

Molecular Characteristics and Genetic Evolutionary Analyses of Circulating Parvoviruses Derived From Cats in Beijing

Yashu Tang

China Agricultural University

Na Tang

China Agricultural University

Jingru Zhu

China Agricultural University

Yang Liu

China Agricultural University Veterinary Teaching Hospital

Yanli Lyu (✉ luyanli@cau.edu.cn)

China Agricultural University

Research Article

Keywords: feline parvovirus (FPV), canine parvovirus (CPV), VP2 gene, evolution, recombination

Posted Date: July 21st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-714489/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Feline parvovirus (FPV) is a member of the Parvoviridae family, which is a major enteric pathogen of cats worldwide. This study aimed to investigate the prevalence of feline parvovirus in Beijing, China and analyze the genetic features of detected viruses.

Results: In this study, a total of 60 parvovirus-positive samples (8.5%) were detected from 702 cat fecal samples using parvovirus specific PCR. The complete VP2 genes were amplified from all these samples. Among them, 55 sequences were characterized as FPV (91.7%), the other five were typed as canine parvovirus type 2 (CPV-2) variants (8.3%), comprised of four CPV-2c and a new CPV-2b strain. In order to identify the origin of CPV-2 variants in cats, we amplified full-length VP2 genes from 7 fecal samples of dogs infected with CPV-2, which were further classified as CPV-2c. Moreover, the sequences of new CPV-2b/MT270586 and CPV-2c/MT270587 detected from feline samples shared 100% identity with previous canine isolates KT156833 and MF467242 respectively, indicating the CPV-2 variants circulating in cats were derived from dogs. Sequence analysis indicated new mutations, Ala91Ser and Ser192Phe, in the FPV sequences, while obtained CPV-2c carried mutations reported in Asian CPV variants, showing they share a common evolutionary pattern with the Asian 2c strains. Interestingly, the FPV sequence (MT270571), displaying four CPV-specific residues, was found to be a putative recombinant sequence between CPV-2c and FPV. Phylogenetic analysis of the VP2 gene showed that amino acid and nucleotide mutations promoted the evolution of different geographical branches of FPV and CPV lineages.

Conclusions: Our findings will be helpful to further understand the circulation and evolution of canine and feline parvovirus in Beijing.

Background

Feline parvovirus infection is a contagious disease characterized by severe leukopenia, vomiting, diarrhea with fever and abdominal pain, and a high rate of morbidity in young cats (1). This disease is caused by feline parvovirus (FPV), a small, non-enveloped single-stranded DNA virus, belongs to the Parvoviridae family, along with canine parvovirus (CPV) and other parvoviruses of carnivores (2).

Recent studies showed that in domestic felines, FPV remains the prevalent cause of parvovirus infection in domestic felines, while CPV infection became increasingly common. Molecular surveillance in China demonstrated that FPV and CPV variants (CPV-2a/2b and new CPV-2a/2b) are co-circulating in cats in Northeast China (3), while new CPV-2a was the predominant CPV variant in domestic cats in Beijing (4) and Henan province (5). After its emergence, CPV-2c was found with high incidence in European feline population, but was relatively rare in Asia. In China, CPV-2c infection was first reported in Jilin province in 2009 (6). Later, this variant was also identified in dogs from other provinces (7-9). However, there has not been any report of CPV-2c infection from cats in China before.

Although showed a completely different pattern, genetic recombination played an important role in the evolution of both FPV and CPV. In FPV, evolution was mainly forced by random genetic drift (10). The

antigenic and biological properties of FPV have not experienced significant changes since its first identification in 1920 (11). On the contrary, CPV evolved by positive selection and high nucleotide substitution rate (12), which leads to the emergence of new antigenic variants (2a, 2b and 2c) and enables them to expand their host-range to cats. Genetic recombination is generally considered as a major key mechanism for virus evolution, especially for RNA viruses such as feline calicivirus (13), feline immunodeficiency virus (14), and canine distemper virus (15). Recently, Shackelton *et al.* pointed out that genetic recombination should be considered as an essential mechanism for the evolution of parvoviruses in nature (12). Subsequently, they described natural recombination among porcine parvovirus, Aleutian mink disease, and several rodent parvoviruses (16). More recently, studies in Japan, Uruguay and China reported the natural recombination between the vaccine CPV-2 and either field CPV-2a or 2b (17), CPV-2c and CPV-2a (18) as well as FPV and CPV-2 (19), respectively, further indicating the important role of genetic recombination in the natural evolution of parvoviruses. Moreover, some recombination events possibly went undetected due to the high genome homology (98%) between FPV and CPV.

Previous studies provided information on FPV and/or CPV strains spreading in domestic dogs in some provinces of China, while there was limited information regarding parvovirus in cats, suggesting the need of an epidemiological survey to evaluate the parvovirus circulation and evolution in cat population in China. In this study, we characterized VP2 sequences of FPV and CPV strains collected from domestic cats in Beijing, and compared the feline CPV VP2 sequences to those obtained from dogs in the same area, to investigate the prevalence of parvovirus amongst cats in Beijing and to gain insights into the evolution of the detected VP2 genes.

