

MANAGEMENT OF LEUKOPENIA ASSOCIATED WITH PARVOVIRUS GASTROENTERITIS IN A LABRADOR RETRIEVER PUPPY - A CASE REPORT

M.A. Ensha Lomiya¹, *R. Raguvaran², Renu Motwani³, Narayani Yadav⁴ and D. B. Mondal⁵

*Division of Medicine
Indian Veterinary Research Institute
Izatnagar, Bareilly – 24311, Uttar Pradesh, India*

ABSTRACT

A four-month-old male Labrador retriever puppy was presented to Referral Veterinary Polyclinic, Indian Veterinary Research Institute with the history of anorexia, vomiting, haemorrhagic diarrhoea and signs of hypovolaemic shock. The case was diagnosed as parvovirus gastroenteritis by rapid antigen detection test. Shock was managed by infusion of polyionic isotonic fluid. Haematological examination revealed severe leukopenia with WBC count of $0.3 \times 10^3/\mu\text{L}$, which was treated with administration of recombinant human granulocyte colony stimulating factor (Filgrastim) at 5 mcg/kg subcutaneously along with supportive therapy. Dog showed elevated WBC count and improvement in clinical signs after 48 h. Administration of granulocyte colony stimulating factor along with routine therapy enhanced the survivability in canine parvovirus gastroenteritis.

Keywords: Canine parvovirus, Filgrastim, Leukopenia.

Received : 14.06.2023

Revised : 31.07.2023

Accepted : 25.08.2023

Canine parvovirus (CPV) enteritis is a contagious disease affecting young puppies, reported to have high morbidity and mortality despite of aggressive therapy (Nandi and Kumar, 2010). CPV targets the haemopoietic progenitor cells of bone marrow and can

cause direct destruction of active myeloblasts which result in leukopenia. Leukopenia is also related to massive loss of neutrophils through inflamed intestinal wall as well as neutrophil margination in response to sepsis (Prittie, 2004). Marked leukopenia even after initiation of treatment in CPV enteritis is attributed to poor prognosis as it makes the animal more susceptible to secondary bacterial infection and leading to septicemia (Goddard *et al.*, 2008).

¹M.V.Sc Student

²Scientist (SS), Corresponding author Email id: raguivri@gmail.com

³B.V.Sc & AH final year

⁴Ph.D. Scholar

⁵Principal Scientist

The production, maturation, and discharge of neutrophils from the bone marrow are all influenced by granulocyte colony stimulating factor (G-CSF) (Dale *et al.*, 1993). G-CSF is being used in dogs for the management of pancytopenia, cyclic hereditary neutropenia, neutropenia induced by chemotherapy and certain infections (Pothiappan *et al.*, 2020). Administration of G-CSF along with routine therapy was shown to improve the survival rate of CPV affected dogs in previous studies (Armenise *et al.*, 2019; Punia *et al.*, 2021). This report briefly describes the management of leukopenia associated with CPV enteritis.

A four-month-old male Labrador retriever puppy was presented to Referral Veterinary Polyclinic, Indian Veterinary Research Institute with the complaint of anorexia, vomiting and bloody diarrhoea since four days. Dog had its 1st dose of multicomponent vaccine and was dewormed one month back. Anamnesis also revealed death of another puppy which showed similar clinical signs. Dog had received supportive therapy with intravenous fluids, antibiotics, antihistamine, antiemetic and vitamin B complex for two days with no improvement.

The dog was recumbent at the time of presentation and developed signs of hypovolemic shock. Clinical examination revealed congested and dry mucous membrane, pyrexia (103.3° F), extreme dehydration (>12%), sunken eyes, appreciable loss of skin turgor, elevated capillary refill time (>2 sec) and feeble pulse. The respiratory rate was 30/min and heart rate was 102 bpm.

Blood examination revealed packed cell volume of 37 %, haemoglobin- 11.7 g%, RBC count- $5.33 \times 10^6/\mu\text{L}$ and severe leukopenia with WBC count of $0.3 \times 10^3/\mu\text{L}$. Faecal sample was collected using sterile swabs and it was negative for parasitic oocysts and ova. Considering the age, history and clinical symptoms the case was tentatively diagnosed as parvoviral gastroenteritis and further confirmed by using rapid CPV antigen detection kit (Bionote Inc, Korea).

