



Detection of *Rickettsia africae* in ticks and cattle in Côte d'Ivoire by real-time PCR

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ABSTRACT

Objective: Since ticks capable of transmitting *Rickettsia africae*, which causes African tick-bite fever, are present in Côte d'Ivoire, this study aims to investigate the circulation of this bacterium in ticks and cattle in Côte d'Ivoire.

Methodology and Results: Ticks and blood samples collected from cattle in all cardinal points of Côte d'Ivoire were tested for the presence of *Rickettsia africae*. Samples were tested for the presence of *R. africae* by real-time PCR using a primer and probe set targeting the *poT15-dam2* gene. Although no blood sample tested positive for *R. africae*, 43.67% (138/316) of the tick pools were positive for the same pathogen. The majority of positive pools came from Korhogo (53/138), followed by Bondoukou (39/138), and Abidjan (33/138). On the contrary, Bouaflé (6/138) and Man (7/138) had the lowest number of positive pools. Of the positive tick pools, 69.56% were *Amblyomma variegatum*, 13.77% *Rhipicephalus (Boophilus) microplus*, 10.86% *Rhipicephalus sanguineus*, 0.72% *Hyalomma impeltatum*, 0.72% *Rhipicephalus (Boophilus) decoloratus*, 1.45% *Hyalomma spp* and 1.45% *Rhipicephalus spp*.

Conclusion and applications of results: The results of this study show that *Rickettsia africae* circulate in a wide range of tick species from the five geographic areas of the Côte d'Ivoire and clinicians should be aware that this rickettsia has the potential to cause febrile illness in the human population. Indeed, better diagnostic capacities such as rapid tests, which could be used in high-risk rural areas, would make it possible to detect and treat these rickettsioses. It would also reduce the morbidity associated with febrile illnesses and overtreatment with ineffective drugs used for the treatment of febrile illness syndrome, such as antimalarials and arboviruses in Côte d'Ivoire. In addition, it is necessary to strengthen collaboration between veterinarians and clinicians and biologists while contributing to the dissemination of the so-called « One Health » approach. Finally, make people at risk aware of the role of tick vectors.

Key words: *R. africae*, tick, bovine, zoonotic, Côte d'Ivoire

INTRODUCTION

Tick-borne rickettsiae are pathogens belonging to the spotted fever group (SFG). Recent studies provide new insights into the zoonotic role and diversity of these agents, while also updating their geographical distribution (Pillay *et al.*, 2020). Some zoonotic rickettsiae that were previously considered limited to a specific geographical area have now been detected on different continents (Parola *et al.*, 2013). The disease in humans is usually characterised by fever, malaise, arthralgias, lymphadenopathies, myalgias, and headaches (Boulgosa, 2020). An inoculation chancre can also be present, while clinical manifestations vary among *Rickettsia* species (Faccini-Martinez *et al.*, 2014). Furthermore, the same spectrum of clinical symptoms can be observed in other diseases such as malaria, influenza and typhoid fever (Richards *et al.*, 2010), making the clinical diagnosis of *Rickettsia* infections very difficult. Ticks are the main vectors and reservoirs of SFG *Rickettsia*, and they transmit these bacteria to synanthropic and sylvatic animals, and humans (Aubry & Gaüzère, 2016). Nowadays, an increasing number of reports indicate the widespread presence of *Rickettsia* spp. in ticks, animals and humans in Africa (Tomassone *et al.*, 2016, Abanda1 *et al.*, 2019). In addition,

