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Clinical Article

MOLECULAR DIAGNOSIS AND THERAPEUTIC MANAGEMENT OF *BABESIA GIBSONI* INFECTION IN ROTTWEILER: A CASE STUDY

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SUMMARY

A five years old male Rottweiler dog, exhibiting clinical signs including anorexia, weakness, depression, mild fever, and pale mucous membranes, was presented to the Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Jabalpur. As the clinical sings of the ailing dog was suggestive of haemoprotozoan infection, a blood sample was collected for parasitological and haematological examinations. Microscopic examination of thin blood smear stained with Giemsa stain revealed the presence of small form of *Babesia* species. Haematological analysis revealed a significant reduction in haemoglobin (Hb), packed cell volume (PCV) and total erythrocyte count (TEC). The blood sample was further subjected to polymerase chain reaction (PCR) targeting 619 bp fragment of the 18s rRNA gene, confirmed the presence of *Babesia gibsoni*. The dog was successfully treated with a combination of diminazene aceturate at the recommended dose and antibiotics along with supportive medications and recovered to normal as the clinical signs disappeared and the absence of piroplasm in post-treatment examination of the stained thin blood smear. As no published reports were available, this investigation accounts for the first molecular confirmation of *B. gibsoni* in Madhya Pradesh, India.

Keywords: *Babesia gibsoni*, Dog, Molecular detection, Therapeutic management, Madhya Pradesh

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Canine babesiosis, a clinically significant haemoprotozoan disease of dogs, is caused by intra-erythrocytic protozoa belonging to the genus *Babesia* transmitted by Ixodid ticks (Karasova *et al.*, 2022). In India, two documented species of *Babesia* in dogs are *B. canis vogeli* and *B. gibsoni*, recognized as large and small form, respectively (Sarma *et al.*, 2019). In particular, *B. gibsoni* exhibits higher pathogenicity than *B. canis vogeli*, primarily presenting in chronic forms of infection and causing severe illnesses with the potential fatality in affected animals (Liu *et al.*, 2022). Clinical manifestations of the ailment encompass a wide spectrum of clinical symptoms, including general weakness, pale mucus membranes, fever, jaundice, splenomegaly, thrombocytopenia and lymphadenopathy (Karasova *et al.*, 2022). The management of *B. gibsoni* infections poses substantial challenges, including treatment effectiveness varying from case to case. The commonly employed medications include imidocarb dipropionate, diminazene aceturate, doxycycline, clindamycin and atovaquone (Baneth, 2018). However, the choice and combination of medications depend on individual cases, the severity of the infection, and the response to treatment (Karasova *et al.*, 2022). Due to the complexity in managing *B. gibsoni* infections, early and sensitive detection is crucial for effective control.

In December 2022, a five years old male Rottweiler dog was presented to the Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Jabalpur (M.P.) with the history of lethargy and anorexia over the past three weeks, accompanied by profuse vomiting in the last two days. Upon clinical examination, the dog appeared dull and depressed with pale mucous membranes. The dog was passing dark yellow urine and had slightly increased respiration and temperature (102.3°F) along with lymph node swelling and tick infestation. As the clinical sings of the ailing dog were suggestive of haemoprotozoan infection, a blood sample was collected aseptically for parasitological and haematological examinations. Thin blood smear was prepared, stained using routine Giemsa staining procedure, and subsequently examined under a microscope. Haematological parameters, comprising Hb, PCV, TEC and total leukocyte count (TLC), were quantified using a semi-auto haematology analyzer (MODELABCUS).

For molecular detection, genomic DNA was extracted from this sample using the DNeasy Blood and Tissue Kit (Qiagen, Germany) as per the manufacturer's protocol. The extracted DNA was subjected to PCR for amplification of a 619 bp fragment of 18s rRNA gene using primers Ba103F (5'-CCAATCCTGACACAGGGA GGTAGTGACA-3') and Ba721R (5'-CCCCAGA ACCCAAAGACTTTGATTTCTCTCAAG-3') as per Brahma *et al.* (2019). The reaction mixture consisted of 12.5 µl of Dream Taq Green PCR Master Mix (2X) (Fermentas), 1.0 µl of each primer (10 µM), 3.0 µl of

