



## RESEARCH PAPER

## OPEN ACCESS

# Distribution of cattle tick-borne haemoparasites in 54 Departments of Côte d'Ivoire after the invasion of *Rhipicephalus microplus*

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**Key words:** *Rhipicephalus microplus*, *Anaplasma marginale*, *Anaplasma centrale*, *Babesia bovis*, Côte d'Ivoire

<http://dx.doi.org/10.12692/ijb/20.1.123-132>

Article published on January 10, 2022

## Abstract

The *Rhipicephalus microplus* tick is a major economic and veterinary concern on livestock production in tropical regions of the world. In Côte d'Ivoire, this invasive and proliferating tick has occupied almost the entire territory. To assess the prevalence of tick-borne haemoparasites in cattle following this invasion, a parasitological study was carried out in 179 farms in 54 departments. Blood and tick samples were collected from 895 cattle over one year of age, with 5 individuals per farm. Ten tick species were identified, of which *Rhipicephalus microplus* the most found. Microscopic analysis of blood smears identified 3 tick-borne haemoparasites: *Anaplasma marginale*, *Anaplasma centrale* and *Babesia bovis*, with prevalences between 4% and 24% according to departments. Only the Southern, Central, and Northern zones were infested with tick-borne haemoparasites of cattle. The highest prevalence of *Babesia bovis* (8%) was found in the Southern zone. Agboville's department was the most infested by *A. marginale* (24%) and *B. bovis* (20%). Parasitological analyses revealed a low prevalence of tick-borne haemoparasites in Côte d'Ivoire. A molecular study should be conducted to confirm these results.

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## Introduction

Ticks are obligate haematophagous acarines that parasitize all class of vertebrates in all regions of the world, particularly in Africa (Brites-Neto *et al.*, 2015). During their blood meal in a vertebrate host, they can transmit a wide variety of pathogens (viruses, bacteria, and protozoa) that can be the cause of several infectious diseases (De La Fuente *et al.*, 2017). In cattle, ticks are the most important ectoparasites, with about 80% of cattle globally infested (Kasozi *et al.*, 2014).

The cattle tick *Rhipicephalus microplus* is a major threat to the improvement of livestock production, which has an important economic impact in addition to animal health issue, particularly under the tropics (Hüe *et al.*, 2016; Sato *et al.*, 2020). In Brazil, the cattle tick *Rhipicephalus microplus* is distributed throughout the country and it is estimated that 80% of the national cattle herd is infested (Politi *et al.*, 2019). The economic loss caused by this tick is estimated at about \$3 billion per year to the Brazilian cattle industry, representing 75% of the total economic loss in this sector (Sato *et al.*, 2020).

In West Africa, *Rhipicephalus microplus* was first discovered in 2007 in southern Côte d'Ivoire (Madder *et al.*, 2007); then in Benin (Madder *et al.*, 2012), and more recently in Burkina Faso, Mali, and Togo (Adakal *et al.*, 2013). In Côte d'Ivoire, it has invaded almost department now (Boka *et al.*, 2017). *Rhipicephalus microplus* is a serious threat to livestock breeding in West Africa (Madder *et al.*, 2011). Thus, this tick is known to be the cause of the increased prevalence of anaplasmosis and babesiosis (Adjou Moumouni *et al.*, 2018). Anaplasmosis, babesiosis, and theileriosis are among the most diagnosed tick-borne haemoparasitosis in sheep and cattle in West Africa (Djakaridja *et al.*, 2014). In the North and South of Benin, the different prevalences obtained in the cattle were 30.88% and 86.20% for *B. bovis* and *A. marginale*, respectively. In Southwest Burkina Faso, 57.24% of cattle were infected by *A. marginale* and 12.52% by *B. bovis* (Biguezoton, 2016). In Côte d'Ivoire, *A. marginale* and *B. bovis* were identified with prevalences of 76.94 and 45.83% in the North and 55 and 59.43% in the Center of the

country (Djakaridja *et al.*, 2014; Yéo *et al.*, 2017a). Diagnosis of haemoparasites relies primarily on microscopy for its low cost and broad-spectrum (trypanosoma, anaplasma, babesia, etc.) (Djakaridja *et al.*, 2014; Ganguly *et al.*, 2020). Therefore, this study was conducted to assess the level of infection of cattle by tick-borne haemoparasites using microscopy technic, following the colonization of the entire country by *R. microplus*. Specifically, it was to:

