


Original Research Article

<https://doi.org/10.20546/ijcmas.2022.1110.028>

## Diagnosis and Incidence of Canine Parvovirus Gastroenteritis

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### ABSTRACT

The present study conducted to carry out incidence of canine parvovirus gastroenteritis at Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat, India. A total of 1540 dogs were presented at the Veterinary Clinical Complex, Anand during the study period from October 2021 to February 2022. Faecal samples were for confirmation of canine parvovirus gastroenteritis using the polymerase chain reaction (PCR). Out of 1540 dogs, 145 (9.42%) dogs were found positive for Canine Parvo Virus (CPV) by PCR. The age-wise incidence was the highest 66.20 % (96/145) in 0-3 months followed by 22.06 % (32/145) in 4-6 months, 8.96% (13/145) in 7-12 months and 2.76 % (4/145) in >12 months of age. The breed-wise incidence was the highest 51.03% (74/145) in non-descript breeds followed by Labrador 18.62% (27/15), German shepherd 8.96% (13/145), Doberman 7.59% (11/145), Pomeranian 5.52% (8/145), Rottweiler 2.76% (4/145), Pug 2.76% (4/145), and Golden Retriever, Lhasa Apso, Beagle and Mongrel 0.86% (1/145) each. The sex-wise incidence was higher 62.07% (90/145) in males as compared to females 37.93% (55/145). The vaccination status wise incidence was the highest 80.69% (117/145) in unvaccinated dogs followed by vaccinated dogs 17.24% (25/145) and partially vaccinated dogs 2.07% (3/145).

#### Keywords

PCR, Incidence,  
Age wise, Breed  
wise, Sex wise,  
Vaccination status  
wise

#### Article Info

**Received:**  
05 September 2022  
**Accepted:**  
30 September 2022  
**Available Online:**  
10 October 2022

### Introduction

Haemorrhagic gastroenteritis (HGE) in dogs is a life-threatening condition. HGE is a severe form of diarrhoea that affects dogs of all breeds and ages, though small breeds are more likely to be affected than large breeds (Kumar *et al.*, 2014). Incidence is higher in animal shelters, pet stores and breeding kennels. Canine parvovirus (CPV) can affect dogs at

any age. Severe infection is most common in puppies between 6 weeks to 4 months. All breeds of dogs are susceptible to CPV infection. In comparison to pure breeds such as Rottweilers, Doberman Pinchers, English Springer Spaniels and German Shepherds, the crossbreeds, Toy Poodles and Cocker Spaniels are less vulnerable (Houston *et al.*, 1996). Puppies are predisposed to parvovirus infection because of a lack of maternal immunity,

gastrointestinal parasites, unsanitary environments and stress (Hong *et al.*, 2007; Mylonakis *et al.*, 2016). CPV causes acute gastroenteritis in puppies with a high fatality rate (Carmichael and Binn, 1981). In young puppies, the death rate might be as high as 69 per cent (Horner, 1983). Adults are assumed to be immune to CPV infection due to their decreasing susceptibility as they get older and the presence of particular immunity acquired by vaccination or past (typically subclinical) infections (Ukwueze *et al.*, 2020).

Serological tests for CPV detection include ELISA, haemagglutination, slide inhibition test, slide agglutination test, immunochromatographic strip test and PCR and its variants such as nested PCR, insulate isothermal PCR and real-time PCR in the current days. LAMP (Loop-Mediated Isothermal Amplification) and Biosensors are two of the quickest testing. The immune-chromatographic strip, on the other hand, is the most often utilized (Khatri *et al.*, 2017).

## **Materials and Methods**

The incidence of CPV was assessed for parameters, *viz.*, age, sex, breed and vaccination status. The dogs were grouped into 4 classes for the age-wise incidence, *viz.*, 0 to 3 months, 4 to 6 months, 7 to 12 months and >12 months, into two classes for sex-wise incidence, *viz.*, male and female and into three classes based on vaccination status, *viz.*, vaccinated, partially vaccinated (only primary vaccination done, booster dose not done) and non-vaccinated. For breed-wise incidence the dogs were categorized according to different breeds presented at the Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, India.

A total 168 faecal samples were collected using sterile swabs from diarrhoeic dogs reported to Veterinary Clinical Complex, Anand, Gujarat with clinical signs of dullness, diarrhea or haemorrhagic diarrhoea, vomition, dehydration and loss of appetite. Sterile swabs and swabs were stored in

phosphate buffer saline (pH=7.2), vortexed, and stored at -20°C until further use. The viral DNA was extracted according to Antony *et al.*, (2006) by boiling method. Samples suspended in 100 µL milli Q water were boiled for 15 minutes. Centrifugation was employed to remove the cell debris and 5 µL of the supernatant was employed as a template DNA and then they were examined for the presence of CPV DNA using traditional PCR using published primers (Table 1). Faecal samples were also examined for endoparasites in all dogs CPV affected dogs as per the method described.

