International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(2): 1349-1352

Received: 03-01-2019
Accepted: 07-02-2019

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Incidence of canine parvovirus type 2c in a puppy with haemorrhagic gastroenteritis in Tamil Nadu, India

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Abstract
Canine parvovirus enteritis is caused by canine parvovirus -2 (CPV-2), which is highly contagious and often fatal disease, characterized by vomiting, fowl smelling bloody diarrhoea and myocarditis in young dogs. In the present study, a total of 150 faecal and blood samples were collected from dogs with the symptoms of haemorrhagic gastroenteritis from Madras Veterinary College Teaching Hospital (MVCTH), Chennai to study the molecular epidemiology and haemato-biochemical changes in CPV-2 infected dogs respectively. Seventy one (47.33 per cent) dogs were positive for CPV-2 by PCR assay. Strain specific assay reveal CPV-2a and CPV-2c variants were recorded in 70 and 01 dogs respectively. Incidence of CPV-2c is the first report in Tamil Nadu. DNA sequencing was done for 8 PCR positive samples, out of which three were characterized as CPV-2c, indicating that this CPV type 2c is currently circulating in India.

Keywords: Canine parvovirus-2, PCR, CPV-2c, genotyping, mutants

Introduction
Canine parvoviral enteritis is caused by canine parvovirus-2 (CPV-2). CPV-2 is a highly contagious and often fatal disease, characterized by vomiting and hemorrhagic enteritis in dogs of all age (Appel *et al*., 1979) [2] and myocarditis leading to heart failure in pups of less than 3 month of age (Appel *et al*., 1978) [1]. Canine parvovirus (CPV) is a small (diameter of 25 nm), non-enveloped virus with a single-stranded DNA molecule of a approximately 5000 bases infecting vertebrates. The parvovirus virion consists of a spherical capsid, which is composed by three structural proteins namely VP1, VP2 and VP3 (Muzyczka and Berns, 2001) [7] and two non-structural (NS1 and NS2) proteins.

The virus primarily originated as a host variant from feline panleukopenia virus (FLV), which was sooner adapted to the canine host via wild carnivores, such as foxes and minks (Truyen *et al*., 1992) [16]. After few years of spreading, the original CPV-2 virus was replaced completely replaced by CPV-2a, a newer type virus that can infect both dogs and cats (Truyen *et al*., 1996a). The difference between CPV-2a from CPV-2 was five amino acid changes in the VP2 coat protein. (Truyen *et al*., 1995) [17]. In 1984, yet another antigenic CPV variant emerged and was designated as CPV-2b, which is in cocirculation with the CPV-2a within the dog population all around the world.

The first confirmatory incidence of CPV-2 in dogs was reported at United States in 1978 (Appel *et al*., 1979) [2]. Ever since the emergence of CPV-2 in 1978, its strain have been continuously mutating with the emergence of CPV-2a in 1979, CPV-2b in 1984 (Parrish *et al*., 1985) [10] and CPV-2c in 2000 (Italy) (Buonavoglia *et al*., 2001) [4].

In India, the disease was first reported at Madras in 1981 by Balu and Thangaraj. The incidence of CPV-2 variants in dogs were reported from different states *viz*., Puducherry (Parthiban *et al*., 2011) [11], Kerala (Deepa and Saseedrannath, 2000) [5], Haryana (Sanjukta *et al*., 2008) [14], Uttar Pradesh (Nandi *et al*., 2009) [8] and Assam (Phukan *et al*., 2004) [13] and West Bengal (Biswas *et al*., 2006). The first confirmatory of CPV-2c was reported by Nandi *et al.*, 2010 [9] at New Delhi.

Polymerase Chain Reaction (PCR)

Stool DNA Extraction kit (Bio Basic), 2X Red dye Master Mix (Lot No: 5200300-12L4, Ampliqon), DEPC treated nuclease free water (Bio Basic) and oligonucleotide primer (Table 2) were used in this study.

