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Epidemiological Situation of Bovine Haemoparasites in the Peri-Urban Area of Bouaké in Côte d'Ivoire

Laciné Kalo Bamba [1], Nawolo Yéo [2,*], Gouagoua Severin Kouadja [1], Adam Camille Kouamé [1], Kouakou Eugene Kouadio [1] and Kouabena Kreman [1]

[1] Livestock Production Research Station, Livestock Production Program, National Center for Agricultural Research (CNRA), 01 BP 633 Bouaké, Côte d'Ivoire

[2] Biological Sciences Training and Research Unit, Department of Animal Biology, Université Peleforo Gon COULIBALY, BP 1328, Korhogo, Côte d'Ivoire.

* Correspondence: yeonawolo81@gmail.com

Abstract

A parasitological study conducted in the peri-urban area of Bouaké, aims to control the main haemoparasites in cattle. A total of 240 blood samples were taken in 2021 from cattle of different breeds and age groups for the production of blood smears. These blood smears were fixed in 96% methanol for 3 to 5 minutes, then stained with diluted 10% Giemsa for 20 to 30 minutes and observed under a light microscope at immersion objective 100. Four species of haemoparasites specifically caused by ticks were observed: *Anaplasma marginale* (48.33%), *Theileria spp* (21.25%), *Babesia bigemina* (9.58%) and *Anaplasma centrale* (4.58%). *Trypanosoma spp* were not encountered. With the exception of *B. bigemina*, the statistical difference in prevalence was significant at the p-value threshold ≤ 0.05 at the sampling sites. In addition, *A. marginale* and *Theileria spp* differed significantly at p-value ≤ 0.05 according to season, with infection levels higher in the rainy season than in the dry season. Among cattle breeds, *Theileria spp* was more prevalent in the N'dama breed (28.8%). The difference in prevalence for this blood parasite is considered significant at p-value ≤ 0.05 at breed level. Further studies on haemoparasite vectors are needed for more effective integrated pest management.

Keywords: prevalence; haemoparasites; cattle; Bouaké; Côte d'Ivoire

Introduction

In Côte d'Ivoire, the livestock production sub-sector, contributing 4.5% to the agricultural GDP (and only 2% to the national GDP), occupies a marginal position in the Ivorian economy **(MIRAH, 2022)**. Animal production from these livestock systems remains underexploited. The deficits in meat and dairy products are estimated at 55.4% and 87.4%, respectively.

Like the major cities of sub-Saharan Africa, large Ivorian cities are experiencing rapid urbanization. The urbanization rate is 52% (INS, 2022) according to the 2021 general population and housing census. Peri-urban livestock farming occupies a key position in meeting the needs of urban populations for meat, dairy, and poultry products. Numerous food and health constraints limit the development of livestock production. Among these constraints, blood parasites, particularly those of the genera *Trypanosoma, Anaplasma, Babesia*, and *Theileria*, occupy a prominent place.

Hemoparasites constitute a major obstacle to the development and improvement of cattle breeding worldwide, particularly in Africa (Roy et al., 2018). Furthermore, hemoparasitoses of cattle can have a negative impact on animal health, the livestock industry and, sometimes, on humans. Parasitic diseases such as babesiosis, thelleriosis and anaplasmosis are widespread in tropical and subtropical regions, including Côte d'Ivoire. Infections can be fatal to livestock but are also known to cause fever, anorexia, jaundice, increased abortion rates and infertility (Abdallah et al., 2019). These hemoparasitoses caused by protozoa (*Theileria* and *Babesia*) and bacteria (*Anaplasma* / *Ehrlichia*)

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constitute a serious challenge for the health and welfare of livestock, particularly in tropical and subtropical regions.

To our knowledge, few previous reports illustrate the distribution and microscopic identification of hemoparasites in cattle in the city of Bouaké. In order to improve livestock productivity in peri-urban areas while effectively combating food insecurity and reducing economic losses caused by parasitic diseases, this study was initiated to inventory the different hemoparasites and assess their prevalence in cattle raised in parks located in the peri-urban area of Bouaké.