Results

Detection and characterization of FPV and CPV VP2 gene

Sixty (8.5%) samples from 702 cats and seven samples from dogs were tested positive for parvovirus. 1755 bp fragments covering the entire VP2 gene were obtained from all parvovirus positive samples. The obtained full-length VP2 sequences were submitted to GenBank, with accession numbers MT270531-90 (feline sequences) and MT270591-97 (canine sequences) (Table 2).

Analysis of the key aa residues of the VP2 protein showed that the 60 sequences from cats were typed as FPV (91.7%; n = 55), new CPV-2b (1.7%; n = 1) or CPV-2c (6.7%; n = 4) (Table 2). No type CPV-2, CPV-2a or new CPV-2a was found in this study. Meanwhile, seven sequences from dogs were all carried residue 426Glu and therefore were typed as CPV-2c variants.

Comparison of the obtained VP2 sequences revealed genetic diversity between the detected FPV and CPV sequences, with a 97.7%-98.6% nucleotide identity. Nucleotide pairwise identity revealed that the tested 55 FPV VP2 sequences were highly identical, ranging from 98.6% to 100.0% in nucleotide similarity, and sixteen of them showed 100% identity. Interestingly, the CPV-2c sequences collected from cats were found to be closely related to the 2c-type sequences isolated from dogs in this study, sharing a nucleotide identity of 99.7-100%. Moreover, the two CPV-2c sequences from cats, MT270587 and MT270589, were

complete identical to the sequences from dogs, MT270594 and MT270595, respectively. Furthermore, the feline 2c-type sequence (MT270587) also shared 100% identity with a CPV-2c strain (MF467242) isolated from a dog in Guangxi of China in 2015. Meanwhile, the detected new CPV-2b sequence (MT270586) from cats showed 100% identity with the strain (KT156833) derived from a dog in Heilongjiang of China in 2014.

Sequence analysis of VP2 gene

Analysis of deduced amino acid (aa) sequences of the FPV VP2 gene revealed that all the 55 obtained FPV sequences (100%) had the Ile232Val mutation, 39 (70.9%) sequences had the Ala91Ser mutation, and one (1.8%) sequence had the Ser192Phe mutation (Table 2). Notably, FPV/MT270571 (BJ-A240) was detected as a highly different sequence, with one 2c-specific residue, Asn426Glu, and three 2a/2b/2c-specific residues Ala300Gly, Asn564Ser and Ala568Gly (Table 2).

When compared with the reference strains, six aa mutations were observed in the CPV VP2 sequences (Table 2). All the 11 CPV-2c sequences from cats and dogs and the one new CPV-2b sequence presented the Phe267Tyr and Tyr324Ile mutations, which were observed in 100% of recently analyzed Asian CPVs (32). The other two mutations detected at Ser297Ala and Thr440Ala in the obtained new CPV-2b, as well as the Ala5Gly and Gln370Arg in the 11 CPV-2c sequences (from cats and dogs), were identical to recent Asian new CPV-2b (32) and CPV-2c strains (32-34), respectively.

Phylogeny

On phylogenetic analysis, all 111 sequences from this study and GenBank clustered mainly depending on their virus type, formed two branches. In the FPV branch, the 71 FPVs fell into three clades (Figure 1). The clade A consisted of 27 FPV viruses with 91Ala in VP2, while sequences presented Ser at residue 91 clustered separately in FPV clade B. Notably, the FPV sequence (MT270571), displaying one 2c-specific and three 2a/2b/2c-specific substitutions, separated into a monophyletic clade (clade C). Thereafter, the FPV clade A and clade B were further divided into two subclades, respectively, according to several synonymous substitutions in VP2. The 15 obtained FPV sequences and three Asian strains, which presented C or A at nt residues 1185, clustered in the A- \square subclade (Asian subclade). While the European strains clustered with two Asian isolates in the A- \square subclade, and they all had 1185T. Similar to clade A, the clade B also subdivide into subclade B- \square (Asian subclade) and subclade B- \square (European subclade) depending on the nt residues 750 and 1572. The only exception in these clusters is MT270534, which showed 91Ala in VP2, located within the FPV clade B rather than clade A.

In the CPV branch, the 40 CPV sequences clustered into five clades according to the variants type of CPV (Figure 1). In the 2c clade, 21 CPV-2c sequences expressed 267 Tyr, 324 Ile, and 370Arg in VP2, forming the 2c-II subclade (Asian subclade), which included 2c-type sequences obtained from cats and dogs in this study. Six strains showed 267Phe, 324Tyr, and 370AGln clustered in the 2c-I subclade (European subclade), contained CPV-2c detected from Spain and Italy. However, the MT270593 displayed 370Gln in VP2 protein, clustered in the 2c-II subclade. During the last few decades, CPV-2a/2b carrying Ser297Ala

were designated new CPV-2a/2b (21, 35). Therefore, eight of the variants that had been initially named as CPV-2a/2b in the NCBI database were updated to new CPV-2a/2b in the ML phylogenetic tree. Consequently, the new CPV-2b sequences from China (MT270586 in this study) and South Korea, new CPV-2a isolates from China, Thailand and India established the new CPV-2a/2b clade based on the Ser297Ala change, distinguished from the prototype CPV-2a (M24003) and CPV-2b (M74849).

