



**Original Paper**

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**Epidemiology of heartwater disease in West Africa: similar infection rate of *Ehrlichia ruminantium* evidenced in adults of *Amblyomma variegatum* and *Rhipicephalus microplus* in peri-urban villages of Bobo-Dioulasso, Burkina Faso**

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**ABSTRACT**

Heartwater disease, is a virulent tick-borne disease of ruminants caused by *Ehrlichia ruminantium* (ER), biologically transmitted by *Amblyomma* spp. However, in West Africa, the potential of *Rhipicephalus microplus* (*R. microplus*) larvae to transmit this bacterium has been demonstrated. Although previous studies have been conducted on heartwater and ER in Burkina Faso, data on infection rate of the bacterium in *A. variegatum* and *R. microplus* is lacking in the peri-urban areas. Hence, this study aimed to compensate this gap, focusing on five peri-urban villages around 10-30 km from Bobo-Dioulasso. A total of 359 cattle was examined for ticks collection. However, only adult specimens of the two tick species collected on the same animals were included in the molecular detection of ER by semi-nested PCR. Results revealed a global contact rate of 19% in *A. variegatum* and 26% in *R. microplus* without a significant difference between these rates. Thus, while *R. microplus* invasion in West Africa is accompanied by the transmission of ER to animals, its contact rate with the causal agent of heartwater disease is similar to that with the previous known vector. Such context could impact the heartwater disease epidemiology, mainly in the peri-urban areas where the study was carried out. From previous knowledge and literature, that is the first time such study focusing on *E. ruminantium* infection rate in ticks in peri-urban areas is conducted. Results suggest the necessity to improve tick control methods in the study areas since both ticks species can transmit the causal agent of heartwater disease to animals.

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**Keywords:** Heartwater, *Ehrlichia ruminantium*, ticks, peri-urban villages, livestock.

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## INTRODUCTION

In Burkina Faso, peri-urban livestock plays a key role in providing the needs of urban populations for meat, milk, dairy and poultry products (Kiendrebeogo et al., 2012; Zampaligre et al., 2019). As in rural areas, peri-urban livestock are negatively impacted by animals' mortality. The death of a single animal can have dramatic consequences on a vulnerable household by reducing its ability to withstand food crisis and lift itself out of poverty (FAO, 2012). Heartwater disease, with its mortality rate of up to 80% in small ruminants (Stachurski et al., 2019) is one of the animal diseases causing this household vulnerability. It is caused by *Ehrlichia ruminantium* which is transmitted by *Amblyomma* spp ticks (*Amblyomma variegatum* in West Africa) (Jongejan and Uilenberg, 2004) with the acute form of the disease often found in susceptible animals (Stachurski et al., 2019). The infection rate determined in this tick-vector in West Africa is 9%-26% (Faburay et al., 2007; Farougou et al., 2012) and was assessed mainly in rural areas. However, following the recent establishment of *R. microplus* in West Africa (Madder et al., 2011; Adakal et al., 2013; Touré et al., 2012, 2014), it has been shown that 29% of *R. microplus* adults collected in various west-African countries (i.e. Benin, Burkina Faso and Ivory Coast) appeared to be naturally infected by *E. ruminantium* with transovarial transmission occurring from infected adult females to their offspring (Biguezoton et al., 2016a). Therefore, since these data resulted mainly from works carried out in rural areas and given the importance of the peri-urban areas in livestock production during last decades, it was necessary to investigate the infection rates of the causal agent of heartwater disease in both tick species focusing on such areas. Since the infection rate of a given pathogen in its vector is one of the parameters which influence the caused disease epidemiology in an area, this study therefore

aimed at appreciating the risk of infection of sensitive animals in the targeted areas. For this purpose, five peri-urban villages around 10-30 Km from Bobo-Dioulasso, the economic capital of Burkina Faso was visited during rainy season of 2020.

## MATERIALS AND METHODS

### Study areas

Ticks sampling was carried out in the province of Houet (Figure 1), which had the highest cattle and small ruminant production in the "Hauts Bassins" region in Burkina Faso. This province is one of the most humid in Burkina Faso, presenting favourable conditions of ticks development. Five (05) villages located within a 10-30 km radius of Bobo-Dioulasso (that is Darsalamy, Manengue, Nasso, Niamandougou and Tolotama), were randomly selected for tick collection.

