Indian J. Vet. Pathol., 50(1) : 36-39, 2026: DOI: https://doi.org/10.56093/cr49qj17

RESEARCH ARTICLE

Canine Parvovirus-2a infection in a juvenile pup: Bone marrow findings and clinical implications

J. Thilahayswari, K. Nagarajan*, S. Hemalatha and M. Chandrasekar[1]
Department of Veterinary Pathology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India; [1]Professor and Head, Resident Veterinary Officer

Address for correspondence:
Dr. K. Nagarajan, Professor, Department of Veterinary Pathology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India; E-mail: nagavet@gmail.com

Received: 02.11.25; Accepted: 21.11.25

ABSTRACT

A 2 month old non descriptive pup was presented to the Emergency and Critical Care Unit (ECCU) at Madras Veterinary College Teaching Hospital with a 4-day clinical history of bloody diarrhoea, vomition and neurological manifestations suggestive of severe systemic involvement. Haematological analysis revealed anaemia and profound leukopenia, indicative of marked immunosuppression. Necropsy findings demonstrated extensive hemorrhagic enteritis and multi-organ pathology, including fatty enlarged liver and epicardial pallor. Histopatholgical examination revealed hepatic fatty degeneration, myocardial fibre degeneration, and intestinal crypt cell necrosis. Bone marrow examination showed severe hypocellularity with depletion of myeloid and erythroid cell lines that lead to leukopenia. Molecular detection and characterization of the VP2 gene confirmed infection with the CPV-2a variant, genetically related to strains reported from India, Bangladesh, and Nigeria. The study highlights the importance of bone marrow evaluation in diagnosing and managing CPV-2 infection, and underscores the value of integrating molecular and pathological findings for accurate diagnosis and improved treatment outcomes.

Keywords: Bone marrow, Canine Parvovirus, Colony-Stimulating Factors, Hypocellularity, Leukopenia, Phylogeny, VP2 Gene

INTRODUCTION

Canine parvovirus type 2 (CPV-2) is a significant cause of illness and mortality in young dogs, with a history of evolution and adaptation since its discovery in 1978[1]. Classified within the family Parvoviridae, CPV-2 is a single-stranded DNA virus with a 5.2 kb genome encoding two structural proteins (VP1 and VP2) and two non-structural proteins (NS1 and NS2)[2]. The VP2 protein is crucial for receptor recruitment, host-specificity, and antigenicity[3]. The virus being reported to have evolved from feline panleukopenia virus (FPV), CPV-2 shares over 98% nucleotide similarity with FPV but has six amino acid changes in VP2 that enable it to infect dogs while losing its capacity to infect cats[4]. CPV-2 has three variants: CPV-2a (426Asn), CPV-2b (426Asp), and CPV-2c (426Glu), classified based on the amino acid configuration of the capsid protein.

In India, CPV-2 infection was first documented in 1982[5], followed by the detection of all three variants - CPV-2a, CPV-2b, and CPV-2c across various regions, with CPV-2c being the least frequently detected[6]. The virus primarily replicates in highly mitotically active tissues such as bone marrow, lymphoid tissue, and gastrointestinal tract epithelium, making young growing dogs more susceptible to infection. The destruction of hematopoietic progenitor cells in bone marrow and lymphoproliferative organs leads to notable hematobiochemical alterations. This study aims to elucidate the presence of parvovirus in bone marrow and its molecular differentiation, addressing the lack of comprehensive knowledge on the entire genome of CPV-2 in circulation in India.

How to cite this article: Thilahayswari, J., Nagarajan, K., Hemalatha, S. and Chandrasekar, M. Canine Parvovirus-2a infection in a juvenile pup: Bone marrow findings and clinical implications. Indian J. Vet. Pathol., 50(1) : 36-39.

MATERIALS AND METHODS

A 2-month-old female pup, weighing 1.5 kg, was presented to the Emergency and Critical Care Unit (ECCU) at Madras Veterinary College Teaching Hospital with a 4-day clinical history of bloody diarrhoea and vomition. Born to a vaccinated dam, the pup was part of a litter of five that had received a single vaccination against canine distemper, adenovirus type-2, leptospirosis, parainfluenza, and parvovirus. Notably, one week after vaccination, four of the five littermates developed lethargy, vomition, and diarrhoea, and despite treatment, all four pups succumbed to the illness. Upon presentation, the last surviving pup was found to be dull, recumbent, and hypothermic, with foul-smelling bloody faeces adherent to the anal region. Laboratory findings revealed severe panleukopenia, microcytic anaemia, and hypoalbuminemia that indicated a critical condition.

