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## Incidence of canine parvovirus in different breeds of dogs in Ludhiana during 2020-2021

**Koppu Vasavi, Poloju Deepa, Kavitha Madineni, Sri Sai Charan Manchikanti, Mudasir M Rather, Bhavani Puvvala, Shilpa Balaji and Chandana MS**

### Abstract

Canine Parvovirus (CPV) is a highly contagious and potentially fatal viral infection that primarily affects domestic dogs, particularly puppies and young adults. This research study aimed to investigate the incidence of Canine Parvovirus in various dog breeds residing in Ludhiana, India, during the period spanning 2020 to 2021. The prevalence of CPV in different breeds was assessed using a Polymerase Chain Reaction (PCR)-based approach, offering both qualitative and quantitative insights into the virus's distribution and concentration within the canine population.

A total of 67 dogs from diverse breeds, ages, and backgrounds were enrolled in this study. Fecal samples were collected from each dog and subjected to PCR analysis to detect the presence of CPV DNA. Additionally, clinical data such as age, sex, vaccination status, and presenting symptoms were recorded for each dog to understand potential risk factors and clinical outcomes associated with CPV infection.

The results revealed an overall incidence rate of CPV infection in Ludhiana's dog population at 50% by PCR during the study period. Among the different breeds studied, German Shepherds, Labrador Retrievers, and Pomeranians exhibited the highest CPV prevalence rates, while other breeds displayed varying susceptibility. Age appeared to be a significant factor, with puppies and young dogs being more vulnerable to CPV infection. Unvaccinated dogs were also more susceptible to the virus, highlighting the importance of timely vaccination in preventing CPV outbreaks.

**Keywords:** Canine parvovirus, PCR, German shepherd, Pomeranian, Labrador

### Introduction

Canine parvovirus type 2 (CPV-2) is one of the most important viral disease encountered in dogs. This virus is contagious, causing high level of morbidity with more incidence observed in pet stores, shelters and breeding kennels. The disease has a rapid clinical course with mortality occurring within 2-3 days after the clinical signs appear in unvaccinated dogs (Carman and Povey, 1985) [22]. Dogs of all ages are susceptible but severity of infection is common in puppies between 6 weeks to 6 months of age (Houston *et al.*, 1996) [21]. Besides all breeds of dogs being susceptible to the disease, the mixed breeds are less susceptible than pure-breeds. Labrador Retrievers, Rottweilers, Doberman Pinschers, English Springer Spaniels, German Shepherds and American Pit Bull Terriers are the pure-breeds that have higher risk for CPV gastroenteritis (Glickman *et al.*, 1985) [20].

The CPV-2 is categorized under the Protoparvovirus genus and belongs to the Parvoviridae family. It has been classified as part of the Carnivore protoparvovirus 1 species, along with Feline panleukopenia virus (FPV), Mink enteritis virus (MEV), and Raccoon parvovirus (RPV) by the International Committee on Taxonomy of Viruses (Tijssen *et al.*, 2011) [18]. Parvoviruses are characterized by their small, non-enveloped icosahedral capsids with a diameter of approximately 25 nm.

The virus possesses a single-stranded negative-sense DNA genome, which is approximately 5.2 kilobases in length. This genome consists of two open reading frames (ORFs): one that encodes non-structural proteins NS1 and NS2, and another that encodes the structural proteins VP1 and VP2. Palindromic hairpin structures, each around 150 bases long, are present at both ends of the genome and play a crucial role in the replication of the viral DNA (Reed *et al.*, 1988; Parrish, 1999) [16, 17].

The canine parvovirus (CPV) has a predilection for specific target tissues where it replicates, including the intestinal crypts and lymphoid organs. However, it has the capacity to spread to various tissues throughout the body, including the brain (Elia *et al.*, 2007; Decaro *et al.*,

2009a) [15, 14]. Upon initial infection through the oronasal route, CPV replicates in the lymphoid tissues associated with the gastrointestinal tract. Infected leukocytes then carry the virus to the germinal epithelium of the small intestine's crypts, resulting in diarrhea. In particular, infection of leukocytes, predominantly circulating and tissue-associated lymphocytes, leads to acute lymphopenia, often accompanied by neutropenia (Pollock, 1982) [19].

