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Research Article

COMPLETE CASE STUDY OF *HEPATOZOON CANIS*: CLINICAL, HAEMATO-BIOCHEMICAL, MOLECULAR ANALYSIS AND THERAPEUTIC MANAGEMENT

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ABSTRACT

A three-year-old GSD dog arrived at the Veterinary Clinical Complex, Veterinary College, Junagadh, with a history of malnutrition, a significant tick infestation and severe thigh pain. Physical exams showed that the animal was hyperthermic (104.5°F) and mildly dehydrated. During the examination, a blood sample was taken for hematology, microscopic analysis and PCR for diagnosis of hemoprotozoan infection and tick samples were taken for morphological identification. Additionally, a serum sample was also collected for biochemical analysis. When a thin blood smear was made and stained with Giemsa stain, it revealed that the neutrophil contained a gamont of *Hepatozoon canis*. Using morphological traits, ticks were recognized as female *Rhipicephalus sanguineus*. Haematological investigation shows neutrophilia, lymphocytopenia, thrombocytopenia, and severe anemia. Biochemical investigation showed an elevated AST/SGOT and AKP value and a low level of total protein, hypoalbuminemia. Whole blood PCR analysis revealed amplification at 666 bp for *H. canis* infection, but all other hemoparasites, including *B. vogeli*, *B. gibsoni*, *T. evansi* and *E. canis* were negative. Following the injection of doxycycline and imidocarb dipropionate, the dog's clinical condition was improved. Drugs were administered intramuscularly (IM) at a dose of 5-6 mg/kg; imidocarb dipropionate is repeated every 14 days. Doxycycline is frequently recommended in combination with imidocarb because of its broad-spectrum antibacterial qualities and capacity to lower parasitemia. This combined therapy lowers the organism burden, enhances health and manages the clinical indications of hepatozoonosis.

Keywords: Anemia, Haemato-biochemical, *Hepatozoon canis*, PCR, *Rhipicephalus sanguineus*

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Canine hepatozoonosis is an important tick-borne and widely prevalent disease of dogs caused by *H. canis*, and transmission of *H. canis* to dogs occurs by ingestion of an infected tick, *Rhipicephalus sanguineus* (Baneth *et al.*, 2001). The brown dog tick, *R. sanguineus*, is a globally prevalent ectoparasite, particularly common in tropical and subtropical regions. In India, this tick species is widespread and poses significant concerns for canine health. In Gujarat, research highlights a significant prevalence of *R. sanguineus* among the canine population. A study conducted in the middle zone of Gujarat found that 58.11% of dogs harbored clinical tick infestations, with *R. sanguineus* being the predominant species (Raval *et al.*, 2014). These findings underscore the tick's widespread presence in Gujarat, necessitating regular monitoring and control measures to safeguard canine health. Ingestion of infected ticks or tick parts containing mature oocysts with infectious sporozoites through the oral route is the main cause of *H. canis* infection (Aktas *et al.*, 2013). Dog leucocytes are impacted by this internal parasite, which resembles malaria. In peripheral blood smears, leucocytes harboring *H. canis* gamonts are typically observed in neutrophils (Hendrix, 2006; Dhankar *et al.*, 2011).

The widespread presence of *R. sanguineus* in India's tropical and subtropical regions facilitates the maintenance and transmission of *H. canis* among the canine population (Agnihotri *et al.*, 2012). While *H. canis* infections in India are typically subclinical or mild, dogs may exhibit clinical signs such as intermittent fever, anemia, lethargy and weight loss in cases with heavy parasite loads or concurrent infections. The immune response to the parasite involves inflammation and granuloma formation in affected tissues, which can contribute to pathology. PCR analysis for confirmation and blood smear analysis for gamont detection are essential for an accurate diagnosis. Implementing suitable control methods to lessen the burden of this disease on dogs requires an understanding of the occurrence, transmission and pathophysiology of *H. canis* in India (Singh *et al.*, 2013; Gondal & Kaur, 2014). In this study, a dog infected with *H. canis* was conventionally and molecularly diagnosed along with haemato-biochemical analysis. Additionally, control measures were also explained.

