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Incidence of *Babesia gibsoni* in dogs in and around Shivamogga

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Abstract

Canine babesiosis is an important tick-borne hemoprotozoan disease in India, predominantly caused by *Babesia gibsoni*. The present study aimed to detect babesiosis in clinically suspected dogs using microscopic examination (8.62 per cent) and PCR (31.03 per cent) and analyze associated clinical patterns and prevalence factors. A total of 58 dogs exhibiting signs such as fever, anemia, lethargy and tick infestation were screened from January to June 2025. Genomic DNA extracted from EDTA blood samples was subjected to PCR using *Babesia* genus-specific and *B. gibsoni*-specific primers. Further, Species-specific diagnosis on 18 blood samples revealed positivity in 5 (27.77%), 8 (44.44%) and 12 (66.66%) samples by microscopy, rapid antibody kit and PCR respectively. The most common clinical signs included fever and anorexia (94.44%), tick infestation (83.33%), lethargy (66.66%) and pale mucous membranes (55.55%). Breed-wise analysis showed the highest prevalence in Labrador Retrievers (44.44%), followed by non-descript dogs (27.77%). Dogs aged 1-3 years constituted the major affected group (50%), indicating higher susceptibility in young adults. Male dogs (61.11%) were more frequently positive than females (38.88%). The study highlights the utility of PCR for definitive diagnosis and emphasizes the role of tick control and early detection in managing canine babesiosis effectively.

Keywords: *Babesia*, *Babesia gibsoni*, Canine babesiosis, PCR

Introduction

Canine babesiosis is a tick-borne hemoprotozoan disease caused by intraerythrocytic parasites of the genus *Babesia*, belonging to the phylum Apicomplexa. Among the various species, *Babesia gibsoni* is the predominant causative agent of canine babesiosis in India, with prevalence rates ranging from 6% to 64% depending on geographic location and diagnostic method employed (Kumar *et al.*, 2015; Mahalingaiah *et al.*, 2017) [8]. The disease is transmitted primarily by *Rhipicephalus sanguineus* and *Dermacentor* species tick and presents with a wide spectrum of clinical signs including anemia, thrombocytopenia, fever, lethargy, splenomegaly and multi-organ dysfunction (Mittal *et al.*, 2019; Perna *et al.*, 2021) [9].

Materials and Methods

A total of 58 samples were collected from dogs with a history of tick infestation and exhibiting clinical signs of high fever, pale / icteric mucous membrane, inappetence, anaemia, lethargy, vomiting, diarrhoea, hemoglobinuria from different breeds, gender and age group of dogs during the period from January 2025 to June 2025, which were presented to Veterinary clinical practice Shivamogga. Blood samples were collected in vacutainer containing EDTA as anticoagulant and stored at -20 °C for further processing. Blood samples were screened by PCR.

Polymerase Chain Reaction (PCR)

The extraction of genomic DNA from whole blood was done by using HiPurA® Blood Genomic DNA Miniprep Purification Kit (Himedia, HiGenoMB® Catalogue no. MB504) as per the manufacturer's protocol. DNA samples were amplified using *Babesia* and *B. gibsoni* species specific primers (Hilpert Hauser *et al.*, 2007, Jain *et al.*, 2018) [4, 6].

The specific amplicon size of 440 bp and 488 bp was analysed by 1.2% agarose ethidium bromide gel electrophoresis.

Primers: The primers for *Babesia* genus and *Babesia gibsoni* were procured from procured from Bio serve biotechnologies India Pvt. Ltd., Hyderabad.

Table 1: Description of Primer for detection of *Babesia* genus and *Babesia gibsoni*

Sl. No	Target gene	Primer Sequence (5' - 3')	Product size	Reference
1	18S rRNA	F: GTTCTGCCCCATCAGCTTGAC R: CAAGACAAAAGTCTGCTTGAAAC	440bp	Hilpertshauser et al. (2007) [4]
2	18S rRNA	BAGI F: TTGGCGGCGTTTATTAGTTC BAGI R: AAAGGGGAAAACCCCAAAAG	488bp	Jain et al. (2018) [6]
F: Forward Primer and R: Reverse Primer				

Table 2: Composition of PCR reaction mixture

Contents	Quantity
PCR master mix	12.5 µl
Forward Primer (10pmol/µl)	1µl
Reverse Primer (10pmol/µl)	1µl
Template DNA	5µl
Nuclease free water	5.5µl
Total	25.0 µl

Table 3: Polymerase chain reaction cycling conditions for *Babesia* genus

	Steps	Duration	No. of cycles
Step1	Initial denaturation	94 °C for 10 min	1 cycle
Step2	Denaturation Annealing of primer Extension	94 °C for 30 sec 62 °C for 30 sec 72 °C for 45 sec	40 cycles
Step3	Final extension	72 °C for 10 min	1 cycle
	Hold	4 °C	

