

MOLECULAR DIAGNOSIS BY PCR AND THERAPEUTIC BENEFIT OF FILGRASTIM IN CANINE PARVOVIRUS INFECTION

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ABSTRACT

Canine parvoviral gastroenteritis is highly contagious and a potentially fatal disease of dogs, particularly puppies. The present study evaluated the efficacy of diagnostics and therapeutic interventions towards canine parvovirus (CPV) infection. The fecal samples from 59 dogs presenting with a complaint of vomiting and bloody diarrhea were screened using Ubio Quick VET CPV Antigen Rapid Test Kit and polymerase chain reaction (PCR). PCR detected CPV in 100% (59/59) of dogs, while the rapid test diagnosed only 25.42% (15/59), yielding a sensitivity of 25.42% and specificity of 100%. The clinical signs of the affected dogs were vomiting (100%), hemorrhagic diarrhea (100%), dehydration (100%), and inappetence (100%). Hematological alterations in the affected dogs included elevation of hemoglobin, total erythrocyte count, and packed cell volume with decreases in total leukocyte count, neutrophils, lymphocytes, and platelets. Biochemical changes revealed decrease in total protein, albumin, globulin and A/G ratio, with an increase in creatinine and BUN. Twelve PCR-positive dogs were divided into two groups: Group I (n=6) dogs received filgrastim (recombinant human granulocyte colony-stimulating factor) as an adjuvant therapy, and Group II (n=6) dogs received a placebo, alongside standard supportive care (antibiotics, antiemetics, fluids). Post-treatment (day 5), Group I showed normalization of hematobiochemical parameters 100% (17/17) and recovery in 5 days, compared to 70.05% (12/17) in Group II with recovery period of 7 days. PCR proved as superior for diagnosis, and filgrastim enhanced the therapeutic efficacy by accelerating leukocyte recovery.

KEYWORDS: Parvovirus, dogs, PCR, filgrastim, hematology, biochemistry

1. INTRODUCTION

Gastroenteritis is one of the major causes of morbidity and mortality in canine population throughout the world. Among the viral diseases, canine parvovirus (CPV) is considered as the major cause of severe gastroenteritis in dogs (Sakulwira et al., 2003). Canine parvovirus gastroenteritis, an acute, fatal and contagious disease is mainly seen in puppies belonging to the age group of 6 to 20 weeks (Carmichael, 2005). Two distinct parvoviruses are now known to infect dogs, mild or non-pathogenic CPV-1 or the minute virus of canine (MVC) and the pathogenic CPV-2. CPV-2 is a single-stranded DNA virus with an icosahedral symmetry. CPV-2 is one of the most important pathogenic viruses with high morbidity (100%) and frequent mortality up to 10% in adult dogs and 91% in pups (Nandi and Kumar, 2010). Vaccination is the most efficient strategy for the control of the CPV-2 and CAV-1 infections (Thomas et al., 2014). In spite of vaccination, the incidence of CPV-2 infection has been increased since last few decades because of emergence of a number of variants like CPV-2a, CPV-2b and CPV-2c that is present throughout the world (Thomas et al., 2017; Gaykwad et al., 2018). In spite of significant impact of these infections on canine health, there is a paucity of information regarding epidemiological aspects of their occurrence because a large proportion of canine gastroenteritis remains undiagnosed either because the disease is self-limiting or due to financial constraints on diagnostic procedures. Therefore, the purpose of the present study was to investigate hemato-biochemical variations, diagnostic and therapeutic aspects of parvo viral infection in dogs.

MATERIALS AND METHODS

Selection of animals and collection of samples

The study was conducted at the Department of Veterinary Medicine, Veterinary Clinical Complex, Veterinary College Bidar, Karnataka, India. Dogs with a history of pyrexia, weakness, low appetite, diarrhea with or without blood, vomiting and dehydration were included in the present study. The affected dogs were clinically examined for the rectal temperature (°F), heart rate and pulse rate (Beats/min), respiration rate (Breaths/min), mucous membrane (graded as pale, congested and icteric) and degree of dehydration (%). Around 5 ml of blood was collected aseptically from the saphenous vein for hematological and biochemical examination. The faecal samples from diarrheic dogs were collected in a sterile swab and transported on ice to the laboratory.

The present study was conducted in strict accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC), Veterinary College, Bidar, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, India. The experimental protocol was duly reviewed and approved by the IAEC.

Collection of Blood

Around 2 ml of blood was collected aseptically from the saphenous vein into an EDTA-coated vial for hematological examination. For serum extraction, 3ml of blood was collected into a clot activator tube, allowed to clot at room temperature for 30 minutes and centrifuged at 5000 rpm for 10 minutes. The serum samples were used for the estimation of biochemical parameters.

Hematological parameters

Estimation of hemoglobin [Hb (g/dL)], packed cell volume [PCV (%)], total erythrocyte count [TEC ($\times 10^6/\mu\text{L}$)], total leucocyte count [TLC ($\times 10^3/\mu\text{L}$)], differential leukocyte count [DLC (%)] and platelets count [PLT ($\times 10^3/\mu\text{L}$)] were carried out in ERMA PCE-210 fully automatic blood cell counter (ERMA PCE-210 fully automatic blood cell counter, manufactured by ERMA Inc., Tokyo, Japan).

