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Isolation of Canine Parvovirus from a Cat Manifesting Clinical Signs of Feline Panleukopenia

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Twenty-seven feline parvovirus (FPV) isolates were recovered from cats clinically diagnosed with feline panleukopenia (FPL) for assessing antigenic and genomic properties of FPL viruses (FPLV) recently prevalent among cats in Japan. All isolates, with the exception of one novel isolate, FPV-314, possessed homologous properties, and their subgroups in FPVs were identified as FPLV. The FPV-314 isolate, which was from a 1.5-year-old cat which manifested clinical signs of FPL and died on the 13th day after the first medical examination, was finally identified as canine parvovirus (CPV) because it lacked a specific antigenic epitope commonly detected in FPLV and mink enteritis virus and because the nucleotide sequence of the capsid protein gene was almost identical to those of CPV-2a and -2b antigenic type strains recently prevalent among dogs in Japan. The present result together with our previous findings (M. Mochizuki, R. Harasawa, and H. Nakatani. Vet. Microbiol. 38:1–10, 1993) indicates the possibility that CPV and FPLV undergo mutual interspecies transmission between dogs and cats, and it is postulated that they may cause disease in some adventitious hosts.

Feline panleukopenia (FPL) is an acute viral infection of domestic as well as wild felids and is characterized by acute depression, gastroenteric symptoms such as diarrhea and vomiting, and lymphopenia, with a high mortality among nonimmune kittens (1, 15). The causative agent, FPL virus (FPLV), is a host-range variant of the feline parvovirus (FPV) subgroup consisting of canine parvovirus (CPV), mink enteritis virus (MEV), and racoon parvovirus (2). These are at least 98% homologous in DNA sequence (9, 16, 20), and their antigenic properties are almost identical. The classification of FPVs, therefore, has traditionally been based on the hosts from which they were isolated. That is, FPV strains isolated from cats are classified as FPLV, and those from dogs are classified as CPV. However, it has been shown that some VP2 amino acids are responsible for the specificity of the host range as well as antigenic epitopes of CPVs (3, 5, 14, 16, 23) hence subgrouping of FPVs is possible.

Previously, we detected three CPV-like viruses from healthy cats and one FPLV-like virus from a dog clinically diagnosed with CPV infection and pointed out the possibility that FPVs have been mutually transmitted between cats and dogs in nature (11). In this study, we isolated 27 FPVs from cats clinically diagnosed with FPL and analyzed them for their antigenic and genomic properties by hemagglutination inhibition with monoclonal antibodies (MAbs) and by PCR based restriction fragment length polymorphism (RFLP) (PCR-RFLP) assays, respectively. The nucleotide sequence of the capsid protein gene of one novel isolate was compared with those of the reference FPV strains.

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MATERIALS AND METHODS

Viruses and cell cultures. The following previously characterized viruses were used as references (5, 7, 8, 11, 12, 21): strains Cp49, 29F, KS5701, CPK-12, Y1, and Ob1 of CPV; strains TU3, TU5, TU7, and TU10 of FPLV; and strain Abashiri of MEV. The antigenic types of strains Cp49 and 29F, strains KS5701 and CPK-12, and strain Y1 are CPV-2, CPV-2a, and CPV-2b, respectively (12, 22), and strain Ob1 is either CPV-2a or CPV-2b.

Crandell feline kidney (ATCC CCL94) cells were cultured and used for cultivation of the reference viruses and for isolation of viruses from the swab specimens by methods that have been described previously (8, 12).

Field isolates examined. From November 1993 to May 1994, 27 FPV isolates were obtained from rectal swabs of 34 cats clinically diagnosed with FPL based on the following clinical signs: fever, depression, anorexia, diarrhea, and/or vomiting, and leukopenia (<3,000 cells per μl). These cats were presented at animal hospitals located in various areas of Japan. For virus isolation and PCR assays, the swab extraction was done by methods described previously (13), and as a result, 30 cases were diagnosed as FPV positive by the PCR assay, with FPV being recovered from 27 of them (10). These samples were passaged several times in the cell culture before examination.

Serology. Antigenic analysis was performed by hemagglutination inhibition assay with MAbs 2D9 and 4G1 (11, 12) and MAb P2-215 (6) as described previously (12). MAbs 2D9 and 4G1 discriminate between antigenic types CPV-2 and CPV-2a. MAb P2-215 recognizes an FPLV- and MEV (FPLV/MEV)-specific conformational epitope involving amino acid (aa) 93 of VP2 (6).