several rickettsiae have been detected in ticks collected from cattle in the north-eastern transhumance corridor of Côte d'Ivoire (Ehounoud *et al.*, 2016). The same authors showed that 46% of the ticks collected carried *R. africae*. It is noteworthy that in Côte d'Ivoire, farmers and herdsman live in close promiscuity with the animals on the farms. Moreover, almost all farmers practice extensive livestock rearing, which favours the dissemination of ticks and increases their contact with the human population (Pfaffle *et al.*, 2013). As these human communities interact closely with cattle, the risk of contracting tick-borne infections is high. To date, there are no studies in Côte d'Ivoire that examines the burden of rickettsial infections in human communities despite the presence of risk indicators like including the widespread presence of tick species that can transmit these bacteria and livestock practices. Unfortunately, rickettsial diseases are difficult to diagnose in these communities due to the widespread clinical presentation and the lack of routine or robust diagnostics tools in local hospitals. Thus, this study aimed to analyse the presence and prevalence of *R. africae* in various tick species collected in several areas in Côte d'Ivoire.

MATERIALS AND METHODS

Study areas and designs: This study was conducted in Côte d'Ivoire (4°5 and 10°5 North latitude and between 2°5 and 8°5 West longitude) from January 2019 to April 2021. Côte d'Ivoire is an ecologically diverse territory (Sudanian zone, sub-Sudanese zone, Lower Côte d'Ivoire zone, forest zone, Middle Côte d'Ivoire zone). The selected study areas belong to the five geographical points of Côte d'Ivoire and four agro-ecological zones as follows : Korhogo in the North (Sub-Sudanese zone), Bondoukou in the East (Sub-Sudanese

zone), Abidjan in the South (Lower Côte d'Ivoire zone), Man in the West (forest zone) and Bouaflé in the Centre (Middle Côte d'Ivoire zone) (Figure 1). From January 2018 to July 2019, ticks and a blood sample were collected from cattle in all five zones. A total of 10 yards were visited, with 2 yards per zone and 20 cattle per yard selected. Ticks were collected from cattle over one year of age, regardless of sex, for 10 minutes. At least three to nine ticks were collected from an animal.

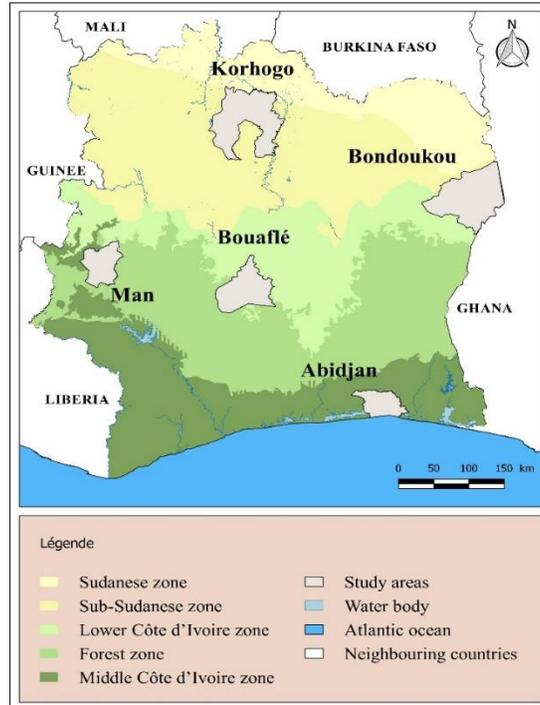


Figure 1: The geographical positioning of the five study areas

The identification of the ticks was done at the species level using a binocular magnifying glass OPTIKA (G x 10 or G x 20), and a digital microscope BA-210 LED FULLHD with an x100 magnification. The dichotomous identification keys of Apanaskevich and Horak (2007), Meddour *et al.* (2006), and Walker *et al.* (2003) were used for tick identification. Ticks were then grouped into pools of 1-9 according to species, developmental stage, host and location area, (Tomassone *et al.*, 2012). Pools were supplemented with phosphate-buffered saline (PBS). Additionally, three 2mm diameter beads were added in every tube and then placed in a Tissue Lyser adapter 2 X 24 for grinding at 5500 rpm for 3 minutes. The grindings were then stored at -80°C. Blood collection was performed with a 5cc syringe from the jugular vein of the cattle and stored into EDTA tubes. The blood samples were then preserved in liquid nitrogen and transported to the laboratory of the Institut Pasteur de Côte d'Ivoire and stored at -80°C.

