

Original Research Article

Prevalence of Major Haemoparasites in cattle at Department of Kounahiri, Côte d'Ivoire

ABSTRACT

Aims: This study was initiated to inventory the various haemoparasites and assess their prevalence in animals in this department.

Place and Duration of Study: This work took place from May 2020 to December 2022 in the department of Kounahiri.

Methodology: 400 randomly selected cattle were used for the samples. Using a lancet, the ear was pricked to draw blood while immobilizing the animal. A drop was then placed on a slide and another slide was beveled at an angle of around 30° and brought into contact with the drop of blood. The smear is then taken on clean, dry slides. The smears are fixed in 95% methanol and stained with Giemsa diluted 1:10 in distilled water, then observed under a light microscope.

Results: The results showed that three types of blood parasites were identified through microscopic observations. The respective infection rates were 87.25% for *Anaplasma*, 64% for *Babesia*, and 21.75% for *Theileria*. Among the observed species of blood parasites, *Anaplasma marginale* was the most widespread with a significant infection prevalence of 77.5% in cattle. Furthermore, within all age groups, this pathogen (*Anaplasma marginale*) had the highest infection rate. The respective prevalence rates were 73.58% in cattle aged between 01-12 months, 78.90% in those of 13-24 months, and 78.92% in cattle aged more than 25 months. No difference in infection rates was observed based on the age of the animals. Additionally, no difference was detected in the seasonal distribution of *Anaplasma marginale*. The infection prevalencies during the two seasons were 73% at the beginning of the rainy season and 82% at the beginning of the dry season.

Conclusion: It will be essential to promote training for livestock farmers in terms of livestock health management.

Keywords: Côte d'Ivoire, blood parasite, *Anaplasma marginale*, cattle, infection

1. INTRODUCTION

Tick-borne haemoparasites are a major obstacle to the development and improvement of cattle breeding worldwide, especially in Africa (1). In Kounahiri department, no studies have been conducted up to date to assess the prevalence of tick-borne haemoparasites of cattle. Thus, our study is the first to provide information on their prevalence, based on smear analyzed with parasitological methods. In addition, most of haemo parasitosis transmitted by ticks can have a negative impact on animal health, the livestock industry and, sometimes, on humans. Bovine blood parasitic diseases, such as babesiosis, theileriosis, and anaplasmosis, are widely distributed throughout tropical and sub-tropical regions including Côte d'Ivoire. Infections can be deadly to farm animals but are also known to be the cause of fever, anorexia, jaundice, increased abortion rates, and sterility (2). *Anaplasma marginale* infection can also lead to reduced milk production,

abortion in pregnant females and, in extreme cases, death of the animal (3). According to studies carried (4), ticks of the genus *Dermacentor*, *Ixodes*, *Amblyomma* and *Rhipicephalus* are important vectors that cause a great deal of damage to cattle in the Punjab region of Pakistan. This was the case in recent studies by (5), in which numerous cases of co-infection by *Babesia* spp, *Theileria* spp and *Anaplasma* spp were reported in cattle. A variety of parasite species belonging to these genera are common and widespread blood parasites worldwide, with significant economic, medical, and veterinary consequences (6). Although some are benign or low pathogenic, others can cause a wide range of symptoms and even death. A range of prevalence of the piroplasmids in different animals was recorded worldwide (7-8). Accordingly, there have been many reports of *Babesia* spp., *Theileria* spp., and *Anaplasma* spp. co-infections in cattle (5). They can be adversely impacting animal health, the livestock industry, and on occasion, human beings (2). These haemo parasitosis caused by protozoa (*Theileria* and *Babesia*) and bacteria (*Anaplasma/Ehrlichia*) pose a serious challenge to the health and well-being of livestock, particularly in tropical and subtropical regions. In Côte d'Ivoire, the distribution of hosts, pathogens and vectors overlap (9). As a result, a parasitological study was conducted throughout the country (Côte d'Ivoire) to map the distribution of tick-borne haemoparasites. Furthermore, three haemoparasites were identified in all livestock areas in Côte d'Ivoire, with very high prevalences for *A. marginale* and *B. bovis*. However, in a previous, we found low prevalences for these haemoparasites using light microscopy in three livestock areas (below 10%) (10). Furthermore, data on haemoparasites in Côte d'Ivoire are scarce and limited to a few departments within the different districts (11; 12-13). On the diagnostic front, microscopic examination of blood smear has been widely used to detect the piroplasm stage of parasites in erythrocytes (14). In order to ensure that cattle farming is conducted in a sensible and sustainable manner, it is therefore necessary that the diversity of *Anaplasma* and other blood parasites be documented for the control of the causative pathogens. To the best of our knowledge, fewer previous reports illustrate the distribution and microscopic identification of haemoparasites in cattle. In order to improve cattle productivity while effectively combating food insecurity and reducing economic losses caused by parasitic diseases, this study was initiated to inventory the various haemoparasites and assess their prevalence in animals in this department.

