Prevalence of *Babesia bovis* and *Babesia bigemina* in Mali

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Abstract

This study, carried out between June 2019 and September 2020 in the localities of Kadiolo, Dioïla and Diéma, aimed to determine the prevalence of *Babesia bovis* and *Babesia bigemina*. It involved blood analysis of 648 cattle samples (327 females and 321 males) and tick collection for *Babesia bigemina* and *Babesia bovis* parasites and their vectors. During the dry season, 72 cattle tested positive for *Babesia bovis* out of a total of 324 examined, giving a prevalence rate of 22.22%. On the other hand, during the rainy season, 128 cattle were positive for *Babesia bovis* out of a total of 324 examined, for a prevalence rate of 39.50%. The study revealed that the vectors of *Babesia bigemina* and *Babesia bovis* are more abundant during the rainy season than in the dry season.

Keywords: *Babesia bigemina*; *Babesia bovis*; Prevalence; Mali

1. Introduction

Malian livestock production contributed around 15.2% to the country’s gross domestic product in 2013[3]. Animal pathologies have always been a major constraint on the development of livestock farming in general. Among these pathologies, ticks and tick-borne diseases occupy an important place. In sub-Saharan Africa, babesiosis, anaplasmosis, theileriosis and cowdriosis (heartwater) are the main tick-borne livestock diseases, causing major economic losses [5]. Very few studies have been carried out on bovine babesiosis in Mali. The studies carried out have mainly focused on the parasites responsible for babesiosis and on the vectors. Serological surveys carried out during the 1980s revealed that *B. bigemina* was the dominant parasite species in Mali, with a maximum serological prevalence of 57.5% for *B. bigemina* in the Sikasso region, compared with 38.1% in the District of Bamako [11]. During the same period, three tick species were identified as the only vectors of bovine babesiosis in Mali. These are Rhipicephalus (Boophilus) geigyi, R.(B) annulatus and R.(B) decoloratus. Recent entomological studies have described the presence of a new tick, R.(B) microplus, which has joined the three tick species already known to be vectors of bovine babesiosis in Mali. This new tick originates from South Asia, where *B. bovis* is the dominant species. It was first identified in Mali in the Sikasso region in 2013. The R. (B) microplus tick is known to be the most invasive vector and the most resistant to all conventional acaricides available on the market [13]. However, very few data are available on the seasonal prevalence of *Babesia bovis* and *Babesia bigemina* in 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 localities: the cercles of Kadiolo, Dioïla and Diéma. The cercle covers an area of 6,640 km² and borders Burkina Faso and Côte d'Ivoire. It comprises 120 villages grouped into 9 communes, including the urban commune of Kadiolo. The cercle of Kadiolo has a particularly agropastoral rural economy, with cotton cultivation playing an important role in the household economy. The climate is Sudano-Guinean, with dry and rainy seasons. It is one of Mali's wettest circles, with an average annual rainfall of 841.4 mm and an average annual temperature of 27.6°C. Vegetation is of the wooded savannah and gallery forest type [9].

The cercle of Dioïla is located in the center of southern Mali, and used to be part of the Koulikoro region (Mali's second-largest administrative region), but is now part of the Dioïla region. The population is 95% agro-pastoral. The climate is humid tropical Sudanian. It is marked by a dry season from March to June, a rainy season from June to October and a cold season from November to February. Vegetation cover is more or less dense and varied (Sudanian savannah) [1, 10]. Annual rainfall is 700 mm in the north and almost 1000 mm in the south.

The Diéma district is located in the Kayes region. The Diéma district is typical 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 [12].

2.2.2. Sampling and collection of samples
Two Communes were chosen per Cercle, and 3 villages per Commune. Then 2 3 cattle were sampled per herd of at least 50 head.

The sample size for this study was 648 cattle. This figure breaks down as follows: 216 cattle per agro-ecological zone, 108 per Commune, 36 per village and 3 cattle per herd. Blood sampling and tick collection were carried out at the end of the dry season and during the rainy season. Blood was drawn from the jugular vein of the cattle. Blood was drawn into vacuum Vacutainer tubes containing EDTA anticoagulant. 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 were carried out on cattle from which blood had been 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 were then preserved in Falcon tube 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 were then placed in a tube crate for transport to the laboratory.

