American Journal of

Biochemistry and Molecular Biology

ISSN 2150-4210

Academic
Journals Inc.

www.academicjournals.com

American Journal of Biochemistry and Molecular Biology 3 (2): 264-270, 2013
ISSN 2150-4210 / DOI: 10.3923/ajbmb.2013.264.270
© 2013 Academic Journals Inc.

Detection of Canine Parvo Virus by Polymerase Chain Reaction Assay and its Prevalence in Dogs in and Around Mathura, Uttar Pradesh, India

[1]Deepti Singh, [2]Amit Kumar Verma, [3]Amit Kumar, [4]Mukesh Srivastava, [4]Shanker Kumar Singh, [4]Arvind Kumar Tripathi, [4]Ashish Srivastava and [2]Iftekhar Ahmed

[1]College of Biotechnology, Uttar Pradesh Pandit Deen Dayal Upadhayay Pashu Chikitsa Vigyan Vishvidhyalaya Ewam Go-Anusandhan Sansthan (DUVASU), Mathura, 281001, India

[2]Department of Veterinary Epidemiology and Preventive Medicine, [3]Department of Veterinary Microbiology and Immunology, [4]Department of Clinical Veterinary Medicine, College of Biotechnology, Uttar Pradesh Pandit Deen Dayal Upadhayay Pashu Chikitsa Vigyan Vishvidhyalaya Ewam Go-Anusandhan Sansthan (DUVASU), Mathura, 281001, India

Corresponding Author: Deepti Singh, College of Biotechnology, Uttar Pradesh Pandit Deen Dayal Upadhayay Pashu Chikitsa Vigyan Vishvidhyalaya Ewam Go-Anusandhan Sansthan (DUVASU), Mathura, 281001, India

ABSTRACT

The early detection of the Canine Parvo Virus (CPV) is of paramount importance. The present study was aimed to know the molecular epidemiology of Canine parvo virus. Canine faecal samples from 100 dogs showing the clinical signs of gastroenteritis in and around Mathura, Uttar Pradesh, India were collected and DNA was extracted by phenol-chloroform method. CPV vaccine strain was used as a positive control. Polymerase Chain Reaction (PCR) was carried out to amplify VPI/VP2 gene using a set of 20-mer primers [pCPV-RT (Forward): 5'-CAT TGG GCT TAC CAC CAT TT-3'; (Reverse): 5'-CCA ACC TCA GCT GGT CTC AT-3')] from position 3136-3155 to 3276-3295 of VP1/VP2 gene. A PCR product of approximately 160 bp was generated with positive faecal samples and CPV vaccine strain. After screening, 63 dogs were found positive for CPV but no sex variation was noticed amongst the CPV positive cases. Dogs, of the age group of ≤6 months were more susceptible in comparison to of >6 months and highest occurrence was noted in unvaccinated dogs and dogs in co-habitation with other dogs. Breed wise distribution of CPV in dogs revealed that the prevalence of CPV was the highest in Doberman (77.78%), followed by Spitz (78.57%), German shepherd (70.00%), Labrador (68.75%), Pomeranian (45.45%). It is concluded that CPV is prevalent in the Mathura and nearby area and it is more common in pups of age less than 6 months old and more prevalent in German shepherd, Labrador and Pomeranian breeds of dog.

Key words: Canine parvo virus, dog, risk factors, polymerase chain reaction, Mathura, India

INTRODUCTION

Dogs have been domesticated by human since a long time and are supposed to be the companion of human since civilization. They have very close association with human beings and most prominent example of the same is in Mahabharata where dog followed the eldest Pandav Yudhistir till its life lasted. In present time tremendous change in socio-economic structure in India has increased the demand for pet dogs and it has started showing an increasing trend as dogs are now a day status symbol. This is reflected by the 3.93% growth rate in population from 1987-1992. In 1992 pet dog population in India has been recorded 21.76 million in 1992 (Anonymous, 1997).