DNA extraction and real-time PCR amplification: DNA extraction from tick

homogenates was performed using the DNeasy Blood and Tissue kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. For the blood samples, a total of 5 ml of EDTA blood was centrifuged and 200 µl of the buffy coat was collected. Then the DNA was extracted with the previously mentioned kit and further stored at 70/80°C until amplification. Molecular detection was performed by real-time PCR targeting the *poT15-dam2* gene of *R. africae*. The Ambion kit with forward primer: TGCAACACGAAGCACAAAAC, reverse primer: CCTCTTGCGAAACTCTACTT and the specific FAM-TGACGTGGATTTCGAGCACCGGA-TAMRA (Ehounoud *et al.*, 2016 and Luce-Fedrow *et al.*, 2015) probe were used. PCRs were performed in a final volume of 20 µL comprising Ambion Applied Biosystems kit reagents (10 µL of 2X with 1 µL of buffer and 5.75 µL of water-free nuclease), 0.5 µL of forward and reverse primer (10 µM), 0.25 µL of specific probe (10 µM), and 2 µL of DNA in a 96-well matrix. PCR assays were performed

in an Applied biosystems 7500 thermal cycler using the following amplification program: 50°C for 10 minutes, 95°C for 15 minutes followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute.

Statistical analysis: Proportions of real-time PCR positive batches per study area were calculated, with exact binomial 95%

confidence intervals (95% CI). Proportions of infection in tick pools were also calculated at the 95% confidence level. The analyses were performed using R software. For the different cartographic figures, a Geographic Information System (GIS) software, in this case QGIS version 2.16 was used.

RESULTS

Tick species diversity by sampling area: A total of 1240 engorged ticks were collected from 200 cattle in 10 parks in the five study areas. Overall, 12 tick species were identified as follows: *A. variegatum* (n=544), *H. impressium* (n=51), *H. m. marginatum* (n=2), *H. marginatum* (n=3), *H. impeltatum* (n=6), *H. truncatum* (n=9), *H. rufipes* (n=2), *R.(B.) annulatus* (n=36), *R.(B.) decoloratus* (n=21), *R.(B.) geigy* (n=18), *R.(B.) microplus* (n=484), *R. sanguineus* (n=30), *Rhipicephalus spp* (n=18), *Hyalomma spp* (n=16). Then, a total of 316 pools were formed in the different study areas. Table 1 shows that the Korhogo zone had the highest number of pools, followed by the Abidjan and Bondoukou

zones, which had almost equal numbers. The Bouaflé and Man zones have the lowest and almost equal number of pools (Table 1).

Distribution of ticks in the study areas: Based on the tick species identified in the different study areas, a spatial distribution map was established. This distribution showed that all the 12 tick species detected were present in Korhogo. The Abidjan and Bondoukou were similar in terms of tick species diversity and number. The map also shows that species of the genus *Hyalomma* are present in Korhogo and Bondoukou. Finally, only two species, namely *A. variegatum* and *R. (B.) microplus* were recorded in the Bouaflé and Man zones (Figure 2).

Table 1. Number of pools according to tick species and study areas

Tick species	Abidjan	Bondoukou	Bouaflé	Korhogo	Man	TOTAL
<i>A. variegatum</i>	36	35	9	37	6	123
<i>H. impressium</i>	-	1	-	16	-	17
<i>H.m.marginatum</i>	-	-	-	1	-	1
<i>H.marginatum</i>	-	-	-	1	-	1
<i>H. impeltatum</i>	-	-	-	2	-	2
<i>H.truncatum</i>	-	-	-	3	-	3
<i>H.rufipes</i>	-	-	-	1	-	1
<i>Hyalomma spp</i> (nymph)	-	2	-	10	-	12
<i>R.(B.) annulatus</i>	6	5	-	1	-	12
<i>R. (B.) decoloratus</i>	1	4	-	2	-	7
<i>R. (B.) geigy</i>	3	2	-	1	-	6
<i>R. (B.) microplus</i>	23	8	32	9	36	108
<i>R. sanguineus</i>	-	1	-	14	-	15
<i>Rhipicephalus spp</i> (nymph)	3	4	-	1	-	8
TOTAL	72	62	41	99	42	316

