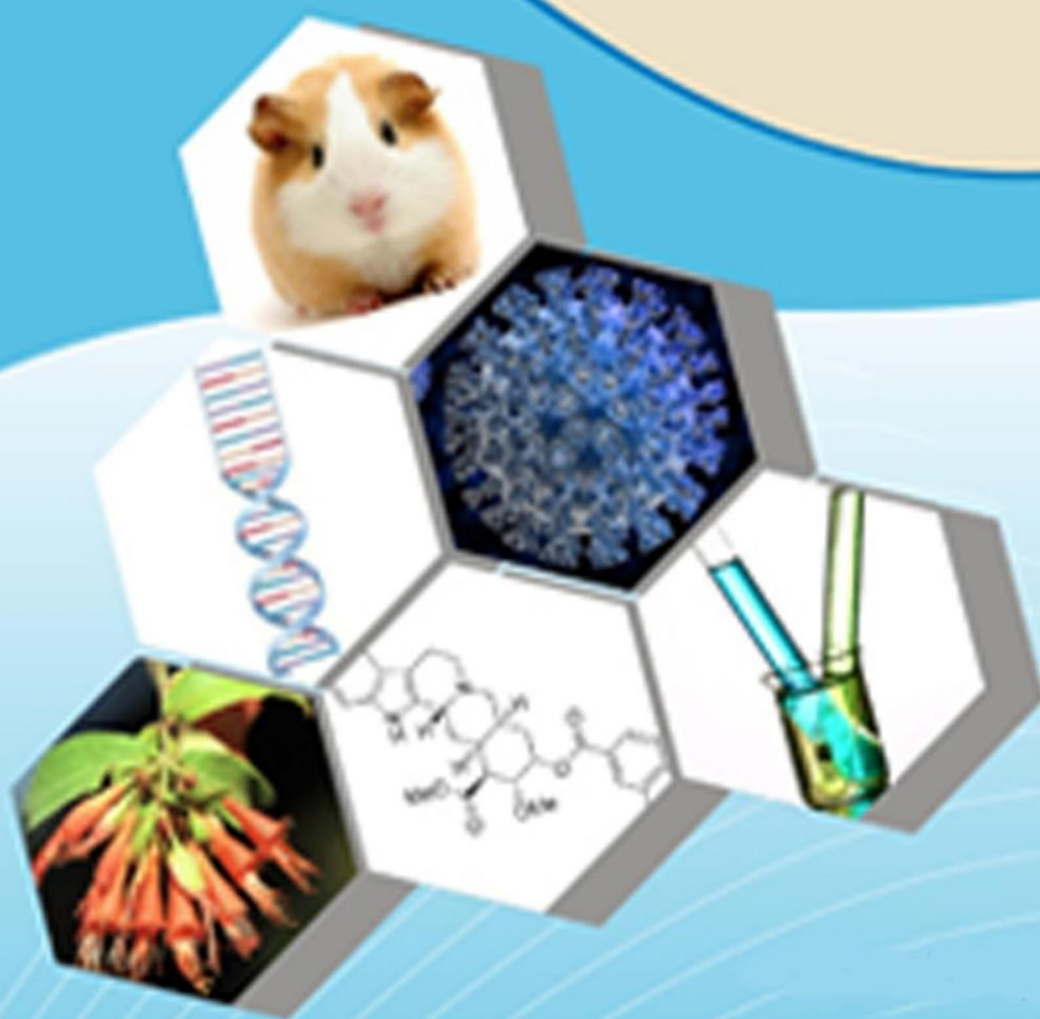




ISSN : 2347-2251

**Indo-American Journal of
Pharma and Bio Sciences**



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Malian babesia bovis and bigemina prevalence as a function of ecoclimatic zone variation

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Received on: 03 Apr 2025 Accepted Date: 10 May 2025 Published on: 25 June 2025