Canine parvo virus is a highly contagious and fatal disease characterized by vomition and haemorrhagic gastroenteritis in dogs of all age (Appel *et al.*, 1978; Verma *et al.*, 2009; Kumar *et al.*, 2010) and myocarditis (Han *et al.*, 2011; Ying *et al.*, 2012) and subsequently heart failure in puppies of less than 6 months of age (Zhou *et al.*, 2009). The disease was first recognized in CPV-2 form in U.S. in 1978 as a cause of new disease in dogs and in one or two years spread worldwide in domestic dog populations as well as in wild dogs with high morbidity (100%) and frequent mortality (up to 10%) which may reach to 91%, if untreated (Appel *et al.*, 1978). In India, CPV-2 was first reported in by Ramadass and Khader (1982) and then had drawn major concerns of animal health professionals. The disease is transmitted from dog to dog by direct or indirect contact with their infected feces. Therefore, early and rapid diagnosis is necessary so that infected dogs can be isolated and supportive treatment can be administered to reduce morbidity and mortality (Cho *et al.*, 2006). Clinical diagnosis of CPV infection is difficult, because the main clinical signs of the disease (vomiting and diarrhea) are common to other enteric diseases as well (De Castro *et al.*, 2007). However, Polymerase Chain Reaction (PCR) technique has been widely applied for early and confirmatory laboratory diagnosis of the disease, due to high sensitivity and specificity (Schunck *et al.*, 1995; Nandi *et al.*, 2009, 2010). Hence, the present study was conducted to detect canine parvo virus by polymerase chain reaction assay and its prevalence in dogs in and around Mathura, Uttar Pradesh, India.

MATERIALS AND METHODS

Animals and collection of samples: During the present study (February, 2012 to July, 2012), dogs suffering from diarrhea and vomiting and suspected of CPV infection were selected for the study. The faecal samples were collected with the help of sterilized swabs from the dogs presented to the Teaching Veterinary Clinical Complex, DUVASU and places near to Mathura and a total of 100 samples were collected, out of which 31 were nondescript, 16 Labrador, 14 Spitz, 11 Pomeranian, 10 German Shepard, 9 Doberman, 4 Boxer, 3 Rottweiller and 2 were Bull Mastiff. The information of infected dogs according to risk factors viz., sex age, vaccination status and co-habitation with other dogs was collected in a pre designed performa (Annexure 1). The faecal samples were collected in the form of a rectal swab using pre-sterilized swabs and immediately transferred to the labeled sterile vials containing Hank's Balanced Salt Solution (HBBS) in a ratio of 1:9, containing streptomycin (100 mg L^{-1}) and penicillin (1 lakh IU^{-1}). Collected samples were then stored in -20°C refrigerator till further processing.

Preparation of faecal sample: All the faecal samples stored in -20°C refrigerator were brought at room temperature. Then swabs were properly rinsed with HBSS of vials and taken out. Remaining faecal contents in HBSS were centrifuged at 10,000 rpm at 4°C for 3 min in a refrigerated centrifuge (Remi centrifuge). The supernatants were pipette out and filtered through a disposable syringe filter (0.45 µm) (Millex, Millipore) syringe filter.

Genomic DNA extraction: The genomic DNA from the faecal samples was extracted by phenol chloroform method (Sambrook and Russell, 2001) with slight modification like to remove inhibitory substances, 200 µL of sample was treated with Sodium Dodecyl Sulphate (SDS) and proteinase K with a final concentration of 1% and 250 µg mL^{-1} and kept at 56°C for 30 min in water bath GFL, Germany).

Polymerase chain reaction (PCR) assay: The primer set pCPV-RT (F) 5'-CAT TGG GCT TAC CAC CAT TT-3' (20-mer) and pCPV-RT (R) 5'-CCA ACC TCA GCT GGT CTC AT-3' (20-mer) from

position 3136-3155 to 3276-3295 of VP1/VP2 gene of CPV-2 was used to yield an amplicon of 160 bp in PCR (Nandi *et al*., 2009). The PCR was conducted in a thermocycler (Techne, USA) using a reaction volume of 50 μL containing 5 μL of *Taq* DNA polymerase buffer with 15 mM $MgCl_2$ (10x), 200 μM dNTPs, 10 pmol of each primer, 5 μL of processed sample as source of template DNA and 1 μL of *Taq* DNA polymerase (1 IU μL^{-1}). The thermal conditions consisted of initial denaturation at 94°C for 3 min, 30 cycles consisting of denaturation at 94°C for 30 sec, annealing at 52°C for 1 min and extension at 72°C for 30 sec and a final extension at 72°C for 5 min. The PCR products were electrophoresed along with 100 bp DNA ladder in 1% agarose gel containing 0.5 μg mL^{-1} ethidium bromide and progress of the mobility was monitored by migration of dye.

Statistical analysis: chi-square analysis: The data obtained was tested for significant ($p<0.05$) difference between age, sex, breed, vaccination status and co-habitation with another dog collected with respect to prevalence using chi-square test as described by Snedecor and Cochran (1967).