2.3. Experimental protocol
At the Laboratoire Central Vétérinaire in Bamako, smears were prepared on glass slides with a margin at one end. The smears were fixed in methanol solution for 5 minutes, then stained in Rapid GIEMSA solution for 5 minutes. They were then 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 X100 objective of a binocular electric microscope to test for Babesia bovis and Babesia bigemina in red blood cells.

Ticks collected during both seasons were identified and counted by species at the Laboratoire Central Vétérinaire in Bamako. Identification was made using an electric binocular magnifier. Ticks were placed in Petri dishes under the magnifying lens. The key of WALKER et al [15] was used to identify the ticks.
Tick identification focused on the search for the vectors *Babesia bovis* and *Babesia bigemina*. Identification was based primarily on morphological characteristics. After identification, ticks not carrying *Babesia bovis* and *Babesia bigemina* were placed in vials containing 70°C alcohol. These vials were then placed in tick boxes and stored at the "Tiques et Maladies Transmises par les Tiques" (TMT) laboratory for posterity. Tick vectors *Babesia bovis* and *Babesia bigemina* were counted by species. After counting, the numbers were entered into the database. The ticks were returned to the vials containing the 70°C alcohol solution and stored in the tick boxes.

2.4. Data analysis

Data were entered using Microsoft EXCEL 2010 and analyzed using Stata version 12.1. The "chi2" test was used to compare the different variables.

3. Results

The prevalence of *Babesia bovis* and *Babesia bigemina* varied in the three circles according to the season, age and sex of the cattle.

3.1. Seasonality of *Babesia bovis*

During the dry season, 72 cattle were positive for *Babesia bovis* out of a total of 324 examined, giving a prevalence rate of 22.22%. In contrast, during the rainy season, 128 cattle were positive for *Babesia bovis* out of a total of 324 examined, giving a prevalence rate of 39.50%. The difference between the dry and rainy seasons was statistically significant using the chi2 test (chi2= 45.5462, p<0.05). The prevalence of *Babesia bovis* therefore shows a decreasing variation from dry to rainy season (Table 1).

Table 1 Seasonal variations in the prevalence of *Babesia bovis*

<table>
<thead>
<tr>
<th>Season</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
<th>Prevalence (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry season</td>
<td>252</td>
<td>72</td>
<td>324</td>
<td>22.22</td>
<td>0.00</td>
</tr>
<tr>
<td>Rainy season</td>
<td>196</td>
<td>128</td>
<td>324</td>
<td>39.50</td>
<td></td>
</tr>
</tbody>
</table>

Pearson chi2(2) = 45.5462 Pr = 0.000

3.1.1. Seasonality of *Babesia bigemina*

The study showed that out of a total of 324 cattle examined during the dry season, 64 were positive for *Babesia bigemina*, giving a prevalence rate of 19.75%. On the other hand, 92 cattle were positive for *Babesia bigemina* out of a total of 324 examined during the rainy season, for a prevalence rate of 28.39%. The difference observed between the dry and rainy seasons was statistically significant using the chi2 test (chi2= 6.6191, p<0.05). The prevalence of *Babesia bigemina* therefore shows a decreasing variation from dry to rainy season (Table 2).

Table 2 Seasonal variations in the prevalence of *Babesia bigemina*

<table>
<thead>
<tr>
<th>Season</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
<th>Prevalence (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry season</td>
<td>260</td>
<td>64</td>
<td>324</td>
<td>19.75</td>
<td>0.01</td>
</tr>
<tr>
<td>Rainy season</td>
<td>232</td>
<td>92</td>
<td>324</td>
<td>28.39</td>
<td></td>
</tr>
</tbody>
</table>

