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Ticks and haemoparasites in cattle at the port bouet abattoir in Abidjan (South-East, Cote d'ivoire)

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Abstract
Tick-borne infections occur worldwide and have been well known for over a century. Their incidence varies according to the time of year and geographical location, and depends on a variety of circumstances such as animal reservoirs, climate, ecological conditions and the lifestyle of infected individuals. Most of them are zoonotic (transmissible from animals to humans), but some only affect animals, with a very significant economic impact. A study was carried out in July 2022 at the Port-Bouet slaughterhouse in Abidjan, where an entomological survey was carried out to collect ticks and blood from cattle destined for slaughter. Samples were collected from 62 cattle aged between 2 and 5 years. A total of 372 tick specimens were collected, comprising 93.82% females, 5.91% males and 0.27% nymphs. Three tick species were identified, and *Rhipicephalus* (*Boophilus*) *microplus* was the predominant species with 80.37% of cumulative numbers. After Giemsa staining, analysis of the 62 blood smears revealed blood parasites in 40 samples, representing a prevalence of 64.51%. The frequencies observed were 17.74% *Trypanosoma* sp, 30.64% *Babesia bovis*, 30.64% *Anaplasma marginale* and 29.03% *Theileria* sp. Polyparasitism has been marked by bi- and tri-infestations of these haemoparasites in blood samples. It is therefore necessary to make the Port Bouet slaughterhouse a sentinel site for entomological and microbiological surveillance.

Keywords: Tick, *Rhipicephalus* (*Boophilus*) *microplus*, Haemoparasite, *Babesia*, *Anaplasma*, *Theileria*

Introduction
The major constraint on livestock farming in tropical countries is internal parasitosis (gastro-intestinal) and external parasitosis (ticks and tsetse flies), with the pathogens they transmit. Ticks are strictly hematophagous mites that parasitize almost all vertebrates worldwide. More importantly, ticks can also transmit serious infections, as they can carry a variety of pathogens [1, 2]. Tick-Borne Diseases (TBD) are also emerging and/or re-emerging due to the geographical expansion of ticks, particularly in North America, Europe, Asia and Japan, where they are responsible for the majority of vector-borne diseases [3, 4, 5]. Some of the pathogens transmitted by ticks are poorly understood, but can cause infections with very harmful consequences for health [6]. In West Africa, tick-borne diseases have often been overshadowed by major viral or bacterial epizootics. As a result, there is still a great deal of confusion when it comes to understanding their epidemiology and pathogenicity. Cattle farming in Côte d'Ivoire is still extensive. The animals are fed on natural grazing, by roaming through savannahs and areas that have been developed or are suitable for this purpose. The scarcity of grass during the dry season in certain areas forces livestock farmers, particularly those in the north of the country, to move their herds to central locations. This practice is also used by herders in Sahelian countries. Indeed, during the dry season, many herds from Burkina Faso and Mali come to Côte d'Ivoire in search of grazing sites. It should also be noted that the cattle encountered on the farms and at the livestock market mainly come from the various sub-Saharan countries bordering Côte d'Ivoire. The practice of moving animals from one country to another, in search of pasture, is not without consequences for the distribution of ticks and the pathogens they harbor. With no quarantine zones in neighboring countries, incoming animals can carry disease vectors or even be sources of contamination and/or contagion for animals in the host country. At the same time, they can also contract diseases in that country and spread them back home. This phenomenon of cross-border diseases has also been applied to ticks, as southern

Burkina Faso has been infested with *Rhipicephalus* (*Boophilus*) *microplus* by animals returning from transhumance in Côte d'Ivoire [7]. Since 2007, this tick species has been discovered in Côte d'Ivoire through imports of live animals from Brazil [8]. This tick is specific to cattle and is characterized by its ability to replace native *Rhipicephalus* (*Boophilus*) species, its resistance to commonly used acaricides and its ability to transmit pathogens. The present study aims to contribute to a better understanding of the impact of ticks infesting animals by determining tick species and Haemoparasite transmitted to cattle in cattle destined for slaughter at the abattoir in Port Bouet, Abidjan.

Materials and Methods

Study areas

The study was conducted in July 2022 at the Port-Bouet abattoir in Abidjan, where an entomological survey was carried out to collect ticks and blood samples from cattle destined for slaughter. The Port-Bouet abattoir is located in the commune of Port-Bouët, in the southeast of the autonomous district of Abidjan. It is a peninsula located between the Atlantic Ocean and the Ebrié Lagoon, stretching from west to east along the maritime coastline for some thirty kilometers (Fig 1). It covers an area of 111 km² with a relief of coastal plain with a sandy and hydromorphic soil, with depressions in places [9]. This commune includes the municipal abattoir, the largest in Abidjan and the country. The abattoir comprises three sections: the livestock market, the butchery area and the small ruminant park. The livestock market covers an area of 3.2 hectares, where only cattle from various sub-Saharan countries and Côte d'Ivoire are sold.