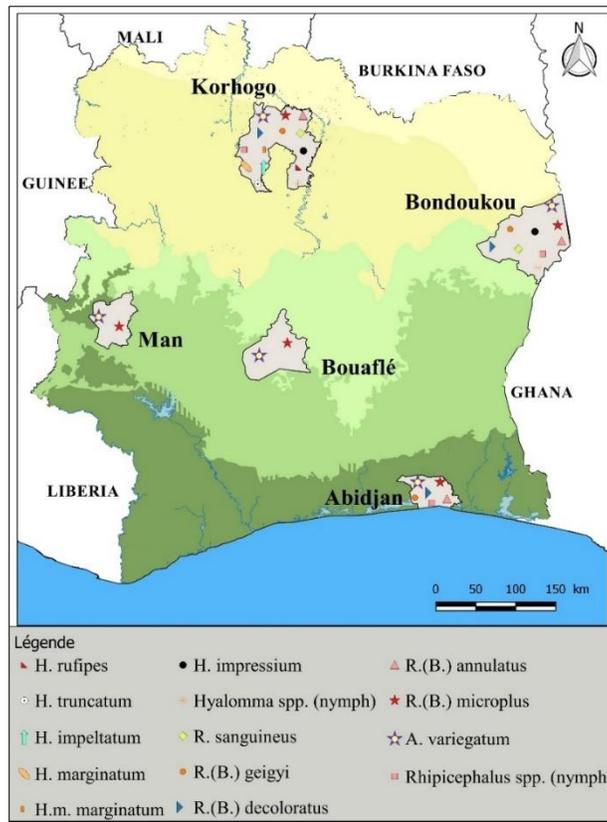


Figure 2: Distribution of tick species in the five study areas

Prevalence of *Rickettsia africae* according to study areas: The overall prevalence of pools positive for *R. africae* was 43.67% (138/316). The maximum number of positive pools for *R. africae* came from Korhogo (53/138), followed by Bondoukou (39/138), and Abidjan (33/138). On the contrary, Bouaflé (6/138) and Man (7/138) displayed the lowest prevalence of all the locations (Table 2). The prevalence

of *R. africae* varied between the study areas. The prevalence observed in Korhogo (53.53%, 95% CI: 43.23-63.62%), Bondoukou (62.9%, 95% CI: 49.69-74.83%) and Abidjan (45.83%, 95% CI: 33.02-57.99%) were higher than those in Bouaflé (14.63%, 95% CI: 5.56-29.17%) and Man (16.67%, 95% CI: 6.97-31.36%) (Table 2).

Table 2: Prevalence of *Rickettsia africae* in tick pools according to study areas

Study area	N	n _{pos}	%.	95% CI
Abidjan	72	33	45,83	33,02 - 57,99
Bondoukou	62	39	62,9	49,69 - 74,83
Bouaflé	41	6	14,63	5,56 - 29,17
Korhogo	99	53	53,53	43,23 - 63,62
Man	42	7	16,67	6,97 - 31,36
TOTAL	316	138	43,67	38,13 - 49,33

N: number of pool, n_{pos}: number of pool Infested, %: Prevalence, CI: Confidence Interval