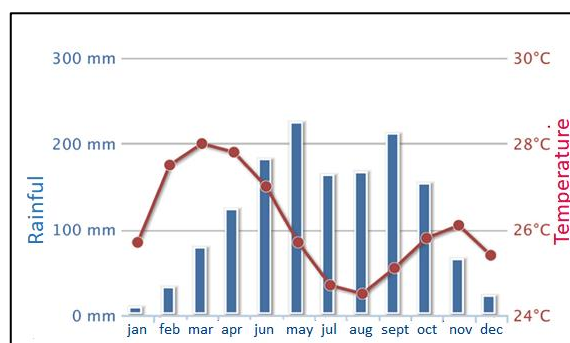
- (i) Determine tick species and their intensity per animal;
- (ii) Determine the distribution of tick-borne haemoparasites in cattle;

## Material and methods

### Study areas

This study was conducted in 59 localities of 54 departments in Côte d'Ivoire from April 2014 to May 2015 exclusively during the rainy season. Côte d'Ivoire is a West Africa country, located between latitudes 5° and 11°N, and longitudes 3° and 9°W. The climate is hot with average monthly temperature from 24-28°C and monthly rainfall from 10-230mm. The North of the country has one short rainy season from the beginning of June to the end of September, with a high precipitation in August.

The Central and Southern regions have two rainy and two dry seasons per year. The main rainy season in the Centre goes from March to the end of June, and the short, from September to October. In the South, the main rainy season goes from April to the end of July, and the shorter rainy from the beginning of October to the end of November (Fig 1).



**Fig. 1.** Average monthly temperature and rainfall for Ivory Coast from 1900 to 2012 (Boka *et al.*, 2017).

### Sampling

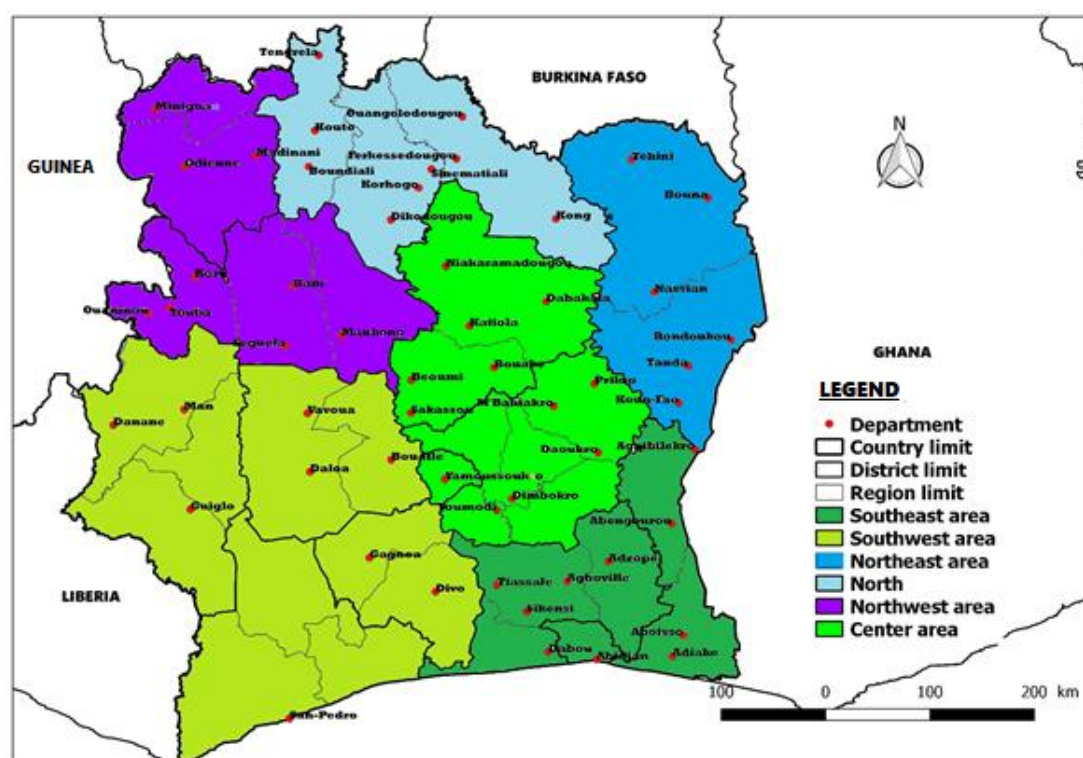
The sampling method was carried out with the assistance of the “Ministère des Ressources Animales et Halieutiques” (MIRAH) according to the administrative division (Fig 2) consisting of 19 regional departments subdivided into 77 departments. The regions have been divided into 6 geographical areas (South-East, South-West, Centre, North, North-East, and North-West) according to cattle density. The sample size was calculated according to the following formula (Thrusfield *et al.*,

