VETERINARIA ITALIANA
RIVISTA DI SANITÀ PUBBLICA VETERINARIA

Paper

Isolation and genetic characterization of parvoviruses from domestic cats reveals emergence of CPV-2c in India: A first report

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Veterinaria Italiana, Vol. 60 No. 2 (2024) DOI: 10.12834/VetIt.3430.23463.2

Abstract

The objective of the present study was to isolate and characterize the VP2 gene of parvoviruses from domestic cats in India. For that, 38 fecal samples were screened by PCR with 36.84% positivity. Sequence analysis of those isolates showed canine parvovirus type-2c (CPV-2c) as the predominant variant, followed by feline panleukopenia virus (FPV) and 2a. Phylogenetic analysis of the CPV-2c sequences revealed clustering with Singaporean, South Korean, Mongolian and Bangladeshi dog 2c sequences. Phylogenetic analysis of the 2a isolate (MZC 2) was found to be clustered with Indian, Thai and Singaporean dog 2a isolates. Similarly, all the four FPV sequences were ancestrally related to Indian dog and cat FPV sequences hinting towards interspecies transmission between dogs and cats. Both synonymous and non-synonymous mutations were evident in CPV-2c, 2a and FPV sequences indicative of active evolution. In cell culture medium, CPV-2 showed cytopathogenic effects at the third passage level. In conclusion, the study provided the first report of CPV-2c in cats from India, which demands for extensive epidemiological surveillance to monitor interspecies spread and to shed more light on viral phylogenomics, their distribution in the country and in the Southeast Asian region and usage of current vaccines.

Keywords

CPV-2, cats, CPV-2c, CPV-2a, epidemiology, FPV, Mutations, VP2 protein

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Introduction

Canine parvovirus-2 (CPV-2) and Feline panleukopenia virus (FPV) are members of the genus Protoparvovirus, variants of the species *Protoparvovirus carnivoran 1* and family *Parvoviridae*, and are major pathogens of domestic and wild carnivores causing fatal haemorrhagic gastroenteritis (Milićević *et al.* 2023). CPV-2 after its emergence in the late 1970s as a host variant of FPV evolved into newer antigenic variants such as CPV-2a, 2b and 2c; New CPV-2a, 2b and 2c; Asp-300 (2a/2b), which then completely replaced the original antigenic type and are now distributed worldwide (Decaro and Buonavoglia 2012). In the 1980s, CPV-2a had emerged from CPV-2 as the first antigenic variant, which differs from the original type in 5-6 amino acid (aa) positions of the VP2 protein (Decaro *et al.* 2011). A second antigenic variant, CPV-2b, had a further mutation (asparagine to aspartic acid at aa residue 426) in the VP2 protein (Parrish *et al.* 1991). In 2000, a third antigenic variant, CPV-2c, was detected which showed aa change asparagine/aspartic acid to glutamic acid at residue 426 of the VP2 protein (Buonavoglia *et al.* 2001). Further, the CPV-2a and CPV-2b variants showing amino acid change 297 Ser→Ala have been designated as the new CPV-2a and new CPV-2b, respectively (Decaro *et al.* 2009). The original CPV-2 was unable to infect cats but new variants of CPV-2 have the capability to infect both cats and dogs (Clegg *et al.* 2012).

Among the CPV-2s, CPV-2a was first detected in cats in the late 1980s from non-symptomatic cats in Japan (Mochizuki *et al.* 1993). Later on, CPV-2b was detected in cats from USA (Truyen *et al.* 1996). Subsequently, CPV-2 variants were found to spread across all continents and retrieved from cats with gastroenteritis in Italy, Thailand, Germany, USA, India, Portugal, and Spain (Truyen *et al.* 1996, Gamoh *et al.* 2003, Decaro *et al.* 2010, Decaro *et al.* 2011, Mukhopadhyay *et al.* 2016, Balboni *et al.* 2018, Charoenkul *et al.* 2019, Calatayud *et al.* 2020). CPV-2 variants have also been isolated from the feces of clinically healthy domestic and wild felines, suggesting that the virus was either shed long after infection or that it causes subclinical or very mild disease in this species (Markovich *et al.* 2012). In addition, there has been a report of coinfection by multiple CPV variants in a cat with CPV-2a and the 426 Glu variant (Battilani *et al.* 2007). Mixed infections by FPV and CPV-2 or FPV and CPV-2a have also been reported (Urb*et al.* 2003; Battilani *et al*. 2013).

Underscoring the importance of cats as a potential source of genetic heterogeneity and recombination for parvoviruses, and as very few reports are available with respect to molecular characterization of parvoviruses in domestic cats from India, the current study was conceptualized for isolation, VP2 sequence analysis and the phylogenomics of CPV-2/FPV virus strains which were detected in suspected cats. Further, to the best of the author's knowledge this is the first report of CPV-2/FPV in cats from North eastern states of India like Mizoram and Manipur.

Materials and methods

Study area, population, sample collection and epidemiological data

A total of 38 rectal swabs were collected from suspected (cats with hemorrhagic gastroenteritis, anorexia and depression) cases irrespective of age, sex and breed from four different states (Assam, Maharashtra, Manipur, and Mizoram) of India between 2019 and 2022 (Table 1). The samples were initially screened for CPV-2 infection by rapid antigen detection kit (Genbody, South Korea) followed by confirmation by polymerase chain reaction (PCR). Epidemiological data with respect to age, sex, breed, vaccination status and clinical signs of the affected cats were collected. Clinical signs were scored as per Van Nguyen et al. (2006) with minor modifications (Table 2).

