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Occurrence of canine babesiosis: A comparative study of microscopy, PCR and LFA

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Abstract

Canine babesiosis is a tick-borne hemoprotozoan disease caused by intra-erythrocytic parasites of the genus *Babesia*, mainly *B. canis vogeli* and *B. gibsoni* in tropical and subtropical countries, including India. In the present study, dogs showing clinical signs such as fever, anorexia, vomiting, lethargy, ascites, hind limb weakness, haemoglobinuria and tick infestation were diagnosed using a rapid antibody test kit (LFA), blood smear examination and Polymerase Chain Reaction (PCR). Out of 40 suspected cases of canine babesiosis, 27 (67.5%) were positive by LFA, 10 (25%) by microscopic examination and 24 (60%) by PCR. Among diagnostic techniques, LFA showed the higher number of positive cases, followed by PCR and blood smear examination. However, LFA is prone to false positives as it detects antibodies from both past and ongoing infections, whereas PCR remains the most sensitive and specific diagnostic tool for detecting the organism in low parasitaemia.

Keywords: *Babesia canis vogeli*, *Babesia gibsoni*, PCR, lateral flow assay and blood smear examination

1. Introduction

Canine babesiosis is a clinically significant tick-borne protozoan disease caused by intraerythrocytic parasites of the genus *Babesia* (Abdoli *et al.*, 2024; Allsopp, 2015) [1, 2]. The disease is distributed worldwide and is transmitted predominantly by tick vectors such as *Rhipicephalus sanguineus*, *Haemaphysalis leachi* and *Dermacentor reticulatus* (Dantas-Torres *et al.*, 2012; Beugnet *et al.*, 2016) [9, 5]. In India, canine babesiosis is frequently reported and remains an important cause of morbidity and mortality in dogs (Ancy *et al.*, 2020; Brahma *et al.*, 2019) [3, 6].

The disease manifests with variable severity, ranging from mild to life-threatening illness, characterized by fever, anemia, hemoglobinuria, icterus, splenomegaly, hepatomegaly and multi-organ dysfunction (Baneth, 2018; Zygnier *et al.*, 2010; Court *et al.*, 2023) [4, 20, 8]. Diagnosis is routinely performed through microscopic examination of stained blood smears, but low-level parasitemia often results in false negatives (Mukartal *et al.*, 2024; Sai Sindhu *et al.*, 2020) [15, 18]. Hence, molecular methods such as PCR provide superior sensitivity and specificity (Birkenheuer *et al.*, 2003; Duarte *et al.*, 2008; Inokuma *et al.*, 2004) [7, 10, 11]. Antibody-based rapid tests are widely used in clinical practice but may yield false positives due to the persistence of antibodies from past infections (Jia *et al.*, 2007; Kalaivanan *et al.*, 2018) [12, 13].

2. Materials and Methods

Dogs presented to the Department of Veterinary Medicine, Veterinary College, Hebbal, Bengaluru with clinical signs such as fever, tick infestation, anorexia, vomiting, lethargy, ascites, hind limb weakness, melena, or haemoglobinuria were included in the study. A total of 40 suspected cases were examined. Approximately 2 mL of blood was collected aseptically from the cephalic or saphenous vein of each dog into EDTA vials for PCR, and a drop of blood from the ear-tip was used for blood smear examination. Diagnostic evaluation included a rapid antibody test using *Babesia canis* and *Babesia gibsoni* kits (PetX) as per manufacturer's instructions, with results interpreted after 10 minutes, microscopic examination of Giemsa-stained blood smears for intraerythrocytic *Babesia* organisms and PCR analysis.

Genomic DNA was extracted from EDTA blood using the QiAamp DNA Blood Mini Kit (Qiagen, Germany), and PCR amplification was carried out using species-specific primers, with amplified products analyzed by agarose gel electrophoresis. The primers used for PCR included BAB-1 (5'-GTG AAC CTT ATC ACT TAA AGG-3') and BAB-4 (5'-CAA CTC CTC CAC GCA ATC-3') for *Babesia canis vogeli* (600 bp product; Duarte *et al.*, 2008) [10], and Gib 599 (5'-CTC GGC TAC TTG CCT TGT C-3') and DGib 1270 (5'-GCC GAA ACT GAA ATA ACG GC-3') for *Babesia gibsoni* (670 bp product; Inokuma *et al.*, 2004) [11].

