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# Therapeutic management of concomitant canine parvoviral gastroenteritis and hepatozoonosis in a crossbreed dog-first report from Mizoram, India

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**Abstract**
The present case study depicts first report of concomitant canine parvoviral gastroenteritis and hepatozoonosis in a crossbreed dog from Mizoram, India. A crossbreed female dog was presented with the complaints of anorexia, lethargy, vomiting and bloody diarrhoea. The physical and clinical examination revealed fever, tachycardia, tachypnoea, slightly pale mucous membranes, dehydration, and enlargement of popliteal and sub-mandibular lymph nodes. Haemato-biochemical examination showed anaemia, thrombocytopenia, leukocytosis with neutrophilia, eosinophilia, hypoproteinaemia, hypoalbuminemia, hypoglycaemia, and elevated liver-specific enzymes. The faecal and blood samples were found positive for canine parvovirus type 2 (CPV-2) and *Hepatozoon* spp., respectively. The animal was treated with doxycycline and supportive therapy for 28 days. An uneventful recovery was noticed after the completion of treatment.

**Keywords:** Anaemia, CPV-2, *Hepatozoon* spp., doxycycline

## 1. Introduction
Canine parvovirus (CPV) infection is one of the most prevalent enteric viral infections in domestic dogs, which causes acute gastroenteritis with a high global morbidity and fatality rate (Thakur *et al*., 2020; Chethan *et al.*, 2022) [24, 6]. CPV, a *Carnivore Protoparvovirus 1*, is a non-enveloped single-stranded DNA virus that belongs to the *Protoparvovirus* genus under the subfamily *Parvovirinae* of the family *Parvoviridae* (Canuti *et al*., 2021) [4]. Even with widespread vaccination, young pups may still be at serious risk from canine parvovirus type 2 (CPV-2) (Decaro *et al.*, 2020) [9]. Canine parvoviral gastroenteritis (CPVGE) is mainly seen in puppies upto six months of age, and significant clinical symptoms include anorexia, lethargy, depression, fever/sub-normal body temperature, vomiting and foul smelling bloody diarrhoea (Chethan *et al*., 2016) [8]. Several infections (bacterial, viral, or parasitic) are often observed during the course of CPVGE, and frequently exacerbated by the immunosuppressive condition brought on by CPV-2, deteriorating the overall state of the patient (Tuteja *et al.*, 2022) [25].
*Hepatozoon* is an apicomplexan vector-borne protozoan parasite in the family *Hepatozoidae*. It primarily affects mammalian leukocytes. Canine hepatozoonosis is mainly transmitted by ingestion of a brown dog tick (*Rhipicephalus sanguineus*) belonging to the family *Ixodidae* (Shalini *et al.*, 2023) [20]. Symptoms of the infection are typically pre-clinical and obvious, including anaemia and lethargy (Spolidorio *et al.*, 2009) [21]. Unless there is concomitant infection with other pathogens, sub-clinical infection may go undetected. The severity of hepatozoonosis can range from sub-clinical in apparently healthy dogs to severe and potentially fatal outcomes in canine patients that exhibit extreme anaemia, cachexia, and lethargy (Baneth, 2011) [1]. Microscopically, *Hepatozoon* gamonts are found in neutrophils and macrophages. However, molecular techniques like polymerase chain reaction (PCR) are found to be highly sensitive (Thakur *et al*., 2018) [23]. Earlier case reports have documented combined infection of CPV-2 with vector-borne diseases like babesiosis and ehrlichiosis (Bhanuprakash

*et al.*, 2015; Chethan *et al*., 2016; Thakur *et al.*, 2020) [2, 8, 24]. The present report describes the first study of concomitant canine parvoviral gastroenteritis and hepatozoonosis in a crossbreed dog from Mizoram, India, and its therapeutic management.