The most distinctive clinical manifestation of CPV infection is hemorrhagic enteritis, and the severity of this condition is frequently influenced by the maternal antibody (MDA) titers present in infected puppies at the time of exposure. Clinical symptoms typically appear after an incubation period of 3 to 7 days and include anorexia, depression, vomiting, and mucoid or bloody diarrhea. Dehydration and fever are commonly observed. Leukopenia, characterized by a decrease in white blood cell (WBC) counts to levels below 2000–3000 cells/ $\mu$ L of blood, is a consistent finding. However, total WBC counts may fall within normal ranges due to CPV-induced lymphopenia, with neutrophilia often resulting from concurrent infections by opportunistic bacteria. Additionally, concurrent pulmonary infections can lead to respiratory distress. Subclinical and asymptomatic infections are frequently identified, particularly in puppies with intermediate MDA titers and adult dogs (Decaro *et al.*, 2005a) [13]. Mortality rates can be substantial, reaching up to 70% in puppies but generally remaining below 1% in adult dogs.

## Materials and Methods

### Sample collection from suspected cases of Canine Parvovirus dogs

The total of 67 Samples (rectal swabs) were collected from dogs suspected of Canine parvovirus infection during the period from October, 2020 to February, 2021 from Multi Speciality Veterinary Hospital, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab (Mentioned in Table 1). The samples were collected using the sterile swabs dipped in 4ml of Phosphate buffered saline having pH of 7.4. Further, the samples were kept at refrigeration temperature (4 °C) for further processing of samples like DNA extraction and processing for isolation in MDCK cells.

**DNA Isolation:** (From samples and vaccine Nobivac DHPPi (Intervet, Pvt. Ltd)

The DNA was extracted from the CPV samples and commercially procured vaccine using the phenol-chloroform extraction method (Sambrook and Russell, 2001) [12].

- To extract DNA first 2 ml of sample was Centrifuged at 3000 rpm for 5 min at 4 °C
- Then the supernatant was collected approximately 600  $\mu$ l
- Then to this supernatant 20  $\mu$ l proteinase K (20 mg/ml) was added (Thermo Fischer Scientific)
- Again to this supernatant also add 20  $\mu$ l of 10% SDS (Sodium Dodecyl Sulphate)
- Then the tubes were Vortexed for 10-15secs
- The tubes were incubated at 56 °C for two to three hours in water bath
- After 2-3 hrs, the mixture of phenol-chloroform-isoamyl alcohol (25:24:1) was added
- Then the mixture was Vortexed for 10-15 secs
- To collect the aqueous layer of supernatant the tubes were Centrifuged at 13000 rpm for 15 min at refrigeration temperature of 4 °C

- Then another mixture of chloroform and isoamyl alcohol (24:1) was added approximately 600  $\mu$ l volume
- After adding this mixture, it was gently mixed and again centrifuged at 13000 rpm for 15 min at refrigeration temperature of 4 °C
- After centrifugation the supernatant was collected and 1/10<sup>th</sup> volume of sodium acetate was added (3M, pH 5.2)
- To precipitate the DNA 600ul of isopropanol was added to the mixture and was mixed gently thereafter.
- The mixture was Kept overnight at -20 °C for complete precipitation of DNA
- Next morning the mixture was Centrifuged at 12000 rpm for 10 min at refrigeration temperature of 4 °C
- The pellet was collected and washed twice with 70% ethanol
- It was then kept for drying in the incubator at 37 °C
- Finally, the pellet was suspended in 50 $\mu$ l Nuclease free water and stored at -20 °C to be used in various molecular detection assays.

## Polymerase chain reaction

### Procedure for PCR

PCR reaction mixture was made for the confirmation of virus by adding 5  $\mu$ l of 10X PCR buffer (with 15mM MgCl<sub>2</sub>) (TaKaRa), 1.0  $\mu$ l of forward and reverse primer each (20pm/ $\mu$ l each) as per Mizak and Rzezukta (1999) [23] (Table 2) 1.0  $\mu$ l of dNTP's (10mM each) (TaKaRa), 0.2  $\mu$ l of Taq polymerase (5 units/ $\mu$ l) (QIAGEN), 15.0  $\mu$ l of the template DNA and 26.8 $\mu$ l of Nuclease free water making a total of 50 $\mu$ l of the reaction mixture (Table 3). The DNA from the vaccine was used as a positive control and the rectal swab from a healthy dog was used as negative control.

