RESEARCH ARTICLE

Ozone as an Adjunct Therapy in Canine Parvo Virus Infection in Dogs

Pranay Nagarkar, Balaji Ambore*, Ravindra Jadhav, Mahesh Ranganekar, Chandrakant Mote, Sameer Jadhav

Abstract

Canine Parvovirus infection is highly infectious disease of younger dogs with high mortality rates. A total of 1026 dogs presented at Veterinary Clinical Complex of the College in Shirwal were screened, out of which 33 dogs (3.2%) were confirmed positive for CPV using rapid antigen test, which were further confirmed by PCR. Twenty affected dogs were selected and divided into two treatment groups: Group 2 (standard ceftriaxone + supportive therapy) and Group 3 (ozonated RL as an adjunct therapy along with standard treatment), each comprising 10 animals, while 10 healthy dogs served as control Group-I. Haematological findings revealed a significant ($p<0.05$) decrease in Hb, PCV, TEC, TLC and platelet values in all the affected dogs compared with the healthy control group. Biochemical findings revealed significantly ($p<0.05$) increased ALT, AST, ALP, bilirubin, creatinine and BUN values, while significant decrease in total protein, Na, K, Cl and glucose levels in CPV affected dogs. Dogs received ozone therapy (Group 3) showed better recovery with mean recovery period of 4.6 days as compared to 5.9 days in Group 2 dogs. Further dogs treated with ozone therapy reported faster restoration of clinico-haemato-biochemical values compared to standard antibiotic therapy in CPV infected dogs.

Key words: Adjunct therapy, Canine parvo virus, Ozone.

Ind J Vet Sci and Biotech (2026): 10.48165/ijvsbt.22.4.16

Introduction

Canine parvoviral gastroenteritis, a deadly disease in dogs, was first emerged in 1978 in North America, Europe, and Australia. Canine parvovirus is now the most prevalent viral gastroenteritis affecting dogs worldwide. This disease has become a major concern for pet owners and veterinarians, particularly with the rise of viral gastroenteritis in purebred dogs. Parvoviruses are primarily categorized into two types: Type I and Type II, with Type II being the more common form found in nature. Parvovirus infections manifest mainly in two forms: enteritis and myocarditis. Parvoviral myocarditis occurs specifically when puppies contract the infection very close to their birth, during the period when their myocardial cells are undergoing rapid proliferation.

Acute parvoviral enteritis can occur in dogs of any breed, age, or sex; however, puppies between 6 weeks and 6 months of age are particularly vulnerable. The main predisposing factor for parvoviral infection in puppies is insufficient protective immunity, especially due to inadequate maternal antibodies, incomplete vaccination (Khare *et al*., 2020). Common clinical symptoms include sudden onset of vomiting, haemorrhagic diarrhoea, dehydration, anorexia, and depression. The primary causative agent responsible for haemorrhagic gastroenteritis in dogs is canine parvovirus, a highly contagious viral pathogen that targets rapidly dividing cells of the intestinal crypt epithelium and leads to severe enteritis (Khatri *et al.,* 2017).

Conventional therapy for parvo-viral gastroenteritis includes the use of antimicrobials like amoxicillin or ampicillin, intravenous fluid therapy with Ringer's lactate, antiemetics like ondansetron and maropitant, and gastroprotectants like omeprazole and sucralfate. Gastrointestinal diet or probiotics are recommended (Dupont *et al*., 2021). Ozone has anti-inflammatory, antimicrobial and antioxidant properties. Ozone has different antimicrobial activity, like inactivation of bacteria, viruses, fungi (Elvis and Ekta, 2011). Ozone therapy serves as an effective alternative intervention, seeing extensive clinical use throughout Europe and Asia. Within the veterinary field, it is increasingly utilized as a cost-effective and successful integrative treatment for various pathologies. In the management of canine parvovirus, ozone therapy serves as a supportive integrative modality that may shorten the duration of gastrointestinal distress.

Department of Veterinary Medicine, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara-412801, Maharashtra Animal & Fishery Sciences University, Nagpur, India

Corresponding Author: Dr. Balaji Ambore, Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara-412801, MAFSU, Maharashtra, India. e-mail: balajiambore@mafsu.ac.in

How to cite this article: Nagarkar, P., Ambore, B., Jadhav, R., Rangnekar, M., Mote, C., & Jadhav, S. (2026).Ozone as an Adjunct Therapy in Canine Parvo Virus Infection in Dogs. Ind J Vet Sci and Biotech, 22(4), 88-92.