PCR. The primers used in this study are listed in Table 1. A set of primers, FMF and FMR, was first used in the present study for distinguishing FPLV/MEV from CPV. As shown in Fig. 1, they were designed according to the nucleotide differences between FPLV/MEV and CPV detected at nucleotides (nt) 3132 and 3791 (5). A fragment of 698 bp from nt 3113 to 3810 was amplified from FPLV/MEV but not from CPV.

The conditions for the PCR assays have been described previously (5, 7, 13). Briefly, the sample was diluted 1:100 with sterile distilled water and applied in the PCR. Amplification was performed with 30 cycles of denaturation at 94°C for 30 s, primer annealing at 55°C for 2 min, and extention at 72°C for 2 min with a recombinant *Taq* DNA polymerase (Takara Shuzo Co., Ltd., Shiga, Japan).

PCR-RFLP analysis of capsid protein genes. The capsid protein VP1/VP2 gene, 2246 bp from nt 2285 to 4530 (9, 20), was amplified with a set of primers, VPF and VPR, and was digested with *Afa*I and *Hin*cII.

The genetic nature of the FPV-314 isolate was analyzed more specifically by the PCR-RFLP, because six amino acid residues within the VP2 have been identified as CPV specific (conversely, FPLV/MEV specific) to date (16). Of the six coding differences, two amino acid differences at aa 93 and aa 103 in the VP2 between CPV and FPLV or MEV were monitored by PCR-RFLP analysis with *Hin*cII and *Rsa*I. The PCR product of primers 1 and 52 was digested with the enzymes for examining aa 93 and aa 103.

TABLE 1. Primers used in PCR

Primer	Sequence	Binding site
VPF	5′-ATGGCACCTCCGGCAAAGA-3′	nt 2285–2303
VPR	5′-TTTCTAGGTGCTAGTTGAG-3′	nt 4512–4530
FMF	5′-GCTTTAGATGATACTCATGT-3′	nt 3113–3132
FMR	5′-GTAGCTTCAGTAATATAGTC-3′	nt 3791–3810
1	5′-GTACATTTAAATATGCCAGA-3′	nt 3029–3408
5	5′-AGCTATGAGATCTGAGACA-3′	nt 3388–3406
8	5′-AATACAAACTATATTACTGAAG-3′	nt 3785–3806
9	5′-TCCTGCTGGATATCTTCCT-3′	nt 4042–4060
10	5′-TGTCATCTAAAGCCATGT-3′	nt 3108–3125
22	5′-TGTCAAAATAATTGTCCTG-3′	nt 4292–4310
23	5′-CTTTCCTCCAAAAATCTGA-3′	nt 4397–4415
41	5′-ATTGTATACCATATAACAAACC-3′	nt 4738–4759
51	5′-CCAACTAAAAGAAGTAAACC-3′	nt 2726–2745
52	5′-ATTAATGTTCTATCCCATTG-3′	nt 3461–3480

TABLE 2. Reactivities of MAbs against reference FPV strains and a novel FPV-314 isolate by hemagglutination inhibition assay

Virus strain	MAb titer against FPLVs		
	2D9	4G1	P2-215
Reference strains			
CPV Cp49	256	32	<100
CPV 29F	256	32	<100
CPV KS5701	64	2	<100
CPV CPK-12	64	2	<100
FPLV TU3	1,024	128	3,200
FPLV TU5	1,024	64	3,200
FPLV TU7	1,024	64	3,200
MEV Abashiri	1,024	128	3,200
Novel FPV isolate FPV-314	256	<2	<100

The resulting restricted fragments were resolved by 1.8 or 2% agarose gel electrophoresis, and the bands were visualized after staining with ethidium bromide.

DNA sequencing. The nucleotide sequence of the VP2 gene (nt 2825 to 4579) of isolate FPV-314 was determined by direct sequencing of PCR products as described previously (5), with a slight modification. To obtain the templates for DNA sequencing, PCR was directly performed with a sample prepared from a rectal swab from the cat. The region from nt 2726 to 4760 was amplified by PCR in five sections, with five sets of primers: primers 10 and 51, primers 1 and 52, primers 5 and 9, primers 8 and 23, and primers 22 and 41. The sequence was determined either with Sequenase (U.S. Biochemical, Cleveland, Ohio) by using [^{32}P]dCTP and single-stranded DNA generated from the PCR product as a template or by using a *Taq* cycle sequencing kit and an ABI-373A automated DNA sequencer (Applied Biosystems, Foster City, Calif.). The sequences were analyzed with GENETYX-MAC/CD software (Software Development Co., Ltd., Tokyo, Japan). The nucleotide numbering followed the nucleotide sequence of the MEV Abashiri strain (7).