A total 5 µL of DNA sample containing 30 ng/µL concentration was used as a template for PCR reaction (Table 2). 6.25µL of PCR master mix (2X concentration), 1 µL forward and reverse primer and 5.5 µL nuclease free water. Cyclic conditions for CPV VP2 gene primer included one cycle of initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec., annealing at 58°C for 15 sec. and extension at 72°C for 1 min. The PCR products were analyzed in 2% agarose gel electrophoresis and visualized using UV trans-illuminator or Gel Documentation System.

## **Results and Discussion**

### **Incidence**

#### **Age Wise**

The age-wise incidence of CPV is depicted in Table 2. Similar the highest incidence of canine parvovirus gastroenteritis in less than 6 months of age puppies were observed by Mehta *et al.*, (2017); Roy *et al.*, (2018); Sharma *et al.*, (2019); Saravanan *et al.*, (2020); Bhattacharjee *et al.*, (2021) and Kushwaha *et al.*, (2021).

The higher prevalence in puppies of 0-3 months may be due to greater susceptibility of the enterocytes to viral tropism. According to Houston *et al.*, (1996), enterocytes of the intestinal crypts have a higher mitotic index during weaning as a result of changes in bacterial flora and diet and are thus more

susceptible. Thus, the higher prevalence of CPV infection in young dogs (0-3 months) was likely due to the close affinity of CPV virus for rapidly dividing intestinal cells. In addition, for lack of protective immunity from maternally derived antibodies or ineffective vaccination responses, the puppies are the most vulnerable to canine parvovirus infection. Above the age of one year, there was a very low incidence, which could be due to the development of antibodies in adults, either as a result of the vaccination schedule used or as a result of mild virus exposure leading to the development of antibodies in the host or for other reasons that need to be investigated (Patterson *et al.*, 2007).

### **Breed Wise**

The breed wise incidence is depicted in Table 3. Considering the breeds, non-descript breeds of dogs were found to have a maximum infection as compared to other breeds. This is in agreement with Behera *et al.*, (2015) who reported highest incidence of canine parvovirus gastroenteritis in non-descript breeds. It has been observed that non-descript breeds and stray dogs are free roaming, without vaccination and deworming. This might be the reason for high incidence of CPV gastroenteritis in stray dogs.

### **Sex Wise**

The sex wise incidence is depicted in Table 4. The result was in agreement with Rawat and Sumathi (2017); Roy *et al.*, (2018); Sharma *et al.*, (2019); Bhattacharjee *et al.*, (2021); Subramanian *et al.*, (2021) and in disagreement with Francis *et al.*, (2020); Chethan *et al.*, (2021) and Kushwaha *et al.*, (2021) who reported higher incidence of canine parvovirus gastroenteritis in males.

Male dogs have been suggested to be at higher risk for several diseases due to their behavior of roam more frequently thus increasing the risk of exposure to infectious agents and the selective desire of pet owners for male dogs (Anderson, 1980).

### **Vaccination Status Wise**

The vaccination status wise incidence is depicted in Table 5. These findings of Sharma *et al.*, (2019); Francis *et al.*, (2020); Tanwar *et al.*, (2020) and Kushwaha *et al.*, (2021), who also found that unvaccinated dogs are at an increased risk of parvovirus infection and that CPV vaccination is required to protect young puppies from this lethal infection. To protect young puppies from this deadly virus, CPV immunization is required.

### **Diagnosis**

#### **Direct faecal examination for parasitic infection**

The results of direct faecal sample examination depicted in table 6 and figure of endoparasites depicted in 1 to 4.

#### **Polymerase Chain Reaction (PCR)**

The results of PCR is depicted in Table 7. PCR assay is useful as a standard diagnostic tool for detecting CPV from faecal material, according to recent investigations (Andrea *et al.*, 2017; Agnihotri *et al.*, 2018; Kumari *et al.*, 2019). On agarose gel electrophoresis, it could identify 10 fg of the viral replicative form (RF) DNA and the PCR technique has recently become more widely employed to diagnose canine parvoviral infection.

