

Evidence for Recombination Between Feline Panleukopenia Virus and Canine Parvovirus Type 2

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ABSTRACT. Canine parvovirus type 2 (CPV) is a virulent pathogen that emerged in the late 1970s, probably originating from feline panleukopenia virus (FPLV) or a closely related carnivore parvovirus belonging to the feline parvovirus (FPV) subspecies. In contrast to FPLV, CPV has evolved rapidly since its emergence. The original antigenic type of CPV disappeared more than two decades ago and several new antigenic as well as genetic CPV variants have appeared and spread in the field. Both high mutation rate and positive selection of mutations in the capsid gene appear to be the driving force for such rapid evolution. In addition, genetic recombination has been assessed as a factor in parvovirus evolution. Recently, we provided the first evidence of inter-antigenic type recombination of CPV in nature. Here, an inter-FPV subspecies recombinant was revealed by analyzing the genetic data deposited in databases with several recombination detection programs, and by phylogeny. FPLV strain XJ-1, submitted by Su et al., Harbin, China in 2007 (GenBank accession no. EF988660), was most likely generated by recombination between CPV and FPLV. Its genome was generally composed of the NS1 gene of CPV origin and the VP1 gene of FPLV origin. This is the first demonstration of recombination between different FPV subspecies in nature. Consequently, recombination should be considered as an element in the generation and evolution of parvoviruses of the FPV subspecies.

KEY WORDS: canine parvovirus, cat, feline panleukopenia virus, parvovirus, recombination.

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Parvoviruses are small spherical viruses which have a linear single-stranded DNA genome with a characteristic length of about 5 kb and unique hairpin structures at both ends. They infect many animal species including man and cause a variety of diseases that are sometimes fatal for newborn animals. Canine parvovirus type 2 (CPV), one of the feline parvovirus (FPV) subspecies, is a parvovirus that first appeared in the late 1970s as an emerging pathogen [1]. In the early 1980s, the first CPV (antigenic type 2: CPV-2) disappeared from the field having been replaced by a new antigenic variant designated CPV-2a, and another antigenic variant CPV-2b appeared soon afterwards. During the period between the late 1980s and the early 1990s these prototype CPV-2a and 2b viruses were replaced further by “new CPV-2a and 2b” variants in which the amino acid (aa) residue at site 297 of the VP2 protein was changed from Ser to Ala [3, 36]. This is a non-synonymous substitution that does not result in an antigenic change and consequently new CPV-2a and 2b are genetic but not antigenic variants of the prototype CPV-2a and 2b. The new CPV-2a and 2b have subsequently become the predominant CPV throughout the world; for example, in Japan the new CPV-2b has been predominant since 1997 [22].

The origin of CPV is still not clear but it is arguable that CPV originated from feline panleukopenia virus (FPLV) or a very closely related carnivore parvovirus [34]. Although a DNA virus, CPV has a nucleotide (nt) substitution rate

closer to that of RNA viruses than to that of double-stranded DNA viruses: the mean substitution rates for the CPV and FPLV clades were 1.7×10^{-4} and 9.4×10^{-5} substitutions per site per year, respectively [32]. Both high mutation rate and positive selection of mutations in the capsid protein gene appeared to be the driving force in the emergence and subsequent evolution of CPV [32]. Genetic recombination was not proposed as being involved [32].

On the other hand, genetic recombination is generally considered as a major key mechanism for virus evolution, especially for RNA viruses such as influenza viruses [26]. Recombination may modify viral properties and aid adaptation to new host species. This applies also to RNA viruses infecting cats and dogs; for example, group 1 feline and canine coronaviruses [8, 21], feline calicivirus [4], feline immunodeficiency virus [2, 7, 10, 29], and canine distemper virus [6]. Recently Shackelton *et al.* [31] found some evidence of natural recombination among porcine, mink, and rodent parvoviruses by analyzing the genetic data deposited in databases, and discussed the possibility that recombination may be important in the natural evolution of parvoviruses, especially in circumstances where parvoviruses cause persistent infections. However, in their previous study they found no evidence for recombination of any carnivore parvovirus, including CPV and FPLV [32].

More recently, we provided the first clear evidence of recombination events between CPV antigenic types in natural conditions: that is, between vaccine CPV-2 and field CPV-2a, and vaccine CPV-2 and field CPV-2b viruses, in dogs vaccinated with live combined vaccine containing CPV-2 [20]. These results suggested that recombination is

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a more frequent event than previously believed for the FPLV subspecies that generally cause acute infection. This observation prompted us to search for further evidence of natural recombination among FPLV subspecies using similar methods to those employed by Shackelton *et al.* [31]. As a result we found evidence for inter-FPLV subspecies recombination between FPLV and CPV.