Source of support: Nil

Conflict of interest: None

Submitted 29/04/2026 **Accepted** 23/05/2026 **Published** 10/07/2026

Whether administered systemically or locally, specifically through rectal routes, ozone has shown therapeutic utility. Nevertheless, the validation of its effectiveness in canine subjects has thus far been documented largely through qualitative clinical improvements rather than quantitative laboratory validation (Dos Santos *et al*., 2023). This study was aimed to validate effectiveness of ozone as an adjunct therapy through clinical and haemato-biochemical evaluation of parvovirus infected dogs.

Materials and Methods

Dogs (n=1026) above 6 months of age presented to Veterinary Clinical Complex at Krantisinh Nana Patil College of Veterinary Science, MAFSU, Shirwal (India), during May 2025 to January 2026 with the complaint of anorexia, vomition, diarrhoea with and without blood, dehydration, and lethargy were included in the study. Blood samples from CPV affected dogs for haematological analysis were collected from the cephalic vein in a clean, sterile tube containing EDTA@ 2 mg/mL of blood. Dogs showing signs of CPV were considered for confirmation of CPV using ELISA-based rapid antigen test (Bionote® CPV Ag test kit), a rapid, qualitative, immunochromatographic assay for the detection of specific CPV antigen, and fecal samples were subjected for further confirmation by PCR.

Confirmation of CPV by PCR

For confirmation of CPV by PCR, faecal samples of animals positive for CPV using an ELISA-based rapid antigen test kit were taken directly from the rectum of animals using swab, added with 2 mL phosphate buffer saline (PBS), vortexed for 1 min till proper mixing of the sample and then stored at -20°C until further processing. For DNA extraction, a 200 µL sample was taken in an Eppendorf tube, vortexed for 2 min, put in water bath set to 91°C for 10 min, and then snap-chilled on ice for 10 min and then centrifuged at 16000 x g for 10 min, and the supernatant was used as a DNA template for molecular detection of 719 bp amplicon using forward and reverse primers 5'CTGCTACTCAGCCACCAACT'3 and 5'AGGTGTTTCT CCTGTTGTGGT'3 (Desai *et al*., 2020). The PCR was carried out in 25 µL reaction volume consisting of PCR master mix 12.5 µL, forward and reverse primers 1 µL each, Template DNA 3 µL and nuclease free water 7.5 µL. The PCR thermal cycling conditions used were: initial denaturation at 94°C for 5 min, followed by 35 cycles each of Denaturation at 94°C for 60 s, Annealing at 59°C for 60 s, Extension at 72°C for 150 s, and Final Extension at 72°C for 10 min. To visualize the amplified viral capsid protein (CPV-2) PCR products, horizontal electrophoresis was performed at a constant 90V using a 2% agarose gel added with ethidium bromide (0.5 µg/mL) for nucleic acid staining with 1x TAE buffer along with a DNA ladder (Promega, USA), loaded into the wells and visualized under a UV transilluminator (Desai *et al*., 2020), which revealed a 719 bp product using the Gel Documentation System (Fig. 1).

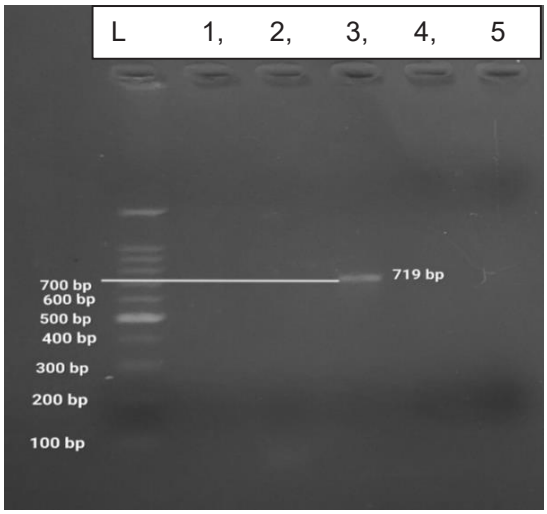

Fig. 1: PCR positive fecal sample for CPV on agarose gel. Lane L: 719 bp DNA ladder, Lane 1-2: CPV -ve control, Lane 3-4 CPV +ve samples.