Biochemical parameters

Biochemical estimations of the following parameters were carried out by utilizing the biochemical diagnostic reagent kit (ERBA® diagnostic reagent kit) and semi-automatic biochemical analyzer (MICROLAB-300®, Eli Tech Group) for estimation of Serum glutamic pyruvic transaminase (SGPT) (IU/L), Blood urea nitrogen (BUN) mg/dL, Creatinine (mg/dL), Serum total protein (g/dL), Albumin (g/dL), Globulin (g/dL) and Albumin/globulin (A/G) ratio.

Diagnosis

Screening of fecal samples

The screening was done by Ubio Quick VET CPV Antigen Rapid Test Kit1 and Polymerase Chain Reaction (PCR) techniques.

Detection of Parvo Viral Antigen in dog fecal Sample Using Canine Parvo Viral Antigen Rapid Test Kit:

Canine Parvo viral antigen in the fecal sample was detected using Ubio Quick VET CPV Antigen Rapid Test Kit based on an immunochromatographic sandwich lateral flow assay as per the procedure indicated:

A fresh fecal sample was collected from the affected dogs by inserting the wet swab into the rectum of the affected dogs. The swab was then inserted into the provided assay diluent tube and rotated to ensure good sample extraction and all the sediments were allowed to settle down in the tube. The test card was taken out of the foil pouch and placed horizontally. Three drops of the sample were added to the sample area of the test card with the help of a dropper and the test was read after 5 minutes.

Result Interpretation

Control and test band visible- positive as shown in Figure 1 (Left).

Only control band visible- negative as shown in Figure 1 (Right).

No control band visible or only test band visible- Invalid.

Polymerase Chain Reaction

The fecal samples were collected from the dogs suspected to be suffering from diarrhea due to canine parvoviral enteritis in PBS (Phosphate Buffer Saline) and stored at -20°C for DNA extraction and polymerase chain reaction.

Extraction of DNA from the faecal samples

Total DNA was extracted from fecal samples using QIAamp® Fast DNA Stool Mini Kit as per the manufacturer's protocol (QIAGEN QIAamp® Fast DNA Stool Mini Kit-51604) (QIAGEN N.V, Hilden, Germany).

Around 180-220 mg of a stool sample was taken in a 2 ml microcentrifuge tube and placed on ice. 1ml InhibitEX Buffer was added to each stool sample and vortexed continuously for 1 minute or until the stool sample was thoroughly homogenized. This suspension was heated for 5 min at 70°C and then vortexed for 15 seconds. The Sample was centrifuged for 1 minute to pellet stool particles. 15 μL of Proteinase K is pipetted into a new 1.5 ml microcentrifuge tube. 200 μL supernatant from step 4 was pipetted into the 1.5 ml microcentrifuge tube containing Proteinase K. 200 μL Buffer A1 was added and vortex for 15seconds and incubated at 70°C for 10 min. 200 μL of ethanol (96-100%) was added to the lysate and mixed by vortexing. 600 μL lysate was carefully added to the QIAamp spin column and centrifuged for 1 minute. QIAamp spin column is placed in a new 2 ml collection tube, and the tube containing the filtrate was discarded. After carefully opening the QIAamp spin column, 500 μL Buffer AW1 was added and centrifuged for 1 minute. QIAamp spin column is placed in a new 2 ml collection tube, and the tube containing the filtrate was discarded. After carefully opening the QIAamp spin column 500 μL Buffer AW2 was added, centrifuged for 3 minutes and then the collection tube containing the filtrate was discarded. QIAamp spin column was then placed in a new 2 ml collection tube and the old collection tube with the filtrate was discarded, Centrifuged for 3 minutes. QIAamp spin column was transferred into a new, labeled 1.5 ml microcentrifuge tube and pipetted 200 μL Buffer ATE directly onto the QIAamp membrane. Incubated for 1 minute at room temperature, then centrifuged for 1 min to elute DNA.

Primers and PCR mixture composition used for the study are depicted in Table 1 and Table 2.

Polymerase chain reaction assay was carried as outlined below: The reactions were carried out in a thermal cycler as per the following program:

Step I Initial denaturation: 94°C for 5 min (1 cycle)

Step II Denaturation: 94°C for 30 sec

Annealing: 56°C for 30 sec (35 cycles)

Extension: 72°C for 30 sec

Step III Final extension: 72°C for 10 min (1 cycle)

Table 1: Published primer used for amplification of the pCPV-2ab gene

Name	Primer	Amplicon size (bp)
pCPV-2ab (F)	5' GAAGAGTGGTTGTAAATAATT 3'	681
pCPV-2ab (R)	5' CCTATATAACCAAAGTTAGTAC 3'	

Table 2: Components of PCR mix

Sl. No.	Reagent	Volume
1	PCR Master MIX	6.1 µl
2	Forward Primer	2.0 µL
3	Reverse Primer	2.0µL
4	DNA	2.0µL
5	NFW	12.9 µL
Total		25 µL

Analysis of PCR products

PCR products were subjected to gel electrophoresis in a horizontal gel electrophoresis apparatus as described by Sambrook and Russel (2001). Electrophoresis was carried out at 100 voltage for 30 minutes and then the gel was visualized using a UV transilluminator (GelDoc) (Fig. 2).