Sl. No.	Isolate number	Sample details	Place of collection	Year of collection	Accession no.	Variant	Breed	Age (month)	Sex	Clinical presentation
1.	MZC 02	Faeces	Mizoram	2020	OM891547	2a	Mixed	4	M	Gastroenteritis
2.	MZC 16	Faeces	Mizoram	2021	OP729179	FPV	Non-descript	11	M	Enteritis
3.	MZC 35	Faeces	Mizoram	2022	OP729184	2c	Mixed	12	F	Gastritis
4.	MZC 36	Faeces	Mizoram	2022	OP729180	FPV	Mixed	3	M	Gastritis
5.	MZC 37	Faeces	Mizoram	2022	OP729181	FPV	Non-descript	3	F	Gastritis
6.	MZC 38	Faeces	Mizoram	2022	OP729182	FPV	Mixed	2	F	Gastritis
7.	MHC 18	Faeces	Maharashtra	2021	OQ024194	2c	Domestic short-haired	3	F	Gastritis
8.	MHC 20	Faeces	Maharashtra	2021	OQ024195	2a	Domestic short-haired	1	M	Gastritis
9.	MNC 25	Faeces	Manipur	2021	OQ024196	2c	Mixed	6	M	None
10.	MNC 27	Faeces	Manipur	2021	OQ024197	2c	Mixed	6	F	Gastritis
11.	MNC 29	Faeces	Manipur	2021	OP729183	2c	Mixed	5	F	Gastritis
12.	MNC 30	Faeces	Manipur	2021	OQ024198	2c	Mixed	7	M	Gastritis
13.	MNC 31	Faeces	Manipur	2021	OQ024199	2c	Mixed	8	M	None
14.	MNC 32	Faeces	Manipur	2021	OP961983	2c	Mixed	24	F	None
15.	Mizoram 17*	Faeces	Mizoram	2020	OP778053	FPV	Mixed	3	M	Gastroenteritis

Table I. Summary of descriptive data of the cat (n=14) and dog FPV samples (OP778053) used for the present study. * Dog FPV sample.

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Score	Attitude	Appetite	Vomiting	Feces	Dehydration	Stool frequency	Temperature
1	Normal	Normal	Absent	Well-formed or absent	Normal eyes and bright	1-2/day	100-102°F
2	Mild to moderate depression	Inappetence	Mild (once per12 hrs)	Soft/pasty feces	Mild/Slight loss of skin elasticity, STT< 3 sec.	3-4/day	98-99°F
3	Severe depression	Anorexia	Moderate (2-5 times/12 hrs)	Watery diarrhea, non-bloody	Moderate (STT> 3 sec but < 10 sec.	5-6/day	<98 °F
4	Collapsed or moribund	Not offered	Severe (>6times per12 hrs)	Watery, bloody diarrhea	Unable to stand, Severe dehydration, STT> 10 sec.	>6times	>103°F

Table II. Clinical score card used for the cats with CPV-2/FPV gastroenteritis.

PCR screening and amplification of VP2 gene fragment

Viral DNA was extracted from stool samples using commercially available kit (QIAamp Fast DNA Stool Mini Kit, Qiagen). The quantity and the purity of DNA were checked in a microvolume spectrophotometer (Eppendorf BioSpectrometer basic). The DNA thus extracted was kept at –20ºC until further use. The extracted template DNA was screened for the presence of CPV-2 using the Hfor/Hrev primer pair listed in Table 3, which amplifies a 630 base pair (bp) fragment of the VP2 gene encoding capsid protein (Buonavoglia et al. 2001). The DNA prepared from CPV-2 vaccine strain (Canigen DHPPiL, Virbac) was used as positive control in the PCR assay. PCR amplification was carried out in 25 µL reaction mixture containing 50-100 ng template DNA, 12.5 µL of 2x PCR master mix [Thermo Fisher Scientific containing Taq DNA polymerase (0.05 U/µL), reaction buffer, 4 mM MgCl2, and 0.4 mM of each dNTP], 1 µL each of forward and reverse primer (10 pmol/µl) and nuclease free water to adjust the volume. PCR amplification consisted of initial denaturation at 95ºC for 5 mins followed by 35 cycles of denaturation at 95ºC for 30 s, annealing at 55ºC for 30 s and extension at 70ºC for 1 min and final extension at 70ºC for 10 mins. The PCR-amplified products were electrophoresed on 1.5% agarose gel in Tris acetate EDTA buffer and visualized under UV light and documented by gel documentation system (Vilber Bioprint, France).

Primer	Primer sequence (5'- 3')	Position (nt)	Amplicon size (bp)	Reference
H for	CAGGTGATGAATTTGCTACA	3556–3575	630	Buonavoglia *et al.* (2001)
H rev	CATTTGGATAAACTGGTGGT	4185-4166		

Table III. Primers for the amplification of partial VP2 gene of CPV-2/FPV.

Cloning of PCR product

Positive PCR products were purified from agarose gel by GeneJet Gel Extraction Kit (Thermo Scientific, K0702) followed by cloning with CloneJET PCR Cloning Kit (Thermo Scientific, K1232) as per the instructions given by the manufacturer.

Genotyping of CPV-2 isolates

The recombinant plasmid carrying correct insert, isolated from the representative clone was sent to Central Instrumentation Facility, Biotech Center, University of Delhi South Campus, India for sequencing. The sequence chromatogram was visualized and aligned in BioEdit v 7.2.5 analysis software (Isis Therapeutics, Carlsbad, CA, USA) to get a clean sequence of 630 nucleotides followed by BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast) to confirm the presence of CPV-2/FPV. The deduced aa sequences obtained were aligned with corresponding sequences available in GenBank using Clustal W of Mega 11 (Tamura *et al.* 2011) with default parameters. The aligned sequences (both nucleotide and aa) of VP2 genes of all the isolates were then submitted to GenBank for allotment of accession numbers.

Phylogenetic analysis

All the sequences obtained from this study along with the reference and vaccine sequences retrieved from GenBank were aligned using ClustalW and the phylogenetic analysis was performed employing the neighbor joining approach with Tamura 3-parameter model using MEGA 11 programme and deduced aa sequences of VP2, percentage homology and differences were analysed using DNA Star sequence analysis software (Tamura *et al.* 2011).