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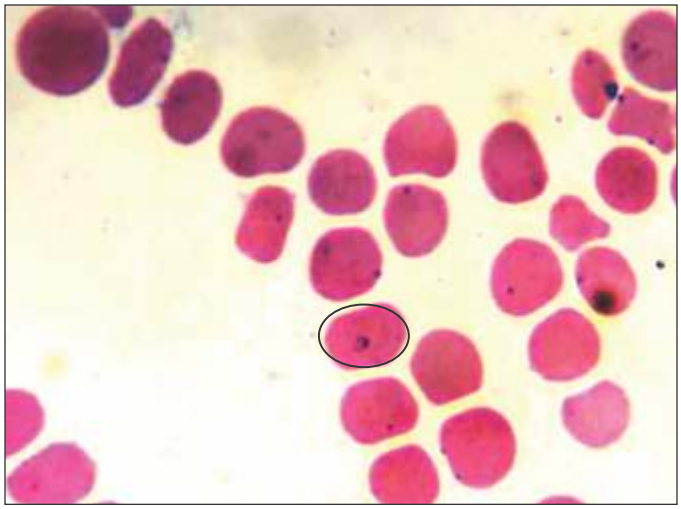

Fig. 1. Giemsa-stained thin blood smear illustrating *B. gibsoni* (red circle) infection in erythrocytes (100X)

template DNA (15 ng) and nuclease-free water up to 25 µL. The PCR was performed using thermal cycler (Applied Biosystems Veriti 96 well thermal cycler, Thermo Fisher USA) with the following cycling conditions: an initial denaturation at 94° C for 10 min, followed by 40 cycles of denaturation at 94° C for 30 s, annealing at 61° C for 45 s, elongation at 72° C for 45 s, and a final elongation at 72° C for 10 min. The amplicon was seen on 1.5% agarose gel containing ethidium bromide.

Examination of the stained thin blood smear revealed the presence of small, ring-shaped organisms within the erythrocytes (Fig. 1) which were recognized as *Babesia gibsoni*. Concurrently, alterations in haematological parameters were noted in the affected animal, as mentioned in Table 1. Subsequent to the extraction of DNA from the blood sample, conventional PCR targeting the 18s rRNA gene was employed for the detection of *B. gibsoni*, yielding a 619 bp DNA fragment (Fig. 2).

Based on the clinical manifestations and laboratory findings, the disease was diagnosed as canine babesiosis caused by *B. gibsoni*. Treatment commenced with an injection of Diminazene aceturate @ 5mg/kg b.wt. deep intramuscularly, repeated on the 3rd day. Antibiotics therapy included oxytetracycline @ 10 mg/kg b.wt. and metronidazole @ 15 mg/kg b.wt. intravenously twice daily for 5 days. Supportive therapy included dextrose normal saline @ 120 ml, ringer's Lactate @ 100 ml intravenously twice a day, ondansetron @ 0.5 mg/kg b.wt., omeprazole @ 0.5 mg/kg b.wt. intravenously daily and meloxicam @ 0.5 mg/kg b.wt. intramuscularly daily for 5 days, along with a single iron dextran @ 1.5 mg/kg b.wt. intramuscularly on day one. The dog responded to therapy and the owner was advised for oral administration of doxycycline @ 5 mg/kg b.wt. and metronidazole @ 15 mg/kg b.wt. twice a day,

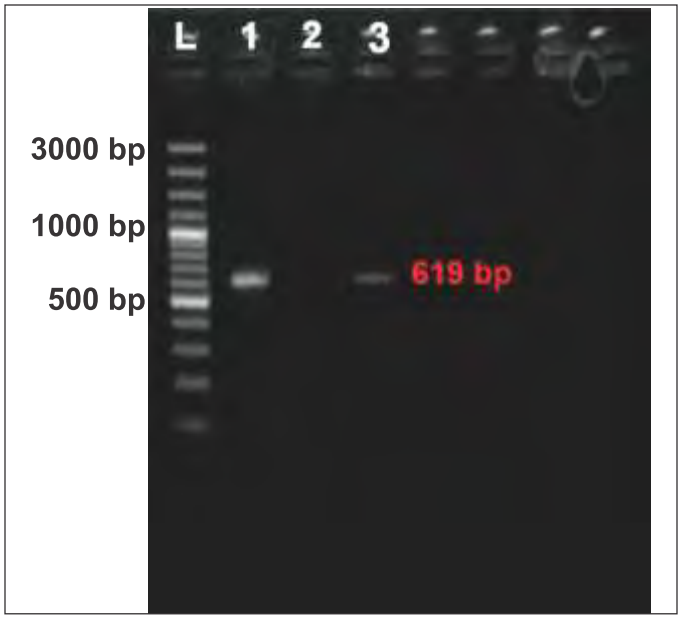

Fig. 2. Agarose gel electrophoresis (1.5%) revealing 619 bp fragment from the 18s rRNA gene of *B. gibsoni*. Lane assignments: L-100 bp DNA ladder, 1-positive sample, 2-negative control and 3-positive control

omeprazole @ 0.5 mg/kg b.wt once a day and oral haematinics (Syrup Sharkoferrol) @ 1 teaspoon twice a day for 21 days. After 21 days, the dog recovered completely as the clinical symptoms disappeared and no piroplasms were detected in stained thin blood smear examinations.