### Tick sampling

Ticks were collected from six herds in each of the five selected villages. In each herd, 12 cattle were examined for tick collection. Ticks were collected in August 2020 during the rainy season when there is significant population growth of three-host species such as *A. variegatum* (Farougou et al., 2013). For tick sampling, each animal was kept with one flank on the ground for no more than 15 min to allow collection according to Biguezoton et al. (2016b). Ticks were stored in labelled vials containing 70% ethanol and brought to laboratory for identification. Sampling date, host ID and number were recorded.

### Ticks identification and samples for *E. ruminantium* screening

The identification of the different ticks species collected was performed using the standard taxonomic identification keys (Walker et al., 2003). The identification and enumeration of ticks were carried out using a

binocular magnifying glass, forceps and petri dishes.

### Molecular detection of *E. ruminantium*

Prior to *E. ruminantium* DNA identification, DNA extraction was performed according to the manufacturer's instructions. The semi-nested PCR for the detection of *E. ruminantium* DNA was implemented as described in previous studies (Biguezoton et al., 2016a). A positive and a negative control were systematically included for each PCR (Molia et al., 2008). The positive control was a

DNA previously tested positive for *E. ruminantium*, while the negative control was a distilled water sample.

### Statistical analysis

The data recorded were analyzed using R statistical software version 4.2.1 for windows (R Core Team, 2022). The fisher's exact test for count data of Rcommander package was performed to compare *E. ruminantium* infection rate in *A. variegatum* and in *R. microplus*.

