Indian Journal of Animal Sciences **82** (3): 237–239, March 2012

Detection of antigenic variation of canine parvovirus strains of Tamil Nadu using differential PCR

M PARTHIBAN[1], R SARANYA[2], KURUNCHI C DIVYA[3] and K KUMANAN[4]

Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu 600 051 India

Received: 4 January 2011; Accepted: 18 October 2011

ABSTRACT

Canine parvovirus 2 (CPV2) is a highly contagious and fatal disease of dogs causing acute hemorrhagic enteritis and myocarditis. In this study, faecal samples were initially screened using haemagglutination test followed by differential PCR assay using CPV2a and 2b antigenic type specific primers. Out of 53 samples screened, 17 samples were found positive by CPV2b specific primers and no amplification was observed with CPV 2a primers. The PCR products were sequenced and the phylogenetic analysis and amino acid aspartic acid at 426th position clearly indicates the prevalence of CPV antigenic type 2b isolates in Tamil Nadu, India.

Key words: Antigenic types, Canine parvovirus, Differential polymerase chain reaction, Phylogenetic analysis

Canine parvovirus a member of the genus autonomous parvovirus, is a non-enveloped single-stranded DNA virus of approximately 5000 bases encoding structural (VP-1 and VP-2) and non-structural (NS1 and NS2) protein genes (Notomi *et al*. 2000). Canine parvovirus pathogen emerged in early 1978 in USA with signs of myocarditis and gastroenteritis (Appel *et al.* 1979). Analysis of CPV isolates using monoclonal antibodies and restriction enzyme analysis showed that after the first emergence of CPV in early 1980, it evolved to give a new variant termed as CPV-2a (Parrish *et al*. 1991). This new variant underwent further antigenic change to give a new variant termed as CPV-2b (Parrish *et al.* 1991) and CPV- 2c (Decaro *et al.* 2007). The characterization of CPV-2 antigenic type is exclusively based on the differential PCR (Pereira *et al.* 2000) and identification on the amino acid residue at position 426 of the capsid protein (Nakamura *et al*.2004). Till date, there has been no information regarding the antigenic types of CPV prevailing in state of Tamil Nadu, India. Hence in this study, differential polymerase chain reaction (PCR), sequencing and phylogenetic analysis and identification of hot spot aminoacid in capsid protein were used to type CPV strains prevalent in Tamil Nadu state, India during the period of 2002 and 2010.

Present address: [1]Associate Professor, [2,3]Senior Research Fellow, [4]Professor and Head (drparthiban66@yahoo.co.in; saranr77@gmail.com; kurunchidivya@gmail.com; kumananrani @hotmail.com), Department of Animal Biotechnology, Madras Veterinary College, Chennai 600 007 India.

MATERIALS AND METHODS

Faecal samples (53) were collected from dogs showing clinical signs such as diarrhoea, persistent vomiting and hemorrhagic enteritis, were brought to the Department of Clinics, Madras Veterinary College, Tamil Nadu, India during the year 2002 and 2010.

Processing of fecal samples: Faecal samples (1g) were suspended in 10 ml of phosphate-buffered saline (PBS. pH 7.2), centrifuged at 10,000 rpm for 10 min, the supernatant of each sample was filtered using 0.45µm filter and stored at –20°C until use. The filtered samples were used for PCR assay.

