

Molecular Diagnosis and Haemato-Biochemical Alterations in Canine Parvoviral Infection in Dogs

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ABSTRACT

In the present study, a total of 12 dogs suspected of parvovirus infection were subjected to a definitive diagnosis. The canine parvovirus (CPV) infection was detected in the faeces of affected dogs by using lateral flow-based immunochromatographic assay rapid canine parvovirus (CPV) antigen test kit and polymerase chain reaction (PCR). All 12 dogs were found positive by a rapid antigen test kit and VP2 gene of CPV PCR assay using the CPV-2ab primer pair and expected size of 681 bp. The haemato-biochemical study revealed a decrease in Hb, TEC, serum total protein, albumin, and globulin, whereas the increase in absolute neutrophil count and BUN indicated anaemia and hypoproteinemia in parvovirus infected dogs.

Key words: Canine Parvovirus, Haematological-biochemical alterations, PCR

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INTRODUCTION

Canine parvovirus is a highly contagious viral disease that causes severe, acute haemorrhagic gastroenteritis and myocarditis mostly in puppies between 6 and 24 weeks old, although it affects dogs of all age groups. The excessive loss of fluid during vomiting and diarrhoea causes major fluid and electrolyte abnormalities marked by dehydration, hypokalemia, hypochloremic metabolic alkalosis, hypoglycemia, hypoproteinemia and loss of oncotic pressure in circulation that results in the development of hypovolemic shock (Crawford and Sellon, 2010; Goddard and Leisewitz, 2010).

In CPV, there is protein losing enteropathy that attributes to pronounced hypoalbuminemia and/or hypoproteinemia resulting in peripheral edema, and pleural or abdominal effusions (Singh *et al.*, 2022). Apart from diarrhoea and vomition, respiratory distress, pulmonary congestion, alveolar and bronchiolar haemorrhage and convulsions are other clinical signs occasionally manifested due to hypovolemia, endotoxic and septicemic shock (Prittie, 2004; Goddard and Leisewitz, 2010). PCR-based methods, specifically real-time PCR, have been considered the most sensitive and specific than other conventional tests for the diagnosis of CPV (Goddard and Leisewitz, 2010; Decaro *et al.*, 2013). The present research work was carried out to diagnose and assess the haematological and biochemical alterations in parvoviral gastroenteritis in dogs.

MATERIALS AND METHODS

In the present study, total of 12 dogs positive for CPV infection were selected and subjected to haematological & biochemical parameters before initiation of treatment. The canine parvovirus (CPV) infection was detected

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in the faeces of affected dogs by lateral flow-based immunochromatographic assay rapid canine parvovirus (CPV) antigen test kit (ACCUVET Vet Cetera Animal Health) and polymerase chain reaction (PCR) technique. The DNA was extracted with the help of DNA extraction kit with the standard procedure given with the kit and tested by a VP2 gene of CPV PCR assay as per the method developed by Pereira *et al.* (2000).

The PCR was performed in a conventional PCR machine which was programmed as initial denaturation at 95 °C for 5

min, followed by 30 cycles of 94 °C for 1 min, annealing at 53 °C for 1 min, extension at 72 °C for 1 min, and final extension at 72 °C for 10 min. The VP2 gene of CPV was amplified by using the following primers (Table 1).

Table 1: Forward and Reverse primers sequences

Primers	Sequence 5'-3'	Product size
CPV-2ab-F	GAAGAGTGGTTGTAAATAATA	681 bp
CPV-2ab-R	CCTATATACCAAGGTTAGTAG	

The 10 µL of the amplified PCR products were electrophoresed on 1% agarose gel and stained with ethidium bromide in Tris-acetate-EDTA (TAE Buffer) (Sheikh *et al.*, 2017) and then visualized under a UV transilluminator as per the procedure described by Sambrook and Russel (2006).

Blood samples were collected before initiation of treatment from cephalic or saphenous vein of all dogs without and with anticoagulant for haematology and serum biochemistry. Haematological parameters, *viz.*, haemoglobin, packed cell volume, total leukocyte count, total erythrocyte count and absolute DLC were determined by using automated haematology analyzer (ABAXIS VetScan HM5). Biochemical parameters, *viz.*, total protein, albumin, globulin, ALT, AST, BUN, creatinine, serum sodium, serum potassium, serum chloride were estimated by using ready-made kits on biochemical auto-analyzer (Rapid Diagnostic Pvt. Ltd. STAR 21 plus).

RESULTS AND DISCUSSION

In the present study, the CPV was detected by immunochromatographic-based rapid canine parvovirus (CPV) antigen test kit and confirmed by PCR technique in the faeces of affected dogs. In all 12 samples were found positive by rapid antigen test kit. These 12 samples were tested by a VP2 gene of CPV PCR assay and found positive using the CPV-2ab primer pair and expected size of 681 bp (Fig. 1). PCR techniques have been considered the most sensitive and specific for detection of virus particles in faeces or from oropharyngeal swabs than other conventional tests (Goddard and Leisewitz, 2010; Decaro *et al.*, 2013).

