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Short Communication

Polymerase chain reaction as a diagnostic tool for the detection of canine parvovirus infection in dogs

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Abstract

Canine parvovirus (CPV) is a highly contagious viral pathogen affecting dogs, primarily targeting rapidly dividing cells of the gastrointestinal tract (GIT), which leads to haemorrhagic gastroenteritis. Several variants include CPV-2, CPV-2a, CPV-2b and CPV-2c. CPV can be prevented by timely and complete vaccination, and can be treated effectively if timely diagnosis is done. In the present study, polymerase chain reaction (PCR) was conducted on 100 samples, and the total percent positivity was calculated to be 39%. It was observed that the maximum number of cases were in the age group of 1- 6 months, while dogs aged more than 1 year were found to be less positive. Males were found to be more positive than females, and maximum cases were found in German Shepherd, Retriever and Pomeranian. It was also found that the incidence rate and the positivity rate were both high in stray animals. It can be concluded that canine parvovirus infection is quite prevalent in dogs, and care should be taken for its diagnosis and treatment.

Keywords: Canine parvovirus, Dog, Haemorrhagic gastroenteritis, Polymerase chain reaction

Highlights

- CPV causes haemorrhagic gastroenteritis.
- Rectal swabs are an appropriate sample for detection.
- PCR is an effective technique to detect CPV.
- Percent positivity is high in young dogs.
- German Shepherd, Retriever and Pomeranian breeds are more susceptible.

Canine parvovirus (CPV) belongs to the *Parvovirus* genus under *Parvoviridae* family. It is a linear, single-stranded, negative-sense virus (Parrish *et al*., 1982). It is an icosahedral non-enveloped virus that is around 20 nm in size (Reed *et al*., 1988). Two structural proteins, VP-1 and VP-2, as well as two non-structural proteins, NS-1 and NS-2, are encoded by the 5323 bases of single-stranded DNA that make up the genome (Reed *et al*., 1988). In the 5323 base CPV genome, the VP-1 gene is found between 2285 and 4537 (2253 bases) and the VP-2 gene between 2783 and 4537 (1755 bases).

Diagnosis of CPV is very important, and subsequent treatment can prevent mortality in dogs. A number of laboratory diagnostic tools are available and can be used for its diagnosis. However, molecular techniques like PCR are rapid and highly sensitive techniques. These techniques are well-established for the diagnosis of CPV and can be used routinely for the surveillance of the disease.

It has been discovered that dogs of all sexes, ages, and breeds are vulnerable to CPV-2 infection (Castro *et al*., 2007; Gombac *et al*., 2008). During the acute phase of infection, the virus is shed in the feces of infected dogs, and infected feces serve as the primary source of infection (Carmichael and Binn, 1981). Thus, the feco-oral route is the primary means of transmission. The virus can spread to people's hands, shoes, clothing, food bowls, and other objects, which can then infect dogs (Pollock and Carmichael, 1982; Carmichael, 1994; Decaro *et al*., 2005). The transmission takes place directly or indirectly, and the virus is hardy and can survive in the environment for long periods.

Therefore, in this study, PCR was used to detect canine parvovirus infection in dogs as this is a sensitive technique for detecting CPV.

To detect the percent positivity of canine parvovirus infection in clinical cases of dogs, 100 samples (Table 1) were collected from the University Veterinary Hospital, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, all aged from 1 week to 1 year and above. The samples were collected from February 2022 to February 2023, with maximum samples available from November to February. Rectal swabs were collected using sterile swabs, which were

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Table 1. Details of samples collected from the Veterinary Hospital of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana

Age group	Number of samples	Sex (M/F)	Vaccination status	Breed
1-3 months	42	M= 29 F= 13	Complete= 3 Partial= 5 Not done= 34	Non-descript/Stray= 11 Others= 31
3-6 months	26	M= 17 F= 9	Complete= 5 Partial= 6 Not done= 15	Non-descript/Stray= 7 Others= 19
6-9 months	6	M= 5 F= 1	Complete= 2 Partial= 2 Not done= 2	Non-descript/Stray= 1 Others= 5
9-12 months	8	M= 4 F= 4	Complete= 2 Partial= 3 Not done= 3	Non-descript/Stray= 3 Others= 5
1 year and above	18	M= 13 F= 5	Complete= 7 Partial= 4 Not done= 7	Non-descript/Stray= 2 Others= 16

M: Male; F: Female; Partial/Incomplete Vaccination: animals vaccinated only first time

placed in 4 mL phosphate-buffer saline, pH 7.4. The samples were processed by centrifugation at 3000 x g for 5 minutes, supernatant collected, and stored at a temperature of -20°C for further processing, such as DNA extraction. Clinical signs for the suspected patients included haemorrhagic gastroenteritis, nausea, vomiting, fever, lethargy, in appetence, dehydration, and abdominal pain (Sykes, 2014). Samples were collected, DNA was extracted, and PCR was performed on the samples.