Treatment Protocol

Among the 33 (3.2%) CPV confirmed cases, 20 dog were selected for different treatment protocols each group containing 10 animals (Group 2: Standard antibiotics and supportive therapy group and Group 3: Ozone therapy along with standard therapy group) keeping Group 1 as healthy control group as described in Table 1 below. Common Supportive and Nutritional therapy in both the groups included Fluid therapy (DNS), Antacid (Pantoprazole @ 0.7-1 mg/kg), Antiemetics (Ondansetron @ 0.2 mg/kg), Haemocoagulant (inj. Botropase)

Intravenous Ozone Therapy

In the present research work, ozone was used in the treatment of parvoviral gastroenteritis in the form of ozonated Ringer's lactate solution using a medical ozone generator. The medical ozone generator uses pure quartz dielectrics, high-frequency electrical corona discharge technology and latest

Table 1: Treatment protocol

Sr. No	Groups	No. of Animals	Treatment	Sample collection frequency
1	Group 1	10	Healthy animals with no treatment	0 Day
2	Group 2	10	Ringer's lactate I/V based on dehydration status plus Inj. Ceftriaxone 15-25mg/kg, I/V	0 day and 7th day
3	Group 3	10	Ozonated Ringer's lactate @ 50 mL/kg (Ozone @ 41 µg/mL RL) for 2-3 days plus Inj. Ceftriaxone 15-25 mg/kg, I/V	0 day and 7th day

electronic components to deliver 05 to 65 µg/mL ozone using a pure oxygen supply. Another important part of the ozone generator machine is the oxygen pressure flow controller and silicone rubber tubing.

Ozone therapy was performed for 2 days only; afterwards, regular fluid therapy was given. Ozonated Ringer's Lactate solution was prepared by using an ozone generator machine. A 500 mL bag of Ringer's lactate was coupled to a medical ozone generator through a previously sterilized silicone hose. For 5 min, the Ringer Lactate solution was ozonated at a concentration of 41µg/mL at a flow rate of 0.125 L/min. After this period, the solution was administered intravenously through the cephalic vein, at a rate of 30 mL/kg/20 min, and then reduced to a rate of 10 mL/kg/h for 4 h (Dos Santos *et al*., 2023).

The results of comparative therapeutic efficacy of standard antibiotic protocol and ozone with standard antibiotic protocol in canine parvo virus infected dogs were evaluated by assessing clinic-haemato-biochemical analysis before and after treatment using standard statistical procedure (ANOVA) and unpaired 't' tests (Snedecor and Cochran, 1994).

Results and Discussion

Haematological parameters in both treatment groups showed improvement by Day 7, indicating recovery from CPV infection. The increase in Hb, PCV, and TEC ($p<0.05$) observed in both groups suggests restoration of erythropoietic activity following therapy, which is in agreement with the findings of Salem (2014) and Ogbu *et al*. (2022), who reported anaemia in CPV infection due to bone marrow suppression and intestinal blood loss, followed by improvement after treatment. The significant rise in TLC ($p<0.05$) in both groups, particularly in the ozone-treated group, indicates recovery from virus-induced leukopenia. Similar observations were reported by Shruti and Ajay (2023), who described leukopenia as a characteristic feature of early CPV infection, with leukocytosis occurring during recovery or secondary bacterial involvement. Neutrophil percentage increased significantly ($p<0.05$), while lymphocytes decreased in both groups ($p<0.05$), indicating inflammatory response and infection control. These findings are consistent with reports of Ramprabhu *et al*. (2002) and Saravanan *et al*. (2020), who observed neutrophilia and lymphopenia in CPV cases. Platelet count increased post-treatment, indicating recovery from thrombocytopenia, as also reported by Castro *et al*. (2013) and Dogra *et al*. (2016).

