



Molecular typing of canine parvovirus with PCR assay based on genetic markers of the antigenic variants

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ABSTRACT

The canine parvovirus (CPV) is prevalent in India but only scanty literature is available on molecular differentiation of its strain using polymerase chain reaction. In the present study, 100 faecal samples from dogs suspected for canine parvo virus infection (gastroenteritis) were collected and processed for isolation of viral genomic DNA. Molecular test like PCR assay using 3 specific pairs of primers (pCPV-RT, pCPV-2ab and pCPV-2b) was conducted for differential diagnosis of the virus type. Out of 100 faecal samples, 63 were positive for presence of CPV, of which 54 were CPV-2b and 9 were CPV-2a, indicating the predominance of CPV-2b virus type.

Key words: Canine parvovirus, Dog, India, Polymerase chain reaction

Canine parvovirus (CPV) was first identified in 1978 in USA and became endemic worldwide and responsible for high morbidity (up to 100%) and mortality (Zhao *et al.* 2011, Decaro and Buonavoglia, 2012). The infection spreads from dog to dog by direct or indirect contact with their infected faeces (Singh *et al.* 2013). The genome is of non-enveloped single-stranded DNA of 5.2kb, expressing 2 structural (VP1 and VP2) and 2 non-structural (NS1 and NS2) proteins (Notomi *et al.* 2000). The diagnosis of the disease on basis of clinical signs (vomition and diarrhoea) is difficult because these signs are common to other enteric diseases too (Verma *et al.* 2009, Kumar *et al.* 2010). Although, the canine parvovirus (CPV) is prevalent in India but only scanty literature is available on molecular differentiation of its strain using polymerase chain reaction. For effective development of control measures, detection and molecular characterization of presently circulating strains, are very necessary (Zhao *et al.* 2011). Therefore, the present study was carried out for typing and characterization of Indian CPV strains based on genetic markers of the antigenic variants.

MATERIALS AND METHODS

Sample and its preparation: During the present study period (February 2012 to July 2012), a total of 100 faecal

samples of dogs suffering from diarrhea and vomition and suspected of CPV infection attended Teaching Veterinary Clinical Complex, DUVASU and places near to Mathura, were collected with the help of sterilized swabs and immediately transferred to the labelled sterile vials containing Hank's balanced salt solution (HBBS) in a ratio of 1:9 containing streptomycin (100 mg/l) and penicillin (1 lakh IU/l). These were centrifuged at 10,000 rpm at 4°C for 3 min in a refrigerated centrifuge and filtered through a disposable syringe filter (0.45 µm).

Polymerase chain reaction (PCR) assay: The genomic DNA of virus from the faecal samples was extracted by phenol chloroform method (Sambrook and Russel 2001) with slight modifications such as to remove inhibitory substances, 200µl of sample was treated with sodium dodecyl sulphate (SDS) and proteinase K with a final concentration of 1% and 250µg/ml and kept at 56°C for 30 min in water bath. An attenuated CPV live vaccine prepared by Indian Immunological Limited, Hyderabad for vaccination trial and stool sample from a healthy dog were used as a positive and negative control respectively. The PCR was standardized for the primer set pCPV-RT (Nandi *et al.* 2009), pCPV-2ab and pCPV-2b (Pereira *et al.* 2000). The details of primers are given in Table 1.

RESULTS AND DISCUSSION

In this study, out of 100 faecal samples tested, an amplicon of 160bp size was obtained using primer set (pCPV-2RT) in 63 samples indicating the presence of CPV. In the positive control, there was also amplification of template DNA whereas, in the negative control, no amplification of template DNA was visualized on an agarose

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Table 1. Details of primer sets used, their product sizes and annealing temperatures