2. MATERIAL AND METHODS

2.1 Study site

This work took place from May 2020 to December 2022 in the department of Kounahiri in the Béré region. Kounahiri is located 520 km far away from Abidjan. It is located in the central-northern part of Côte d'Ivoire between 7°30' and 8°10' N latitudes and 5°40' and 6°20' W longitudes, covering an area of 2,110 km², with an estimated population of 101,111 inhabitants (15). The majority of the population is rural and engaged in agriculture. The department lies between the medium forest agro-climatic zones and the low forest zone (Figure 1).

from the auricular vein. Thus, the animal was immobilized and the base of the ear was compressed. A lancet was then used to prick the ear and collect the blood sample. The blood was collected using a microcapillary tube. A drop of blood is then placed on a slide and another slide is beveled at a 30° angle. The beveled slide is then brought into contact with the blood drop, and as it spreads by capillary action and slides forwards, the smear is applied to clean, dry slides. The smears were fixed in 95% methanol and stained with Giemsa diluted at 1/10 with distilled water. The smears were then observed under an optical microscope to detect *Anaplasma* and other erythrocytic forms of piroplasm. The microscopic observation was performed 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 Korhogo Regional Laboratory (LRK), a branch of the National Agricultural Development Support Laboratory (LANADA).

2.4 Statistical Analyses

The obtained results were recorded using Excel software. Descriptive analysis was conducted to calculate prevalences and frequencies for qualitative variables. Subsequently, the chi-square statistical test was performed using R software version 4.1.3 to compare the prevalences and frequencies of the different parameters studied, and the obtained values (percentages) were compared against the 5% threshold.

3. RESULTS AND DISCUSSION

3.1 Parasitological research of *Anaplasma* spp and other haemoparasites

Microscopic examination of 400 samples (Table 1) revealed that 386 smears were positive for at least one blood parasite. The overall prevalence of cattle infected with at least one blood parasite was 96.5%, while 3.5% of animals were free from any haemoparasite. A total of 05 species belonging to 03 genera of blood parasites were identified. These included the genus *Anaplasma* (87.25%) with two species, *A. centrale* and *A. marginale*, with respective proportions of 60.75% and 77.5%; *Babesia* (64%), which comprised the species *B. bovis* and *B. bigemina*; and the genus *Theileria* spp. The prevalences of *B. bovis*, *B. bigemina*, and *Theileria* spp were estimated at 55.75%, 26.5%, and 21.75%, respectively. The statistical analysis revealed a high significant difference ($P=0.0001$) among the prevalence of the haemoparasites.

Table 1: Prevalence of *Anaplasma* spp. and other blood parasites

Species and/or Genus of Parasites	Number of Parasitized Cattle (N=400)	Prevalence (%)	P-Value
<i>Anaplasma</i> spp	349	87.25% ^a	0.0001*
<i>Babesia</i> spp	256	64% ^b	
<i>Theileria</i> spp	87	21.75% ^c	
<i>Anaplasma centrale</i>	243	60.75% ^b	
<i>Anaplasma marginale</i>	310	77.5% ^a	
<i>Babesia bovis</i>	223	55.75% ^b	
<i>Babesia bigemina</i>	106	26.5% ^c	

Note: The values in the same column with different superscript letters significantly differ at the 0.05 threshold for each parameter. * highly significant