Biochemical parameters showed marked improvement by Day 7 in both groups. Elevated ALT, AST, and ALP levels during the acute phase returned towards normal values ($p<0.05$), indicating restoration of hepatic function. Similar trends have earlier been reported by Shah *et al*. (2013), Salem (2014), and Saravanan *et al*. (2020).The increase in total protein, albumin, and globulin levels post-treatment suggests recovery from protein-losing enteropathy and improved liver function, as supported by Ramprabhu *et al*. (2002) and Khare *et al*. (2020). Reduction in bilirubin, creatinine, and BUN levels ($p<0.05$) indicates improvement in hepatic and renal function and correction of dehydration, which agrees with Castro *et al*. (2013) and Salem (2014). Electrolyte imbalances (hyponatremia, hypokalemia, hypochloremia) and hypoglycaemia observed on Day 0 were corrected after treatment ($p<0.01$), reflecting restoration of fluid and metabolic balance. Similar findings were reported by Haque and Tayyaba (2011) and Abdullaziz *et al*. (2022).

Comparatively, ozone therapy group showed better improvement in TLC, electrolyte balance, and recovery period, suggesting enhanced therapeutic response. This may be attributed to improved oxygen delivery, immunomodulatory effects, and anti-inflammatory properties of ozone therapy, as reported by Saini (2011), Elvis and Ekta (2011).

Table 2: Hematological changes in CPV affected dogs before and after standard antibiotic and ozone with standard antibiotic treatment (Mean ± SE, n=10)

		Standard antibiotic		Ozone with std antibiotic	
Parameter	**Healthy group**	**Day 0**	**Day 7**	**Day 0**	**Day 7**
Hb (g/dL)	14.01±0.31^{b}	11.18±0.66^{a}	12.02 ±0.63*	11.59 ±0.79^{a}	12.42±0.78*
PCV (%)	40.77±0.51^{b}	28.42±2.20^{a}	36.55±1.93*	27.81±1.20^{a}	37.41±2.61*
TEC (×10^{6}/µL)	7.59±0.29^{b}	5.98±0.58^{a}	6.52±0.40*	5.66±0.35^{a}	6.74±0.43**
TLC (×10^{3}/µL)	8.53±0.33^{a}	5.58±0.32^{b}	8.85±0.44**	5.33±0.27^{b}	13.2±2.36**
Platelet (×10^{3}/µL)	5.41±0.45^{b}	3.89±0.76^{a}	4.78±0.54**	3.92±0.55^{a}	4.33±0.49*
Neutrophils (%)	70.40±1.01^{b}	48.00±2.30^{a}	74.90±2.99	51.30±6.26^{a}	70.10±1.24*
Lymphocytes (%)	28.70±1.17^{a}	49.90±2.61^{b}	20.60±2.64*	46.20±5.52^{a}	25.50±2.01
Monocytes (%)	0.00±0.00^{a}	1.40±0.27^{b}	1.40±0.72	2.00±0.56^{b}	1.50±0.43
Eosinophils (%)	1.10±0.35^{ab}	0.60±0.34^{a}	2.30±1.00	2.40±0.64^{b}	2.90±1.05
MCV(fL)	54.27±1.66^{a}	49.43±2.56^{a}	53.33±2.43	50.20±2.30^{a}	53.33±2.43*
MCH (pg)	18.60±0.55^{a}	21.09±3.36^{a}	19.37±1.03	20.91±1.43^{a}	19.37±1.03
MCHC	39.54±2.31^{a}	34.60±1.66^{a}	35.97±1.04	36.84±1.11^{a}	35.97±1.04

The means bearing different superscripts (a,b) differ significantly between groups;*$p<0.05$, **$p<0.01$.

Table 3: Biochemical changes in CPV affected dogs before and after standard antibiotic and ozone with standard antibiotic treatment (Mean ± SE, n=10)