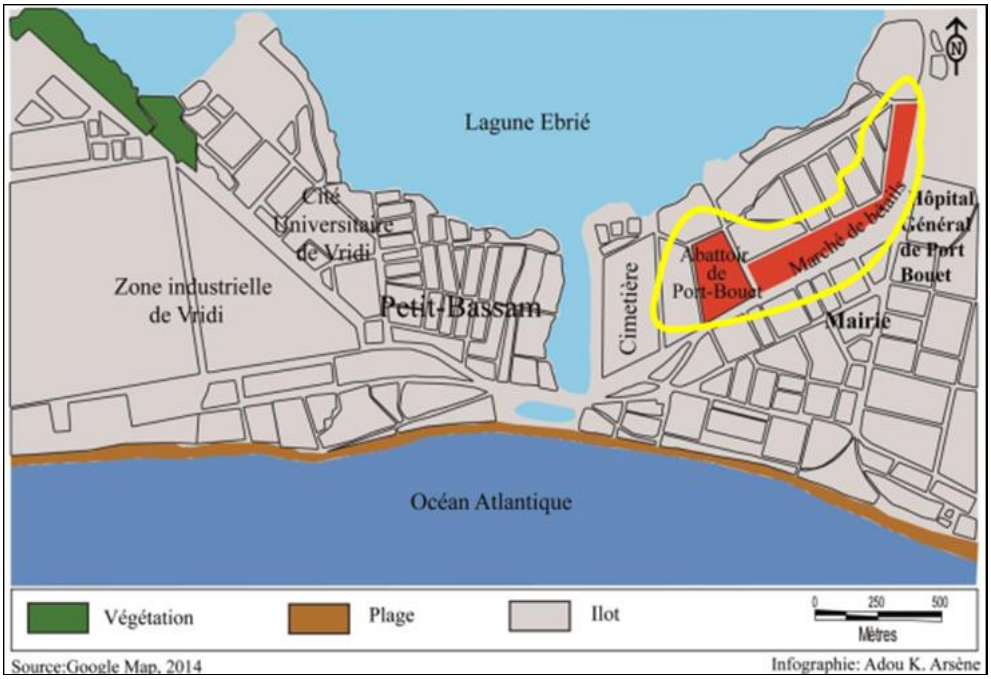

Fig 1: Location of the Port Bouet abattoir

Tick and blood sampling

Ticks were collected from 62 cattle and stored alive. Ticks collected from each bovine were kept in a collection tube until identification in the laboratory. The collection technique consisted of a visual examination by searching the fur of the different parts of the body of each animal. The aim was to find any ticks present on the animals. For each animal searched, all the ticks collected were kept in a collection tube. Each collection tube was marked with information such as the animal's code, date of collection and place of collection. In addition to this information, a survey form was sent to pet owners to collect other data such as the origin of the animals (country, region, locality), as well as other information such as the different treatments used against ticks, and the frequency of these treatments. Blood samples were taken from the same animals, then smears were taken on object slides. Blood was also collected in EDTA tubes and dry tubes for subsequent pathogen detection using the PCR molecular characterization tool.

Tick identification

After each harvest, all samples were taken to the laboratory of the Institut Pasteur de Côte d'Ivoire, where the ticks were identified. Specimens were identified using OPTIKA binocular magnifiers at 4X magnification and dichotomous identification keys [10, 11, 12]. Ticks were identified according to stasis, genus and species. Following identification, the collected ticks were grouped into mono-specific batches and then stored for subsequent arbovirus research.

Parasitological analysis

Hematological examination of blood samples was performed using the 10% Giemsa-stained blood smear technique. Blood smears were taken on object slides and dried for 20 min. Fixation was then carried out with methanol and Giemsa staining was performed for 30 minutes. An electron microscope was used to observe the haemoparasites. For this purpose, a drop of immersion oil was applied to the stained smears.

Data processing

The data collected were entered using Microsoft Office Excel version 2013. We also used Microsoft Office Excel to calculate proportions and create figures. The non-parametric Wilcoxon-Mann-Whitney statistical test was used to test the hypothesis that the distributions of each population are close.