PCR assay is widely employed standard diagnostic tool for diagnosis of canine parvoviral gastroenteritis from faecal material. A total 86.31% dogs were found positive for CPV using PCR. The overall incidence of CPV was 9.42% (145/1540) at Veterinary Clinical Complex, College of Veterinary science and A.H., Anand, Gujarat, India. The age-wise incidence was the highest 66.20 % in 0-3 months followed by 22.06 % in 4-6 months, 8.96% in 7-12 months and 2.76 % in >12 months of age.

**Table.1** Primers used for amplification of target VP2 gene fragment of viral DNA

Name of the target organism	Primer Sequence (5'-3')		Size of amplified products (bp)	References
VP2 gene of Canine Parvovirus	(F)	TCCAGCAGCTATGAGATC	747	Sakulwira <i>et al.</i> , (2003)
	(R)	GATCTGTTGGTAGCAATAC		

**Table.2** Age-wise incidence of canine parvovirus gastroenteritis

Age	No. of Cases	Percentage
0-3 months	96	66.20
4-6 months	32	22.06
7-12 months	13	8.96
>12 months	4	2.76
<b>Total</b>	<b>145</b>	<b>100</b>

**Table.3** Breed-wise incidence of canine parvovirus gastroenteritis

Breed	No. of Cases	Percentage
Non-descript	74	51.03
Labrador	27	18.62
German shepherd	13	8.96
Doberman	11	7.59
Pomeranian	8	5.52
Rottweiler	4	2.76
Pug	4	2.76
Golden Retriever	1	0.89
Lhasa Apso	1	0.89
Beagle	1	0.89
Mongrel	1	0.89
<b>Total</b>	<b>145</b>	<b>100</b>

**Table.4** Sex-wise incidence of canine parvovirus gastroenteritis

Sex	No. of Cases	Percentage
Male	90	62.07
Female	55	37.93
<b>Total</b>	<b>145</b>	<b>100</b>

**Table.5** Vaccination status wise incidence of canine parvovirus gastroenteritis

Vaccination Status	No. of Cases	Percentage
Vaccinated	25	17.24
Unvaccinated	117	80.69
Partially Vaccinated	3	2.07
<b>Total</b>	<b>145</b>	<b>100</b>

**Table.6** Results of direct faecal samples examination

Name of eggs of endoparasite	Total no. and Per cent
<i>Ancylostoma caninum</i>	51 (35.17)
<i>Isosporaspp.</i>	2 (1.38)
<i>Toxocaracanis</i>	1 (0.69)
<b>Total</b>	<b>54 (37.24)</b>

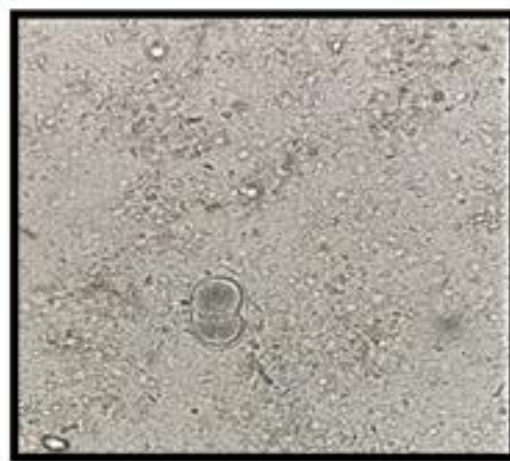
**Table.7** Detection of canine parvoviral VP2 gene in faeces of dogs by PCR

Polymerase Chain Reaction results	Percentage
Positive	145 (86.31)
Negative	23 (13.69)
<b>Total</b>	<b>168 (100)</b>

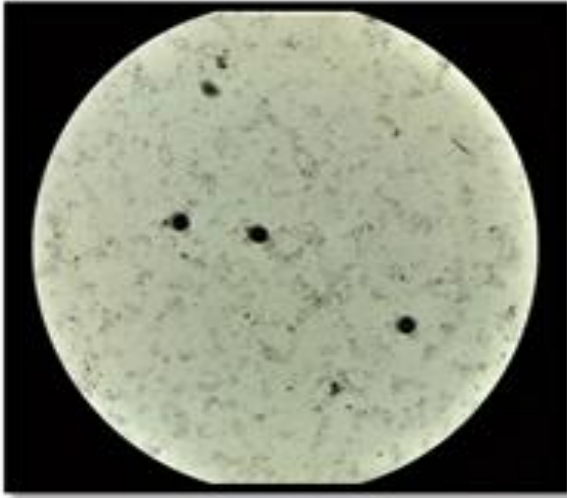
**Fig.1** Egg of *Ancylostoma caninum*(40X)