		Standard antibiotic		Ozone with std antibiotic	
Parameter	**Healthy group**	**Day 0**	**Day 7**	**Day 0**	**Day 7**
ALT (U/L)	22.07±2.71[a]	41.79±9.09[b]	30.73±3.94	39.02±11.87[b]	36.66±4.07*
AST (U/L)	21.76±2.16[a]	53.72±8.4[b]	34.98±3.25[*]	53.3±8.4[b]	34.59±5.93*
ALP (U/L)	73.03±1.89[a]	177.54±18.39[b]	122.5±8.74[**]	177.30±25.19[b]	132.90±12.75*
Total protein (g/dL)	6.90±0.18[b]	4.47±0.33[a]	5.16±0.35[**]	4.90±0.21[a]	5.11±0.15
Albumin (g/dL)	3.21±0.11[b]	2.38±0.14[a]	2.59±0.12[*]	2.13±0.17[a]	2.13±0.16
Globulin (g/dL)	3.69±0.13[b]	2.09±0.26[a]	2.57±0.36[**]	2.77±0.19[a]	2.98±0.21[*]
Bilirubin (mg/dL)	0.11±0.02[a]	1.10±0.11[b]	0.38±0.11[**]	1.11±0.13[b]	0.36±0.08**
Creatinine (mg/dL)	0.25±0.05[a]	1.22±0.06[b]	0.52±0.05[**]	1.30±0.14[b]	0.57±0.08**
BUN (mg/dL)	13.85±1.45[a]	62.01±8.49[b]	22.57±4.41[**]	56.37±10.01[b]	23.09±3.33**
Sodium (mEq/L)	143.37±1.72[b]	125.42±3.63[a]	136.80±2.35[**]	121.96±3.95[a]	141.60±2.32**
Potassium (mEq/L)	4.06±0.06[b]	3.24±0.22[a]	3.88±0.14[**]	3.05±0.17[a]	3.99±0.07**
Chloride (mEq/L)	115.00±1.38[b]	103.18±1.31[a]	111.42±2.27[**]	105.29±2.84[a]	115.66±2.60**
Glucose (mg/dL)	106.89±2.70[b]	69.45±4.41[a]	82.82±5.69[**]	69.48±6.04[a]	91.19±10.26**

The means bearing different superscripts (a,b) differ significantly between groups;*p<0.05, **p<0.01.

Recovery Period

Recovery period in group 2 dogs who received standard antibiotic treatment was 5.90±0.18 day and recovery period in group 3 dogs who received ozone therapy along with standard antibiotic treatment was 4.60±0.31 days. The shorter recovery period in dogs of Group 3 who received ozone therapy shows a clear benefit over conventional treatment alone. Canine parvoviral gastroenteritis leads to severe gastrointestinal damage, dehydration, vomiting, bloody diarrhea, and overall illness, which all contribute to longer recovery times (Kalli *et al*., 2010; Dos Santos *et al.,* 2023) found that recovery time for canine parvovirus infection can range from 3 to 7 days, depending on the severity of the infection and how well the dog responds to treatment.

Conclusion

The present study demonstrated that canine parvoviral gastroenteritis is associated with significant haemato-biochemical alterations, including anaemia, leukopenia, elevated hepatic enzymes, electrolyte imbalance, and hypoglycaemia. Both standard therapy and ozone therapy in combination with standard treatment resulted in marked improvement in these parameters by Day 7, indicating effective recovery. However, dogs treated with ozone therapy showed comparatively better improvement in total leukocyte count, electrolyte balance, and overall clinical status with faster recovery, suggesting enhanced therapeutic efficacy. Thus, ozone therapy can be considered as a beneficial adjunct to conventional treatment in the management of canine parvoviral gastroenteritis, contributing to faster recovery and improved clinical outcomes. Further studies with larger sample sizes are recommended to validate its therapeutic potential.

Acknowledgement

The authors acknowledge the hospital authorities for permitting the study to be conducted, as well as the hospital staffs and trained students who assisted in restraining the dogs during sample collection. Also, thanks to the Department of Animal Reproduction , Gynaecology and Obstetrics who made possible to use ozone generator for ozone therapy in dogs.

References

Abdullaziz, I., Aly, M., & ElShahawy, I. (2022).Clinical, haemato-biochemical alterations with acute phase response in canine parvoviral enteritis. *Damanhour Journal of Veterinary Sciences*, *7*(1), 23-27.

Castro, T.X., Rita de Cássia, N., Gonçalves, L.P., Costa, E.M., Marcello, G.C., Labarthe, N.V., & Mendes-de-Almeida, F. (2013).Clinical, hematological, and biochemical findings in puppies with coronavirus and parvovirus enteritis. *The Canadian Veterinary Journal, 54*(9), 885.

