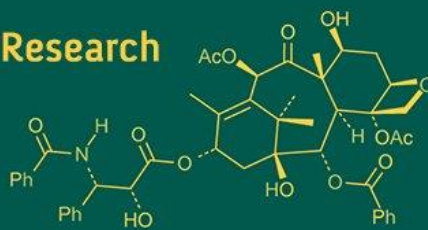


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Molecular detection of canine parvovirus (CPV-2) in dogs in Jabalpur

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Abstract

Canine parvovirus (CPV-2) is one of the most important viral pathogens of dogs, causing acute haemorrhagic enteritis, severe dehydration, immunosuppression, and high mortality, particularly in young puppies. The present study was undertaken to determine the presence of CPV-2 in dogs presented with gastroenteritis in Jabalpur using polymerase chain reaction (PCR). A total of 111 rectal swab samples were collected from dogs presented at the Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Jabalpur, and private veterinary clinics between October 2024 and October 2025. Viral DNA was extracted using the QIAamp DNA Mini Kit, and PCR targeting the VP2 gene was performed using specific primers to amplify a 747 bp fragment. Of the 111 samples examined, 109 were positive for CPV-2, indicating an overall percentage positivity of 98.19%. Age-wise analysis revealed the highest positivity (100%) among puppies aged 3-6 months, followed by 97.91% in puppies below three months and 95.23% in dogs aged 6-9 months. Male dogs exhibited a slightly higher rate (98.66%) than females. Breed-wise evaluation showed that non-descript stray dogs constituted the majority of positive cases, while Labrador Retrievers were the most frequently affected among pedigree breeds. Assessment of vaccination and deworming history indicated CPV infection in both vaccinated and unvaccinated dogs, suggesting possible vaccine failure, incomplete vaccination schedules, or inadequate booster administration. The high percentage positivity observed in the present study may also be attributed to poor vaccination coverage, free-roaming stray dog populations, and increased environmental contamination with the virus. PCR proved to be a rapid, sensitive, and reliable diagnostic tool for confirming CPV infection. The findings highlight the endemic nature of CPV-2 in the Jabalpur region and emphasize the need for timely diagnosis, effective vaccination programmes, improved owner awareness, and regular monitoring to reduce disease incidence and associated economic losses.

Keywords: Canine parvovirus, CPV-2, PCR, VP2 gene, prevalence, dogs, Jabalpur

1. Introduction

Dogs have earned a special place in human life as loyal companions, offering affection, support, and companionship. They play diverse roles, ranging from pets and sporting partners to valuable helpers in stress reduction, security, tracking, hunting, bomb detection, and assistance for blind individuals. They also contribute to human well-being by supporting both physical and emotional health. Canine parvovirus (CPV) is one of the most important viral disease causing significant economic impact loss. It causes acute enteritis in dogs and can lead to severe damage to the intestinal barrier. It is a DNA virus (small, icosahedral and non-enveloped with single-stranded genome) that belongs to the family Parvoviridae under the subfamily Parvovirinae and the genus *Protoparvovirus*. This genus consists of some important viruses of dogs and cats which include CPV and feline panleukopenia virus (FPV), that share a close genetic and antigenic relationship, so that they are now included in unique species *Carnivore protoparvovirus-1* (Cotmore *et al.*, 2019) [6]. CPV-2 was first identified during mid 1978 as an emerging pathogen (Appel, 1978) [2]. It was referred to as CPV-2 to distinguish it from CPV-1 (Parrish and Carmichael, 1983) [22]. *Canine parvovirus type 1* (CPV-1, minute virus) is usually only considered pathogenic in puppies less than 6 weeks of age, whereas the more commonly encountered CPV-2 can cause disease in dogs of any age. Regarding its genome, it is approximately 5.2 kbp long and contains two main open reading

frames (ORFs). ORF1 encodes the non-structural proteins NS1 and NS2, while ORF2 encodes the structural proteins VP1 and VP2. VP2 plays a key role in determining viral antigenicity and host range.