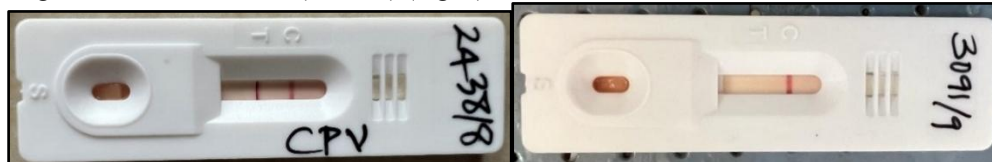


Fig. 1: The CPV Rapid Antigen Test Kit showing both Test and Control bands indicating Positive result (Left) and Negative result (Right)



Fig. 2: BIO-RAD Gel Doc™ XR+ with Image Lab™ Software

Therapeutic study

Twelve dogs positive for parvoviral enteritis either by CPV Rapid Test Kit or PCR were considered for treatment protocol and randomly divided in two groups: Group I and Group II, each group consisting of six animals (Table 3).

Six healthy dogs well vaccinated and dewormed without any clinical signs suggestive of Canine Parvo viral Enteritis were kept as healthy control group for studying the haemato-biochemical parameters. A common treatment protocol for all the affected dogs with antibiotic, antiemetic, antacid, hemostat, fluid and electrolyte supplementation was carried out.

Table 3: Group-wise treatment protocol

Protocol		Group-I (n=6)	Group-II (n=6)
Comparative therapeutic evaluation		Filgrastim @ 10 µg/kg body weight S/C OD	Placebo (NS) S/C OD
Common treatment protocol	Antimicrobials	Ceftriaxone @ 25mg/kg body weight IV OD Tazobactam @ 25mg/kg body weight IV BD Metronidazole @ 25mg/kg body weight IV BD	
	Anti-emetic	Ondansetron @ 0.1-0.2mg/kg body weight IV BD	
	Antacid	Pantoprazole @ 1mg/kg body weight IV OD	
	Haemostat	Tranexamic Acid @ 10mg/kg body weight IV OD	
	Fluids and electrolyte	Ringer's lactate and D.N.S at a rate of 10ml/kg body weight IV OD	
	Prebiotic and probiotic	Gutwell Powder @ 2grams PO BD	

Statistical Analysis

Statistical difference between respective means for various parameters was evaluated using appropriate statistical tests. Comparisons within and between the affected groups and healthy control groups were made. Statistical analysis of the data was done using ANOVA (two-tailed) technique according to the method described by Snedecor and Cochran (1967). A statistically significant difference was considered at the 5 percent level.

RESULTS

Physical parameters and clinical manifestations of Canine Parvoviral gastroenteritis

Vital parameters on the day of presentation (day 0) are presented in Table 4. Mean $\pm$ SE rectal temperature in CPV-affected dogs was significantly higher (102.73 ± 0.38 °F) than in healthy controls (101.63 ± 0.20 °F; $p \leq 0.05$). Heart rate was markedly elevated in affected dogs (135.22 ± 2.88 bpm) compared with controls (87.33 ± 2.53 bpm; $p \leq 0.05$), consistent with compensatory tachycardia secondary to hypovolemia, dehydration and possible endotoxemia. Respiratory rate was also significantly increased (34.05 ± 0.87 breaths/min versus 26.33 ± 1.33 breaths/min in controls; $p \leq 0.05$), reflecting fever, pain, metabolic acidosis and compensatory efforts.

Table 4: Physiological parameters in dogs affected with Parvoviral gastroenteritis

Sl. No.	Parameters	Healthy control	Parvo viral Group (n=59)
1.	Rectal Temperature (° F)	101.63 ± 0.2^a	102.73 ± 0.38^b
2.	Heart Rate (beats per min)	87.33 ± 2.53^a	135.22 ± 2.88^b
3.	Respiration rate (breaths per min)	26.33 ± 1.33^a	34.05 ± 0.87^b

The clinical signs observed in 59 dogs with PCR-confirmed canine parvoviral enteritis are summarized in Table 5. Vomiting (100%), hemorrhagic diarrhea (100%), inappetence (100%) and dehydration (100%) were recorded in all affected dogs and represented the predominant clinical features. Dehydration was classified as moderate in 69.5% and severe in 30.5% of cases. Mucous membrane changes included congestion (52.54%), pallor (25.42%) and pink appearance (22.04%).