GenBank accession numbers. The nucleotide sequence of the VP2 gene of isolate FPV-314 has been assigned DDBJ accession no. D78585. Those of MEV Abashiri strain (D00765) and FPLV TU10 strain (D78584) refer to the studies by Kariatsumari et al. (7) and Horiuchi et al. (6), respectively, and those of CPVs Y1 (D26079), Ob1(D26080), and Cp49(D26081) refer to the studies by Horiuchi et al. (5).

RESULTS

Antigenic characteristics of field isolates. All isolates except one, FPV-314, reacted with MAbs 2D9 and 4G1, showing levels of hemagglutination inhibition antibody titer similar to those of the reference FPLV strains (data not shown). They also reacted with MAb P2-215, indicating that they possess the FPLV/MEV-specific common epitope. On the other hand, isolate FPV-314 did not react with MAbs 4G1 and P2-215, differing from the other 26 isolates, but its reactivity was similar to those of the reference CPV strains (Table 2), indicating the possibility that it was not FPLV.

Genomic characteristics of field isolates. The results of PCR-RFLP analysis of the VP1/VP2 genes of the 27 isolates are shown in Fig. 2. When the results obtained by *Afa*I digestion were compared, all isolates except the FPV-314 isolate were very similar to each other as well as to the reference FPLV and MEV strains (Fig. 2A). The FPV-314 isolate resembled the reference CPV-2a strain rather than CPV-2, FPLV, or MEV strains.

With *Hin*cII digestion, (i) the FPV-314 isolate again differed from other FPVs including the reference strains and (ii) a clearly visible RFLP pattern was not obtained for three other isolates, FPV-273, -274, and -275 (Fig. 2B). The reason for the latter finding was not clear, but the RFLP pattern of the FPV-314 isolate suggested that one cleavage site at nt 3098 in the VP2 gene (9, 20) had disappeared.

The isolates were then examined to see whether they possess an FPLV/MEV-specific region in the VP2 gene. The fragment of 698 bp from nt 3113 to 3810 was amplified in all isolates except FPV-314 (Fig. 3), indicating that the FPV-314 isolate is not FPLV.

Molecular properties of FPV-314. The PCR-RFLP analysis revealed that the FPV-314 isolate has a CPV-like genetic nature. A PCR product (451 bp) between nt 3029 and 3480 of

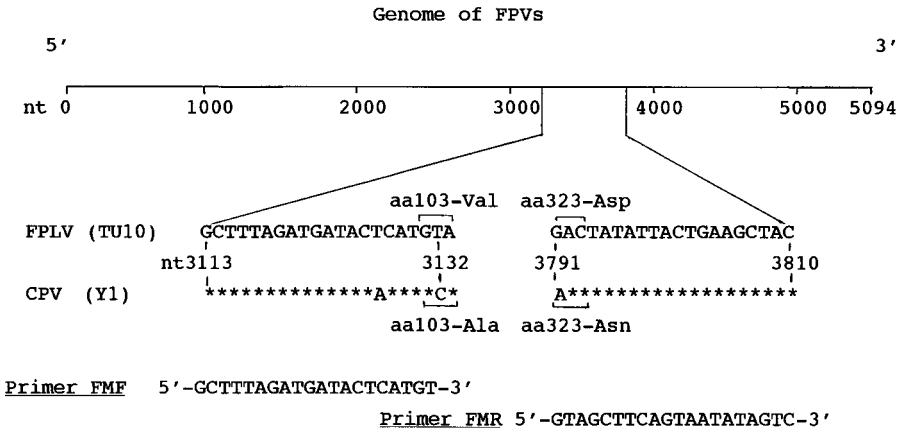

FIG. 1. Primers FMF and FMR for distinguishing FPLV/MEV from CPV.