After preparing the PCR mix, the reaction was put in a Thermocycler (BioRad) which was subjected to the following thermocycling conditions i.e. for the step of denaturation a temperature of 94 °C was kept for 60 seconds, for the step of annealing a temperature of 55 °C was kept for 60 seconds, the extension step was set up at 72 °C for 150 seconds and these were carried out for a total of 35 cycles and final extension was kept at 72 °C for 10 minutes (Table 4).

## Gel electrophoresis

The PCR product was run in a 1.5% gel in 1X TBE at 90 volts. The TBE was prepared by adding 10.8 gm Tris hydroxyl methyl amino methane, 5.5 gm boric acid (10X), 0.938 gm EDTA and distilled water upto 100 ml (Table 5).

## Results

### Vaccination status of CPV by PCR

When the vaccination status was evaluated, it was found that out of 34 PCR positive CPV samples, 15 (44.11%) were vaccinated and 19 (55.88%) were not vaccinated (Fig. 1). Thus, some of the dogs which were positive for CPV in PCR were vaccinated indicating that it might be possible that vaccination of pups against CPV is not conferring immunity against CPV. This might be due to the mismatching of vaccine strain and the CPV strain causing infection in dogs.

### Age-Wise Status of CPV by PCR

When the age wise status among positive by PCR was evaluated (Fig. 2), it was found that out of 34 PCR positive samples:

- 18 (52.94%) samples were from dogs below 3.5 months of age

- 16 (47.05%) samples were from dogs above 3.5 months of age

Thus, maximum animals affected by CPV were below 3 months of age substantiating the already established fact that the infection caused by CPV is more prevalent in young animals (Deepa and Saseendranath, 2000, Banja *et al.*, 2002, Biswas *et al.*, 2006, McCaw *et al.*, 2006, Deepti *et al.*, 2013, Kaur *et al.*, 2015, Kushwaha *et al.*, 2018) [8, 9, 10, 11, 6, 4, 3].

#### Breed-Wise Status of CPV by PCR

When the breed wise prevalence was evaluated, it was found that out of 34 PCR positive CPV samples:

- 12 (35.29%) were Mongrel dogs (non-descript breed)
- 5 (14.70%) were Pomeranian dogs
- 3 (8.82%) were German shepherd dogs, etc.

Breed wise comparison indicated that among pedigree breeds,

German shepherds, Labrador Retrievers and Mongrel were mostly affected by CPV as detected by PCR (Fig.3). These observations too are in tandem to the earlier reported facts in which, Kumar *et al.* (2011) [7], Deepti *et al.* (2013) [6], Kaur *et al.* (2015) [4], Kushwaha *et al.* (2018) [3] reported that in India, German Shepherd, Labrador Retriever and Pomeranian breeds of dogs are most predisposed for CPV.

#### Detection of CPV by PCR

Canine Parvovirus was detected by above mentioned PCR primers (Table 2) and positive samples were found in the agarose gel electrophoresis at 1198 bp which is compared with the 100 bp plus DNA marker along with positive control (Nobivac DHPPi vaccine) and negative control (faecal swab collected from healthy dog (Figure 4). Out of 67 samples suspected for CPV, 34 samples (50%) were found to be positive in our study.