Recombination analyses

With respect to the genetic recombination forced evolution of parvovirus (12), two genetic recombination detection modules annotated the FPV/MT270571 as a potential recombinant sequence. The RDP4 identified evidence of genetic recombination in the VP2 protein of FPV/MT270571, supported by Maxchi, SiScan, and 3Seq with a p -value of 4.263×10^{-3} , 4.085×10^{-6} , and 8.115×10^{-4} , respectively. The recombinant FPV/MT270571 sequence had a CPV-2c strain KT156832 isolated from a dog in China and a FPV strain MK570646 derived from a cat in Australia as its putative major and minor parents, respectively. The obtained RDP4 results were then further examined using the SimPlot software, which confirmed that the FPV/MT270571 was a recombinant sequence (Figure 2A). The generated similarity plot and bootscan analysis for the FPV/MT270571 sequence suggested it had a high nucleotide similarity to the FPV strain MK570646 (blue line) at the beginning of the VP2 gene, but also had high nucleotide identity to the CPV-2c strain KT156832 (red line) at the latter part of VP2 gene (Figure 2A). The potential recombination event was also confirmed by phylogenetic tree construction of different genome segments (Figure 2B).

Discussion

In recent years, despite the widespread use of vaccines, total number of FPV cases has been increased in Beijing with the expansion of pet cat population. This study revealed that cats in Beijing are still primarily infected by FPV, along with the new CPV-2b and CPV-2c variants co-circulating in the population. The present study also showed that CPV-2c has emerged for the first time as a dominant antigenic CPV variant prevalent in cats in Beijing. Recently, studies have shown that the new CPV-2a variant was prevalent in dogs of some provinces of China, including Beijing (2014-2015) (7), Sichuan (2011) (36), Gansu (2014) (37), Shangdong (2015) and Heilongjiang (2014-2015) (32), whereas the CPV-2c is relatively rare. However, this situation has changed as the detection of seven CPV-2c sequences from seven dogs in this study, suggesting the 2c variant probably has replaced new CPV-2a become the predominant strain in Beijing.

Moreover, we found that the CPV-2c sequence (MT270587) obtained from a cat displayed 100% identity with the CPV-2c sequence (MT270594) detected from a dog in this study as well as a published 2c-type sequence (MF467242) from a dog in Guangxi of China in 2015, which is consistent with previous studies by Decaro *et al.* (38) and Wu *et al.* (4). Meanwhile, the CPV-2c sequence (MT270589) and the new CPV-2b sequence (MT270586), detected from cats, were identical to the obtained sequence (CPV-2c/MT270595) and a reported sequence (new CPV-2b/KT156833) from dogs in Heilongjiang of China, respectively.

These results indicated that the 2c-type sequences are co-circulating in Chinese cat and dog populations, and the CPV sequences detected in cats were derived from dogs.

Based on the sequence analysis, Ala91Ser, and Ser192Phe were first identified in FPV VP2 sequences. Besides, all FPV sequences tested in this study carried a reported (39) mutation, Ile232Val, which probably represented a novel pattern of VP2 genetic evolution in FPV strains in Beijing, China. The potential functional consequence of these mutations remains unknown, but the Ala91Ser worked as the hallmark to separate FPV sequences in the phylogenetic analysis, as shown in Figure 1. Thereafter, several synonymous substitutions served as the following evolutionary force, further divided the FPV clade A/B into two subclades with diverse geographical origin. On the other hand, mutations observed in obtained CPV-2c and new CPV-2b VP2 sequences have been elaborated in other CPV variants (2, 10, 21, 32, 33, 35-37, 40-55). Among these, Phe267Tyr, Tyr324Ile, Gln370Arg mutations acted like the Ala91Ser in FPV clades, subdivided the 2c clade into Asian/European subclades. Moreover, the exceptions in these geographically distinct clusters may result from trading or transport of animals and contaminated equipment (56). Taken together, changes in the VP2 gene might play a significant role in the evolution of FPV and CPV viruses.

Interestingly, we detected high genetic complexity of the sequence FPV/MT270571, which formed a monophyletic clade in phylogeny. This sequence was further subjected to recombination analysis, which was proven to be a recombinant sequence between CPV-2c and FPV in VP2 gene. Recently, several studies have reported the natural recombination events between the vaccine CPV-2 and either field CPV-2a or 2b (17), FPV and new CPV-2b (19), as well as CPV-2c and CPV-2a (18), showing the important role of genetic recombination in the natural evolution of parvoviruses. In the present study, the MT270571 sequence was most closely related to FPV/MK570646, indicating the likely origin (strain) from which the 1-1,129 nt region in the VP2 gene of the recombinant MT270571 circulating in Beijing came from. Therefore, we assumed that recombination during a co-infection was the most likely origin of this recombinant FPV VP2 sequence, since cats are susceptible to both FPV and CPV variants (57, 58), and co-infection of FPV and CPV-2a/2c in domestic cats were reported before (1, 59, 60).