Prevalence of *R. africae* according to tick species: The majority of positive pools were represented by *A. variegatum* ticks (96/138), and the lowest prevalence was detected in *Rhipicephalus (Boophilus) microplus* (19/138), or *Rhipicephalus sanguineus* (15/138) tick pools. On the contrary, the pools of other infested species are very small and do not exceed 2 per species (Table 3). From this analysis, a high prevalence was observed between the infestation of *Amblyomma variegatum* pools (69.56%, 95% CI: 49.69-74.83%) and the pools of other tick species, for

example *Rhipicephalus (Boophilus) microplus* 13.77% (95% CI: 8.49-20.66%) Table 3. It is also useful to know the distribution of tick species infested with *Rickettsia africae* in the different study areas. *A. variegatum* ticks highlighted the highest rate of infection in the five study areas. Several tick species were positive for the presence of *R. africae* in Korhogo and Bondoukou, compared to Bouaflé, Man and Abidjan where only *A. variegatum* and *R. (B.) microplus* ticks were infected (Figure 3).

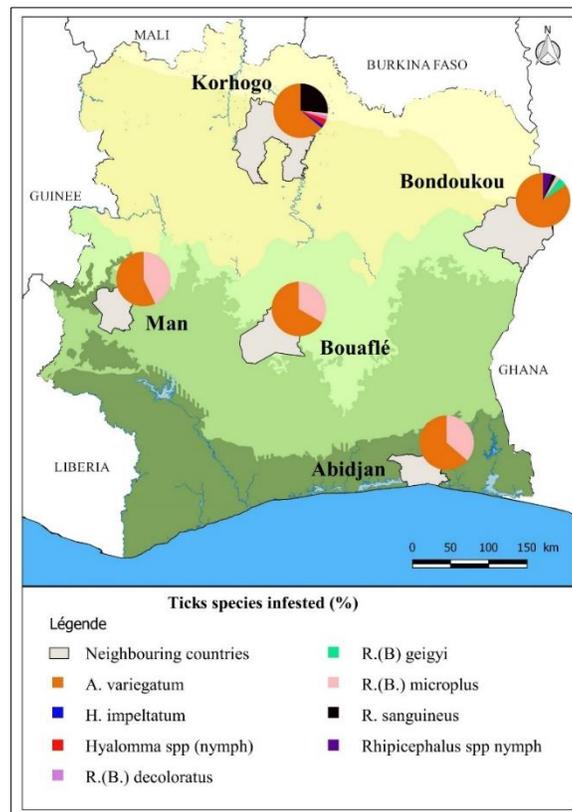


Figure 3: Distribution of tick species infested by *R. africae*

Prevalence of cattle infested with *Rickettsia africae*: Molecular analysis of cattle blood

samples revealed an overall prevalence of 0% of *R. africae* for the gene (poT15-dam2).

Table 3: Prevalence of *R. africae* according to tick species

Tick species	N	n _{pos}	%.	95% CI
<i>A. variegatum</i>	138	96	69,56	61,16-77,1
<i>H. impeltatum</i>	138	1	0,72	0,02-3,97
<i>Hyalomma spp</i> (nymph)	138	2	1,45	0,17-5,14
<i>R. (B.) decoloratus</i>	138	1	0,72	0,02-3,97
<i>R. (B.) geigy</i>	138	2	1,45	0,17-5,14
<i>R. (B.) microplus</i>	138	19	13,77	8,49-20,66
<i>R. sanguineus</i>	138	15	10,86	6,21-17,29
<i>Rhipicephalus spp</i> (nymph)	138	2	1,45	0,17-5,14
TOTAL	138	138	100	97,36-100

N: number of pool, n_{pos}: number of pool Infested, %: Prevalence, CI: Confidence Interval