Results

Tick genera and species identified

Ticks were collected from 62 cattle aged between 2 and 5 years. 67.74% were male. A total of 372 tick specimens were collected, comprising 349 (93.82%) females, 22 (5.91%) males and 1 (0.27%) nymph. Three tick species belonging to two genera have been identified, namely *Amblyomma variegatum* (Fabricius, 1794), *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini, 1888) and *Rhipicephalus sanguineus* (Latreille, 1806). *R.* (*B.*) *microplus* was the predominant species, with a frequency of 80.37% (Table 1).

The Wilcoxon-Mann-Whitney test performed on our data gives us $p>0.05$. There is therefore no significant difference between the *Amblyomma variegatum* and *Rhipicephalus* (*Boophilus*) *microplus* populations.

Table 1: Relative abundance of stasis and tick species identified

Ticks	Number		Abondance %
	F	M	
Rhipicephalus (*Boophilus*) *microplus*	290	9	299 (80.37)
Amblyomma variegatum	59	10	69 (18.55)
Rhipicephalus sanguineus	0	3	3 (0.81)
Nymph	1	0	1 (0.27)
Total	350	22	372 (100)

Prevalence of heamoparasites

Blood smear analysis revealed blood parasites in 40 bovine blood samples, representing a prevalence of 64.51%. These blood parasites belong to the genera *Trypanosoma* (17.74%), *Babesia* (30.64%), *Anaplasma* (30.64%) and *Theileria* (29.03%) (Table 2). The proportions of mono infestations in the blood samples, in order of numerical importance, are as follows: *Babesia bovis* 7 (11.29%), *Theileria sp* 5 (8.06%), *Anaplasma marginale* 4 (6.45%) and *Trypanosoma sp* 2 (3.23%).

Polyparasitism was observed in blood samples. The proportions of bi-infestations are as follows: Bb_Am 4 (6.45%), Bb_Trysp 2 (3.23%), Am_Thsp 6 (9.68%), Trysp_Thsp 1 (1.61%), Am_Trysp 3 (4.84%) and Bb Thsp 4 (6.45%).

The analyses revealed two types of tri-infestation combination in similar proportions. Thus, the Bb_Trysp_Thsp and Am_Trysp_Thsp associations each obtained frequencies of 1.61% (Table 2).

Table 2: Co-infestation of Haemoparasites observed in cattle

Type of infestation	Haemoparasites	Prevalences (%)
Mono-infestation	*Babesia bovis*	7 (11.29)
	Anaplasma marginale	4 (6.45)
	Trypanosoma sp	2 (3.23)
	Theileria sp	5 (8.06)
Bi infestation	Bb_Am	4 (6.45)
	Bb_Trysp	2 (3.23)
	Am_Thsp	6 (9.68)
	Trysp_Thsp	1 (1.61)
	Am_Trysp	3 (4.84)
	Bb_Thsp	4 (6.45)
Tri infestation	Bb_Trysp_Thsp	1 (1.61)
	Am_Trysp_Thsp	1 (1.61)

Babesia bovis (Bb); *Anaplasma marginale* (Am); *Trypanosoma sp* (Trysp); *Theileria sp* (Thsp)

Discussion

This study highlighted the tick species and haemoparasites present in cattle at the Port Bouet abattoir in Abidjan. The two tick genera identified, *Amblyomma* and *Rhipicephalus*, have also been highlighted in several previous studies in Côte d'Ivoire [8, 13, 14]. Of the three species collected, the predominant species was *R.* (*B.*) *microplus* with a proportion of 80.37%, followed by *A. variegatum* (18.55%). It should be noted that, long before the introduction of *R.* (*B.*) *microplus* on cattle farms in Côte d'Ivoire [15], *A. variegatum* was the predominant tick species on cattle farms [16, 17]. The other species of the *R.* (*Boophilus*) subgenus, *R.* (*B.*) *decoloratus*, *R.* (*B.*) *annulatus* and *R.* (*B.*) *geigyi*, are increasingly absent from the distribution of ticks in farm animals. The species newly introduced to Côte d'Ivoire, *R.* (*B.*) *microplus*, is said to be gaining the upper hand. Observations made in the present work show that the other species of *R.* (*Boophilus*) could almost all have been replaced by *R.* (*B.*) *microplus*. In fact, this exotic *R.* (*B.*) *microplus* tick has invaded all the country's livestock farms and is now the main cattle tick, followed by *A. variegatum* [13, 14]. This new distribution of tick species in Côte d'Ivoire since the introduction of *R.* (*B.*) *microplus* should attract the attention of decision-makers. Female ticks were collected in this study in a proportion of 93.82%. In fact, the tick species found on cattle farms are Ixodidae, which show a sufficiently marked sexual dimorphism. During sampling, only the most visible ticks are collected from the animals' bodies, and most of these are female ticks. These female ticks are very large, as they gorge themselves abundantly on blood.