Oligonucleotides primers used in this study

Four pairs of primers were used for PCR amplification (Sigma-Aldrich, Bangalore). The details of the primers are given in the table

Table 1: Reverse primers Primer Sequence

S. No	Forward and Reverse primers	Primer Sequence 5'------3'	Product size	Reference
1	CPV2(FP)	CAGGTGATGAATTTGCTACA	630bp	Buonavoglia *et al.* 2001 [4].
	CPV2 (RP)	CATTTGGATAAACTGGTGGT		
2	CPV2a(FP)	AGAGCATTGGGCTTACCACC	379bp	Kaur *et al.* 2014 [6].
	CPV2a(RP)	ATCTTCCTGTATCTTGATGTGCT		
3	CPV2b(FP)	CTTTAACCTTCCTGTAACAG	427bp	Peraira *et al.* 2000 [12].
	CPV2b(RP)	CATAGTTAAATTGGTTATCTAC		
4	CPV2c(FP)	GTGGTTCTGGGGGTGTGG	470bp	Kaur *et al.* 2014 [6].
	CPV2c(RP)	AGCTGCTGGAGTAAATGGCA		

DNA extraction from faecal samples

DNA was extracted from collected faecal samples by using tissue DNA extraction kit (Bio Basic) as per the protocol of manufacturer guidelines

PCR assay for full length VP1/VP2 gene (2.2 kb)

PCR for amplification of full length VP1/VP2 gene (2.2 kb) was carried out as per Nandi *et al.* (2009) [8] for confirmation of canine parvovirus infection in dogs presented with gastroenteritis as well as for apparently healthy dogs for checking canine parvovirus carrier status.

PCR reaction mixture

The PCR was performed in an Eppendorf thermal cycler. All the reactions were carried out in volume of 25µl in 0.2 ml PCR tubes. The reaction mixture contains PCR master mix (12.5µl), forward primer (FP) (1.0 µl), reverse primer (RP) (1.0 µl), nuclease free distilled water (7.5 µl) and template DNA (3.0 µl).

PCR cyclic conditions

Details of PCR cyclic conditions

Table 2: Initial denaturation annealing extension final extension

	Initial denaturation	Denaturation	Annealing	Extension	Final extension	No. of cycles
CPV2	95^{O}C for 5 mins	95^{O}C for 1 min	55^{O}C for 2 mins	72^{O}C for 30 sec	72^{O}C for 10 mins	30
CPV2a	94^{O}C for 20sec	94^{O}C for 60 sec	55^{O}C for 60 sec	72^{O}C for 2min 30 sec	72^{O}C for 10 mins	34
CPV2b	95^{O}C for 5 mins	95^{O}C for 1 min	55^{O}C for 2 mins	72^{O}C for 30 sec	72^{O}C for 10 mins	30
CPV2c	94^{O}C for 60 sec	94^{O}C for 60 sec	55^{O}C for 60 sec	72^{O}C for 2min 30 sec	72^{O}C for 10 mins	34

Result and Discussion

In the present study, a total of 150 faecal and blood samples were collected from dogs with the symptoms of haemorrhagic gastroenteritis from Madras Veterinary College Teaching Hospital (MVCTH), Chennai to study the molecular epidemiology and haemato-biochemical changes in CPV-2 infected dogs respectively. Seventy one (47.33 per cent) dogs were positive for CPV-2 by PCR assay

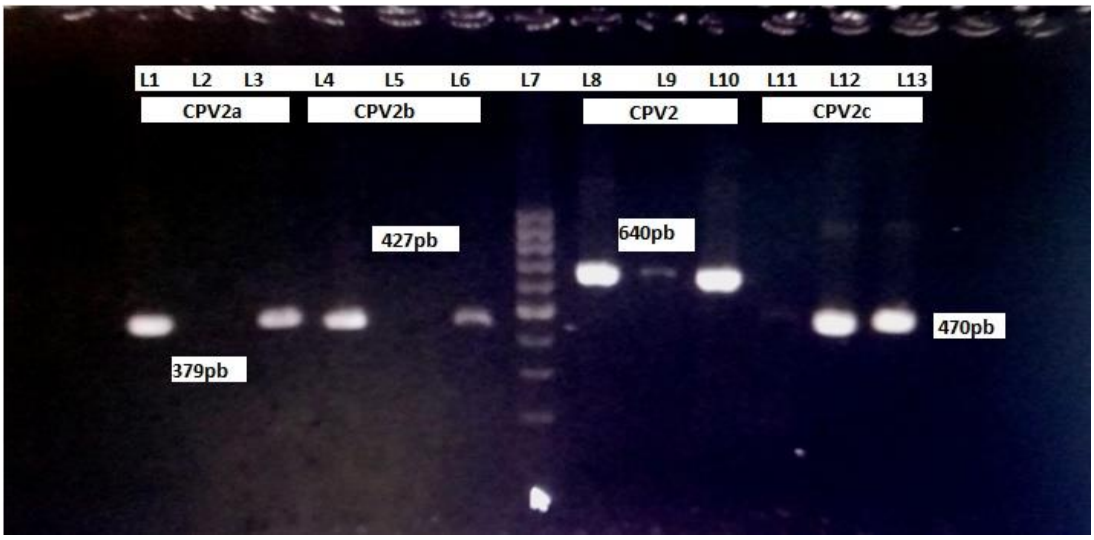

Fig 1: L1 - Positive Control 2a (Vanguard), L2 - Negative Control, L3 - Sample, L4 - Positive Control 2b (Vanguard), L5 - Sample, L6 - Positive Control (Puppy DP), L7 - Ladder, L8 - Positive Control (Puppy DP), L9- Sample, L10- Positive Control (Puppy DP), L11 - Sample, L12 and 13 - Positive Controls