3.2 Prevalence of identified parasites by animal sex

The inventory of Anaplasma species and other associated blood parasites in cattle was also conducted, taking into account the sex of the animals. Among a total of 143 male subjects, the species *A. centrale*, *A. marginale*, and *B. bovis* were the most prevalent. Their respective prevalences were 62.24% (89/143), 76.22% (109/143), and 53.8% (77/143). Notably, the species *Babesia bigemina* represented the lowest prevalence (26.57%), with 38 infected male cattle out of the 143 male subjects. Among the 257 females, *A. centrale* and *A. marginale* were the most numerically dominant species, with prevalences of 78.2% and 59.9%, respectively. As for *Theileria* spp, it had the least infectivity (18.7%) in females (Figure 2). Overall, the chi-square test showed that the presence of parasites was not sex-related ($p>0.05$).

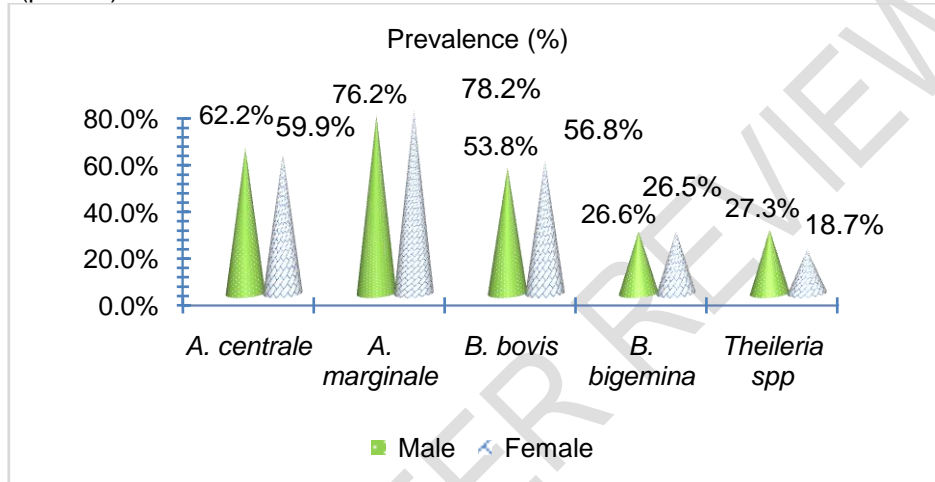


Figure 2: Variation of the prevalence of parasites by animal sex

3.3 Prevalence of parasite species according to the age of animals

The parasite *A. marginale* showed respective proportions of 73.58% (youngest age group), 78.89% (juveniles), and 78.91% (adults) across all age categories. Additionally, *A. centrale* also recorded significant prevalence rates, estimated at 54.71% in animals aged 01 to 12 months, 67.18% in those aged 13-24 months, and 59.63% in adults (25 months and older). Similarly, 55.66% of the youngest animals (01-12 months), 53.90% of juveniles (13-24 months), and 57.22% of animals aged 25 months and older were infected with *B. bovis*. In contrast to these pathogens, the prevalence of *B. bigemina* showed lower proportions among the youngest (22.64%), 31.25% among juvenile animals, and 25.30% in those aged 25 months and older. Likewise, *Theileria* spp. was less represented, with distinct prevalences of 12.26% (01-13 months), 24.21% (13-24 months), and 25.90% (25 months and older). The following graphs summarize and illustrate these details (Figure 3).