These observations confirm that acute-onset vomiting, hemorrhagic diarrhea, profound dehydration, complete anorexia, fever, tachycardia and tachypnea are the hallmark clinical manifestations of canine parvoviral enteritis. Hemato-biochemical alterations in parvoviral-affected dogs

Table 5: Clinical signs observed in parvoviral gastroenteritis affected dogs

Sl. No.	Clinical signs	Total number of cases	Percentage (%)
1.	Inappetence	59	100

2.	Vomiting	59	100
3.	Diarrhea	59	100
4.	Mucus Membrane		
	Congested	31	52.54
	Pale Pink	15	25.42
	Pink	13	22.04
5.	Dehydration		
	Absent	0	0
	Moderate	41	69.50
	Severe	18	30.50
	Shock	0	0
6.	General body condition		
	a. Excellent	0	0
	b. Good	29	49.15
	c. Poor	30	50.85

Hematological parameters in parvoviral gastroenteritis-affected dogs

Hematological and biochemical findings in CPV-affected dogs on the day of presentation, compared with healthy control dogs, are presented in Tables 6 and 7

Hemogram: Marked hemoconcentration was evident in all CPV-affected dogs. Mean $\pm$ SE hemoglobin (15.97 ± 0.93 g/dL), total erythrocyte count ($8.00 \pm 0.21 \times 10^6/\mu\text{L}$), and packed cell volume ($55.1 \pm 1.07\%$) were significantly higher ($p \leq 0.05$) than in healthy controls (13.36 ± 0.37 g/dL, $6.53 \pm 0.20 \times 10^6/\mu\text{L}$, and $42.88 \pm 0.63\%$, respectively).

Total and differential leukocyte counts: Profound leukopenia was a consistent feature. Total leukocyte count was drastically reduced ($2.54 \pm 0.31 \times 10^3/\mu\text{L}$) compared with controls ($12.70 \pm 0.18 \times 10^3/\mu\text{L}$; $p \leq 0.05$). Differential counts revealed severe neutropenia (1.37 ± 0.24 vs. $8.21 \pm 0.20 \times 10^3/\mu\text{L}$) and lymphopenia (0.97 ± 0.17 vs. $3.98 \pm 0.15 \times 10^3/\mu\text{L}$) (both $p \leq 0.05$). Eosinophil, monocyte, and basophil counts showed no significant differences between groups.

Platelet count: Thrombocytopenia was observed in affected dogs ($2.10 \pm 0.28 \times 10^5/\mu\text{L}$) compared with controls ($3.39 \pm 0.15 \times 10^5/\mu\text{L}$; $p \leq 0.05$).

Biochemical findings in parvoviral gastroenteritis-affected dogs

Serum proteins

Significant hypoproteinemia was recorded. Total protein (5.16 ± 0.12 g/dL), albumin (2.22 ± 0.05 g/dL), and globulin (2.93 ± 0.07 g/dL) were markedly lower than control values (7.14 ± 0.15 , 3.81 ± 0.08 , and 3.32 ± 0.10 g/dL, respectively; $p \leq 0.05$). Consequently, the albumin/globulin (A/G) ratio was significantly reduced (0.74 ± 0.01 vs. 1.14 ± 0.03).

Renal parameters

Mild azotemia was present, with significantly elevated creatinine (1.20 ± 0.21 vs. 0.96 ± 0.09 mg/dL) and blood urea nitrogen (22.13 ± 2.95 vs. 18.91 ± 1.22 mg/dL) (both $p \leq 0.05$), consistent with prerenal mechanisms secondary to dehydration and hypovolemia.

Liver enzymes

The mean SGPT (ALT) activity was slightly higher in CPV-affected dogs (32.21 ± 2.80 U/L) than in controls (28.43 ± 1.49 U/L), although the difference did not reach statistical significance and remained within physiological limits.

These hematological and biochemical alterations reflect the characteristic pathophysiology of canine parvoviral enteritis of severe gastrointestinal fluid and protein loss, dehydration with hemoconcentration, bone marrow suppression leading to panleukopenia and thrombocytopenia, and prerenal azotemia.

Table 6: Changes in Hematological parameters of parvoviral gastroenteritis-affected dogs

Parameter	Healthy Control	CPVGE affected Animal
Hb(g/dL)	13.36 ± 0.37^b	15.97 ± 0.93^a
TEC ($\times 10^6/\mu\text{L}$)	6.53 ± 0.2^b	8.00 ± 0.21^a
PCV (%)	42.88 ± 0.63^b	55.1 ± 1.07^a
TLC ($10^3/\mu\text{L}$)	12.7 ± 0.18^b	2.54 ± 0.31^a

Neutrophil ($\times 10^3/\mu\text{L}$)	8.21 ± 0.20^b	1.37 ± 0.24^a
Lymphocyte ($\times 10^3/\mu\text{L}$)	3.98 ± 0.15^b	0.97 ± 0.17^a
Eosinophil (%)	0.83 ± 0.3	1.48 ± 0.43
Monocyte (%)	2.66 ± 0.33	2.40 ± 0.24
Basophil (%)	0.5 ± 0.22	0.58 ± 0.14
PLT ($\times 10^5/\mu\text{L}$)	3.39 ± 0.15^b	2.10 ± 0.28^a

Mean $\pm$ SE bearing a,b superscripts differ significantly ($P \leq 0.05$) between the groups.