Materials and Methods

Study Areas

The study is carried out on cattle farms located in urban and peri-urban areas of Bouaké. Bouaké is located in the center of Côte d'Ivoire in the Gbêkê region on the major road and rail axis linking Abidjan to the north of the country **(Figure 1).**

With a population of 832,371 inhabitants (INS, 2021), Bouaké presents a forest-savannah transition zone (Sudanese-Guinean) characterized by temperatures oscillating around 27°C with differences of the order of 3 to 5°C. Average decadal evaporation varies between 35 and 55 mm during the rainy season and relative humidity fluctuates between 70 and 80%. The rainfall pattern is bimodal, with irregular precipitation reaching an average annual total of around 1,100 mm (Brou *et al*., 2018). Night parks located in the neighborhoods along the different corridors of the city were visited.

In each site, four farms were chosen for sampling. The sites are as follows:

- ✓ Site 1 located in the southern corridor (Air France 3, cemetery and Kongodekro);
- ✓ Site 2 located in the eastern corridor (Olienou, Belleville, and Kouassibilekro);
- ✓ Site 3 located in the western corridor (Tchelekro, CIDT estate, Adjeyaokro);
- ✓ Site 4 located in the North corridor (CNRA breeding station, Tollakouadiokro).

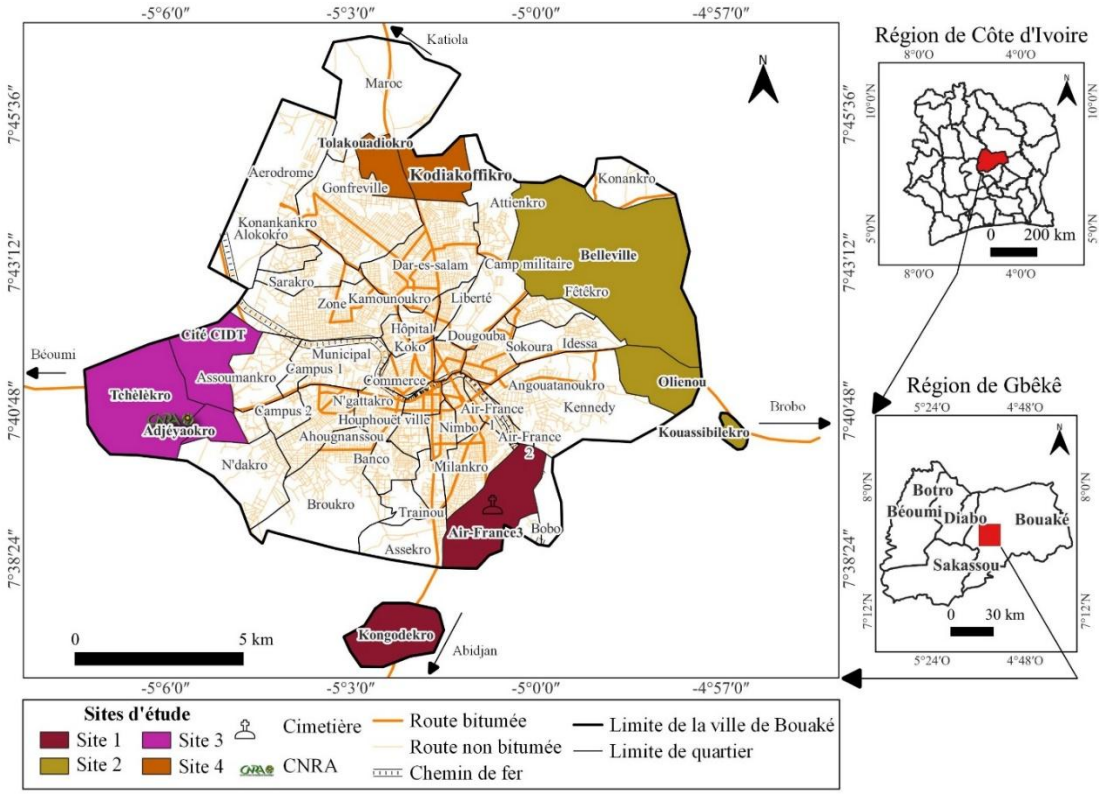

Figure 1. Location of the study area.

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Materials

Cattle of all ages, breeds and both sexes belonging to different age classes were sampled as shown in Table 1. Standard equipment for identification of blood parasites was used, which consisted of a light microscope, immersion oil, GIEMSA stain, microscope slides, 95% methanol, lancet, microcapillary tube, microscope slide and gloves.