In India, prevalence of canine parvovirus (CPV) was first reported by Balu and Thangaraj (1981) ^[3] in Madras and Ramadass and Khader (1982) ^[24] were the first to confirm that CPV-2 is the etiological agent of disease in dogs. After that, a large number of CPV outbreaks have been reported from different parts of India. The incidence of CPV-2 variants in dog was reported from different states viz. Kerala, Tamil Nadu, Karnataka, Andhra Pradesh, Odisha, Pondicherry (Mukhopadhyay *et al.*, 2014), Haryana (Savi *et al.*, 2009) ^[26] and Uttar Pradesh (Nandi *et al.*, 2010) ^[20]. Regarding the clinical diagnosis of CPV-2 infection, it is difficult because the main clinical signs of the disease are vomiting and diarrhoea, which are common to other enteric diseases. The diagnostic tests which were employed earlier include Haemagglutination test (HA), Electron Microscopy, Virus isolation using MDCK, CRFK or A 72 cell line, Enzyme Linked Immunosorbent Assay (ELISA), Latex agglutination Test, Fluorescent Antibody Test (FAT), Counter Immuno Electrophoresis (CIE) test, Virus Neutralization Test, Polymerase Chain Reaction (PCR), Restriction Enzyme (RE) digestion, Real time PCR (RT-PCR), Loop-mediated isothermal amplification (LAMP), Nucleic acid hybridization or dot blot, in situ hybridization, nucleic acid sequencing etc., but these have varying degree of sensitivity and specificity and sometimes may yield false positive cases. Out of these, DNA-based detection using PCR is one of the most precise and rapid method for the detection of CPV-2. This assay requires only 2 to 4 hours for the detection of viral nucleic acid (Schunck *et al.*, 1995) ^[27]. With this background, the present work has been designed for molecular detection of canine parvo virus in faecal samples of dogs in Jabalpur.

2. Material and Method

The proposed work was conducted in the Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur. The study was conducted for a period of 12 months.

2.1. Faecal sample collection

The dog rectal swabs (n=111) in phosphate buffer saline (pH=7.2) were collected from Veterinary Clinical Complex (VCC), College of Veterinary Science, Jabalpur and/or private clinics, Jabalpur. They were collected in a sterile vial of 2 ml and transported on ice to the laboratory and stored at -20°C until DNA extraction.

A 10% suspension of faecal material was prepared with phosphate buffer saline (PBS, pH 7.2) by dissolving one gram of faeces in 10 ml PBS. Centrifugation was carried out, after thorough vortex, at 7500 rpm for 20 min to remove the coarse debris. Supernatant was subjected to further processing (Gupta *et al.*, 2023) ^[12].

2.2. Extraction of DNA from faecal samples

The DNA from the faecal and scat samples were extracted using QIAamp® DNA Mini Kit (Qiagen, Germany) following manufacturer's protocol. 200 µl of the faecal suspension with PBS was placed in a 1.5 ml microcentrifuge tube. To this, 180 µl Buffer ATL and 20 µl proteinase K

were added, mixed by vortexing and incubated at 56°C (1.5 hrs). Following lysis, 200 µl Buffer AL was added and mixed thoroughly by vortexing for 15 seconds. The sample was incubated at 70°C for 10 minutes. Absolute ethanol (96-100%) of 200 µl was added and the mixture was vortexed for 15 seconds. The mixture was pipetted onto the QIAamp Mini spin column, which was placed in a 2 ml collection tube. It was then centrifuged at 6000 x g (8000 rpm) for 1 minute and the flow-through along with the collection tube were discarded. The QIAamp Mini spin column was placed in a new 2 ml collection tube, 500 µl of Buffer AW1 was added and the column was centrifuged at 6000 x g (8000 rpm) for 1 minute, the flow-through and collection tube were discarded. The QIAamp Mini spin column was placed in a new 2 ml collection tube, 500 µl of Buffer AW2 was added and the column was centrifuged at full speed (20,000 x g; 14,000 rpm) for 3 minutes, the flow-through was discarded. The column was placed back into the same collection tube and centrifuged again at full speed for 1 minute to eliminate any potential carryover of Buffer AW2. The column was then transferred to a new 1.5 ml microcentrifuge tube and 200 µl of Buffer AE was added. The mixture was incubated at room temperature for 2-3 minutes, followed by centrifugation at 6000 x g (8000 rpm) for 1 minute to elute the DNA. The extracted DNA samples were stored at -20°C for further use.

