Detection of Canine Parvovirus-2c Variants from Dogs using Amplification Refractory Mutation System Polymerase Chain Reaction

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ABSTRACT

Canine Parvovirus-2c (CPV-2c) infection in dogs has been a growing concern due to its severe clinical manifestations. This study aims to determine the occurrence of CPV-2c among dogs in Kerala using amplification refractory mutation system polymerase chain reaction (ARMS-PCR). A total of 60 dogs exhibiting signs of CPV-infection were included in the study, and faecal samples collected from these dogs were subjected to ARMS-PCR. Out of 52 samples positive for CPV 34 were positive for CPV-2c, and sequencing was performed to confirm the presence of CPV-2c. This marks the second study investigating the occurrence of CPV-2c in Kerala, highlighting the significance of this variant in the region.

Keywords: Canine, Parvovirus, Arms-PCR, CPV-2C

INTRODUCTION

Canine parvovirus type 2 (CPV-2), which emerged around the 1970s from a proto-parvovirus that infected wild or domestic carnivores (Parrish, 1999), is known to be the causative agent of acute haemorrhagic enteritis and potentially fatal myocarditis in dogs. Currently, CPV-2 has been replaced by its variants CPV-2a, CPV-2b and CPV-2c (Gaykwad *et al*., 2018). In regions like Kerala, where CPV-2c has recently emerged, there is an urgent requirement for a detection method that is both simpler and more cost- effective, while also providing rapid results. Amplification Refractory Mutation System PCR (ARMS-PCR) effectively meets this need by simultaneously detecting and typing haplotypes or single-nucleotide polymorphisms (Newton *et al*., 1989). The present study reports the occurrence of CPV-2c variants in parvoviral enteritis cases among dogs using ARMS-PCR.

MATERIALS AND METHODS

A total of 60 dogs showing clinical signs suggestive of canine parvoviral enteritis, such as pyrexia, anorexia, vomiting, bloody or mucoid diarrhoea, presented to the University Veterinary Hospitals (UVH), Mannuthy and Kokkalai formed the subjects of the study. Faecal samples were collected from the animals, and total genomic DNA was extracted using a commercial stool DNA extraction kit (Qiagen, Germany). The extracted DNA was subjected to ARMS-PCR for typing of CPV-2 variants (Chander *et al*., 2016). Sequence analysis was conducted on representative CPV positive samples. The compositions of PCR mixtures are given in Tables I and II):

Table I: PCR reaction mixture used for detecting CPV-2a and CPV 2b/2c

Name of the reagent	Concentration	Quantity (µl)
Template DNA	50-150µg/µl	5
Master mix	2X	15.0
Forward Primer (FP)	10 pmol/µl	1.20
Reverse Primer (RP)	10 pmol/µl	1.20
CPV-IR (2a)	10 pmol/µl	1.20

Name of the reagent	Concentration	Quantity (µl)
CPV-IF (2b)	10 pmol/µl	1.20
Nuclease-Free Water (NFW)	-	5.2
Total		30

Table II: PCR reaction mixture used for differentiating CPV-2b from CPV-2c

Name of the reagent	Concentration	Quantity (µl)
Template DNA	50-150µg/µl	5
Master mix	2X	15.0
Forward Primer (FP)	10 pmol/µl	1.20
Reverse Primer (RP)	10 pmol/µl	1.20
CPV-IR (2c)	10 pmol/µl	1.20
Nuclease Free Water (NFW)	-	6.40
Total		30

RESULTS AND DISCUSSION

A two-step reaction strategy was utilized for ARMS-PCR, with the initial reaction aimed at distinguishing CPV-2a from CPV-2b/CPV-2c. In this first step, using FP, RP, CPV-IR (2a) and CPV-IR (2b), 34 animals were found to be positive for CPV-2b/2c (amplicon size ~ 179 bp) in addition to the common 631 bp product. In the second step, using FP, RP and CPV-IR(2C), these 34 animals (54.33 per cent) displayed an amplicon size of 495 bp along with a common 631 bp, indicating CPV-2c (Figure 1). Sequencing was performed on three canine samples, where one sample produced an amplicon of approximately 631 base pairs, corresponding to the presence of CPV, and two samples yielded amplicons of approximately 495 base pairs, which correspond to the CPV-2c variant. The resulting sequence data were submitted to the National Centre for Biotechnology Information (NCBI) GenBank, where they were assigned unique accession numbers, PQ037858, PQ037857, and PQ037856.

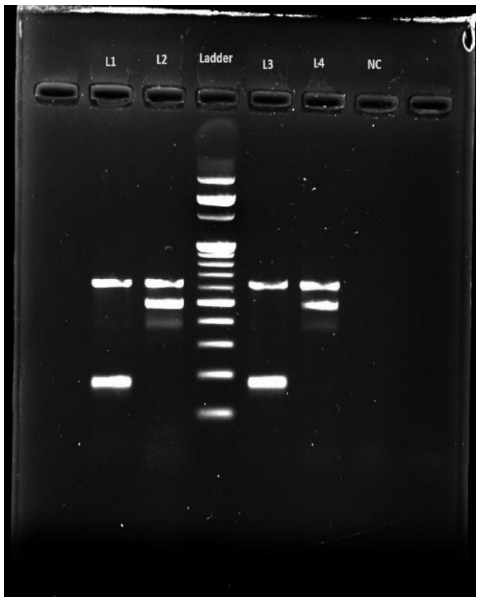

Figure 1: Agarose gel (1.5%) showing PCR amplified product from positive CPV-2c antigenic types. Lane1 and Lane 2: Positive control (Lane 1: 631+ 179; CPV-2b/2c and Lane 2: 631+ 495; confirming CPV-2c), Ladder: 100 bp, Lane 3 and Lane 4: Representative sample tested positive for CPV-2c, NC: Negative control.

Similar to the findings on CPV-2c emergence in the present study, Harikrishnan *et al*. (2023) identified the CPV-2c antigenic variant in Tamil Nadu, using ARMS-PCR, whereas Krishna *et al*. (2024) reported its presence in Kerala via conventional PCR followed by sequencing. However, these observations are at odds with the findings reported by Robin (2017), who identified new CPV-2a as the predominant antigenic variant in and around Thrissur, Kerala. This notable difference emphasizes variations in the occurrence of CPV-2c across different geographic regions and study populations. The higher prevalence of CPV-2c in the present study, when compared to recent reports of Umar *et al*. (2024), warrants the need for adoption of appropriate control measures against the new variants.

SUMMARY

Among the various PCR-based assays available for typing CPV-2 variants, ARMS-PCR was found to be an affordable, fast, and reliable method for the identification of CPV- 2 variants. The increasing dominance of CPV- 2c necessitates a re-evaluation of current vaccination protocols and biosecurity measures to ensure adequate protection against CPV-2c.

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