In this study, all haemoparasites were obtained in similar proportions apart from *Trypanosoma* sp (17.74%). We have *Babesia bovis* with a rate of 30.64%, followed by *Anaplasma marginale* with a rate of 30.64% and *Theileria* sp with a rate of 29.03%. This finding suggests that ticks are among the main vectors of livestock diseases [18]. Berte and collaborator showed that, with the exception of trypanosome prevalence, tick-borne disease (TBD) prevalences were also very high, both in the north and center of the country. In Côte d'Ivoire, when rearing domestic ruminants, the search for pasture through grassy and wooded savannahs is a factor that could favor the animal's contact with the vector, particularly tsetse flies and ticks. The result is the transmission of pathogens to animals by these vectors. This results in the onset of general symptoms such as fever, inappetence and, in some cases, death. Because of the similarity of these symptoms, the tsetse fly or glossina is still considered the only vector harmful to animals. Breeders are therefore unaware that ticks can also be the cause of certain diseases. This study shows that, in addition to Trypanosomiasis, several other infections can be transmitted by ticks. Studies carried out in Cameroon have shown that the genera *Anaplasma*, *Babesia* and *Theileria* are the most frequent hemoparasites in small ruminant farms [19]. Blood parasites, in particular the *Anaplasma*, *Babesia* and *Theileria* genera, occupy a prominent position. This is because these hemoparasites are responsible for significant mortality on farms, and the presence of tick-borne hemoparasites is strongly linked to the presence of tick vectors [20]. The prevalence of pathogens (64.51%) is high in this study. Indeed, ticks are increasingly resistant to the acaricides used [21]. Observed a similar rate (66.3%) in samples from Morocco's major livestock farming regions, with *Theileria annulata* (35.4%), *Anaplasma marginale* (20.5%), *Babesia bigemina* (13.6%) and *Babesia bovis* (12%) as pathogens. Also in north-central Nigeria, examination of Giemsa-stained thin blood smears showed an overall prevalence of 25.7%, with *Babesia bigemina* and *Babesia*

bovis accounting for 16.0%, followed by *Theileria mutans* (3.1%), *Trypanosoma spp* (2.8%), *Anaplasma marginale* (1.9%), Microfilaria (1.4%) [22]. Since the introduction of *R.* (*B.*) *microplus* to Côte d'Ivoire and several other parts of West Africa [15, 8, 7, 13], the presence of this tick species on farms has changed the diversity of ticks and, more importantly, the pathogens they transmit. One example is babesiosis, which is causing major economic damage; this disease is caused by livestock arthropods worldwide, with vast areas of the world under constant threat [23].

Information gathered from breeders showed that the cattle came from countries bordering Côte d'Ivoire: Burkina Faso, Mali, Ghana and the northern towns of Bondoukou and Korhogo. In recent years, the results of several studies have shown that these haemoparasites are endemic in cattle in several study areas, which can lead to serious illness when these animals are subjected to stressful conditions. Unfortunately, these tick-borne diseases are poorly controlled in sub-Saharan Africa due to a lack of understanding of the infection biology of the pathogen species. Understanding the species composition of pathogens in infected cattle will enable us to formulate cost-effective interventions that will develop effective control strategies and improve the ability to control tick-borne diseases [24]. Research efforts and financial support should be focused in particular on haemoparasitic diseases (babesiosis, anaplasmosis, theileriosis, ehrlichiosis and trypanosomiasis). For the diagnosis of febrile diseases of unknown origin, we need to make more effective use of molecular tools for the detection of these rapidly expanding animal diseases.

Conclusion

The results of this study show that animals reared at the Port Bouet slaughterhouse are not immune to tick and hemoparasite infestation. The study identified the tick species present in cattle destined for slaughter. Three tick species were identified: *Amblyomma variegatum, Rhipicephalus* (*Boophilus*) *microplus* and *Rhipicephalus sanguineus*. *Rhipicephalus* (*Boophilus*) *microplus* was the dominant species with 80.37%. Anaplasmosis, babesiosis and theileriosis are the main tick-borne hemoparasitoses affecting cattle at the Port Bouet abattoir. A number of key findings emerged from this study. The association of several species in addition to trypanosomiasis is a factor contributing to the persistence of animal resource insecurity. In terms of future prospects, it would be interesting to initiate a study using more modern techniques such as serology and molecular biology to better assess the prevalence of hemoparasites and the real impact of the different species on livestock health, with a view to taking them fully into account. It is therefore necessary to set up an entomological and microbiological surveillance system in order to effectively combat ticks and tick-borne diseases in Côte d'Ivoire.

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Competing interests

Authors have declared that no competing interests exist.

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