2.3. Quantity and purity check of DNA by nanodrop spectrophotometer

The purity of the genomic DNA was evaluated using Nanodrop spectrophotometry. For each sample, 1 µl of genomic DNA was measured at OD260 and OD280, with 1 µl of elution buffer used as the blank. Only the genomic DNA samples lying in the ranges of OD ratio (260:280) between 1.8 to 2 were considered good and were used for subsequent PCR amplification.

2.4. Molecular detection of canine parvovirus-2 in faecal samples of dog by PCR

Isolated DNA was subjected to PCR using VP2 gene-specific primers as per Sakulwira *et al.* (2001) ^[25] (Table 01, Plate 01) and produced an amplicon size of 747bp. PCR-amplified products had been analyzed by agarose gel electrophoresis by employing 1.5 per cent (w/v) agarose gel in 1X Tris Acetate EDTA buffer, Gel documentation unit was employed to visualize PCR products (Alpha Innotech).

1. Result and discussion

The canine parvovirus is a highly contagious viral disease of dogs and they get exposed to the infection while sniffing, licking, or consumption of CPV contaminated objects. There was no latest data available of the incidence of CPV infection in dogs in Jabalpur so this study help in detection of CPV in faecal samples of dogs presented in VCC (Veterinary Clinical Complex), Jabalpur and/or from private clinics in Jabalpur.

Out of 111 samples of dogs, 109 samples showed amplification in PCR of 747 bp, considered positive for the presence of CPV-2.

Percent positivity of CPV-2 was found around 98.19% (Table 02 and Figure 01) in clinical cases in Jabalpur. The findings follow similar higher results for Singh *et al.* (2013) ^[28] having 63% (63/100) in Mathura. During previous studies done in the Gujrat, More (2010) ^[18] reported a

similar results (82% i.e. 14/17), whereas, some other researchers found a low percentage as in case of Desai *et al.* (2020) with 33.94% (37/109). Studies from across the globe have reported lower rates than our present study. Some of those studies include around 15.62% (15/96) in Bangladesh by Alam *et al.* (2021) ^[1]; 41.7% by Navarro *et al.* (2020) ^[21] and 46% (30/65) by Castillo *et al.* (2020) ^[5] in Chile, with an exception of a higher rate of 71.8% (56/78) was reported in Egypt by El-Neshwy *et al.* (2019) ^[9].

Major factors for an increase in number of CPV positive cases during the present study maybe due to improper or inappropriate vaccination or lack of vaccination as in our study where out of 111 dogs, only 22 were vaccinated and rest of them were unvaccinated which supports the reason for such high result. The majority of the population was of non-descript local breeds and with/ without its owners, thus plausible for the out of reach for vaccination.

3.1. Age group-wise categorization of cpv-2 in dogs

Rectal swab samples from dogs of various age groups were collected. The highest percent positivity of CPV-2 was found 100% (39/39) in pups between 3-6 months of age and same (3/3) in age 9-12 months. The percent positivity of 97.91% and 95.23% was seen in 0-3 and 6-9 month old pups respectively. Overall, 42.34% (47/111) percent positivity was observed in 0-3 month old pups while lowest 2.71% (3/111) in 9-12 month old pups (Table 03 and Fig. 02). The above findings were in unison with the results observed by Harikrishnan *et al.*, 2023 ^[12] reported age-wise percentage of parvo virus infection being more in the age group of 3-6 months 42% (63/150) followed by 0-3 months 38.66% (58/150) followed by 6-9 months 8.6% (13/150) and a lowest percent positivity in age groups above 9-12 months 4.6% (7/150). Similarly, Kumar *et al.*, 2025 ^[17] in Uttarakhand region stated similar findings of higher percentage in 3-6 months aged dogs, i.e. 49.9% (4030/8110) followed by 27.94% (2266/8110) in less than 3 month puppies. Another study conducted by Daneshwari *et al.*, 2026 ^[7] reported highest percentage in dogs aged between three and six months (36.02%, 58/161), followed by 0 to 3 months (32.29%, 52/161), 6 to 12 months (23.60%, 38/161). This is probably due to the maternal antibody inter-reference and gut flora alteration through dietary change during weaning time period, that increased the chances of CPV in more than three months of age. Fall in maternal antibody after three months makes three to six months age group more vulnerable in endemic areas.