To our knowledge, this is the first demonstration of FPV and CPV-2c recombination within VP2 genes in field. However, the effects of this mutation on FPV are unknown. Previously identified recombination breakpoints occur within the VP1/VP2 (18) or NS1/VP1 (19) gene boundary, therefore ongoing researches on the potential recombinant events in the NS and VP1 genes of sample BJ-A240 are necessary.

Conclusion

In conclusion, these results revealed that the FPV is still the predominant parvovirus infecting cats, with the new CPV-2b, CPV-2c, and the recombinant FPV are co-circulating in Beijing of China. Besides, this study provided the first evidence of CPV-2c emerged as the dominant antigenic CPV variant circulating in domestic cats and dogs in Beijing, which had a common evolutionary pattern in VP2 protein with other Asian CPV-2c strains. Moreover, the FPV and CPV lineages likely evolve in local populations by changes

in nt and aa composition of VP2 gene, and the identification of the genetic recombination in VP2 may contribute to the evolution of parvovirus diversity. Further in-depth studies of the pathology of the recombinant infection are required.

Methods

Sample Collection

A total of 702 fecal samples from both healthy and parvoviruses-suspected cats were collected in Beijing during 2019. Meanwhile, seven samples from domestic dogs diagnosed with CPV infection using colloidal gold test strips were obtained in the same region for comparison. All samples were kept at -80°C for later investigation.

DNA extraction and VP2 sequencing

For each sample, DNA was extracted from specimens using the Aidlab Virus DNA Kit (Beijing Aidlab Biotech Company, Beijing, China) according to the manufacturer's instructions. Detection of the presence of FPV/CPV in extracted viral DNA was determined by parvovirus polymerase chain reaction (PCR) using F1 primer pairs (Table 1). Amplification was carried out in 25- μ L reactions, consisting of 12.5 μ L extensor PCR master mix (Aidlab), 9.5 μ L of nuclease-free water, 0.5 μ L of each primer (10 μ M; F1F and F1R) and 2 μ L of DNA template. Negative controls (water) were processed alongside fecal samples throughout all stages. The PCR cycling conditions were 5 min at 94°C, followed by 30 cycles of denaturation at 94°C for 10 s, annealing at 53°C for 15 s and extension at 72°C for 20 s, followed by a final extension at 72°C for 10 mins. A 5 μ L aliquot of each PCR product was analyzed by electrophoresis using a 1% agarose gel and ethidium bromide staining. Subsequently, the parvovirus PCR positive samples were further subjected to full-length VP2 sequencing using F2 primer pairs (Table 1). The PCR amplification and thermal cycling conditions for the F2 primers were prepared as for the F1 primers, with minor modifications: 6.5 μ L of nuclease-free water, plus 3 μ L of MgCl₂ (25mM).

Sanger sequencing of PCR products was performed at a commercial laboratory (Tianyihuiyuan Beijing, China).

Sequence analysis

All external primer sites of the obtained sequences were removed, giving a final consensus sequence of 1755 bp for the full-length VP2 gene. The derived full-length VP2 sequences were aligned to the published FPV and CPV strains, translated into aa sequences, and analyzed using the MEGA software package version X. Viral typing of tested sequences was based on the analysis of key VP2 aa residues discriminating the viral type (FPV/CPV) and the CPV variants (2, 20-22)

Phylogenetic analysis

To elucidate the evolutionary history of FPV and CPV VP2 sequences identified in this study, a phylogenetic tree was constructed with 67 sequences obtained in this study and 44 sequences corresponding to the full-length VP2 gene from CPV and FPV strains (GenBank No.: FPV: EF988660, AB054226, JX048608, JX048608, EU498716, EU498720, M24004, MK570646, AF015223, AF015223, AF015223, EU498713, EU252146, EU252147, FJ440712, EU360958; CPV: EU914139, FJ011098, M24003, M74849, FJ005214, FJ005245, FJ005233, HQ025913, KP682522, FJ222821, MH329284, GQ379042, KP090138, MT488457, KT156833, KY418607, MN453226, JX048607, KT162014, MK357727, MK895487, MN453228, KP749854, KY937662, MT488453, MF467242, MK357735, MF467229). The tree reconstruction was performed with MEGA X software package (23) using the maximum-likelihood (ML) according to the Tamura3-parameters model with discrete Gamma distribution (T92 + G), which was selected using the find-best-fit model algorithm in MEGA X. A total of 1000 replicates were used to generate bootstrap values.

Detection of recombination

To explore the role of genetic recombination in the evolution of FPV and CPV VP2 gene, a dataset of alignments used in the phylogenetic analysis (section 2.4) were examined using various recombination detection methods. The dataset was examined for recombination events using the incorporated recombination detection program 4 (RDP4) package v. 4.101 software, which contains a collection of methods: Bootscan (24), Chimera (25), GeneConv (26), MaxChi (27), RDP (28), SiScan (29), and 3 Seq (30). The highest acceptable *P* value was set at 0.05. Only sequences that showed a positive recombination event in 3 or more different methods within the same general region of the alignment were considered potential recombination sequences. Detected recombination events in the RDP4 were then confirmed using the similarity plot and bootscanning analysis in the SimPlot software package v. 3.5.1 (31), with a window and step sizes of 200 bp and 20 bp, respectively. The recombination breakpoints were detected and evaluated by the Kimura-2 parameter (K2P) and the GapStrip models for bootscan analysis similarity plot, respectively. The potential recombination breakpoints were further identified by constructing maximum-likelihood phylogenetic trees with MEGA X, as adopted in the section 2.4.