DISCUSSION

Pool size and distribution of tick species in the study areas: The tick species identified in the herein study have also been highlighted by several other studies in Côte d'Ivoire (Touré *et al.*, 2014; Achi *et al.*, 2001). The Korhogo area had the highest number and diversity of tick species compared to the other study areas. This could be explained by the intense transhumance activity that takes place in this location, as according to Boka *et al.* (2015), the transhumance corridor contains more tick species compared to the species counted in Côte d'Ivoire. Additionally, according to the FAO (2012), the transhumance phenomenon forces herds to move over longer distances and wider areas during the dry season. This would increase the risk of dissemination of tick species throughout the transhumance area, possibly contributing to the diversity of the tick species present in these three areas. On the other hand, only two tick species were collected in Bouaflé and Man. A scarcer diversity of tick species was previously reported in these areas (Touré *et al.* 2014). This could be explained by the low level of cattle farming in these two areas in contrast to the other study areas. Furthermore, the same authors have shown that in Côte d'Ivoire, the most numerous species of the genus *Hyalomma* are only found in the north.

Prevalence of *Rickettsia africae* according to study areas: A high prevalence (43.67%) of *R.*

africae was detected in the tick pools analysed from cattle in five geographical areas of Côte d'Ivoire with positive pools in each area. This outcome is similar to that reported (46%) in a study that examined ticks from cattle from twenty-five livestock farms in the north-eastern transhumance zone of Côte d'Ivoire (Ehounoud *et al.*, 2016). The high number of cattle and their high migratory activity in this area could explain this. This confirms that the presence of *R. africae* is likely to be linked to the tick-harboring cattle (Brouqui *et al.*, 2004). Ticks from Korhogo, Bondoukou and Abidjan had significantly higher infection rates with *Rickettsia africae* than those from Bouaflé and Man. These results could be related to the diversity of tick species collected in the different areas. Furthermore, the species *Amblyomma variegatum*, a preferred reservoir of *Rickettsia africae* (Socolovschi *et al.*, 2009), was more abundant in Korhogo, Bondoukou and Abidjan. In contrast, it was not very abundant in Bouaflé and Man.

Prevalence of *R. africae* positive pools according to tick species: Several tick species pools were positive for *Rickettsia africae* with varying prevalence. The prevalence of *Amblyomma variegatum* tick infection was higher than previously reported in Burkina Faso by Tomassonea *et al.* (2016). Nonetheless, even higher prevalence rates (90%) of *R. africae* have been reported in A.

variegatum ticks from cattle in the northern departments of the Côte d'Ivoire including Korhgo by Ehounoud *et al.* (2016). In addition, variable infection rates (62-100%) with *R. africae* are reported in *A. variegatum* in other sub-Saharan African countries. The high prevalence of infection might be explained by the transstadial and transovarial transmission of *R. africae* in *A. variegatum* (Socolovschi *et al.*, 2009), which is considered the main vector of this bacterium. In addition, this rickettsia species has been detected in other tick species, including *H. impeltatum*, *R. (B.) decoloratus*, *R. (B.) microplus*, *R. (B.) geigy*, *R. sanguineus* in our study. To our best knowledge, this is the first report of *R. africae* in these tick species in

CONCLUSION

The results of this study indicate that *R. africae* circulates in a wide range of ticks species in all five geographical locations assessed in Côte d'Ivoire. Clinicians should be aware that this bacterium has the potential to cause a febrile illness in the human population. Improved diagnostic methods such as rapid kits, which

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Côte d'Ivoire. which indicates a wide range of widely distributed ticks that could transmit this pathogen.

Prevalence of cattle infested with *Rickettsia africae*: None of the cattle blood samples collected in Côte d'Ivoire was positive for the presence of *R. africae*. These results are in line with other studies that report a 0% prevalence of this pathogen in cattle blood (Edwards *et al.*, 2011, Barradasa *et al.*, 2021). A possible explanation might be that the cattle only play an essential role in the feeding process and transportation of ticks and providing habitat for ticks, the bacterium circulates through co-feeding mechanisms, without the host showing an active infection Edwards *et al.* (2011).

could be used in rural public health facilities, would allow detection and treatment of these infections. This would also reduce the morbidity associated with febrile illness and the overtreatment with ineffective drugs such as antimalarials used in the case of febrile illness syndrome.

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