Table 7: Changes in Biochemical parameters of parvoviral gastroenteritis-affected dogs

Parameter	Healthy control dogs	CPVGE affected Dogs
Total protein (g/dL)	7.14 ± 0.15^b	5.16 ± 0.12^a
Albumin (g/dL)	3.81 ± 0.08^b	2.22 ± 0.05^a
Globulin (g/dL)	3.32 ± 0.1^b	2.93 ± 0.07^a
A/G	1.14 ± 0.03^b	0.74 ± 0.01^a
SGPT (U/L)	28.43 ± 1.49	32.21 ± 2.8
Creatinine (mg/dL)	0.96 ± 0.09	1.2 ± 0.21
BUN (mg/dL)	18.91 ± 1.22	22.13 ± 2.95

Mean $\pm$ SE bearing a,b superscripts differ significantly ($P \leq 0.05$) between the groups.

Diagnosis

Efficacy of various Diagnostics in canine parvoviral gastroenteritis Diagnosis The diagnostic performance of a commercial CPV antigen rapid test kit (lateral flow immunochromatography) was compared with conventional PCR in 59 dogs with clinical signs of parvoviral enteritis (Table 8). PCR detected CPV in all 59 cases (100% efficacy) and was therefore considered the gold standard. The rapid antigen test was positive in only 15 of these 59 PCR-confirmed cases, yielding a sensitivity of 25.42% (95% CI: 15.3–37.9%) and specificity of 100%. These results demonstrate the markedly superior diagnostic reliability of PCR over the rapid antigen test kit in naturally occurring canine parvoviral enteritis.

Sensitivity and Specificity of Lateral flow assay

Since PCR demonstrated 100% diagnostic efficacy (59/59 positive), it was established as the reference standard. Comparative evaluation of the commercial CPV antigen rapid test kit was performed using PCR as the gold standard (Table 8) (Fig 3). The rapid test exhibited a sensitivity of 25.42% (15/59 true positives) and a specificity of 100%. These findings confirm the poor sensitivity and limited clinical reliability of the lateral flow antigen test for confirming canine parvoviral enteritis, particularly in cases with low fecal viral shedding.

Table 8: Comparative evaluation of CPV Rapid Antigen test with PCR for diagnosis of canine parvoviral gastroenteritis

Screening test results (CPV Rapid Antigen test)	Diseased	Non-diseased	Total	Sensitivity (%)	Specificity (%)
Positive	15	0	15	25.42	100
Negative	44	1	45		
Total	59	1	60		

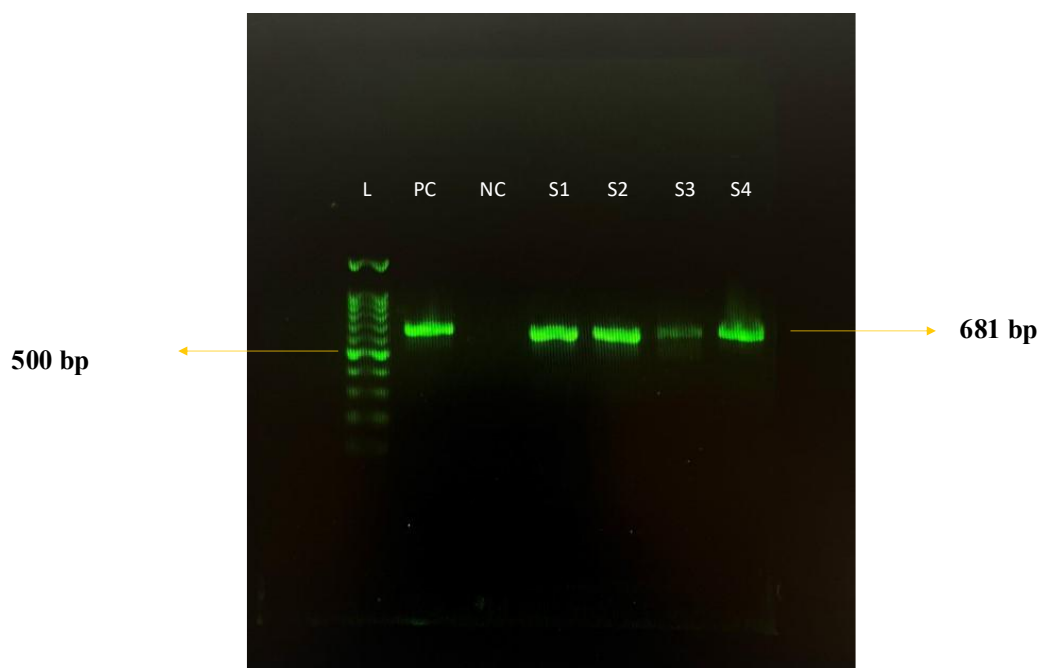


Fig. 3: Amplification of Vp2 Gene of canine parvovirus from Faecal Sample

Hematological parameters of Group I and Group II parvoviral gastroenteritis-affected dogs before and after treatment in comparison with healthy control group dogs:

