

## Impact of ecoclimatic zone variation on the prevalence of *Babesia bovis* and *Babesia bigemina* in Mali

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### Abstract

The study took place from June 2019 to September 2020 in the Sudanoguinian, Sudanian and Sahelian ecoclimatic zones of Mali. Its aim was to determine the prevalence of *Babesia bovis* and *Babesia bigemina* in each of these zones. A sample of 648 cattle, including 327 females and 321 males, examined for *Babesia bigemina* and *Babesia bovis* parasites and their vectors, using blood and tick sampling. Microscopic examination of the blood smears revealed the presence of the *Babesia bovis* parasite in 200 cattle out of 648 examined, i.e. an overall prevalence rate of 30.86%, and the presence of *Babesia bigemina* in 156 cattle, i.e. an overall prevalence rate of 24.07%. The present study showed a significant difference in *Babesia bovis* prevalence between ecoclimatic zones ( $p < 0.05$ ). On the other hand, statistical analysis of *Babesia bigemina* prevalence results revealed no significant difference ( $p > 0.05$ ) between zones. There was no significant difference between male and female prevalence of *Babesia bovis* and *Babesia bigemina* ( $P > 0.05$ ). The same was true for the prevalence of *Babesia bovis* and *Babesia bigemina* observed in the different age groups, ( $p > 0.05$ ). The study revealed that the *Boophilus microplus* species is absent in the Sahelian zone.

**Keywords:** Prevalence; *Babesia Bigemina*; *Babesia bovis*; Mali.

### 1. Introduction

Mali is the leading livestock producer in the UEMOA (West African Economic and Monetary Union) region, with a herd of 11,758,377 cattle and 43,494,990 small ruminants. It contributes 15.2% to the gross domestic product and is the third largest export after gold and cotton. Animal diseases have always been a major constraint to the development of livestock farming in general. Among these pathologies, ticks and tick-borne diseases, notably *bovine babesiosis*, occupy an important place. Annual economic losses caused by the R. (B.) *microplus* tick alone in southern Africa estimated at US\$160 million <sup>[5]</sup>. In sub-Saharan Africa, *babesiosis*, *anaplasmosis*, theileriosis and cowdriosis (heart water) are the main tick-borne livestock diseases, causing major economic losses <sup>[6]</sup>. Very few studies have been carried out on bovine babesiosis in Mali is the leading livestock-producing country in the UEMOA (West African Economic and Monetary Union) region, with a herd of 11,758,377 cattle and 43,494,990 small ruminants. It contributes 15.2% to the gross domestic product and is the third largest export after gold and cotton. Animal diseases have always been a major constraint to the development of livestock farming in general. Among these pathologies, ticks and tick-borne diseases, notably Studies have focused on the parasites responsible for babesiosis and on the vectors. Serological surveys carried out during the 1980s revealed that *Babesia bigemina* was the dominant parasite species in Mali, with a maximum serological prevalence of 57.5% for *Babesia bigemina* in the Sikasso region, compared with 38.1% in the District of Bamako <sup>[15]</sup>. During the same period, three tick species identified as the sole vectors of bovine babesiosis in Mali. These were *Rhipicephalus (Boophilus) geigy*, R (B) *annulatus* and R. (B) *decoloratus*. During the same period, a new tick, R.(B)

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*microplus*, added to the three tick species already known to be vectors of bovine babesiosis in Mali. This new tick originates from South Asia, where *Babesia bovis* is the dominant species. It first identified in Mali in the Sikasso region in 2013. The R. (B) microplus tick known to be the most invasive vector and the most resistant to all conventional acaricides available on the market [17]. This tick species is therefore a serious threat to cattle farming in the West African sub-region. The concern of Mali's veterinary services about the threat of a new, more dangerous vector of bovine babesiosis than those already known at national level prompted the need to undertake research on *Babesia bovis* and its vectors in Mali. As a result, we decided to carry out surveys to improve our knowledge of the situation of *Babesia bovis* babesiosis and *Babesia bigemina* babesiosis in Mali. More specifically, our surveys aimed to determine the prevalence of babesiosis and its vectors in the Sudano-Guinean, Sudanian and Sahelian zones of Mali.

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## 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 cercle 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

The sample size for the present study was 648 cattle. This figure breaks down as follows: 216 cattle per ecoclimatic zone, 108 cattle per Commune, 36 cattle per village and 3 cattle per herd. Blood sampling and tick collection from cattle were carried out at the end of the dry season and during the rainy season between June 2019 and September 2020. Blood drawn from the jugular vein of cattle. The blood drawn into vacuum Vacutainer tubes containing EDTA anticoagulant using a sampling needle. Each tube was marked with the site code, animal identification number and date of collection. The tubes containing the collected blood were placed in a rack and then stored in a cooler containing ice cubes. Tick collections carried out on cattle from which blood drawn. Ticks were collected from both sides of the animal in 7 predefined anatomical regions: "ear", "head-neck", "dorsal region", "abdomen-legs", "ano-genital region", "tail" and "feet". Ticks then preserved in Falcon tubes containing a 70°C ethanol solution. The tubes containing ticks collected from the same animal were marked with the site code, animal identification number and collection date corresponding

to the tube containing the blood sample. The tubes containing ticks then placed in a tube crate for transport to the laboratory.