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Virus isolation

Ten CPV-2 PCR positive fecal samples were filtered and used for virus isolation in the Madin darby canine kidney (MDCK) cell line. The infected monolayers were harvested on day 3 post inoculation (with or without a cytopathic effect [CPE]) by three cycles of alternative freezing, thawing and centrifuged at 6000 g for 15 mins in a refrigerated centrifuge. The supernatants were collected in a microcentrifuge tube and stored at −80ºC until further use. Finally, the virus in the cell culture fluids was confirmed at the third passage level by PCR assay using the Hfor/Hrev primer pair.

Statistical analysis

Variant-wise clinical score comparison was done using Independent-Samples Kruskal-Wallis Test. The results were considered significant when P was < 0.05. Statistical analyses were carried out using statistical software (SPSS version 27.0).

Results

Polymerase chain reaction and epidemiological data

Out of total 38 fecal samples screened for CPV-2, 14 (36.84%) samples were found positive by PCR. Signalment data and descriptive statistics of the CPV-2/FPV positive cats are summarized in table 1. The median age of the cats was found to be between 3-6 months (range 1 month – 02 years). None of the cats were found to be vaccinated against FPV. Breed-wise occurrence was found to be highest among mixed breed (71.42%) followed by domestic short-haired (14.28%) and non-descript cats (14.28%). Major clinical signs observed (n=14) were anorexia (85.71%), mild to moderate depression (85.71%), moderate vomiting (71.42%) and hemorrhagic watery diarrhea (14.28%). Variant-wise clinical score comparisons (Table 4) revealed significant (P<0.05) difference in the degree of severity when CPV-2a, 2c and FPV affected cats were compared against healthy control. Similarly, mean clinical score comparison of FPV (9.5±2.61) affected cats were significantly (P<0.05) altered compared to CPV-2a (11.5±3.32) and 2c (12.14±3.51) affected cats. However, non-significant difference was observed when mean clinical score of CPV 2a was compared against CPV 2c.

SL. NO	Groups Compared	Mean±S.E.	Sig
1	Healthy control- FPV	8.81±4.58	S
2	Healthy control- CPV 2a	18.25±4.51	S
3	Healthy control- CPV 2c	22.43±4.54	S
4	FPV - CPV 2a	9.437±2.71	S
5	FPV - CPV 2c	13.62±3.48	S
6	CPV 2a- CPV 2c	5.18±1.61	NS

Table IV. Variant-wise clinical score (Mean±S.E.) comparison against healthy control on the day of presentation. S- significant at P>0.05 NS- Non-significant.

Sequencing and amino acid mutation

In order to characterize the detected CPV-2 virus, the coding VP2 gene sequence of 630 nucleotides was obtained from all the isolates. Based on the 426 amino acid (aa) residues of the deduced VP2 protein, 08/14 (57.14%) CPV-2s were classified as 2c, 04/14 (28.57%) was found to be FPV and 02/14 (14.28%) viruses were characterized as the 2a variant. The original CPV type 2 was not found. Several synonymous and non-synonymous mutations were noticed in all the types of CPV-2 variants (2a and 2c) and FPV under study when compared against database and the amino acid substitutions are summarized in Table 5. Non-synonymous mutations observed by CPV-2c isolates were Ser292Asn, Val294Leu, His309Gln, His318Gln, Gln370Arg, His404Gln, Leu404Gln, Glu424Val, Gln426Glu, Tyr427Asp, Val436Ile and Leu447Ile. Non-synonymous mutations observed by FPV isolates were Val401Ile and Ile466Asn. Similarly, non-synonymous mutations observed by CPV-2a isolates were Ser292Asn, Pro293Ser, Ile336Val, His404Gln, His426Asn and Ala440Thr.

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Isolate number	Amino acid position	292	293	294	309	318	336	370	401	404	424	426	427	436	440	466	Variant
790312	[a]M38245	Asn	Ser	Leu	Gln	Gln	Val	Gln	Ile	Gln	Val	Asn	Asp	Ile	Thr	Asn	CPV-2
CPV-LZ2	[b]JQ268284	Asn	Ser	Leu	Gln	Gln	Val	Gln	Ile	Gln	Val	Asp	Asp	Ile	Ala	Asn	2b
CU-4	[c]M38246	Asn	Ser	Leu	Gln	Gln	Val	Gln	Ile	Gln	Val	Asn	Asp	Ile	Thr	Asn	FPV
MZC 16	OP729179	-	-	-	-	-	-	-	Val	-	-	-	-	-	-	-	FPV
MZC 36	OP729180	-	-	-	-	-	-	-	Val	-	-	-	-	-	-	Ile	FPV
MZC 37	OP729181	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	FPV
MZC 38	OP729182	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	FPV
*Mizoram 17	OP778053	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	FPV
V154	[d]AB054217	Asn	Ser	Leu	Gln	Gln	Val	Gln	Ile	Gln	Val	Asp	Asp	Ile	Thr	Pro	2a
MHC 20	OQ024195	Ser	-	-	-	-	-	-	-	His	-	His	-	-	Ala	-	2a
MZC 02	OM891547	-	Pro	-	-	-	Ile	-	-	-	-	-	-	-	Ala	-	2a
cat234/08	[e]GU362935	Asn	Ser	Leu	Gln	Gln	Val	Gln	Ile	Gln	Val	Glu	Asp	Ile	Thr	Asn	2c
MHC 18	OQ024194	-	-	Val	His	His	-	-	-	His		Gln	Tyr	Val	-	-	2c
MNC 25	OQ024196	-	-	-	-	-	-	-	-	His	-	-	-	-	-	-	2c
MNC 27	OQ024197	Ser	-	-	-	-	-	-	-	Leu	-	-	-	-	-	-	2c
MNC 29	OP729183	-	-	-	-	-	-	Gln	-	-	-	-	-	-	-	-	2c
MNC 32	OP961983	-	-	-	-	-	-	-	-	-	Glu	-	-	-	-	-	2c
MZC 35	OP729184	-	-	-	-	-	-	-	-	-	Glu	-	-	-	-	-	2c

Table V. Amino acid variations in the CPV-2a, 2b and 2c VP2 capsid protein of feline isolates from the present study against reference, dog FPV sequence (Mizoram 17; OP778053) and vaccine strains. * Dog FPV sequence for comparison. Superscript a, b, c, d and e are indicative of reference strains for CPV-2, CPV-2b, FPV, CPV-2a and 2c, respectively