Abstract

Research in Mali's Sudano-Guinean, Sudanian, and Sahelian ecoclimatic zones occurred between June 2019 and September 2020. Babesia bovis and Babesia bigemina prevalence in these zones was the target of this study. The parasites Babesia bigemina and Babesia bovis, as well as the ticks that carry them, were investigated in a sample of 648 cattle (327 females and 321 males). Among the 648 calves tested, 200 tested positive for Babesia bovis, for a total prevalence rate of 30.86 percent; 156 tested positive for Babesia bigemina, for a total prevalence rate of 24.07%, as shown by microscopic analysis of the blood smears. The incidence of Babesia bovis varied significantly between ecoclimatic zones, according to the current research ($p < 0.05$). However, there was no statistically significant difference ($p > 0.05$) across zones when it came to the prevalence of Babesia bigemina. The prevalence of Babesia bovis and Babesia bigemina did not vary significantly between males and females ($P > 0.05$). Additionally, there was no significant difference in the prevalence of Babesia bovis and Babesia bigemina across the various age groups ($p > 0.05$). According to the research, the Sahelian zone does not have any Boophilus microplus species.

Topics: Mali; Babesia bovis; Babesia bigemina; prevalence.

1. Introduction

With a herd of 11,758,377 cattle and 43,494,990 small ruminants, Mali is the top livestock producer in the UEMOA area. It ranks third in exports, behind only cotton and gold, and adds 15.2% to GDP. A key barrier to the expansion of livestock husbandry in general has always been animal illnesses. A significant position is held by ticks and illnesses transmitted by ticks, most notably bovine babesiosis. R. (B.) microplus ticks are responsible for an estimated US\$160 million in annual economic losses in southern Africa [5]. The most common tick-borne livestock illnesses in sub-Saharan Africa are babesiosis, anaplasmosis, theileriosis, and cowdriosis (heart water), which result in significant economic losses [6]. In the West African Economic and Monetary Union (UEMOA) area, Mali is the top livestock producer with a herd of 11,758,377 cattle and 43,494,990 small ruminants; yet, there has been a dearth of research on bovine babesiosis in the nation. It ranks third in exports, behind only cotton and gold, and adds 15.2% to GDP. A key barrier to the expansion of livestock husbandry in general has always been animal illnesses. Ticks and illnesses transmitted by ticks are among these diseases, most notably Babesiosis parasites and vectors have been the primary foci of research. While the District of Bamako had a serological prevalence of 38.1% for Babesia bigemina, the Sikasso area had a highest serological prevalence of 57.5% for this parasite species, according to serological studies conducted in the 1980s [15]. At the same time, three tick species were found to be the only ones in Mali that could transmit bovine babesiosis. Boophilus geigy, R. annulatus, and R. decoloratus were the species in question. At the same time, R.(B) microplus joined the three tick species previously identified as bovine babesiosis vectors in Mali. The new tick species, Babesia bovis, is native to South Asia. It wasn't until 2013 that it was discovered in Mali's Sikasso area. The R. (B) microplus tick is notorious for being both an invasive vector and an acaricide superbug [17]. Therefore, this tick species poses a significant risk to cattle husbandry in the sub-region of West Africa. Research on Babesia bovis and its vectors in Mali was necessary due to the worry of the country's veterinary services over the possibility of a novel and more hazardous bovine babesiosis vector than those previously identified at the national level. So, to better understand the state of Babesia bovis and Babesia bigemina babesiosis in Mali, we decided to conduct surveys. Specifically, we conducted surveys in the Sudano-Guinean, Sudanian, and Sahelian zones of Mali to ascertain the vectors and incidence of babesiosis.

2. Materials and methods

2.1. Materials

2.1.1. Biological material

Blood and ticks collected from cattle are the main biological material.

2.2. Method

2.2.1. Characteristics of the study area

The study took place in three ecoclimatic zones: the Sahelian zone, the Sudanian zone and the Sudano-Guinean zone.

The Diema circle, which represents the Sahelian zone, is located in the Kayes region. This circle has the characteristics of the Sahel, with shrub-savanna vegetation cover and average rainfall of 550 to 750 mm (distributed over 45 to 55 days), with varying degrees of fluctuation from one year to the next.

The circle of Dioïla represents the Sudanian zone. It is located in the center of southern Mali. Formerly part of the Koulikoro region (Mali's second-largest administrative region), it is now part of the Dioïla region [11].

Vegetation cover is more or less dense and varied (Sudanian savannah) [2,12]. Annual rainfall is 700 mm in the north and almost 1000 mm in the south.

