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Molecular Characterization of Canine Parvo Virus Variant-2 in Cats in Chennai by Amplification-Refractory Mutation System Polymerase Chain Reaction

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ABSTRACT

Background: Feline panleukopenia virus and canine parvovirus infections are highly contagious and serious enteric diseases of cats and dogs with high fatality rate. Canine parvo viral enteritis (CPV) is caused by CPV-2 antigenic variants (CPV-2a, 2b, 2c) is frequently reported in dogs worldwide leading to morbidity and mortality Infection in cats by canine parvo virus variants causes clinical signs similar to feline panleukopenia virus. CPV-2 variants have recently acquired the feline host rage, allowing it to infect both cats and dogs. Feline Panleukopenia virus is not the only parvovirus species affecting cats, in addition to Mink enteritis virus, the new variants of canine parvovirus, CPV-2a, 2b and 2c have also penetrated the feline host-range, and able to infect and replicate in cats, causing diseases indistinguishable from feline panleukopenia. The present study was taken to identify the canine parvovirus variants among domestic cats in Chennai and its role in transmission of CPV-2 between dogs through Amplification refractory mutation system. These findings suggest that species jump of CPV from dog to cats as well as CPV had presumably started a new process of readapting in feline hosts and confirmed the importance of viral host switching as a mechanism for the emergence of new viruses.

Methods: In this study around 166 cat faecal swabs were collected during 2020-2022 in and around Chennai which were brought with the clinical signs of vomition and diarrhoea. Collected faecal samples were subjected to DNA extraction and Amplification Refractory Mutation System Polymerase Chain Reaction and 50 cat samples showed positivity towards canine parvovirus infection.

Result: Of 166 faecal samples, 50 samples showed positive for the CPV-2a variant. By ARMS-PCR In this case, the 50 samples responding to outer forward and inner reverse primers and yielding a 492 bp amplicons in addition to the 631 bp common outer product as CPV-2a/2. This study suggested that CPV infection in cat showed that CPV has started a new process of readaptation in the feline host and confirming the importance of viral host switching mechanism as a mechanism for the emergence of new viruses. The introduction of CPV into the feline population raises concern about the efficacy of FPV-based vaccines in preventing CPV infection and point out the necessity for intensifying surveillance of parvovirus infection in cats.

Key words: ARMS-PCR, Canine parvovirus, CPV-2a, Canine parvovirus variant in cats, Feline parvovirus.

INTRODUCTION

Feline panleukopenia virus and canine parvovirus infections are highly contagious and serious enteric diseases of cats and dogs with high fatality rate. Canine parvo viral enteritis is caused by CPV-2 antigenic variants (CPV-2a, 2b, 2c) is frequently reported in dogs worldwide leading to morbidity and mortality Infection in cats by canine parvo virus variants causes clinical signs similar to feline panleukopenia virus. CPV-2 variants have recently acquired the feline host rage, allowing it to infect both cats and dogs. Canine parvovirus belongs to the genus protoparvovirus, family Parvoviridae. Parvoviruses of carnivores include three closely related parvoviruses: Canine parvovirus (CPV), feline panleukopenia virus (FPV) and mink enteritis virus (MEV). FPV is not the only parvovirus species affecting cats, in addition to MEV, the new variants of canine parvovirus, CPV-2a, 2b and 2c have also penetrated the feline host-range and able to infect and replicate in cats, causing diseases indistinguishable from feline panleukopenia. CPV-2a like strains was isolated from non-symptomatic cats in Japan (Mochizuki *et al*., 1993).

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CPV was also detected in cats with the identification of 2b strains in the USA (Truyen *et al*., 1996) and CPV-2a/2b strains in Germany (Truyen *et al*., 1996). In cats, CPV causes a mild to subclinical disease where the symptoms are less severe than those in dogs (Mochizuki *et al*., 1993).