Immunochromatographic assay based rapid CPV faecal antigen test kit is the most common method for initial screening and detection of canine parvovirus in faecal samples of dogs. This test has been used by several workers to identify CPV infection from faecal samples of dogs and is reported as a reliable, rapid, sensitive, simple, easy to perform the diagnostic test for the detection of CPV antigen in faecal samples (Khare *et al.*, 2020, Harizan *et al.*, 2021, Abdullaziz *et al.*, 2022), although it has intermediate to low specificity as compared with PCR (Vakili *et al.*, 2014, Tinky *et al.*, 2015, Mazzaferro, 2020). Several workers reported the highest sensitivity of PCR in detecting parvovirus infection as compared to other diagnostic tests (Bhargavi *et al.*, 2017, Pandya *et al.*, 2017, Dorlikar, 2018, Khadse *et al.*, 2023). PCR and RT-PCR techniques are widely used for the confirmation of

CPV infection which provides rapid, sensitive and accurate diagnosis of the CPV.

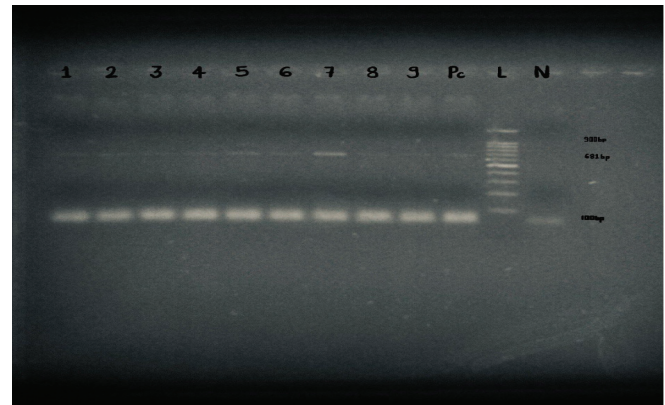


Fig. 1: Agarose gel electrophoresis stained with ethidium bromide showing amplification of the partial VP-2 gene (681 bp). Positive samples: 1 to 9, Pc: Positive control, L: Ladder, N: Negative

Haematological study revealed a decrease in Hb, PCV and TEC in parvovirus infected dogs as compared to normal reference values (Table 2). Similar observations were also reported by Bhargavi *et al.* (2017), Arora *et al.* (2018), Khare *et al.* (2020), Harizan *et al.* (2021) in CPV infected dogs. The low Hb, PCV and TEC in parvovirus affected dogs might be due to the virus-induced destruction of the enterocyte, leading to mucosal barrier disruption and villous atrophy, which causes profuse haemorrhagic diarrhoea (Van den Berg *et al.*, 2018; Bhargavi *et al.*, 2017; Bhalekar, 2020; Harizan *et al.*, 2021). Low Hb and TEC could also be due to the suppression of erythropoiesis as a result of the direct inhibitory effect of CPV on the bone marrow (Elsayed *et al.*, 2020). Some researchers observed an increase in Hb and PCV in CPV infected dogs, which might be due to haemoconcentration as a result of dehydration (Kataria *et al.*, 2020; Patel *et al.*, 2022).

The mean TLC was in the normal range in parvovirus infected dogs (Table 2). Similar findings were also reported by Bhargavi *et al.* (2017). It might be attributed to concomitant lymphopenia and neutrophilia induced by parvovirus consequent to bacterial infections as also suggested by Decaro and Buonavoglia (2012). In the present investigation, the absolute differential leucocyte count (AbDLC) study showed an increase in neutrophil count, whereas no alterations in lymphocyte, monocyte, eosinophil and basophil counts in parvovirus infected dogs. Many studies reported a significant increase in neutrophils (%) and a decrease in lymphocytes (%) and monocytes (%) in parvovirus affected dogs, while there was no significant changes in eosinophil (%) as compared to normal healthy dogs (Ramprabhu *et al.*, 2002; Sagar *et al.*, 2008; Dongre *et al.*, 2013). Abdullaziz *et al.* (2022) showed significant leukopenia with marked lymphopenia and neutropenia, while eosinophils, basophils and monocytes showed non-significant changes between CPV infected dogs and healthy dogs. However, some researchers recorded leukocytosis and neutrophilia that might be due to severe inflammatory reactions

caused by secondary bacterial complications in parvoviral enteritis (Ramprabhu *et al.*, 2002; Kubesy *et al.*, 2019; Bhargavi *et al.*, 2017).