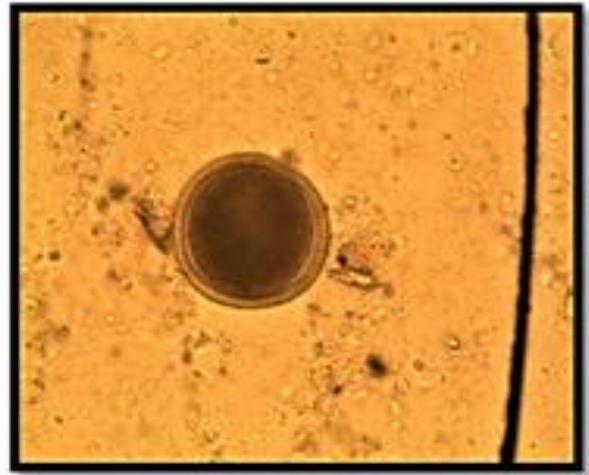
**Fig.2** Egg of *Isosporaspp.* (40X)



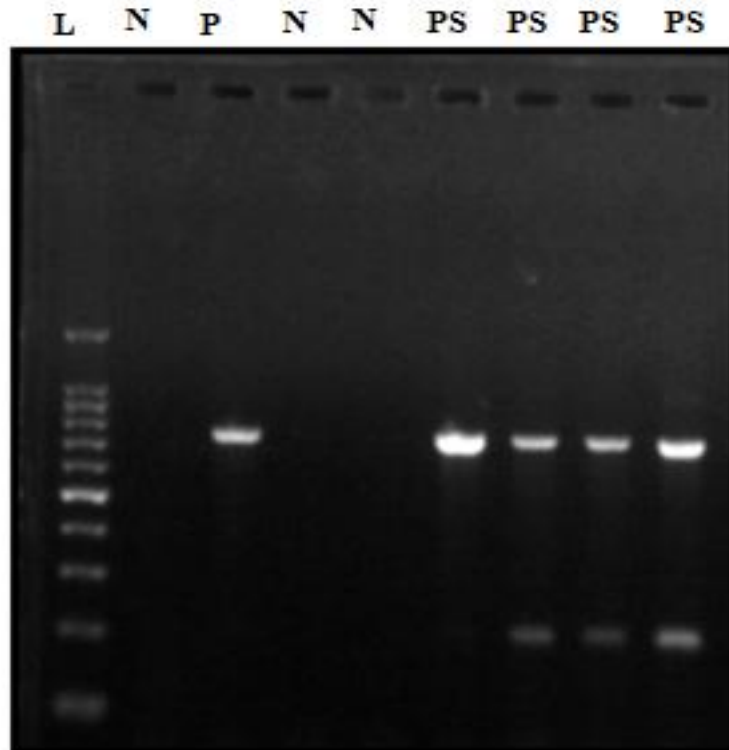
**Fig.3** Eggs of *Toxocaracanis* (10X)



**Fig.4** Egg of *Toxocaracanis* (40X)



**Fig.5** VP<sub>2</sub> gene amplicon of CPV2 isolates from Veterinary Clinical Complex, Kamdhenu University, Anand.



L=Ladder, NC= Negative Control, PC= Positive Control, NS=Negative Sample, PS=Positive Sample

The breed-wise incidence was the highest 51.03% in non-descript breeds followed by Labrador 18.62%, German shepherd 8.96%, Doberman 7.59%, Pomeranian 5.52%, Rottweiler 2.76%, Pug 2.76%, and Golden Retriever, Lhasa Apso, Beagle and Mongrel 0.86% each. The sex-wise incidence was higher 62.07% in males as compared to females 37.93%. The vaccination status wise incidence was the highest 80.69% in unvaccinated dogs followed by vaccinated dogs 17.24% and partially vaccinated dogs 2.07%.

### **Acknowledgement**

Authors thanking the Dean of the College and Professor & Head of Department of Veterinary Medicine, College of Veterinary science and Animal Husbandry, Kamdhenu University, Anand, Gujarat, India.

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**How to cite this article:**

Hemali Anilbhai Patel, Neha Rao, Bharatbhai B. Bhanderi, Shima Saiyad, Kamlesh K. Hadiya and Nidhi Patel. 2022. Diagnosis and Incidence of Canine Parvovirus Gastroenteritis. *Int.J.Curr.Microbiol.App.Sci*. 11(10): 222-229. doi: <https://doi.org/10.20546/ijcmas.2022.1110.028>