MATERIALS AND METHODS

A three-year-old, female German shepherd breed dog was brought to the Veterinary Clinical Complex, Veterinary College, Junagadh during the month of May 2025 with the history of high fever, tick infestation, having pain at thigh region and anorexia. Clinical examination of the dog revealed that pyrexia (104.5 °F), pale conjuctival and oral mucous membranes. The animal was suspected

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for the haemoprotozoan infection based on the clinical examination. A thin peripheral blood smear, whole blood, serum sample and ticks were received to the clinical diagnostic laboratory for the parasitological examination. During collection, the animal was thoroughly examined, physically and clinically and details such as age, breed, sex and history were recorded. Before sample collection, owner consent was taken. Standard collection procedure was followed for the collection of blood sample from animals following bio-safety measures. Blood sample was collected from the dog in EDTA coated vaccutainer & serum vaccutainer. Animals were thoroughly examined for tick infestations, illness and clinical signs and then ticks were also collected in vial for identification and sent to Department of Veterinary Parasitology for identification.

RESULTS AND DISCUSSION

Microscopic Examination

The thin blood smear was methanol-fixed, stained with 1:20 diluted Giemsa for 40-45 minutes, washed, dried and examined under oil immersion. Microscopy examination revealed rectangular, capsule-like bodies within neutrophils, indicating the infection (Soulsby, 1982) as shown in Figs. 1. & 2. Canine hepatozoonosis is a disease that is currently very common in India. The infection is known to be caused by the species *H. canis* (Palanivel *et al.*, 2010). In this case, the haemato-biochemical alterations and molecular detection of other haemoparasites were detected. The results indicate that *H. canis* is responsible for causing pathogenicity. The variations observed in the haemogram and leukogram parameters as well as in serum biochemical parameters of the infected dog may be related to parasitaemia and response of animal.

Tick Identification

Tick samples were also collected from infected dog. Collected ticks were identified under stereo-zoom microscope based on morphology using standard identification key (Walker *et al.*, 2003) at department of Veterinary Parasitology, Veterinary College, Kamdhenu University, Junagadh as shown in Figs. 3 & 4.

Haemato-biochemical analysis

Haematological analysis of the blood sample was done by using Automatic Whole Blood Analyzer (Mindray BC-2800 Vet) and biochemical analysis of the serum samples was done by Serum Auto-analyzer (MicroLab-300) with commercial kit (Randox, UK) at the College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh. haematological investigation shows

Table 1. Key points for identification of tick at genus level.

Sr. No.	Major tick genera of dog	Main identification points
1.	*Rhipicephalus* (*Boophilus)*	Bravirostrate, Festoon absent, Coxae 1 have small paired spurs.
2.	*Rhipicephalus sanguineus*	Bravirostrate, eye present, Hexagonal basis capitulum, Festoon present.
3.	*Haemaphysalis*	Bravirostrate, Eye absent, Ventral plates are absent, Festoon present.

Table 2. Haematological finding of the present case.

Sr. No.	Parameters	Result of positive case	Reference Value
1.	Hb (g/dl)	3.0	10-16
2.	RBCs $\times 10^6$	2.0	05-08
3.	WBCs	13400	5500-19500
4.	Neutrophils (%)	85	35-70
5.	Eosinophils (%)	00	0-1.0
6.	Basophils (%)	00	0-1.0
7.	Lymphocytes (%)	17	20-55
8.	Monocytes (%)	07	1.0-04
9.	Platelates $\times 10^5$	01	03-07
10.	PCV (%)	14.0	30-50
11.	MCV (fl)	70.0	55-75
12.	MCH (pg)	19.0	19-24
13.	MCHC (g/dl)	27.0	30-36

Table 3. Result of serum biochemistry of the present case.

