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Cattle ticks population and prevalence of *Babesia spp* amongst it vector: *Rhipicephalus (Boophilus) microplus* in a zone of Ivory Coast

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ABSTRACT

The aim of this survey is to evaluate prevalence of *Babesia spp* in *Rhipicephalus (Boophilus) microplus* and the importance of ticks species existing in Bingerville area. In this cross sectional study, thirty six cattle from 6 farms have been sampled in Bingerville area (Ivory coast Southeast). From 511 ticks collected, 102 *Ambyomma spp* (19.97%) and 409 *Rhipicephalus (Boophilus) microplus* (80.03%) were identified. Then, the prevalence of *Babesia spp* parasites amongst *Rhipicephalus* (*Boophilus) microplus was focused*. With 120 engorged females of *Rhipicephalus (Boophilus) microplus*, 24 pools of 5 ticks each were done. The Giemsa stain test had shown prevalences of 8.33% and 1.73% of *Babesia spp* respectively in pool and individual levels.

Keywords: Cattle,Ticks, *Ambyomma spp*, *Rhipicephalus (Boophilus) microplus*, *Babesia spp*, prevalence.

INTRODUCTION

Babesiosis due to *Babesia bovis* is one of the major causes of loss in cattle farm. For instance, in queensland -an australian region- ($ 132 millions of losses in term of treatments) (Mc Leod, 1995), and in United state of America (from 1906 to 1943): $ 3 billions of losses in terms of productivity, diseased animals, deaths. The disease has been eradicated in USA. (CFSPH, 2007). Some African areas like western part were free of its main vector agent: *Rhipicephalus (Boophilus) microplus* (Gragnon 2005; Chegou, 2005). A few years ago, this vector has been clearly discovered in west Africa: Ivory Coast, Benin, etc. (Madder et al., 2007; Toure, 2009). In Ivory Coast, cattle babesiosis due to another species *Babesia bigemina* is endemic, but rare study on cattle babesiosis prevalence is available. Surveys on prevalence could help in control strategy of the disease. Gragnon (2005) found by PCR-RFLP method in a bigger region including Bingerville area, a prevalence of 41.6% of *Babesia bigemina*. The double aim of the present survey is to assess the presence or absence of this new

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vector and then to determine the prevalence of *Babesia spp* in *Rhipicephalus (Boophilus) microplus* in southeast cattle farm of Ivory Coast.

MATERIALS AND METHODS

Presentation of study area or zone

This survey was a cross sectional study lead in Bingerville, 17 km away from Abidjan (Ivory Coast). Bingerville is geographically located at latitude north 5° 22 ‘, longitude east 3°53 ‘. Its surface is 664 km² and located in southeast (Figure 1). Temperatures vary between 24 and 29 °C, and mean temperature was 26 °C during 2010. We collected ticks on 36 cattle in the 6 farms of Bingerville from June 10th to June 29th (Table 1).

Choice of cattle farm used and animals sampling

All traditional and semi modern cattle farms were concerning by the survey. Therefore, six (6) out of ten (10) farms were chosen. Modern farms are not concerned. Preferably, if exist, we sampled five animals whatever the age, that looks the most infested with ticks. In order to have more accurate results, we looked for recent (four weeks ago) acaricide treatment, dosage and way of use. We also, checked for recent (four to six weeks ago) drugs (Oxytetracycline, Imidocarb dipropionate, Diminazene aceturate) used, and for what purpose (treatment, prophylactic, or sterilize) against *Babesia spp*. These foregoing practices could be confounding factors because they influence results like: presence of no ticks or presence of more species of ticks than others, with no kinetes of *Babesia spp* shown by the Giemsa smear test. After catching the animal, they were spent ten to fifteen minutes sampling ticks. Additional information on the farm was also taken: age of animal sampled, sex, acaricid use, etc.

Every single part of the animal was analysed and the ticks were removed with forceps and we put in phials containing alcohol 70%. For each animal, the phial containing corresponding ticks was clearly identified.

We determined the importance (expressed in percentage: %) of each genus and species of cattle ticks. Also, we estimated the prevalence of the parasite *Babesia spp* in it vector (female of *Rhipicephalus* (*Boophilus*) *microplus*). The prevalence is also expressed in percentage (%) with corresponding confident interval.

Laboratory essay

Using Stereo microscope, we determined genus and species of ticks collected.

