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Molecular Occurrence of CPV-2 Infection in HGE Affected Dogs

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ABSTRACT

Background: Canine parvovirus is the most prevalent infectious disease affecting dogs globally. Canine parvovirus type-2 is regarded as the causal factor of canine parvoviral enteritis (PVE). The objective of the study was to determine the occurrence of parvovirus in dogs in Jabalpur from June, 2023 to December, 2023.

Methods: Faecal samples form suspected cases were handled using sterile swab and immersed immediately in phosphate buffer saline (1X PBS; pH 7.3±0.1). Polymerase chain reaction, conducted to confirm the cases of parvovirus infection.

Result: Out of 125 faecal samples of dogs affected with HGE, 78 (62.4%) samples were found to be positive for VP-2 gene. The detailed investigation of the positive samples showed that the occurrence was highest among dogs of 0-3 months age group (79.06%). Non-descript breed had the highest occurrence of parvovirus infection (78.57%). Males showed a higher occurrence (71.64%) than female.

Key words: Non-descript, Parvoviral enteritis, Phosphate buffer saline, Polymerase chain reaction.

INTRODUCTION

Canine parvovirus type-2 is the causal factor of canine parvoviral enteritis (PVE), a critical disease that causes nearby 100% morbidity and up to 10-90% mortality in unvaccinated adults and puppies, respectively (Nandi and Kumar, 2010). Young animals (6 weeks to 6 months, but especially those less than 12 weeks of age) are more susceptible to develop serious illness. However, disease may also occur in adult dogs who did not receive the recommended vaccinations or who have received them inadvertently (Greene *et al.*, 2012). Parvovirus can be transmitted via the faecal-oral or oronasal routes after being exposed to the virus in vomitus or faeces or by the virus remaining on fomites. Lack of immune defence, intestinal parasites and crowded, unhealthy and stressful environments are risk factors for puppies to contract parvovirus infection.

Two significant clinical forms of the illness stand out: enteritis, which causes vomition and diarrhoea in dogs of all ages and myocarditis, which causes heart failure in puppies younger than three months of age (Nandi and Kumar, 2010). These rapidly multiplying viral cells bind to crypt epithelium of the small intestine, bone marrow precursor cells, lymphoid cells and cardiac muscle cells (Cenk and Mahamut, 2015). Objectives were to study the occurrence of parvovirus infection in dogs and to assess the effect of cardiac troponin T and faecal calprotectin in dogs suffering from parvovirus infection.

MATERIALS AND METHODS

Selection of animals

The study was conducted for a period of six months (July – December, 2023) at Nanaji Deshmukh Veterinary Science University (NDVSU), Jabalpur, Madhya Pradesh, India. Dogs suspected of parvovirus infection were screened for the presence of vomition and blood in motion along with anorexia or inappetence. During the study, 325 dogs with gastroenteritis were screened and amongst them, 125 dogs with haemorrhagic gastroenteritis (HGE) were selected for the analysis of molecular occurrence of parvovirus with the help of polymerase chain reaction.

Collection of samples

Faecal samples (n=125) of dogs with HGE were collected in a sterile swab and immersed immediately in a tube filled with phosphate buffer saline (1x pbs; ph 7.3±0.1) and stored at -20°C.

Extraction of DNA

The DNA was extracted from sterile faecal swab sample stored in PBS with the help of PurelinkTM Viral RNA/DNA Mini kit (INVITROGEN) according to the manufacturer's protocol.

Primers

To detect VP-2 gene in the faeces, polymerase chain reaction was carried out with the following primers: 555 (forw) - 5′ - CAGGAAGATATCCAGAAGGA – 3′ and 555 (rev) - 5'-

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GGTG CTAGTTGA TATGTAATAAACA- 3' Navarro *et al.* (2020) (Table 1).

Reaction mixture

The reaction mixture (25µL) composed of the following (Table 2).

Polymerase chain reaction procedure

The PCR programme conducted on ProFlex thermal cycler (ABI). The protocol followed for the detection of VP-2 gene was in accordance with Navarro *et al*. (2020) (Table 3).

Hundred base pair ladder (ThermoScientific) in the agarose gel used for measuring the product size. The amplified product with a band of 580 base pair was visualised as a single compact band under UV light.

RESULTS AND DISCUSSION

The PCR product positive for VP-2 gene yielded a band of 580 base pair (Fig 1). The overall occurrence of parvovirus infection among dogs with HGE was 62.40% (78/125). The results resembled closely with that of Sakulwira *et al*. (2003) and Kataria *et al*. (2020). The results of present study reflected parvovirus infection in dogs which might be due to the absence of vaccination or incomplete vaccination among the dogs. Out of 125 cases of HGE, the age wise occurrence was significantly higher in dogs of 0-3 months of age *i.e*., 79.06% (34/43) and lowest in dogs of 3-6 months of age *i.e*., 53.65% (44/82).

The results of the present study are in accordance with Naveenkumar *et al*. (2019); Chetan *et al*. (2021); Patel *et al*. (2022). Mahato *et al*. (2023). Whereas, in accordance with Behra *et al*. (2015), the occurrence of parvovirus was highest in dogs of age 3-6 months *i.e*., 41.37%.

The possible reason of high occurrence of parvovirus infection among dogs of age group 0-3 months might be due to loss of immune system pertaining in the young ones due to improper weaning and transfer of the virus from infected dam to the foetus. Since the virus has more affinity towards rapidly growing intestinal crypt cells and myocardial cells, the young dogs of age 0-3 months are most vulnerable (Houston *et al*., 1996). The dogs of age group 3-6 months may encounter the virus due to declining maternal antibody, paving a way to infection.