## 2. Case History and Diagnosis

A six month old crossbreed female dog weighing 5.8 Kg was reported to the Veterinary Clinical Complex (VCC), College of Veterinary Sciences and Animal Husbandry (CVSc & AH), Central Agricultural University (CAU), Selesih, Aizawl, Mizoram with the chief complaints of anorexia, lethargy, vomiting and bloody diarrhoea. Physical and clinical examination revealed an increase in body temperature, tachycardia, tachypnoea, slightly pale conjunctival and oral mucous membranes, dehydration, and popliteal and sub-mandibular lymphadenopathy. The haematological examination results revealed anaemia, thrombocytopenia, leukocytosis with neutrophilia and eosinophilia (Table 1). Analysis of serum biochemical parameters revealed hypoproteinaemia, hypoalbuminemia, hypoglycaemia and elevated liver-specific enzymes [alanine aminotransferase (ALT) and alkaline phosphatase (ALP)] activity.

**Table 1:** Hematologic and serum biochemical parameters on day 0 and day 28

| Parameters | 0 day | 28 day | Reference Range | Key Findings |
|---|---|---|---|---|
| **Haematological Parameters*** | | | | |
| TEC (× $10^6$ cells/µL) | 4.02 | 5.35 | 5.0-7.9 | |
| Hb (g/dL) | 8.78 | 12.26 | 12.0-19.0 | Anaemia |
| PCV (%) | 24.63 | 37.89 | 35.0-57.0 | |
| TLC (× $10^3$ cells/µL) | 23.46 | 13.98 | 5.0-14.1 | Leukocytosis |
| Neutrophil count (%) | 88.00 | 67.00 | 58.0-85.0 | Neutrophilia |
| Lymphocyte count (%) | 8.0 | 21.00 | 8.0-21.0 | |
| Monocyte count (%) | 2.0 | 2.0 | 2.0-10.0 | |
| Basophil count (%) | 0.0 | 0.0 | 0-1 | |
| Eosinophil count (%) | 2.0 | 2.0 | 0-9 | Eosinophilia |
| Total platelet count (%) | 1.71 | 2.28 | 2.11-6.21 | Thrombocytopenia |
| **Serum Biochemical Parameters#** | | | | |
| Total protein (g/dL) | 5.2 | 6.5 | 5.4-7.5 | Hypoproteinemia |
| Albumin (g/dL) | 2.0 | 2.9 | 2.3-3.1 | Hypoalbuminemia |
| Total bilirubin (mg/dL) | 0.27 | 0.12 | 0-0.3 | |
| Direct bilirubin (mg/dL) | 0.19 | 0.07 | 0-0.2 | |
| Indirect bilirubin (mg/dL) | 0.08 | 0.05 | 0-0.1 | |
| ALT (U/L) | 182.00 | 42.00 | 10.0-109.0 | Elevated liver enzymes |
| AST (U/L) | 16.00 | 17.00 | 13.0-15.0 | |
| ALP (U/L) | 185.00 | 95.00 | 1.0-114.0 | |
| BUN (mg/dL) | 27.00 | 18.00 | 8.0-28.0 | |
| Creatinine (mg/dL) | 1.30 | 1.25 | 0.5-1.7 | |
| Random blood glucose (mg/dL) | 52.00 | 96.00 | 76.0-119.0 | Hypoglycaemia |

*March 2012: Haematology reference ranges, 10th edn. The Merck Veterinary Manual

#March 2012: Serum biochemical reference ranges, 10th edn. The Merck Veterinary Manual

Upon microscopic examination of the faecal sample, no parasite ova or oocysts were found. Rapid immunochromatographic antigen test (PetX™ Canine Parvo Virus Ag Rapid Test, J&G Biotech Ltd, United Kingdom) was performed by using the faecal sample and showed positive result for CPV-2 infection (Fig. 1).

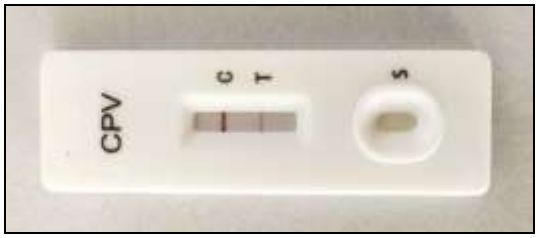
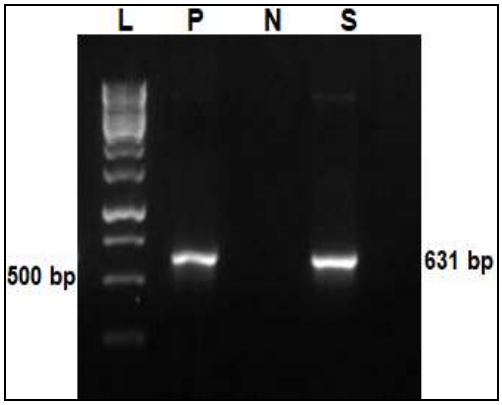