In this study, *B. gibsoni* was identified using both microscopic examination and PCR techniques. The presence of *B. gibsoni* infection has been extensively documented across India, as evidenced by prior studies (Abd Rani *et al.*, 2011; Sarma *et al.*, 2019; Chauhan *et al.*, 2022; Patel *et al.*, 2023) underscores its endemic nature. Despite microscopic examination being the gold standard for diagnosis, its reliance on technical expertise presents challenges, especially in identifying the chronic or subclinical forms of infections, which are more prevalent and pose additional complexities (Karasova *et al.*, 2022). Moreover, the potential for undiagnosed multiple infections in the same host further complicates the diagnostic landscape. Serological tests, including indirect fluorescent antibody tests and indirect enzyme-linked immunosorbent tests, are acknowledged for their high sensitivity (Karasova *et al.*, 2022). However, their specificity is moderately compromised due to antigenic cross-reactions with other *Babesia* species (Yamane *et al.*, 1993). Recent advancements in diagnostic methods have witnessed the increasing application of PCR-based assays, which offer distinct advantages over both microscopic and serological tests in terms of enhanced sensitivity and specificity (Deepa *et al.*, 2021).

Table 1. Altered haematological parameters in infected Rottweiler dog

Haematological parameter	Sample values	Reference range (Studdert *et al.*, 2020)
Haemoglobin (Hb) (g/dL)	6.8	12-18
Packed cell volume (PCV) (%)	21.4	37-55
Total erythrocyte count (TEC) (10^6/µL)	3.06	5.5-8.5
Mean corpuscular volume (MCV) (fL)	71	60-75
Mean corpuscular haemoglobin (MCH) (pg)	22.3	20-28
Mean corpuscular haemoglobin concentration (MCHC) (g/dL)	31.8	32-36
Platelets (10^6/µL)	1.2	2-5
Neutrophils (%)	45	60-77
Lymphocytes (%)	52	12-30
Monocytes (%)	1	3-10
Eosinophil (%)	1	2-10

The clinical manifestations observed in this study, including anaemia, dullness, depression, pale mucous membranes, dark yellow urine, slightly increased respiration and a temperature of 102.3° F, coupled with reductions in haematological parameters such as Hb, PCV, TEC and thrombocytopenia, align with characteristic clinical presentations associated with *B. gibsoni* infection in dogs (Kandasamy *et al.*, 2021; Liu *et al.*, 2022; Karasova *et al.*, 2022; Teodorowski *et al.*, 2022). These findings emphasize the significant impact of the parasitic infection on the hematological profile and overall health of the affected animal.

The successful treatment of the dog with Diminazene aceturate, complemented by other antibiotics and supportive medications, highlights its therapeutic potential against *B. gibsoni*. However, recognizing associated side effects and the risk of recurrence is paramount (Karasova *et al.*, 2022). Our results align with previous studies by Lin and Huang (2010) and Chauhan *et al.* (2022) substantiating diminazene aceturate's efficacy with antibiotics such as doxycycline, clindamycin, metronidazole and enrofloxacin, along with supportive medications. Conversely, the efficacy of imidocarb dipropionate against *B. gibsoni* infection appears unsatisfactory (Karasova *et al.*, 2022; Patel *et al.*, 2023). Atovaquone, combined with antibiotics like azithromycin, emerges as a more commonly recommended therapeutic approach for small form *Babesia* infection, reflecting evolving treatment strategies (Karasova *et al.*, 2022). The choice and combination of medications should be tailored to individual cases, considering infection severity and treatment response. The severity of the observed clinical and haematological alterations underscores the importance of timely and accurate diagnosis, as well as appropriate therapeutic interventions in managing *B. gibsoni* infections.

The present study contributes to the existing knowledge by reporting the first molecular detection of *B. gibsoni* in Madhya Pradesh, filling a gap in the published literature regarding molecular detection in this region.

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