2.2.2. Site selection

A preliminary field visit carried out to conduct interviews with the heads of veterinary services in the various project zones. This visit also used to select the communes, villages and herds studied.

The sampling plan was as follows:

- 3 Cercles: Diema, Dioïla, Kadiolo ;
- 2 Communes chosen per Cercle, and 3 villages chosen per Commune (i.e., a total of 18 villages);
- 3 cattle sampled per herd of at least 50 head, for a total sample of 648 cattle for the entire study.

2.2.3. Survey type and period

This is a cross-sectional survey conducted over the period from June 2019 to September 2020.

2.2.4. Sample size, blood sampling and tick collection

A total of 648 cattle were used as a sample in this investigation. The components of this figure are as follows: There are 216 cattle in each ecoclimatic zone, 108 in each commune, 36 in each hamlet, and 3 in each herd. During the rainy season (June 2019–September 2020), blood samples and tick collections were taken from cattle as the dry season came to a close. Animal blood collected from the jugular vein. The blood was collected into EDTA-containing vacuum Vacutainer tubes using a sample needle. There was a unique identifier for each tube as well as the date of collection and the place of collection. A refrigerator with ice cubes was used to keep the tubes with the collected blood after they had been put on a rack. Blood samples were taken from animals after ticks were collected. Seven anatomical regions—"ear," "head-neck," "dorsal region," "abdomen-legs," "ano-genital region," "tail," and "feet"—were used to gather ticks from the animal's flanks. Ticks were then kept in ethanol solutions maintained at 70°C using Falcon tubes. Like the tube with the blood sample, the one with the ticks was labeled with the site code, animal identification number, and collection date. All of them were taken from the same animal. Before being transported to the lab, the tubes containing ticks are packed in a tube crate.

2.2.5. Questionnaire

A questionnaire administered to herd owners at each site visited. The questions concerned the type of farming, herd size, prophylaxis and cross-border transhumance.

2.3. Experimental protocol

At the Central Veterinary Laboratory in Bamako, smears were prepared on glass slides with a margin at one end. Smears fixed in methanol solution for 5 minutes, then stained in Rapid GIEMSA solution for 5 minutes. They washed under a stream of tap water and dried. After receiving 2 or 3 drops of immersion oil, the dried smears were placed under the " objective X100 binocular electric microscope for *Babesia bovis* and *Babesia bigemina* in red blood cells.

Ticks collected during both seasons identified and counted by species at the Central Veterinary Laboratory in Bamako. Identification made using an electric binocular magnifier. Ticks placed in Petri dishes under the magnifying lens. The key of WALKER and al ^[19] used to identify the ticks. Tick identification focused on the search for the vectors *Babesia bovis* and *Babesia bigemina*. Identification based primarily on morphological characteristics. After identification, ticks not carrying *Babesia bovis* and *Babesia bigemina* placed in vials containing 70°C alcohol. These vials placed in tick boxes and stored at the "Tick and tick borne diseases" (TMT) laboratory for posterity. Tick vectors *Babesia bovis* and *Babesia bigemina* counted by species. After counting, the numbers entered into the database. The ticks returned to the vials containing the 70°C alcohol solution and stored in the tick boxes.

2.4. Data analysis

The raw database entered using Microsoft EXCEL 2010 and analyzed using Stata version 12.1. The "chi 2" test used to compare the different variables, notably the prevalence of *Babesia bovis* and *Babesia bigemina*. The variables compared were *Babesia bovis* and *Babesia bigemina* prevalence rates in the three zones, and as a function of cattle age and sex.

3. Results

3.1. Prevalence of *Babesia bovis* in ecoclimatic zones

200 cattle tested positive for *Babesia bovis* out of 648 examined in the ecoclimatic zones. A sample of 216 cattle tested in each ecoclimatic zone. 100 cattle tested positive for *Babesia bovis* in the Sudano-Guinean zone, compared with 89 in the Sudanian zone and 11 in the Sahelian zone. In descending order, the prevalence rates recorded were 46.29% in the Sudanoguinian zone, 41.20% in the Sudanian zone and 5.09% in the Sahelian zone (Table 1). Statistical analysis revealed a significant difference between the three zones, ($P < 0.05$).