The pup underwent intensive care, including stabilization on a heating pad, administration of IV fluids comprising Ringer's Lactate with 5% dextrose supplementation, and a regimen of medications consisting of Ceftriaxone (20 mg/kg) to combat bacterial infections, Ondansetron (0.5 mg/kg) to prevent vomition, and Pantaprazole (1 mg/kg) to protect the gastrointestinal tract was followed. A worse, marked decline in white blood cell count and onset of neurological signs, including vocalization, cervical hyperesthesia, seizures and nystagmusdevelopedthat led to collapse and deathof the pup.A detailed necropsy was conducted, and bone marrow aspirates were collected in a sterile manner for laboratory investigation. Representative tissue samples and cut section of the femur with bone marrow were collectedin 10% neutral buffered formalin. Tissuesamples were routinely processed, bone tissue decalcified and 3-5 micron thick tissue sections were stained with Haematoxylin and eosin[7].

Bone marrow tissue sample of about 25 mg was used for the extraction of genomic DNA, using commercially standardized DNeasy Blood and Tissue Kit (QIAGEN, Germany). The CPV genome was confirmed by Polymerase Chain Reaction (PCR), targeting a portion of the VP2 gene. The amplification was carried out under standard conditions using the specific primer set: Forward Primer (CPV-F: AAG ACG TGC AAG CGA GTC C) and Reverse Primer (CPV-R: GAG CGA AGA TAA GCA GCG TAA). The resulting PCR product was then subjected to electrophoresis and analyzed on a 1.5% agarose gel. The final analytical stages involved nucleotide sequencing of the purified VP2 gene product. The sequence data were used for a detailed comparison against VP2 reference sequences retrieved from the National Centre for Biotechnology Information (NCBI) database, utilizing MEGA12 software to construct phylogenetic trees.

RESULTS

A detailed necropsy revealed a carcass poor in condition with pallor of visible mucous membranes. Liver appeared enlarged, yellow with rounded borders (Fig. 1). Lungs were pale with watery fluid flowing out on incision. Epicardium revealed patchy areas of pale discolouration (Fig. 2). The kidneys appeared pale. Large roundworms, approximately 5-8 cm in diameter, in the lumen of intestine was observed (Fig. 3). The mucosa of intestine was dark red with blood mixed contents. The stomach revealed serosal haemorrhage with blood tinged contents. Additionally, faecal examination confirmed the presence of *Toxocara canis* eggs .

Microscopically, liver shared fatty degeneration of hepatocytes, characterized by intracytoplasmic clear vacuoles leading to cytoplasmic rarefaction and peripheral displacement of nuclei, indicating secondary hypoxic injury (Fig. 5). The kidneys showed glomerular atrophy (Fig. 4) and mild tubular degeneration. The myocardium revealed degeneration of muscle fibres with loss of cross striations. Affected cardiac muscle fibers appear swollen, fragmented, and hypereosinophilic with mild mononuclear cell infiltration. Lungs revealed edema of alveoli. The spleen revealed marked lymphoid depletion in the white pulp. The intestine showed