$$2018): n = \frac{(Z^2 \times p(1-p) \times c)}{d^2}$$

Where  $Z=1,96$ ;  $d=5\%$  absolute precision,  $p=77\%$  known prevalence, and  $c=3$  the correction coefficient considering the three main zones of Côte d'Ivoire

(North, Center, and South). The total number of cattle in the study was estimated at 895 with  $n=816$ . Therefore, a total of 895 bovines from 54 departments and 179 farms were randomly selected in the six livestock area using a database of farms listed by MIRAH regional offices (Fig 2).

A total of 150 cattle were sampled in each livestock area, except in the Northeast zone where 145 cattle were sampled. Ticks and blood samples were collected from cattle aged at least one year. Blood samples were collected from the cattle's auricular vein. Samples were collected during the colder times of the day, either early in the morning before the departure of the animals to the pasture or at least one week after the last acaricide treatment.



**Fig. 2.** Location of departments visited for the country-wide survey of Côte d'Ivoire. Software QGIS version 2.16.0" Nodebo" (QGIS development team).

After immobilising the animal, blood samples were collected from the auricular vein of cattle at least one year old at a rate of 5 individuals per farm. Blood collected in a hematocrit tube was used to realize blood smears, thick drops, and confetti for each cattle according OMS (2015). Tick species were collected for 10 minutes, according Socolovski *et al.* (2008).

The farms were georeferenced using a Garmin GPSMAP 64-Multicolored GPS. Farmers provide information from a questionnaire that to capture farm animal details (the number of animals according to sex, breeding system and nature of the last treatment, farmers' perception of ticks, and their resistance to acaricides).

### Parasitological examination

Blood smears and thick drops were taken according to the method published by the OMS, 2015. Parasitological analyses were carried out under an optical microscope at x100 magnification after staining with 10% Giemsa. The species and developmental stage of all collected ticks were identified using a stereomicroscope (80-fold magnification), following identification standards (Walker *et al.*, 2003; Madder *et al.*, 2012).

### Data analysis

The Microsoft ACCESS database software was used to enter the data that was extracted to the Microsoft EXCEL spreadsheet software. Firstly, the average parasite intensity (Ip) of the tick species was calculated using the software (STATA version 14) using the following formula:  $I_p = n_i/N$ ; where  $n_i$  is the total number of individuals of a tick species and N is the number of infested cattle in the total sample. Also, the ANOVA test was carried out to compare the different parasite intensities of each species in the different livestock areas. Then the prevalence of the identified haemoparasites was calculated and the confident intervals were performed. Finally, QGIS version 2.16.0 software was used to map the distribution and prevalence of the haemoparasites.