Haemagglutination test was performed using pig erythrocytes as per Senda *et al.* (1986). The CPV vaccine virus was used as a positive control and the faecal samples from healthy dogs were used as negative control

Differential primers for polymerase chain reaction: The filtered faecal samples were boiled for 5–10 min and snap chilled on ice immediately. 10µl of the boiled samples were used as template for PCR. The CPV vaccine virus was used as a positive control and the faecal sample from healthy dogs and canine adenovirus sample were used as negative controls. CPV antigenic types 2a and 2b primers, designed by Pereira *et al.* (2000) were used for this study. Primer pair 2a sense (5'-GAAGAGTGGTTGTAAATAATA-3') and 2a anti-sense (5'-CCTATATCACCAAAGTTAGTAG-3') located, respectively, at 3025±3045 and 3685±3706 of the CPV genome, yields a 681 bp product, while 2b sense (5'-CTTTAACCTTCCTGTAACAG-3') and 2b anti-sense (5'-

CATAGTTAAATTGGTTATCTAC-3') located, respectively, at 4043±4062 and 4449±4470 yields a 427 bp product. The temperature profile consisted of an initial denaturation at 94°C for 5 min, followed by 30 cycles comprised denaturation at 94°C for 30 sec, annealing at 55°C for 2 min and extension at 72°C for 2 min. The final elongation was done at 72°C for 10 min. Ten microlitres (10µl) of each amplified DNA samples were loaded onto a 1.5% agarose gel stained with ethidium bromide. Electrophoresis was performed at 100V for 40 min.

Sequencing and phylogenetic analysis: Two PCR products from the year 2002 and 2010, samples were purified using gel extraction kit. The purified PCR products were sequenced using genetic analyzer. The obtained sequences were aligned and BLAST analysis was performed .The strains with similar homology were selected for Multiple Sequence Alignment using Clustal 1.8 and Phylip software packages (Parthiban *et al.* 2005). Phylogenetic analysis was done to analyze the homology between our strains with other CPV isolates using MEGA software.

Amino acid analysis: The nucleotide sequences were then translated into amino acid sequence using DNA star to find out the antigenic type of CPV.

RESULTS AND DISCUSSION

Out of 53 samples collected from various breeds of dog, 22 samples were positive by HA with titres range between 1:16 to 1:1024. Out of 53 samples, 17 samples were found CPV-positive using PCR assay (Table 1). No amplification was observed using 2a primers. The amplification was observed only in 2b primers and the expected amplicon size of 427 bp was observed in agarose gel electrophoresis (Fig. 1). All the 17 samples were found positive by HA test also. Nucleotide sequence analysis revealed 98% homology with other CPV2b antigenic type (GU380298, GU212792, DQ025997, GU569937, GQ379049) The phylogenetic analysis showed the relationship of our strains with other 2b isolates of other countries (Fig. 2). The amino acid at the position 426th of local strains were compared with other CPV strains. The accession number and antigenic type of local isolates were presented in Table 2.

Table 1. Screening of field samples by HA and PCR assay

Year of collection	No of samples	Positive by HA	Positive by PCR
2002	25	10	6
2010	28	12	11

Table 2. Antigenic type of CPV based on amino acid position

Sample name and year of collection	Accession number	CPV type	Amino acid at 426th position
CPV TN A, 2002	HQ008323	CPV2b	Aspartic acid
CPV TN B, 2002	HQ008324	CPV2b	Aspartic acid
CPV TN A, 2010	HQ259077	CPV2b	Aspartic acid
CPV TN B, 2010	HQ259076	CPV2b	Aspartic acid

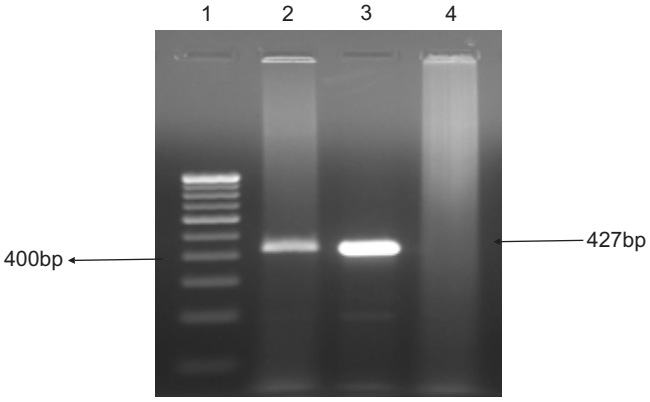

Fig. 1. Agarose gel electrophoresis showing the amplified product of VP2 gene of CPV faecal samples. Lane 1, 100bp ladder; lane 2, CPV suspected faecal sample; lane 3, positive control; lane 4, negative control.