Table 1. Distribution of the number of cattle collected from the collection sites according to sex, age classes and physiological status of the animals.

Collection Site	Sex		age Classes			Physiological status					
	M	F	JUV (≤ 1 year)	SAD (1-3 years)	ADU (≥ 3 years)	Vx	Ve	Tn	Gn	Tx	Vc
Southern corridor	20	40	14	20	26	9	5	7	13	4	22
Eastern corridor	20	40	10	21	29	6	4	10	11	4	25
West corridor	21	39	22	15	23	14	8	3	12	4	19
northern corridor	16	44	16	19	25	6	10	8	11	2	23
Total	**77**	**163**	**62**	**75**	**103**	**35**	**27**	**28**	**47**	**14**	**89**

M: male; F: female; JUV: juvenile; SAD: subadult; ADU: adult; Vx: calf; Ve: female calf; Tn: young bull; Gn: heifer; Tx: bull; Vc: cow.

Methods

The samples were collected in February and October, corresponding to the dry and rainy seasons respectively. For a more representative sample, 60 samples were taken per site. In total, 240 cattle were sampled, including 163 females and 77 males. One hundred and twenty (120) samples were taken during the dry season (February 2021) and 120 during the rainy season (October 2021). The age of the cattle ranged from 1 to 12 months for calves and she-calves, from 13 to 36 months for young bulls and heifers and from 37 months and over for bulls and cows. The distribution of cattle numbers collected at the collection sites is shown in **Table 1**.

Blood samples were collected from the ear vein. The animal is immobilized and the base of the ear is compressed. A lancet is then used to prick the ear and collect the blood sample. A drop of blood was then placed on one slide and another slide was beveled at a 30° angle. The beveled slide was then brought into contact with the drop of blood, and when it spread by capillary action and slid forward, the smear was applied to clean, dry slides. Smears were fixed in 95% methanol and stained with GIEMSA diluted to 10% in distilled water. The smears were then observed under a light microscope to detect different species of hemoparasites. Microscopic observation was carried out at 100x magnification using immersion oil and the results were recorded. After observing 3/4 of the observable fields, the smears were considered negative if no parasites were detected. All analyses were carried out at the Bouaké Regional Laboratory (LRB), a technical sub-unit of the National Laboratory for Agricultural Development Support (LANADA).

Statistical Analysis

The results were recorded using Excel software. A descriptive analysis was carried out to calculate the prevalences according to the following formula:

$$\textbf{Prevalence}\ (\%) = \frac{\textbf{Number of smears positive for a parasite}}{\textbf{Total number of smears examined}} \times \mathbf{100}$$

The Chi-square statistical test was performed using STATISTICA version 7.0 software to compare the prevalence of the different parameters studied, and the values obtained (percentages) were compared with the 5% threshold.

Results

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Parasitic Screening for Blood Parasites

Microscopic examination of the 240 samples revealed that 201 smears were positive for at least one blood parasite. The frequency of cattle infected with at least one blood parasite was 83.75%, while 42.91% of animals were free of any blood parasites. Figure 2 shows the prevalence of the different species of hemoparasites encountered in the study area. A total of 04 species belonging to 03 genera of blood parasites were identified. This is the genus *Anaplasma* with two species, *A. marginale* (48.33%) and *A. centrale* (4.58%); *Babesia* includes the species *B. bigemina* with a prevalence of 9.6%; and the genus *Theileria*. The prevalence of *Theileria spp* was estimated at 21.25%. Statistical analysis revealed a significant difference (P=0.0001) between the prevalences of these hemoparasites. Hemoparasites of the genus *Trypanosoma* were not encountered.

