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Raisudin M Sherasiya

Department of Veterinary Medicine,
College of Veterinary Science and
Animal Husbandry, Kamdhenu
University, Junagadh, Gujarat, India

Arshi A Vagh

Department of Veterinary Medicine,
College of Veterinary Science and
Animal Husbandry, Kamdhenu
University, Junagadh, Gujarat, India

Avinash K Bilwal

Department of Veterinary Medicine,
College of Veterinary Science and
Animal Husbandry, Kamdhenu
University, Junagadh, Gujarat, India

Jayendra R Damor

Department of Veterinary Clinical
Complex, College of Veterinary
Science and Animal Husbandry,
Kamdhenu University, Junagadh,
Gujarat, India

Vijay L Parmar

Department of Veterinary Clinical
Complex, College of Veterinary
Science and Animal Husbandry,
Kamdhenu University, Junagadh,
Gujarat, India

Jaynudin H Khorajiya

Department of Veterinary Pathology,
College of Veterinary Science and
Animal Husbandry, Kamdhenu
University, Navsari-362001, Gujarat,
India

Vinay R Baria

Department of Veterinary Medicine,
College of Veterinary Science and
Animal Husbandry, Kamdhenu
University, Junagadh, Gujarat, India

Priyanshi V Patel

Department of Veterinary Medicine,
College of Veterinary Science and
Animal Husbandry, Kamdhenu
University, Junagadh, Gujarat, India

Corresponding Author:

Raisudin M Sherasiya

Department of Veterinary Medicine,
College of Veterinary Science and
Animal Husbandry, Kamdhenu
University, Junagadh, Gujarat, India

Evaluation of various alterations in clinical signs and clinical parameters in canine parvo viral infection

Raisudin M Sherasiya, Arshi A Vagh, Avinash K Bilwal, Jayendra R Damor, Vijay L Parmar, Jaynudin H Khorajiya, Vinay R Baria and Priyanshi V Patel

Abstract

A comprehensive study conducted at the Veterinary Clinical Complex, Kamdhenu University, Junagadh, Gujarat, from October 2022 to March 2023, involved screening 817 dogs for canine parvoviral infection using PCR, revealing 39 positive cases. Notably, non-hemorrhagic vomiting was predominant, observed in 38 dogs, while only one dog exhibited hemorrhagic vomiting. Hemorrhagic diarrhoea was reported in 29 dogs, contrasting with 10 cases of non-hemorrhagic diarrhoea. Anorexia was observed in 31 dogs. Assessment of dehydration severity revealed varying degrees, with 18, 11, 7, and 3 dogs experiencing mild, moderate, severe, and shock dehydration, respectively. Mucous membrane examination identified 11, 23, and 5 dogs with pale, pink and congested mucous membranes, respectively. Clinical parameters including rectal temperature, heart rate, respiration rate, and capillary refill time were evaluated, with significant increases noted in heart rate, respiration rate, and capillary refill time, while rectal temperature remained unchanged.

Keywords: Dogs, gastro-enteritis, parvo virus, PCR, clinical sign, clinical parameter

1. Introduction

The pet population in India is on the rise, with dogs comprising nearly 95% of the total pet population. Dogs were the first domesticated animals and have formed close bonds with humans. Their devotion, fidelity, obedience, and charming personalities have made them dependable friends over the years (Young, 1985) [25]. Gastroenteritis is a common affliction in dogs, caused by various infectious and non-infectious agents. Among these, parvoviral gastroenteritis is a significant concern for dog owners, necessitating vaccination against specific viral diseases (Salem, 2014) [21].

Dogs are vulnerable to contagious canine parvovirus (CPV) infection, caused by canine parvovirus-2 (CPV-2), a single-stranded DNA virus. CPV-2, recognized in 1977, swiftly spreads among susceptible canine populations, posing high pathogenicity to canines worldwide (Haque and Tayyaba, 2011; Appel *et al.*, 1979) [12, 4]. Dogs of all ages can contract the disease, but puppies under three months are particularly vulnerable (Behera *et al.*, 2015) [6]. Clinical symptoms typically manifest 3 to 5 days after infection and persist for 5-7 days. Stool characteristics vary, ranging from watery consistency to yellow hue or presence of frank blood in severe cases (Fletcher *et al.*, 1979) [9].