**Fig 1:** Confirmation of CPV infection by using rapid CPV Ag test kit (Positive sample-Presence of both test (T) line and control (C) line in the result window)

For further confirmation, the faecal sample was subjected to PCR for the identification of CPV-2 by using diagnostic primers (FP-TGATTGTAAACCATGTAGACTA and RPTAAGTCAGTATCAAATTCTTTATC) targeting VP2 gene (Chander *et al.*, 2016) [5]. The presence of expected 631 bp result in agarose gel under UV transillumination confirmed CPV-2 infection (Fig. 2).




Lane L: 250 bp DNA ladder
Lane P: Positive control
Lane N: Negative Control
Lanes S: Sample (631 bp)

**Fig 2:** 1.5% agarose gel electrophoresis of PCR amplified products for the identification of CPV-2

The dog was treated with symptomatic and supportive therapy but had persistent high body temperature. A peripheral blood smear was prepared to rule out haemoparasitic diseases. Microscopic examination of the peripheral blood smear stained with Giemsa revealed the presence of *Hepatozoon* spp. gamonts in the neutrophils (Fig. 3) indicating concomitant canine parvoviral gastroenteritis and hepatozoonosis.

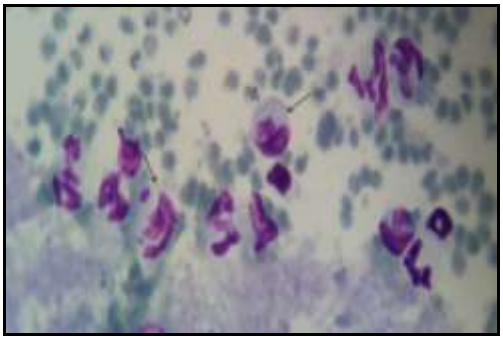


**Fig 3:** Demonstration of *Hepatozoon* spp. gamonts in neutrophils by using Giemsa stain (× 1000)

## 3. Treatment and Discussion

The dog was initially treated by using intravenous fluids (normal saline, Ringer's lactate), ceftriaxone-tazobactam (Intacef-Tazo™, Intas) at 25 mg/kg b.wt. (IV, BID), meloxicam (Melonex™, Intas) at 0.2 mg/kg b.wt. (IM, BID), ondansetron (Emigo™, Zuventus) at 0.2 mg/kg b.wt. (IV, BID), pantoprazole (Pantop™, Aristo) at 1 mg/kg b.wt. (IV, OD) and ethamsylate (Hemsyl™, Indoco Remedies Ltd.) at 5 mg/kg b.wt. (IV, BID) for 3 days. Oral electrolytes, and pre and probiotics supplementation (GuttyPet™, SkyEC) were also provided. After the confirmation of hepatozoonosis, doxycycline injection (DOXYPET MONO™, SavaVet) at 5 mg/kg b.wt. (slow IV, OD) was given for 3 days. Once the animal was stabilized, it was kept on doxycycline (Doxypet™, SavaVet) at 5 mg/kg b.wt. (PO, BID) and pantoprazole (Pantop™, Aristo) at 1 mg/kg b.wt. (PO, OD) for another 25 days. Supportive medications were given which included hepatoprotective silymarin (Hepamust™, Pet Mankind) at 1 tsf/10 kg b.wt., PO, BID; haematinic (Sharkoferrol Pet™, Alembic) at 1 tsf/10 kg b.wt., PO, BID for 4 weeks. The dog responded well to treatment and made a full recovery after four weeks of treatment. After the complete treatment, haemato-biochemical values were found to be within the normal reference range and peripheral blood smear analysis was found to be negative for any haemoparasite.