**Table 1:** History of the samples collected from the suspected cases of dogs

| Date of sample collection | Lab No. | History  | Age        | Sex    | Breed                     | Vaccination status              |
|---------------------------|---------|--|------------|--------|---------------------------|---------------------------------|
| 27/10/2020                | L1      | Blood mixed diarrhea, White frothy vomit, Retching, Anorexia since 1 day                             | 1 year     | Female | American Pit Bull terrier | Not done                        |
| 27/10/2020                | L2      | Loose faeces, initially frothy vomition now whitish, inappetance, no water intake, urination reduced | 4 months   | Female | NA                        | Not done                        |
| 27/10/2020                | L3      | No appetite, vomition reduced from 6 times to 2 times a day, Blood mixed diarrhea                    | 7 months   | Female | American Pit Bull terrier | Not done                        |
| 29/10/2020                | L4      | Bloody diarrhea, vomition  | 4 months   | Female | pomeranian                | Not done                        |
| 3/11/2020                 | L5      | Anorexia, bloody diarrhea, vomition mixed with mucous  | 4 months   | female | Labrador retriever        | Not done                        |
| 8/11/2020                 | L6      | Bloody diarrhea, vomition, dull and depressed  | 4 months   | Male   | Labrador Retriever        | Done, booster dose-not done     |
| 11/11/2020                | L7      | Acute vomition since a week, haematochezia with mucus in it  | 2 ½ months | Female | NA-dog                    | Not done                        |
| 11/11/2020                | L8      | Bloody diarrhea, vomition, yellowish urine, anorexia   | 4 months   | Male   | Border collie             | Done, booster- not done         |
| 12/11/2020                | L9      | Bloody diarrhea, no vomition, deworming done 2 months back   | 9 years    | Male   | Daschund                  | Done, booster- not done         |
| 16/11/2020                | L10     | Diarrhoea, vomition 2-3 times a day, weakness  | 8 months   | Male   | NA- dog                   | Not done                        |
| 16/11/2020                | L11     | Loose black faeces, vomition, high fever   | 6 years    | Male   | American Pit bull terrier | Done                            |
| 16/11/2020                | L12     | Bloody diarrhea, vomition  | 3 ½ months | Male   | NA dog                    | Not done                        |
| 16/11/2020                | L13     | Foul smelling diarrhoea, vomition, dull  | 3 months   | Male   | NA- dog                   | Not done                        |
| 16/11/2020                | L14     | Loose faeces 2 times a day, vomition after water intake, dehydration++, weight loss                  | 4 months   | Male   | NA dog                    | Not done                        |
| 16/11/2020                | L15     | Blood in vomit, diarrhoea, pica, retching  | 5 months   | Male   | German shepherd           | Done                            |
| 17/11/2020                | L16     | Watery faeces with blood and mucus, vomition from 3 days, dehydration+++                             | 3 months   | Male   | NA dog                    | Not done                        |
| 17/11/2020                | L17     | Loose faeces, vomition 10 times a day since 4 days, urination yellowish, No water intake             | 1 year     | Female | Labrador Retriever        | Not done                        |
| 18/11/2020                | L18     | Vomition from 4 days, Loose faeces with blood from 4 days(Foul smelling), Dehydration 5+             | 2 months   | Male   | Rottweiler                | Not done                        |
| 20/11/2020                | L19     | Foul smelling diarrhea, Vomition since 2 days  | 6 months   | Male   | German Shepherd           | Done                            |
| 19/11/2020                | L20     | Bloody diarrhea, Vomitions, Anorexia   | 3 months   | Male   | German shepherd           | Not done                        |
| 19/11/2020                | L21     | Dark faeces, Vomition 5-6 times a day  | 1 year     | Female | NA - dog                  | Not done                        |
| 19/11/2020                | L22     | Blood in faeces, Foul smelling, Vomitions from 2 days, No feed intake                                | 3 months   | Female | NA- dog                   | Not done                        |
| 21/11/2020                | L23     | Vomitions, Bloody diarrhea   | 45 days    | Female | Bull dog                  | Mother Vaccinated with Vencosix |
| 23/11/2020                | L24     | Vomitions, Bloody loose faeces   | 40 days    | Male   | Bull dog                  | Mother vaccinated with vencosix |
| 23/11/2020                | L25     | Vomitions, Bloody diarrhea   | 45 days    | Male   | Bull dog                  | Mother vaccinated with vencosix |
| 23/11/2020                | L26     | Vomitions, Bloody diarrhea   | 45 days    | Male   | Bull dog                  | Mother vaccinated               |