FPLV causes systemic infection in cats, in dogs FPLV only replicates to a very low level in lymphoid tissues such as the thymus, not in gut and it is not shed, nor does it induce clinical signs (Truyen and Parrish, 1992). CPV strains can replicate in both canine and feline cells in cell culture, whereas FPLV strains can replicate efficiently only in feline cells (Truyen and Parrish, 1992). New variants of CPV (2a, 2b, 2c) can bind both the feline transferrin receptor (Hueffer and Parrish, 2003) and canine transferrin receptors that infect cats (Ikeda *et al*., 2002) as well as dogs.

ARMS-PCR is a technique designed for the detection of known sequence polymorphisms (Newton *et al*., 1989). In this technique, two pairs of primers in a single PCR tube can simultaneously amplify different alleles as well as amplify in internal DNA. The inner forward and inner reverse primers with outer reverse and outer forward primers respectively generate allele-specific amplicons which will be of different sizes and hence easily discriminated on an Agarose gel (Ye *et al*., 2001).

The present study was taken to identify the canine parvovirus variants among domestic cats in Chennai and its role in transmission of CPV-2 between dogs. A Simple one-step ARMS-PCR assay was used to find out the circulating antigenic variants of CPV-2 involved in the cat population by partial amplification of VP2 gene. These findings suggest that species jump of CPV from dog to cats as well as CPV had presumably started a new process of re adapting in feline hosts and confirmed the importance of viral host switching as a mechanism for the emergence of new viruses.

MATERIALS AND METHODS

Sample collection

A total of 166 faecal swabs were collected from cats presented with the symptoms of anorexia, fever, ocular discharge, oral ulcer, yellowish diarrhoea with dehydration at different age groups of cats in and around Chennai. The faecal samples/rectal swabs were collected in Hi-media swabs and stored at 4°C till further use.

DNA extraction

DNA extraction was carried out for the all 166 samples as per the manufacturer guidelines (Qiagen). DNA concentration was checked by Nano drop method. A minimum of 45 ng/ul concentration DNA was used for Tetra arm PCR to find out the CPV-2 variants. The filtrate was collected and stored at -80°C until further use. The supernatant was used as a template in the PCR assay.

ARMS PCR

All the 166 samples were screened for ARMS –PCR to detect CPV-2 variants in cats using two pairs of primers (outer forward and reverse primers and inner forward and reverse primers) (Kalyani *et al*., 2021) (Table 1). The ARMS-PCR mix composition consist of (20ul) PCR master Mix (2x) 10 µl, Forward primer-outer 0.6 ul, Reverse primer-outer 0.6 µl, Forward primer-Inner 0.6 ul, Reverse primer-Inner 0.6 µl , Template DNA 2 ul, Nuclease free water 5.6 µl.

The cycling parameters for ARMS-PCR consistof initial denaturation at 95°C for five minute followed by 35 cycles of denaturation at 95°C for 30s, annealing at 45°C for 30s and extension at 72°C for one minute, with a final extension step at 72°C for ten minutes.

All the PCR amplified products were resolved on 3% Agarose gel in 1× Tris-acetate-EDTA (TAE) buffer and visualized under UV light in a gel documentation system (Bio-rad, India) and sequenced at Eurofins Genomics India Pvt. Ltd, Bangalore using VP2 gene specific primers used for amplification.

Genotyping of canine parvo variants (CPV-2a) in cats

Three randomly selected positive PCR products of (CPV-2a) cats were sequenced at Eurofins Genomics, Bengaluru, India.

RESULTS AND DISCUSSION

ARMS-PCR

Of 166 faecalsamples, 50 samples showed positive for the CPV-2a variant (Fig 1). By ARMS-PCR. The outer primers (OF and OR) targeting the conserved region of VP2 631 bp in all antigenic variants of CPV-2 (Chander *et al*., 2016). The Inner forward primer targeting SNP at 1276 nucleotide position at its 3′ end yields an amplicons size of 178 bp with the outer reverse primer specifically in CPV-2b/2c. The Inner reverse primer was designed targeting the SNP at 1278 nucleotide position at its 3′ end yielding an amplicons size of 492 bp with the outer forward primer specifically in CPV-2/2a/2b but not in CPV-2c. In this case, the 50 samples responding to outer forward and inner reverse primers and yielding a 492 bp amplicons in addition to the 631 bp common outer product as CPV-2a/2.

