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Epidemiological and haemato-biochemical study on bovine babesiosis in and around Bidar district, Karnataka

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Abstract

Bovine babesiosis, a tick-borne haemoprotozoan disease caused primarily by *Babesia* species, leads to severe economic challenges in the livestock industry. This study investigated the prevalence of bovine babesiosis in Bidar, Karnataka, alongside corresponding changes in haematological and biochemical indices in affected animals. Out of 954 cattle cases presented to the Veterinary Clinical Complex, Veterinary College, Bidar, between April 2021 and March 2024, 13 cases were confirmed positive for babesiosis, denoting an overall prevalence of 1.36%. Epidemiological evaluation indicated higher point prevalence in female cattle (53.85%) compared to male cattle (46.15%), and within young cattle aged under 2 years (46.15%). Non-descript breeds represented 69.23% of cases. Seasonally, the highest incidence coincided with the rainy season (61.54%). Predominant clinical manifestations included haemoglobinuria (100.0%), tick infestation (95.83%), and pyrexia (87.50%). Haematological profiling of infected cattle revealed highly significant reductions in haemoglobin (5.34 ± 0.35 g/dL), total erythrocyte count ($2.90 \pm 0.16 \times 10^6/\mu\text{L}$), packed cell volume ($18.06 \pm 1.06\%$), and platelets ($153.33 \pm 11.88 \times 10^3/\mu\text{L}$). Significant biochemical changes involved elevated serum levels of blood urea nitrogen (33.14 ± 1.54 mg/dL), creatinine (1.96 ± 0.08 mg/dL), total bilirubin (1.86 ± 0.07 mg/dL), and serum transaminases (AST: 153.26 ± 1.89 U/L; ALT: 51.94 ± 2.69 U/L). These findings demonstrate intensive intravascular haemolysis and multi-organ distress in clinical bovine babesiosis.

Keywords: Babesiosis, cattle, epidemiology, haemato-biochemical

1. Introduction

Bovine babesiosis, a tick-borne disease caused by intraerythrocytic protozoa of the genus *Babesia* (phylum Apicomplexa), results in significant economic losses globally, affecting both domestic and wild bovids (Almazan *et al.*, 2022) [1]. The key etiological agents include *Babesia bovis*, *Babesia bigemina*, *Babesia divergens* and *Babesia major* (Santos *et al.*, 2023) [33]. *Babesia bigemina* ranks first among the top ten economically important parasitic diseases of domestic cattle in India (Minjauw and McLeod, 2003) [25].

The clinical manifestations of an acute babesiosis generally run a course of three to seven days. Fever ($>40^\circ\text{C}$) is usually present for several days before other clinical signs become apparent, followed by inappetence, depression, increased respiratory rate, weakness, and reluctance to move. Haemoglobinuria is an overt clinical sign, hence the disease is known as red water. Anaemia and jaundice develop especially in protracted cases, while muscle wasting, tremors, and recumbency develop in advanced stages followed terminally by coma (DeVos and Potgieter, 1994) [9]. Keeping in view the significance of bovine babesiosis, the present study was conducted to evaluate the prevalence and haemato-biochemical parameters in cattle affected with babesiosis.

2. Materials and Methods

The present study was conducted to analyze the prevalence of babesiosis in cattle in and around Bidar, Karnataka, alongside tracking corresponding haemato-biochemical biomarkers in babesiosis-affected cattle. For the epidemiological prevalence component, official clinical records of the Out-Patient Ward of the Veterinary Clinical Complex (VCC, Medicine), Veterinary College, Bidar, spanning from April 2021 to March 2024, were systematically evaluated.

Blood samples were collected from cattle presented to the VCC and peripheral Veterinary clinics exhibiting clinical symptoms suggestive of haemoprotozoan infections, namely pyrexia, haemoglobinuria, pale or icteric mucous membranes, and severe tick infestation. Of these, 24 clinical cases of bovine babesiosis confirmed by blood smear microscopy were selected for intensive clinical, haematological and biochemical tracking.