RESULT

In this study, out of 100 faecal samples from dogs showing the clinical signs of gastroenteritis tested, an amplicon of 160 bp size was obtained using primer set (pCPV-2RT) in 63 samples (63.00%) indicating the presence of CPV in all of these samples. In the positive control, there was amplification of template DNA whereas, in the negative control, no amplification of template DNA was visualized on an agarose gel (Fig. 1). The epidemiological factors which are also known as

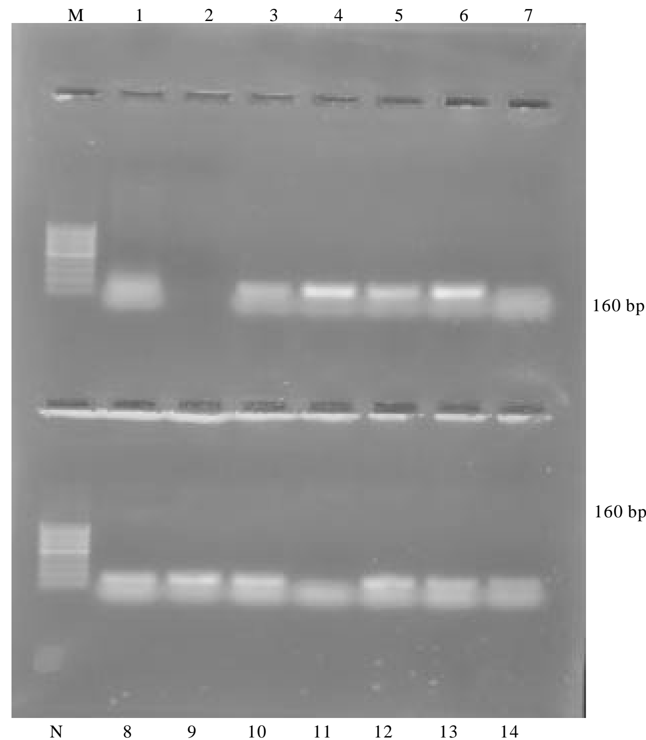

Fig. 1: Agar gel electrophoreses showing the amplified product of 160 bp of CPV (F and R) Primers, Lane M, N: 100 bp DNA ladder, Lane 1: Positive control (vaccine), Lane 2: Negative control (faecal sample of healthy dog), Lane 4, 5, 6, 8, 9, 10, 12, 13: CPV suspected faecal sample, Lane 7, 11, 14: CPV negative faecal samples

predisposing factors for the CPV infection were also analyzed from the information collected on performa. Various informations regarding the association of risk factors like breed, sex, age, vaccination status and cohabitation with other dogs and canine parvo virus infection status is shown in Table 1-5, respectively. In breed wise prevalence analysis we did not include all the breeds because in some breeds numbers of dogs are very low. Breed wise prevalence analysis revealed that distribution of canine parvo virus infection varied significantly ($p<0.05$) among different breeds (Table 1). It was the highest in Doberman (77.78%) and the lowest in Pomeranian (45.45%).

Table 1: Breed-wise distribution of canine parvo virus infection in dogs

Breed	Total No.	No. of positive	Positivity (%)
Boxer	4	1	25.00
Bull mastiff	2	2	100.00
Doberman	9	7	77.78*
German shepherd	10	7	70.00*
Labrador	16	11	68.75*
Pomeranian	11	5	45.45*
Rottweiller	3	3	100.00
Spitz	14	11	78.57*
Nondescript	31	16	51.61*
Total	100	63	63.00

*Significant at $p<0.05$

Table 2: Sex-wise distribution of canine parvo virus infection in dogs

Sex	Total No.	No. of positive	Positivity (%)
Male	78	49	62.82
Female	22	14	63.64
Total	100	63	63.00

Table 3: Age-wise distribution of canine parvo virus infection in dogs

Age	Total No.	No. of positive	Positivity (%)
<6 months	58	38	65.51*
≥6 months	42	24	57.14*
Total	100	63	63.00

*Significant at $p<0.05$

Table 4: Vaccination status and canine parvo virus infection in dogs

Vaccination Status	Total No.	No. of positive	Positivity (%)
Vaccinated	10	5	50.00*
Unvaccinated	90	58	64.00*
Total	100	63	63.00

*Significant at $p<0.05$

Table 5: Co-habitation with other dog and canine parvo virus infection in dogs

Co-habitation with other dog	Total No.	No. of positive	Positivity (%)
Yes	17	12	70.58*
No	83	51	61.44*
Total	100	63	63.00

*Significant at $p<0.05$

Sex wise prevalence of the canine parvo virus infection was equally common in males and females dogs and was 62.82 and 63.64%, respectively (Table 2). Dogs were grouped into two age groups viz., 0-<6 months and ≥6 months. Age wise prevalence results (Table 3) revealed that the prevalence of the canine parvo virus infection was higher in pups (0-<6 months age group) in comparison to dogs in the age group of ≥6 months and it was 65.51 and 57.14%, respectively. Association of canine parvo virus infection and vaccination status of dogs was compared (Table 4). The results revealed that prevalence of canine parvo virus infection was higher in unvaccinated dogs (64.00%) in comparison to that of vaccinated dogs (50.00%). Dogs were grouped into two groups on the basis of co-habitation with other dogs (Table 5). The results showed that the dogs in cohabitation with other dogs which were positive for canine parvo virus infection, were significantly associated with canine parvo virus infection. The prevalence of canine parvo virus infection was higher in dogs which shared their habitat with other dogs (70.58%) in comparison to those of which do not share their habitat with other dogs (61.44%).