Table 4: Polymerase chain reaction cycling conditions for *Babesia gibsoni*

	Steps	Duration	No. of cycles
Step 1	Initial denaturation	94 °C for 10 min	1 cycle
Step 2	Denaturation Annealing of primer Extension	94 °C for 30 sec 59 °C for 30 sec 72 °C for 45 sec	35 cycles
Step 3	Final extension	72 °C for 10 min	1 cycle
	Hold	4°C	

Table 5: Prevalence of babesiosis by blood smear examination and polymerase chain reaction

Diagnostic method	No. of dogs Examined (N=58)	No. of dogs positive	Percent prevalence (%)
Blood smear examination	58	05	08.62
PCR	58	18	31.03

Table 6: Diagnosis of *Babesia gibsoni* infection by blood smear examination, rapid antibody kit test and polymerase chain reaction in dogs

Diagnostic method	No. of dogs examined	No. of dogs positive	Percent (%) prevalence
Giemsa staining	18	05	27.77
Rapid antibody kit test	18	08	44.44
PCR	18	12	66.66

Results

A total of 58 dogs suspected for canine babesiosis of which 18 dogs were positive for babesiosis by PCR were studied and are documented in Table 5.

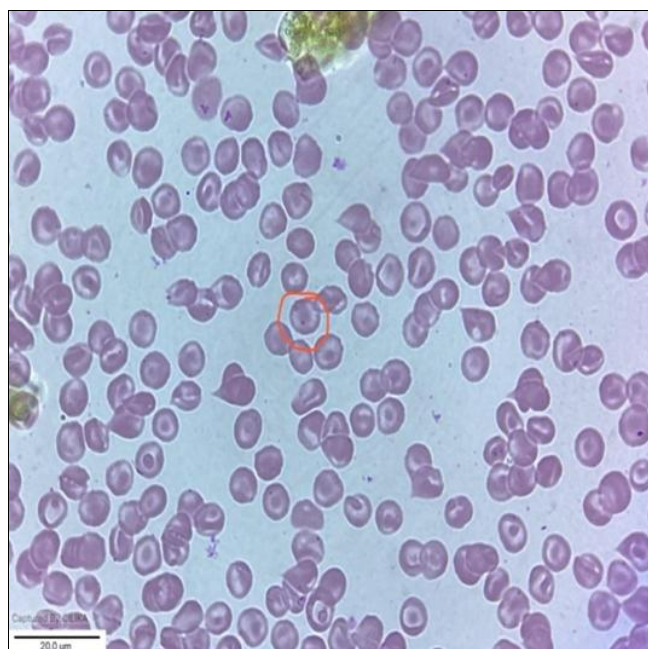
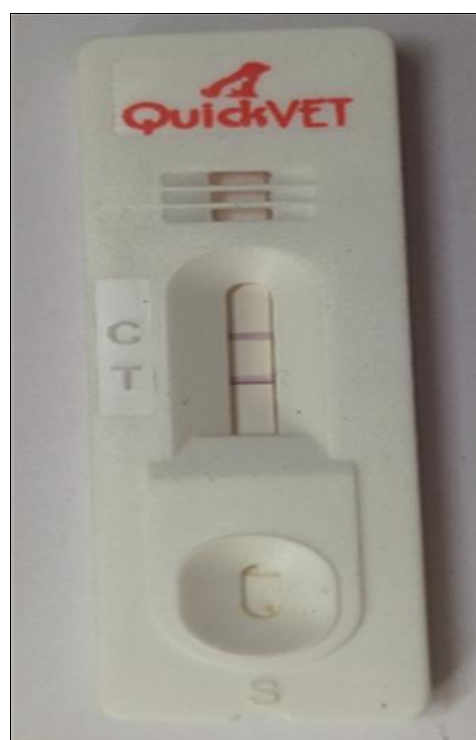
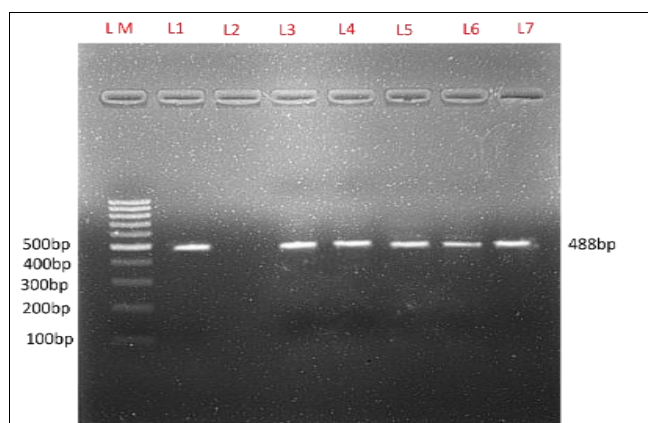
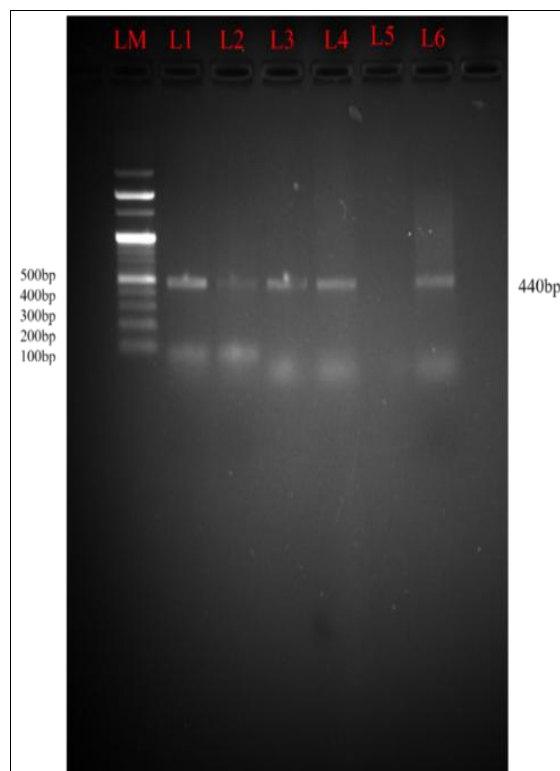
**Plate 1****Plate 2****Plate 3****Plate 4**