|            |      |   |            |        |                    |                                  |
|------------|------|---|------------|--------|--------------------|----------------------------------|
|            |      |   |            |        |                    | with vencosix                    |
| 23/11/2020 | L27  | Vomitions, Bloody diarrhea  | 3 months   | Female | NA- dog            | Not done                         |
| 21/11/2020 | L28  | Vomitions, Bloody diarrhea, Anorexia, No water intake   | 5 months   | Male   | Pomeranian         | Not done                         |
| 24/11/2020 | L29  | Vomitions, bloody diarrhea  | 2 ½ months | Male   | Labrador retriever | Vaccinated                       |
| 24/11/2020 | L30  | Vomitions, Bloody diarrhea  | 2 ½ months | Female | Labrador retriever | Vaccinated                       |
| 24/11/2020 | L31  | Vomitions, Bloody diarrhea  | 30 Days    | Male   | Pomeranian         | Not done                         |
| 24/11/2020 | L32  | Vomitions, bloody diarrhea  | 30 days    | Female | Shitzu             | Not done                         |
| 25/11/2020 | L33  | Vomitions, bloody diarrhea  | 9 years    | Female | NA-dog             | Not done                         |
| 25/11/2020 | L34  | Watery faeces, Vomitions  | 6 months   | Female | Chow chow          | Not done                         |
| 28/11/2020 | L35  | Foul smelling bloody diarrhea   | 11 months  | Female | Pomeranian         | Not done                         |
| 28/11/2020 | L36  | Blood in vomit, diarrhea, depressed, dull   | 4 months   | Female | German shepherd    | Vaccinated                       |
| 5/12/2020  | L37  | Foul smelling faeces, vomition 5-6 times a day  | 5 months   | Male   | Dachshund          | vaccinated                       |
| 5/12/2020  | L38  | Vomitions, loose faeces containing mucus  | 2 months   | Male   | Pomeranian         | Not done                         |
| 7/12/2020  | L39  | Vomitions, black loose faeces   | 3 months   | Male   | Labrador retriever | Not done                         |
| 8/12/2020  | L40  | Foul smelling bloody diarrhea, vomitions, polydypsia  | 9 months   | Female | shihtzu            | Not done                         |
| 8/12/2020  | L41  | Animal is not defecated, followed by blood urine  | 2 months   | Male   | Belgian shepherd   | done                             |
| 6/12/2020  | L42  | Bloody faeces, froathy vomition 4-5 times a day   | 6 months   | Female | Pomeranian         | Not done                         |
| 8/12/2020  | L43  | Vomitions, watery faeces with blood   | 9 months   | Male   | shihtzu            | Mother vaccinated with DHPPL     |
| 11/12/2020 | L44  | Vomitions, bloody diarrhea  | 5 months   | Male   | NA- dog            | Not done                         |
| 8/12/2020  | L45  | Vomitions, inappetance  | 3 months   | Female | NA- dog            | Done                             |
| 11/12/2020 | L46  | Vomitions 2-3 times a day, bloody foul smelling diarrhea, bilateral facial swelling                                 | 4 months   | Male   | Pomeranian         | Not done                         |
| 13/12/2020 | L47  | Vomitions, Mild gastritis   | 5 months   | Male   | Beagle             | Done                             |
| 13/12/2020 | L48  | Frank blood in faeces from 3-4 days, haematemesis, dehydration +  | 6 months   | Female | Rottweiler         | Done                             |
| 21/12/2020 | L49  | White froathy blood tinged Vomitions& diarrhea(8-10) times a day  | 45 days    | Male   | French bulldog     | Done                             |
| 21/12/2020 | L50  | Blood faeces, vomiting from 2 days  | 2 months   | Male   | Labrador retriever | Done with Vencosix               |
| 19/12/2020 | L51  | Melena &haematochezia, frequent vomitions   | 2 months   | Male   | NA-dog             | Not done                         |
| 19/12/2020 | L52  | Whitish diarrhea, vomition since yesterday 2 times a day  | 3 months   | Male   | NA-dog             | Not done                         |
| 22/12/2020 | L53  | Haematemesis, haematochezia, blood in urine, vomitions  | 9 months   | Male   | German shepherd    | Done, booster not done           |
| 21/12/2020 | L54  | Loose faeces since 5 days, haematochezia, polydypsia, dark yellow urine, anorexia, dull, congested mucous membranes | 2 yrs      | Male   | NA-dog             | Not done                         |
| 22/12/2020 | L55  | Bloody diarrhea &vomitions  | 3 Months   | Male   | NA-dog             | Not vaccinated                   |
| 22/12/2020 | R1   | Vomitions& bloody diarrhea  | 38 days    | Male   | American bully     | Not vaccinated                   |
| 1/2/2021   | L56  | Vomitions& bloody faeces  | 3 years    | Male   | Pug                | Done                             |
| 2/2/2021   | L57  | Vomitions& melena since 5 days, anorexia & decreased water intake   | 2 months   | Male   | NA-dog             | Not vaccinated                   |
| 3/2/2021   | L58  | Bloody faeces &vomitions  | 2 months   | Male   | NA-dog             | Not vaccinated                   |
| 1/2/2021   | L59  | Loose faeces white in colour, vomitions   | 1 month    | Male   | Pomeranian         | Done                             |
| 1/2/2021   | L60  | Vomitions 2 times a day, fowl smelling faeces, hypersalivation, anorexia and dull                                   | 7 months   | Female | Pug                | Done, but booster dose not given |
| 2/2/2021   | L61  | Bloody diarrhea, recumbent & dull   | 8 months   | Male   | Pomeranian         |                                  |
| 2/2/2021   | L62  | Vomiting from 4 days (7-8 ) times fraothy, whitish color faeces   | 2 months   | Male   | NA-dog             | Not vaccinated                   |
| 2/2/2021   | L63  | Acute onset of vomition, haematochezia  | 2 months   | Female | Pomeranian         | Done                             |
| 2/2/2021   | L64  | Bloody faeces since 1 month   | 8 months   | Female | German shepherd    |                                  |
| 3/2/2021   | L65  | Black loose faeces, vomition 2-3 times, abdominal distension from 10-12 days  | 1 year     | Male   | Golden retriever   | Not done                         |
| 4/2/2021   | L66  | Melena, vomitions from 5 days, yellowish, whitish with foamyness  | 2 months   | Male   | Labrador retriever | Done                             |
| 5/2/2021   | L 67 | Acute onset of vomitions from 3 days, loose faeces yellowish  | 3 months   | Male   | Pomeranian         | Done                             |