## Results and discussion

### Tick species collected and parasite intensity per bovine

In total, 24,031 ticks of three genus were collected and identified. These were *Rhipicephalus* (including

those of the subgenus *Boophilus*), *Amblyomma* and *Hyalomma*. A total of 15,575 ticks of the subgenus *Rhipicephalus* (*Boophilus*), representing approximately 65% ticks was collected. A total of 10 species of ticks were identified. These are: *Rhipicephalus senegalensis*, *Rhipicephalus sanguineus*, *Rhipicephalus lunulatus*, *Rhipicephalus* (*Boophilus*) *microplus* or *R. microplus*, *Rhipicephalus* (*Boophilus*) *decoloratus* or *R. decoloratus*, *Rhipicephalus* (*Boophilus*) *annulatus* or *R. annulatus*, *Rhipicephalus* (*B.*) *geigy* or *R. geigy*, *Hyalomma truncatum* and *Hyalomma marginatum rufipes*. *R. microplus* has been the main species with 15,291 specimens, which represented 98.2% of all species of subgenus *Boophilus* and 63.6% of all collected ticks.

The distribution of parasite intensity in the livestock areas was statistically significant for four species. These were *A. variegatum* ( $F=56.5$ ;  $p<0.001$ ), *R. microplus* ( $F=22.04$ ;  $p<0.001$ ), *R. annulatus* ( $F=3.05$ ;  $p=0.022$ ) and *R. senegalensis* ( $F=3.2$ ;  $p<0.001$ ). Two ticks species were the most infesting to livestock and were reported in all livestock areas. These are *R. microplus*, the majority species, followed by *A. variegatum*. The highest parasite infestations by *R. microplus* were found in the south and center of the country. In the South of the country, 84% of cattle were infested with  $41.86 \pm 77.08$  ticks/cattle and in the Center, 99.33% of cattle have  $34.17 \pm 31.99$  ticks/cattle. In the case of *A. variegatum*, the Northern zone was the most infested. A total of 89.33% of cattle were infested with  $26.16 \pm 23.8$  ticks (Table 1).