Plate 1: *Babesia gibsoni* (arrow) in microscopy of Giemsa-stained blood smear (100X)

Plate 2: Affected dog showing positive for *Babesia gibsoni* rapid antibody kit test

Plate 3: Agarose gel (1%) electrophoresis showing amplified DNA from *Babesia gibsoni* (488bp)

- **Lane M:** 100bp marker
- **Lane 1:** Positive control
- **Lane 2:** Negative control
- **Lane 3, 4, 5, 6, 7:** Samples positive for *Babesia gibsoni*.

Plate 4: Agarose gel (1%) electrophoresis showing amplified DNA from *Babesia* genus (440bp)

- **Lane M:** 100bp marker
- **Lane 1, 2, 3, 4:** Samples positive for *Babesia* genus.
- **Lane 5:** Negative control
- **Lane 6:** Positive control

Table 7: Clinical signs of canine babesiosis

Clinical signs	No. of animals affected (n=18)
Fever	17 (94.44 %)
Anorexia	17 (94.44 %)
Tick infestation	15 (83.33 %)
Depression and lethargy	12 (66.66 %)
Pale CMM	10 (55.55 %)
Respiratory distress	09 (50.00 %)
Vomiting	09 (50.00 %)
Lymphadenopathy	07 (38.88 %)
Weakness	07 (38.88 %)
Ascites	04 (22.22 %)
Jaundice	04 (22.22 %)
Dark Urine	04 (22.22 %)
Icteric oral mucous membrane	04 (22.22 %)
Melena	03 (16.66 %)
Papery white CMM	01 (5.55%)
Petechial hemorrhages	01 (5.55%)

Out of 18 dogs, examined, the most evident clinical signs reported were fever and anorexia (94.44%), followed by tick infestation (83.33%), depression and lethargy (66.66%), pale conjunctival mucous membranes (55.55%), respiratory distress and vomiting (50.00%), lymphadenopathy and weakness were (38.88%), ascites, jaundice, dark urine and icteric oral mucous membranes (22.22%) and the least frequently noted clinical signs were melena (16.66%), papery white conjunctival mucous membranes and petechial hemorrhages (5.55%) were present (Table 5).

Table 8: Breed wise prevalence

Breed	Number of positive	Percent (%) prevalence
Labrador Retriever	8	44.44
Nondescript	5	27.77
Golden Retriever	2	11.11
German shepherd	1	05.55
Mudhol hound	1	05.55
Siberian Husky	1	05.55
Total	18	100.00

Out of 18 PCR-positive cases of canine babesiosis, Labrador Retrievers showed the highest occurrence (44.44%), followed by non-descript dogs (27.77%), Golden Retrievers (11.11%) and German Shepherds, Mudhol Hounds and Siberian Huskies (5.55% each) (Table 6).