3. Results and Discussion

In the present study on Canine Babesiosis, among 40 suspected cases, 27 dogs (67.5%) tested positive using the lateral flow assay (LFA), 24 dogs (60%) were positive by PCR and 10 dogs (25%) tested positive by microscopic examination. Details were depicted in the Table 1. A higher occurrence of Canine Babesiosis was recorded using LFA, followed by PCR and then microscopy. In contrast to our findings, Ravi *et al.* (2016) [17], Mahalingaiah *et al.* (2017) [14], Shil *et al.* (2022) [19] and Raju (2023) [16] reported a higher occurrence of Canine Babesiosis using PCR. PCR is recognized as the gold standard due to its high sensitivity and specificity in detecting *Babesia* organisms, especially in cases of low parasitaemia. The higher occurrence observed with LFA in the present study may be attributed to its antibody-based nature, which can detect antibodies from both previous and ongoing infections, potentially increasing positive cases, although false-positive results are also possible. Only 25% of dogs were positive by microscopic examination, which may be due to false-negative results where *Babesia* organisms are not detected in low or very low parasitaemia. Thus, while PCR is highly reliable for diagnosing canine babesiosis, LFA can indicate higher occurrence due to antibody detection and microscopic examination may give false negative results when parasitaemia is low.

Table 1: Diagnostic results of dogs suspected of babesiosis (n = 40)

Diagnostic method	Number of dogs positive (n = 40)	Percentage (%)
LFA Test Kit	27	67.50
Microscopic examination	10	25.00
PCR (Total Positive)	24	60.00