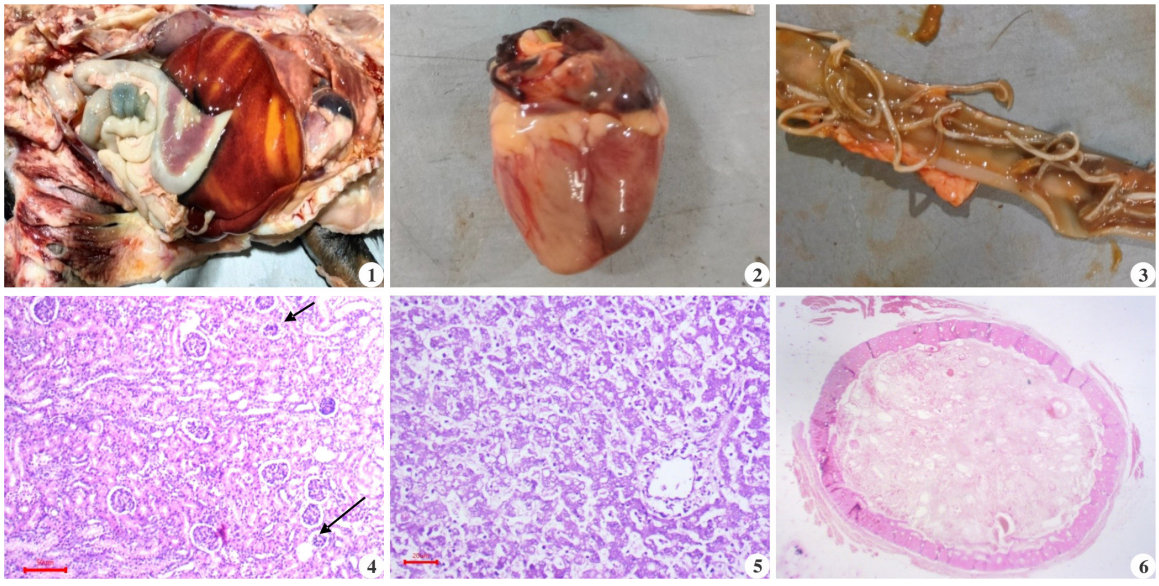

Fig. 1: Liver- enlarged with rib impressions; soft and rounded borders; Fig.2: Heart- pallor of epicardium; Fig.3: Intestine- Lumen occluded with Toxocara canis; Fig.4: Kidney- Glomerular atrophy; 100X; Fig.5:Liver-Fatty change in hepatocytes; H&E stain;200X; Fig.6: Cross section of femur with hypocellular bone marrow inside; 12.5X-H&E

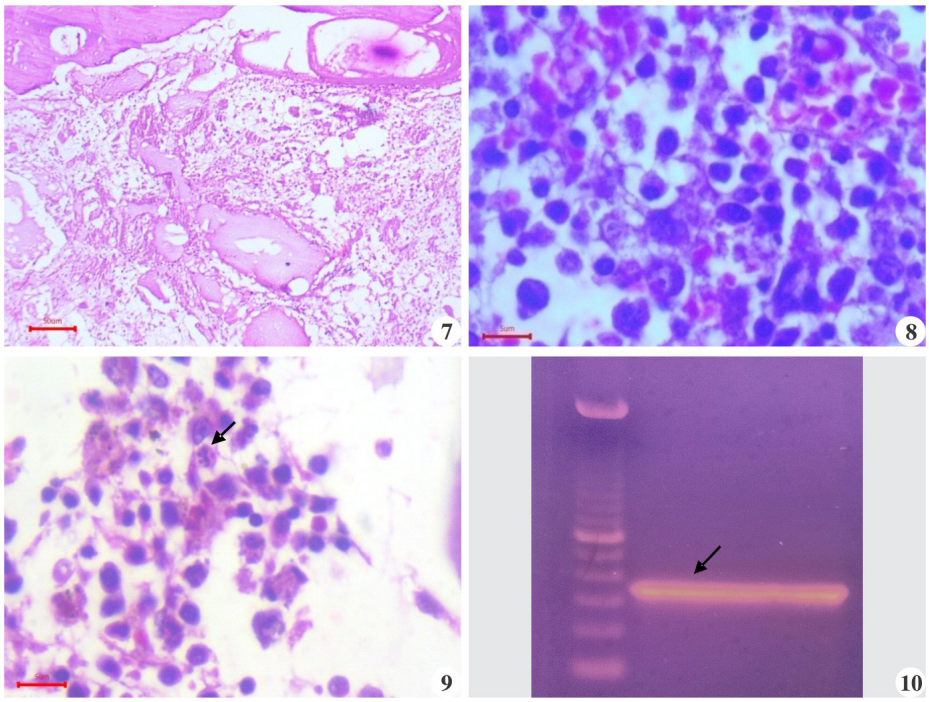

Fig.7. Bone marrow- hypocellularity with adipose tissue and stroma; 100X-H&E; **Fig.8.** Bone marrow- A pool of erythroid blast cells without maturation; 1000X-H&E; **Fig.9.** Bone marrow- Dysplastic neutrophil with fragmented nuclei (black arrow); 1000X; **Fig. 10:** PCR- A lane showing a positive thick band at 377 bp

