the international databases of FPLV from wild carnivores and constitute the first laboratory diagnosis of FPLV of large Felidae in Portugal.

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