S. No.	Primer type		Sequences 5' to 3'	Position of genome	Product size (bp)	Annealing temp
1	pCPV-RT	Forward	5'-CAT TGG GCT TAC CAC CAT TT-3' (20mer)	3136–3155	160bp	52°C
		Reverse	5'-CCA ACC TCA GCT GGT CTC AT-3' (20mer)	3276–3295		
2	pCPV-2ab	Forward	5'-GAA GAG TGG TTG TAA ATA ATT-3' (21mer)	3025–3045	681bp	55°C
		Reverse	5'-CCT ATA TCA CCA AAG TTA GTA G-3' (22mer)	3685–3706		
3	pCPV-2b	Forward	5'-CTT TAA CCT TCC TGT AAC AG-3' (20mer)	4043–4062	427bp	55°C
		Reverse	5'-CAT AGT TAA ATT GGT TAT CTA C-3' (22mer)	4449–4470		

gel. The high prevalence of CPV in diarrhoeic dogs is in concurrence to the findings of other workers (Nandi *et al.* 2009, Panda *et al.* 2009, Pinto *et al.* 2012) in India. The main source of the infection appears to be the faeces of infected dogs because more than 10^9 virus particles per gram of faeces can be shed during the acute phase of the enteric disease. Therefore, faeces are accepted as a suitable material to detect the virus in the enteric form of the disease (Carmichael and Binn 1981).

After confirmation of CPV by primers pCPV-2RT, another PCR was carried out with all the 100 faecal samples to amplify VP1/VP2 structural gene of CPV genome using 2 primer sets, i.e. pCPV-2ab and pCPV-2b (Table 1). Out of 100 faecal samples, amplicons of 681bp were amplified from all the 63 CPV positive samples by pCPV-2ab primer set (Fig. 1), whereas amplicons of 427bp were amplified in 54 samples with pCPV-2b primer set (Fig. 2). The pCPV-2ab primer set amplified portion of VP1/VP2 gene of both CPV-2a and CPV-2b variants (3025 to 3706 nucleotide position of CPV genomic DNA) to yield a product of 681bp whereas, pCPV-2b primer set amplified specific portion of VP1/VP2 gene of only CPV-2b (4043 to 4470 nucleotide position of CPV genomic DNA) to yield a product of 427bp and thereby differentiate between CPV-2a and CPV-2b. So,

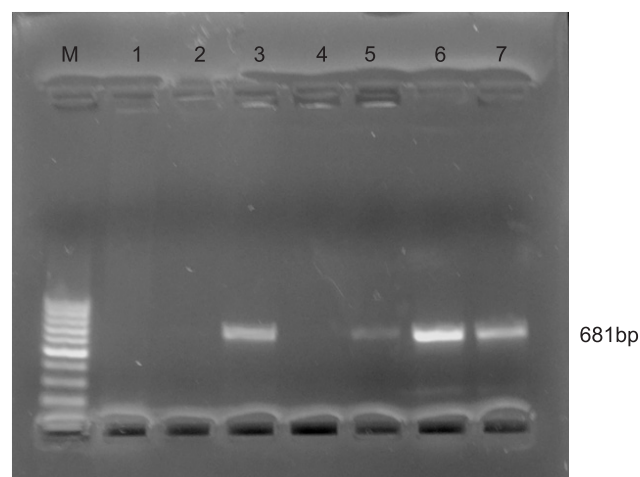


Fig. 1. Agar gel electrophoreses showing the amplified product of 681 bp of CPV-2a and CPV-2b positive samples using CPV-2ab (F and R) primers. Lane M: 100 bp DNA ladder; Lane 1: Negative control; Lane 3: positive control; Lane 2& 4: CPV negative faecal sample; Lane 5,6,7: 681 bp PCR product of CPV positive faecal sample.