Pearson chi2(1) = 6.6191 Pr = 0.010

3.2. Seasonality of *Babesia bovis* and *Babesia bigemina* tick vectors

A total of 1,310 ticks of all species were collected. Of these, 9727 *Babesia bovis* and *Babesia bigemina* vector ticks belonging to four species were identified. These were Rhipicephalus (*Boophilus*) microplus (3,766 individuals), R.(B) annulatus (2,747 individuals), R.(B) geigyi (2,567 individuals) and R.(B) decoloratus (647 individuals). All tick species carrying *Babesia bovis* and *Babesia bigemina* were identified in both seasons. The study revealed that individuals of all four species are more abundant in the rainy season than in the dry season (Tables 3, 4,5 and 6).
Table 3 Seasonal abundance of *R. microplus*

<table>
<thead>
<tr>
<th>Season</th>
<th><em>R. microplus</em></th>
<th>Total tiques</th>
<th>Abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry season</td>
<td>471</td>
<td>3958</td>
<td>11.89</td>
</tr>
<tr>
<td>Rainy season</td>
<td>3295</td>
<td>7352</td>
<td>44.81</td>
</tr>
<tr>
<td>Total</td>
<td>3766</td>
<td>11310</td>
<td>33.29</td>
</tr>
</tbody>
</table>

Table 4 Seasonal abundance of *R. annulatus*

<table>
<thead>
<tr>
<th>Saisons</th>
<th><em>R. decoloratus</em></th>
<th>Total tiques</th>
<th>Abondances (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry season</td>
<td>197</td>
<td>3958</td>
<td>4.97</td>
</tr>
<tr>
<td>Rainy season</td>
<td>450</td>
<td>7352</td>
<td>6.12</td>
</tr>
<tr>
<td>Total</td>
<td>647</td>
<td>11310</td>
<td>6.65</td>
</tr>
</tbody>
</table>

Table 5 Seasonal abundance of *R. geigyi*

<table>
<thead>
<tr>
<th>Season</th>
<th><em>R. annulatus</em></th>
<th>Total tiques</th>
<th>Abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry season</td>
<td>527</td>
<td>3958</td>
<td>13.31</td>
</tr>
<tr>
<td>Rainy season</td>
<td>2220</td>
<td>7352</td>
<td>30.19</td>
</tr>
<tr>
<td>Total</td>
<td>2747</td>
<td>11310</td>
<td>24.28</td>
</tr>
</tbody>
</table>

Table 6 Seasonal abundance of *R. decoloratus*

<table>
<thead>
<tr>
<th>Season</th>
<th><em>R. geigyi</em></th>
<th>Total tiques</th>
<th>Abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry season</td>
<td>647</td>
<td>3958</td>
<td>16.34</td>
</tr>
<tr>
<td>Rainy season</td>
<td>1920</td>
<td>7352</td>
<td>26.11</td>
</tr>
<tr>
<td>Total</td>
<td>2567</td>
<td>11310</td>
<td>26.39</td>
</tr>
</tbody>
</table>

4. Discussion

*Babesia bigemina* and *Babesia bovis* prevalence rates were significantly higher in the rainy season than in the dry season in the study area. This could be explained by a variation in rainfall and vegetation cover in the same direction, which are important factors for tick survival and development. Similar results were obtained by YEO et al. [16] who revealed that the prevalence rates of *Babesia bovis* and *Babesia bigemina* during the rainy season are higher than during the dry season. The same trends were obtained by DJAKARIDJA et al. [4] who showed that the prevalence rate of *Babesia bovis* is higher during the rainy season than in the dry season. Similar results to ours were also obtained by BIPIN et al. [2] who showed that the prevalence of *Babesia bigemina* is higher during the rainy season. The study revealed that vectors are more abundant during the rainy season than in the dry season for *Babesia bovis* and *Babesia bigemina*. The same trend was observed by FAROUGOU et al. [7], TAKELE et al. [14] who recorded high tick infestations during the period of heavy rains.

5. Conclusion

The results of the study showed that the prevalence rates of *Babesia bigemina* and *Babesia bovis* hemoparasites are higher in the rainy season than in the dry season. Similarly, the vectors of these parasites were found to be more abundant in the rainy season than in the dry season.
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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 Sakho, Aliou Traore and Sekouba Bengaly participated in the design, planning and implementation of the study; Amadou Sery participated in the statistical analysis of the data.

Références

[1] AQUASTAT, 2005