The DNA extraction from the collected samples was done using the QIAMP® DNA mini kit (Germany). PCR was run on 100 samples using the primers as per Buonavoglia *et al*. (2001). The primers targeted the VP2 and the amplicon size was 611bp. The reaction mixture and thermal cycling conditions are given in Tables 2 and 3, respectively.

Table 2. Components and their respective volumes for PCR master mixture

Components	Volume (μ L)
*Forward primer (20 pmol/μ L)	0.8
*Reverse primer (20 pmol/μ L)	0.8
10x PCR Buffer	2.0
dNTP (10 mM)	1.0
$MgCl_2$ (50 mM)	0.5
Taq Polymerase (5 units/μ L)	0.2
Template	1.0
Nuclease free water	13.7
Total	20

After PCR, the samples were then subjected to agarose gel electrophoresis using 1.5% agarose at 90 volts for 1 hour with a thermo fisher ladder plus 100bp and then examined under a gel documentation system (Syngene, USA). The positive control used in PCR was the vaccine (Nobivac DHPPi) procured commercially, and the negative control was the sample from a healthy dog.

To detect the percent positivity of canine parvovirus infection in clinical cases of dogs, PCR was done, and the number of samples positive for CPV was 39 out of 100. Therefore, the total percent positivity for CPV infection came out to be 39% (Fig. 1).

It was observed that the maximum number of cases were in the age

Table 3. Thermal cycling conditions used for PCR in the thermal cycler (Bio Rad)

Condition	Temperature	Time	Cycles
Initial denaturation	94°C	3 minutes	1
Denaturation	94°C	30 seconds	40
Annealing	54°C	45 seconds	
Extension	72°C	1 minute	
Final extension	72°C	7 minutes	1

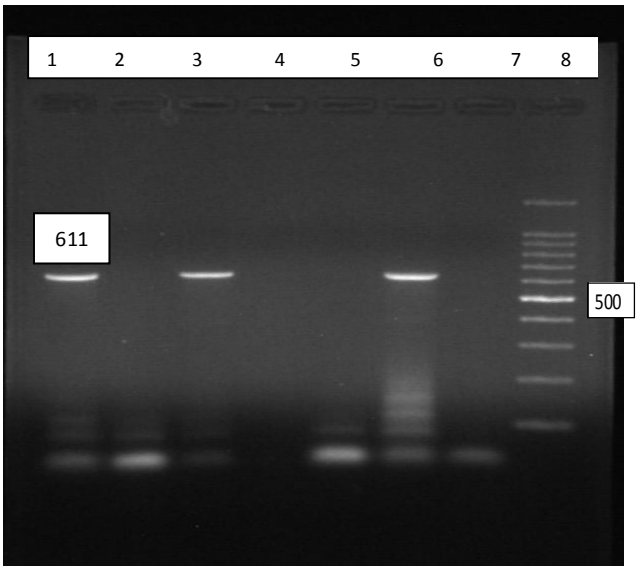

Fig. 1. CPV Positive samples in lane 1, 3, 6 at 611bp; 8th lane depicting ladder

group of 1 month to 6 months, while dogs aged more than 1 year were found to be less positive (Fig. 2). Also, a comparison was made on the basis of sex, and it was found that males (62%) were found to be more positive than females (38%). Samples were collected from a number of breeds and the maximum positive cases came from German Shepherd, Retriever and Pomeranian. It was also found the incidence rate and the positivity rate were both high in stray animals.