Table 1: List of primers used in ARMS-PCR.

Primer code	Sequence of the primer (5′-3′)	Amplicons size (bp)	Reference
CPV-outer FP	TGATTGTAAACCATGTAGACTA	631	Chander *et al*., 2016
CPV-outer RP	AAGTCAGTATCAAATTCTTTATC		
CPV-inner FP	ACTTTAACCTTCCTGTAAGAG	179	
CPV-inner RP	GTTGGTAGCAATACATTAGCA	492	Kalyani *et al*., 2021

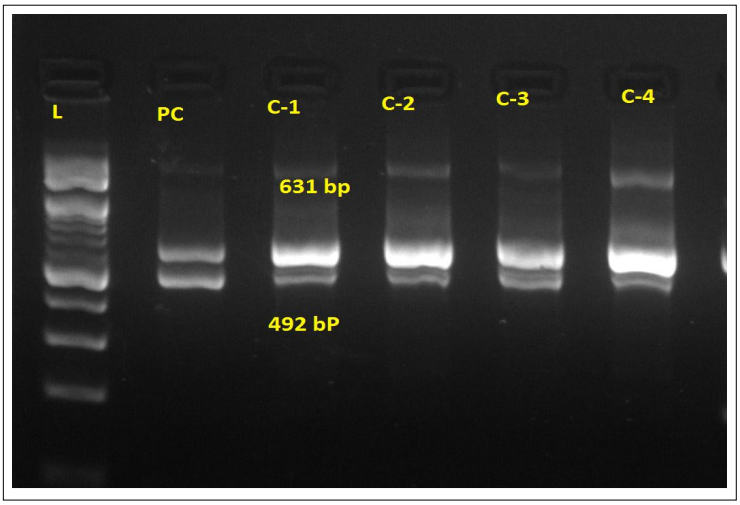

Fig 1: PCR gel image depicting the amplification CPV-2a gene (631 bp+492bp). PC- Positive content. C_1, C_2, C_3, C_4- Ca= Cpv- 2a positive samples.

Sequencing and blast analysis

A nucleotide basic local alignment search tool (BLAST) analysis was performed with the obtained sequences using the NCBI nucleotide database to confirm the genus specificity to canine parvovirus. VP2 partial gene sequences of canine parvovirus (CPV-2a) variant in cats from different parts of the world were retrieved from the NCBI nucleotide database and sequence assembly alignment was performed using the DNA baser assembler.

Upon BLAST analysis, the specificity of the sequences of partial VP-2 gene of canine parvovirus amplified from cat faecal samples representing 98-99 per cent query coverage with canine parvovirus variant and related to 99 per cent matched with canine parvovirus variant. This CPV-2 variant in cat from Chennai was found to be maximally identical with VP2 gene sequences of canine parvovirus strains and feline parvovirus available in GenBank.

Nucleotide sequence

The sequence of two cat isolates (C91 and C92) had maximum identity (99%) with Feline parvo virus partial VP2 gene, Feline parvovirus strain C-Dy6, Feline parvo isolates cat1 NS1, NS2,VP1 and VP2 genes and 98.33% identity with feline parvovirus isolates cat 2 NS1, NS2 gene complete sequence - VP1 and VP2 gene partial and 97% identity with feline parvovirus strain C27 VP2 gene partial sequence, feline parvo virus strain C26 VP2 gene partial sequence, Feline parvovirus strain C31 VP2 gene partial sequence, Feline parvo strain 25-2 VP2 gene partial sequence, Feline parvo strain 25-1 VP2 gene partial sequence, Feline parvo strain C10- 2 VP2 gene partial sequence.