Aggressive fluid therapy was given at the dose rate of 90 ml/kg intravenously (IV) with Inj. Ringer lactate and Inj. Normal Saline. Since, the puppy was severely dehydrated hypertonic saline was avoided due to unfavourable effect of cellular dehydration and diuresis before adequate expansion of plasma volume. The dog also received symptomatic and supportive treatment with antibiotics: amoxicillin-sulbactam @ 8 mg/kg q 12 hrs IV, metronidazole @ 20 mg/kg q 12 hrs IV, and proton pump inhibitor- pantoprazole @ 1 mg/kg q 24 hrs IV, antiemetic-ondansetron @ 0.5 mg/kg q 12 hrs IV, hemostat - hemocoagulase @ 1 ml IV q 24 hrs, vitamin B complex @ 1ml intramuscularly q 24 hrs for 5 days. Acute neutropenia was treated with single injection of filgrastim (recombinant human granulocyte colony stimulating factor) @ 5 mcg/kg subcutaneously (Duffy *et al.*, 2010). The dog was kept under observation and complete haematology was performed after 48 hrs and 96 hrs of filgrastim administration. Peripheral blood smear was prepared and differential leucocyte count was done after 48 hrs and 96 hrs of therapy.

Animal showed elevation in total leucocyte count to $27 \times 10^3/\mu\text{L}$ on day 3 and $24 \times 10^3/\mu\text{L}$ on day 5. Marked improvement in clinical condition was evident after 48 hrs of filgrastim administration and had uneventful recovery at the end of treatment. Haematological examination on day 3 revealed anaemia with RBC count of $3.87 \times 10^6/\mu\text{L}$ and haemoglobin concentration of 7.5 g%, which was improved on 5th day of treatment. Details of the clinico-hematological parameters before and after therapy are depicted in the table 1 and 2.

Unvaccinated puppies are highly susceptible to CPV infection. In this case, the dog had received one dose of vaccine prior to infection. Lack of protective immunity (Carr-Smith *et al.*, 1997) and vaccine failure could be the possible reason for the development of severe clinicopathological abnormalities. Anaemia and leucopenia are the consistent findings associated with CPV enteritis (Chalifoux *et al.*, 2021). In this case, the puppy was presented with extreme dehydration and relative values of hematocrit, hemoglobin and RBC count was found to be normal which possibly due to hemoconcentration and a reduction in all these values was observed on day 3. Haemorrhage of the intestinal tract and suppression of erythropoiesis in the bone marrow are attributed to low haematocrit and less numbers of circulating red blood cells during the period of infection. Similar haematological changes were reported in previous studies by Areshkumar *et al.* (2017) and Punia *et al.* (2021).

Onset of clinical signs in CPV enteritis usually preceded the onset of neutropenia. Cohn *et al.* (1999) reported that plasma concentration of endogenous G-CSF remained undetectable during the onset of neutropenia and apparent when neutrophil nadir occurs. The administration of exogenous G-CSF at the onset of neutropenia or clinical signs may be of benefit in ameliorating leukopenia induced by CPV. Filgrastim is a recombinant human granulocyte colony stimulating factor reported to enhance the production and release of active neutrophils from the bone marrow within 24 hrs of administration. According to Goddard *et al.* (2008) improvement in total leukocyte count in 24 hrs of hospital admission increases the likelihood of survivability. In the present study the puppy had severe leukopenia and suffered from dehydration, pyrexia and hypovolemic shock at the time of hospitalization. The animal showed improved total leucocyte count and recovered from clinical signs after filgrastim and supportive medications. Although monocytes or lymphocytes are not target sites for the action of G-CSF, acute rise in number of these cells was observed in this study with single injection of filgrastim followed by increase in the neutrophil count, which was similar to the findings of Duffy *et al.*, 2010 and Armenise *et al.*, 2019. Researchers also explored the therapeutic value of antioxidants in CPVE cases and found that supplementations of N-acetylcysteine and resveratrol markedly ($P < 0.05$) improved the total leukocyte count and neutrophil count in CPVE-affected dogs. NAC

Table 1. Pre and post treatment clinical examination findings

S. No.	Parameters	0 th day	3 rd day	5 th day	Reference range
1	Rectal temperature	103.3°F	102.4°F	102.1°F	99.5-102.5°F
2	Colour of mucous membrane	Congested and dry	Congested	Pale	Pale roseate
3	Respiration per min	30	Severe panting	30	10-30
4	Heart rate per min	102	120	110	80-120
5	Hydration status	Extreme dehydration (12%)	Hydrated	Hydrated	Hydrated

(Reference range: Saunders Manual of Small Animal Practice, 3rd edition)