Blood sampling was performed via aseptic jugular vein puncture into three sets of tubes: (1) EDTA tubes (2 mL) processed for peripheral thin blood films, automated haematology validation, and PCR studies; and (2) clot activator tubes (4 mL) allowed to clot for serum separation to facilitate semi-automated biochemistry profiling.

Haematological parameters were quantified using the Merilyzer CelQuant 360 VET fully automated blood cell counter to determine haemoglobin (Hb), total erythrocyte count (TEC), packed cell volume (PCV), total leukocyte count (TLC), and total platelet count (PLT). Manual differential leukocyte count (DLC) was carried out under oil immersion (100×) on Giemsa-stained thin blood smears.

Serum biochemical indices including total protein, albumin, globulin, blood urea nitrogen (BUN), creatinine, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were processed on a semi-automated biochemical analyser (Microlab 300, Eli Tech Group) using ERBA diagnostic reagent kits.

3. Results

Out of 954 cattle presented to the medicine ward over the three-year timeline, 13 were definitively confirmed positive for babesiosis, yielding an overall prevalence of 1.36% (Table 1). Year-wise distribution indicated a peak point prevalence during 2022-23 (46.15%). Risk factor stratification highlighted a distinct gender predisposition, with female cattle manifesting a higher point prevalence (53.85%) than males (46.15%), a variation strongly correlated with the physiological stresses of lactation and reproduction. Age-wise distribution showed the highest infection rates in cattle under 2 years of age (46.15%), followed by the 2-6 years cohort (30.77%), reflecting relative immunological immaturity in young stock. Breed evaluation revealed that non-descript indigenous cattle

comprised 69.23% of cases, while crossbred Holstein Friesian (HF) cattle comprised 30.77% (Table 1). Seasonally, a major surge occurred during the monsoon/rainy season (61.54%).

Table 1: Epidemiological Distribution and Point Prevalence Risk Factors of Bovine Babesiosis (n = 13)

Risk Factor Category	Sub-category Variables	Positive Cases (n)	Point Prevalence (%)
Temporal Evaluation (Yearly)	2021-2022	4	30.77
	2022-2023	6	46.15
	2023-2024	3	23.08
Gender Identification	Male	6	46.15
	Female	7	53.85
Age Cohorts	< 2 Years	6	46.15
	2-6 Years	4	30.77
	> 6 Years	3	23.08
Breed Stratification	Non-descript (Indigenous)	9	69.23
	Holstein Friesian (HF) Cross	4	30.77
Seasonal Variance	Winter Season	4	30.77
	Summer Season	1	7.69
	Monsoon/Rainy Season	8	61.54

Infected animals demonstrated acute disruptions across their haematological landscape (Table 2). A severe state of normocytic normochromic anaemia was evidenced by a substantial collapse in haemoglobin values (5.34 ± 0.35 g/dL), alongside reductions in total erythrocyte counts ($2.90 \pm 0.16 \times 10^6/\mu\text{L}$) and packed cell volume ($18.06 \pm 1.06\%$). Absolute values for MCV, MCH, and MCHC remained statistically unaltered compared to healthy cattle, indicating consistent normocytic normochromic architecture. Widespread thrombocyte destruction was reflected in intense thrombocytopenia ($153.33 \pm 11.88 \times 10^3/\mu\text{L}$). Total leukocyte counts dropped significantly to $4.01 \pm 0.21 \times 10^3/\mu\text{L}$, indicating transient leukopenia associated with systemic stress or lymphoid exhaustion. Differential leukocyte percentages demonstrated no statistically significant variance.