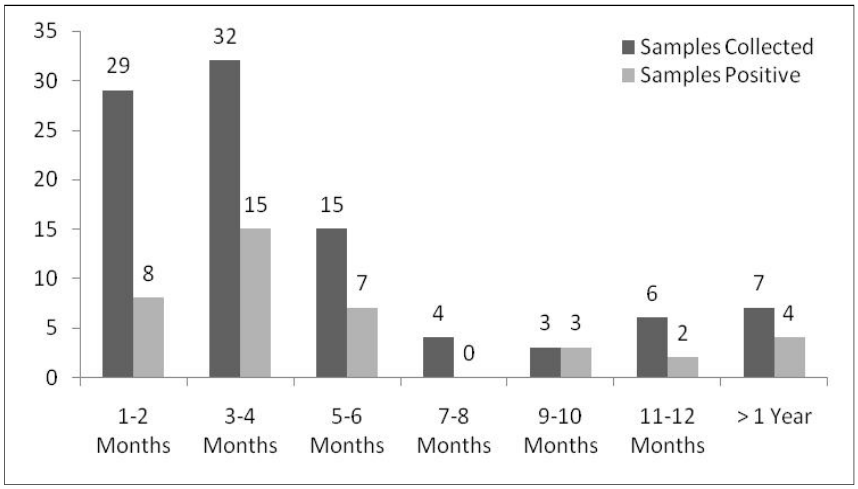

Fig. 2. Comparison on the basis of age of dogs

Canine parvovirus causing gastroenteritis in dogs is highly prevalent in the dog population and is also responsible for heavy mortality in young dogs. It is a small DNA virus that has two open reading frames, and the VP2 protein is responsible for antigenic variability in the virus types. Similar to the present study Özkul *et al*. (2002) also used PCR for the detection of canine parvovirus (CPV) in a fecal sample from a dog with enteritis in Turkey. The final PCR product was analyzed using the restriction fragment length polymorphism (RFLP) technique. RFLP analysis using Apa LI and Eco RV restriction endonucleases revealed homology in the nucleotide sequence in at least the VP2 coding region of the virus DNAs detected in the fecal specimen and prepared from attenuated vaccine virus as a positive control. A similar study was conducted to understand the antigenic types of the virus prevailing in northern India. For this, rectal swabs were collected from suspected cases of dogs, i.e. dogs having clinical signs of gastroenteritis. PCR and Nested PCR showed 28% and 70% positivity, respectively, and when 13 samples out of these positive samples were sequence analysed on the basis of VP2 gene of the virus, CPV-2a type was found to be predominant. It was also observed that 24.10% of positive dogs had been vaccinated for the disease (Singh *et al*., 2021).

Further the studies on the same lines was done on a total of 410 rectal swabs from canines exhibiting signs of haemorrhagic gastroenteritis by Kour *et al*. (2023). Individual data such as age, breed, and immunization history were also recorded for each dog. The CPV-2 positives showed a band at the expected size of 611 bp. The researchers found in their study a total of 225 samples to be positive, with an overall prevalence of 54.88%. Female dogs (66.89%) were found to be affected more commonly than male dogs (48.09%), contrary to our study. Higher percent positives were found in puppies under 3 months of age (65.83%). Data analysis by breed revealed that the exotic dog breeds showed a bit higher percent positivity (55.89%) than indigenous and non-descript breeds (53.08%). Unvaccinated dogs were infected with CPV at a higher rate (72.88%) than the vaccinated dogs (52.55%). Researchers have used Real Time PCR too for the detection of CPV, as revealed by Kour *et al*. (2022), who performed Real Time PCR on a total of 178 samples collected from Punjab, Assam, J&K, Chandigarh and Delhi to assess number of positive samples for each CPV-2, CPV-2a and CPV-2b. Out of the total 178 samples, 27 were positive for CPV-2, 86 were positive for CPV-2a and 20 samples for CPV-2b. Another interesting fact discovered was that a few samples were positive for more than one CPV variant simultaneously.

The study encompassed the detection of CPV in the rectal swabs of dogs showing symptoms of gastroenteritis, and it was found to be 39% with maximum positive cases in pups of 1-6 months of age belonging to German Shepherd, Pomeranian and Retrievers. It can be concluded that canine parvovirus infection is quite prevalent in dogs, and care should be taken for its diagnosis and treatment.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: KK: Involved in the investigation, data generation and preparing original draft; GK: Engaged in conceptualization, supervision and final editing; MC: Involved in methodology and editing.

Data availability statement: Raw data of this study are available from the corresponding author.

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