MATERIALS AND METHODS

Sequence data obtained from GenBank: The entire genomic sequences of 6 FPLV and 16 CPV strains that were available for the present study are presented in Table 1. Throughout, nucleotide numbering follows the sequence of the strain CPV-b of CPV-2 [24, 25].

Recombination and phylogenetic analyses: Alignments were screened to identify potential recombination breakpoints by using different recombination detection programs: Genetic Algorithms for Recombination Detection (GARD) [12] and RDP3 [16]. RDP3 is composed of the following 6 separate programs and enables automated analyses of a nucleotide sequence alignment using all of the programs concomitantly: Bootscan [14], Chimaera [27], GENECONV [23], MaxChi [18], RDP [15], and SiScan [5]. For recombination analysis, a 4,269 bp alignment corresponding to nt positions 273 to 4,541 of the strain CPV-b was constructed, which includes both NS1 (nt positions 273 to 2,279) and VP1 (nt positions 2,286 to 4,541) genes.

Phylogenetic analysis was performed using MEGA version 3.1 [13]. Multiple alignments were carried out using CLUSTAL X version 1.83 [33]. A phylogenetic tree was constructed using the neighbor-joining method [30] and distances were determined using the Kimura two-parameter

method [11].

RESULTS

A survey of possible recombination sequence in databases: Both programs GARD and RDP3 detected a putative recombination breakpoint in the nucleotide sequence alignment of a FPLV strain XJ-1 deposited recently from China (EF988660). There was no evidence supporting recombination events in the genome sequences of the remaining 21 strains of FPLV or CPV.

Amino acid (aa) and nucleotide sequence characterization of FPLV XJ-1 strain: A comparison of the predicted aa sequences of the NS1 and VP1 proteins of the strain XJ-1 with those of reference FPLV and CPV is presented in Table 2, in which some critical nt variations are also shown. The NS1 (from N-terminal to at site 595) had aa characteristics of CPV and the VP1 (from at site 223 to C-terminal) had those of FPLV. In addition, some nucleotides of each NS1 (at positions 1,926 and 1,959) and VP1 (at positions 2,110, 3,681, and 4,137) sequence of the strain XJ-1 showed those of CPV and FPLV characteristics, respectively. They were synonymous substitutions.

Characterization of recombination detected in the genome of FPLV XJ-1 strain: GARD identified nt position 2,752 as a putative breakpoint for recombination. The alignment was cut at this point and separate phylogenies for each section were constructed (Fig. 1). The first half of the fragment (1–2,752) belonged to the clade with the same nt regions of CPV (Fig. 1A), while the latter fragment (2,753–4,269) belonged to the clade of FPLV (Fig. 1B).

On the other hand, MaxChi identified nt position 2,554 as an ending breakpoint with a Bonferroni corrected P-Value

Table 1. Nucleotide sequence accession numbers of FPLV and CPV strains analysed in this study

Strains	Accession number	Submitted year	Genetic type of VP1 gene	Reference
CU-4	M38246	1996	FPLV	[24], [25]
193/70	X55115	2005	FPLV	[17]
FPLV_XJ-1	EF988660	2007	FPLV	Su <i>et al.</i> , unpublished
FPV-3.us.67	EU659111	2008	FPLV	Hoelzer <i>et al.</i> , unpublished
FPV-4.us.64	EU659112	2008	FPLV	Hoelzer <i>et al.</i> , unpublished
FPV-kai.us.06	EU659115	2008	FPLV	Hoelzer <i>et al.</i> , unpublished
CPV-N	M19296	1995	CPV-2	[28]
CPV-b	M38245	1996	CPV-2	[24], [25]
Y1	D26079	2002	prototype CPV-2a	[9]
CPV2a	AJ564427	2004	new CPV-2a	Rastogi, A. K., unpublished
CPV-193	AY742932	2005	new CPV-2b	[32]
CPV-339	AY742933	2005	new CPV-2a	[32]
CPV-447	AY742934	2005	new CPV-2b	[32]
CPV-U6	AY742935	2005	new CPV-2a	[32]
CPV-395	AY742936	2005	new CPV-2b	[32]
B-2004	EF011664	2006	new CPV-2a	He <i>et al.</i> , unpublished
CPV-5.us.79	EU659116	2008	CPV-2	Hoelzer <i>et al.</i> , unpublished
CPV-6.us.80	EU659117	2008	CPV-2	Hoelzer <i>et al.</i> , unpublished
CPV-13.us.81	EU659118	2008	prototype CPV-2a	Hoelzer <i>et al.</i> , unpublished
CPV-410.us.00	EU659119	2008	new CPV-2b	Hoelzer <i>et al.</i> , unpublished
CPV-411a.us.98	EU659120	2008	new CPV-2b	Hoelzer <i>et al.</i> , unpublished
CPV-411b.us.98	EU659121	2008	new CPV-2b	Hoelzer <i>et al.</i> , unpublished