3.2. Prevalence of *Babesia bigemina* in ecoclimatic zones

156 cattle were positive for *Babesia bigemina* out of 648 examined in the ecoclimatic zones. In the Sudanoguinian zone, 51 cattle tested positive for *Babesia bigemina*, compared with 55 positive cases in the Sudanian zone and 50 in the Sahelian zone. The prevalence rates recorded were, in descending order, 25.46% in the Sudanian zone, 23.51% in the Sudanoguinian zone and 23.14% in the Sahelian zone (Table 2). Statistical analysis revealed no significant difference between the three zones ($P > 0.05$).

Table 1 Variation in the prevalence of *Babesia bovis* in different ecoclimatic zones

Ecoclimatic zones	Negative	Positive	Total	Prevalence (%)	p-value
Sahelian	205	11	216	5.09	0.00
Sudanese	127	89	216	41.20	
Sudano-Guinean	116	100	216	46.29	
Total	448	200	648	30.86	

Pearson chi2 = 45.5462 Pr = 0.000

Table 2 Variation in the prevalence of *Babesia bigemina* in different ecoclimatic zones

Ecoclimatic zones	Negative	Positive	Total	Prevalence (%)	p-value
Sahelian	166	50	216	23.14	0.83
Sudanese	161	55	216	25.46	
Sudano-Guinean	165	51	216	23.51	
Total	492	156	648	24.07	

Pearson chi2 = 0.3546 Pr = 0.838

3.3. Prevalence of *Babesia bovis* by sex of cattle

The prevalence rate of *Babesia bovis* was higher in male cattle (32.08%, 103/321) than in female cattle (23.54%, 77/327). Statistical analysis of the results showed no significant difference between *Babesia bovis* prevalence rates in male and female cattle ($P > 0.05$), (Table 3).

3.4. Prevalence of *Babesia bigemina* by sex of cattle

The highest *Babesia bigemina* prevalence rate recorded in male cattle (24.61%, 79/321) and the lowest in females (23.54%, 77/327). Analysis of the results revealed no significant difference between the prevalence rates of *Babesia bigemina* in male and female cattle ($P > 0.05$), (Table 4).

Table 3 Variation in the prevalence of *Babesia bovis* according to the sex of the cattle

Sexe des bovins	Negative	Positive	Total	Prevalence (%)	p-value
Female	230	97	327	29.66	0.50
Male	218	103	321	32.08	
Total	448	200	648	30.86	

Pearson chi2 (1) = 0.4459 Pr = 0.504

Table 4 Variation in the prevalence of *Babesia bigemina* according to the sex of the cattle

Sexe des bovins	Negative	Positive	Total	Prevalence (%)	p-value
Female	250	77	327	23.54	0.75
Male	242	79	321	24.61	
Total	492	156	648	34.07	

Pearson chi2 (1) = 0.1002 Pr = 0.752

3.5. Prevalence of *Babesia bovis* according to age of cattle.

The highest prevalence of *Babesia bovis* in cattle of different age groups was recorded in adults (33.33%, 43/129), followed by calves (30.91%, 98/317) and bulls/heifers (29.20%, 59/202). Analysis of the results revealed no statistically significant difference ($p > 0.05$) between the prevalence of *Babesia bovis* in cattle of different age groups, (Table 5).

3.6. Prevalence de *Babesia bigemina* according of cattle.

The highest prevalence of *Babesia bigemina* in cattle of different age groups was recorded in adults (25.58%, 33/129), followed by calves (24.29%, /77317). And bull calves/heifers (22.77 %, 46/202). Analysis of the results revealed no statistically significant difference ($p > 0.05$) between the prevalence of *Babesia bigemina* in cattle of different age groups, (Table 6).