Phylogenetic analysis

The phylogenetic tree based on aa sequences of the present isolates against reference, field and the vaccine strains is depicted in Figure 1. The 2c isolates from the present study formed 3 separate clusters (*viz.* OP961983 and OP729184; OQ024198 and OQ024199; OQ024197, OQ024195, OQ024196, OQ024194 and OP729183). Besides, the 2c isolates were grouped together along with Indian (OP125772), Singaporean (ARR75645), Mongolian (QBB89700), South Korean (UID86069) and Bangladeshi (WCD68703) 2c isolates from the database (aa homology 98.9% - 100%) following the same evolutionary pattern, albeit, forming separate lineages. The aa homology within the 2c isolates was between 95.1% to 100%. Further, all the 2c sequences were closely grouped with dog 2c sequence (Figure 2) from our previous study (unpublished) with 99.5% to 100% aa homology indicating existence of possible transmission of CPVs between dogs and cats.

In the present study, isolate MHC 20 (CPV-2a) was found to be clustered with 2c isolate, MNC 27. Another 2a isolate (MZC 2) was found to be clustered with Indian, Thai and Singaporean 2a isolates with aa homology of 98.4%-98.9%. Both the 2a isolates (MHC 20 and MZC 2) had aa homology of 97.4% with each other. In addition, 2a isolates were also found to be clustered with dog 2a sequence from our previous study (unpublished) (Figure 2) with aa homology of 98.4%-98.9% which was indicative of possible transmission of CPVs between dogs and cats.

Similarly, all the four FPV sequences formed two separate clusters (MZC 37 and MZC 38; MZC 16 and MZC 36) and had amino acid homology of 99.5%-100% with each other and were ancestrally related to Indian dog (OP778053) and cat FPV sequences (aa homology of 98.9% to 100%) from the database hinting towards transmission of FPV between dogs and cats.

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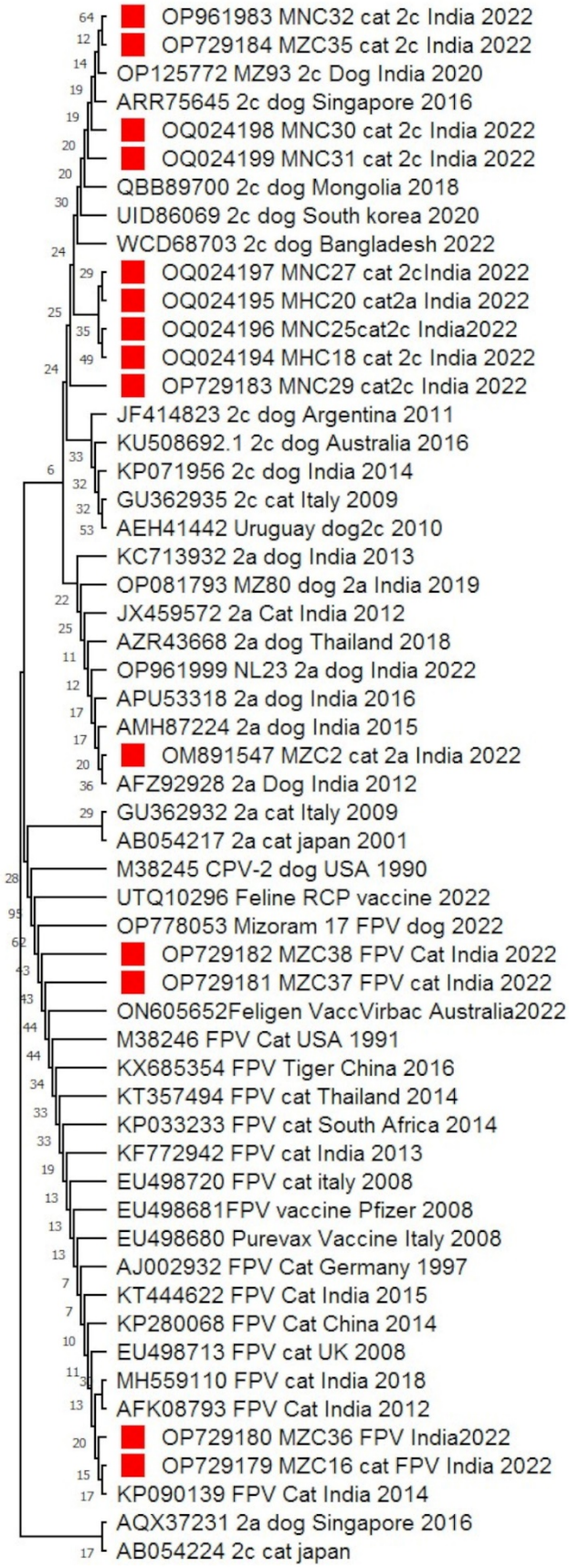

Figure 1. Phylogenetic analysis of partial VP2 sequences of CPV-2 variants and FPV isolates from the cats against the reference and vaccine strains using the Neighbour-joining method with Bootstrap consensus tree (1000 replications). The Tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The isolates obtained in this study are red coloured with accession number, followed by sample number, strain identity, host and year of isolation and. This analysis involved 55 amino acid sequences. Drawn using MEGA version 11.0.