Table 2. Amino acid and nucleotide sequence variations in the NS1 and VP1 of FPLV XJ-1 strain

< NS1 >								
nt position in the alignment ^{a)}	67	741	743	1327	1633	1785	1926	1959
aa site in the alignment	23	247	248	443	545	595	* ^{b)}	*
FPLV	N/D	H	T	I/V	Q	H	a	t
XJ-1 strain	N	Q	T	I	E	Q	g	c
CPV	N	Q	I	I	E	Q	g/a	c

< VP1 >											
nt position in the alignment	2110	2355	2753	2773	2793	2816/2817	2822	3208	3413	3427	3481
aa site in the alignment	*	*	223	230	236	233	246	375	443	448	466
FPLV	c	a	K	M	K	T/I	V	V	A	D	D
XJ-1 strain	c	g	K	M	K	T	V	V	A	D	D
CPV	t	g	R	M/L	N	T/I	A	I	A/G	D/Y	N

< VP1 continued >						
nt position in the alignment	3637	3681	3790	4137	4205	4217
aa site in the alignment	518	*	569	*	707	711
FPLV	D	t	N	a	N	A
XJ-1 strain	D	t	N	a	N	A
CPV	D/N	c	N/D	c	S	G

a) In the alignment nt positions 1–2,007 and 2,014–4,269 are corresponding to NS1 and VP1 genes, respectively, and a square outlined shows aa and nt similarities between XJ-1 strain and either FPLV or CPV.

b) An asterisk indicates synonymous substitution of coding region.

