International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
NAAS Rating (2025): 4.61
VET 2025; 10(11): 353-356

www.veterinarypaper.com
Received: 25-08-2025
Accepted: 28-09-2025

Abhijith SP
M.V. Sc Scholar, Department of Veterinary Medicine, Veterinary College, Bengaluru, Karnataka, India

Lathamani VS
Assistant Professor, Department of Veterinary Medicine, Veterinary College, Bengaluru, Karnataka, India

Ramesh PT
Registrar, Karnataka Veterinary, Animal & Fisheries Sciences University, Bidar, Karnataka, India

Sharada R
Professor, Department of Veterinary Microbiology, Veterinary College, Bengaluru, Karnataka, India

Girish BC
Associate Professor, Department of Veterinary Pathology, Veterinary College, Hassan, Karnataka, India

Srinivasa Murthy KM
Professor, Department of Veterinary Surgery & Radiology, Veterinary College, Bengaluru, Karnataka, India

Corresponding Author:
Abhijith SP
M.V. Sc Scholar, Department of Veterinary Medicine, Veterinary College, Bengaluru, Karnataka, India

Haematological and biochemical alterations in dogs naturally infected with *babesia gibsoni* in Bengaluru

Abhijith SP, Lathamani VS, Ramesh PT, Sharada R, Girish BC and Srinivasa Murthy KM

DOI: https://www.doi.org/10.22271/veterinary.2025.v10.i11f.2752

Abstract
Babesia gibsoni is a small, intraerythrocytic protozoan parasite that causes canine babesiosis, a tick-borne disease primarily transmitted by *Rhipicephalus sanguineus* and through dog bites, blood transfusions, or vertically. Resistance to commonly used drugs like Imidocarb dipropionate and even the standard Atovaquone-Azithromycin protocol has been increasingly reported, especially in endemic areas. The emergence of resistant strains and frequent treatment failures make *B. gibsoni* a growing therapeutic concern in veterinary practice. In this study, we aim to potentiate Artesunate with Atovaquone-Azithromycin therapy and evaluate its efficacy in achieving parasitaemia clearance.
The present study was conducted to compare the efficacy of three treatment regimens for canine babesiosis. A total of 18 dogs infected with *Babesia gibsoni*, diagnosed by microscopy and PCR assay, were randomly allocated into three groups: Group I (n=6) received Atovaquone @13.3mg/kg TID P.O & Azithromycin @10mg/kg OD P.O for 10 days; Group II (n=6) received Artesunate @ 12.5mg/kg SID P.O for 10 days; and Group III (n=6) received Atovaquone @13.3mg/kg TID P.O & Azithromycin @10mg/kg OD P.O & Artesunate @ 12.5mg/kg SID P.O for 10 days. Clinico-pathological examinations and parasite detection were performed on Day 0, Day 10 & Day 21 post-treatment to assess efficacy. The major abnormalities observed at presentation included tachypnea, anorexia, pale or abnormal mucous membranes, lethargy, fever, thrombocytopenia, and anemia. Dogs in Group III showed significant improvement in most clinical and hematological parameters compared with those in Groups I and II. Complete parasitological clearance was not achieved, and 4 out of 18 dogs (2 in Group I, 2 in Group II,) remained positive for *B. gibsoni* on microscopy. Based on the clinico-pathological recovery, the regimen used in Group III may provide better therapeutic outcomes under field conditions.

Keywords: *Babesia gibsoni*, artesunate, atovaquone, pcr

Introduction
Babesia gibsoni is a small intraerythrocytic protozoan parasite that infects canine red blood cells, transmitted primarily through tick bites or direct blood exposure. It is endemic in Asia, the Americas, and parts of Europe (Birkenheuer *et al.,* 2004) [2]. Once inside erythrocytes, the organism replicates by binary fission, causing both intravascular and extravascular hemolysis, leading to chronic, low-level parasitemia and immune-mediated erythrocyte destruction (Sakuma *et al.,* 2009) [9]. Affected dogs may show fever, cachexia, anemia, thrombocytopenia, splenomegaly, and potentially life-threatening complications like acute kidney injury, liver dysfunction, and immune-mediated hemolytic anemia (IMHA) (Baneth *et al.,* 2018) [1].
Pathologically, *Babesia gibsoni* infection causes erythrophagocytosis, immune-mediated thrombocytopenia, splenic activation, and systemic inflammation. These events may culminate in Multiple Organ Dysfunction Syndrome (MODS), particularly in immunosuppressed dogs (Köster *et al.,* 2015) [8]. MODS is characterized by liver and kidney dysfunction, pulmonary involvement, and coagulopathies, driven by cytokine storms, oxidative stress, and endothelial damage (Irwin *et al.,* 1991; Baneth *et al.,* 2018) [6, 1]. Laboratory findings often include elevated ALT, AST, creatinine, BUN, prolonged PT/aPTT, and increased acute-phase proteins such as CRP.
Compounding this is the frequent development of immune-mediated hemolytic anemia