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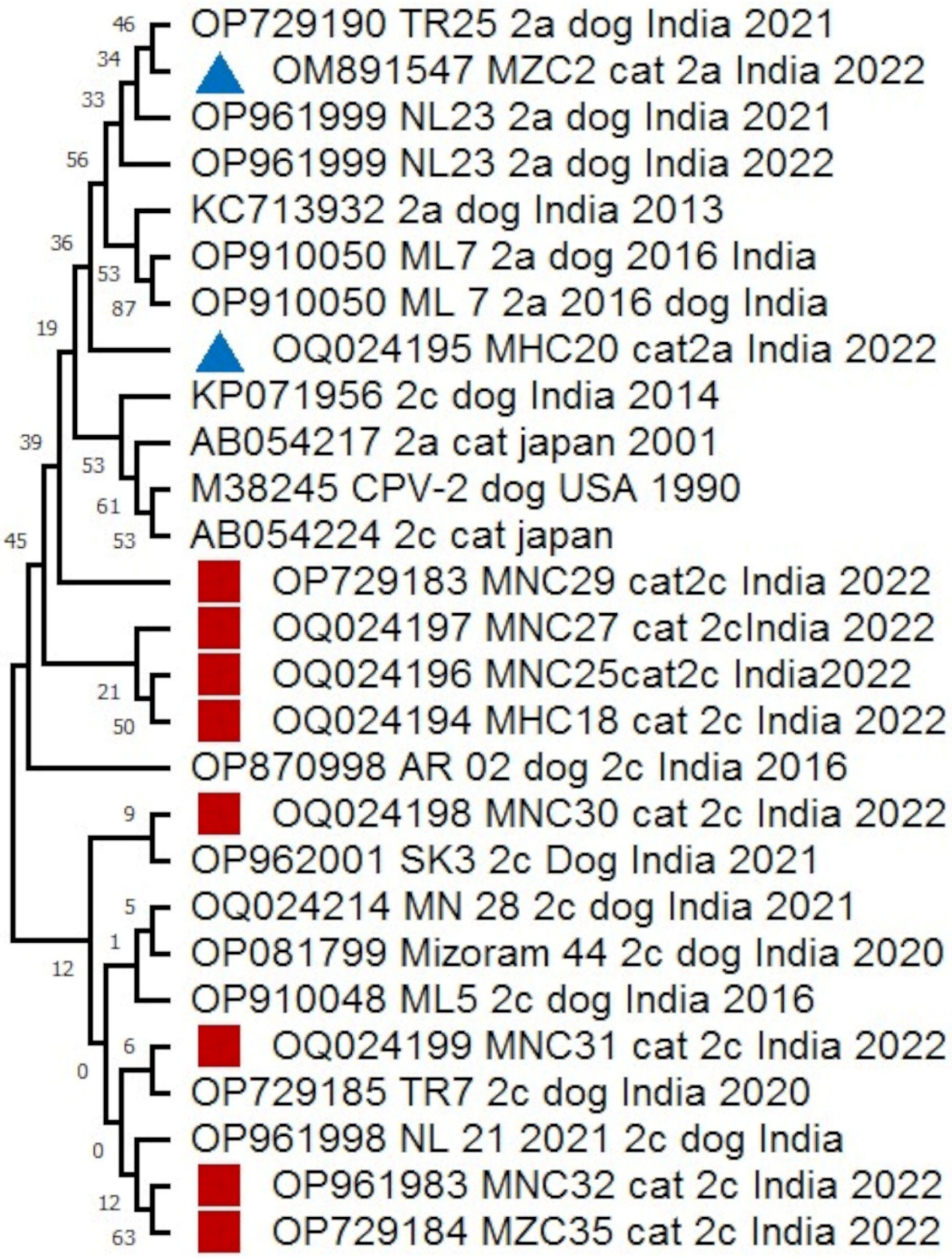

Figure 2. Phylogenetic analysis of partial VP2 sequences of CPV-2 variants from the cats against the reference and dog CPV-2 strains from our previous study using the Neighbour-joining method with Bootstrap consensus tree (1000 replications). The Tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The isolates obtained in this study are red coloured for 2c isolates and blue coloured for 2a isolates. This analysis involved 27 amino acid sequences. Drawn using MEGA version 11.0.

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Virus isolation

In MDCK cell culture, typical cytopathogenic effects (Figure 3) (rounding and detached cells) were observed for seven out of 10 CPV-2 positive cat samples at the third passage level (72 hours of incubation) and confirmed with PCR. No effort was made to revive FPV because of lack of feline specific cell lines.

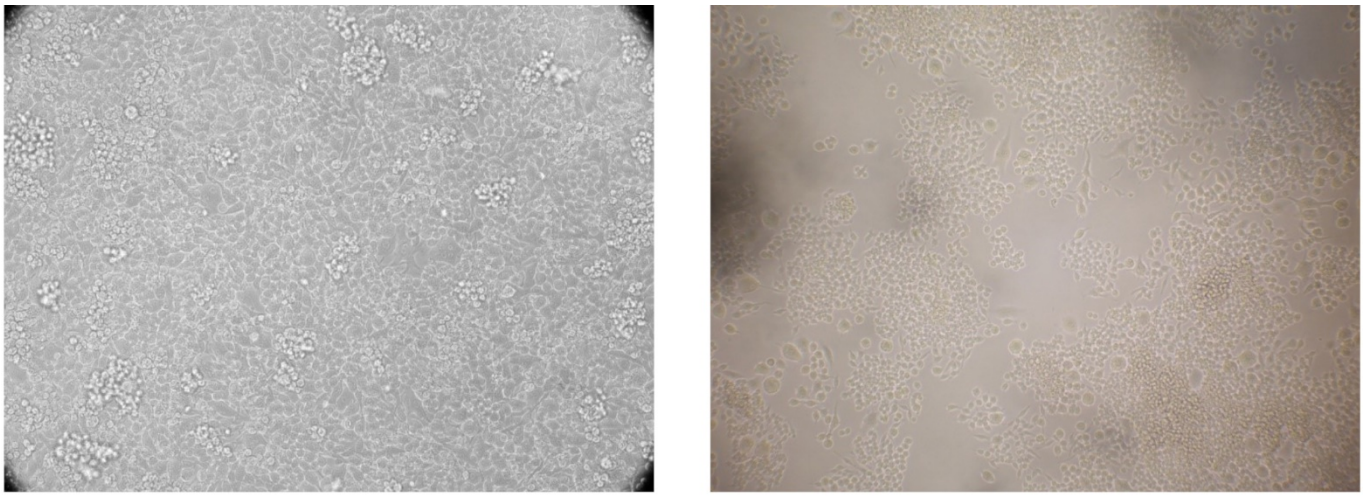

Figure 3. Citopathic effect of CPV-2c on MDCK cell culture. Left: Uninfected cells, Right: MDCK infected with CPV-2c (MHC 18) showing rounding and sloughing of cells (200X magnification).