Concerning species of *Rhipicephalus (Boophilus*), we discriminated with microscope at 100 × magnification; indeed we highlighted on lay out of dentition, the existence of ventro-internal protuberance bearing setae near the roster. As far as larvae and nymphs are found, we precise their genus. (Walker et al., 2003)

Amongst adults females of *Rhipicephalus (Boophilus) microplus*, we performed GIEMSA stain of haemolymph after fixation in Methanol. Haemolymph was obtained by breaking legs of ticks and the haemolymph drops on slide according to Burgdorfer (1970). We constituted 24 pools of 5 ticks each. So one slide stain is made from 5 ticks’ haemolymph. Finally, at 100 × magnification, we looked for kinetes of *Babesia spp* as described by Gugliemone et al. (1995 A positive case was considered when kinetes was present in a slide. When we didn’t observe any kinetes, it was a negative case.

Calculation of a genus or species importance

Genus 1 (in percentage: %) = Total of genus 1 collected / Total of ticks collected

Species 1 (in percentage %) = Total of species 1 collected / Total of ticks collected

Statistical calculation of Prevalence

According to the formula of Katholi et al. (1995) which is:

$P = 1 - (K/M)^{1/N}$, we programmed it in Microsoft Excel software (version 2003).

P is the prevalence of infection; K the number of negatives pools to GIEMSA Stain essay, M the number of total pools, and N the pool size.

The Confident interval at 95% is shown by:

$1- Wu (5\%)^{1/N} \leq P \leq 1 - Wl (5\%)^{1/N}$,

With $Wl (5\%) = V1 \times F2.5\% (V1, V2) / (V2 + V1 \times F2.5\% (V1, V2))$

Where $V1 = 2 K$, and $V2 = 2 (M-K)$,

and $Wu (5\%) = V1 \times F 1\text{-}2.5\% (V1, V2) / (V2 + V1 F 1\text{-}2.5\% (V1, V2))$

Indeed $V1 = 2 (K+1)$, $V2 = 2 (M-K)$.

In the expression of Wl (5%) and Wu (5%), F is the critical value given by a standard statistical table of F distribution.