**Table 2:** Primers specific for VP2 gene of CPV used in PCR

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



**Table 3: PCR Master Mix**

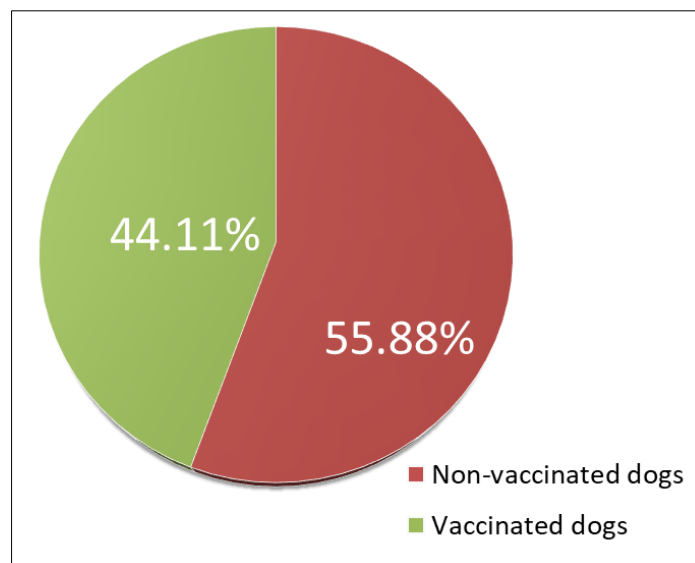
| Components                                     | Volume |
|--|--------|
| 10X PCR buffer (with 15 mM MgCl <sub>2</sub> ) | 5.0µl  |
| Forward Primer (20 pm/µl)                      | 1.0µl  |
| Reverse Primer (20 pm/µl)                      | 1.0µl  |
| dNTPs (10 mM each)                             | 1.0µl  |
| Taq polymerase (5 units/µl)                    | 0.2µl  |
| Template DNA                                   | 15.0µl |
| Nuclease free water                            | 26.8µl |
| Total  | 50µl   |