The disease presents in two forms: enteric and cardiac. The enteric form is characterized by bloody diarrhoea, along with depression, loss of appetite, vomiting, and high fever. Initially, there's a rise in temperature, but later, due to severe vomiting and diarrhoea, subnormal temperatures are observed (Kramer *et al.*, 1980) [17]. The cardiac form, predominantly observed in dogs under three months old, presents as myocarditis syndrome. Within eight weeks of age, approximately 70% of affected pups succumb to cardiac failure, while the remaining 30% may experience fatal pathological changes months or even years later. The sudden death of newborn puppies, typically at around four weeks old, is a hallmark symptom of CPV-2 myocarditis. Affected pups exhibit cold extremities, pale mucous membranes, and may demonstrate gasping respiration or terminal convulsions (Mochizuki *et al.*, 1996) [18]

2. Materials and Methods

From October 2022 to March 2023, a total of 817 dogs were presented to the Veterinary Clinical Complex, Veterinary College, Junagadh (India), for screening for Canine parvovirus infection. Dogs exhibiting chief complaints such as anorexia, foul-smelling diarrhoea, vomiting, dehydration, and depression, suspected of Canine parvovirus infection, underwent clinical examination followed by confirmation using polymerase chain reaction screening.

Clinical signs including anorexia, dehydration, vomiting, diarrhea, dysentery, and melena were observed. The degree of dehydration was determined using the dehydration index established by Chakrabarti (2014) [8], as outlined in Table 1. Parameters such as rectal temperature (°F), respiratory rate (breaths/minute), heart rate (beats/minute), conjunctival mucous membrane coloration (pink, pale, or congested), and capillary refill time (seconds) were recorded.

Upon presentation, fecal samples were aseptically collected from suspected dogs and placed in sterile swab containers. These samples were preserved at -20 °C in 10% phosphate-buffered saline (PBS) solution with the aim of detecting the CPV virus using Polymerase Chain Reaction (PCR).

2.1 PCR

The genomic DNA of CPV-2 was extracted from stool samples using the DNASure tissue micro kit. PCR was performed with primers detailed in Table 2. Each 25 µL reaction mixture contained 2 X PCR Master mix (12.5 µL), DNA template (3 µL), forward and reverse primers (1 µL each), and nuclease-free water (7.5 µL). PCR cycling conditions included initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 52 °C for 30 sec, and extension at 72 °C for 45 sec, with a final extension at 72 °C for 3 min.

2.2 Statistical analysis

The data was analysed using GraphPad Prism 9.0 software. The value was expressed as mean ± SE and significant level was kept as 0.05 ($P < 0.05$) and statistical significance was determined by unpaired t test before treatment.

Table 1: Dehydration index

Degree of dehydration (%)	Retention of skin fold (fold/sec.)
4-6 (mild)	Absent
6-8 (moderate)	2-4
8-10 (severe)	6-10
10-12 (shock)	20-45

Table 2: Primer used for amplification of target VP2 gene fragment of viral DNA

Target gene	Primer sequence (5' to 3')	Size of amplicon
VP2 gene of canine parvo virus	F TCCAGCAGCTATGAGATC	747 bp
	R GATCTGTTGGTAGCAATAC	

3. Results

3.1 Clinical Sign

All dogs in the healthy group A exhibited normal behaviour, alertness, and activity. They appeared healthy, without any signs of diarrhoea or vomiting, and demonstrated a good appetite.

A total of 39 dogs diagnosed with CPV infection were included in this study, and all cases exhibited symptoms of vomiting and foetid diarrhoea (100%). Among the CPV-infected dogs, both haemorrhagic and non-haemorrhagic

forms of diarrhoea were observed in this study (Table 3) (Fig 1, 2, 3, 4).



Fig. 1: Watery Vomition



Fig. 2: Haemorrhagic Vomition



Fig. 3: Non Haemorrhagic diarrhoea



Fig. 4: Haemorrhagic diarrhoea

This study included a total of 39 dogs diagnosed with canine parvovirus (CPV) infection, among which 31(79.48%) cases exhibited clinical signs of anorexia (Table 3). Also, many dogs show symptoms of dull and depressed.

Mucous membrane color varied as follows: pale (28.20%), pink (58.97%), and congested (12.82%) Shrunken eyeballs and dehydration levels were categorized as mild (46.15%), moderate (28.20%), severe (17.94%), and shock (7.69%) in CPV-infected cases (Table 3).

Table 3: Clinical signs in canine parvovirus affected dogs (n=39)

Sr. No.	Clinical signs	No. of dog	Percent (%)
1.	Vomition		
	a. Hemorrhagic	1	2.56
	b. Non-hemorrhagic	38	97.43
2.	Anorexia	31	79.48
3.	Diarrhea		
	a. Hemorrhagic	29	74.35
	b. Non-hemorrhagic	10	25.64
4.	Dehydration (%)		
	a. Mild (4-6%)	18	46.15
	b. Moderate (6-8%)	11	28.20
	c. Severe (8-10%)	07	17.94
	d. Shock (10-12%)	03	07.69
5.	Mucous membrane (Eyes/Gums)		
	a. Pale	11	28.20
	b. Pink	23	58.97
	c. Congested	5	12.82

3.2 Clinical Parameters

3.2.1 Rectal Temperature

The mean± SE temperature values of 101.97±0.43 and 101.36±0.26°F were observed in CPV affected dogs and apparently healthy dogs respectively (Table 4). Also, CPV affected dogs show variation in body temperature according to severity of disease (Table 3).