Table 2: Haematological Profile in Babesiosis Affected Cattle (Mean ± SE)

Haematological Parameter	Healthy Control (n = 6)	Babesiosis Infected (n = 24)	Statistical Significance
Haemoglobin (g/dL)	10.61 ± 0.20	5.34 ± 0.35	Significant Reduction ($p \leq 0.05$)
Total Erythrocyte Count ($\times 10^6/\mu\text{L}$)	5.21 ± 0.52	2.90 ± 0.16	Significant Reduction ($p \leq 0.05$)
Packed Cell Volume (%)	34.83 ± 1.35	18.06 ± 1.06	Significant Reduction ($p \leq 0.05$)
MCV (fl)	71.02 ± 9.14	63.30 ± 2.06	Non-Significant ($P \geq 0.05$)
MCH (pg)	21.36 ± 2.07	18.57 ± 0.71	Non-Significant ($P \geq 0.05$)
MCHC (%)	30.60 ± 1.22	29.37 ± 0.62	Non-Significant ($P \geq 0.05$)
Total Platelet Count ($\times 10^3/\mu\text{L}$)	296.83 ± 9.70	153.33 ± 11.88	Significant Reduction ($p \leq 0.05$)
Total Leukocyte Count ($\times 10^3/\mu\text{L}$)	6.88 ± 0.18	4.01 ± 0.21	Significant Reduction ($p \leq 0.05$)
Neutrophils (%)	28.33 ± 1.24	33.87 ± 1.09	Non-Significant ($P \geq 0.05$)
Lymphocytes (%)	61.83 ± 1.22	57.62 ± 0.93	Non-Significant ($P \geq 0.05$)
Monocytes (%)	3.00 ± 0.22	3.25 ± 0.30	Non-Significant ($P \geq 0.05$)
Eosinophils (%)	4.16 ± 0.20	3.37 ± 0.28	Non-Significant ($P \geq 0.05$)
Basophils (%)	2.50 ± 0.21	1.91 ± 0.15	Non-Significant ($P \geq 0.05$)

Serum analysis reflected advanced multi-organ strain induced by acute babesiosis parasitaemia, anaemic hypoxia, and haemoglobinuric filtration stress (Table 3). Serum total proteins (5.80 ± 0.14 g/dL) and globulin fractions ($2.41 \pm$

0.10 g/dL) were significantly depressed, indicating compromised hepatic function secondary to parasite-derived toxic metabolites. Extreme haemolysis resulted in marked hyperbilirubinaemia (1.86 ± 0.07 mg/dL), confirming

extensive post-haemolytic icterus. Renal damage was evidenced by significant spikes in blood urea nitrogen (33.14 ± 1.54 mg/dL) and serum creatinine (1.96 ± 0.08 mg/dL). Marked hepatocellular leak and tissue necrosis

were verified by massive increases in serum transaminases and alkaline phosphatase—AST surged to 153.26 ± 1.89 U/L, ALT to 51.94 ± 2.69 U/L, and ALP to 258.15 ± 8.72 U/L.

Table 3: Serum Biochemical Profile in Babesiosis Affected Cattle (Mean $\pm$ SE)

Biochemical Parameter	Healthy Control (n = 6)	Babesiosis Infected (n = 24)	Statistical Significance
Total Protein (g/dL)	6.80 ± 0.10	5.80 ± 0.14	Significant Reduction ($p \leq 0.05$)
Serum Albumin (g/dL)	3.41 ± 0.02	3.39 ± 0.06	Non-Significant ($P \geq 0.05$)
Serum Globulin (g/dL)	3.20 ± 0.04	2.41 ± 0.10	Significant Reduction ($p \leq 0.05$)
Albumin:Globulin (A:G) Ratio	1.008 ± 0.02	1.46 ± 0.06	Significant Elevation ($p \leq 0.05$)
Blood Urea Nitrogen (mg/dL)	16.61 ± 0.10	33.14 ± 1.54	Significant Elevation ($p \leq 0.05$)
Serum Creatinine (mg/dL)	0.98 ± 0.04	1.96 ± 0.08	Significant Elevation ($p \leq 0.05$)
Total Bilirubin (mg/dL)	0.18 ± 0.003	1.86 ± 0.07	Significant Elevation ($p \leq 0.05$)
AST (U/L)	84.00 ± 3.11	153.26 ± 1.89	Significant Elevation ($p \leq 0.05$)
ALT (U/L)	29.83 ± 3.04	51.94 ± 2.69	Significant Elevation ($p \leq 0.05$)
ALP (U/L)	89.00 ± 3.74	258.15 ± 8.72	Significant Elevation ($p \leq 0.05$)