**Table 1.** Parasite intensity per bovine in Côte d'Ivoire.

|                          | Centre (N=150) |       |        | North (=150) |        |        | Northeast (N=145) |       |       | Northwest (N=150) |       |       | South (N=150) |       |        | Southwest (N=150) |      |       |       |       |
|--------------------------|----------------|-------|--------|--------------|--------|--------|-------------------|-------|-------|-------------------|-------|-------|---------------|-------|--------|-------------------|------|-------|-------|-------|
|                          | n              | Means | SD     | n            | Means  | SD     | n                 | Means | SD    | n                 | Means | SD    | n             | Means | SD     | F                 | P    |       |       |       |
| <i>A. variegatum</i>     | 93             | 6.12  | ±8.12  | 134          | 26.16  | ±23.8  | 127               | 5     | ±4.3  | 131               | 7.56  | ±4.99 | 114           | 14.02 | ±15.94 | 115               | 4.5  | 3.81  | 56.5  | 0     |
| <i>R. (B.) annulatus</i> | 37             | 1.84  | ±1.21  | 4            | 1.75   | ±0.96  | 1                 | 1     | 0     | 0                 | 0     | 0     | 30            | 5.93  | ±7.79  | 3                 | 1    | 0     | 3.05  | 0.022 |
| <i>R. (B.) geigy</i>     | 5              | 1     | 0      | 0            | 0      | 0      | 3                 | 1     | 0     | 0                 | 0     | 0     | 6             | 1     | 0      | 2                 | 1    | 0     |       |       |
| <i>R. (B.) microplus</i> | 149            | 34.17 | ±31.99 | 83           | 11.51  | ±18.83 | 88                | 5.32  | ±5.85 | 91                | 3.89  | ±3.07 | 126           | 41.86 | ±77.08 | 113               | 6.39 | ±6.98 | 22.04 | 0     |
| <i>R. (B.) deco</i>      | 0              | 0     | 0      | 1            | 2      | 0      | 0                 | 0     | 0     | 0                 | 0     | 0     | 4             | 1.75  | ±0.96  | 109               | 6.27 | ±6.88 | 1.4   | 0.357 |
| <i>H. truncatum</i>      | 0              | 0     | 0      | 29           | 1.93   | ±1.33  | 5                 | 1.6   | ±0.89 | 2                 | 1     | 0     | 0             | 0     | 0      | 1                 | 1    | 0     | 0.54  | 0.657 |
| <i>H. m. rufipes</i>     | 0              | 0     | 0      | 20           | 1      |        | 20                | 1     | 0     | 5                 | 1     | 0     | 5             | 1     | 0      | 0                 | 0    | 0     | 0.37  | 0.775 |
| <i>R. sanguineus</i>     | 0              | 0     | 0      | 8            | 116.83 | ±95.64 | 1                 | 0     | 0     | 2                 | 2     | ±1.41 | 0             | 0     | 0      | 0                 | 0    | 0     | 1.81  | 0.225 |
| <i>R. senegalensis</i>   | 5              | 1.4   | ±0.89  | 5            | 2.4    | ±1.14  | 0                 | 0     | 0     | 5                 | 1     | 0     | 10            | 81.6  | 0      | 0                 | 0    | 0     | 3.2   | 0     |
| <i>R. lunulatus</i>      | 10             | 1     | 0      | 30           | 1      | 0      | 0                 | 0     | 0     | 0                 | 0     | 0     | 0             | 0     | 0      | 0                 | 0    | 0     | 1.7   | 0.199 |

N = number of cattle; n = number of infested cattle.

Ten species of ticks belonging to three genus were identified. These are *Amblyomma* spp., *Hyalomma* spp., and *Rhipicephalus* spp. However, Diaha-

Kouamé (2017) identified in addition to the ten species *H. m. marginatum* and *H. impressum* in the North-East Ivorian Transhumance Corridor (NITC).

This difference could be explained by the fact that this was a longitudinal study over two years in the NITC. The NITC is an area with an intense flow of transhumant herds during the dry season and therefore an exchange of ectoparasites (Diaha-Kouamé, 2017).

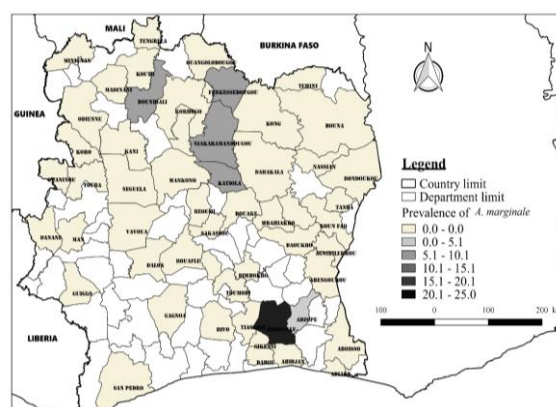
In terms of parasite intensity, *Rhipicephalus microplus* was the most infesting tick and present in all livestock areas. Since the accidental introduction of *Rhipicephalus microplus* in Côte d'Ivoire, its overplanted *Amblyomma variegatum*, a potential cowdriosis vector that was more abundant in West Africa (Madder *et al.*, 2007). *Rhipicephalus microplus* is a serious threat to livestock breeding in West Africa (Madder *et al.*, 2011). This tick is invasive and has the ability to compete and establish itself at the expense of other native *Rhipicephalus* species (*R. B. geigy*, *R. B. decoloratus* et *R. B. annulatus*) (Madder *et al.*, 2011; Touré *et al.*, 2012; Boka *et al.*, 2017). The highest infestations were in the South and the Center of the country. Indeed, the introduction of this species seems to have been made between 2002 and 2004 cattle imported from Brazil (Madder *et al.*, 2011) in the South of Bingerville and the Center of Toumodi and Dabakala (Boka *et al.*, 2017). Also, the highest intensity of infestation was recorded in the southern zone due to the cattle breeds collected from the dairy eco-farms in the town of Azaguié (Agboville department). These farms contain exotic Montbeliard and Hostein crossbred cattle. These breeds are more susceptible to tick and tick-borne haemoparasites infestations than the local breeds (Simuunza *et al.*, 2011; Adjou Moumouni *et al.*, 2018). The standard deviation for the southern livestock area is very high ( $\pm 77.1$ ) due to the disproportionate number of ticks on cattle on some farms visited. Indeed, while the parasite intensity was 41.8 Tick/cattle in 10 minutes, some cattle had up to 515 and 171 adult ticks respectively on a farm in Azaguié and Bingerville. This high parasite intensity of *R. microplus* in the south and north is expected to lead to increased prevalences of anaplasmosis and babesiosis in these areas. This has been observed in South Africa, where invasive waves of this tick have been accompanied by outbreaks of the pathogen *B. bovis*. Similarly, in Brazil, the invasion of *R. microplus* has generated a