(IMHA). Here, the immune system targets both infected and uninfected erythrocytes, resulting in spherocytosis, a positive Coombs' test, and persistent regenerative anemia (Wulansari *et al.,* 2003) [13]. This autoimmune response is likely triggered by parasite-induced antigen exposure, cross-reactivity, or bystander activation (Birkenheuer *et al.,* 2005) [2]. IMHA may continue even after the parasitemia resolves, indicating long-term immune dysregulation and risk of relapse (Birkenheuer *et al.,* 2004; Solano-Gallego *et al.,* 2016) [2, 12].

Managing such complex cases requires more than antiparasitic therapy. Supportive interventions such as blood transfusions, corticosteroids for IMHA, fluid therapy, and extended antimicrobial protocols are essential (Baneth *et al.,* 2018) [1]. Early intervention is critical, as mortality rates increase significantly in dogs with MODS or IMHA (Karasová *et al.,* 2024) [7].

The emergence of multi-drug-resistant *Babesia gibsoni* strains poses a serious therapeutic challenge. Resistance, primarily caused by mutations in CYTb, undermines the efficacy of standard drugs including atovaquone (Sasaki *et al.,* 2019) [10]. Additionally, B. gibsoni persists in subclinical carriers who can relapse during immunosuppression or stress, continuing the transmission cycle (Fukumoto *et al.,* 2001; Singh *et al.,* 2022) [5, 11].

To address resistance and relapse, recent studies emphasize alternative protocols and drug potentiation. The AAA protocol; Atovaquone (13.3 mg/kg PO q8h), Azithromycin (10 mg/kg PO q24h), and Artesunate (12.5 mg/kg PO q24h)-has been successful in reducing parasitemia and improving hematological recovery in relapsed cases (Karasová *et al.,* 2024; Singh *et al.,* 2022) [7, 11]. Artesunate enhances the effect by generating reactive oxygen species, amplifying mitochondrial stress in the parasite.

Materials & Methods

The present study was undertaken to investigate the clinical, haematological, biochemical, and molecular aspects of canine babesiosis, with a particular focus on evaluating the therapeutic efficacy of different drug regimens against *Babesia gibsoni*. Dogs presented to the Department of Veterinary Medicine, Veterinary College Hospital, Hebbal, Bengaluru, between January and June 2025, showing clinical signs such as fever, inappetence, anaemia, lethargy, and/or tick infestation, were considered for inclusion. Pregnant or lactating bitches and dogs with concurrent vector-borne infections were excluded. This study was designed to compare the hematological and biochemical changes associated with three therapeutic regimens in PCR-confirmed cases of *Babesia gibsoni* infection in dogs.

A total of 18 dogs naturally infected with *Babesia gibsoni*, confirmed by PCR, were enrolled and randomly allocated into three treatment groups (n=6 per group). Group I received Atovaquone and Azithromycin combination therapy, Group II was administered Artesunate monotherapy, and Group III received a triple regimen comprising Atovaquone, Azithromycin, and Artesunate.

For diagnosis, thin blood smears were prepared and giemsa-stained to identify intra-erythrocytic *Babesia* organisms. Molecular confirmation was achieved by PCR targeting the ITS1 region of *Babesia gibsoni* using species-specific primers, with positive and negative controls. PCR products were visualized by agarose gel electrophoresis. The PCR assay developed by Do *et al.* (2021) [4] specifically amplifies the internal transcribed spacer 1 (ITS1) region of *Babesia gibsoni*, selected for its high species specificity and reliability in molecular detection.