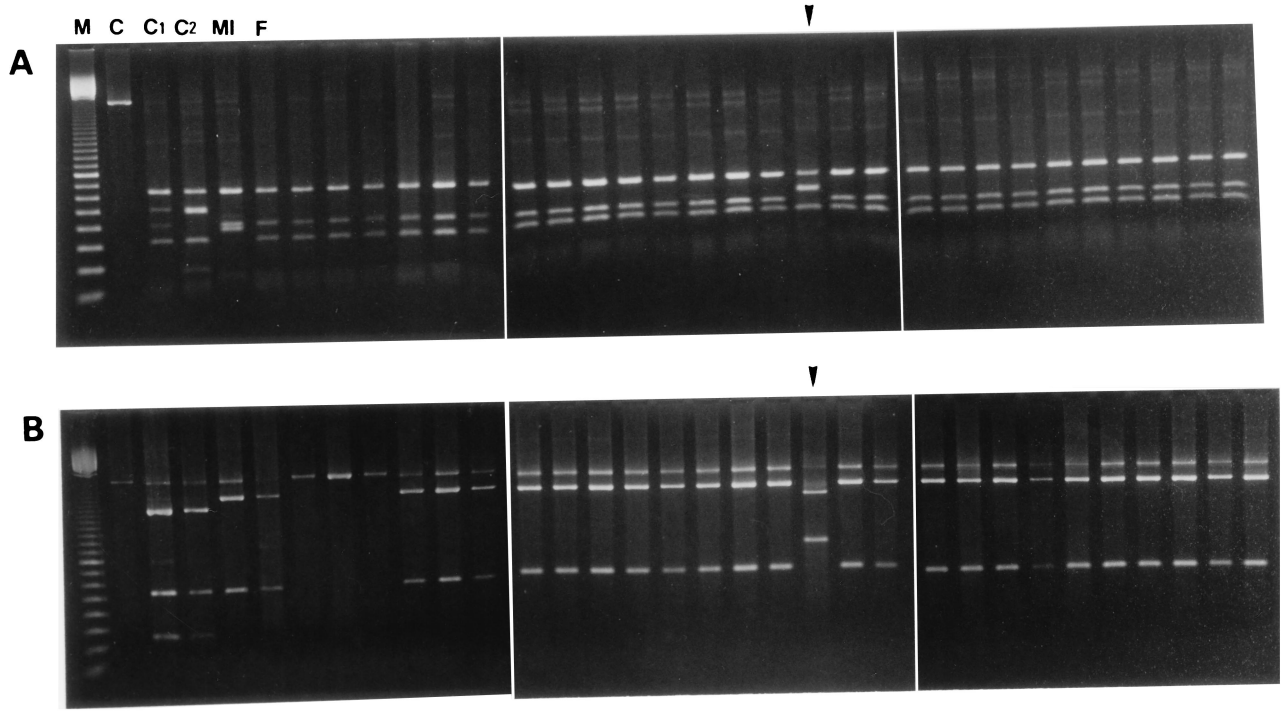

FIG. 2. PCR-RFLP of VP1/VP2 genes of 27 FPV isolates by *Afa*I (A) and *Hin*cII (B) digestion. Lanes: M, 100-bp ladder size markers; C, undigested control VP1/VP2 gene of FPLV TU5 strain; C1, CPV 29F strain (CPV-2 type); C2, CPV CPK-12 strain (CPV-2a type); MI, MEV Abashiri strain; F, FPLV TU5 strain. The remaining lanes are FPV field isolates. Arrowheads indicate the FPV-314 isolate.

FPV-314 was cleaved into two fragments (69 and 382 bp) by *Hin*cII, but not by *Rsa*I, in the same way as those of the reference CPV strain (Fig. 4). This pattern implies that amino acid residues at aa 93 and aa 103 of isolate FPV-314 are asparagine and adenine, respectively, in agreement with the known properties specific for CPVs.

Amino acid variations between FPV-314 and several reference FPV strains based on the DNA sequencing results are summarized in Table 3. With regard to the CPV-specific versus FPLV/MEV-specific amino acid residues, FPV-314 possessed all six CPV-specific changes as indicated by underlining (aa 80, 93, 103, 323, 564, and 568). FPV-314 aa 87, 300, and 305 are found to be CPV-2a- and CPV-2b-specific amino acids. Furthermore, aa 297 of FPV-314 is identical to that of the Ob1 strain, which is a recent CPV isolate in Japan. These genomic properties of FPV-314 are sufficient to consider it CPV.