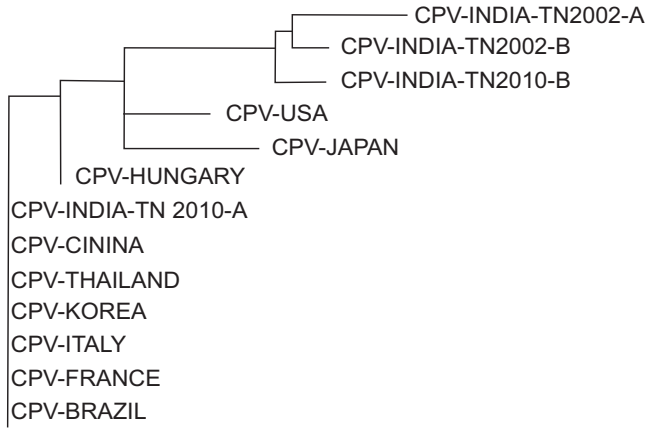

Fig. 2. Phylogenetic analysis of CPV strains of Tamil Nadu, India.

Out of 53 samples, more number of samples were positive in HA test due to non specific agglutination reaction. On the other hand PCR was highly specific assay which avoid non specific reaction because samples were analyzed at nucleic acid level. The appearance of expected size band in agarose gel is not sufficient to confirm the presence of viral genome in clinical samples. Hence, PCR products needs to be further confirmed by one of the several methods available such as nested PCR, southern blot, hybridization and sequencing (Schwarz *et al.* 1995). In this study, sequencing was done to confirm the PCR products and it showed 98% homology with other CPV isolates.

The proportions of the new antigenic types of CPV vary in different countries. In the USA and Southern Africa, CPV-2b was the predominant virus type responsible for most outbreaks of CPV infection (Parrish *et al.* 1991, Steinel *et al.* 1998). The new strains also replaced the original one in Taiwan and Italy, though the CPV-2a strain is still

predominant (Chang *et al.*1996, Sagazio *et al.* 1998.). In our study, CPV-2b is predominant in the year 2002 and 2010 and the results coincide with previous work in India (Nandi *et al.* 2010).

However, Punducherry isolates were phylogenetically closely related to new CPV-2a strains of Japan and India (Mohan Raj *et al.* 2010). The major viral proteins of canine parvovirus are VP2 gene 4 on which type or group determinants are located (Parrish and Carmichael 1986). Currently, 3 antigenic types CPV–2A (asparagine N^{426}), CPV–2B (aspartic acid D^{426}) and CPV 2C (glutamic acid E^{426}) are circulating worldwide (Nakamura *et al.* 2004). In this study, the presence of amino acid, viz. aspartic acid at 426^{th} positions clearly indicates the prevalence of antigenic type CPV- 2b in dog population in Tamil Nadu, India. At present, CPV-2c is progressively replacing other CPV types in the Italian and Indian dog populations as reported by Martella *et al*. (2005) and Kumar and Nandi (2010) respectively. Although on phylogenetic analysis our isolates are closely related to Italian isolate and new antigenic type 2c was not found in our study and the results coincide with other recent reports in India (Nandi *et al.* 2009)

REFERENCES

Appel M J, Scott F W and Carmichael L E. 1979. Isolation and immunization studies of a canine parvo-like virus from dogs with hemorrhagic enteritis. *Veterinary Record* **105**: 156–59.

Chang W L, Chang A C H and Pan M J. 1996. Antigenic types of canine parvoviruses prevailing in Taiwan. *Veterinary Record* **138**: 447–47.