the results showed that out of 63 positive samples, 54 (85.71%) were CPV-2b variants, while 9 (16.67%) were CPV-2a strain indicating CPV-2b as the predominant virus type. These findings are in agreement with the earlier reports from India in which CPV-2b has been reported the major antigenic variant of CPV-2 along with CPV-2a at a smaller proportion. (Nandi *et al.* 2009, Panda *et al.* 2009, Parthiban *et al.* 2012). Similarly, various researchers reported that CPV-2b is the predominant virus type responsible for most of outbreaks in US (Truyen 2006), Brazil (Pereira *et al.* 2000), China (Zhang *et al.* 2010), Taiwan (Wang *et al.* 2005), Japan (Soma *et al.* 2013), South Africa (Steinel *et al.* 1998) and Iran (Firoozjahi *et al.* 2011), Ireland (McElligott *et al.* 2011), while CPV-2a is the predominant virus type in Italy (Martella *et al.* 2006), India (Chinchkar *et al.* 2006) and Korea (Kang *et al.* 2008). However, in UK

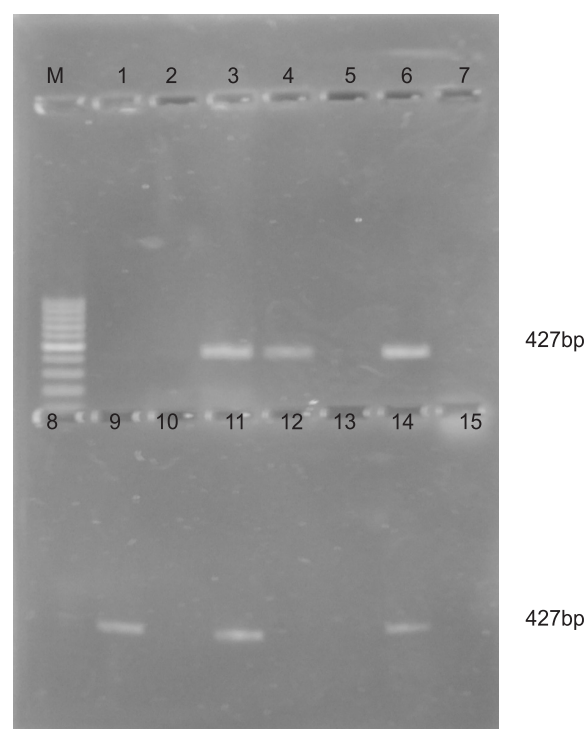


Fig. 2. Agar gel electrophoreses showing the amplicon of 427 bp CPV-2b positive samples using CPV-2b (F and R) primers. Lane M: 100 bp DNA ladder; Lane 1: Negative control; Lane 3: positive control; Lane 4, 6, 9, 11, 14: CPV Suspected faecal sample; Lane 5,7,10,12,13,15: CPV negative faecal sample.

(Greenwood *et al.* 1996), Germany (Truyen *et al.* 2000), Spain (de Ybanez *et al.* 1995), Italy (Battilani *et al.* 2001), Bulgaria (Filipov *et al.* 2011) and Australia (Meers *et al.* 2007), both CPV-2a and CPV-2b occur with similar frequencies. The reasons to the different distribution of CPV types in various countries are unknown, and there is a need to address this question.

Most vaccines used in India and other developing countries are based on original CPV-2 (Firoozjahi *et al.* 2011). Since, 1980, the canine parvovirus evolves into CPV-2a, CPV-2b and CPV-2c. Truyen (2006) reported the presence of cross-protection among these new strains but the protection with current homologous types is more efficient and potent way to prevent the disease and limit its spread in an area. Beside this, attention is also required towards maternal antibody titre. Under field conditions, vaccination against CPV starts at the age of 6 weeks, without considering the maternal antibody titre, which interferes with the development of immunity. This problem can be ruled out with either testing of antibody titre using haemagglutination or ELISA tests or administration of modified live canine parvovirus type 2b vaccine in pups in early age (Martella *et al.* (2005). So, a special attention from veterinarians and animal health professional to animal vaccination appears to be priority to prevent and control the disease (Singh *et al.* 2014).

It can be concluded that CPV-2b is the major canine parvovirus antigen type prevalent in this area along with smaller portion of CPV-2a circulating in the field.

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