DISCUSSION

In the present study, the prevalence of CPV in diarrheic dogs was found as 63.00% indicating the higher presence of CPV in diarrheic dogs. The high prevalence of CPV in diarrheic dogs is in concurrence to the findings of other workers (Nandi *et al.*, 2006, 2009; Biswas *et al.*, 2006; Panda *et al.*, 2009) in India. The main source of the infection seems to be the feces of infected dogs because more than 10^9 virus particles per gram of feces can be shed during the acute phase of the enteric disease. Therefore, feces are accepted as a suitable material to detect the virus in the enteric form of the disease (Carmichael and Binn, 1981).

The epidemiological factors which are also known as predisposing factors for the CPV infection were also analyzed from the information collected on proforma. Various informations regarding the association of risk factors like breed, sex, age, vaccination status and cohabitation with other dogs and canine parvo virus infection status is shown in Table 1-5, respectively. In breed wise prevalence analysis all the breeds were not included because in some breeds numbers of dogs were very low (Table 1). Breed wise distribution of CPV in dogs revealed that the prevalence of CPV was the highest in Doberman (77.78%), followed by Spitz (78.57%), German shepherd (70.00%), Labrador (68.75%), Pomeranian (45.45%) (Table 1). The effect of breed has also been noticed earlier in dogs by Kumar *et al.* (2011). They reported that the percent positivity was highest in German shepherd (69.2%), followed by nondescript breed (63.1%), Pomeranian (61.5%) and Doberman (28.5%) with overall percent positivity of 59.6%. These findings are almost in concurrence with the findings of present study where overall positivity is 63%.

Sex wise prevalence of the canine parvo virus infection revealed no discrimination with percentage positivity in males (62.82%) and females (63.64%) dogs (Table 2) indicating that sex had no influence on the prevalence of CPV in diarrhoeic dogs. Biswas *et al.* (2006) also found no sex variation amongst the CPV positive cases, thus CPV infect/invade both the sexes uniformly and physiological differences viz., pregnancy, lactation, etc. have no influences in infectivity of virus or the susceptibility of dogs.

Dogs were grouped into two age groups viz., 0-<6 months and ≥6 months. Age wise prevalence results (Table 3) revealed that the prevalence of the canine parvo virus infection was higher in pups (0-<6 months age group) in comparison to dogs in the age group of ≥6 months and it was 65.51 and 57.14%, respectively. Similarly, Biswas *et al.* (2006) also reported that dogs of 0-6 months were more susceptible with highest mortality rate. The probable reason for low prevalence of CPV

in adult dogs might be the repeated subclinical infection or the exposure of virus in less number and the prevalent CPV might be boosting the antibody titre high enough to protect the animal.

Association of canine parvo virus infection with vaccination status of dogs revealed that prevalence of canine parvo virus infection was higher in unvaccinated dogs (64.00%) in comparison to vaccinated dogs (50.00%) (Table 4). Although the 50.00% prevalence of CPV in vaccinated dogs was quite high and it might be because of irregular vaccination or the use of improperly maintained vaccines. However, vaccination helped in controlling the disease in dogs and reduced the incidence. The prevalence of canine parvo virus infection was higher in dogs which shared their habitat with other dogs (70.58%) in comparison to those which were reared only (61.44%) (Table 5). It indicated that Canine parvo virus appeared to be more prevalent in areas of intensive animal husbandry practices such as kennels and dog training centres, thus unhygienic conditions and improper management also seems to be key factors.

CONCLUSION

From the present study, it can be concluded that CPV is prevalent in the dogs of Mathura and nearby area and more common in pups of age less than 6 months old and more prevalent in German shepherd, Labrador and Pomeranian breeds of dog and more common in the dogs that share their habitat with another dog. This will be helpful in formulating the control strategies for controlling this highly contagious disease.