4. Discussion

4.1 Prevalence

The overall prevalence of bovine babesiosis in the present study was found to be 1.36%. Similarly, lower prevalence rates of 1.56% were observed by Singh *et al.* (2012) [37] and Sharma *et al.* (2016) [34]. Kumar *et al.* (2016) [20] reported a prevalence of 54.0%; Guswanto *et al.* (2017) [14] noted 27.1% seroprevalence; Siddique *et al.* (2020) [36] reported 26.86%; Haidy *et al.* (2023) [15] reported 13.88%; and Aziz *et al.* (2025) [3] reported a prevalence of 14.5%. The lower prevalence in the present study may be attributable to the assessment of a smaller number of samples presented to VCC, Veterinary College, Bidar.

The gender-wise point prevalence study revealed a higher prevalence in female cattle (53.84%) compared to male cattle (46.15%), in accordance with Hamsho *et al.* (2015) [16], Mahmud *et al.* (2015) [23], Zia and Nazir (2019) [42], and Mushahary *et al.* (2020) [27]. This difference is strongly correlated with the physiological stress of reproduction and lactation. However, Casa *et al.* (2025) noted maximum cases in male cattle, and Aziz *et al.* (2025) [3] highlighted the importance of considering local geographical factors, specific parasite species, and management systems while assessing disease risk.

The age-wise point prevalence study revealed the highest prevalence in animals under 2 years, in accordance with Mahmud *et al.* (2015) [23], Hussain *et al.* (2017) [18], Bary *et al.* (2018) [6], and Aziz *et al.* (2025) [3], while Siddique *et al.* (2020) [36] reported higher prevalence in adult cattle. The highest prevalence in young animals may be attributed to immunological immaturity.

The breed-wise prevalence study revealed that non-descript cattle comprised the majority of cases (69.23%), while crossbred HF cattle comprised 30.77%, yielding a breed-specific prevalence of 14.33% in crossbreds. Higher susceptibility of exotic/crossbred cattle is largely attributable to their lack of natural resistance to tropical ticks and pathogens when introduced into endemic areas (Hussain *et al.*, 2017; Bary *et al.*, 2018; Mushahary *et al.*, 2020) [18, 6, 27]. Anbu *et al.* (2020) [2] reported higher prevalence (5.55%) in non-descript animals exposed to high tick challenge environments, which can override genetic resistance.

The season-wise prevalence study revealed the highest prevalence during the rainy season, in agreement with Bhatnagar *et al.* (2015) [7], Parmar and Upadhyay (2017) [30],

Barman *et al.* (2018) [5], and Kumar *et al.* (2018) [21]. High humidity and moderate-to-high temperatures during this period are ideal for tick hatching, development, and questing behaviour. Cold temperatures in winter slow tick metabolic activity and prolong the *Babesia* life cycle, leading to a significant drop in disease transmission (Bhatnagar *et al.*, 2015; Kumar *et al.*, 2018) [7, 21].

4.2 Haematological findings

Values of haemoglobin, total erythrocyte count, and packed cell volume were significantly decreased in babesiosis-affected cattle compared to healthy controls, consistent with anaemia. These findings accord with Mervat *et al.* (2013) [24], Mahmoud *et al.* (2015) [22], Tufani *et al.* (2015) [40], El-Hamed *et al.* (2016) [11], Aziz *et al.* (2020) [4], and Charaya *et al.* (2021). In babesiosis, major alteration in total RBC osmotic fragility causes even intact unaffected red cells to become easily destroyed, creating haemolysis (Kakoma and Ristic, 1984) [19].