Sr. No.	Parameters	Result of positive case	Reference Value
1.	ALT/SGPT	58.28	25-92
2.	AST/SGOT	124.34	10-52
3.	AKP	103.4	10-94
4.	Total protein	4.42	5.0-7.0
5.	Albumin	0.91	2.5-4.0
6.	Urea nitrogen (mg/dl)	35.68	8.0-25
7.	Cholesterol (mg/dl)	97	110-250

neutrophilia, lymphocytopenia, thrombocytopenia and severe anemia. The severity of the infection may have contributed to the dog's anemia in this investigation. According to several experts, the most prevalent and primary haematological indication seen in the majority of cases is anemia (Baneth, 2006). The main findings of this study was anemia. This could be the result of a persistent blood loss-induced iron shortage. Neutrophilia, lymphocytopenia, and thrombocytopenia were found in the study, which is in line with the results of other researchers who regularly documented these alterations (Voyoda *et al.*, 2004). These changes could result from the parasite's multiplication in the animal's organs, which

Table 4. PCR primers used for detection of haemoparasites of dogs in Gujarat

Sr.No.	Name of Parasite	Primer information (5′ → 3′)	Expected amplicon size	Gene	Reference
1.	*B. vogeli*	5' gtgaaccttatcacttaaagg3' 5' caactcctccacgcaatcg3'	610 bp	18S *rRNA*	Kumar *et al.*, 2022
2.	*B. gibsoni*	5' aagccaacatcaaggaaagc3' 5' ttctggtatgcggcagtgta3'	679 bp	*TRAP*	
3.	*T. evansi*	5' gcacaaatgccgacggta 3' 5' gtcgttgccggttattgct 3'	200 bp	RoTat1.2	Kumar *et al.*, 2021
4.	*H. canis*	5' atacatgagcaaaatctcaac 3' 5' cttattattccatgctgcag 3'	666 bp	18S *rRNA*	Kumar *et al.*, 2018
5.	*E. canis*	5' caattatttatagcctctggctctggctatagga3' 5' tataggtaccgtcattatcttccctat'	389 bp	16S *rRNA*	Murphy *et al.*, 1998

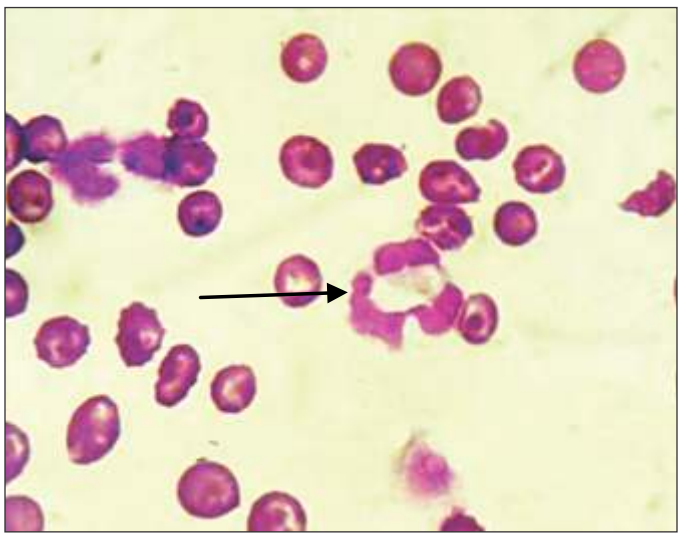

Fig. 1. *H. canis* gamonts in neutrophils (100X)