Table 5 Variation in *Babesia bovis* prevalence as a function of cattle age

Age of cattle	Negative	Positive	Total	Prevalence (%)	p-value
Adult	86	43	129	33.33	0.73
Taurillon/Genisse	143	59	202	29.20	
calves/Velles	219	98	317	30.91	
Total	448	200	648	30.86	

Pearson chi2(2) = 0.6286 Pr = 0.730

Table 5.1 Variation in *Babesia bigemina* prevalence as a function of cattle age

Age of cattle	Negative	Positive	Total	Prevalence (%)	p-value
Adult	96	33	129	25.58	0.83
Taurillon/Genisse	156	46	202	22.77	
calves/Velles	240	77	317	24.29	
Total	492	156	648	24.07	

Pearson chi2 (2) = 0.3557 Pr = 0.837

3.7. Abundance of *Babesia bovis* and *Babesia bigemina* tick vectors in ecoclimatic zones

1,310 ticks of all species collected. Of these, 9727 ticks carrying *Babesia bovis* and *Babesia bigemina*, belonging to four species, identified. These were *Rhipicephalus* (*Boophilus*) *microplus* (3,766 individuals), *R.(B) annulatus* (2,747 individuals), *R.(B) geigy* (2,567 individuals) and *R.(B) decoloratus* (647 individuals). The different species of tick's vectors of *Babesia bovis* and *Babesia bigemina* identified in the three-ecoclimatic zones. The study revealed that the *Boophilus microplus* species is absent from the Sahelian zone. (Tables).

3.7.1. Abundance of *Boophilus microplus* in ecoclimatic zones

The overall abundance of *Boophilus microplus* in all ecoclimatic zones is 33.29% (3766/11310). The Sudano-Guinean zone came out on top with an abundance rate of 44.44% (2364/5319). This zone followed by the Sudanian zone, with an abundance of 32.48% (1402/4316) of the *R. microplus* species. This tick is absent from the Sahelian zone (Table 6).

Table 6 *Boophilus microplus* abundance in ecoclimatic zones

Ecoclimatic zone	<i>Boophilus microplus</i>	Total tick's	Abondances (%)
Sahelian	0	1675	0.00
Soudanese	1402	4316	32.48
Sudano-Guinean	2364	5319	44.44
Total	3766	11310	33.29

3.7.2. Abundance of *Boophilus annulatus* in ecoclimatic zones

The overall abundance of *Boophilus annulatus* in the ecoclimatic zones was 24.28% (2747/11310). The Sudano-Guinean zone was more dominant than other zones, with an abundance of 27.99% (1489/5319). This zone followed in order by the Sudanian zone: 21.17% (914/4316) and the Sahelian zone: 20.53%. (344/1675).

Table 6a *Boophilus annulatus* abundance in ecoclimatic zones

Ecoclimatic zone	<i>R. annulatus</i>	Total ticks	Abundance (%)
Sahelian	344	1675	20.53
Soudanese	914	4316	21.17
Sudano-Guinean	1489	5319	27.99
Total	2747	11310	24.28

3.7.3. Abundances of *Boophilus geigy* in ecoclimatic zones

The overall abundance of *Boophilus geigy* in all ecoclimatic zones is 22.69% (2567/11310). The Sahelian zone has the highest abundance of this tick, at 34.98% (586 /1675). It followed by the Sudanian zone: 29.12% (1257/4316) and the Sudano-Guinean zone: 13.61% (724/5319). (Table 7).

Table 7 *Boophilus geigy* abundance in ecoclimatic zones

Ecoclimatic zone	<i>Boophilus geigy</i>	Total ticks	Abundance (%)
Sahelian	586	1675	34.98
Soudanese	1257	4316	29.12
Soudano-guinean	724	5319	13.61
Total	2567	11310	22.69

3.7.4. Abundances of *Boophilus decoloratus* in ecoclimatic zones

The *Boophilus decoloratus* tick had an overall abundance of 5.72% (647/11310) in all ecoclimatic zones. This species was most abundant in the Sahelian zone, with a rate of 20.65% (346/1675). In the Sudano-Guinean zone, the abundance of this tick was 3.25% (173/5319). The Sudanian zone recorded the lowest abundance of *Boophilus decoloratus* at 2.96% (128/4316),(Table 8).