Clinical features of the cat from which isolate FPV-314 was recovered. The patient was a 1.5-year-old female domestic cat and was presented with complaints typical of FPL, such as vomiting, anorexia, and bloody diarrhea. Leukopenia (about 3,000 cells per μl) was also detected during the first medical examination. She was intravenously given 9 MU of interferon (Intercat; Toray Industries, Inc., Tokyo, Japan) for 3 consecutive days from the second day of hospitalization, and the leukocyte count recovered to 6,700 cells per μl on the sixth day. She was hospitalized in a routinely disinfected cage which had been used for quarantine and for dogs infected with CPV in the hospital. However, the clinical conditions did not improve significantly, and the leukocyte count again decreased after the eighth day, to about 300 cells per μl when she died on the 13th day despite intensive care with antibiotics, hematopoietic growth factor (granulocyte colony-stimulating factor), and fluid therapies. Packed cell volume also decreased gradually. Neither FPV in the feces nor feline leukemia virus in the blood was detected on the second day, but FPV was detected by PCR and was isolated from a cell culture of a swab specimen taken during the second examination on the eighth day. The anti-feline immunodeficiency virus antibody was negative by enzyme-linked immunosorbent assay.

According to the postmortem examination by the practitioner, the prominent pathological findings were hemorrhage at the mucous membrane of the colon and an anemic appearance

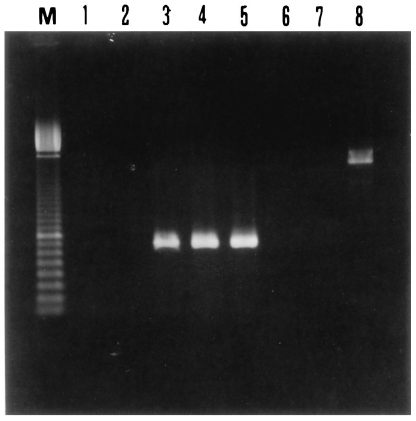

FIG. 3. Detection of FPLV/MEV-specific region (a fragment of 698 bp) in VP2 gene. Lanes: M, 100-bp ladder size markers; 1 to 8, CPV 29F strain, CPV KS5701 strain, FPLV TU5 strain, FPLV TU7 strain, MEV Abashiri strain, the fluid of the 2nd passage of Crandell feline kidney cell culture infected with FPV-314 isolate, the supernatant of FPV-314 swab sample, a fragment of 2246 bp of the VP1/VP2 gene amplified by using a set of primers (VPF and VPR) from the same swab sample run in the lane 7, respectively.

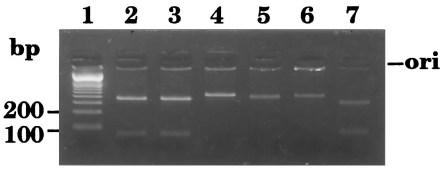

FIG. 4. PCR-RFLP of VP2 gene of FPV-314 isolate. A PCR product (451 bp) between nt 3029 and 3480 was obtained with primers 1 and 52 and digested with each enzyme. Lanes: 1, 100-bp ladder size markers; 2 and 5, *Hin*cII and *Rsa*I digests of PCR product of FPV-314 isolate, respectively; 3 and 6, *Hin*cII and *Rsa*I digests of PCR product of CPV Y1 strain, respectively; 4 and 7, *Hin*cII and *Rsa*I digests of PCR product of MEV Abashiri strain, respectively. ori, original PCR product.

of abdominal organs. Microscopic changes were observed in the liver, spleen, kidneys, and gut. In the liver, Kupffer cells were activated and Glisson's capsule and sinusoid were infiltered by lymphocytes and plasma cells. Atrophy of splenic lymph follicles was noted. The kidneys were diagnosed with slight interstitial nephritis. Part of the small intestinal villi showed petechial hemorrhages in the lamina propria mucosae, accompanied by loss of mucosal epithelia. In the colon, the number of goblet cells increased, epithelium loss was prominent, and scattered erosions were present in the lamina propria mucosae on which necrotic cellular substances formed a pseudomembrane (pseudomembranous colitis). Diffused hemorrhages were observed between the tunica muscularis coli and submucosa. Intranuclear inclusions were not detected in the samples submitted.

DISCUSSION

The data in the present study together with those from our previous one (11) have revealed two significant points in the epizootiology of FPV infections in cats and dogs. One is that FPLVs compose a more homologous genetic and antigenic group than CPVs: as is well-known, new antigenic variants CPV-2a and CPV-2b were identified shortly after the emergence of CPV-2 (17, 19). On the other hand, little antigenic variation has been detected among FPLV isolates (11, 12, 18) over the last 30 years at least. Although some genetic variations among them have recently been reported (23), their significance is obscure.

