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Research Article

PREVALENCE AND MOLECULAR CHARACTERIZATION OF CANINE PARVOVIRUS IN ASSAM AND PUNJAB

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Abstract- Canine parvovirus a single stranded DNA virus causes hemorrhagic gastroenteritis in dogs and is the major cause of mortality in pups due to gastroenteritis. The virus has three major antigenic types CPV 2a, CPV 2b and CPV 2c which are prevalent in dog population worldwide. The virus is excreted heavily in feces of affected dogs, so can be easily detected in the feces of the affected dogs. Therefore, in the present study fecal samples were collected from the dogs suspected of CPV, DNA extracted from them and subjected to PCR and nested PCR to study the prevalence of CPV in two regions *viz*. Punjab and Assam regions of India. The prevalence of CPV was found to be 75% in Punjab and 80.55% in Assam by nested PCR with overall prevalence being 77%. Positivity for CPV was also observed in dogs which were vaccinated for the disease with percent positivity in Punjab being 84.6% and in Assam being 72.7% by nested PCR. The overall percent positivity in vaccinated dogs was found to be 79.16%. Therefore, it can be concluded that CPV is highly prevalent in the regions under study.

Keywords- *Canine Parvovirus, dogs, PCR, Nested PCR*

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Introduction

Canine parvovirus is a single stranded DNA non-enveloped icosahedral virus with approximate diameter of 20nm that belongs to the genus Parvovirus under the family Parvoviridae [1]. Currently, the three major antigenic variants of CPV-2 have been found among the dog population are *i.e*. 2a, 2b and 2c [2]. The genome size of the CPV is about 5.3 Kb and has terminal palindromic sequences and two open reading frames (ORF). The ORF1 encodes for two non-structural (NS) proteins NS1 and NS2 mRNA and ORF2 encodes for two capsid proteins VP1 and VP2 [3]. CPV-2 mainly causes hemorrhagic gastroenteritis and myocarditis in dogs and spreads rapidly in both domestic as well as wild population of canines. The disease is widely prevalent in Asia [4]. In India disease was first reported in Madras [5]. The analysis of CPV strains in India revealed the evolution of an unusual CPV-2 mutant, with a change (Asp-426 to Glu) occurring in the strategic residue 426 and previously designated as CPV-2c [6]. Occurrence of different antigenic types of CPV have been reported from different parts of India *viz*. Kerala, Assam, Tamil Nadu, Orissa, West Bengal, Pondicherry, Punjab, Haryana and Uttar Pradesh.A number of methods are being used to diagnose the disease *viz*. virus isolation in cell culture using Madin Darby Canine Kidney (MDCK), Crandle Feline Kidney (CRFK) and A-72 cell lines, Haemagglutination (HA), Haemagglutination inhibition (HI), Agar Gel Precipitation Test (AGPT), Electron Microscopy, Indirect Fluorescent Antibody Test (IFT), Enzyme Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR) [7].However, their sensitivity, like other traditional diagnostic methods, has been proven to be inferior to molecular assays. Besides these, PCR and nested-PCR (NPCR) could be used for CPV detection as these have reported high sensitivity and specificity [8, 9]. Therefore, the present study was envisaged with the objective to study the prevalence and molecular characterize CPV in parts of Assam and Punjab using PCR and nested PCR.

Materials and Methods

The institutional animal ethics committee permission was taken to perform the experiments (GADVASU/2018/IAEC/47/19).

A total of 100 rectal swabs were collected in phosphate buffer saline (pH=7.2) from the dogs showing clinical symptoms of Canine Parvovirus *viz*. vomition, diarrhea, hemorrhagic diarrhea during the period from January 2018 to April 2019. The samples were collected from two different states, n=64 from Punjab and n=36 from Assam along with the history of vaccination of the dogs for CPV. The DNA from all the samples as well as the vaccine was extracted using conventional method (phenol-chloroform extraction method) [10]. The extracted DNA were then subjected to PCR as well as nested PCR (NPCR) to study the prevalence of the disease.