Hemogram

As depicted in Table 9, On day 0, marked hemoconcentration was evident in both treatment groups (Group I and Group II), with significantly higher hemoglobin (15.10 ± 0.72 and 16.85 ± 1.73 g/dL), total erythrocyte count (8.40 ± 0.21 and $7.96 \pm 0.40 \times 10^6/\mu\text{L}$), and packed cell volume ($54.5 \pm 1.52\%$ and $55.7 \pm 1.63\%$) compared to healthy controls ($p \leq 0.05$). By day 5 post-treatment, all erythron parameters had returned to values statistically indistinguishable from controls (Hb: 12.85 ± 1.26 and 14.06 ± 0.64 g/dL; TEC: 6.70 ± 0.32 and $6.63 \pm 0.29 \times 10^6/\mu\text{L}$; PCV: $38.73 \pm 2.17\%$ and $41.6 \pm 1.8\%$, respectively), with a significant decline from day 0 values ($p \leq 0.05$) in both groups.

Total Leukocyte Count ($\times 10^3/\mu\text{L}$) and Differential Leucocyte Count

Severe leukopenia observed on admission ($2.30 \pm 0.57 \times 10^3/\mu\text{L}$ in Group I; $2.78 \pm 0.28 \times 10^3/\mu\text{L}$ in Group II) resolved rapidly. By day 5, total leukocyte count in Group I normalized completely ($12.78 \pm 0.93 \times 10^3/\mu\text{L}$; no significant difference from controls) and showed a significant increase from day 0 ($p \leq 0.05$). Neutrophil and lymphocyte counts followed the same pattern: profound neutropenia (1.11 ± 0.26 and $1.63 \pm 0.19 \times 10^3/\mu\text{L}$) and lymphopenia (0.93 ± 0.24 and $1.01 \pm 0.08 \times 10^3/\mu\text{L}$) on day 0 returned to control levels by day 5, with significant recovery in both groups ($p \leq 0.05$). Eosinophil, monocyte, and basophil counts remained unaffected throughout the study period

Platelets ($\times 10^5/\mu\text{L}$)

Thrombocytopenia present on day 0 ($\approx 2.10 \times 10^5/\mu\text{L}$ in both groups) resolved by day 5, with platelet counts rising to $3.03 \pm 0.16 \times 10^5/\mu\text{L}$ (Group I) and $2.87 \pm 0.19 \times 10^5/\mu\text{L}$ (Group II), showing no significant difference from healthy controls and a significant increase from admission values ($p \leq 0.05$).

Table 9: Hematological parameters of Group I and Group II CPV-affected dogs before and after treatment in comparison with healthy control dogs

Parameter	Control	G1		G2	
		Day 0 Before Treatment	Day 5 After Treatment	Day 0 Before Treatment	Day 5 After Treatment
Hb(g/dL)	13.36 ± 0.37^a	15.1 ± 0.72^{bB}	12.85 ± 1.26^{aA}	16.85 ± 1.73^{aB}	14.06 ± 0.64^{bA}
TEC ($\times 10^6/\mu\text{L}$)	6.53 ± 0.20^a	8.04 ± 0.21^{bB}	6.70 ± 0.32^{aA}	7.96 ± 0.40^{bB}	6.63 ± 0.29^{aA}
PCV (%)	42.88 ± 0.63^a	54.5 ± 1.52^{bB}	38.73 ± 2.17^{aA}	55.7 ± 1.63^{bB}	41.6 ± 1.8^{aA}

TLC ($\times 103/\mu\text{L}$)	12.70 ± 0.18^b	2.3 ± 0.57^{aA}	12.78 ± 0.93^{bB}	2.78 ± 0.28^{aA}	9.25 ± 0.58^{aB}
Neutrophils ($\times 103/\mu\text{L}$)	8.21 ± 0.20^b	1.11 ± 0.26^{aA}	8.53 ± 0.66^{bB}	1.63 ± 0.19^{aA}	5.61 ± 0.45^{aB}
Lymphocytes ($\times 103/\mu\text{L}$)	3.98 ± 0.15^b	0.93 ± 0.24^{aA}	3.70 ± 0.38^{bB}	1.01 ± 0.08^{aA}	3.14 ± 0.17^{aB}
Eosinophils (%)	0.83 ± 0.30	1.5 ± 0.33	1.16 ± 0.16	1.66 ± 0.56	1.33 ± 0.21
Monocytes (%)	2.66 ± 0.33	2.16 ± 0.40	2.5 ± 0.22	2.33 ± 0.30	2.83 ± 0.30
Basophils (%)	0.5 ± 0.22	0.5 ± 0.22	0.66 ± 0.21	0.33 ± 0.21	0.5 ± 0.22
PLT ($\times 105/\mu\text{L}$)	3.39 ± 0.35^a	2.10 ± 0.32^{bA}	3.03 ± 0.16^{aB}	2.11 ± 0.49^{bA}	2.87 ± 0.19^{aB}

Mean $\pm$ SE bearing a,b superscripts differ significantly ($P \leq 0.05$) between the groups. Mean $\pm$ SE bearing A,B superscripts differ significantly ($P \leq 0.05$) within the groups.