Table 2. Pre and post treatment haematology findings

Sl. No.	Parameters	0 th day	3 rd day	5 th day	Reference range
1	Packed cell volume (%)	37.2	22.5	24.2	35-57
2	Haemoglobin (g%)	11.7	7.5	7.8	11.9-18.9
3	Total erythrocyte count (10 ⁶ /μL)	5.33	3.87	4	4.95-7.87
4	Total leukocyte count (10 ³ /μL)	0.3	27	24	5.0-14.1
5	Differential leukocyte count (%)	Unable to count	N: 40; L: 53; M: 7; E: 0; B: 0	N: 79; L: 18; M: 2; E: 1; B: 0	N: 58-85; L: 18-21; M: 2-10; E: 0-9; B: 0-1

(Reference range: The Merck Veterinary Manual, 10th edition)

and RES could serve as better antioxidants for the amelioration of oxidative stress in CPVE

The study suggests that G-CSF causes marked leukocytosis, increase in neutrophil count and can be effectively used as an adjunct in the treatment protocol of CPV gastroenteritis.

REFERENCES

- Areshkumar, M., Vijayalakshmi, P., Venkatesa Perumal, S. and Selvi, D. (2017). Effect of Filgrastim in a Severe Leucopenia associated Parvoviral Enteritis in Rottweiler. *International Journal of Current Microbiology and Applied Sciences* **4**: 1060 - 1065.
- Armenise, A., Trerotoli, P., Cirone, F., De Nitto, A., De Sario, C., Bertazzolo, W., Pratelli, A. and Decaro, N. (2019). Use of recombinant canine granulocyte-colony stimulating factor to increase leukocyte count in dogs naturally infected by canine parvovirus. *Veterinary Microbiology*, **231**: 177 - 182.
- Carr-Smith, S., Macintire, D.K. and Swango, L.J. (1997). Canine parvovirus: Part 1. Pathogenesis and vaccination. *Compendium on Continuing Education for the Practising Veterinarian*, **19**: 125 - 133.
- Chalifoux, N.V., Parker, S.E. and Cosford, K.L. (2021). Prognostic indicators at presentation for canine parvoviral enteritis: 322 cases (2001- 2018). *Journal of Veterinary Emergency and Critical Care*, **31**(3): 402 - 413.
- Cohn, L.A., Rewerts, J.M., McCaw, D., Boon, G.D., Wagner-Mann, C. and Lothrop Jr, C.D. (1999). Plasma granulocyte colony - stimulating factor concentrations in neutropenic, parvoviral enteritis - infected puppies. *Journal of Veterinary Internal Medicine*, **13**(6): 581 - 586.
- Dale, D.C., Bonilla, M.A., Davis, M.W., Nakanishi, A.M., Hammond, W.P., Kurtzberg, J., Wang, W., Jakubowski, A., Winton, E., Lalezari, P. and Robinson, W. (1993). A randomized controlled phase III trial of recombinant human granulocyte colony-stimulating factor (filgrastim) for treatment of severe chronic neutropenia. *Blood*, **81**(10): 2496 - 2502.
- Duffy, A., Dow, S., Ogilvie, G., Rao, S. and Hackett, T. (2010). Haematologic improvement in dogs with parvovirus infection treated with recombinant canine granulocyte-colony stimulating factor. *Journal of Veterinary Pharmacology and Therapeutics*, **33**(4): 352 -356.
- Goddard, A., Leisewitz, A.L., Christopher, M.M., Duncan, N.M. and Becker, P.J. (2008). Prognostic usefulness of blood leukocyte changes in canine

- parvoviral enteritis. *Journal of Veterinary Internal Medicine*, **22**(2): 309 - 316.
- Nandi, S. and Kumar, M. (2010). Canine parvovirus: current perspective. *Indian Journal of Virology*, **21**(1): 31-44.
- Pothiappan, P., Nagarajan, B., Selvaraj, P., Vairamuthu, S. and Tirumurugaan, K.G. (2020). Novel Granulocytic Colony Stimulating Factor based Therapy for Morbidity Reduction in Pancytopenic Dogs with Babesia gibsoni. *Journal of Animal Research*, **10**(2): 199 -204.
- Prittie, J. (2004). Canine parvoviral enteritis: a review of diagnosis, management, and prevention. *Journal of Veterinary Emergency and Critical Care*, **14**(3): 167 - 176.
- Punia, S., Kumar, T., Agnihotri, D. and Sharma, M. (2021). A study on effect of filgrastim in severe leukopenia associated with hemorrhagic gastroenteritis in dogs. *The Pharma Innovation*, **10**(11): 868 - 870.