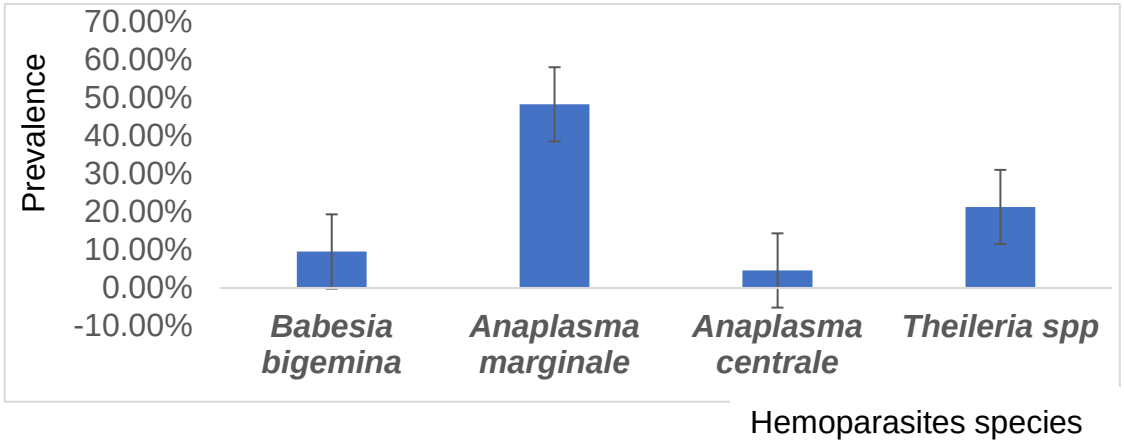

Figure 2. Prevalence of the different species of blood parasites encountered.

Prevalence of Parasites Identified According to the Sex of the Animal

The inventory of hemoparasite species in cattle was also carried out taking into account the sex of the animals. Of a total of 77 male subjects, the species *A. marginale*, *Theileria spp* and *B. bigemina* were the most prevalent. Their respective prevalences were 50.65%, 19.48% and 12.99%. *A. centrale* represented the lowest prevalence encountered (6.49%), with 5 infected male cattle out of 77 male subjects. Among the 163 females, *A. marginale, Theileria spp* and *B. bigemina* were the most numerically dominant species, with respective prevalences of 42.24%, 22.08% and 7.97%. *A. centrale* had the lowest infectivity (7.97%) in females (Figure 3). Overall, the Chi2 test showed that the presence of parasites was not related to sex (p>0.05).

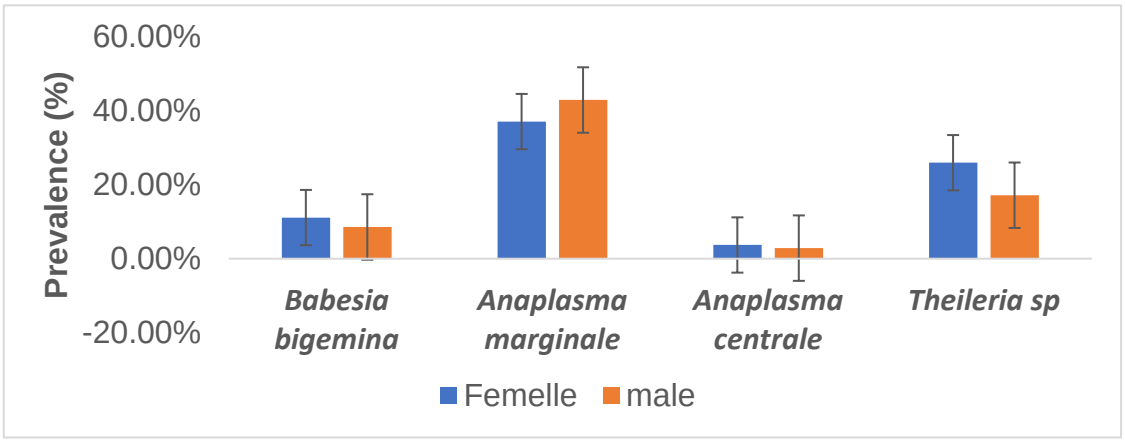

Figure 3. Variation in parasite prevalence according to the sex of the animal.

Prevalence of Parasite Species According to Animal Age Class

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Figure 4 shows the prevalence of hemoparasites encountered according to the age class of the cattle sampled. A. marginale infected more cattle identically regardless of their physiological stage followed by *Theileria spp*. *Babesia bigemina* and *A. centrale* weakly infected cattle. The prevalence of *A. marginale* infection in these categories of cattle was 51.46% in adults, 50.66% in juveniles and 40.32% in young cattle.