Discussion

The present study revealed the presence of both CPV-2 and FPV infections among the cat population from four states of India. Of the 38 suspected cats screened by conventional PCR assay, 14 (36.84%) were found positive for CPV-2 variants/FPV. The PCR positivity of 36.84% was found to be in agreement with previous reports (Parthiban *et al.* 2014, Jing *et al.* 2015, Mukhopadhyay *et al.* 2016, Karanam *et al.* 2022). As such there are only few reports of CPV-2/FPV infection among cats in India and to the best of the author's knowledge, this is the first report of CVP-2c in cats from India and first report of CPV-2/FPV infection in cats from states like Mizoram and Manipur. So, the study provided in depth molecular insights into the distribution and evolution dynamics of the CPV-2/FPV from different geographical regions of India.

The median age of the cats was found to be between 3-6 months (range of 1month to 2 years) and was in accordance with earlier report (Stuetzer and Hartmann 2014). This might be attributed to viral replication in mitotically active tissues such as intestinal mucosa, lymphoid tissue and bone marrow (Parker *et al.* 2001).

The most prominent clinical signs among the affected cats were anorexia (85.71%), depression (71.42%), vomition (71.42%) and diarrhea (50%) which was in agreement with earlier report (Miranda et al. 2014; Nelson and Couto 2014). To assess the severity of clinical signs across variants, mean clinical score of FPV, 2a and 2c affected cats were compared against healthy control as well as against each other on the day of presentation. There was significant increase in severity of clinical signs in all the affected cats compared to healthy control. Severity of clinical signs was also significantly more pronounced in 2a and 2c affected cats compared to FPV affected cats. 2c has been reported to cause severe disease in adult dogs and also in dogs that have completed the vaccination protocols (Decaro and Buonavoglia 2012). However, non-significant alteration of clinical severity among 2a and 2c affected cats might be due to small sample size and need to be repeated in larger sample size. As such many studies noted a striking variety in the clinical course of CPV-2 infection in dogs and cats ranging from subclinical infection to acute fatal illness (Miranda *et al.* 2014). This variation was largely attributed to age of infection, lack of protective immunity, stress level and having a higher number of dividing cells and concurrent infections (Schoeman *et al.* 2013).

One striking finding in the current investigation was lack of vaccination of 100% of the cats, which raised a major concern about the screening population's lack of disease-protective immunity and was in agreement with previous report (Mukhopadhyay *et al.* 2016).This further complicates the scenario as cats act as carriers and can easily transmit the infection to unprotected dogs.

CPV-2c was the predominant variant (57.14 %) in the study population which was indicative of shifting trend of CPV-2 antigenic variant in this part of the country as earlier reports from different parts of India reported FPV or CPV-2a or new CPV-2a as the predominant variant (Parthiban *et al.* 2014, Mukhopadhyay *et al.* 2014, Karanam *et al.* 2022) among feline population. Further, our recent large-scale study to determine the prevalence of CPV-2 variants in canine

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population (unpublished) also revealed CPV-2c as the predominant variant (61.26%). At present, CPV-2c represents only a small percentage of parvovirus in canine and feline populations in India; however, looking into the current trend from the present study, authors presumed a major antigenic shift in favor of CPV-2c both in canine and feline populations. This presumption will be confirmed in future studies from the country as similar to what has happened in the past in other countries, the new variant may likely become the predominant variant in the field (Pereira *et al.* 2007). To make the matter worse, CPV-2c have already revealing itself as the predominant feline variant in some of the Asian countries along with FPV, CPV-2a, new CPV-2a and new CPV-2b (Pan *et al.* 2023, Tang *et al.* 2022).

All the CPV-2c isolates from the present study showed a synonymous mutation with aa Glu (type 2c) at residue 426 of the VP2 protein confirming them as CPV-2c variant (Decaro and Buonavoglia 2012). Further, highest number of non-synonymous mutations (12) were observed by CPV-2c isolates such as Ser292Asn, Val294Leu, His309Gln, His318Gln, Gln370Arg, His404Gln, Leu404Gln, Glu424Val, Gln426Glu, Tyr427Asp, Val436Ile and Leu447Ile compared to six in 2a and two in FPV isolates. This could be attributed to higher sequence variability of CPV-2c due to its acquisition of multiple nucleotides and amino acid changes over time (Battilani *et al.* 2019). Mutation Gln370Arg was shown by 2c sequence (MNC 29) from the present study had also been reported from Italian, Chinese, Taiwanese, Thai, and Japanese canine 2c strains (Yi *et al.* 2016, Geng *et al.* 2017, Zhao *et al.* 2017, Mira *et al.* 2018, Jiang *et al.* 2021). As per the existing information, residue 370 may be necessary for a conformational shift or may influence receptor binding via neighboring residues (Buonavoglia *et al.* 2001). Non-synonymous mutations observed by FPV isolates were Val401Ile and Ile466Asn. Similarly, non-synonymous mutations observed by CPV-2a isolates were Ser292Asn, Pro293Ser, Ile336Val, His404Gln, His426Asn and Ala440Thr. Amino acid mutation 440 Thr→Ala was reported in both the 2a isolates (MHC 20 and MZC 2) from the present study and had been reported in canine CPV-2a, 2b and 2c sequences from different parts of the world (Chinchkar*et al.* 2006, Clegg *et al.* 2012, Calderón *et al.* 2011, Battilani *et al.* 2019). The 440 residue sits at the peak of the threefold spike and considered to be the primary antigenic site of the virus (Chapman and Rossmann 1993). The remaining non-synonymous mutations were unique to this study and had not been reported till date. These non-synonymous mutations were recorded in the GH loop of VP2 protein (267–498 residues located between βG and βH strands) of the virus which were exposed on the surface of capsid and likely to undergo mutation aiding in evolvement of new variants (Kang *et al.* 2008).