The PCR products of five C PV-2a and one CPV-2c positive samples were purified and 25ul of this was sent for sequencing by primer walking at Eurofins Genomics India Pvt Ltd, Bangalore. The sequencing was carried out with the help of an automated DNA Sequencer.

Screening of the samples for presence of CPV-2 variants was carried out by PCR using CPV-2 strain specific primers for all the 150 samples and revealed 70 and 01 sample were positive for CPV-2a and 2c respectively.

On analysis of nucleotide sequences, T to A substitution at the third codon of 4064 position of VP2 gene and aspartic acid in CPV-2b has been replaced by glutamic acid at amino acid sequence position of 426 in CPV-2c.

>PCR_CPV_2a_CPV_2A_F-D02.ab1

GCAACGGGCAATTAACTATACTAATATATTTAATAC
TTATGGTCCTTTAACTAGCATTAAATAATGTACCACC
AGTTTATCCAAATGGTCAAATTTGGGATAAAGAATT
TGATACTGACTTAAAACCAAGACTTCATGTAAATGC
ACCATTTGTTTGTCAAAATAATTGTCCTGGTCAATTA
TTTGTGAAAGTTGCGCCTAATTTAACAAATGAATAT
GATCCTGATGCATCTGCTAATATGTCAAGAATTGTA
ACTTACTCAGATTTTTGGTGGAAAGGTAAATTAGTAT
TTAAAGCTAAACTAAGAGCCTCTCATACTTGGAATC
CAATTCAACAAATGAGTATTAATGTAGATAACCATT
TA

>PCR_CPV_2c_CPV_2C_F-E02.ab1

TTTCTCGAGATAACGGATGGGTGGAAATCACAGCAA
ACTCAAGCAGACTTGTACATTTAAATATGCCAGAAA
GTGAAAATTATAGAAGAGTGGTTGTAAATAATTTGG
ATAAAACTGCAGTTAACGGAAACATGGCTTTAGATG
ATACCCATGCACAAATTGTAACACCTTGGTCATTGGT
TGATGCAAATGCTTGGGGAGTTTGGTTTAATCCAGG
AGATTGGCAACTAATTGTTAATACTATGAGTGAGTT
GCATTTAGTTAGTTTTGAACAAGAAATTTTTAATGTT
GTTTTAAAAAATGTTTCAGAATCTGCTAGTCTGCCGC
CAACTAAAGATTATAATAATGACGTAACTGCAGCAT
TGATGGTGGCTTTAGAAAGTACCAATA

>PCR_CPV_2a_2A1F_B03.ab1

GTAGCATAATGCCACGAATGAAACCCTCTTACTTTA
GCTCTAATACTAATTTCCCTTTCCCCAAAATTCGGAT
CTGTTACAGTTCTTGACATATTATCAAATGCCTCAGT
ATCAAATTCCTTTGTTAAATTAGGCGCAACTTTTACA
TAATTGACCAGGACAATTATTTTGACAGACAAATGG
TGGTTTACATGAAATCTTGGTTTTGGTCAGATCAAAT
TCTTTATCCCAAATTTGACCATTTGGATAAACTGGTG
GTACATTGTTTAATGCCGTTAAAGGACCATAAGTATT
ACATATGTTGGTATAGTTAATTCCTGTTTTACCTCCA
ATTGGATCCCGTGCTAACAATACATTATCATCTGTTA
CAGGAATGCTGAATGAGAAGGTATACTCAAAA

>PCR_2A3_2AF_C03.ab1

TTACCCATACTGCAGGAATCACACCTTGCCTACAATT
CACCGGTGAAATGAATCTGAGGTCCCATAAATTGGT
GTTTAAATGGCCCTCAAACTATTCTCATGGACCCTAG
GTGGAAATTAATTTGCTAAACCACGCCGTAACCCTA
ACCCTCCGTTAGCAGGACAATCCTTTTGAAAAACAA
AAGGGGGGGACACATGTAAACCCGGGGTTCGCCAA
GATTACATAATTTTTACCCAAAGTGACCGCTTGGATA
ATAAGGGGGTGGATTATCTATTGCGGGTTTTGGGGC
AGAAGGGTTAAATATTTTTTTAAAGCTATATTCCCCC
CTTCCCCCCCCAATAGAACACGTTGCGGGAGGAGGAT
TTTTGTGCCAGACCCCTGATTGGTTAGAGGAAAA