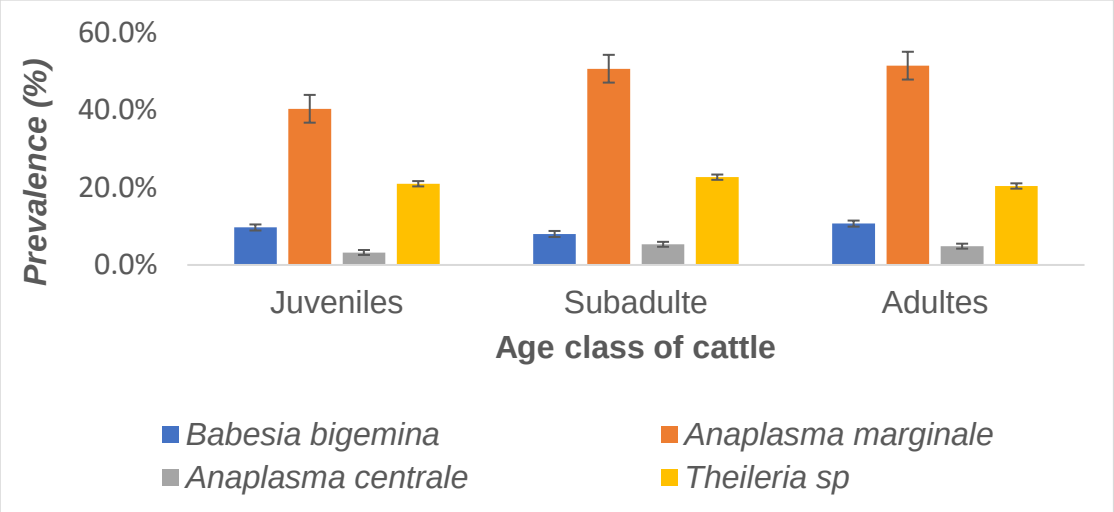

Figure 4. Prevalence of parasite species according to animal age class.

Parasite Prevalence According to the Physiological Stage Cattle

All blood parasites identified during this study were found in cows, with high prevalences of over 49.44% for *A. marginale*, 22.47% for *Theileria spp*., 7.86% for *B. bigemina*, and 5.61% for *A. centrale*. *A. centrale* was not encountered in heifers and bulls. The prevalence of parasites encountered according to the physiological stage of the cattle sampled are presented in Figure 5.

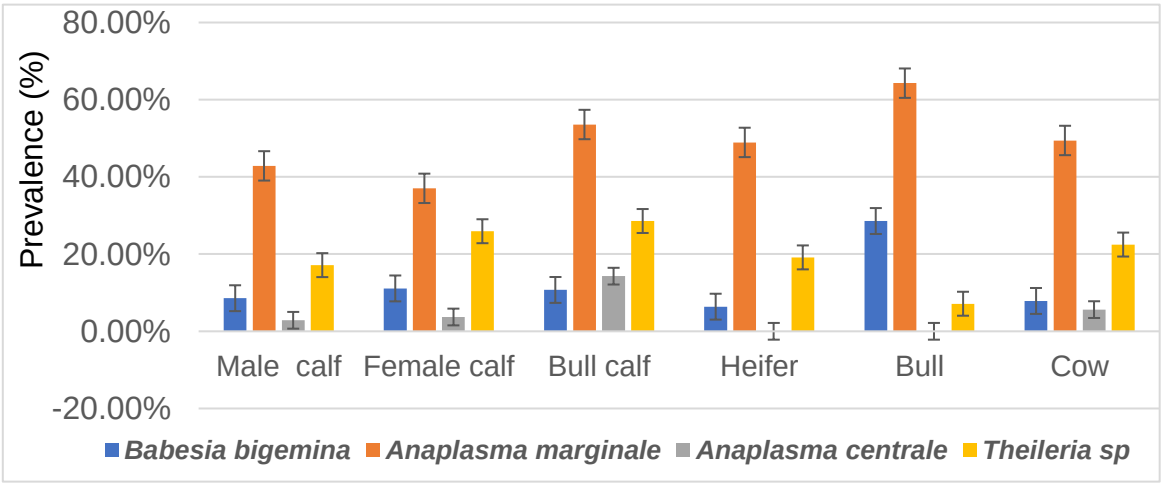

Figure 5. Variation in parasite prevalence according to the physiological stage of the cattle sampled.