Another point is that some novel strains may circulate, mixing themselves among FPLVs in cats and CPVs in dogs. There is no doubt that the FPV-314 isolate studied here was CPV itself rather than an FPLV variant. The three previously described CPV-like viruses (F8, F12, and F13) from healthy cats and one FPLV-like virus (C27) from a dog clinically diagnosed with CPV infection (11) were confirmed to be CPVs and FPLV, respectively, by serological analysis with MAb P2-215 (10). These facts, together with the detection of FPV-314 from the cat with FPL in the present study, may describe one aspect of the properties of FPVs, i.e., that they mutually transmit between dogs and cats once in a while.

Since the emergence of CPVs it is well-known that CPVs grow in both feline and canine cells in vitro, but little is known about CPV infections of cats, and different findings have been reported (4, 24). In one study, CPV replicated in specific-pathogen-free cats after subcutaneous inoculation which showed antibody production but no clinical signs (4), while in another study CPV-2 did not replicate in any tissue of specific-pathogen-free cats after intramuscular or intravenous inoculation or cause seroconversion (24). One should always be careful about cross-contamination by a contaminated environment during a study, especially on parvoviruses because of their highly resistant nature, as the investigators of the latter study also discussed in their paper. In the present study, only the rectal swab sample was available for the virus examination, and we had no opportunity to examine the intact tissues of the cat from which FPV-314 was recovered. Thus, the suspicion that the isolation of FPV-314 resulted from contamination cannot be disproved. We suspect that the cat was probably infected with CPV from a contaminated cage during hospitalization. Some considerations supporting this idea can be given. (i) Other samples treated at the same time as the sample in question

TABLE 3. Amino acid variations in VP2 between FPV-314 and other FPVs

Amino acid in VP2	Amino acid variation in:						Known specificity
	MEV Abashiri (1978)[a]	FPLV TU10 (1977)	CPV Cp49 (1979)	CPV Y1 (1982)	CPV Ob1 (1992)	FPV-314 (1993)	
7	Q	Q	ND[b]	Q	ND	R	
21	T	A	ND	T	ND	T	
80	K	K	ND	R[c]	ND	R	CPV vs FPLV/MEV
85	N	I	ND	N	ND	N	
87	M	M	ND	L*[d]	ND	L*	CPV-2 vs CPV-2a/2b
93	K	K	N	N	N	N	CPV vs FPLV/MEV
103	V	V	A	A	A	A	CPV vs FPLV/MEV
232	I	V	I	I	I	I	
297	S	S	S	S	A	A	
300	A	A	A	G*	G*	G*	CPV-2 vs CPV-2a/2b
305	D	D	D	Y*	Y*	Y*	CPV-2 vs CPV-2a/2b
323	D	D	N	N	N	N	CPV vs FPLV/MEV
375	D	D	N	D	D	D	
411	A	E	E	E	E	E	
562	L	V	V	V	V	V	
564	N	N	S	S	S	S	CPV vs FPLV/MEV
568	A	A	G	G	G	G	CPV vs FPLV/MEV
582	L	L	L	L	E	L	

[a] Year of isolation.
[b] ND, not determined.
[c] CPV-specific amino acids are indicated with underline.
[d] CPV-2a- and CPV-2b-specific amino acids are indicated with an asterisk.

were not contaminated with CPV, and the templates for DNA sequencing of the FPV-314 isolate were obtained directly from the swab sample by PCR. These facts may deny a possibility of laboratory contamination of the sample. (ii) Although it is uncertain whether FPVs were involved in the clinical signs presented at the first medical examination, the FPV-314 isolate did exist in the rectal swab sample taken on the eighth day when the leukocyte count had again dramatically decreased and the clinical conditions were taking a turn for the worse. (iii) Although the pathological findings did not agree entirely, some of them, such as the splenic and intestinal findings, are consistent with the diagnostic indexes for parvovirus infections (1).

This kind of study tends to end retrospectively, because by the time that the results are obtained the patients have either recovered or died, so that in most cases it is impossible to collect additional samples. We consider the present results to support our previous finding that FPVs may undergo interspecies transmission among dogs and cats (11) and, furthermore, heterologous FPVs to also be potential pathogens in some cases, as observed here. However, to form a convincing conclusion on the question of whether CPV is a pathogen for cats, it is essential to perform experimental infections in cats with CPVs recovered from cats, including the FPV-314 isolate and/or other CPV strains.

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