>PCR_2B1_2BF_D03.ab1

AATCCCATTGGAGGTAAAACAGGAATTAACTATACC
AACATATTTAATACTTATGGTCCTTTAACTGCATTAA
ACAATGTACCACCAGTTTATCCAAATGGTCAAATTT
GGGATAAAGAATTTGATACTGACTTAAAACCAAGAC
TTCATGTAAATGCACCATTTGTCTGTCAAAATAATTG
TCCTGGTCAATTATTTGTAAAAGTTGCGCCTAATTTA
ACAAATGAATATGATCCTGATGCATCTGCTAATATG
TCAAGAATTGTAACTTACTCAGATTTTTGGTGGAAA
GGTAAATTAGTATTTAAAGCTAAACTAAGAGCCTCT
CATACTTGGAATCCAATACAACAAATGAGTATTAAT
GTAGATAACCAATTTAACTATGA

>PCR_2C1_2CF_E03.ab1

CATGAACTTCAAGAACGGATTCCATTCATCCAGGAA
ACGGATGGGTTGAAATCACAGCAAACTCAAGCAGAC
TTGTACATTTAAATATGCCAGAAAGTGAAAATTATA
GAAGAGTGGTTGTAAATAATTTGGATAAAACTGCAG
TTAACGGAAACATGGCTTTAGATGATACTCATGCAC
AAATTGTAACACCTTGGTCATTGGTTGATGCAAATG
CTTGGGGAGTTTGGTTTAATCCAGGAGATTGGCAAC
TAATTGTTAATACTATGAGTGAGTTGCATTTAGTTAG
TTTTGAACAGGAAATTTTTAATGTTGTTTTAAAGACT
GTTTCAGAATCTGCTACTCAACCACCAACTAAAGTTT
ATAATAATGATTTAACTGCATCATTGATGGTTGCATT
AGATAGTAATAATACTATGCCATTTACTCCAGC

>PCR_2C2_2CF_F03.ab1

TCAATAATCAGGACGGATTTAATTTTTGGAAACGGA
TGGGTGGAAATCACAGCAAACTCAAGCAGACTTGTA
CATTTAAATATGCCAGAAAGTGAAAATTATAGAAGA
GTGGTTGTAAATAATTTGGATAAAACTGCAGTTAAC
GGAAACATGGCTTTAGATGATACCCATGCACAAATT
GTAACACCTTGGTCATTGGTTGATGCAAATGCTTGG
GGAGTTTGGTTTAATCCAGGAGATTGGCAACTAATT
GTTAATACTATGAGTGAGTTGCATTTAGTTAGTTTTG
AACAAGAAATTTTTAATGTTGTTTTAAAGACTGTTTC
AGAATCTGCTACTCAGCCACCAACTAAAGTTTATAA
TAATGATTTAACTGCATCATTGATGGTTGCATTAGAT
AGTAATAATACTATGCCATTTACTCCCAGCAGCTA

>PCR_2C3_2CF_G03.ab1

TCATTTATGCAAGACCGGTAATTTCCATTCATCAAGG
AAACGGATGGGTGGAAATCACAGCAAACTCAAGCA
GACTTGTACATTTAAATATGCCAGAAAGTGAAAATT
ATAGAAGAGTGGTTGTAAATAATTTGGATAAAACTG
CAGTTAACGGAAACATGGCTTTAGATGATACCCATG
CACAAATTGTAACACCTTGGTCATTGGTTGATGCAA
ATGCTTGGGGAGTTTGGTTTAATCCAGGAGATTGGC
AACTAATTGTTAATACTATGAGTGAGTTGCATTTAGT
TAGTTTTGAACAAGAAATTTTCAATGTTGTTTTAAAG
ACTGTTTCAGAATCTGCTACTCAGCCACCAACTAAA
GTTTATAATAATGATTTAACTGCATCATTGATGGTTG
CATTAGATAGTAATAATACTATGCCATTTACTCCAGC
C

From the sequence obtained, it can be concluded that sample no. 2 having T to A substitution indicate the presence of CPV-2c mutants. Analysis of CPV strains revealed the onset of an unusual CPV-2 mutant, with a change (Asp-426 to Glu) occurring in the strategic residue 426 (Strassheim *et al*., 1994) [15]. However, further epidemiological surveillance and sequence analysis will help to elucidate if there is any mutation and will provide insights about the prevalence of different antigenic variants of CPV.