Seasonal Variation in the Prevalence of Hemoparasites Found in Cattle

The influence of the season on the prevalence of hemoparasites was studied. The diversity of hemoparasite species found in cattle farms in the city of Bouaké during the rainy season and the dry season remains completely identical. Overall, a decreasing trend in the prevalence of all parasites during the dry season was observed (Figure 6) except for *A. centrale* and *B. bigemina*. *A marginale* responsible for bovine anaplasmosis recorded prevalences of 31.67% during the dry season against

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65% during the rainy season. Prevalences of 3.33% and 39.17% were recorded during the dry and rainy seasons respectively for *Theileria spp*.

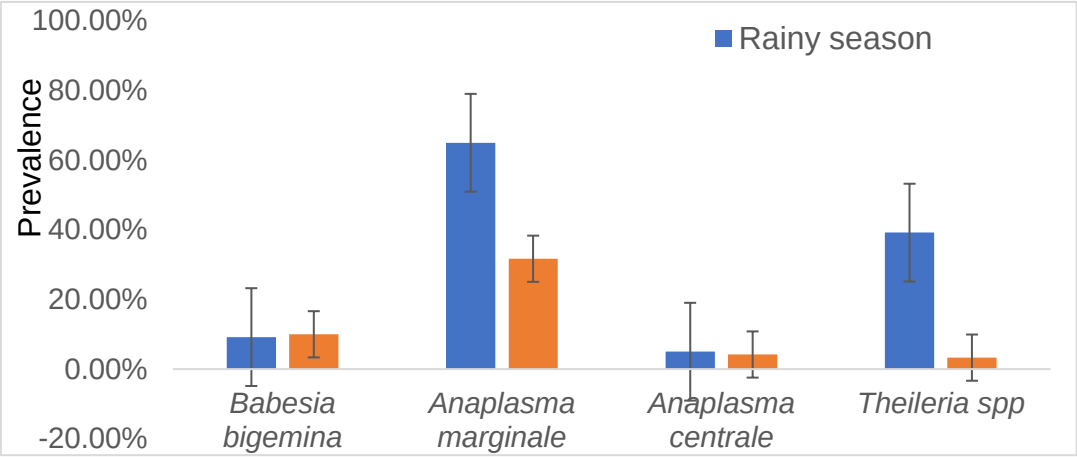

Figure 6. Variation in parasite prevalence depending on the sampling season.

Variation in the Prevalence of Hemoparasites Encountered Depending on the Sample Collection Site in Cattle

The overall prevalence of hemoparasites encountered in the different sampling sites is presented in Table 2. It appears that *A. centrale* was not encountered in the southern districts (Air France 3, cemetery and Kongodekro) and the western districts (Tchelekro, CIDT city and Adjeyaokro) of the city. *A. marginale* was found to be more infectious for cattle with a prevalence of 75% for the eastern districts (Olienou, Belleville and Kouassibilekro), 46.7% in cattle in the northern districts (CNRA station and Tollakouadiokro), 41.7% in the southern districts (Air France cemetery and Kongodekro) and 30% in the western districts (Tchelekro, CIDT city and Adjeyaokro). The prevalence of *A. marginale, A. centrale* and *Theileria spp* varied significantly depending on the area ($p < 0.05$).

Table 2. Variation in the prevalence of hemoparasites encountered according sampling site.

Hemoparasites species	Prevalence by collection site				Significability	X^2	P (%)
	Southern corridor	Eastern corridor	West corridor	Northern corridor			
B. bigemina	6,7 %^{a}	15,0 %^{a}	6,7 %^{a}	10,0 %^{a}	NS	3,222	0,00
A. marginale	41,7% ^{a}	75,0 %^{b}	30,0 % ^{c}	46,7 %^{d}	***	26,296	0,00
A. centrale	0,0 %^{a}	11,7 %^{b}	0,0 %^{c}	6,7 %^{d}	***	13,243	50,00
Theileria spp	16,7 %^{a}	31,7 %^{b}	6,7 %^{c}	30,0 %^{d}	***	15,014	0,00

*** : Significant difference; *NS : No* significant difference The letters **a, b** and **c** indicate a significant difference with a p-value less than or equal to 5%.