ACKNOWLEDGMENTS

The authors of this study are highly thankful to Dean, College of Biotechnology and Hon'ble Vice Chancellor, DUVASU, Mathura; for providing funds and necessary facilities to conduct the study; Smt. Mamta, laboratory staff of Central disease diagnostic laboratory; for providing technical help in the laboratory; the dog owners who gave their permission for their dogs to take part in the study.

REFERENCES

Anonymous, 1997. Directorate of economics and statistics. M/O Agriculture, livestock Census 1987 and 1992, Basic Animal Husbandry Statistics.

Appel, M.J.G., B.J. Cooper, H. Greisen and L.E. Carmichael, 1978. Status report: Canine viral enteritis. J. Am. Vet. Med. Assoc., 173: 1516-1518.

Biswas, S., P.J. Das, S.K. Ghosh and N.R. Pradhan, 2006. Detection of canine parvo virus (CPV) DNA by polymerase chain reaction and its prevalence in dogs in and around Kolkata, west Bengal. Indian J. Anim. Sci., 76: 324-325.

Carmichael, L.E. and L.N. Binn, 1981. New enteric viruses in the dog. Adv. Vet. Sci. Comp. Med., 25: 1-37.

Cho, H.S., J.I. Kang and N.Y. Park, 2006. Detection of canine parvo virus in fecal samples using loop-mediated isothermal amplification. J. Vet. Diagn Invest., 18: 81-84.

De Castro, T.X., C.M.A. Uchoa, M.C. de Albuquerque, N.V. Labarthe and R. de Cassia Nasser Cubel Garcia, 2007. Canine parvo virus (CPV) and intestinal parasites: Laboratorial diagnosis and clinical signs from puppies with gastroenteritis. Int. J. Appl. Res. Vet. Med., 5: 72-76.

Han, S., B. Qi and X. Zhang, 2011. A retrospective analysis on phylogeny and evolution of CPV Isolates in China. Asian J. Anim. Vet. Adv., 6: 1204-1213.

Kumar, A., A.K. Verma and A. Sharma, 2010. Canine viral diseases: An overview. Indian Pet J., 6: 16-21.

Kumar, M., S. Chidri and S. Nandi, 2011. A sensitive method to detect Canine parvoviral DNA in faecal sample by nested Polymerase chain reaction. Indian J. Biotechnol., 10: 183-187.

Nandi, S., A.B. Pandey, K. Sharma, S.D. Audarya and R.S. Chauhan, 2006. Development and standerdization of PCR for the detection of canine parvoviral DNA in the stool samples of canines. J. Immunol. Immunopathol., 8: 141-142.

Nandi, S., R. Anbazhagan, M. Kumar and R.S. Chauhan, 2009. Molecular characterization of canine parvo virus strains in vaccines by polymerase chain reaction and restriction endonuclease analysis. Indian J. Virol., 20: 12-15.

Nandi, S., R. Anbazhagan and M. Kumar, 2010. Molecular characterization and nucleotide sequence analysis of canine parvo virus strains in vaccines in India. Vet. Ital., 46: 69-81.

Panda, D., R.C. Patra, S. Nandi and D. Swarup, 2009. Antigenic characterization of canine parvo virus by polymerase chain reaction. Indian J. Anim. Sci., 79: 876-879.

Ramadass, P. and T.G.A. Khader, 1982. Diagnosis of canine parvo virus infection by agar gel precipitation test and fluorescent antibody techniques. Cheiron, 11: 323-325.

Sambrook, J. and D.W. Russell, 2001. Molecular Cloning: A Laboratory Manual (6. Preparation and Analysis of Eukaryotic Genomic DNA). 3rd Edn., Cold Spring Harbor Laboratoty Press, New York, pp: 545-547.

Schunck, B., W. Kraft and U. Truyen, 1995. A simple touch-down polymerase chain reaction for the detection of canine parvo virus and feline panleukopenia virus in feces. J. Virol. Methods, 55: 427-433.

Snedecor, G.W. and W.G. Cochran, 1967. Statistical Methods. 6th Edn., Oxford and IBH Publishing Co., New Delhi, India.

Verma, Kr. A., Mahima and A. Kumar, 2009. Canine parvo virus: A threat to pets. Indian Pet J. Online J. Canine, Feline Exotic Pets, Vol. 3.

Ying, H., F. Zhong, W. Cao and M. Zhang, 2012. The subcellular localization and the tissue tropism of canine parvo virus based on the co-localization of transferrin receptors. Asian J. Anim. Vet. Adv., 7: 235-242.

Zhou, B., M.H. Ye, R. Chen and J.T. Ding, 2009. Preliminary observations using canine parvo virus-specific transfer factor in the prevention of canine parvo virus disease. Res. J. Vet. Sci., 2: 21-29.