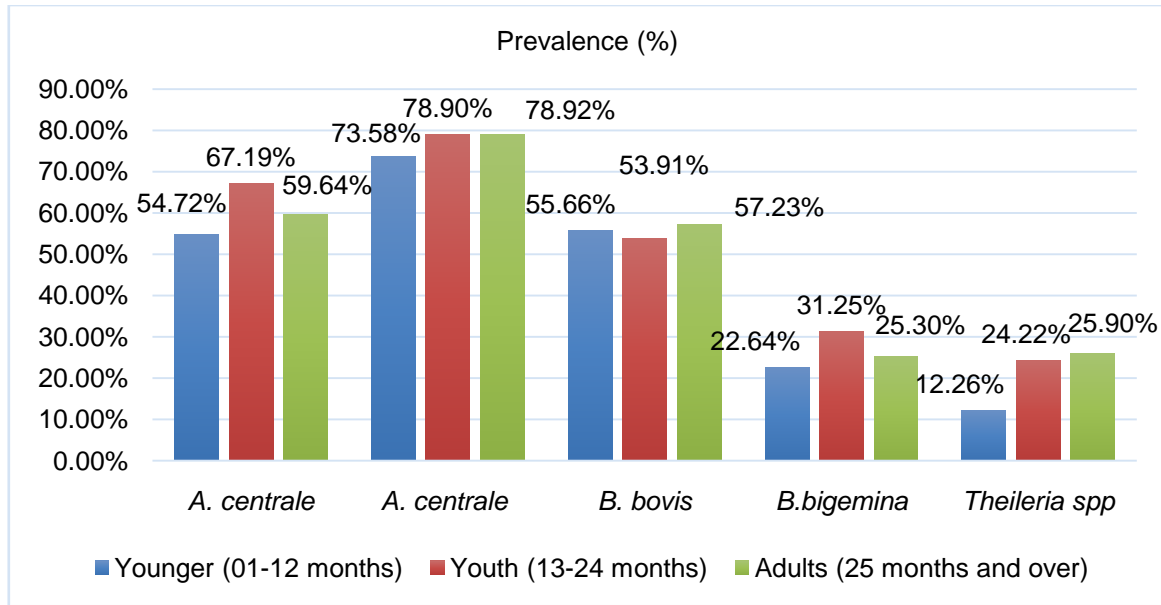


Figure 3: Prevalence of parasite species according to the age of animals

3.4 Prevalence of parasites according to the physiological stage of cattle

Anaplasma centrale was the most infective parasite in yearling bulls (70.58%), while it showed the lowest prevalence in calves (48.93%). As for the infection caused by *A. marginale*, it was highly significant at all stages, with a clear predominance in yearling bulls (77.77%). On the other hand, the haemoparasite *B. bovis* was more prevalent in cows, with a prevalence of 59.50% of infected cows. Additionally, heifers recorded a significant infection of *B. bigemina* with a proportion of 32.46%. Unlike other parasites, infections caused by *Theileria spp* were very low (Table 2). The statistical analysis showed a significant difference ($P=0.0001$) in parasite prevalence with respect to the physiological stage and sex.

Table 2: Prevalence of parasites according to the gender and physiological stage of cattle.

Physiological Stage	Different blood parasites observed					P-Value
	<i>Anaplasma Centrale</i>	<i>Anaplasma Marginale</i>	<i>Babesia Bovis</i>	<i>Babesia Bigemina</i>	<i>Theileria Spp</i>	
Calves N=47	48.93% ^a (23/47)	70.21% ^a (33/47)	51.06% ^a (24/47)	21.27% ^b (10/47)	10.63% ^b (5/47)	0.0001*
Female calves N=59	59.32% ^a (35/59)	76.27% ^a (45/59)	59.32% ^a (35/59)	23.72% ^b (14/59)	13.55% ^b (8/59)	0.0001*
Young bulls N=51	70.58% ^a (35/51)	80.39% ^a (41/51)	58.82% ^a (30/51)	29.41% ^b (15/51)	35.29% ^b (18/51)	0.0001*
Heifers N=77	64.93% ^a (50/77)	77.92% ^a (60/77)	50.64% ^a (39/77)	32.46% ^b (25/77)	16.88% ^c (13/77)	0.0001*
Bulls N=45	66.67% ^a (30/45)	77.77% ^a (35/45)	51.11% ^a (23/45)	28.88% ^b (13/45)	35.55% ^b (16/45)	0.0001*
Cows N=121	57.02% ^a (69/121)	79.33% ^a (96/121)	59.50% ^a (72/121)	23.96% ^b (29/121)	22.31% ^b (22/121)	0.0001*

Note: The values in the same row with different superscript letters significantly differ at the 0.05 threshold for each parameter. * Highly significant

3.5 Seasonal variation in the prevalence of Anaplasma spp and other associated blood parasites

The diversity of haemoparasite species encountered in the cattle farms in the department of Kounahiri during the rainy and dry seasons remains completely identical, with the only difference being their observed proportions (Figure 4). During both seasons, most of the infections were caused by the pathogen *A. marginale* responsible for bovine anaplasmosis, with respective prevalences of 82% at the beginning of the dry season and 73% at the beginning of the rainy season. In contrast, *Theileria* spp had the least impact on animals in this locality, with low seasonal prevalences of 20% and 24%, respectively, at the beginning of the rainy season and the beginning of the dry season. The statistical analysis does not reveal any significant difference ($P=0.05$) between the action of parasites during the seasons.