high prevalence of *B. bigemina* and *B. bovis* in cattle (Zeman and Lynen, 2006; Biguezoton *et al.*, 2016).

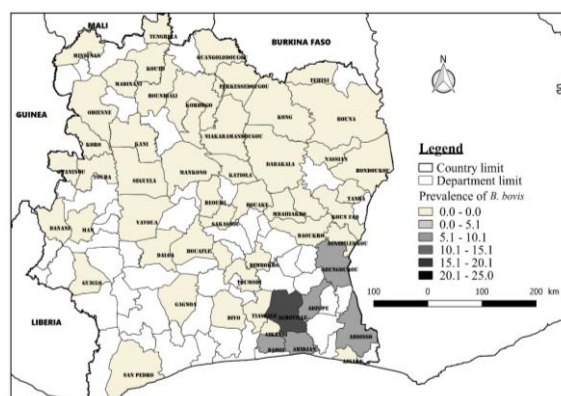
### Prevalence and spatial distribution of tick-borne haemoparasites

#### Prevalence and spatial distribution of tick-borne haemoparasites according to the department

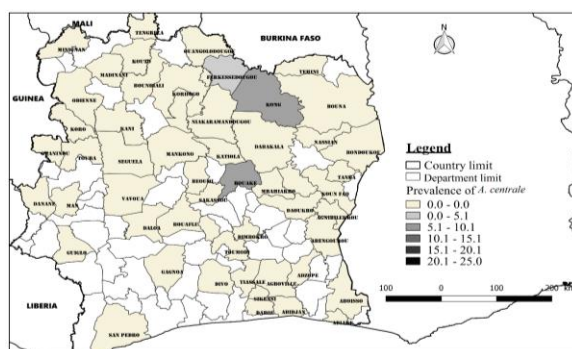
Three tick-borne haemoparasites i.e. *Anaplasma centrale*, *Anaplasma marginale*, and *Babesia bovis* has been identified. The prevalence of the three tick-borne haemoparasites ranged from 4 to 24%. The Northern and Central area of the country recorded low prevalences ( $\sim 10\%$ ) for all haemoparasites. *B. bovis* and *A. marginale* were the most haemoparasites. In addition, their presence was reported in six departments. *B. bovis* and *A. marginale* recorded the highest prevalence in the department of Agboville, 20% and 24% respectively (Fig. 3, Fig.4 and Fig. 5).



**Fig. 3.** Prevalence of *A. marginale* in Côte d'Ivoire. Software QGIS version 2.16.0 "Nodebo" (QGIS development team).



**Fig. 4.** Distribution and prevalence of *B. bovis* in Côte d'Ivoire. Software QGIS version 2.16.0 "Nodebo" (QGIS development team).