3.2.1 Heart Rate

The mean± SE Heart rate values of 116.41±3.23 and 88.66±2.33 Beats/ min. were observed in CPV-affected dogs and apparently healthy dogs respectively (Table 4).

4.3.3 Respiration Rate

The mean± SE Respiration rate values of 33±0.96 and 25.33±0.71 Res./min. were observed in CPV-affected dogs and apparently healthy dogs respectively (Table 4).

Table 4: Comparison of clinical parameters (Mean±SE) value of healthy and CPV

Parameters	Healthy dogs(n=6)	CPV affected dogs (n=12)	P
Temperature (°F)	101.36±0.26°F	101.97±0.43	0.361
Heartrate (/min)	88.66±2.33	116.41±3.23*	<0.0001
Respiration rate (/min)	25.33±0.71	33±0.96**	<0.0001
Capillary refill time (sec.)	1.66±0.21	3.08±0.22*	0.00121

**p<0.01: Highly significant p>0.05 non-significant

4.3.4 Capillary Refill Time

The mean± SE capillary refill time values of 3.08±0.22 and 1.66±0.21 were observed in CPV-affected dogs and apparently healthy dogs respectively. (Table 4).

4. Discussions

4.1 Clinical Sign

CPV-infected dogs, both haemorrhagic and non-haemorrhagic forms of diarrhoea and vomiting were observed in this study

concurrent with various studies conducted by, Godsall *et al.* (2010) [11], Bastan *et al.* (2013) [5], Salem (2014) [21], Khare *et al.* (2020) [15], Kataria *et al.* (2020) [14], and Patel *et al.* (2022) [19]. The presence of diarrhoea and vomiting in dogs infected with canine parvovirus is a result of the damage and breakdown of the intestinal crypts' germinal epithelium. This leads to villous atrophy, affecting the absorption, secretion, and integrity of the mucosal barrier. Secondary bacterial infections further exacerbate intestinal damage, ultimately causing haemorrhagic diarrhoea. These processes have been documented in the study conducted by Bastan *et al.* (2013) [5]. Bacteria can enter the bloodstream, leading to bacteraemia and endotoxemia causing haemorrhagic diarrhoea and the production of cytokines, rather than the viral infection itself (Prittie, 2004) [20].

Alteration in conjunctival mucus membranes and varying degrees of dehydration observed in present study were consistent with results reported by Al- Bayati *et al.* (2010) [2] Kalli *et al.* (2010) [13] and Kocaturk *et al.* (2010) [16] Pale mucous membranes in dogs affected by CPV enteritis may indicate anemia, which is a frequently observed hematological abnormality, particularly in the advanced stages of severe disease. Congested mucous membranes in dogs with parvovirus infection may be associated with secondary bacterial infections. The compromised intestinal barrier caused by the virus allows bacteria to enter the bloodstream and trigger an immune response, resulting in inflammation and congestion of the mucous membranes. Dehydration in dogs is primarily caused by significant fluid (electrolyte) and protein losses through the gastrointestinal tract, attributed to vomiting and diarrhea (Prittie, 2004; Goddord and Leisewitz, 2010) [20, 10].

4.2 Clinical parameters

4.2.1 Rectal Temperature

Fluctuations in body temperature could be attributed to viremia during the early stages of the disease. Subnormal body temperature could be a consequence of severe fluid and electrolyte loss, as stated by Biswas *et al.* (2005). [7] Dogs had to increase rectal temperature in the present study was in accordance with finding of Kalli *et al.* (2010) [13], Vasantha (2011) [24] and Agnihotri *et al.* (2017) [1]

4.2.2 Heart Rate

In line with Saxena *et al.* (2006) [22] and Apoorva *et al.* (2022) [3], the presence of tachycardia in CPV-affected dogs observed in this study could be attributed to the influence of catecholamine and other compensatory mechanisms employed by the heart to ensure an adequate oxygen supply to the tissues.

4.2.3 Respiration Rate

An elevated respiration rate in dogs could be attributed to hypoxia, a condition that leads to an increase in both the depth and rate of breathing. Elevated respiration observed in this study was according to the findings documented by Singh *et al.* (2011) [23], Kalli *et al.* (2010) [13] and Kocaturk *et al.* (2010) [16].

4.2.4 Capillary Refill Time

The prolonged capillary refill time observed in CPV affected dogs were attributed to reduced blood volume and decreased tissue perfusion. The results of this study were consistent with the findings observed by Kalli *et al.* (2010) [13], Kocaturk *et al.* (2010) [16]

5. Conclusions

Canine parvovirus is a deadly disease among dogs, marked by symptoms including vomiting, diarrhea, anorexia, dullness, and varying degrees of dehydration. Clinical parameters such as increased heart rate, respiration rate, and capillary refill time are altered, while rectal temperature typically shows no significant changes.

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