3.2. Sex-wise categorization of cpv-2 in dogs

The percent positivity of CPV-2 according to the sex of the dogs was found to be higher (98.66%) in males compared to females (Table 04 and Fig 03). These findings were in agreement to study done by Khare *et al.*, 2019 ^[16] in Madhya Pradesh, which reported higher percentage in male (7.91%) as compared to female (6.36%). Kataria *et al.*, 2020 ^[15], in Hisar reported 58.06% occurrence of CPV in the male dogs as compared to the 41.94% in female dogs. Harikrishnan *et al.*, 2023 ^[13] in Madras observed percent positivity of 55.33% (83/150) in males and 44.66% (67/150) in females. Likewise, Daneshwari *et al.*, 2026 ^[7] stated CPV was more prevalent in male dogs (55.90%, 90/161) than in females (44.10%, 71/161). This maybe due to the tendency of males to roam more than females and being more prone to infections and the preference of keeping male dogs by pet owners (Kalita *et al.*, 2022) ^[14].

3.3. Percent positivity of CPV-2 in dogs based on vaccination and deworming status

The percent positivity of CPV-2 based on vaccination and deworming history of the affected dogs is presented in Table 05. The prevalence of CPV-2 in vaccinated dogs was 100% (22/22) and dogs with proper deworming done was also the same (31/31). The percent positivity of CPV-2 in non-vaccinated dogs was 97.75% (87/89) and in dogs whose deworming was not done came out to be 97.5% (78/80). Probable-reasons for the occurrence of CPV in vaccinated dogs are, 1) administration of primary dose alone, 2) delayed booster vaccination, 3) lack of awareness on vaccination and 4) affordability of the pet owners (Geetha and Selvaraju, 2023) ^[11].

Findings in disagreement were of Singh *et al.*, (2013) ^[28], who reported the percent positivity of 64% in unvaccinated dogs and 50% in vaccinated population. Likewise, Singh *et al.*, (2021), reported that percent positivity of CPV was 24.10% and 70.9% in vaccinated and non-vaccinated dogs, respectively. The higher rate of CPV in unvaccinated dogs was likely due to the lack of protective passive immunity, which allowed rapid viral multiplication in lymphoid cells of the oropharynx, mesenteric lymph nodes and thymus, resulting in leucopenia (Ettinger and Feldman, 2010) ^[10] and was further influenced by factors such as owner ignorance and high vaccine costs.

3.4. Breed wise categorization of cpv-2 in dogs

Around 9 different breeds of dogs were observed in this study (Table 06 and Fig 04). The majority of cases were from non-descript stray dogs, which might be due to poor nutrition and lack of vaccination. In pet breeds, the highest number of cases were reported in Labrador with percent positivity of CPV-2 around 95% (19/20). A few cases of exotic breeds such as Pug, Beagle, Golden retriever, Shitzu were also reported at the clinic. Almost all the breeds that came for the treatment were found positive for CPV-2 using PCR. Since such being the case, any direct conclusion on the effect of breeds on the prevalence of CPV-2 cannot be interpreted. Maximum cases of non-descript local breeds were more prone to CPV-2 infection as compared to vaccinated pet dogs. These dogs also act as source of infections to other pet/ ND dogs in the vicinity.

Corresponding to our study, Behra *et al.*, 2015 reported 34.48% (10/29) percent positivity of non-descript local breeds followed by German shepherd (17.24%), Labrador retriever (10.34%), Rottweiler and German spitz showed 6.9% each and lower incidences in four breeds (3.45%) such as Dalmatians, Neopolitan mastiff, Pug and Great Dane.