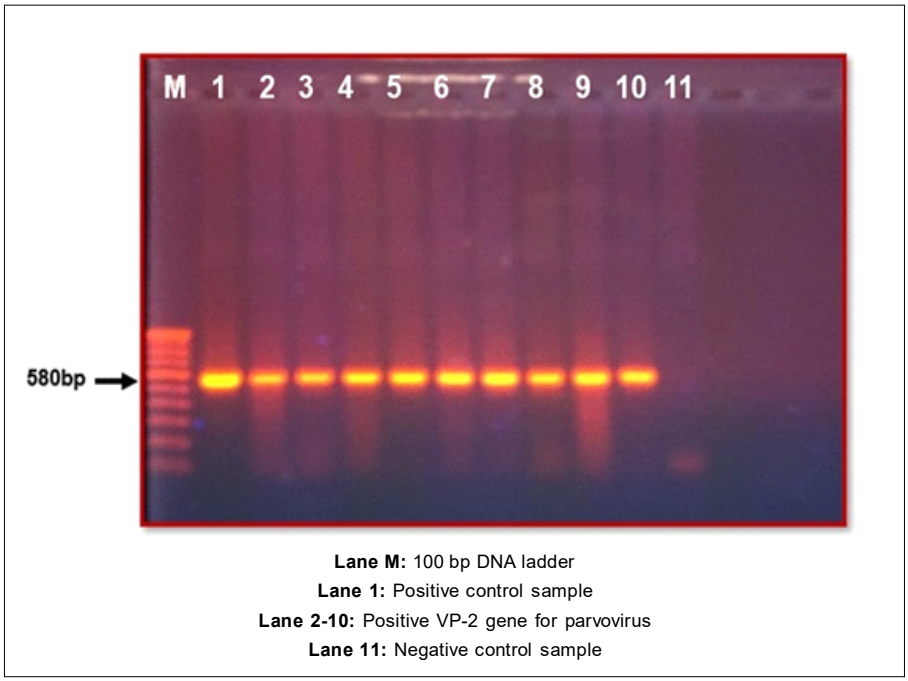
Significantly, higher occurrence of parvovirus infection was seen in nondescript breed *i.e*., 78.57% (33/42) followed by Labrador Retriever *i.e*., 69.69% (23/33) and German Shepherd *i.e*., 68.75% while, occurrence for parvovirus infection was nil among Spitz.

The breed wise occurrence is in accordance with the findings of Sravanthi *et al*. (2019); Naveenkumar *et al*. (2019); Patel *et al*. (2022); Kalita *et al*. (2022); Chetan *et al*. (2021) and Bhujange *et al*. (2024) reported highest occurrence of parvovirus infection in Labrador Retrievers.

The unhygienic conditions to which the dogs may have been exposed earlier, fomite born infection could also be

Table 1: Primer sequences used in the PCR protocol.

Gene	Primer	Oligonucleotide sequence	Product size
"VP-2"	555(forw)	5′- CAGGAAGATATCCAGAAGGA- 3′	
	555(rev)	5′-GGTGCTAGTTGA TATGTAATAAACA- 3′	580 bp



Lane M: 100 bp DNA ladder
Lane 1: Positive control sample
Lane 2-10: Positive VP-2 gene for parvovirus
Lane 11: Negative control sample

Fig 1: Molecular detection of VP-2 gene.

Table 2: Reaction mixture used for the detection of VP-2 gene for parvovirus.

Forward primer (20 picomole)	0.5 µL
Reverse primer (20 picomole)	0.5 µL
Template (50 ng/µL)	2.0 µL
Dream Taq TMGreen PCR master mix	12.5 µL
Bovine serum albumin (BSA)	0.5 µL
Nuclease free water	9.0 µL
Total	25 µL

Table 3: Polymerase chain reaction procedure.

Stages	Temperature	Time	No. of cycles
Stage 1 (Initial denaturation)	94°C	02 minutes	01
Stage 2 (Denaturation)	94°C	30 seconds	40
Stage 3 (Annealing)	48°C	30 seconds	
Stage 4 (Extension)	72°C	01 minute	
Stage 4 (Final extension)	72°C	10 minutes	01

considered as the major factor for disease occurrence among them.

The reduced occurrence of the disease in other breeds like Spitz, Pug, Golden Retrievers might be due to proper vaccination protocols being followed. Among total 125 dogs with HGE, 67 were males and 58 were females. Out of which, 48 male dogs (71.64%) and 30 female dogs (51.72%) were found positive for parvovirus infection by PCR. There was a significant increase in the occurrence of parvovirus infection among males.

The result of the present study closely relates with the findings of Ali *et al.* (2016) and Kataria *et al.* (2020). The findings highlights an increased occurrence among the male dogs, which may be due to males being preferred by the pet owners and hence increased number of their cases being reported.

CONCLUSION

The overall occurrence of parvovirus infection (with haemorrhagic gastroenteritis) in dogs with gastroenteritis was found to be 38.40%. Among dogs with haemorrhagic gastroenteritis, the overall occurrence of parvovirus infection was 62.40% which makes it a pivotal aspect of diagnostic consideration to rule out the disease among dogs with haemorrhagic gastroenteritis. The PCR product positive for VP-2 gene yielded a band of 580 bp.

Conflict of interest

All authors declared that there is no conflict of interest.

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