List Of Abbreviations

FPV: Feline Parvovirus; CPV: Canine Parvovirus; PCR: Polymerase Chain Reaction; ML: maximum-likelihood; RDP4: recombination detection program 4; nt: nucleotide; aa: amino acid; bp: Base Pair; NS: Non-structural protein.

Declarations

Ethics approval and consent to participate

All samples (the gathered feces of cats and dogs) used in this study were those that were submitted to China Agricultural University Teaching Hospital for diagnosis of disease. Ethical approval was not

required as per institutional guidelines and recommendations.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available in GenBank with accession numbers MT270531-97.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors' contributions

YLL and YST participated in the study conception, design, data analysis and interpretation, drafting and revision of the manuscript. JRZ, YL and NT participated in data collection and analysis, and revision of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We are grateful for the help provided by staff in the China Agricultural University Teaching Hospital for collecting animal fecal samples.

References

1. Battilani M, Balboni A, Ustulin M, Giunti M, Scagliarini A, Prospero S. Genetic complexity and multiple infections with more Parvovirus species in naturally infected cats. *Veterinary Research*. 2011;43(1):43.
2. Truyen U. Evolution of canine parvovirus—a need for new vaccines? *Veterinary Microbiology*. 2006;117(1):9-13.
3. Niu J, Yi S, Hu G, Guo Y, Zhang S, Dong H, et al. Prevalence and molecular characterization of parvovirus in domestic kittens from Northeast China during 2016-2017. *Japanese Journal of Veterinary Research*. 2018;66(3):145-55.
4. Wu J, Gao X, Hou S, Guo X, Yang X, Yuan W, et al. Molecular epidemiological and phylogenetic analyses of canine parvovirus in domestic dogs and cats in Beijing, 2010-2013. *Journal of Veterinary Medical Science*. 2015;77(10):1305-10.

5. Li X, Wu H, Wang L, Spibey N, Liu C, Ding H, et al. Genetic characterization of parvoviruses in domestic cats in Henan province, China. *Transboundary and Emerging Diseases*. 2018;65(6):1429-35.
6. Ren-Zhou Z, Song-Tao Y, Hao F, Chang-Sheng C, Xian-Zhu X. The first detection of canine parvovirus type 2c in China. *Journal of Pathogen Biology*. 2010.
7. Wang J, Lin P, Zhao H, Cheng Y, Jiang Z, Zhu H, et al. Continuing evolution of canine parvovirus in China: Isolation of novel variants with an Ala5Gly mutation in the VP2 protein. *Infect Genet Evol*. 2016;38:73-8.
8. Zhao H, Wang J, Jiang Y, Cheng Y, Lin P, Zhu H, et al. Typing of Canine Parvovirus Strains Circulating in North-East China. *Transbound Emerg Dis*. 2017;64(2):495-503.
9. Geng Y, Guo D, Li C, Wang E, Wei S, Wang Z, et al. Co-Circulation of the Rare CPV-2c with Unique Gln370Arg Substitution, New CPV-2b with Unique Thr440Ala Substitution, and New CPV-2a with High Prevalence and Variation in Heilongjiang Province, Northeast China. *PLoS One*. 2015;10(9):e0137288.
10. Horiuchi M, Yamaguchi Y, Gojobori T, Mochizuki M, Nagasawa H, Toyoda Y, et al. Differences in the evolutionary pattern of feline panleukopenia virus and canine parvovirus. *Virology*. 1998;249(2):440-52.
11. Truyen U, Parrish CR. Feline panleukopenia virus: Its interesting evolution and current problems in immunoprophylaxis against a serious pathogen. *Veterinary Microbiology*. 2013;165(1-2):29-32.
12. Shackelton LA, Parrish CR, Truyen U, Holmes EC. High rate of viral evolution associated with the emergence of carnivore parvovirus. *Proceedings of the National Academy of Sciences of the United States of America*. 2005;102(2):379-84.
13. Coyne KP, Reed FC, Porter CJ, Dawson S, Gaskell RM, Radford AD. Recombination of Feline calicivirus within an endemically infected cat colony. *J Gen Virol*. 2006;87(Pt 4):921-6.
14. Hayward JJ, Taylor J, Rodrigo AG. Phylogenetic analysis of feline immunodeficiency virus in feral and companion domestic cats of New Zealand. *J Virol*. 2007;81(6):2999-3004.
15. Han GZ, Liu XP, Li SS. Cross-species recombination in the haemagglutinin gene of canine distemper virus. *Virus Res*. 2008;136(1-2):198-201.
16. Shackelton LA, Hoelzer K, Parrish CR, Holmes EC. Comparative analysis reveals frequent recombination in the parvoviruses. *Journal of General Virology*. 2007;88(12):3294-301.
17. Mochizuki M, Ohshima T, Une Y, Yachi A. Recombination between vaccine and field strains of canine parvovirus is revealed by isolation of virus in canine and feline cell cultures. *The Journal of Veterinary Medical Science*. 2008;70(12):1305-14.