Patel *et al.*, 2022 ^[23] also reported 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.

Geetha and Selvaraju, 2023 ^[11] reported highest representation i.e 55.29% of CPV in non-descript dogs than the pure breed dogs including German Shepherds, Doberman, Spitz, etc. 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.

In contrary to our study, study done by Thomas *et al.*, 2014 ^[30], revealed that the disease was more common in Labrador breed (10/23, 43%) followed by non-descript breed, Spitz, Rottweiler, Great dane etc.

Table 1: Primer used in PCR

S.No.	Forward and Reverse primers	Primer Sequence 5'-3'	Product size	References
1.	CPV VP2 - p1	TCCAGCAGC TATGAGATC	747 bp	Sakulwira <i>et al.</i> (2001) ^[25]
	CPV VP2 - p2	GATCTGTTGGTAGCAATAC		

Table 2: Percentage positivity of CPV-2 in Jabalpur in dogs

S. No.	Result	No. of cases	Percentage positivity
1.	Positive	109	98.19%
2.	Negative	02	
	Total	111	

Table 3: Age- wise categorization of CPV-2 in dogs

S. No.	Age	Positive	Negative	Total	Percentage positivity	Overall Percentage positivity
1.	0-3 month	47	01	48	97.91%	42.34%
2.	3-6 month	39	00	39	100%	35.13%
3.	6-9 month	20	01	21	95.23%	18.01%
4.	9-12 month	03	00	03	100%	2.71%
5.	Total	109	02	111		98.19%

Table 4: Sex- wise categorization of CPV-2 in dogs

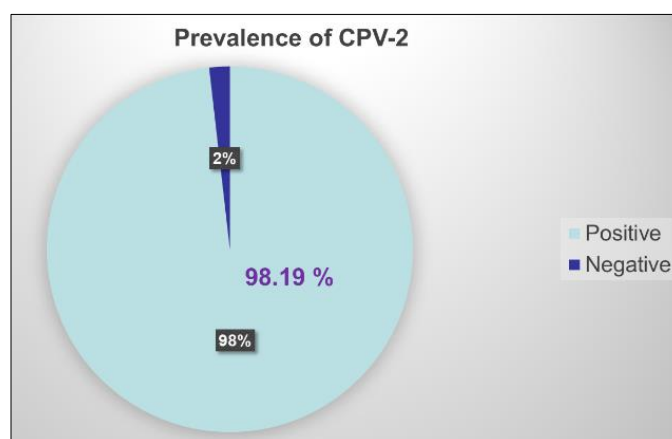
Sex	Positive	Negative	Total	Percentage positivity (%)
Male	74	01	75	98.66%
Female	35	01	36	97.22%
Total	109	02	111	

Table 5: Percentage positivity of CPV-2 in dogs based on vaccination and deworming status

Particulars	Positive	Negative	Total	Percentage positivity (%)
Vaccinated	22	00	22	100%
Non- vaccinated	87	02	89	97.75%
With deworming	31	00	31	100%
Without deworming	78	02	80	97.50%
Total	109	02	111	

Table 6: Breed -wise categorization of CPV-2 in dogs

S. No.	Breeds	Positive	Total	Percentage positivity	Percentage positivity
1.	ND	45	46	97.82%	40.54%
2.	Labrador	19	20	95%	17.12%
3.	Pug	07	07	100%	6.31%
4.	Golden retriever	07	07	100%	6.31%
5.	Rotweiler	08	08	100%	7.21%
6.	Pomerian	09	09	100%	8.11%
7.	Shitzu	05	05	100%	4.50%
8.	German shepherd	07	07	100%	6.31%
9.	Beagle	02	02	100%	1.80%