CPVGE is an acute, contagious and infectious viral disease which mostly affects young, unvaccinated puppies between 6-20 weeks of age (Eregowda *et al*., 2020) [10]. The virus causes the body's defensive mechanism to be rendered ineffective by destroying immature immune system cells, as it multiplies in proliferating cells like bone marrow (Nandi and Kumar, 2010) [17]. One significant haemoprotozoan disease that affects dogs worldwide is canine hepatozoonosis, which is caused by *Hepatozoon* spp. (Ewing and Panciera, 2003) [11]. It is usual for *Hepatozoon* spp. to co-exist with other infections. Earlier reports documented co-infections with *Leptospira interrogans* icterohaemorrhagiae, *Anaplasma phagocytophilum, Ehrlichia canis, Babesia vogeli, Babesia canis* and CPV-2 (Gal *et al.*, 2007; Götsch *et al.*, 2009; Thakur *et al.*, 2019; Urbani *et al.*, 2022) [12, 15, 23, 26] . Age related acute hepatozoonosis has been documented in young dogs, even though the disease usually manifests in a subclinical form. In general, canine hepatozoonosis is thought to be a moderate infection; nevertheless, in young animals, the immune system being immune-deficient, might cause an acute fatality in puppies (Thakur *et al*., 2018) [23]. Concurrent infections can compromise the host immune system and lead to exacerbated clinical signs of hepatozoonosis (Otranto *et al.*, 2011) [18].

In the present case, anaemia and thrombocytopenia were observed in the infected dog. This could be due to bone marrow suppression inflicted by hepatozoonosis (Thakur *et al.*, 2018) [22]. In addition, the anaemia and thrombocytopenia in our case could be justified by the fact that CPV-2 infection causes gastrointestinal bleeding, and depletion of hematopoietic cell lines as a result of the virus's action on the bone marrow (Urbani *et al.*, 2022) [26]. Leukocytosis with neutrophilia, hypoproteinemia, hypoalbuminemia, and elevated liver enzymes such as ALT and ALP could be due to inflammatory response associated with hepatozoonosis (Thakur *et al.*, 2018; Shalini *et al*., 2023) [22, 20]. Liver is the primary organ attacked by canine hepatozoonosis which can lead to hypoproteinemia, hypoalbuminemia and an increase in hepatic enzyme activity, as a result of increased hepatocyte damage (Thakur *et al.*, 2018) [22]. In CPV-2 infection, hypoproteinemia and hypoalbuminemia are likely to be caused by a combination of severe protein loss enteropathy through damaged intestinal villi capillaries, malabsorption, and inappetence/anorexia (Bhat *et al.*, 2013) [3]. Hypoglycaemia is considered a severe complication of CPVGE owing to lesser glycogen stores in juvenile patients, Oral intake is required to maintain euglycemia but it is compromised in CPVGE due to inappetence/anorexia, vomition and diarrhoea (Mylonakis *et al.*, 2016; Sarpong *et al.*, 2017) [19, 20]. Sepsis, hypermetabolism and liver dysfunction are also attributed as potential reasons for hypoglycemia (Goddard and Leisewitz, 2010) [14]. Due to the non-availability of effective antiviral agents, the core treatment criteria for CPVGE is based on symptomatic and supportive therapy targeting correction of fluid and electrolyte imbalance, prevention of secondary bacterial infection and palliation of symptoms (Chethan *et al.*, 2023) [7]. Survival rates can be significantly improved by intensive supportive care (Gaykwad *et al.*, 2018; Chethan *et al.*, 2023) [13, 7]. Gut acting antimicrobials are commonly used to prevent secondary bacterial infections. The present case was treated with doxycycline for the management of hepatozoonosis. Doxycycline is considered the first line of treatment for canine hepatozoonosis (Thakur *et al.*, 2018) [22]. Supportive therapy with poly-ionic electrolytes, antiemetic, antacid, haemostat, hepato-protectant and haematinic hastened the recovery process.

## 4. Conclusion

The present report describes a first study of concomitant canine parvoviral gastroenteritis with hepatozoonosis in a crossbreed dog from Mizoram, India, and its successful therapeutic management with doxycycline, and symptomatic and supportive therapy.

## 5. Acknowledgement

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## 6. Conflicts of Interest

The authors declare no conflicts of interest.

## 7. Financial Support

Not available

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