Fig. 2. Clinical presentation of GSD dog

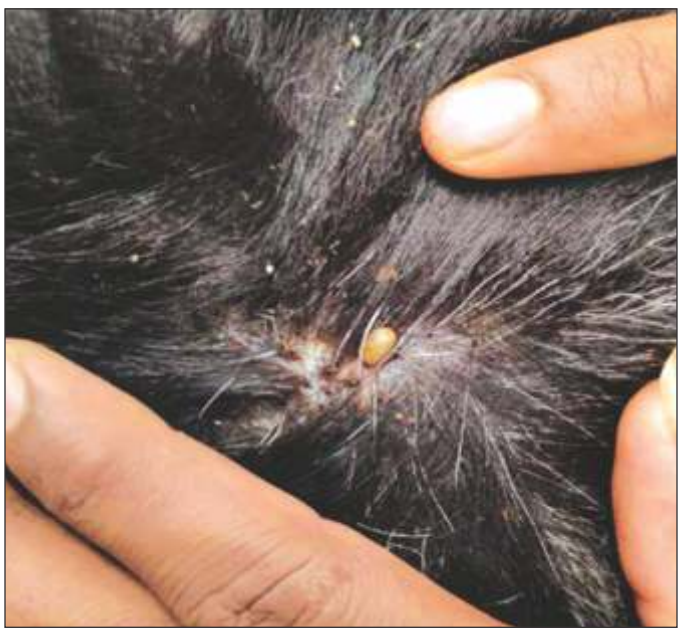

Fig. 3. Infestation of ticks on body coat

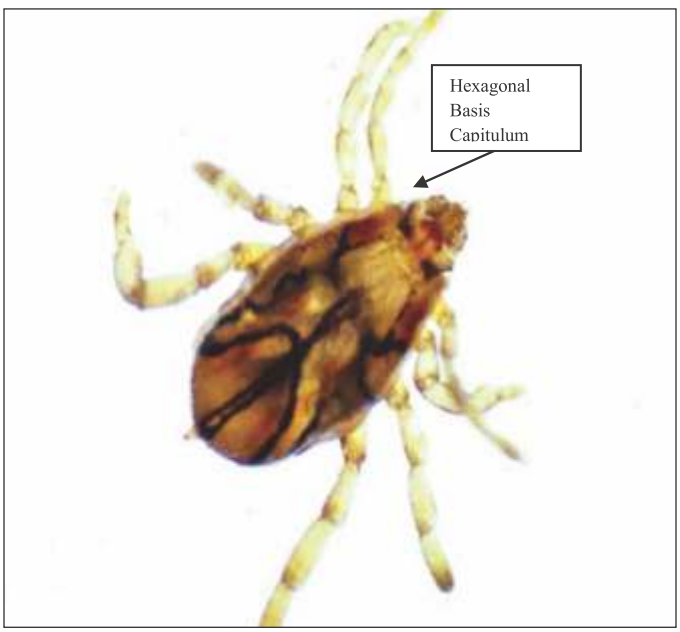

Fig. 4. Rhipicephalus sanguineus tick

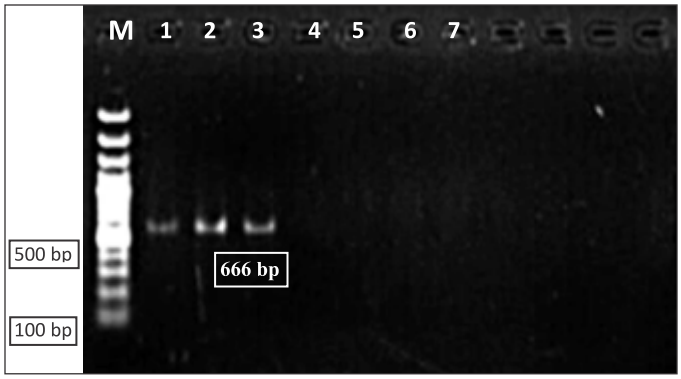

Fig. 5. Amplification of 18S *rRNA* of *H. canis* (666 bp) in dog
M= Marker 100 bp; L1 & L2= Positive control; L3= Sample positive for *H. canis* (666 bp); L4 to L7= Negative for other haemoparasites (*B. vogeli*, *B. gibsoni*, *T. evansi* & *E. canis*)

triggers an inflammatory reaction that could be made worse by the existence of other bacterial diseases. Necrosis and inflammation in the spleen, lymph nodes, liver and lungs are probably the cause of the neutrophilia and anemia (Roopali *et al.*, 2017). Thrombocytopenia was also detected throughout this investigation. While the precise cause of *H. canis*-related thrombocytopenia is uncertain, it may be connected to general thrombocytopenia causes or concomitant infections (Pasa *et al.*, 2009) & it might be also because *H. canis* gamonts cause bone marrow suppression, which could result in decreased platelet production.