Presence of CPV variants (CPV-2a and CPV-2c) in cat from the present study proves the existence of interspecies transmission. Further, close similarity of the present cat FPV sequences with dog FPV sequence (OP778053) from our previous study was indicative of possible transmission of FPV between cats and dogs.

This study confirms that cat population is susceptible to both CPV variants and FPV facilitating interspecies transmission and high genetic heterogeneity. Complete lack of vaccination in the cat population once again highlights the urgent need of educating the owners regarding compulsory vaccination against FPV. Further, studies evaluating the cross protection between FPV vaccines against CPV infection in cats is need of the hour.

In conclusion, the present study provided important update with respect to the evolutionary phylodynamics of parvovirus infections in cats from four states of India. However, further studies with larger sample size over a wider geographical regions incorporating complete VP2 gene analysis are imperative for in-depth understanding of the problem helping in early diagnosis, timely intervention and the development of strategies for feline vaccination in lieu of predominance of CPV-2c. The present finding will also sensitize veterinary practitioners to put more attention on both CPV and FPV infections in light of interspecies transmission. Last but not the least, the role of cats as the source of new variants of CPV-2 needs to be constantly monitored in the country to rule out mutations along with recombination thereby causing emergence and evolution of parvoviruses.

References

Balboni A., Bassi F., De Arcangeli S., Zobba R., Dedola C., Alberti A. & Battilani M. 2018. Molecular analysis of carnivore Protoparvovirus detected in white blood cells of naturally infected cats. BMC Vet Res, 14, 41.

Battilani M., Balboni A., Giunti M. & Prosperi S. 2013. Co-infection with feline and canine parvovirus in a cat. Vet Ital,49, 127–129.

Battilani M., Gallina L., Vaccari F. & Morganti L. 2007. Co-infection with multiple variants of canine parvovirus type 2 (CPV-2). Vet Res Commun,31: 209–212.

Battilani M., Modugno F., Mira F., Purpari G., Di Bella S., Guercio A. & Balboni A. 2019. Molecular epidemiology of

canine parvovirus type 2 in Italy from 1994 to 2017: recurrence of the CPV-2b variant.BMC Vet Res, 15, 1-13.

Buonavoglia C., Martella V., Pratelli A., Tempesta M., Cavalli A., Buonavoglia D., Bozzo G., Elia G., Decaro N. & Carmichael, L. 2001. Evidence for evolution of canine parvovirus type 2 in Italy. J Gen Virol, 82(12), 3021-3025.

Calatayud O., Esperon F., Velarde R., Oleaga A., Llaneza L., Ribas A., Negre N., de la Torre A. Rodriguez A. & Millan J. 2020. Genetic characterization of Carnivore Parvoviruses in Spanish wildlife reveals domestic dog and cat-related sequences. Transbound Emerg Dis, 67, 626–634.

Calderon M.G., Romanutti C., D'Antuono A., Keller L., Mattion N. & La Torre, J. 2011. Evolution of canine parvovirus in Argentina between years 2003 and 2010: CPV2c has become the predominant variant affecting the domestic dog population. Virus research, 157(1), 106-110.

Chapman M.S., & Rossmann M. G. 1993. Structure, sequence, and function correlations among parvoviruses. Virol, 194(2), 491-508.

Charoenkul K.,Tangwangvivat R., Janetanakit T., Boonyapisitsopa S., Bunpapong N., Chaiyawong S., & Amonsin A. 2019. Emergence of canine parvovirus type 2c in domestic dogs and cats from Thailand. Transbound Emerg Dis, 66(4), 1518–1528.

Chinchkar S.R., Mohana Subramanian B., Hanumantha Rao N., Rangarajan P.N., Thiagarajan D. & Srinivasan V.A. 2006. Analysis of VP2 gene sequences of canine parvovirus isolates in India. Arch Virol, 151, 1881-1887.

Clegg S.R., Coyne K.P., Dawson S., Spibey N., Gaskell R.M. & Radford A.D. 2012. Canine parvovirus in asymptomatic feline carriers. Vet Microbiol, 157(1-2), 78-85.

Cotmore S.F., Agbandje-McKenna M., Canuti M., Chiorini J.A., Eis-Hubinger A.M., Hughes J., Mietzsch M., Modha S., Ogliastro M., Pénzes J.J. & Pintel D.J. 2019. ICTV virus taxonomy profile: Parvoviridae. J Gen Virol, 100(3), 367-368.

Decaro N. &Buonavoglia C. 2012. Canine parvovirus – a review of epidemiological and diagnostic aspects, with emphasis on type 2c. Vet Microbiol,155(1), 1–12.

Decaro N., Buonavoglia D., Desario C., Amorisco F., Colaianni M.L., Parisi A., Terio V., Elia G., Lucente M.S., Cavalli A. & Martella V. 2010. Characterisation of canine parvovirus strains isolated from cats with feline panleukopenia. Res Vet Sci, 89(2), 275-278.

Decaro N., Desario C., Amorisco F., Losurdo M., Colaianni M.L., Greco M.F. & Buonavoglia, C. 2011. Canine parvovirus type 2c infection in a kitten associated with intracranial abscess and convulsions. J Feline Med Surg, 13(4), 231–236.

Decaro N., Desario C., Parisi A., Martella V., Lorusso A., Miccolupo A., Mari V., Colaianni M.L., Cavalli A., Di Trani L. & Buonavoglia C. 2009. Genetic analysis of canine parvovirus type 2c. Virol, 385(1), 5-10.

Gamoh K., Shimazaki Y., Senda M., Makie H., Itoh O., Inoue Y. 2003. Antigenic type distribution of parvovirus isolated from domestic cats in Japan. Vet Rec, 153(24), 751–752.

Geng Y., Wang J., Liu L., Lu Y., Tan K and Chang Y. 2017. Development of real-time recombinase polymerase amplification assay for rapid and sensitive detection of canine parvovirus 2. BMC Vet Res, 13, 1-7.