**Fig. 5.** Prevalence of *A. centrale* in Côte d'Ivoire. Software QGIS version 2.16.0 "Nodebo" (QGIS development team).

### Prevalence of tick-borne haemoparasites according to livestock area

Three of the six livestock areas registered the presence of tick-borne haemoparasites in cattle: these were the North, the South, and the Center.

The South was the most infested by *B. bovis* (8%). *A. marginale* was found in the South-eastern (4,7%), Central (1,3%), and Northern areas (2%), whereas *B. bovis* was observed only in the South-eastern area (8%). *A. centrale* was found in the Central (1,3%) and Northern zone (1,3%) (Table 2).

**Table 2.** Prevalence of tick-borne haemoparasites according to farming areas in Côte d'Ivoire.

|                     | Center (N=150) |               | North (N=150) |               | Northeast (N=145) |        | Northwest (N=150) |        | South (N=150) |               | Southwest (N=150) |           |
|---------------------|----------------|---------------|---------------|---------------|-------------------|--------|-------------------|--------|---------------|---------------|-------------------|-----------|
| Haemoparasites      | n              | % (CI)        | n             | % (CI)        | n                 | % (CI) | n                 | % (CI) | n             | % (CI)        | n                 | % (CI)    |
| <i>B. bovis</i>     | 0              | 0             | 0             | 0             | 0                 | 0      | 0                 | 0      | 12            | 8(4.2-13.6)   | 0                 | 0         |
| <i>A. centrale</i>  | 2              | 1.3 (0.2-4.7) | 2             | 1.3 (0.2-4.7) | 0                 | 0      | 0                 | 0      | 0             | 0             | 0                 | 0         |
| <i>A. marginale</i> | 2              | 1.3 (0.2-4.7) | 4             | 2.7 (0.7-6.7) | 0                 | 0      | 0                 | 0      | 7             | 4.7 (1.9-9.4) | 0                 | 2 (0-2.4) |

In the present study, three tick-borne haemoparasites of cattle belonging to two genus were identified from optical microscopy in 54 departments of the country. These are *A. centrale*, *A. marginale*, and *B. bovis* with low prevalence. These tick-borne haemoparasites are known to be transmitted by species of the genus *Rhipicephalus*, *Amblyomma* and *Hyalomma*. (Ziam *et al.*, 2016) also identified in Côte d'Ivoire (Achi *et al.*, 2012; Boka *et al.*, 2017; Diaha-Kouamé, 2017).

These three species of tick-borne haemoparasites were also identified by several authors including Yéo *et al.* (2017a) and Achi *et al.* (2012) in Côte d'Ivoire. However, in addition to these three species, Achi *et al.* (2012) identified in the North of the country *B. bigemina* and *Theileria* spp. The absence of *B. bigemina* is explained by the fact that it is transmitted mainly by *R. annulatus*, which is now practically replaced by *R. microplus*. Also, *B. bigemina* is less present in the blood capillaries unlike *B. bovis* (Thompson and Goodrich, 2018).

The absence of *Theileria* spp. could be due to the fact that the samples were collected during the rainy season, a period unfavorable to the development of its vector (Laamari *et al.*, 2012).

The low prevalence of tick-borne haemoparasites is explained by the treatment and prophylaxis of trypanosomes and tick-borne haemoparasites monitored by farmers (MIRAH 2014). Anti-parasitic agents used by farmers in Côte d'Ivoire, such as diminazene aceturate, are effective against trypanosomes and Babesia (Yéo *et al.*, 2017ba; Matovu *et al.*, 2020).

Also, the use of antibiotics such as tetracyclines by farmers would be at the basis of the reduction of the prevalence of anaplasmosis in cattle (Yeo *et al.*, 2017b; Politi *et al.*, 2019). Thus, these different treatments would have contributed to reduce the parasitaemia rate of haemoparasites in the blood of cattle and make them undetectable by light microscopy. Blood smears and thick drops can be used for acute infection, but they do not allow the detection of healthy carriers, whose parasitemia is too low to be detected (Ganguly *et al.*, 2020). In this case, molecular biology techniques must be used (Ganguly *et al.*, 2020).