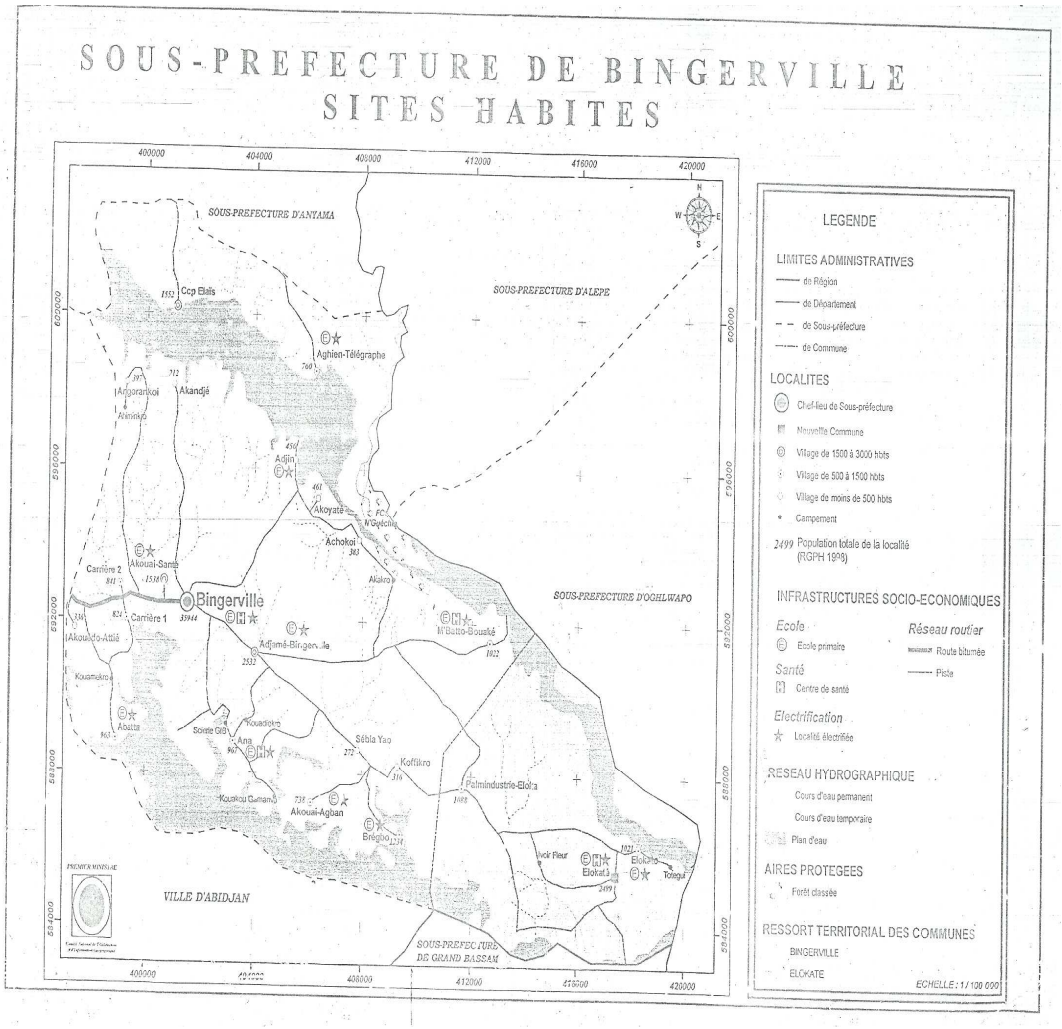


Figure 1: Study region.

RESULTS

All of the traditional and semi modern farms of Bingerville area participated to the survey from beginning to the end. As shown in Table 1, in these six (6) farms, we collected 511 ticks on 36 cows. Amongst these ticks, we identified 102 *Amblyomma spp* and 409 *Rhipicephalus (Boophilus) spp* (Figure 2) that equal respectively to 19.97% and 80.03%. All of the *Amblyomma* genus ticks were *variegatum*. All of the ticks belonging to *Rhipicephalus (Boophilus) genus* were *microplus* species. This population component is presenting as follow:

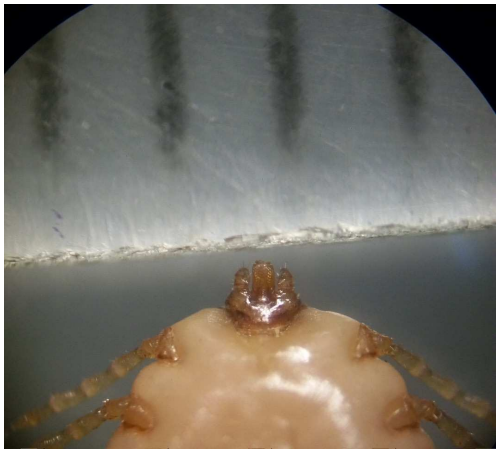
52 males (12.71%), 352 females (86.06%), 4 nymphs (0.98%) and 1 larva (0.25%). Out of 352 females, there were 120 engorged females. (Table 2)

From 120 engorged females, we observed two (2) pools positive to kinetes of *Babesia spp*. So 22 pools were negative to kinetes of *Babesia spp*. (Table 2).

Prevalence's obtained at pool or individual levels are shown in Table 3. We could notice that prevalence is low on individual level as well as on pool level.

A: Ventral view(male).

B: Dorsal view (male).



C: Hypostome.

D: Ventral view (female).

E: Dorsal view (female)

Figure 2: *Rhipicephalus (Boophilus) microplus*. Scale: the space between two lines equals 1 mm.

Table 1: Agenda of ticks' collection.

Date of collection	Name of the farm	Number of animal sampled	Ticks number collected
June 10th 2010	Ex projet laitier sud	5	180
June 14th 2010	Anader	7	111
June 17th 2010	Campement agri	5	51
June 21st 2010	Berlin	5	62
June 24th 2010	Carriere I	8	40
June 29th 2010	Cite feh kesse	6	67
TOTAL		**36**	**511**

Table 2: Types of ticks that predominate in Bingerville area and heamolymph stain results.