Sequencing of the samples was carried out, which revealed that sample no 2 shown in Table 1 were having the T to A mutation. Although limited number of samples have been screened for the presence of CPV-2c mutants, it is to be emphasized that CPV-2c mutants have been evolved to emerge as pathogens of dogs in India. This is the first report of CPV-2c in the South India and it also represents the frequency of this type mutant observed in a dog population. Its presence in India supports the assumption that CPV-2c is reaching a worldwide distribution and provides new information to understand the evolution of antigenic variants of CPV-2.

Reference

1. Appel MJG, Cooper BJ, Greisen H, Carmichael LE. Status report: Canine viral enteritis. J Am. Vet. Med. Assoc. 1978; 173:1516-1518.
2. Appel MJG, Cooper BJ, Greisen H, Scott FW, Carmichael LE. Canine viral enteritis. I. Status report on corona and Parvo–like viral enteritides, Cornell Vet. 1979; 69:123-133.
3. Biswas PJ, Das SK, Ghosh, Pradhan NR. Detection of canine parvovirus (CPV) DNA by polymerase chain reaction assay and its prevalence in dogs in and around Kolkata, West Bengal. Indian J Anim. Sci. 2006; 76(4):324-325
4. Buonavoglia C, Martella V, Pratelli A, Tempesta M, Cavalli A, Buonavoglia D *et al*. Evidence for evolution of canine parvovirus type 2 in Italy. J Gen. Virol. 2001; 82:3021-3025.
5. Deepa PM, Saseendrannath MN. Serological studies on canine parvoviral infection. Indian Vet. J. 2000; 79:643-644.
6. Kaur G, Chandra M, Dwivedi PN. Antigenic typing of canine parvovirus using differential PCR. Indian Virol. Soc. 2014; 25(4):481-487
7. Muzyczka N, Berns KI. Parvoviridae: The Viruses and Their Replication. In: Knipe, D.M., Howley, P.M. (Eds.), Fields Vorology. 4th Edn. Lippincott Williams & Wilkins, Philadephia, PA, 2001, 2327-2359
8. Nandi S, Chidri S, Kumar M. Molecular characterization and phylogenetic analysis of a canine parvovirus isolate in India. Vet. Med. 2009; 54(10):483-490.
9. Nandi S, Chidri S, Kumar M, Chauhan RS, Occurrence of canine parvovirus type 2c in the dogs with haemorrhagic enteritis in India. Res. Vet. Sci. 2010; 288:169–171
10. Parrish CR, O'Connell PH, Evemann JH, Carmichael LE. Natural variation of canine parvovirus. Sci. 1985; 230:1046-1048.
11. Parthiban S, Mukhopadhyay HM, Panneer D, Antony PX, Pillai RM. Isolation and typing of canine parvovirus in CRFK cell line in Puducherry. South India. Indian J. Microbiol. 2011; 51:456-460.
12. Peraira CA, Monezi TA, Mehnert DU, Anglo MD, Durigon EL. Molecular characterization of canine parvovirus in Brazil by polymerase chain reaction assay. Vet. Microbiol. 2000; 75:127-133
13. Phukan A, Deka D, Boro PK. Occurrence of canine parvovirus infection in and around Guwahati. Indian J. Anim. Sci. 2004; 74(4):930-931.
14. Sanjuktha R, Maheshkumar L, Nagappa B, Joken B, Karuna I, Mandakini. Molecular epidemiological study of canine parvovirus in some parts of India in International conference on emerging infectious diseases of animals and biotechnological applications, held at TANUVAS, Chennai, 2008, 65.
15. Strassheim ML, Gruenberg A, Veijalainen P, Sgro JY, Parrish CR. Two dominant neutralizing antigen determinants of canine parvovirus are found on the threefold spike of the virus capsid. Virology. 1994; 198:175-184.
16. Truyen U, Parrish CR. Canine and feline host ranges of canine parvovirus and feline panleucopenia virus: distinct host cell tropisms of each virus *in vitro* and *in vivo*. J Virol. 1992; 66:5399-5408.
17. Truyen U, Gruenberg A, Chang SF, Obermaier B, Veijalainen P, Parrish CR. Evolution of the feline-subgroup parvovirus and the control of canine host range *in vivo*. J Virol. 1995; 60:4702-4710.