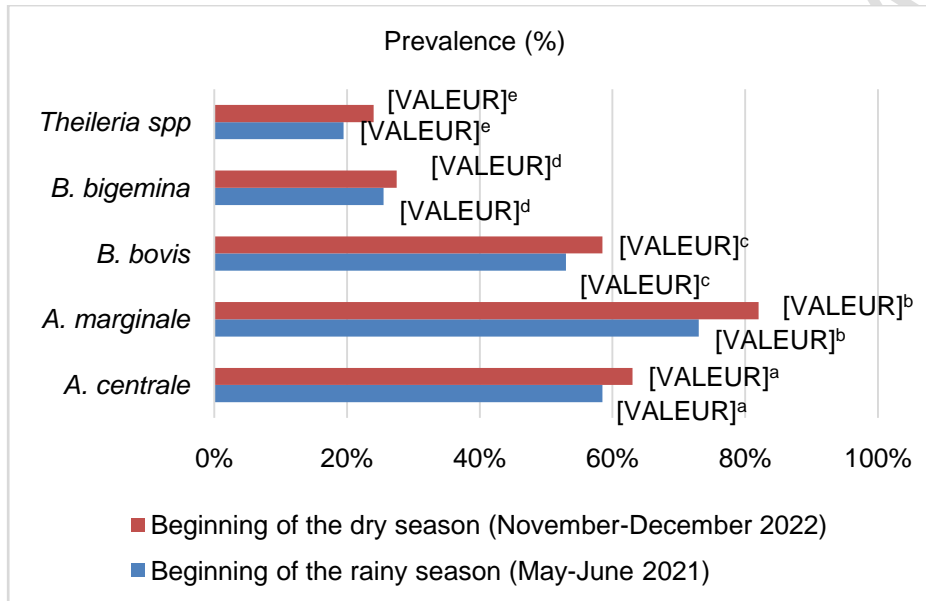


Figure 4: Seasonal dynamics of Anaplasma spp and other associated parasites
 Note: Values with different letters indicate a significant difference in the prevalence of identified parasite species according to the season.

3.6 Discussion

Overall, 96.5% of cattle in this department were infected by at least one haemoparasite. Vector diversity and the lack of an appropriate prophylaxis program would explain this high rate of spread. Our investigations corroborate those of Aké-Bogni et al (10), according to whom a total of 86.1% (155/180) of cattle are infested by one or more haemoparasite species. The search for *Anaplasma* spp and other haemoparasites revealed a significant infection rate of 77.5% for *A. marginale* in bovine samples from Kounahiri. This high prevalence could be attributed to the lack of preventive treatment by farmers. Furthermore, it may be explained by the absence of holding facilities, as well as the size and weight of the animals, which can make the administration of medications (acaricides and other veterinary care) more challenging. Our results are in agreement with those of Turi et al. (16), who reported an infection rate of 41.6% for *A. marginale* in cattle samples from the Lakki Marwat and Peshawar districts in Pakistan. Additionally, Farooq and Ashraf (17) reported significant infection rates of 18.3% for *A. marginale* in samples from cattle in three distinct areas of the Khyber Pakhtunkhwa province and 8.6% for *A. marginale* in cattle from the Layyah district in Pakistan.