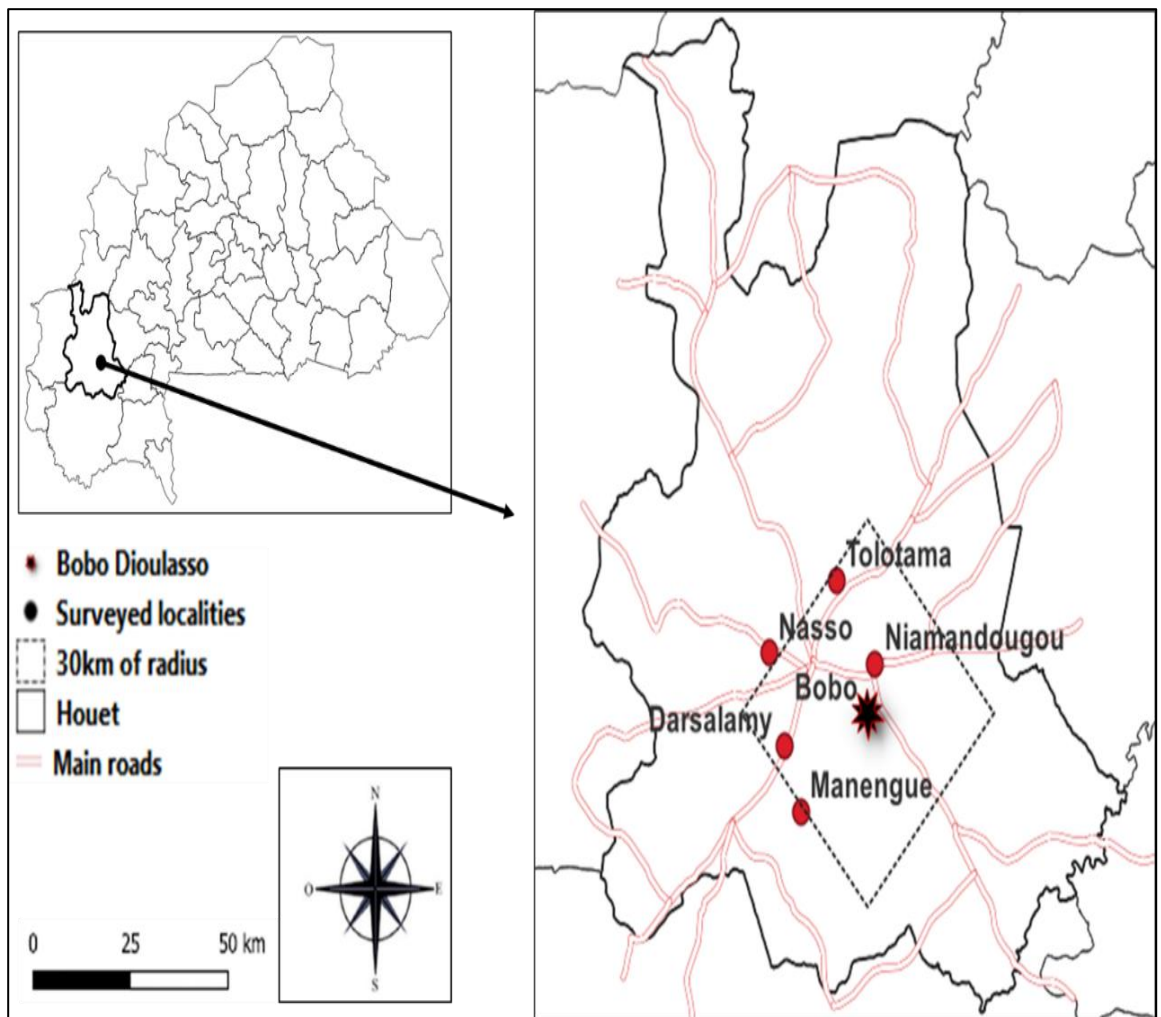
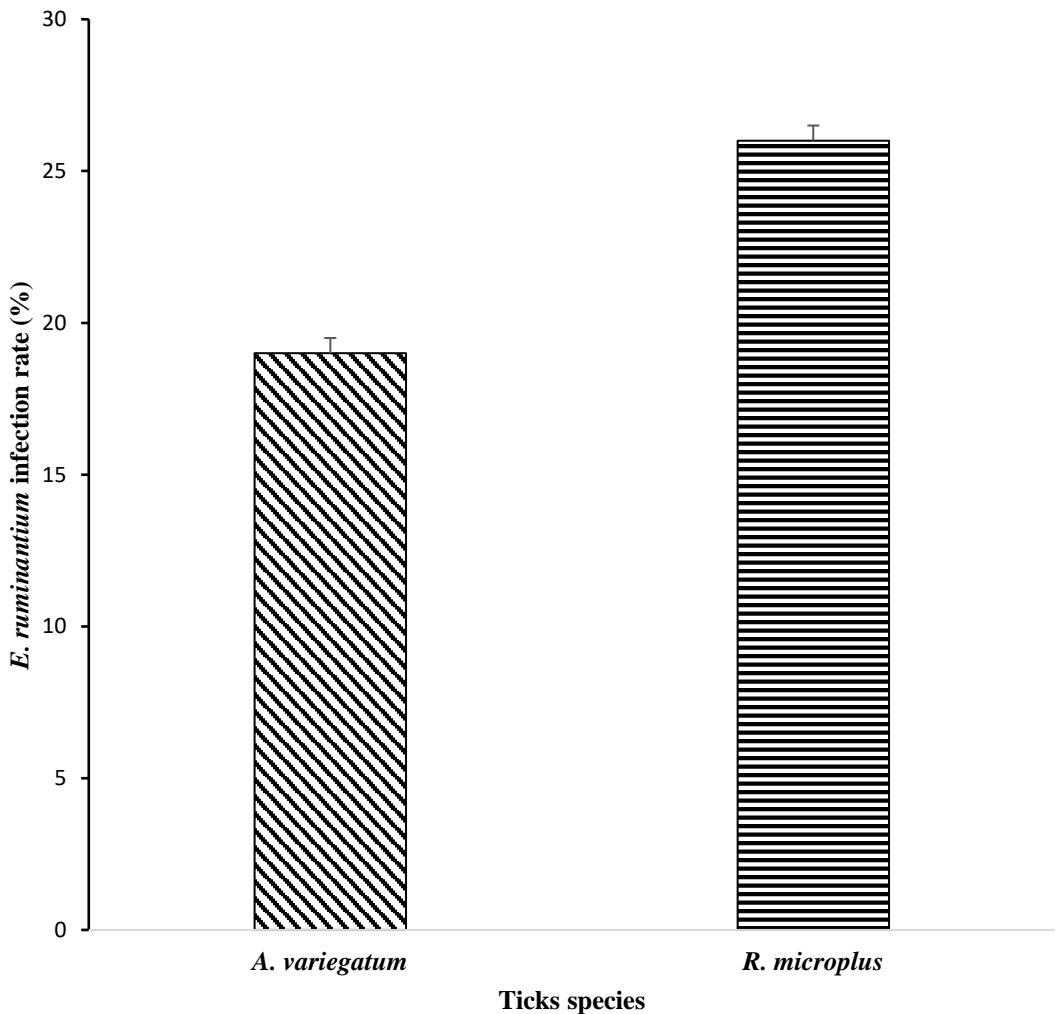