The highest prevalences of *B. bovis* and *A. marginale* were observed in Agneby-Tiassa region, in the Southeast of the country, precisely in the locality of Azaguié. Indeed, in the Southeast region, a large population of *R. microplus*, the main vector of

*A. marginale* and *B. bovis* has been recorded in the Agneby-Tiassa Region (73%) including 64% of the municipality of Azaguié (Boka *et al.*, 2017). The Northern and the Southern parts of the country observed prevalences less than 10% for all haemoparasites in the different departments. These low prevalences in the North due to a low population of *R. microplus* are in this area. The population of *R. (B.) microplus* identified in the Northern part of the country by Boka *et al.* (2017) was 10%. However, in the Central part of the country, Boka *et al.* (2017) identified *R. (B.) microplus* at the level of 50%. According to this high prevalence of *R. (B.) microplus* in the central part, the prevalence of these three tick-borne haemoparasites was expected to be high. This may be due to the different breeds of cattle found in these areas. The cattle herd in the Center is mostly made up of taurines, which are more resistant to hemoparasite infestations than zebus, which are in the majority in the North (MIRAH, 2014).

Three of the six livestock areas registered the presence of tick-borne haemoparasites in cattle: the north, the central, and the south part of the country. The studies of Boka *et al.* (2017) showed more than 100 individuals of this tick in most of the farms in these areas. The highest prevalence was obtained by *B. bovis* only in the southern livestock zone (8%). However, several authors have identified it in the central and northern parts of the country (Achi *et al.*, 2012; Djakaridja *et al.*, 2014; Yéo *et al.*, 2017a). As for *A. marginale*, it is the most common species of livestock and agro-ecological areas. This can be explained by the fact that biological transmission of *A. marginale* is ensured by about twenty species of ticks including those belonging to the genus *Rhipicephalus*, and *Hyalomma* in tropical regions (Silaghi *et al.*, 2017). Also, *Anaplasma marginale* is transmitted via several other ways, including the transplacental ways and the mechanical ways: by biting Diptera and sharp objects (Ringo *et al.*, 2018).

## Conclusion

This study is the first to provide an overview of the level of infection of tick-borne haemoparasites in cattle over the same period and across 54

Departments of the Country. *Rhipicephalus microplus* was more common in all livestock areas. The highest infestations were in the South and the Center of the Country with infestation intensities of 41.86 ticks/cattle and 34.17 ticks/cattle respectively. Also, three tick-borne hemoparasites of livestock were identified in three livestock areas in Côte d'Ivoire with low prevalences: *Anaplasma centrale*, *Anaplasma marginale* and *Babesia bovis*. The three infected livestock areas are North, South and Central. *Anaplasma marginale* and *B. bovis* are the most infective haemoparasites with a reported presence in six departments of the country. The department of Agboville was the most infested by *A. marginale* (24%) and *B. bovis* (20%).

## Recommendation

Thus, it would be interesting to evaluate the prevalence of these tick-borne haemoparasites with a more sensitive method (PCR) to certify its results.

## Acknowledgements

Our sincere thanks go first and foremost to the “Programme d'Appui Stratégique à la Recherche Scientifique” (PASRES) and the Union Économique et Monétaire Ouest-Africaine (UEMOA) through the Regional project of Fight-Against-Tick *Rhipicephalus (Boophilus) microplus* and for the financing of this study. Secondly, we are grateful to the Centre Suisse de Recherche Scientifique (CSRS) in Côte d'Ivoire and the International Research Development Center on farming in Sub-humid Areas (CIRDES) in Burkina Faso for their technical assistance. And finally, we would like to thank KANDE Souleymane, BABA Ouattara, AKOTO Rita Prisca, and KOFFI Kolou Alla Serge Landry for their assistance rendered during the samples collection and laboratory analysis.

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