Out of 166 faecal samples, 50 samples were shown positive in ARMS-PCR (Kalyani *et al*., 2021). ARMS-PCR was highly sensitive for detection and typing of canine parvovirus variant in cats (Kalyani *et al*., 2021). The outer primers targeting the conserved region of VP2 gene amplifies 631 bp in all antigenic variants of CPV-2 (Chander *et al*., 2016). We report that one-step ARMS-PCR assay as a simple, cost effective and sequence independent diagnostic technique for identifying CPV-2a variants in comparison with other available approach. The appearance of expected size products in agarose gel was confirmed at 631 bp and 492 bp in the clinical samples. In this present study, sequencing was done to confirm the PCR products further and it showed 98-99 per cent homology with canine parvovirus strain and feline parvovirus. The present study is the report of detection of parvovirus infection among domestic cat populations in and around Chennai based on partial VP2 gene analysis. This study suggest that the variant of CPV appear to be able to infect cats and other hosts such as coyote, bobcat and raccoon because the variant can bind the feline transferrin receptors and canine receptors (Allison *et al*., 2013).

Battilani *et al*., (2013) reported that CPV in cats showed the clinical signs which was similar in feline panleukopenia or milder clinical signs of parvovirus infection which was showed in dogs. Diarrhea, Vomition, fever, dullness and anorexia correlated with the clinical signs of diarrhea, fever and vomiting, which have also been reported earlier in parvovirus infections in cats and dogs (Qi *et al*., 2020; Kruse *et al*., 2010). These findings correlated with our study showed clinical signs of diarrhea, vomition, anorexia, fever and lethargy among the susceptible cats.

This study reported the CPV-2a positivity among cats in and around Chennai correlated with the findings of Ikeda *et al*. (2002) demonstrated that CPV-2a pand CPV-2b are prevalent in cat populations in Southeast Asia. CPV was detected not only in Asia, it was also reported in many countries in the other continents like Australia, Africa, the Americas as well as Europe (Miranda and Thompson 2016).

This study clearly indicate that positivity of CPV variant among cats in and around Chennai was correlated with Clegg *et al*.,(2012) suggests that cat could potential risk factor for infecting dogs and other cats.

Mukhopadhyay *et al*., (2016) suggest that presence of CPV in both healthy and diarrheic cats emphasizes the possible role of cats as a source of new variants of parvovirus.

These study findings correlated with Batillani *et al*. (2011) suggested that CPV infection in cat showed that CPV has started a new process of readaptation in the feline host and confirming the importance of viral host switching mechanism as a mechanism for the emergence of new viruses. The introduction of CPV-2 variant into the feline population raises concern about the efficasyof FPV-based vaccines in preventing CPV-2 variant infection and point out the necessity for intensifying surveillance of parvovirus infection in cats.

In these present study cats shown positivity were unvaccinated domestic cats in controversy with Ikeda *et al*. (2000) was reported that canine parvovirus virus can infect unvaccinated cats , even in animals that carry neutralising antibodies against the feline parvovirus vaccine.

CONCLUSION

CPV-2 is now known to infect both wild and domesticated feline species. Cat is susceptible to both feline panleukopenia and canine parvo virus infection. The cats also act as carrier and source of infection to dogs. The strain currently circulating in the cat population causing disease is CPV-2a. The presence of CPV-2 variant in cats emphasis the possible role of cats as a source of infection for dogs. Further epidemiological and virological surveillance of the new antigenic strains of CPV-2 variant are clearly needed for controlling parvovirus disease in wild and domestic cats, as well as in dogs. In this case only one variant reported among all the cats. This study provides important results of CPV-2 variant infections in cats, showing that CPV-2 variant has presumably started a new process of readaptation in the feline host and confirming the importance of viral host switching as a mechanism for the emergence of new viruses.

ARMS-PCR assay as a simple, cost effective and sequence independent diagnostic technique for detection and typing of CPV-2 antigenic variants in a single step approach in comparison with other molecular techniques. This study revealed the CPV-2a predominantly circulating in cat population in and around Chennai. This is also emphasizes introduction of parvo virus infection into the feline population raises concerns about the efficacy of Feline pan leukopenia based vaccine in preventing parvo virus infection.

Conflict of interest: None.

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