Molecular detection of Canine Parvovirus

PCR

The PCR reaction mixture was made by adding, 5.0 µl of 10X PCR buffer (with 15 mM $MgCl_2$), 1.0 µl of forward and reverse primer (20 pm/µl) each [Table-1], 1.0 µl of dNTPs mix (10 mM), 0.2 µl Taq DNA polymerase (5units/µl), 15µl of the template DNA and the final volume of the reaction was made up to 50µl using nuclease free water. The rectal swab from a healthy dog was used as a negative control and a DNA from a vaccine (Megavac, Indian Immunologicals Pvt. Ltd.) was used as a positive control.

Nested PCR (NPCR)

NPCR reaction mixture was made by adding 2.5 µl of 10X PCR buffer (with 15 mM $MgCl_2$), 1.0 µl each of forward and reverse primer (20 pm/µl) [Table-2], 1.0 µl of dNTPs (10 mM), 0.2µl Taq DNA polymerase (5 units/µl), 5µl of the PCR product (from above reaction) and the final volume was made up to 25µl by adding nuclease free water. In both PCR and nested PCR, the reaction was put in a thermocycler with 35 cycles of denaturation at 94°C for 60s, annealing at 55°C for 60s, elongation at 72°C for 150s and a final elongation at 72°C for 10 min. PCR and NPCR products were run on 1% agarose gel having ethidium bromide (10 mg/ml) using 1 Kb DNA ladder (Smobio) and visualized and photographed using gel documentation system (Syngene).

Table-1 *Primers specific for VP2 gene of CPV used in PCR[11]*

Primers	Sequence	Position in genome	Product size (bp)
CPV: 2	5'-AGCTATGAGATCTGAGACAT-3'	3350-3369	1198
CPV: 4	5'-AGTATGTTAATATAATTTTCTAGGTGC-3'	4523-4543	

Table-2 *Primers specific for VP2 gene of CPV used in NPCR [11]*

Primers	Sequence	Position in genome	Product size (bp)
CPV: 6	5′-ATACAGGAAGATATCCAGAAG-3′	3350-3396	548
CPV: 4	5′-AGTATGTTAATATAATTTTCTAGGTGC-3′	4523-4543	

Results and Discussion

Out of the total 100 samples subjected to PCR 32 samples were positive for CPV yielding a product size of 1198 bp [Fig-1]. Thus, the prevalence of CPV was found to be 32% by PCR. Further it was observed that out of 64 samples collected from Punjab 20 samples were positive indicating 31.25% positivity in Punjab. Similarly, out of 36 samples collected from Assam 12 samples were positive indicating positivity to be 33.3%. Thus, almost similar status of CPV in northern and north-eastern regions under study was observed.

Table-3 *Positive samples by PCR and Nested PCR from Assam*

SN	Sample No.	Vaccination Status	Booster	PCR	Nested PCR
1	A1	-	Done	-	+
2	A2	-	-	+	+
3	A3	-	-	+	+
4	A4	-	-	+	+
5	A5	-	-	+	+
6	A6	Done	-	+	+
7	A7	-	-	+	+
8	A8	-	-	-	+
9	A9	-	-	-	+
10	A10	Done	-	-	+
11	A35	-	-	+	+
12	A36	Done	Done	-	+
13	A37	-	-	-	+
14	A38	-	-	-	+
15	A39	Done	Done	-	+
16	A40	-	-	-	+
17	A44	Done	Done	-	+
18	A45	-	-	-	+
19	A46	-	-	+	+
20	A64	-	-	-	+
21	A65	Done	-	-	+
22	A66	Done	Done	-	+
23	A67	-	-	-	+
24	A68	-	-	+	+
25	A69	-	-	+	+
26	A70	-	-	-	+
27	A71	Done	Done	-	+
28	A72	-	-	+	+
29	A73	-	-	+	+