No significant differences were observed in MCV, MCH, and MCHC values in babesiosis-affected cattle compared to the healthy control group, in accordance with El-Hamed *et al.* (2016) [11] and Ganguly *et al.* (2017) [13].

There was a significant decrease ($p \leq 0.05$) in platelet values in babesiosis-affected cattle. Similar findings were reported by Tufani *et al.* (2015) [40], Noha *et al.* (2016) [29], Hashem *et al.* (2018) [17], Haidy *et al.* (2023) [15], and Roopali *et al.* (2025) [31]. The thrombocytopenia observed may be attributed to babesiosis-associated immune thrombocytopenia (Narurkar *et al.*, 2017) [28].

There was a significant decrease ($p \leq 0.05$) in total leukocyte count in babesiosis-affected cattle, similar to findings of El-Hamed *et al.* (2016) [11], Noha *et al.* (2016) [29], Sharma *et al.* (2016) [34], and Hashem *et al.* (2018) [17]. No significant differences were observed in differential leukocyte parameters (neutrophils, lymphocytes, monocytes, eosinophils, and basophils).

4.3 Biochemical findings

There was a significant decrease in total protein and globulin levels in cattle presenting with babesiosis, as reported by El-Hamed *et al.* (2016) [11], Noha *et al.* (2016) [29], Hashem *et al.* (2018) [17], Aziz *et al.* (2020) [4], and Roopali *et al.* (2025) [31], attributable to the harmful effect of toxic metabolites of *Babesia* species on liver cells (Mohamed Saied, 2017) [26]. Albumin showed no significant

difference, in accordance with Shinde *et al.* (2019) [35] and Aziz *et al.* (2020) [4]. The A:G ratio was significantly elevated, reflecting complex factors including severe liver dysfunction, dehydration, or co-infections.

Significant elevation in BUN and creatinine was observed, consistent with El-Hamed *et al.* (2016) [11], Ganguly *et al.* (2017) [13], Sivajothi *et al.* (2024) [38], and Roopali *et al.* (2025) [31]. Harmful effects of the parasite's toxic metabolites on kidney cells lead to azotaemia. Degraded byproducts of damaged RBCs deposited in the kidneys reduce creatinine excretion (Talkhan *et al.*, 2010) [39].

Significant elevation in total bilirubin was observed, consistent with multiple prior reports (El-Hamed *et al.*, 2016; El-Diasty *et al.*, 2017; Aziz *et al.*, 2020; Sivajothi *et al.*, 2024) [11, 10, 4, 38]. The increase in total bilirubin results from major alteration in total RBC osmotic fragility causing haemolysis (Kakoma *et al.*, 1984) [19].

Significantly increased values of AST, ALT, and ALP were observed in the present investigation, consistent with Samad *et al.* (2011) [32], El-Hamed *et al.* (2016) [11], Sharma *et al.* (2016) [34], Hashem *et al.* (2018) [17], Haidy *et al.* (2023) [15], Sivajothi *et al.* (2024) [38], and Roopali *et al.* (2025) [31]. The harmful effect of toxic metabolites of *Babesia* spp. on hepatocytes leads to cellular necrosis and elevated serum transaminase and ALP activities (Yeruham *et al.*, 2003; Esmailnejad *et al.*, 2021) [41, 12].

5. Conclusion

The present study confirms the endemic nature of bovine babesiosis in and around Bidar district, Karnataka. Prevalence was highest in female cattle and in the age group of less than 2 years, followed by 2 to 6 years and above 6 years. Prevalence was higher in non-descript cattle and was highest during the rainy season. Haematological investigation showed significant decrease in haemoglobin, total erythrocyte count, packed cell volume, platelets, and total leukocyte count. Biochemical parameters showed significant increase in A:G ratio, BUN, creatinine, total bilirubin, AST, ALT, and ALP, with significant decrease in total protein and globulin in affected cattle.

6. Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing of this manuscript.

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