Decaro N, Desario C, Elia G, Campolo M, Lorusso A, Mari V, Martella V and Buonavoglia C. 2007. Occurrence of severe gastroenteritis in pups after canine parvovirus vaccine administration: A clinical and laboratory diagnostic dilemma. *Vaccine* **25**: 1161–66.

Kumar M and Nandi S. 2010. Molecular typing of canine parvovirus variants by polymerase chain reaction and restriction enzyme analysis. *Transboundary and emerging diseases* **57** (6): 458–63.

Martella V, Decaro N, Elia G and Buonavoglia C. 2005. Surveillance activity for canine parvovirus in Italy. *Journal of Veterinary Medicine Infectious Diseases and Veterinary Public Health* **52**: 312–15.

Mohan Raj J, Mukhopadhyay H K, Thanislass J, Antony P X and Pillai R M. 2010. Isolation, molecular characterization and phylogenetic analysis of canine parvovirus: Infection, genetics and evolution. *Journal of molecular epidemiology and evolutionary genetics in infectious diseases* **10** (8): 1237–41.

Nakamura M, Tohya Y, Miyazawa T, Mochizuki M, Phung H T, Nguyen L T, Nguyen P N, Nguyen P V, Nguyen N P and Akashi H. 2004. A novel antigenic variant of canine parvovirus from a Vietnamese dog. *Archives of Virology* **149**: 2261–69.

Nandi S, Anbuzhagan R and Kumar M. 2010. Strain differentiation and characterization of canine parvovirus by PCR and RE mapping. *Indian journal of Biotechnology* **9**: 38–42.

Nandi S, Chidri S and Kumar M. 2009. Molecular characterization and phylogenetic analysis of a canine parvovirus isolate in India. *Veterinarni Medicina* **54** (10): 483–90.

Notomi T, Okayama H and Masubuchi H. 2000. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research* **28**: e63.

Parrish C R. 1991. Mapping specific functions in the capsid structure of canine parvovirus and feline panleukopenia virus using infectious plasmid clones. *Virology* **183**: 195–205.

Parrish C R, Aquadro C F, Strassheim M L, Evermann J F, Sgro J-Y, Mohammed H O. 1991. Rapid antigenic-type replacement and DNA sequence evolution of canine parvovirus. *Journal of Virology* **65:** 6544–52.

Parrish C R, Carmichael L E. 1986. Characterization and recombination mapping of an antigenic and host range mutation of canine parvovirus. *Virology* **148**: 121–32.

Parthiban M, Manoharan S, Roy P, Chandran N D J, Aruni AW, Koteeswaran A. 2005. Nucleotide sequence analysis of the L1 Loop variable region of Hexon gene of Fowl Adeno Virus 4 isolates from India. *Acta Virologica* **49**: 65–68.

Pereira C A D, Monezia T A, Mehnerta D U, Magali D'Angelob and Durigona E L. 2000. Molecular characterization of canine parvovirus in Brazil by polymerase chain reaction assay. *Veterinary Microbiology* **75**: 127–33.

Sagazio P, Tempesta M, Buonavoglia D, Cirone F and Buonavoglia C. 1998. Antigenic characterization of canine parvovirus strains isolated in Italy. *Journal of Virological Methods* **73**: 197–200.

Schwarz T F, Glilch Jager S and Nitschko H. 1995. Nested RT PCR for detection of sand fly fever virus serotype Toscana, in clinical specimen with conformation by sequence analysis. *Research in Virology* **146**: 355–62.

Senda M, Hirayama N, Yamamoto H and Kurata K. 1986. An improved Haemagglutination test for study of canine parvovirus. *Veterinary Microbiology* **12**(1): 1–6.

Steinel A, Venter E H, Van Vuuren M, Parrish C R and Truyen U. 1998. Antigenic and genetic analysis of canine parvoviruses in southern Africa. *Onderstepoort Journal of Veterinary Research* **65**: 239–42.