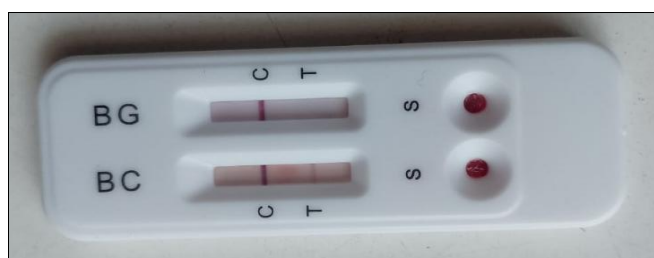


Fig 1: LFA kit positive for *B. canis*

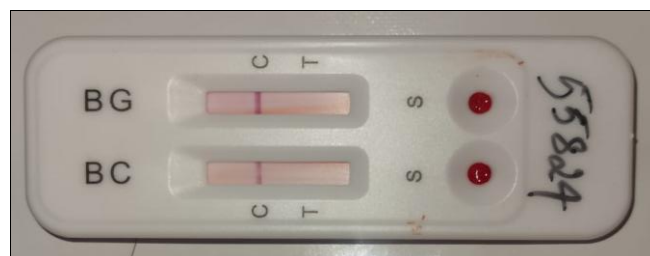


Fig 2: LFA kit negative for both *B. canis* and *B. gibsoni*

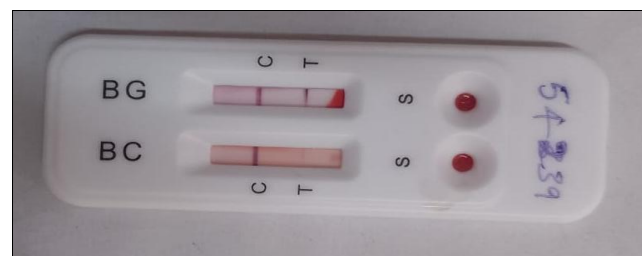


Fig 3: LFA kit positive for *B. gibsoni*

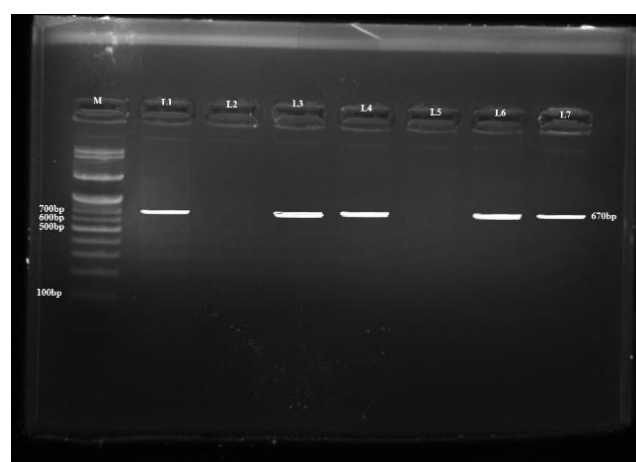


Fig 4: PCR gel picture showing Bands corresponds to *B. gibsoni*

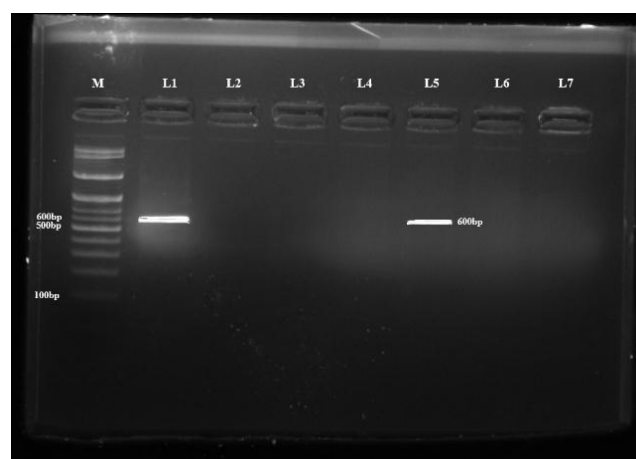


Fig 5: PCR gel picture showing bands corresponds to *B. canis vogeli*

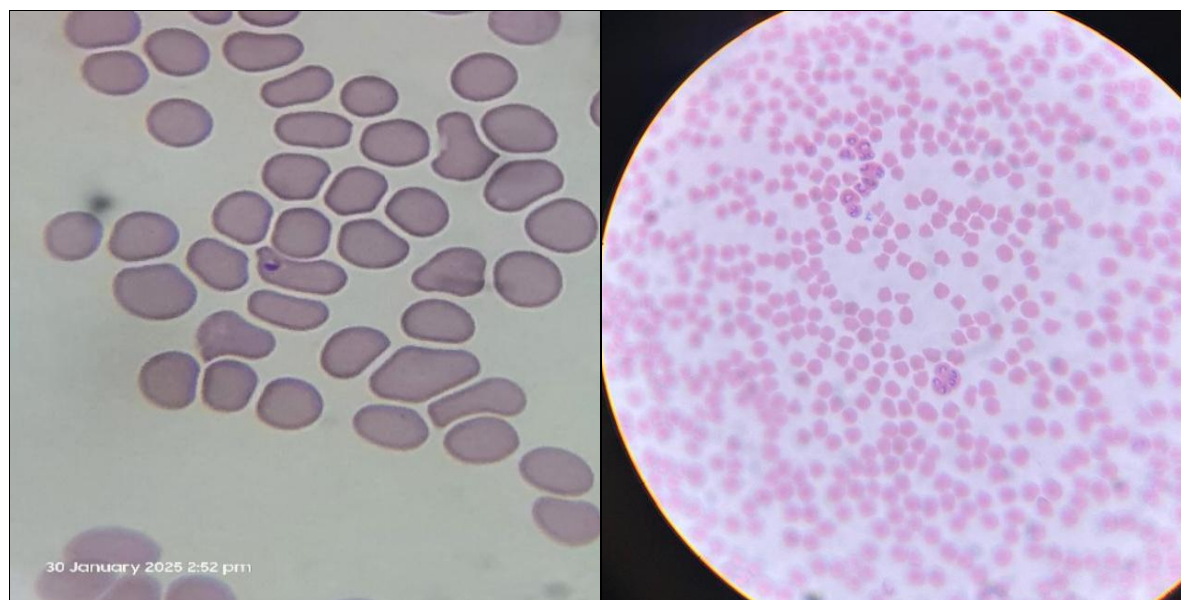


Fig 6: Blood smear examination positive for *B. gibsoni*

4. Conclusion

In the present study on Canine Babesiosis, a higher occurrence of the disease was recorded using LFA, followed by PCR and microscopic examination. The higher detection by LFA may be due to its antibody-based nature, which can identify antibodies from both past and ongoing infections. PCR remains the most sensitive and specific method for detecting *Babesia* organisms, especially in cases of low parasitaemia, whereas microscopic examination detected fewer cases and is suitable mainly when parasitaemia is higher or moderate. These findings suggest that PCR is the preferred diagnostic tool, while LFA can be used as a rapid screening method in clinical practice.

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