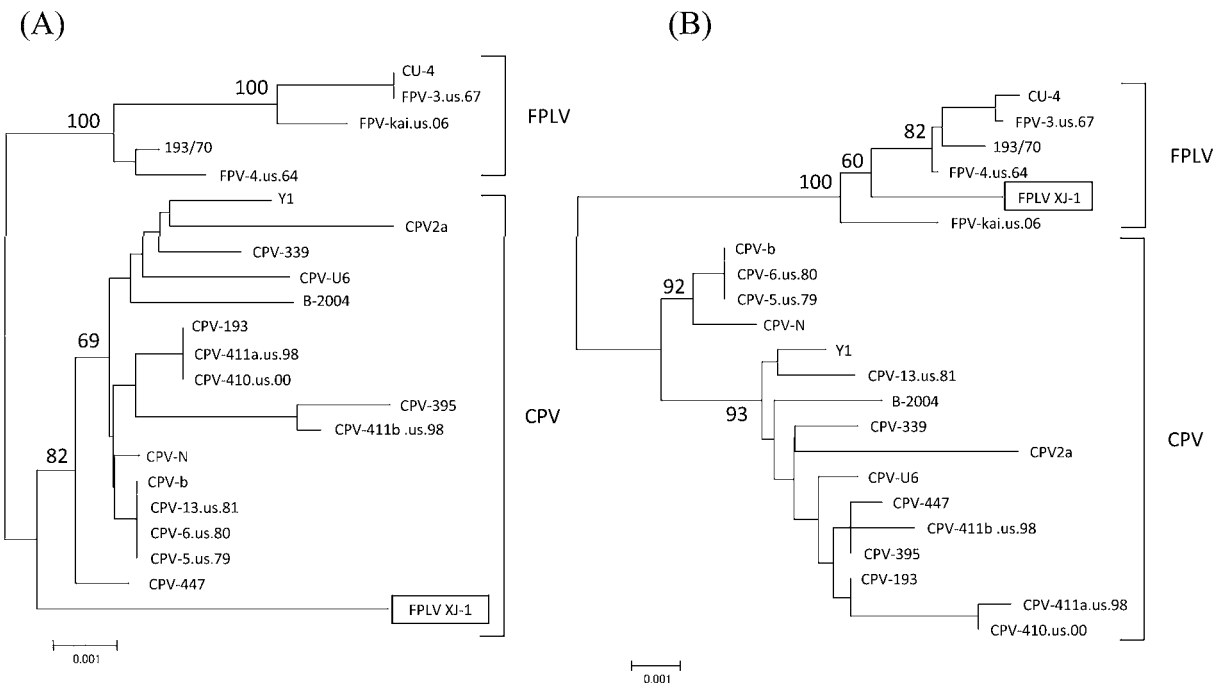


Fig. 1. Phylogeny constructed by the putative breakpoint identified by the recombination detection program GARD. Phylogenetic trees were inferred for nucleotides (A) 1–2,752 and (B) 2,753–4,269. Each bootstrap value indicates the number that each branching was found in 100 bootstrap analyses.

of <0.05, and a predicted recombination region analyzed by RDP3 was located at around positions 1,158 and 2,554 of the alignment. Among the nucleotide sequence data for all 21 strains entered into program RDP3, this part possessed the greatest similarity (99.3%) to the corresponding part of new CPV-2b strain CPV-447. The different breakpoint

determined by MaxChi from that by GARD resulted from MaxChi calculating the midpoint of nt positions 2,355 and 2,753 as the putative breakpoint since no nt difference was detected between the positions of the alignments of FPLV and CPV. The alignment was cut at two points (nt positions 1,158 and 2,554) and separate phylogenies for each three