Biochemical parameters of Group I and Group II parvoviral gastroenteritis-affected dogs before and after treatment in comparison with healthy control group (Table 10)

Serum protein profile

Hypoproteinemia and hypoalbuminemia noted on day 0 (total protein: 5.23 ± 0.18 and 5.09 ± 0.17 g/dL; albumin: 2.30 ± 0.07 and 2.14 ± 0.08 g/dL) improved markedly. By day 5, total protein and albumin in Group I returned to control ranges (6.82 ± 0.29 and 3.48 ± 0.21 g/dL, respectively), while Group II showed substantial but slightly slower recovery. Globulin and A/G ratio also normalized in most animals by day 5, with no significant differences from controls.

Liver and renal parameters

ALT, BUN, and creatinine values, which were either normal or only mildly elevated on admission, showed no significant differences from healthy controls by day 5 in either treatment group, confirming the transient and prerenal/pre-hepatic nature of these alterations.

These findings demonstrate that, with appropriate supportive therapy, complete hematological and biochemical restoration occurs within 5 days in the majority of surviving dogs, with Group I exhibiting faster and more consistent normalization than Group II.

Table 10: Biochemical parameters of Group I and Group II CPV-affected dogs before and after treatment in comparison with healthy control dogs

Parameter	Control	G1		G2	
		Day 0 Before Treatment	Day 5 After Treatment	Day 0 Before Treatment	Day 5 After Treatment
Total protein (g/dL)	7.14 ± 0.15^b	5.23 ± 0.18^{aA}	6.82 ± 0.29^{bB}	5.09 ± 0.17^{aA}	6.25 ± 0.26^{aB}
Albumin (g/dL)	3.81 ± 0.08^b	2.3 ± 0.07^{aA}	3.48 ± 0.21^{bB}	2.14 ± 0.08^{aA}	3.24 ± 0.10^{aB}
Globulin (g/dL)	3.32 ± 0.10^b	2.91 ± 0.11^a	3.34 ± 0.21^b	2.95 ± 0.10^a	3.02 ± 0.21^b
A/G	1.14 ± 0.03^b	0.78 ± 0.01^a	1.06 ± 0.10^b	0.72 ± 0.04^a	1.09 ± 0.07^b
SGPT (U/L)	29.43 ± 2.89	36.81 ± 4.80	31.8 ± 4.18	35.61 ± 3.36	30.6 ± 2.34
Creatinine (mg/dL)	1.08 ± 0.16	1.3 ± 0.15	1.18 ± 0.06	1.25 ± 0.18	1.12 ± 0.10
BUN (mg/dL)	19.91 ± 2.72	26.98 ± 5.44	21.2 ± 1.45	23.28 ± 2.22	21.15 ± 1.34

Mean $\pm$ SE bearing a,b superscripts differ significantly between the groups.

Mean $\pm$ SE bearing A,B superscripts differ significantly within the groups.

Response to treatment

The therapeutic response was evaluated by the degree of normalization of hemato-biochemical parameters by day 5 and the overall clinical recovery time (Table 11). In Group I, 100% (17/17) of the monitored hemato-biochemical parameters returned to values overlapping with those of healthy control dogs by day 5. In contrast, only 70.05% (12/17) of parameters achieved complete normalization in Group II by the same time point. The mean clinical recovery period (time to resolution of all clinical signs, restoration of appetite, and discontinuation of intravenous fluid therapy) was 5 days in Group I and 7 days in Group II, confirming a significantly faster and more complete recovery in Group I. These results indicate superior therapeutic efficacy of the treatment protocol used in Group I compared with Group II.

Table 11: Comparison of treatment by normalcy attained in haemato-biochemical parameters of treatment dogs and recovery period

Group	Hematological parameters	Biochemical parameters	Total	Percent	Total No. recovery Period (days)
Group I	7	10	17	100%	5
Group II	4	8	12	70.05%	7

DISCUSSION

Clinical signs

The clinical presentation in the present study was characteristic of acute canine parvovirus (CPV-2) enteritis. Vomiting, hemorrhagic diarrhea, complete inappetence, and dehydration were observed in 100 % of the 59 PCR-confirmed cases, followed by congested mucous membranes (52.54 %), pale or pink membranes, and poor body condition. These findings align with numerous previous reports (Kalli et al., 2010; Kocaturk et al., 2010; Nwoha, 2011; Singh et al., 2011; Shah et al., 2013; Salem, 2014; Reddy et al., 2015; Surendhar et al., 2018; Abdullaziz et al., 2022).