Figure 1: Map of location of sampling sites.

## RESULTS

A total of 3421 adult ticks were collected from 359 cattle. Six species were identified, *Amblyomma variegatum* being the most frequent (p-values <0.05). From the collected ticks, 137 adults male and female *A. variegatum* (n= 83) and *R. microplus* (n=54) were sampled on 48 cattle which were infested by both tick species for *E. ruminantium* screening. *E. ruminantium* DNA was found in 16 of the 83 (19%) field-collected *A. variegatum* ticks (Figure 2). The variation of the rate of infections between villages was

statistically significant (p-value<0.01). Likewise, the presence of *E. ruminantium* DNA was determined in 14 of the 54 (26%) field-collected *R. microplus* ticks (Figure 2). Considering this later species, the variation of the rate of infections between villages was also statistically significant (p-value=0.02). Interestingly, there was no significant difference between the infection rate of *E. ruminantium* in *A. variegatum* and *R. microplus* (p-value = 0.4>0.05 with 95% confidence interval: 0.28-1.69; odds ratio= 0.68).



**Figure 2:** *Amblyomma variegatum* and *Rhipicephalus microplus* tick infection by *Ehrlichia ruminantium*.

## DISCUSSION

This study provides the first molecular evidence of *E. ruminantium* infection in *A. variegatum* and *R. microplus* ticks in peri-urban herds in Burkina Faso and suggests potential infections of related cattle. Few studies have been conducted on the infection of *R. microplus* by *E. ruminantium* except some studies on its infection by *Ehrlichia minasensis* (Cabezas-Cruz et al., 2016; Carvalho et al., 2016). However, Biguezoton et al. (2016a) determined the presence of *E. ruminantium* DNA in seven of the 24 field-collected *R. microplus* from cattle in Benin, Burkina Faso and Ivory Coast. It is noteworthy that recent experiments highlighted that *R. microplus* larvae can acquire *E. ruminantium* transovarially and transmit it to naïve animals (Somé et al., 2023). But this transmission resulted in a mild subclinical disease whereas severe clinical disease was observed in sheep when infested by *A. variegatum* infected nymphs.

Similar (i.e. not significantly different) infection rates of the bacterium in both tick species could impact the heartwater disease epidemiology in the peri-urban areas where the study was carried out. Nevertheless, the small sample size of the current study represented a limit of solid conclusions. We documented the *E. ruminantium* infection rate in two co-occurring tick species in peri-urban areas in West Africa. Analyses revealed similar infection rates of the bacterium in *A. variegatum* and *R. microplus*. From previous knowledge literature, that was the first time such study focusing on *E. ruminantium* infection rate in peri-urban areas was conducted. Furthermore, results pointed out the necessity to improve tick control methods in the study areas since both ticks species can

transmit the causal agent of heartwater disease to animals.

## COMPETING INTERESTS

The authors declare that there is no competing interests or personal relationships that might have appeared to influence the work reported in this article.

## AUTHORS' CONTRIBUTIONS

MVS and ASB designed the research. MVS, ASB, AZ and SZ conducted the experiments. MVS and ASB performed data analysis and wrote the first draft. All authors contributed to and agreed on the final version.

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