**Fig 1:** Prevalence of CPV-2 in Jabalpur in dogs

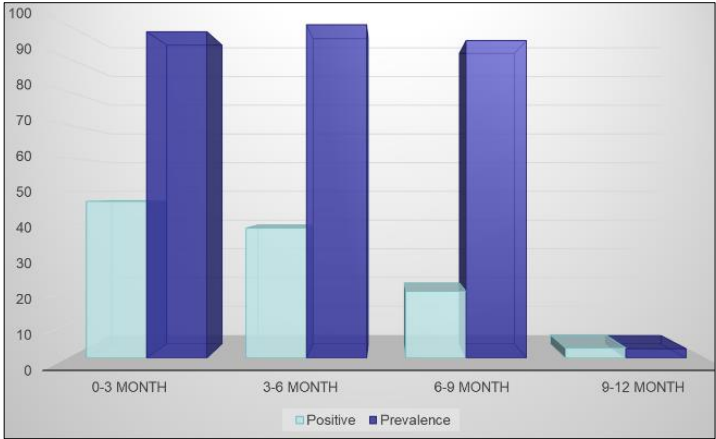


Fig 2: Age- wise categorization of CPV-2 in dogs

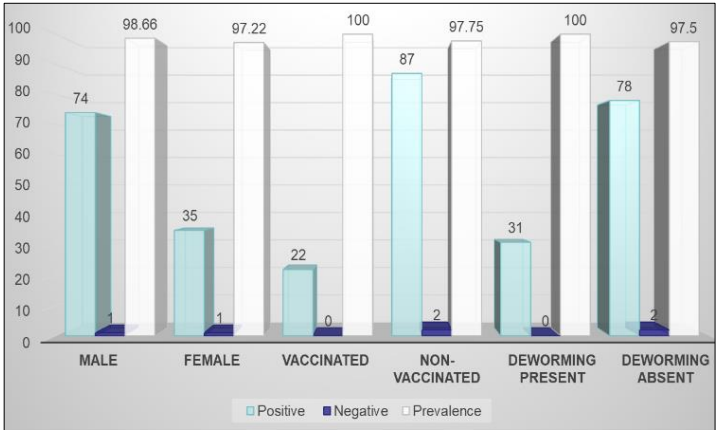


Fig 3: Percentage positivity of CPV-2 in dogs based on sex, vaccination and deworming status



Fig 4: Breed -wise categorization of CPV-2 in dogs

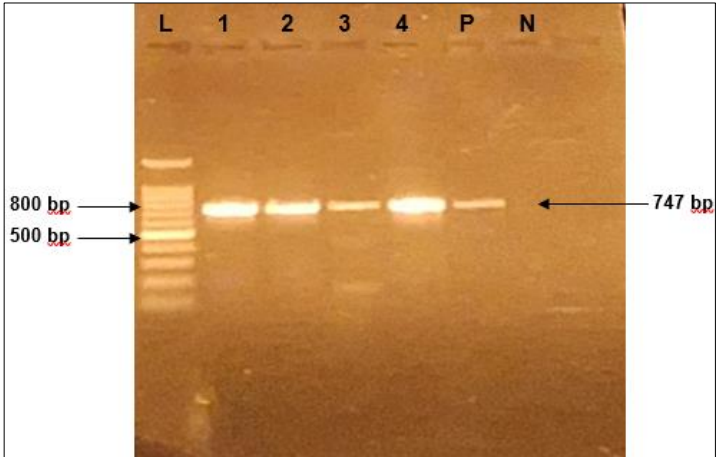


Plate 1: Agarose gel electrophoresis showing 747 bp amplified product of VP2 gene of Canine parvovirus in dogs

- Lane L = 100bp ladder
- Lane P = Positive control
- Lane N = Negative control
- Lane 1-4 shows VP2 gene in positive samples

2. Conclusion

The current investigation could lead to the following findings:

- Percent positivity of CPV-2 in dogs was found to be 98.19% in clinical cases in Jabalpur.
- The highest percent positivity of CPV-2 was found 100% (39/39) in pups between 3-6 months of age.
- The percent positivity of CPV-2 according to the sex of the dogs was found to be higher (98.66%) in males compared to females.
- Around 9 different breeds of dogs were observed in this study and majority of cases were from non-descript stray dogs (45/46).

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