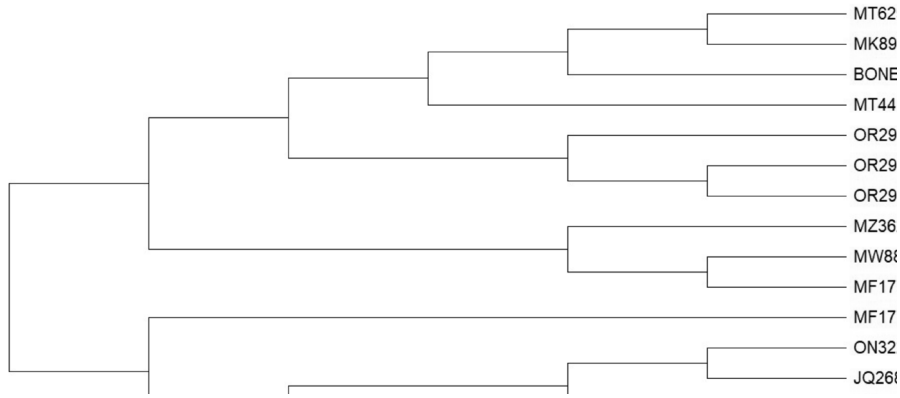

Fig. 11. The phylogeny was inferred using the Maximum Likelihood method and Tamura-Nei (1993) model [10] of nucleotide substitutions and the tree with the highest log likelihood (-556.54) is shown. The initial tree for the heuristic search was selected by choosing the tree with the superior log-likelihood between a Neighbor-Joining (NJ) tree [11] and a Maximum Parsimony (MP) tree. The NJ tree was generated using a matrix of pairwise distances computed using the Tamura-Nei (1993) model [10]. The MP tree had the shortest length among 10 MP tree searches, each performed with a randomly generated starting tree. The analytical procedure encompassed 18 nucleotide sequences with 339 positions in the final dataset. Evolutionary analyses were conducted in MEGA12 [12] utilizing up to 3 parallel computing threads.

extensive sloughing of the mucosal epithelium with hyperplasia of intestinal crypt epithelial cell. The stomach revealed mild multifocal necrosis of crypt epithelium.

Histopathological examination of bone marrow revealed marked hypocellularity. The marrow spaces were largely replaced by adipose tissue and eosinophilic proteinaceous material i.e., 20% cells and 80% stroma and fat (Figs. 6, 7), with pronounced depletion of the myeloid series, particularly granulocytic precursors. Cytologically, erythropoiesis was minimal with an increased proportion of blast cells (Fig. 8). Occasional dysplastic changes in neutrophils (Fig. 9) were evident, indicating disrupted hematopoiesis. These alterations collectively contributed to severe leukopenia, neutropenia, and immunosuppression characteristic of the disease.

The presence of the CPV in the bone marrow was confirmed by PCR (Fig. 10). Subsequent nucleotide sequencing and comparative analysis, allowed for the accurate typing of the CPV variant. The evolutionary relationship and clustering of the Chennai isolate relative to global and regional strains (CPV-2a, 2b, 2c) were confirmed thereby establishing its regional significance within the phylogenetic lineage (Fig. 11).

DISCUSSION

In this study, we have documented severe bone-marrow hypoplasia in a young pup infected with CPV-2 (confirmed by PCR and VP2 sequencing). The histological (hypocellularity, myeloid depletion) and cytological (erythroid blast-cell increase, dysplastic neutrophils) findings indicate the suppression of haematopoiesis alongside intestinal and lymphoid organs. The findings substantiate the significance of bone-marrow evaluation and its significant prognostic and therapeutic implications in CPV-enteritis.

The CPV preferentially infects highly mitotic cells of intestinal crypt epithelium,

lymphoid tissue and bone-marrow progenitor cells. The destruction of hematopoietic progenitor cells in bone marrow leads to leukopenia, neutropenia and lymphopenia, which are recognized markers of severity in CPV infection[8]. The cytological findings in the case studied (marked myeloidcells depletion, adipose/ stroma replacement, and dysplastic neutrophils) align with earlier reports of bone-marrow alterations associated with CPV infection— for example, vacuolated myeloid precursors and bizarre toxic neutrophil forms.

Bone marrow cytological studies are of prognostic significance and are an indicator for the effective immune response[9]. In the present case, the severe bone marrow suppression of haemopoeisiscorresponded with rapid clinical deterioration and death, reinforcing the link between marrow damage and outcome.