The primers used were forward 5′-ACATTGAAACTTGTCGAGCTGCG-3′ and reverse 5′-AGATCCCGCACCCAGCCAC-3′, which successfully amplified a 254 bp fragment serving as a distinct diagnostic marker for *Babesia gibsoni* DNA. The reaction was carried out at an annealing temperature of 60 °C, ensuring optimal binding and amplification specificity. The amplified 254 bp ITS1 products were further sequenced and employed in phylogenetic analysis, showing 100% sequence identity and confirming the robustness of the assay for accurate detection and genetic characterization of *Babesia gibsoni* isolates.

Hematological parameters (PCV, RBC count, Hb concentration, WBC count, platelet count) and serum biochemical markers (ALT, creatinine) were assessed on Days 0, 10, and 21. Clinical recovery, clearance of parasitaemia (by blood smear) and recurrence over a four-month follow-up period were also recorded.

Efficacy of the regimens was determined by clinical improvement, normalization of haematobiochemical values, and absence of recurrence. Data were statistically analysed using descriptive statistics, Chi-square tests, and two-way ANOVA with post-hoc comparisons to evaluate treatment response.

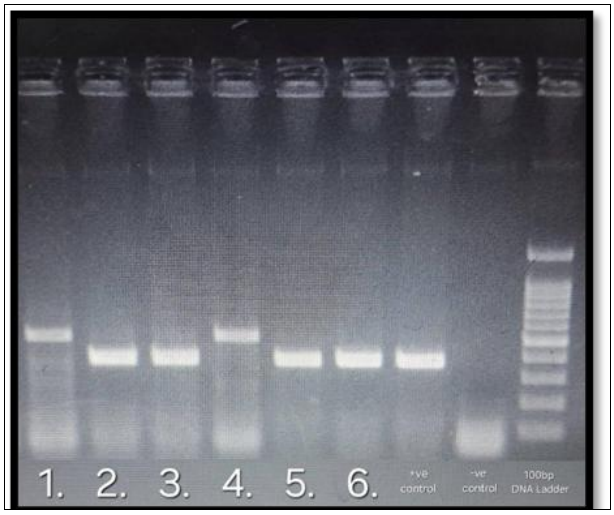

Fig 1: Positive sample yielded a distinct 254 bp band, which was visualized under a UV transilluminator

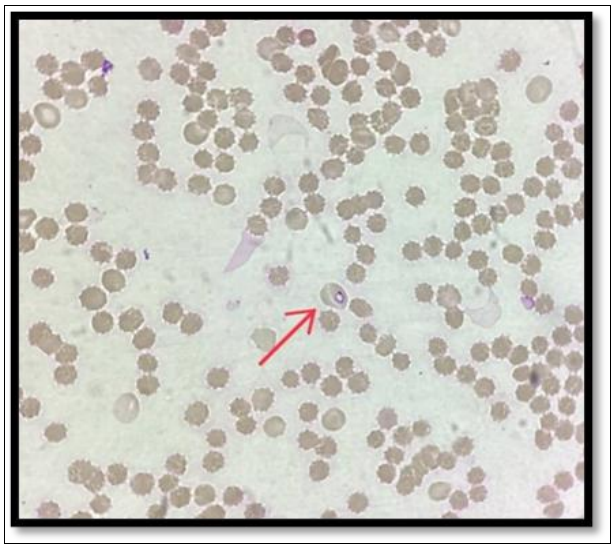

Fig 2: Giemsa-stained Blood smear positive for *Babesia gibsoni*

Results & Discussion

Out of 236 clinically suspected cases of canine babesiosis, 72 showed haematological alterations and were further examined. Microscopy confirmed *Babesia gibsoni* in 32 dogs (44.44%), while PCR confirmed 24 (75%) as true positives, underscoring the superior specificity of molecular methods over smear examination. Breed- and sex-wise distribution revealed higher absolute numbers in Labradors and males, though without statistical significance, while dogs aged 1–6 years showed higher susceptibility. Clinical signs included fever, pale mucous membranes, anorexia, lethargy, lymphadenopathy, and dark-coloured urine, consistent with haemolytic disease.

Haematological analysis demonstrated marked anaemia, leukocytosis, and thrombocytopenia in infected dogs compared to healthy controls, confirming classical features of babesiosis. Serum biochemistry revealed elevated ALT and creatinine, indicating hepatic and renal involvement. These alterations mirror earlier reports attributing erythrocyte destruction to both parasitic invasion and immune-mediated mechanisms.