**Table 4: Thermocycling conditions for PCR**

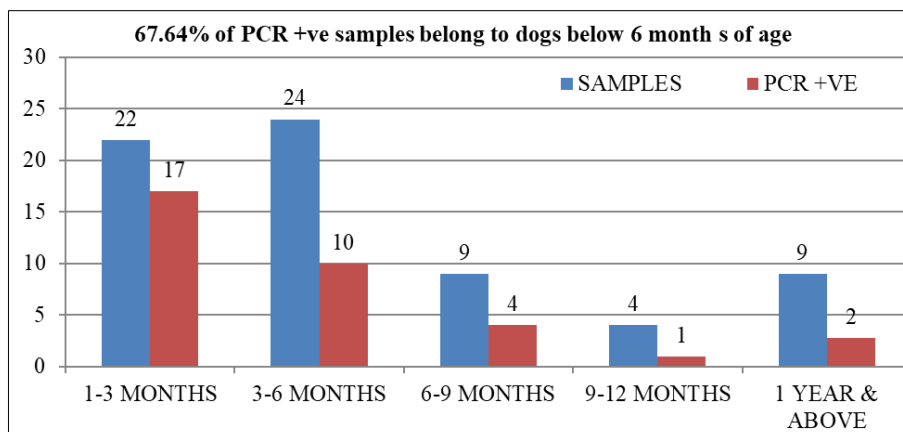
| Steps           | Temperature (°C) | Time  | Cycles |
|-----------------|------------------|-------|--------|
| Denaturation    | 94               | 60s   | 35     |
| Annealing       | 55               | 60s   |        |
| Extension       | 72               | 150s  |        |
| Final extension | 72               | 10min |        |

**Table 5: Preparation of 10X TBE (Tris Borate EDTA) and working buffer (1x TBE)**

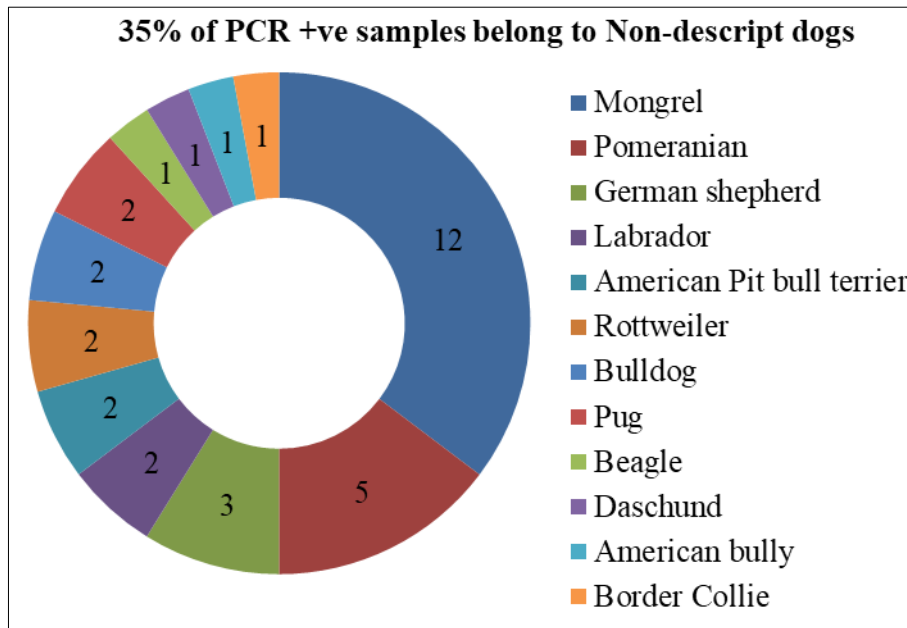
| Components                         | Quantity(10X TBE) |
|------------------------------------|-------------------|
| Tris hydroxyl methyl amino methane | 10.8 gm           |
| Boric acid                         | 5.5 gm            |
| EDTA                               | 0.938 gm          |
| Distilled Water                    | Upto 100 ml       |
| Components                         | Quantity (1X TBE) |
| 10X TBE                            | 100 ml            |
| DDW to make the volume             | 1000 ml           |