Table 9: Age wise prevalence

Age	Number of positive	Per cent (%) prevalence
≤ 1	02	11.11
1 to 3	09	50.00
3 to 6	04	22.22
6 to 9	02	11.11
9 to 12	01	05.55
Total	18	100.00

Among 18 PCR-positive cases of canine babesiosis, 2 cases (11.11%) were less than 1 year of age, 9 cases (50.00%) belonged to 1-3 years old, 4 cases (22.22%) belonged to 3-6 years of age, 2 cases (11.11%) were between 6 to 9 years of age and lowest occurrence in 1 case (5.55%) were between 9-12 years of age (Table 7).

Table 10: Sex wise prevalence

Gender	Number of positive	Percent (%) prevalence
Male	11	61.11
Female	07	38.88
Total	18	100.00

Among 18 PCR-positive cases of canine babesiosis, male dogs showed a higher occurrence (61.11%) compared to female dogs (38.88%) (Table 8).

Discussion

In the present study, the most common clinical signs in *Babesia gibsoni* infected dogs were fever and anorexia, followed by tick infestation, depression, lethargy, pale mucous membranes, respiratory distress and vomiting, which aligns with earlier reports by Furlanello *et al.* (2005) [1], Nandini *et al.* (2016) [10], Sindhu *et al.* (2020), Preena *et al.* (2021) [12], Soundarya *et al.* (2023) [15] and Patel *et al.* (2023) [11]. These signs are largely attributed to erythrolysis, inflammatory mediator release, anemia, hypoxia and liver dysfunction as described by Wadhwa *et al.* (2011) [16], Yogeshpriya *et al.* (2014) and Gonmei *et al.* (2020) [3]. Less frequent findings, such as icterus, ascites, melena and petechial hemorrhages, reflect hemolysis, reduced platelet count and impaired bilirubin clearance (Preena *et al.*, 2021) [12]. The variability in clinical presentation supports the influence of disease stage, immune response and multisystemic involvement.

A significant increase in rectal temperature, heart rate and respiratory rate was observed in affected dogs, likely due to cytokine release and compensatory responses to anemia and hypoxia (Wadhwa *et al.*, 2011) [16]. These findings are consistent with Islam *et al.* (2017) [5], Roopali *et al.* (2018), Gonmei *et al.* (2020) [3] and Patel *et al.* (2023) [11], who also reported elevated physiological parameters in *B. gibsoni*-infected dogs compared to healthy controls.

Breed-wise Prevalence

In the present study, Labrador Retrievers showed the highest prevalence of *Babesia gibsoni* infection (44.44%), followed by non-descript dogs (27.77%), Golden Retrievers (11.11%), and German Shepherds, Mudhol Hounds, and Siberian Huskies (5.55% each). This pattern aligns with earlier reports by Mahalingaiah *et al.* (2017) [8], Gonde *et al.* (2017) and Shinde *et al.* (2021) [14], who also noted Labradors as the most commonly affected breed. Although some studies such as Kunwar *et al.* (2021) [7] and Ranatunga *et al.* (2022) [13] reported higher susceptibility in German Shepherds, these differences likely reflect regional breed popularity, tick exposure and variations in dog populations. Overall, findings suggest a possible breed predisposition, with Labradors appearing more susceptible in the studied region.

Age-wise Prevalence

Babesiosis was most common in dogs aged 1-3 years (50%), followed by 3-6 years (22.22%), with much lower occurrence in dogs below 1 year and 6-9 years (11.11%) and the least in 9-12 years (5.55%). Similar age-related susceptibility was reported by Mahalingaiah *et al.* (2017) [8] and Shinde *et al.* (2021) [14], likely due to higher outdoor activity and tick exposure in young adults. Some studies, however, noted higher prevalence in older dogs, possibly due to long-term tick exposure. Overall, young adult dogs remain the most vulnerable age group.

Sex-wise Prevalence

Male dogs showed a higher prevalence of babesiosis (61.11%) compared to females (38.88%), similar to reports by Mahalingaiah *et al.* (2017) [8], Shinde *et al.* (2021) [14] and others, likely due to greater outdoor exposure and roaming behavior. However, some studies observed no major sex difference, suggesting that management and exposure factors may influence the pattern.

Conclusion

The study confirms *Babesia gibsoni* as a significant cause of canine babesiosis, with fever, anorexia and tick infestation being the predominant clinical signs. PCR proved effective in detecting infection, identifying 18 positive cases. Labrador Retrievers, young adult dogs (1-3 years) and males were more frequently affected, suggesting possible breed, age and sex related susceptibility, likely influenced by outdoor activity and tick exposure. These findings highlight the importance of early diagnosis, tick control and targeted preventive strategies for high-risk groups.

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