Vomiting and hemorrhagic diarrhea result from viral replication in the rapidly dividing crypt epithelium of the small intestine, leading to villous collapse, impaired epithelial barrier function, and secondary bacterial translocation (Greene and Decaro, 2012). Dehydration and inappetence are direct consequences of massive fluid and electrolyte loss, endotoxemia, and release of proinflammatory cytokines such as cachectin/TNF- α and interleukin-1 (Strombeck and Guilford, 1991). The moderate fever, tachycardia, and tachypnea recorded reflect compensatory responses to hypovolemia, metabolic acidosis, and systemic inflammation (Thakur et al., 2020; Hassan et al., 2022).

Hematobiochemical changes in parvoviral gastroenteritis-affected dogs

Hemogram

Significant increases in hemoglobin, total erythrocyte count, and PCV on admission confirmed marked hemoconcentration secondary to severe dehydration and gastrointestinal fluid loss (Bhargavi et al., 2017; Gaykwad et al., 2018; Kataria et al., 2020).

Leukogram

Profound leukopenia, neutropenia, and lymphopenia were consistent findings and are explained by direct CPV-2 destruction of hematopoietic precursors in bone marrow and lymphoid tissues, combined with increased neutrophil consumption in the damaged intestine (Mylonakis et al., 2016; Thakur and Thakur, 2017; Gaykwad et al., 2018; Punia et al., 2021; Abdullaziz et al., 2022). Eosinophil, monocyte, and basophil counts remained within reference ranges.

Thrombocytopenia

A significant reduction in platelet count resulted from viral suppression of megakaryocytes and increased peripheral consumption/disseminated intravascular coagulation at sites of intestinal hemorrhage (Shah et al., 2013; Kataria et al., 2020; Abdullaziz et al., 2022).

Serum proteins and renal/hepatic parameters

Hypoproteinemia, hypoalbuminemia, and hypoglobulinemia with a decreased A/G ratio were attributable to gastrointestinal protein loss, reduced hepatic synthesis due to anorexia, and diversion of amino acids toward acute-phase protein production (Saravanan et al., 2020; Khare et al., 2020; Harizan et al., 2021; Abdullaziz et al., 2022). Mild azotemia was prerenal, and ALT remained largely unaffected, confirming the absence of significant hepatocellular injury in uncomplicated cases.

Diagnosis

PCR detected CPV-2 in all 59 cases (100 % efficacy), whereas the commercial rapid antigen test kit detected only 15/59 cases, yielding a sensitivity of 25.42 % and specificity of 100 %. These results corroborate earlier observations of poor sensitivity of immunochromatographic assays (Tanwar et al., 2020; Walter-Weingartner et al., 2021). False negatives are primarily due to low fecal viral loads (<10⁹ DNA copies/mg) in early or convalescent stages and

interference by intraluminal antibodies (Desario et al., 2005). PCR remains the gold-standard diagnostic tool for reliable confirmation of CPV-2 infection.

Therapeutic efficacy

Supportive therapy (fluid resuscitation, antiemetics, antibiotics) remains the cornerstone of treatment for canine parvoviral enteritis (Gerlach et al., 2020). In the present study, recombinant human granulocyte colony-stimulating factor (filgrastim, 10 µg/kg SC for 3 days) was used as an adjuvant in Group I because severe leukopenia (<1000 leukocytes/µl) is a major predictor of mortality (Greene and Decaro, 2012).

By day 5, all 17 monitored hemato-biochemical parameters in Group I had normalized (100 % overlap with controls), whereas only 70.05 % (12/17 parameters) normalized in Group II (conventional therapy alone). Clinical recovery occurred in 5 days in Group I versus 7 days in Group II. The accelerated leukocyte (especially neutrophil and lymphocyte) recovery in Group I is consistent with the known actions of G-CSF: stimulation of granulopoiesis, enhanced release of mature neutrophils from marrow stores, and immunomodulatory effects on lymphocytes (Duffy et al., 2010; Areshkumar et al., 2017; Armenise et al., 2019; Punia et al., 2021).

The faster restoration of erythron, protein, and platelet parameters in Group I likely reflects earlier cessation of gastrointestinal losses and improved appetite secondary to rapid resolution of leukopenia and systemic inflammation. No adverse effects of short-term filgrastim administration were observed, in agreement with previous canine studies (Schuening et al., 1989; Obradovich et al., 1991; Ogilvie, 1995).

In conclusion, adjunctive use of filgrastim significantly accelerated hemato-biochemical and clinical recovery in dogs with naturally occurring CPV-2 enteritis, supporting its inclusion in the therapeutic protocol for cases presenting with severe leukopenia.

CONCLUSION

PCR proved markedly superior to the commercial rapid antigen test for diagnosing canine parvoviral enteritis. Adjunctive filgrastim (10 µg/kg SC) combined with standard supportive therapy achieved complete normalisation of all haematological and biochemical parameters. Filgrastim significantly accelerated leukocyte recovery and shortened the disease course. These results confirm PCR as the gold-standard diagnostic tool and support routine use of filgrastim as an effective adjuvant therapy in canine parvovirus infection, especially in leukopenic dogs.

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