Total of ticks collected 511						
	Total of rhipicephalus (Boophilus) : 409					
Total of amblyomma : 102	Females engorged 120		Females Not engorged	males	Nymphae	Larvae
	2 Positives pools	22 Negatives pools	232	52	4	1

Table 3: Prevalence of *Babesia spp* in *Rhipicephalus (Boophilus) microplus.*

	Prevalences (%)	**Lower limit of confident interval**	**Upper limit of confident interval**
Individual level	1.73	0.42	6.33
Pool level	8.33	2.09	27.88

DISCUSSION

Most Population ticks remain *Rhipicephalus (Boophilus) spp* mainly *microplus* (80.03%). This species predominates in very high proportion. The current survey confirms persistence and well adaptation to Ivorian ecology. As explanation, It's important to remark that it was still raining during dry season (from October 2010 to end of March 2011) leading to a very humid weather.

This Vector has been recently introduced (Madder et al., 2007) very likely by uncontrolled importations of cattle. Afterward, Madder et al. (2011) found mainly *Rhipicephalus (Boophilus) microplus* (94%) in a more large area including our study region. Indeed, southeast is regularly humid with a mean temperature in Bingerville of 26 °C and relative humidity equals 85%. This vector proliferates very well in such climatic condition. This lead to increase number of generations per year and displacement of other ticks genera and species like *Amblyomma spp, Hyalomma spp Rhipicephalus (Boophilus) spp* (*decoloratus, geigyi,, annulatus*). The more recent acaricide treatment happened nine weeks ago in one farm before our ticks sampling. Thus, we are sure that the acaricide has no confounding

effect on our findings. In 2002, Knopf et al., found in Ivory coast, 96% of *Amblyomma spp* (almost exclusively *variegatum* species), <1% of *Hyalomma spp*, 47% of *Rhipicephalus (Boophilus) spp* (there was not *microplus*). Then, Chegou (2005) evaluated that there were 81.45% of *Amblyomma spp*, 18.54% of *Rhipicephalus (Boophilus) spp* (all were *decoloratus* species). The displacement trend by *Rhipicephalus (Boophilus) microplus* is more and more strengthened. Apart the advantage of humid climate, this strength of displacement deserves furthers research in order to elucidate the reasons: for example growing of *Rhipicephalus (Boophilus) microplus* resistant sub population to acaricides. All very experienced farmers (more than fifteen years doing this job) noticed inefficiency of classical acaricides (Cypermethrin, Amitraz, Deltamethrin, Flumethrin…) at normal dosage. They have started using unauthorized drugs like Fenitrothion that give them satisfaction with drawbacks of animals skin erosion or intoxication (Toure, 2009).

The low prevalence at pool level (8.33%) or at individual level (1.73%) of *Babesia spp* in *Rhipicephalus (Boophilus) microplus* is not astonishing for many reasons. Firstly, we have noticed relative weak sensitivity of GIEMSA stain test. Secondly, depending on immune statue of the animal host leading to lower parasitaemia (Oliveira et al., 2005) so that the females adult would have little chance to infect itself. Cafrune et al. (1993) described the vulnerability of tick severely infected by *Babesia bovis* so that these ticks die.

In such conditions, ticks moderately or less infected by parasite have long lifespan. Amongst the farms studied, the most recent prophylactic treatment of cattle against babesiosis has been done 3 months ago. So this confounding factor (recent drug use) has limited or no effects on our prevalence value. Even if Analytic Method to show *Babesia spp* is highly sensitive, it is not also abnormal to find low prevalence for the same explanations. Toure (2009) with Nested PCR method, found 0% of prevalence. Some authors found low prevalence in natural condition: Mahoney and Mirre (1971) 0.04% for *Babesia bovis* and 0.23% for *Babesia bigemina*; Callow (1984): 1 positive adult female tick on 1000 infesting one cow chronically infected; but others as Cen-Aguilar et al. (1998); Oliveira et al. (2005) respectively assessed 20.3% and 15.4% of prevalence.

Conclusion

The present survey showed that *Rhipicephalus (Boophilus*) *microplus* present in Bingerville (Southeast of Ivory Coast) and predominates (80.03%) amongst ticks population despite recently and unfortunately introduction. Subsequently, Ivory Coast veterinarian authority has to decide efficient control program of tick by taking into account *Rhipicephalus (Boophilus*) *microplus*.

Concerning prevalence of *Babesia spp* parasites in *Rhipicephalus (Boophilus*) *microplus*, we found a low prevalence of 4.7%. Further study of babesiosis surveillance due to Babesia *bovis* and co-infection dynamic between *Babesia bovis* and *Babesia bigemina* are worthwhile.

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