18. Pérez R, Calleros L, Marandino A, Sarute N, Iraola G, Grecco S, et al. Phylogenetic and genome-wide deep-sequencing analyses of canine parvovirus reveal co-infection with field variants and emergence of a recent recombinant strain. *PLoS ONE*. 2014;9(11):e111779.
19. Ohshima T, Mochizuki M. Evidence for recombination between feline panleukopenia virus and canine parvovirus type 2. *The Journal of Veterinary Medical Science*. 2009;71(4):403-8.
20. Martella V, Decaro N, Elia G, Buonavoglia C. Surveillance activity for canine parvovirus in Italy. *Journal of Veterinary Medicine B, Infectious Disease and Veterinary Public Health*. 2005;52(7-8):312-5.
21. Ohshima T, Hisaka M, Kawakami K, Kishi M, Tohya Y, Mochizuki M. Chronological analysis of canine parvovirus type 2 isolates in Japan. *The Journal of Veterinary Medical Science*. 2008;70(8):769-75.
22. Decaro N, Martella V, Desario C, Bellacicco AL, Camero M, Manna L, et al. First detection of canine parvovirus type 2c in pups with haemorrhagic enteritis in Spain. *Journal of Veterinary Medicine B, Infectious Diseases and Veterinary Public Health*. 2006;53(10):468-72.
23. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution*. 2018;35(6):1547-9.
24. Martin DP, Posada D, Crandall KA, Williamson C. A modified bootscan algorithm for automated identification of recombinant sequences and recombination breakpoints. *AIDS Research and Human Retroviruses*. 2005;21(1):98-102.
25. Posada D, Crandall KA. Evaluation of methods for detecting recombination from DNA sequences: computer simulations. *Proceedings of the National Academy of Sciences of the United States of America*. 2001;98(24):13757-62.
26. Padidam M, Sawyer S, Fauquet CM. Possible emergence of new geminiviruses by frequent recombination. *Virology*. 1999;265(2):218-25.
27. Smith JM. Analyzing the mosaic structure of genes. *Journal of Molecular Evolution*. 1992;34(2):126-129. [10.1007/BF00182389](https://doi.org/10.1007/BF00182389).
28. Martin D, Rybicki E. RDP: detection of recombination amongst aligned sequences. *Bioinformatics (Oxford, England)*. 2000;16(6):562-3.
29. Gibbs MJ, Armstrong JS, Gibbs AJ. Sister-Scanning: a Monte Carlo procedure for assessing signals in recombinant sequences. *Bioinformatics (Oxford, England)*. 16(7):573-82.
30. Lam HM, Ratmann Q, Boni MF. Improved algorithmic complexity for the 3SEQ recombination detection algorithm. *Molecular Biology and Evolution*. 2018;35(1):247-51.

31. Lole KS, Bollinger RC, Paranjape RS, Gadkari D, Kulkarni SS, Novak NG, et al. Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. *Journal of Virology*. 1999;73(1):152-60.
32. Geng Y, Guo D, Li C, Wang E, Wei S, Z. W, et al. Co-Circulation of the Rare CPV-2c with Unique Gln370Arg Substitution, New CPV-2b with Unique Thr440Ala Substitution, and New CPV-2a with High Prevalence and Variation in Heilongjiang Province, Northeast China. *PLoS One*. 2015;10(9):e0137288.
33. Chiang S, Wu H, Chiou M, Chang M, Lin C. Identification of a novel canine parvovirus type 2c in Taiwan. *Virology Journal*. 2016;13(1):160.
34. Hoang M, Lin W-H, Le VP, Nga BTT, Chiou M-T, Lin C-N. Molecular epidemiology of canine parvovirus type 2 in Vietnam from November 2016 to February 2018. *Virology Journal*. 2019;16(1):52.
35. Yi L, Tong M, Cheng Y, Song W, Cheng S. Phylogenetic Analysis of Canine Parvovirus VP2 Gene in China. *Transboundary and Emerging Diseases*. 2016;63(2):e262-e9.
36. Xu J, Guo H, Wei Y, Shu L, Wang J, Li J, et al. Phylogenetic analysis of canine parvovirus isolates from Sichuan and Gansu provinces of China in 2011. *Transboundary and Emerging Diseases*. 2015;62(1):91-5.
37. Han S, Guo H, Sun S, Shu L, Wei Y, Sun D, et al. Full-length genomic characterizations of two canine parvoviruses prevalent in Northwest China. *Archives of Microbiology*. 2015;197(4):621-6.
38. Decaro N, Buonavoglia D, Desario C, Amorisco F, Colaianni ML, Parisi A, et al. Characterisation of canine parvovirus strains isolated from cats with feline panleukopenia. *Research in Veterinary Science*. 2010;89(2):275-8.
39. Kwan E, Carrai M, Lanave G, Hill J, Parry K, Kelman M, et al. Analysis of canine parvoviruses circulating in Australia reveals predominance of variant 2b and identifies feline parvovirus-like mutations in the capsid proteins. *Transbound and Emerging Diseases*. 2020.
40. Zhang R, Yang S, Zhang W, Zhang T, Xie Z, Feng H, et al. Phylogenetic analysis of the VP2 gene of canine parvoviruses circulating in China. *Virus Genes*. 2010;40(3):397-402.
41. Chiang SY, Wu HY, Chiou MT, Chang MC, Lin CN. Identification of a novel canine parvovirus type 2c in Taiwan. *Virology Journal*. 2016;13(1).
42. Mukhopadhyay HK, Matta SL, Amsaveni S, Antony PX, Thanislass J, Pillai RM. Phylogenetic analysis of canine parvovirus partial VP2 gene in India. *Virus Genes*. 2014;48(1):89-95.
43. Nakamura M, Tohya Y, Miyazawa T, Mochizuki M, Phung HTT, Nguyen NH, et al. A novel antigenic variant of Canine parvovirus from a Vietnamese dog. *Archives of Virology*. 2004;149(11):2261-9.