In the table'-' depicts negative sample and '+' depicts positive sample

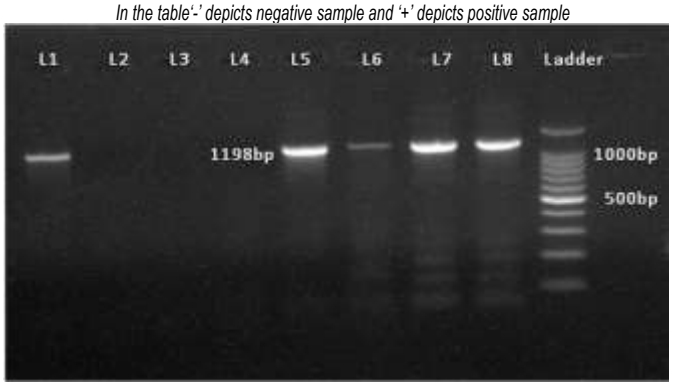

Fig-1 Polymerase Chain Reaction (PCR) for the detection of Canine Parvovirus Lane ladder: 1 Kb ladder, Lane 1, 5, 6, 7: Positive samples, Lane 2, 3: Negative samples, Lane 4: Negative control, Lane 8: Positive control.

When the vaccination status of the dogs was studied it was observed that out of the total of 100 samples collected from dogs; 24 dogs had the history of vaccination for CPV and also nine dogs had received booster vaccination. Out of 24 dogs which had history of vaccination seven dogs were positive for CPV by PCR indicating percent positivity in vaccinated dogs to be 29.16%. When the percent positivity in vaccinated dogs was studied in two states *viz.* Punjab and Assam, it was observed that 46.15% (6/13) were positive in Punjab and 9.0% (1/11) were positive in Assam out of the vaccinated animals in each state. Therefore, CPV positive cases were observed in vaccinated dogs in both the regions under study [Table-3 and 4].

Table-4 *Positive samples by PCR and Nested PCR from Punjab*

SN	Sample No.	Vaccination Status	Booster	PCR	Nested PCR
1	P12	Done	Done	-	+
2	P13	-	-	+	+
3	P14	-	-	-	+
4	P15	-	-	+	+
5	P16	-	-	-	+
6	P23	-	-	+	+
7	P26	Done	-	-	+
8	P27	Done	Done	-	+
9	P28	-	-	-	+
10	P29	-	-	+	+
11	P47	-	-	-	+
12	P48	Done	-	+	+
13	P50	-	-	-	+
14	P53	Done	-	+	+
15	P54	-	-	+	+
16	P56	-	-	+	+
17	P57	-	-	-	+
18	P58	-	-	-	+
19	P59	-	-	-	+
20	P60	-	-	+	+
21	P61	-	-	-	+
22	P63	-	-	+	+
23	P74	Done	-	+	+
24	P75	-	-	+	+
25	P76	Done	-	+	+
26	P77	-	-	-	+
27	P79	-	-	-	+
28	P80	-	-	-	+
29	P81	Done	-	+	+
30	P82	-	-	-	+
31	P83	-	-	-	+
32	P84	-	-	-	+
33	P85	-	-	+	+
34	P86	-	-	+	+
35	P87	-	-	+	+
36	P88	Done	-	+	+
37	P89	Done	-	-	+
38	P90	-	-	-	+
39	P91	-	-	-	+
40	P92	Done	-	-	+
41	P93	-	-	-	+
42	P94	-	-	-	+
43	P95	-	-	+	+
44	P96	-	-	-	+
45	P97	-	-	-	+
46	P98	-	-	-	+
47	P99	-	-	+	+
48	P100	-	-	-	+