Table 8 *Boophilus decoloratus* abundance in ecoclimatic zones

Ecoclimatic zones	<i>Boophilus. decoloratus</i>	Total ticks	Abundance (%)
Sahelian	346	1675	20.65
Soudanese	128	4316	2.96
Soudano-guinean	173	5319	3.25
Total	647	11310	5.72

Discussion

In every ecoclimatic zone that was examined, the hemoparasites *Babesia bovis* and *Babesia bigemina* were found. In all three ecoclimatic zones—the Sudano-Guinean zone, the Sudanian zone, and the Sahelian zone—were the four species of ticks that carried *Babesia bovis* and *Babesia bigemina*: *Boophilus microplus*, *Boophilus annulatus*, *Boophilus geigy*, and *Boophilus decoloratus*. *Boophilus microplus* ticks were not present in the Sahelian zone, however, that was the only exception. The fact that ADAKAL et al. [1] first described the *Boophilus microplus* tick in southern Mali in 2013 explains its presence in the Sudanian zone. The Republic of Côte-d'Ivoire is believed to have been the entry point for these cattle, which are believed to have originated in southern Mali (particularly the cercle de Dioïla) via transhumance. The lack of *Boophilus microplus* in the Sahelian zone could be explained by the fact that cattle from this region often go to Mali's "Baoule" river valley or the Islamic Republic of Mauritania, neither of which have reported

cases of the parasite. In Mali, TEEL and colleagues found three bovine babesiosis vectors—*Boophilus annulatus*, *Boophilus geigy*, and *Boophilus decoloratus*—but our findings contradict theirs [18]. The prevalence percentage of *Babesia bovis* in the Sudanoguinian zone was 46.29%. Based on climate, this finding is similar to those of DJAKARIDJA et al. [4] and YEO et al. [20], who discovered 45.2% and 45.83% of *Babesia bovis* in the northern Côte d'Ivoire zone, respectively. This zone is similar to the Cercle de Kadiolo. *Boophilus microplus*, the principal *Babesia bovis* vector, explains why the Sudanoguinian and Sudanian zones have such a high *Babesia bovis* prevalence [13]. *Babesia bovis* prevalence rates in West Africa have been shown to range from 13.16% to 59.33% in other research. (4, 7). Also falling within this range was the total prevalence rate of *Babesia bovis* (30.86%) that our research documented.

Babesia bovis prevalence rates in male and female cattle were not significantly different according to the statistical analysis ($P > 0.05$). Similar tendencies were noted by DJAKARIDJA and colleagues [4], who demonstrated that there is no statistical evidence to support the idea that sex plays a role in the frequency of hemoparasitosis. Consistent patterns were found by FETHU et al. [9] and BIHONEGN et al. [3]. Nonetheless, a notable disparity in the prevalence rates of *Babesia bovis* and *Babesia bigemina* in male and female cattle was discovered by Hamsho and colleagues [10] ($P < 0.05$). *Babesia bigemina* was found in 24.07% of the population in our research. *Babesia bigemina* has a prevalence of 26.7% in Côte d'Ivoire, as shown in a research by MISHRA and colleagues [16]. *Babesia bigemina* was more common (57%), according to a different research in northern Benin (FAROUGOU et al., 2007). We found a higher prevalence of *Babesia bigemina* than YEO et al. [20], Hamsho et al. [10], and Bihonegn et al. [3], who found prevalences of 13.61%, 7%, and 0.248%, respectively.

Babesia bigemina prevalence rates in male and female cattle were not significantly different ($P > 0.05$), according to the data. Previous research by BIHONEGN et al. [3], DJAKARIDJA et al. [4], FAROUGOU et al. [7], and others reached similar conclusions.

Babesia bovis and *Babesia bigemina* prevalence in cattle of various ages did not vary significantly ($p > 0.05$), according to the data. Both YEO and colleagues [20] and Fethu and colleagues [9] found that the incidence of *Babesia bovis* and *Babesia bigemina* parasites was unaffected by age ($P > 0.05$). According to research carried out in northern Benin, FAROUGOU and colleagues [8] discovered that hemoparasite infestation was higher in adult cattle compared to young calves ($p < 0.05$).

4. Conclusion

The present study determined the prevalence of the hemoparasites *Babesia bovis* and *Babesia bigemina* in three ecoclimatic zones of Mali, namely the Sahelian zone, the Sudanian zone and the Sudano-Guinian zone. The study also enabled us to establish a link between the prevalence of the hemoparasites studied and their vectors. This enabled us to establish that the higher the prevalence of vectors, the higher the prevalence of *Babesia bovis* and *Babesia bigemina*. This factor enables decision-makers to develop a control strategy for these vectors, with a view to reducing the risks associated with the appearance of babesiosis in bovine herds.

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