44. Zhao H, Wang J, Jiang Y, Cheng Y, Lin P, Zhu H, et al. Typing of Canine Parvovirus Strains Circulating in North-East China. *Transboundary and Emerging Diseases*. 2017;64(2):495-503.
45. Guo L, Yang S-L, Chen S-J, Zhang Z, Wang C, Hou R, et al. Identification of canine parvovirus with the Q370R point mutation in the VP2 gene from a giant panda (*Ailuropoda melanoleuca*). *Virology Journal*. 2013;10:163.
46. Moon B, Jang J, Kim S, Kim Y, Lee H, So B, et al. Genetic characterization of canine parvovirus type 2c from domestic dogs in Korea. *Transboundary and Emerging Diseases*. 2020;67(4):1645-53.
47. Yoon SH, Jeong W, Kim HJ, An DJ. Molecular insights into the phylogeny of canine parvovirus 2 (CPV-2) with emphasis on Korean isolates: a Bayesian approach. *Archives of Virology*. 2009;154(8):1353-60.
48. Calderon MG, Romanutti C, A DA, Keller L, Mattion N, La Torre J. Evolution of canine parvovirus in Argentina between years 2003 and 2010: CPV2c has become the predominant variant affecting the domestic dog population. *Virus Research*. 2011;157(1):106-10.
49. Calderón MG, Wilda M, Boado L, Keller L, Malirat V, Iglesias M, et al. Study of canine parvovirus evolution: comparative analysis of full-length VP2 gene sequences from Argentina and international field strains. *Virus Genes*. 2012;44(1):32-9.
50. Battilani M, Ciulli S, Tisato E, Prospero S. Genetic analysis of canine parvovirus isolates (CPV-2) from dogs in Italy. *Virus Research*. 2002;83(1-2):149-57.
51. Decaro N, Desario C, Elia G, Martella V, Mari V, Lavazza A, et al. Evidence for immunisation failure in vaccinated adult dogs infected with canine parvovirus type 2c. *New Microbiologica*. 2008;31(1):125-30.
52. Wang J, Lin P, Zhao H, Cheng Y, Jiang Z, Zhu H, et al. Continuing evolution of canine parvovirus in China: Isolation of novel variants with an Ala5Gly mutation in the VP2 protein. *Infection Genetics & Evolution*. 2016;38:73-8.
53. Hueffer K, Parker JSL, Weichert WS, Geisel RE, Sgro J-Y, Parrish CR. The natural host range shift and subsequent evolution of canine parvovirus resulted from virus-specific binding to the canine transferrin receptor. *Journal of Virology*. 2003;77(3):1718-26.
54. Decaro N, Desario C, Parisi A, Martella V, Lorusso A, Miccolupo A, et al. Genetic analysis of canine parvovirus type 2c. *Virology*. 2009;385(1):5-10.
55. Ikeda Y, Mochizuki M, Naito R, Nakamura K, Miyazawa T, Mikami T, et al. Predominance of canine parvovirus (CPV) in unvaccinated cat populations and emergence of new antigenic types of CPVs in cats. *Virology*. 2000;278(1):13-9.

56. Mira F, Canuti M, Purpari G, Cannella V, Di Bella S, Occhiogrosso L, et al. Molecular Characterization and Evolutionary Analyses of Carnivore Protoparvovirus 1 NS1 Gene. *Viruses*. 2019;11(4):4.
57. Battilani M, Scagliarini A, Ciulli S, Morganti L, Prosperi S. High genetic diversity of the VP2 gene of a canine parvovirus strain detected in a domestic cat. *Virology*. 2006;352(1):22-6.
58. Hoelzer K, Shackelton LA, Parrish CR, Holmes EC. Phylogenetic analysis reveals the emergence, evolution and dispersal of carnivore parvoviruses. *The Journal of General Virology*. 2008;89(Pt 9):2280-9.
59. Balboni A, Bassi F, De Arcangeli S, Zobba R, Dedola C, Alberti A, et al. Molecular analysis of carnivore Protoparvovirus detected in white blood cells of naturally infected cats. *BMC Veterinary Research*. 2018;14(1):41.
60. Battilani M, Balboni A, Giunti M, Prosperi S. Co-infection with feline and canine parvovirus in a cat. *Veterinaria Italiana*. 2013;49(1):127-9.

Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Due to technical limitations, table 2 is not available with this version.