In the table '-' depicts negative sample and '+' depicts positive sample

When the PCR products of the 100 rectal swabs were subjected to NPCR, 77 samples were positive with nested PCR yielding a product size of 548bp [Fig-2]. Thus, the prevalence of CPV was found to be 77% which indicated NPCR to be more sensitive than PCR. Further it was observed that out of 64 samples collected from Punjab 48 samples were positive indicating 75% positivity by NPCR. Similarly, out of 36 samples collected from Assam 29 samples were positive indicating positivity to be 80.55% by NPCR. When the vaccination status of the dogs was studied it was observed that out of the total 100 samples collected from dogs; the 24 dogs which had history of vaccination 19 dogs were positive for CPV by NPCR indicating percent positivity in vaccinated dogs to be 79.16%.

When the percent positivity in vaccinated dogs was studied in two states *viz*. Punjab and Assam, it was observed that 84.6% (11/13) were positive in Punjab and 72.7% (8/11) were positive in Assam [Table-3 and 4].

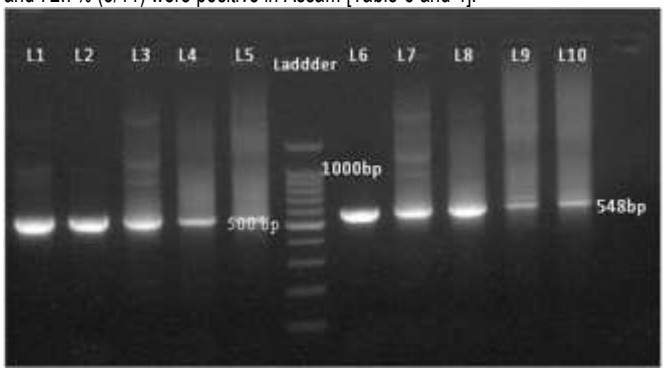

Fig-2 Nested PCR (NPCR) for the detection of Canine Parvovirus

Lane ladder: 1 Kb ladder, Lane 1: Positive control, Lane 2, 3, 4, 5, 6, 7, 8, 9, 10: Positive samples

Many researchers have studied the prevalence of Canine Parvovirus using PCR and NPCR as has been used in the present study. Hirasawa *et al* [12] conducted PCR and nested PCR and showed nested PCR to be 100 times more sensitive than the single PCR and further confirmed the specificity of the method by restriction enzyme analysis and southern hybridization.

Similarly, Singh *et al* [13] screened 100 fecal samples from dogs showing signs of gastroenteritis in Mathura, U.P. and found prevalence to be 63%. Mizak and Rzezutka [11] used nested PCR for detection of canine parvovirus in faeces of dogs and reported that the sensitivity of detection of CPV by nested PCR was increased 60 per cent in comparison with the standard PCR method and it was 30 per cent higher than using isolation of virus in tissue culture. Further, Kumar *et al* [9] developed a nested polymerase chain reaction (n-PCR) using published pCPV-2ab as external primer set and self-designed pCPV-2N as internal primer set for the detection of canine parvovirus in fecal samples of dogs and found nested PCR to be more sensitive than PCR. Kaur *et al* [14] tested 85 animals rectal swabs suspected of CPV using a PCR, nested PCR and a newly designed differential PCR and observed that with PCR seven (8.23 %) and with nested PCR 39 (45.88 %) were positive and 40 (47.05 %) were positive for either one or more than one antigenic types of CPV using differential PCR.

Conclusion

It can be concluded from the study that Canine Parvovirus is highly prevalent in India and further vaccinated dogs are showing positive reaction for the disease. So regular monitoring of the disease is important to have the current status of the disease.

Application of research: Study of prevalence and molecular characterization of canine parvovirus in Assam and Punjab

Research Category: Veterinary Microbiology

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Study area / Sample Collection: Assam and Punjab

Cultivar / Variety / Breed name: Dog

Conflict of Interest: None declared

Ethical approval: Ethical approval taken from Institutional Animal Ethics Committee (IAEC), India.
Ethical Committee Approval Number: GADVASU/2018/IAEC/47/19

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