#### 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 <sup>[19]</sup> 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 | Abundances (%) |
|------------------|----------------------------|--------------|----------------|
| Sahelian         | 0                          | 1675         | 0.00           |
| Soudanese        | 1402                       | 4316         | 32.48          |
| Sudano-Guinean   | 2364                       | 5319         | 44.44          |
| <b>Total</b>     | <b>3766</b>                | <b>11310</b> | <b>33.29</b>   |

#### 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         |
| <b>Total</b>     | <b>2747</b>         | <b>11310</b> | <b>24.28</b>  |

### 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         |
| <b>Total</b>     | <b>2567</b>            | <b>11310</b> | <b>22.69</b>  |

### 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          |
| <b>Total</b>      | <b>647</b>                    | <b>11310</b> | <b>5.72</b>   |

## 4. Discussion

Overall, the results show that the hemoparasites *Babesia bovis* and *Babesia bigemina* are present in all the ecoclimatic zones studied. The four species of tick vectors of *Babesia bovis* and *Babesia bigemina* - *Boophilus microplus*, *Boophilus annulatus*, *Boophilus geigy* and *Boophilus decoloratus* - were found in all three ecoclimatic zones (Sudano-Guinean zone, Sudanian zone and Sahelian zone). The only exception was the absence of the *Boophilus microplus* tick in the Sahelian zone. The presence of the *Boophilus microplus* tick in the Sudanian zone can be explained by the fact that it was first reported in southern Mali in 2013 by ADAKAL and al <sup>[1]</sup>. This introduction into the country is thought to have taken place via cross-border transhumance of cattle herds from southern Mali (including cattle from the cercle de Dioïla) to the Republic of Côte-d'Ivoire. The absence of *Boophilus microplus* in the Sahelian zone may be due to the fact that *cattle* herds in this area generally transhumate to the Islamic Republic of Mauritania, or to the "Baoule" river valley within Mali, where the presence of *Boophilus microplus* has not yet been reported either in Mauritania or in the "Baoule" river valley. Our results differ from those obtained by TEEL and colleagues in Mali <sup>[18]</sup>, who described the presence of three vectors of bovine babesiosis (*Boophilus annulatus*, *Boophilus geigy* and *Boophilus decoloratus*).

The *Babesia bovis* prevalence rate (46.29%) recorded in the Sudanoguinian zone. This result is comparable than obtained by DJAKARIDJA and al [4] and YEO and al [20], who respectively found a 45.2% and 45.83% of *Babesia bovis* in the northern Côte d'Ivoire zone, more or less comparable to the Cercle de Kadiolo from a climatic point of view. The high prevalence of *Babesia bovis* in the Sudanoguinian and Sudanian zones can be explained by the presence of *Boophilus microplus*, the main vector of *Babesia bovis* [13]. Other studies have obtained *Babesia bovis* prevalence rates ranging from 13.16% to 59.33% in West Africa. [4,7]. The overall *Babesia bovis* prevalence rate recorded in our study (30.86%) also fell within this range.

Statistical analysis of the results showed no significant difference between *Babesia bovis* prevalence rates in male and female cattle ( $P > 0.05$ ). The same trends observed by DJAKARIDJA and al [4], who showed that the influence of sex on the prevalence of haemoparasitosis is not statistically proven. Similar trends obtained by FETHU and al [9], BIHONEGN and al [3]. However, Hamsho and al [10] found a significant difference between the prevalence rates of *Babesia bovis* and *Babesia bigemina* in male and female cattle ( $P < 0.05$ ). The overall prevalence of *Babesia bigemina* was 24.07% in our study. In a study conducted in Côte d'Ivoire, MISHRA and al [16] obtained a similar prevalence for *Babesia bigemina* (26.7%). In another study conducted in northern Benin, FAROUGOU and al [7] found a higher prevalence of *Babesia bigemina* (57%). Our figures are higher than those obtained by YEO and al [20], Hamsho and al [10] and Bihonegn and al [3], who obtained prevalences of 13.61%, 7% and 0.248% respectively for *Babesia bigemina*.

Analysis of the results revealed no significant difference between the prevalence rates of *Babesia bigemina* in male and female cattle ( $P > 0.05$ ). Similar results obtained by BIHONEGN, al [3], DJAKARIDJA, al [4], FAROUGOU, and al [7].

Analysis of the results revealed no statistically significant difference ( $p > 0.05$ ) between the prevalence of *Babesia bovis* and *Babesia bigemina* in cattle of different age groups. Similar results were obtained by YEO and al [20] and Fethu and al [9], who showed that the age factor had no influence on the prevalence of *Babesia bovis* and *Babesia bigemina* parasites ( $P > 0.05$ ). In a study conducted in northern Benin, FAROUGOU and al [8] found that adult cattle were more infested with hemoparasites than young cattle ( $p < 0.05$ ).

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## 5. 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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## Compliance with ethical standards

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### Disclosure of conflict of interest

Modibo DIAKITE coordinated the design and planning of the study, the implementation of the laboratory analyses and the writing of the article; Brahim SACKO, and Sekouba BENGALY, participated in the design, planning and implementation of the study; Amadou SERY participated in the statistical analysis of the data.

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