Therapeutic trials highlighted significant differences in efficacy. Artesunate monotherapy improved clinical status but showed incomplete correction of anaemia and slower biochemical recovery. Atovaquone–Azithromycin yielded better haematological improvements, with moderate normalization of ALT and creatinine. The triple regimen (Atovaquone, Azithromycin, and Artesunate) achieved the most consistent results, with marked recovery in haemoglobin, PCV, platelet count, and a near-return to normal liver enzyme levels by day 21, among the three groups. These findings corroborate literature advocating multidrug regimens for superior parasite suppression, reduced relapse, and faster haematological recovery.

Statistical analysis confirmed significant time-dependent improvements in most parameters, with treatment–time interactions favouring the triple regimen. The enhanced efficacy likely stems from synergistic mechanisms, where Atovaquone targets mitochondrial pathways, Azithromycin inhibits protein synthesis, and Artesunate exerts schizonticidal action. Overall, the study emphasizes PCR as the gold standard for diagnosis and supports multidrug therapy as the most effective strategy for managing *Babesia gibsoni* infections in dogs.

Table 1: Mean ± Standard Error in hematological parameters in dogs affected with *Babesia gibsoni* (n=18)

Parameter	Affected (n=18)	Healthy control (n=6)	P value
Haemoglobin (g/dL)	8.17±0.02	12.8±0.33	p<0.001
Packed cell volume (%)	24.26±0.41	38.33±0.88	p<0.001
Total erythrocyte count (x10^6/µL)	3.42±0.20	6±0.14	p<0.001
Total leukocyte count (x10^3/µL)	21.52±1.40	11.42±0.42	p<0.001
Platelet count (x10^3/µL)	135.38±8.51	300.66±29.42	p<0.001

Table 2: Mean ± Standard Error in serological parameters in dogs affected with *Babesia gibsoni* (n=18)

Parameter	Affected (n=18)	Healthy control (n=6)	P -value
Creatinine (mg/dL)	1.53±0.06	1.1±0.09	p<0.001
Alanine Aminotransferase (IU/L)	121.33±12.56	46.16±4.83	p<0.001

Table 3: Hematological parameters (Mean ± SE) in different treatment groups

Parameter	Day	Group I (Atovaquone + Azithromycin)	Group II (Artesunate)	Group III (Atovaquone + Azithromycin + Artesunate)
Total Erythrocyte Count (×10^6/µL)	0	3.76 ± 0.13	3.58 ± 0.21	2.93 ± 0.22
	10	4.85 ± 0.28	4.35 ± 0.16	5.03 ± 0.26
	21	5.33 ± 0.18	5.08 ± 0.15	5.75 ± 0.13
Total Leukocyte Count (×10^3/µL)	0	24.10 ± 2.01	18.26 ± 2.09	22.21 ± 2.37
	10	17.08 ± 1.43	14.08 ± 1.29	13.56 ± 1.24
	21	12.46 ± 0.81	11.90 ± 1.12	10.26 ± 0.67
Hemoglobin (g/dL)	0	8.16 ± 0.40	8.13 ± 0.45	8.21 ± 0.28
	10	10.43 ± 0.94	9.56 ± 0.31	11.48 ± 0.60
	21	11.71 ± 0.40	10.98 ± 0.21	12.65 ± 0.25
Packed Cell Volume (%)	0	23.33 ± 1.22	24.80 ± 1.18	24.26 ± 0.41
	10	31.48 ± 2.90	28.55 ± 0.99	34.60 ± 1.90
	21	34.95 ± 1.42	32.81 ± 0.62	37.63 ± 0.86
Platelet Count (×10^3/µL)	0	156.16 ± 28.29	123.33 ± 23.20	126.66 ± 25.64
	10	198.33 ± 18.61	220.83 ± 16.91	318.00 ± 54.19
	21	310.66 ± 31.64	405.16 ± 52.48	394.33 ± 27.67

Table 4: Serum biochemical parameters (Mean ± SE) in different treatment groups

Parameter	Day	Group I (Atovaquone + Azithromycin)	Group II (Artesunate)	Group III (Atovaquone + Azithromycin + Artesunate)
ALT (IU/L)	0	111.50 ± 8.86	101.00 ± 23.72	151.50 ± 25.89
	10	77.83 ± 7.65	91.83 ± 14.69	88.66 ± 17.03
	21	53.83 ± 5.92	65.50 ± 10.84	57.16 ± 6.62
Creatinine (mg/dL)	0	1.56 ± 0.18	1.40 ± 0.17	1.63 ± 0.17
	10	1.50 ± 0.05	1.36 ± 0.19	1.41 ± 0.15
	21	1.12 ± 0.07	1.41 ± 0.06	1.28 ± 0.09