Variation in the Prevalence of Hemoparasites Encountered According to the Breed of Cattle Sampled

The influence of breed on the receptivity of hemoparasites was studied in the study area. Table 3 shows the prevalence of hemoparasites encountered according to the cattle breeds encountered. A. centrale and Theileria spp were not found in Méré cattle. A. marginale was encountered in all cattle breeds with prevalences of 51.25% in N'dama cattle, 48.48% in zebu cattle and 39.29% in Méré cattle. Comparison of prevalences according to breed revealed no significant difference for Anaplasma marginale, Anaplasma centrale and Babesia bigemina ($p > 0.05$). However, the prevalence of Theileria spp varied significantly depending on the breed ($p < 0.01$) with a high level for N'dama cattle (28.8%) compared to 21.2% for Zebu cattle.

Table 3. Variation in the prevalence of hemoparasites encountered depending on the bovine breed sampled.

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Hemoparasites species	Prevalence by cattle breed			Significability	X^2	P (%)
	Méré	N'dama	Zébu			
B. bigemina	14,29 %[a]	8,75 %[a]	9,09 %[a]	NS	0,816	16,7
A. marginale	39,29 %[a]	51,25 %[a]	48,48 %[a]	NS	1,192	0,00
A. centrale	0,0 %[a]	5,0 %[a]	5,30 %[a]	NS	1,1533	33,33
Theileria spp	0,0 %[a]	28,75 %[b]	21,21 %[c]	***	10,245	0,002

*** : Significant difference; NS : No significant difference The letters **a, b** and **c** indicate a significant difference with a p-value less than or equal to 5%.

Discussion

The study revealed the presence of hemoparasites of the genus *Babesia, Theileria* and *Anaplasma* in cattle farms in the peri-urban area of Bouaké in central Côte d'Ivoire. The presence of these three genera of parasites could be explained by the presence of vectors, particularly ticks, which carry these pathogens. These results corroborate those of Djakaridja et al. (2014) in the Bandama River Valley in central Côte d'Ivoire with similar prevalence rates.

Furthermore, among the 240 blood smears collected, none were positive for parasites of the genus *Trypanosoma*. The absence of parasites of the genus *Trypanosoma* could be explained by the absence in the city of Bouaké of islands of forest called "Bois sacré". These sites, which constitute true tsetse landmarks, contributed to maintaining an environment suitable for the survival of these trypanosome vectors. In addition, the samples were taken specifically in the peri-urban area of the city. In 2014, Djakaridja had not obtained parasites of the genus *Trypanosoma* (*T. vivax* and *T. brucei*). In the northern area of Côte d'Ivoire, on the other hand, the presence of parasites of the genus *Trypanosoma* was demonstrated by Yéo et al. (2017).

Of 240 smears collected, 116 smears were positive for *A. marginale*, *Theileria spp* (51), *B. bigemina* (23), and *A. centrale* (11).

In work on hemoparasites in the central zone, Djakaridja in 2014, observed 2 species of hemoparasites (*B. bovis* and *Anaplasma marginale*). In the northern zone, on the other hand, Yéo et al. (2017), observed in addition to the genus *Trypanosoma*, the species *A. marginale, A. centrale, Babesia bigemina, B.bovis*. These two studies do not take into account the presence of *Theileira spp* in their different investigation zones. In a nearly similar study on hemoparasites of cattle ticks in Benin in 2007, Farougou et al., observed 4 hemoparasites which are *Babesia bigemina, Theileria spp, Anaplasma marginale* and *Anaplasma centrale*. These authors concluded their work by designating ticks of the genus *Rhipicephalus (Boophilus)* as the main vectors of transmission of these blood parasites. Futse et al. (2003) designated ticks as the vector agent of parasites of the genus *Anaplasma*, a rikettsian pathogen of cattle. Among the pathogens isolated in this study, *Anaplasma marginale* was the most common. The prevalence of 48% observed in the present study. This prevalence is lower than the rate recorded in the central zone in 2014 by Djakaridja et al. This work had made it possible to isolate *A. marginale* and *B. bovis* with respective prevalences of more than 55% and 59% in the central areas of Côte d'Ivoire. Yeo et *al.* (2017) obtained prevalences of 85%, 51.67% and 93.33% respectively for the localities of Korhogo, M'bengue and Ferkessédougou in the north of Côte d'Ivoire. The prevalences observed in our study were significantly higher than those obtained by Farhan et al. (2012) and Komoin-Oka et al. (2004) in the central area of the humid savannah of Côte d'Ivoire. The observed prevalences would be around 9.71%. The high presence of *Anaplasma marginale* in this study can be explained by the massive presence of the vector tick *Rhipicephalus (Boophilus)* in livestock farms in the peri-urban area of Bouaké. This tick was identified by Achi et al. (2012) in livestock farms in northern Côte d'Ivoire. Madder et al. (2007) identified *Rhipicephalus (Boophilus) microplus* for the first time in southern Côte d'Ivoire. This tick is widely present in the city's livestock farms. The presence of the parasite of the genus *Babesia* could be explained according to Yeo et al. (2017b), by the presence of the tick species *Rhipicephalus (Boophilus) microplus.*