Since the general clinical examination consists of peripheralblood smear examination and Complete blood counts (RBC, Hemoglobin, PCV, WBC, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Platelets), the inclusion of bone marrow cytology/biopsy may refine the diagnosis and prognosisin CPV infections. For example, if marrow reveals near-complete myeloid progenitor loss, the expected neutrophil recovery may be delayed or absent, signalling a guarded to grave prognosis. In addition, marrow evaluation allows exclusion of concurrent processes (e.g., marrow aplasia of other aetiology, neoplasia) in critical cases.

The observed clustering of the bone marrow CPV isolate within the CPV-2a clade confirms that its evolution is driven by mutations in the VP2 gene. The placement implies that the isolate retains the antigenic and genetic characteristics typical of CPV-2a, which result in differences in pathogenicity and vaccine responsiveness compared to the CPV-2b and CPV-2c variants. Consequently, accurate diagnosis necessitates the integration of clinical, pathological, and molecular findings for reliable confirmation. Finally, effective disease prevention and control rely on the critical recognition of factors contributing to vaccine failure, such as improper vaccination timing, interference by maternally derived antibodies, and, more rarely, vaccine virus reversion.

In present case study, the acute canine parvovirus enteritis that led to systemic shock, immunosuppression, and multi-organ damage was confirmed by histopathological, molecular findings of the viral etiology, with evidence of severe bone marrow hypocellularity underlying the clinical immunosuppression. This case study therefore highlights the prognostic significance of bone marrow cytological studies in CPV infection and highlights the urgent need for integrated diagnostic and preventive approaches—combining pathological surveillance, molecular typing (confirming regional significance of CPV-2a), and aggressive supportive care to effectively reduce morbidity and mortality in affected animals.

ACKNOWLEDGEMENT

The authors are grateful for the support received from the Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai, for providing the necessary facilities and infrastructure to complete this case study.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI) Assisted technology for manuscript preparation: The authors confirm that there was no use of AI – Assisted Technology for assisting in the writing of the manuscript and no images were manipulated using AI

REFERENCES

1. Goddard A, Leisewitz AL. 2010. Canine Parvovirus. *Vet Clin North Am Small Anim Pract* ***40***: 1041–53.
2. Hoelzer K, Shackelton LA, Parris CR,d Holmes EC. 2008. Phylogenetic analysis reveals the emergence, evolution and dispersal of carnivore parvoviruses. *J Gen Virol* **89**: 2280–9.
3. Allison AB, Organtini LJ, Zhang S, Hafenstein SL, Holmes EC, Parrish CR. 2015. Single Mutations in the VP2 300 Loop Region of the Three-Fold Spike of the Carnivore Parvovirus Capsid Can Determine Host Range. *J Virol* **90**: 753–67.
4. Miranda C, Thompson G. 2016. Canine parvovirus: the worldwide occurrence of antigenic variants. *J Gen Virol* **97**: 2043–57.
5. Ramadass P, Khader TGA. 1982. Diagnosis of canine parvovirus infection by agar gel precipitation test and fluorescent antibody techniques. *Cherion* **11**: 323–328.
6. Nandi S, Chidri S, Kumar M, Chauhan RS. 2010. Occurrence of canine parvovirus type 2c in the dogs with haemorrhagic enteritis in India. *Res Vet Sci* **88**: 169–71.
7. Bancroft JD, Gamble M. 2008. Theory and Practice of Histological Techniques. 6th edn. Churchill Livingstone Elsevier.
8. Mihaela-Anca D, Florentina D, Gabriel M, Andreea-Paula C, Șerban M, Oana-Irina T. 2023. Haematological changes in canine parvovirus infection. *Sci Pap J* ***66***: 73–75.
9. Li H, Li S, Pan Y, Shi Q. 2025. Decoding canine parvovirus: biomarkers for diagnosis and advances in vaccine development to address emerging challenges. *Front Vet Sci* **12**
10. Tamura K, Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* **10**: 512–526.
11. Saitou N, Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**: 406–425.
12. Kumar S, Stecher G, Suleski M, Sanderford M, Sharma S, Tamura K. 2024. Molecular Evolutionary Genetics Analysis Version 12 for adaptive and green computing. *Mol Biol Evol* **41**: 1–9.