Figures

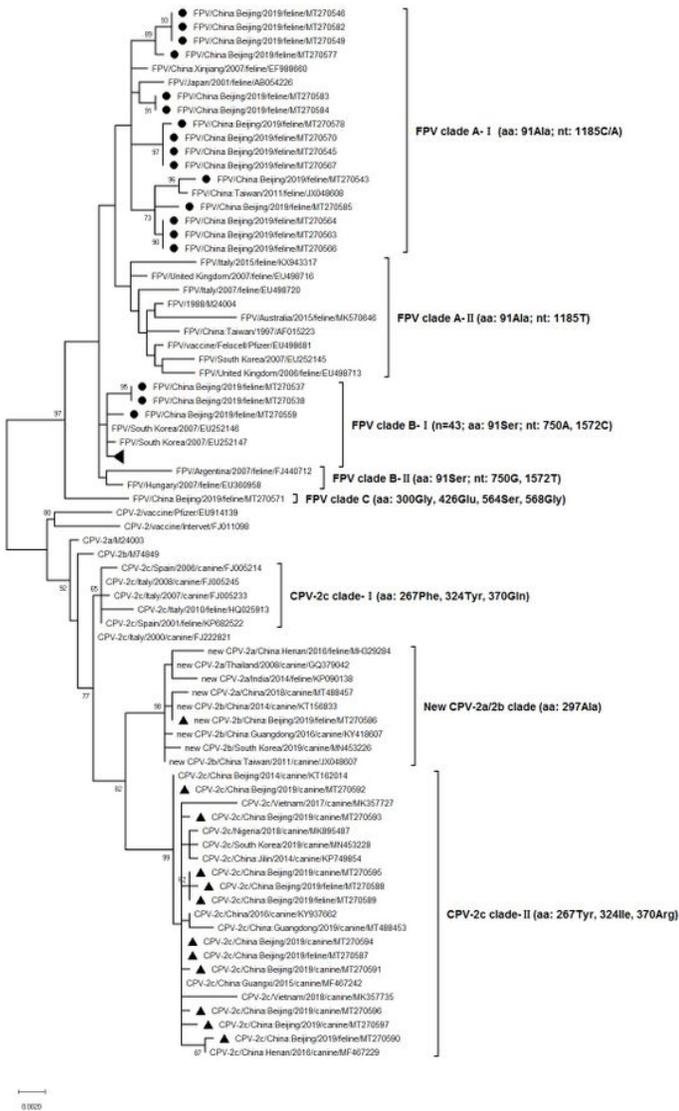


Figure 1

Maximum-likelihood tree showing the genetic relationship of the full-length VP2 gene of feline parvovirus and canine parvovirus strains Maximum-likelihood (ML) tree based on 111 full-length VP2 sequences of FPV and CPV strains. The tree was constructed using the T92 + G model and 1,000 bootstrapping with MEGAX software. Bootstrap values (%) greater than 50 are shown. Sequences used in this analysis are indicated with their respective virus type (FPV/CPV) or variant (CPV-2/2a/2b/2c, new CPV-2a/2b), country and year of collection, origin, and GenBank accession nos. The FPV sequences and CPV sequences detected in this study are indicated by black triangles and black dots, respectively.

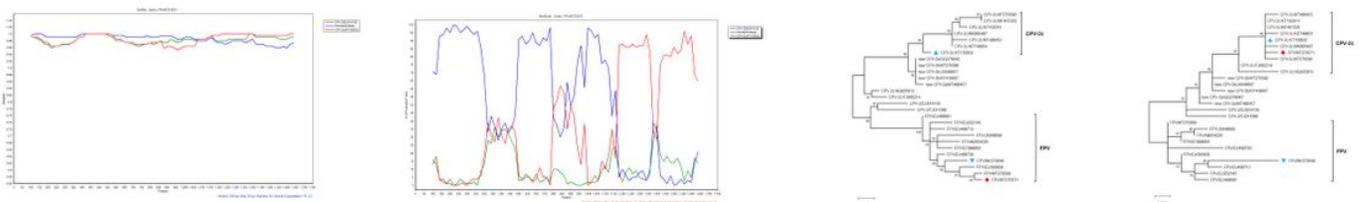


Figure 2

Schematic diagram of the naturally recombinant FPV/MT270571 sequence CPV-2c/KT156832 isolated in China and FPV/MK570646 from Australia served as the putative major and minor parents. (A) The potential recombination event was detected in the VP2 protein gene and was supported by similarity and bootscan analysis, which indicated that CPV-2c/KT156832 (red line) served as the main template of the complete VP2 gene, and the beginning of the VP2 gene was replaced by FPV/MK570646. The FPV/MT270571 sequence served as the query. The y-axis indicated the percentage of nucleotide identity and permutated trees for the similarity plot and boot scanning, respectively, within a 200 bp-wide window with a 20-bp step size between plots. (B) The ML phylogenetic trees of the recombinant MT/270571 strains (◆) and its major (▲) and minor (▼) putative parent strains over two recombinant and non-recombinant segments. Bootstrap (1000 replications) values over 50% are shown for each node.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Additionalfile1.xlsx](#)
- [Table1.xlsx](#)