Although the sample cattle are present in the peri-urban area of Bouaké, the breeding system adopted in these different farms is of the traditional type. The breeders do not have artificial pastures

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to feed their cattle. Sokouri et *al.* (2014) estimated that 5% of breeders have artificial pastures in the northern area of Côte d'Ivoire. To feed their cattle, breeders all turn to the pasture areas scattered in and around homes. In the eastern and western districts of the city, it is not uncommon to see stray cattle grazing in the streets. Peri-urban grazing areas intended for feeding ruminants according to Kouadja et al. (2019), are gradually decreasing due to the rapid urbanization of the city of Bouaké. These grazing areas would represent areas favorable to the transmission of parasitoses according to Djakaridja et al. (2014). For Jorgensen et *al.* (1992), breeding methods are among the factors most influencing the prevalence of Tick-Transmitted Diseases or TTDs. The high recorded prevalence of *A. marginale* reflects the wide distribution of ticks, vectors of these parasites. The massive presence of *A. marginale* on all sites could be explained by a distribution of vector ticks on all the city corridors.

The use of acaricides is strongly recommended for breeders to combat ticks that carry these parasites. The high pressure of acaricides used in tick control in the sampled herds could explain the prevalences obtained. Indeed, regular tick removals carried out with appropriate products can significantly reduce ticks and, consequently, according to Djakaridja et *al.,* (2014). The use of inferior quality acaricides by breeders to combat ticks could explain, according to Yéo et *al.* (2017b), the prevalences observed as in the departments of Dikodougou and M'bengue in the north of Côte d'Ivoire.

During the present study, prevalences were not impacted by the sex of the cattle sampled. Djakaridja et al. (2014), and Komoin-Oka et al. (2004) made the same observation. For all the hemoparasites encountered, no difference in prevalence according to sex was highlighted. However, for Farougou et al. (2007), sex may constitute a risk factor because females would present a greater receptivity linked to their lower resistance during gestation and lactation.

Unlike *A. marginale* and *Theileria spp*, the prevalence of *A. centrale* and *B. bigemina* was not influenced by the seasons of sample collection. This could be explained by the dynamics of the different tick vectors of these parasites. Indeed, Knopf et al. (2004) showed that *Rhipicephalus (Boophilus) microplus* presents a fairly clear seasonality compared to *Rhipicephalus (Boophilus)*. Further studies, particularly on the seasonal dynamics of ticks in the peri-urban area of Bouaké, are necessary for more effective integrated control.

Conclusion

This study confirmed the presence of different species of blood parasites in livestock farms located in the peri-urban area of Bouaké in central Côte d'Ivoire. *Babesia bigemina* is responsible for babesiosis and *Anaplasma marginale* and *Anaplasma centrale* are the etiological agents of anaplasmosis. *Anaplasma marginale* is by far the most common in the city of Bouaké. A decreasing trend in the prevalence of all parasites during the dry season was observed except for *A. centrale* and *B. bigemina*. Parks located in the eastern districts of the city appear to be the most infected by anaplasmosis. Furthermore, all age groups are affected by blood parasites. It is important to undertake rational control measures against ticks, responsible for the transmission of all the parasites encountered. This will reduce parasite loads and improve the profitability of cattle farms in peri-urban areas. These measures must be combined with preventive and curative actions.

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