Desai, D., Kalyani, I., Ramani, U., Makwana, P., Patel, D., & Vala, J. (2020). Evaluation of three different methods of viral DNA extraction for molecular detection of canine parvo virus-2 from faecal samples of dogs. *Journal of Entomology and Zoology studies, 8*(3), 479-481.

Dogra, S., & Sood, N.K. (2016). Haemato-biochemical alterations in canine parvovirus infection. *Indian Journal of Veterinary Pathology, 40*(3), 226-228

Dos Santos, T.G., Orlandin, J.R., de Almeida, M.F., Scassiotti, R.F., Oliveira, V.C., Santos, S.I.P., Pereira, V.M., Pinto, P.A.F., Mariano, C.G., & Ambrósio, C.E. (2023). Ozone therapy: protocol for treating canine parvovirus infection. *Brazilian Journal of Veterinary Medicine, 45,* p.e004622.

Dupont, N., Jessen, L.R., Moberg, F., Zyskind, N., Lorentzen, C., & Bjørnvad, C.R. (2021). A retrospective study of 237 dogs hospitalized with suspected acute hemorrhagic diarrhea

syndrome: Disease severity, treatment, and outcome. *Journal of Veterinary Internal Medicine, 35*(2), 867-877.

Elvis, A.M., & Ekta, J.S. (2011). Ozone therapy: A clinical review. *Journal of Natural Science, Biology, and Medicine, 2*(1), 66.

Haque, S., & Tayyaba, A. (2011).Studies on incidence of parvo gastroenteritis and its treatment by different concentration of fluid in pups. *Indian Journal of Canine Practice, 3*(2), 80-82.

Kalli, I., Leontides, L.S., Mylonakis, M.E., Adamama-Moraitou, K., Rallis, T., & Koutinas, A.F. (2010).Factors affecting the occurrence, duration of hospitalization and final outcome in canine parvovirus infection. *Research in Veterinary Science, 89*(2), 174-178.

Khare, D.S., Gupta, D.K., Shukla, P.C., Das, G., Meena, N.S., & Khare, R. (2020).Clinical and haemato-biochemical changes in canine parvovirus infection. *Journal of Pharmacognosy and Phytochemistry*, *9*(4), 1601-1604.

Khatri, R., Poonam, M.H., & Minakshi, P.C. (2017). Epidemiology, pathogenesis, diagnosis and treatment of canine parvovirus disease in dogs: A mini review abstract. *Journal of Veterinary Sciences and Medical Diagnosis*, *6*(3), 2.

Ogbu, K.I., Chukwudi, C.I., Tion, M.T., Eze, U.U., Nwosuh, I.C., & Anene, B.M. (2022). Haematology and serum biochemistry of dogs naturally infected with canine parvovirus-2. *Sokoto Journal of Veterinary Sciences, 20*(5), 141-152.

Ramprabhu, R., Prathaban, S., Nambi, A.P., & Dhanapalan, P. (2002). Haemorrhagic gastroenteritis in dogs- A clinical profile.*Indian Veterinary Journal, 79*(4), 374-376.

Saini, R. (2011). Ozone therapy in dentistry: A strategic review. *Journal of Natural Science, Biology and Medicine, 2*(2), 151.

Salem, N.Y. (2014). Canine viral diarrhea: clinical, hematologic and biochemical alterations with particular reference to in-clinic rapid diagnosis. *Global Veterinaria, 13*(3), 302-307.

Saravanan, S., Ramprabhu, R., Mohanapriya, T., Chitra, R., & PonnuSwamy, K.K. (2020). Retrospective study on risk factors and haemato-biochemical alterations associated with canine parvo viral enteritis. *Journal of Entomology and Zoology Studies, 8,* 342-346.

Shah, S.A., Sood, N.K., Wani, N., Gupta, K., & Singh, A. (2013). Haemato-biochemical changes in canine parvoviral infection. *Indian Journal of Veterinary Pathology, 37*(2), 131-133.

Shruti, G., & Ajay, K. (2023). Canine parvo viral enteritis in dogs: Diagnostic and therapeutic evaluation. *Journal of Veterinary and Animal Sciences, 54*(1), 71-78

Snedecor, G.W., & Cocharan, W.G. (1994). *Statistical Methods*. 8th edn., Iowa State University Press, Ames, Iowa, USA.