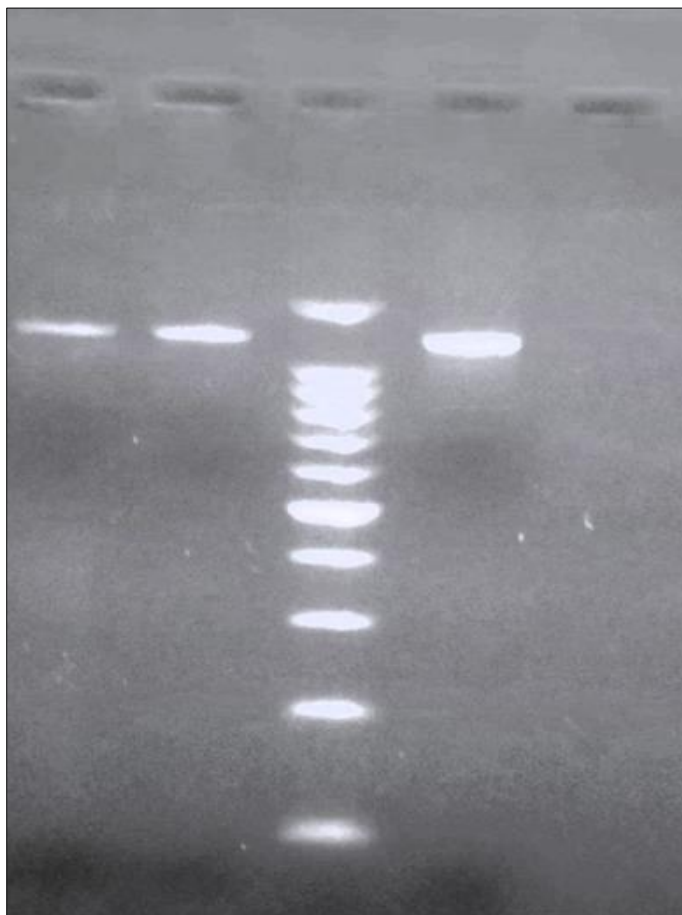
**Fig 1: Vaccination Status of Dogs (PCR positive Samples)**



**Fig 2: Age-Wise Status of Dogs (PCR positive Samples)**



**Fig 3:** Breed-Wise Status of Dogs (PCR+ve Samples)



Lane 1 & 2- CPV positive samples product at 1198 bp  
 Lane 3- 100 bp plus ladder  
 Lane 4- positive control (Nobivac DHPPi vaccine)  
 Lane 5- Negative control (Faecal swab collected from healthy dog)

**Fig 4:** Agarose gel electrophoresis (1.5% gel)- Gel Doc Image

**Conclusions & Discussion**

The PCR analysis revealed that 50% of samples tested positive for Canine Parvovirus (CPV). Notably, a significant proportion of the CPV-positive cases were observed in dogs under the age of 3.5 months, particularly within the Labrador

Retriever, Pomeranian, and German Shepherd breeds. Surprisingly, even among dogs that had received CPV vaccinations, 44.11% still tested positive for the virus. Furthermore, the predominant CPV strain detected in the studied regions was CPV-2a

In a series of studies, researchers investigated the prevalence and characteristics of Canine Parvovirus (CPV) infection among dogs. CPV is a non-enveloped, linear, single-stranded DNA virus belonging to the Parvovirus genus, causing a severe condition known as CPV enteritis, characterized by bloody diarrhea and intestinal hemorrhage.

Behera *et al.* (2015)<sup>[5]</sup> conducted an epidemiological study in Bhubaneswar, Odisha, analyzing 71 samples. Their PCR assay revealed a 40.85% positivity rate for CPV. Kaur *et al.* (2015)<sup>[4]</sup> screened 100 fecal samples using both conventional PCR and nested PCR. The prevalence rate was 11% with conventional PCR and 50% with nested PCR, indicating higher sensitivity for the latter.

Nandi *et al.* (2011)<sup>[7]</sup> employed PCR to detect CPV in 79 clinical samples and identified positive cases in 62 samples, based on the presence of a specific DNA band of 648 bp.

Kushwaha *et al.* (2018)<sup>[3]</sup> analyzed 100 samples using PCR, detecting CPV in 18% of cases. They also applied nested PCR to the same samples, finding a higher incidence of 63% for CPV.

Das *et al.* (2019)<sup>[2]</sup> collected samples from northern India and used both PCR and nested PCR. Out of 100 samples, 77 were positive for CPV via nested PCR.

Singh *et al.* (2021)<sup>[1]</sup> examined 118 rectal swabs from dogs in various regions using nested PCR. They found a high positivity rate of 70%. Interestingly, even dogs with a vaccination history tested positive. Phylogenetic analysis revealed that most samples clustered with CPV 2a isolates.

These studies collectively highlight the prevalence of CPV infection among dogs and the efficacy of PCR-based methods in its detection. The virus was found to affect puppies, including those with vaccination history, and CPV 2a was identified as a prevalent strain in these regions (Behera *et al.*, 2015; Kaur *et al.*, 2015; Kushwaha *et al.*, 2018; Das *et al.*, 2019; Singh *et al.*, 2021)<sup>[5, 4, 3, 2, 1]</sup>.

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