Biochemical investigation showed an elevated AST/SGOT & AKP value and a low level of total protein, hypoalbuminemia. Elevated AST/SGOT and AKP values, as well as a low level of total protein and hypoalbuminemia, were the only changes identified by serum biochemistry in this sick dog; however, this could be explained by the dogs' severe tick infestation. Increased osteoblastic activity, hepatic lesions, or cholestasis are all suggested by elevated liver enzymes. Elevated serum AKP activity has been reported in dogs with *H. canis* because of increased osteoblastic activity (Baneth *et al.*,1997). Elevated AST values in the current study show liver & kidney function impairment linked to *H. canis* merogony in different organs. This study found hypoalbuminemia and decreased total protein levels, which have been previously described by Singh *et al.* (2017) and Gavazza *et al.* (2003). These findings could be caused by lower protein intake, chronic inflammation, or protein-losing nephropathy. Lower protein levels in afflicted dogs could have been caused by renal failure (Pasa *et al.*, 2009).

Molecular analysis

Whole blood genomic DNA was extracted from 200 µL blood sample using commercially available Blood DNA isolation kit (Thermo Scientific, Lithuania; Quigen, Germany) as per the manufacturer's protocol. To rule out the common haemoparasitic infection in dogs, in-house standardized PCR assays was used for detection of *B. vogeli*, *B. gibsoni*, *T. evansi*, *H. canis* & *E. canis*. The PCR reaction was conducted at optimized conditions in Thermal Cycler (Applied BioSystem, USA). The PCR products were resolved in 1% agarose gel and amplicons were visualized under UV light. Genomic DNA isolated from blood sample found microscopically positive for *Hepatozoon* gamont in WBCs of dog was used as positive control. Species specific PCR primers were synthesized for molecular identification of different haemoparasites of dogs (Table 4). In PCR, the sample was positive only for *Hepatozoon canis* (666 bp), while all other haemoparasites tested negative on agarose gel. Thus, the sample was confirmed as a single infection of hepatozoonosis. (Fig. 5). According to Abd Rani *et al.* (2011), the most prevalent canine TBD pathogen infecting dogs in India was *H. canis*, which was followed by *E. canis*, *M. haemocanis* and *A. platys*. The most prevalent tick species in this study was *Rhipicephalus*, which was followed by *Haemaphysalis*. The first study on the molecular detection of *H. canis* in spontaneously infected dogs from Kerala was published by Lakshmanan *et al.* (2018).

Therapeutic Management

Based on microscopic and molecular findings, the dog was diagnosed with *Hepatozoon canis* infection. The appropriate treatment was given as a combination therapy, including a single dose of Imidocarb dipropionate at 5-6 mg/kg intramuscularly and Doxycycline at 5 mg/kg orally for 28 days along with supportive therapy such as fluids, antiemetics and hematinics. Hepatozoonosis is usually chronic, with low-grade parasitemia. Short courses of drugs are ineffective; prolonged therapy is needed to reduce parasite load. Moreover, Imidocarb dipropionate may not be effective or well tolerated in all cases. Hence, Doxycycline (5 mg/kg PO BID for 28 days) is commonly used in field conditions, especially in endemic areas. Additionally, to control ticks, Protector Spot On (Fibronil) was administered topically. Following a four-week course of medication, the dog's condition significantly improved. Once more, an examination of the peripheral blood smear under a microscope revealed no *H. canis*. According to studies by Vijayasarathi *et al.* (2024) and Thakur *et al.* (2018), imidocarb dipropionate and doxycycline combined therapy is effective in treating canine *H. canis* infections.

CONCLUSION

In order to prevent drug resistance today, a thorough case study with a confirmative diagnosis is required. In

order to treat dogs with a confirmed positive case of *H. canis* during their acute stage and stop additional losses, early diagnosis is essential. Additionally, a haemato-biochemical test can aid in the disease's diagnosis. Since microscopic diagnosis might be ambiguous, PCR serves as an excellent tool for both preclinical and confirmative diagnosis for any haemoparasitic condition. The treatment of dogs infected with *H. canis* may benefit from the combination of supportive care and imidocarb dipropionate and doxycycline.

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