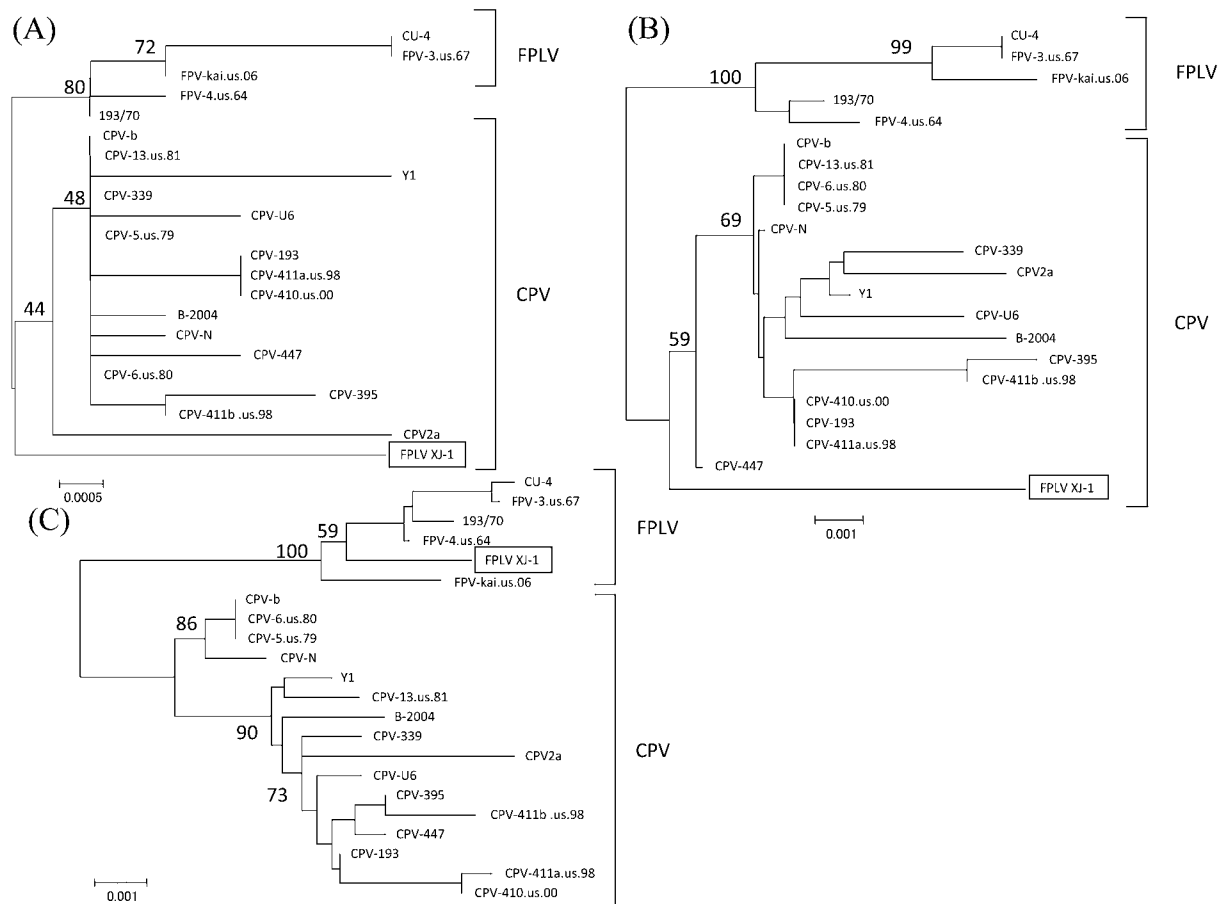


Fig. 2. Phylogeny constructed by the putative breakpoint identified by the recombination detection program MaxChi. Phylogenetic trees were inferred for nucleotides (A) 1–1,158, (B) 1,159–2,554 and (C) 2,555–4,269. Each bootstrap value indicates the number that each branching was found in 100 bootstrap analyses.

section were constructed (Fig. 2). Both the first (1–1,158) and the recombination (1,159–2,554) regions of the alignment belonged to the clade of CPV (Fig. 2A & 2B), and the third fragment (2,555–4,269) belonged to the clade of FPLV (Fig. 2C).

DISCUSSION

Genetic recombination, including intramolecular recombination, reassortment (for segmented genome viruses) and activation (when one of the viruses is inactivated), may occur with both DNA and RNA viruses when two different viruses infect the same cell simultaneously. Usually these events occur more frequently between closely related viruses during replication in the cell; however it is also possible between viruses of different virus families. Consequently, it was natural to consider that a recombinant among FPV subspecies would be discovered in time. As previously discussed by Shackelton *et al.* [31], recombination might be a rare event for FPV subspecies which cause acute disease because early elimination of the virus by an immune

response might reduce the chance of dual infection.

However, over a long period of time no evidence was forthcoming for recombination events among FPV subspecies until our previous study [20] which demonstrated for the first time inter-antigenic type recombinants between CPV-2, CPV-2a, and CPV-2b, found in pups vaccinated with live combined vaccine immediately before developing parvovirus-induced disease. The recombinants were detected in two of six vaccinated pups but none of four unvaccinated pups, suggesting they were recombinants between the vaccine CPV-2 and either field CPV-2a or 2b, because only antigenic type CPV-2 has been included in canine vaccine products in Japan since 1984 [34]. This is clear proof that CPV may be involved in genetic recombination among mutually related viruses in an appropriate environment, in this case in pups that were vaccinated and subsequently infected with field CPV-2a or 2b viruses.

Because of those novel findings, the present study was then performed and further evidence for recombination between FPLV and CPV was revealed. Strain XJ-1 was a FPLV isolate from the feces of a diseased cat. The nucle-

otide sequence of 4,269 bp corresponding to the entire NS1 and VP1 genes were submitted by Su *et al.* of Harbin Veterinary Research Institute of the Chinese Academy of Agriculture Sciences, Harbin, China. In general terms, the genome of FPLV strain XJ-1 was composed of a combination of the NS gene of CPV and the VP gene of FPLV. Although further information on this isolate is not available at present, it may be that strain XJ-1 was generated by a recombination event between FPLV and new CPV-2b in a Chinese cat, possibly when CPV superinfected the cat already infected with FPLV, or vice versa, since new CPV-2a and 2b viruses can infect cats [19, 35]. As in the case of influenza A viruses, it may be essential for such recombination that two, or more, animal species harboring viruses make frequent contact with each other in a given setting.

To our knowledge, this is the first demonstration of inter-FPV subspecies recombination in the field. The frequency of this type of recombination event is uncertain because data from only 22 strains of FPLV and CPV (Table 1) were available for examination in the present study. Subsequently, we intensively searched the genetic data of parvoviruses and bocaviruses of bovine, porcine, mink, rodent, and canine origin deposited in databases for parental parvovirus species of CPV, supposing that FPLV was another parent. However, no significant result was obtained, presumably because of the insufficient data that were available. The XJ-1 strain may be the first recombinant resulting from a cross-species infection. We believe that the inter-FPV subspecies recombination revealed in the present study, together with the inter-antigenic recombination of CPV recently reported in vaccinated pups [20], should be considered as mechanisms for both the emergence of CPV and the evolution of parvoviruses in nature, in addition to the previous "high mutation & positive selection" theory [32].

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