The results concerning the variation in parasite prevalencies with respect to the sex of the animals indicated a significant infection rate of *A. marginale* (53.8%) in males and (59.9%) in females. Consequently, both male and female animals would be susceptible to tick infestations and other vectors, which explains the increased risk of transmission. Our results are not identical to the findings of Yéo et al. (18) on ruminant haemoparasites in the Korhogo and Sinématiali departments. According to these authors, 54.41% of females and 16.18% of males were infected with *A. marginale*. Our results are in line with the work conducted by Djakaridja et al. in the Northern and Central regions of Côte d'Ivoire, where they obtained an infection rate of 78.76% for *A. marginale* in 178 females and a 73.13% infection rate for the same parasite in a population of 98 males (12).

Regarding the diagnosis of parasite species based on the age of the animals, *Anaplasma marginale* prevailed at 78.90% among the young cattle, followed by *Babesia bovis* (57.23%), and *Babesia bigemina* (25.90%) among adult bovines. Several factors could explain this; firstly, young cattle have a diet primarily composed of milk, which may not contain all the necessary nutrients to strengthen their immune system. As a result, calves may be more susceptible to vector infestations. Additionally, young cattle are not treated with antiparasitic agents until a certain age, as these products can be harmful to their health. These results are similar to those obtained by Canever et al. (19) in southern Brazil, where they found that *Babesia bigemina*, *Anaplasma marginale*, and *Babesia bovis* were widespread, with respective infection rates of 63.6%, 60.6%, and 18.2%. According to the researchers, the low prevalence of infection might be related to low parasitaemia during the acute phase of the disease. An important incidence of 52.5% of cattle affected by anaplasmosis was reported in Aberdeen Angus cattle in New Valley, Egypt (20) following a diagnostic study.

At the physiological stage of cattle, high parasite burdens were recorded in yearling bulls. The infection prevalencies were distinctly 70.58% for *A. centrale*, 80.39% for *A. marginale*, and 29.41% for *B. bigemina*. The lack of appropriate management practices, such as pasture rotation and reducing herd density to minimize animal exposure to vectors, could explain this high parasite infection. Our results do not corroborate those of Vieira et al. (21), who observed a low infection rate of 26.76% for *A. marginale* and 35.21% for *B. bovis* in animals aged between 12 and 24 months. Another study conducted in the Balochistan province of Pakistan on the impact of parasite infestations revealed that young animals over one-year-old were the most exposed (22).

Regarding the seasonal variation of *Anaplasma* spp and other associated blood parasites, the pathogen *A. marginale* responsible for bovine anaplasmosis was most prevalent at the beginning of the rainy season and the beginning of the dry season, with respective prevalencies of 82% and 73%. A low distribution of *Theileria* spp, 20% and 24%, respectively, was observed at the beginning of the rainy season and the beginning of the dry season. This high prevalence can be explained by the fact that cattle are more exposed to insect bites and a high burden of tick vectors, which transmit these parasites. Additionally, the humidity may favour certain aspects of the parasites' life cycle and reproductive rate. Similar infection prevalencies have been reported in all cattle farming areas in Côte d'Ivoire, with overall prevalencies of 68.9% for *A. marginale* and 10% for *Theileria annulata* (9). Our results confirm the findings of studies conducted in the Northern and Central regions of Côte d'Ivoire, which estimated 77.7% in the North and 55% in the Center (12). Nwoha et al. (23) also found high proportions of 31% for *A. marginale* in Abia State, southeast Nigeria, although the proportions differ from ours.

4. CONCLUSION

This study of parasite status enabled an exhaustive inventory to be made of the various haemoparasites of cattle in the department of Kounahiri and their prevalence to be assessed. Three genera and four species were identified: *Anaplasma* spp, *Babesia* spp and

Theileria spp. Three genera and four species were identified, namely *Anaplasma spp.*, *Babesia spp.* and *Theileria spp.* The species include *A. marginale*, *A. centrale*, *B. bovis* and *B. bigemina*. The *Anaplasma* genus was the most widespread. Factors such as sex and season had no significant impact on parasite prevalence. However, the physiological stage did influence infection rates. Knowledge of parasites will enable the implementation of health service programs that are more or less appropriate to meet the health needs of livestock, ultimately leading to increased production at all levels.

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

INS : Institut Nation de la Statistique
 A. marginale : *Anaplasma marginale*
 A. centrale : *Anaplasma centrale*

B. bovis : Babesia bovis

B. bigemina : Babesia bigemina

UNDER PEER REVIEW