Conclusion

The combination of Atovaquone, Azithromycin, and Artesunate (Group III) resulted in the most effective parasitemia clearance and hematobiochemical improvement, followed by the dual therapy in Group I. Artesunate monotherapy (Group II) was the least effective, indicating that combination therapy, particularly with triple drugs, offers enhanced therapeutic benefits in the treatment of *Babesia gibsoni*.

Among the three regimens evaluated, the triple drug combination (Group III) achieved the most rapid and sustained parasitemia clearance, with notable hematological and biochemical recovery. The dual combination (Group I) showed moderate efficacy, while Artesunate alone (Group II) was comparatively less effective. These findings support the potential advantage of using potentiated combination therapy in managing canine babesiosis.

Acknowledgement

The authors thank Dean, Veterinary College, Bengaluru, KVAFSU for the services provided.

Conflict of interest

Nil

Reference

1. Baneth G, Florin-Christensen M, Cardoso L, Schnittger L. Reclassification of *Theileria annae* as *Babesia vulpes* sp. nov. Parasites & Vectors. 2018;8:207–218.
2. Birkenheuer AJ, Levy MG, Breitschwerdt EB. Efficacy of combined atovaquone and azithromycin for therapy of chronic *Babesia gibsoni* (Asian genotype) infections in dogs. J Vet Intern Med. 2004;18(4):494–498.
3. Birkenheuer AJ, Correa MT, Levy MG, Breitschwerdt EB. Geographic distribution of canine babesiosis in the United States and association with dog fighting. J Vet Intern Med. 2005;19(6):763–767.
4. Do H, Kim D, Oh J, Lee J. Optimization of PCR detection of *Babesia gibsoni* using species-specific ITS primers. Vet Parasitol. 2021;298:109561.
5. Fukumoto S, Suzuki H, Igarashi I, Xuan X. Fatal experimental transplacental *Babesia gibsoni* infection in dogs. Int J Parasitol. 2001;31(2):175–178.
6. Irwin PJ, Hutchinson GW, Barker EN. Canine babesiosis: from molecular taxonomy to control. Vet Parasitol. 1991;77(1):45–61.
7. Karasová D, Kodym A, Vondráková H, Pejchalová K. Evaluation of triple therapy in multidrug-resistant *Babesia gibsoni* infections in dogs. Veterinary Parasitology. 2024;326:109192.
8. Koster LS, Lobetti RG, Kelly P. Canine babesiosis: a perspective on clinical complications, biomarkers, and treatment. Vet Med Res Rep. 2015;6:119–128.
9. Sakuma M, Endo Y, Endo K. Immune-mediated mechanisms of anaemia in dogs experimentally infected with *Babesia gibsoni*. J Vet Med Sci. 2009;71(4):541–546.
10. Sasaki M, Matsuu A, Ikadai H, Hikasa Y. Mutations in cytochrome b associated with atovaquone resistance in *Babesia gibsoni*. Parasitology International. 2019;70:88–91.
11. Singh A, Verma R, Sharma A, Singh H. Therapeutic challenges and molecular epidemiology of canine babesiosis with emphasis on *Babesia gibsoni*. Veterinary World. 2022;15(4):942–951.
12. Solano-Gallego L, Sainz Á, Roura X, Estrada-Peña A, Miró G. A review of canine babesiosis: the European perspective. Parasites & Vectors. 2016;9:336.
13. Wulansari R, Wijaya H, Tjahajati I. Detection of immune-mediated hemolytic anemia in *Babesia gibsoni*-infected dogs. Vet Clin Pathol. 2003;32(4):181–185.

How to Cite This Article
Abhijith SP, Lathamani VS, Ramesh PT, Sharada R, Girish BC, Srinivasa Murthy KM. Haematological and biochemical alterations in dogs naturally infected with *babesia gibsoni* in Bengaluru. International Journal of Veterinary Sciences and Animal Husbandry. 2025; 10(11): 353-356.