Table 2: Haemato-biochemical parameters in CPV infected dogs (n=12)

Parameters	CPV in- fected dogs	Normal range (Brar <i>et al.</i> , 2004) and Tilley and Smith 2016).
Haematological profile		
Haemoglobin (g/dL)	9.95 ±0.16	10-16
Packed cell volume (%)	32.1±0.83	35-50
Total leukocyte count (x 10 ³ /μL)	13.52 ±1.42	6-16
Total erythrocyte count (x 10 ⁶ /μL)	5.0 ±0.11	6.5-8
Neutrophil count (x 10 ³ /μL)	10.28±1.23	2.9-10
Lymphocyte count (x 10 ³ /μL)	2.21 ±0.25	0.4-2.9
Monocyte count (x 10 ³ /μL)	0.45 ±0.07	0.1-1.4
Eosinophil count (x 10 ³ /μL)	0.49±0.1	0-1.3
Basophil count (x 10 ³ /μL)	0.11 ±0.08	0-0.14
Serum biochemical parameters		
Serum total protein (g/dL)	3.87±0.15	5-7
Albumin (g/dL)	2.39 ±0.08	3-4
Globulin (g/dL)	1.61±0.14	2.5-3
SGOT (AST) (IU/L)	39.97 ±5.53	10-62
SGPT (ALT) (IU/L)	53.31±1.22	25-92
BUN (mg/dL)	36.72±3.34	8-25
Creatinine (mg/dL)	0.73 ±0.07	0.5-1.6
Sodium (Na) (mmol/L)	137.17 ±1.22	137-149
Potassium (K) (mmol/L)	4.0 ±0.2	3.7-5.8
Chloride (Cl) (mmol/L)	107.18 ±2.52	103-115

The biochemical study revealed a decrease in serum total protein, albumin and globulin, whereas an increase in BUN in parvovirus infected dogs as compared to normal reference values (Table 2). Similar findings were also reported by Bhalekar (2020), Saravanan *et al.* (2020), Harizan *et al.* (2021) and Abdullaziz *et al.* (2022). The low serum total protein, albumin and globulin level in parvoviral enteritis might be due to the leakage of serum proteins through the damaged capillaries of the villi of the intestine and may also be due to less absorption of protein through the damaged villi of intestines and altered gastrointestinal mucosal barrier with protein losing-enteropathy (Grigonis *et al.*, 2002; Biswas *et al.*, 2005; Kumar *et al.*, 2020). Anorexia with decreased food consumption, reduced protein synthesis in the liver and malabsorption in parvovirus infection may also be one of the causes of decreased serum total protein in CPV infected dogs (Sagar *et al.*, 2008; Bhargavi *et al.*, 2017; Mazzaferro, 2020).

The mean ALT and AST level were within normal range in parvovirus infected dogs (Table 2), which concurred with Brar *et al.* (2004) and Tilley and Smith (2016). On the contrary, others (Arora *et al.*, 2018; Kataria *et al.*, 2020; Patel *et al.*, 2022; Harizan *et al.*, 2021) observed increase in ALT and AST levels in parvovirus infected dogs as compared to healthy dogs.

In the present study, the mean serum creatinine was within normal range, but BUN was increased in parvovirus infected dogs as compared to normal reference levels (Table 2). Bhargavi *et al.* (2017), Saravanan *et al.* (2020), Abdullaziz *et al.* (2022) and Patel *et al.* (2022), recorded an increase in BUN as well as creatinine in parvovirus infected dogs as compared to healthy dogs. The elevated levels might be due to prerenal azotemia as a result of decreased renal blood flow (reduced glomerular filtration rate) associated with dehydration and hypovolemia as a consequence of vomiting and diarrhoea (Biswas *et al.*, 2005; Shah *et al.*, 2013; Kataria *et al.*, 2020).

The mean sodium, potassium and chloride levels were found within the normal range in parvovirus infected dogs (Table 2). Many researchers recorded a decrease in electrolyte profile in parvovirus infected dogs (Arora *et al.*, 2018, Kataria *et al.*, 2020, Abdullaziz *et al.*, 2022). According to Abdullaziz *et al.* (2022) the reduction in sodium, potassium and chloride level in CPV infected dogs could be attributed to marked fluid and electrolytes loss through vomiting and diarrhoea, reduced absorption from the disrupted mucosal barrier, a poor appetite which mainly contributes to depression and general muscular weakness (Burchell *et al.*, 2014).

CONCLUSION

In the present study, parvovirus infected dogs confirmed by rapid antigen test kit and VP2 gene of CPV PCR assay showed a decrease in Hb, TEC, serum total protein, albumin, and globulin, and an increase in absolute neutrophil count and BUN. These findings indicated anaemia and hypoprotinaemia in parvovirus infected dogs.

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