Jiang H., Yu Y., Yang R., Zhang S., Wang D., Jiang Y., Yang W., Huang H., Shi C., Ye L., Yang G., Wang J., & Wang C. 2021. Detection and molecular epidemiology of canine parvovirus type 2 (CPV-2) circulating in Jilin Province, Northeast China. Comp Immunol Microbiol Infect Dis, 74, 101602.

Kang B.K., Song D.S., Lee C.S., Jung K.I., Park S.J., Kim E.M. & Park B.K. 2008. Prevalence and genetic characterization of canine parvoviruses in Korea. Virus Genes, 36,127–133.

Karanam B., Srinivas M.V., Vasu J., Xavier A.P., Karuppiah R., Shanmugam V.P.& Mukhopadhyay H.K. 2022. Phylodynamic and genetic diversity of parvoviruses of cats in southern India. Virusdisease, 33(1),108-113.

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Markovich J.E., Stucker K.M., Carr A.H., Harbison C.E., Scarlett J.M. & Parrish C.R. 2012. Effects of canine parvovirus strain variations on diagnostic test results and clinical management of enteritis in dogs. J Am Vet Med Assoc, 241(1), 66-72.

Milićević, V., Glišić, D., Veljović, L. Vasić A., Milovanović B., Kureljušić B. & Paunović M. 2023. Protoparvovirus carnivoran 1 infection of golden jackals Canis aureus in Serbia. Vet Res Commun, https://doi.org/10.1007/s11259-023-10249-0

Mira F., Purpari G., Di Bella S., Colaianni M.L., Schirò G., Chiaramonte G., Gucciardi F., Pisano P., Lastra A., Decaro N. & Guercio A. 2019. Spreading of canine parvovirus type 2c mutants of Asian origin in southern Italy. Transbound Emerg Dis, 66(6), 2297-2304.

Miranda C., Parrish C.R. & Thompson G. 2014. Canine parvovirus 2c infection in a cat with severe clinical disease. J Vet Diagn Invest, 26(3), 462-464.
Mochizuki M., Harasawa R. & Nakatani, H. 1993. Antigenic and genomic variabilities among recently prevalent parvoviruses of canine and feline origin in Japan. Vet Microbiol, 38(1-2), 1–10.

Mukhopadhyay H.K., Matta S.L., Amsaveni S., Antony P.X., Thanislass J. & Pillai R.M. 2014. Phylogenetic analysis of canine parvovirus partial VP2 gene in India. Virus Genes, 48, 89-95.

Mukhopadhyay H.K., Nookala M., Thangamani N.R., Sivaprakasam A., Antony P.X., Thanislass J., Srinivas M.V. & Pillai R.M. 2016. Molecular characterisation of parvoviruses from domestic cats reveals emergence of newer variants in India. J Feline Med Surg, 19, 846–852.

Nelson R.W. & Couto C.G. 2014. Feline Parvoviral Enteritis. In Small animal Internal Medicine, 5th Ed. Elsevier Mosby, Missouri, 459-460.
Pan S., Jiao R., Xu X., Ji J., Guo G., Yao L., Kan, Y., Xie Q. & Bi Y. 2023. Molecular characterization and genetic diversity of parvoviruses prevalent in cats in Central and Eastern China from 2018 to 2022. Front Vet Sci, 10.

Parker J.S., Murphy W.J., Wang D., O'Brien S.J. & Parrish C.R. 2001. Canine and feline parvoviruses can use human or feline transferrin receptors to bind, enter, and infect cells. J Virol, 75(8), 3896-3902.

Parrish C.R., Aquadro C.F., Strassheim M.L., Evermann J.F., Sgro J.Y. & Mohammed H. 1991. Rapid antigenic-type replacement and DNA sequence evolution of canine parvovirus. J Virol, 65(12), 6544-6552.

Parthiban M., Aarthi K.S., Balagangatharathilagar M. & Kumanan K. 2014. Evidence of feline panleukopenia infection in cats in India. Virus Dis, 25, 497-499.

Pereira C.A., Leal E.S., & Durigon E.L. 2007. Selective regimen shift and demographic growth increase associated with the emergence of high-fitness variants of canine parvovirus. Infect Genet Evol, 7(3), 399-409.

Schoeman J.P., Goddard A. & Leisewitz A.L. 2013. Biomarkers in canine parvovirus enteritis. N Z Vet J 61(4), 217-222.

Stuetzer B. & Hartmann K. 2014. Feline parvovirus infection and associated diseases. Vet J, 201(2), 150-155.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M. & Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol, 28(10), 2731-2739.

Tang Y., Tang N., Zhu J., Wang M., Liu Y. & Lyu Y. 2022. Molecular characteristics and genetic evolutionary analyses of circulating parvoviruses derived from cats in Beijing. BMC Vet Res, 18(1), pp.1-11.

Truyen U., Evermann J.F., Vieler E. & Parrish C.R. 1996. Evolution of canine parvovirus involved loss and gain of feline host range. Virol, 215, 186–189.

Url A., Truyen U., Rebel-Bauder B., Weissenböck H. & Schmidt P. 2003. Evidence of parvovirus replication in cerebral

neurons of cats. J Clin Microbiol, 41(8), 3801-3805.

Wu J., Gao X.T., Hou S.H., Guo X.Y., Yang X.S., Yuan W.F., Xin T., Zhu H.F. & Jia H. 2015. Molecular epidemiological and phylogenetic analyses of canine parvovirus in domestic dogs and cats in Beijing, 2010–2013. J Vet Med Sci, 77(10), 1305-1310.

Yi L., Tong M., Cheng Y., Song W. & Cheng S. 2016. Phylogenetic Analysis of Canine Parvovirus VP 2 Gene in China. Transbound Emerg Dis, 63(2), 262-e269.

Zhao H., Wang J., Jiang Y., Cheng Y., Lin P., Zhu H., Han G., Yi L., Zhang S., Guo L. & Cheng S. 2017. Typing of canine parvovirus strains circulating in North-East China. Transbound Emerg Dis, 64(2), 495-503.

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