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# Molecular Diagnosis of *Rickettsia aeschlimannii* in Febrile Patients in Côte d'Ivoire

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**Abstract:** *R aeschlimannii* is a bacterium that causes Mediterranean spotted fever. It is a rickettsial disease, which is an acute febrile illness characterized by the appearance of skin pimples and bedsores. *R. aeschlimannii* is mainly transmitted by ticks of the genus *Hyalomma*, which are present throughout the African continent, including Côte d'Ivoire. Despite the presence of the pathogen and its potential vectors in Côte d'Ivoire, the disease is not yet well-known or even undiagnosed in our health centers. Consequently, it is a neglected disease. The aim of this study is to search for *R. aeschlimannii* bacteria in febrile patients in order to improve the management of febrile illnesses in Côte d'Ivoire. Blood samples taken from patients to test for yellow fever virus and stored in the Institut Pasteur of Côte d'Ivoire biobank were also used to test for *R. aeschlimannii* by quantitative PCR. The 5 to 14-year-olds patients from Korhogo were infested with *R. aeschlimannii* with a relatively low prevalence of 9.10%. Our results underline the need to continue the study to control certain tick-borne diseases transmitted to both animals and humans. In the north of the country, the age group most vulnerable to Mediterranean spotted fever is the pre-adolescent age.

**Keywords:** *Rickettsia aeschlimannii*, Mediterranean Spotted Fever, Ticks, Febrile Patients, Côte d'Ivoire

## 1. Introduction

Hard ticks (Acari: Ixodidae) are obligate blood-sucking parasitic arthropods that are vectors for a wide range of zoonoses, such as tick-borne encephalitis; Lyme bor-reliosis; and Anaplasma, Coxiella, Ehrlichia and Rickettsia and Babesia infections [16]. Tick-borne rickettsiae are pathogens belonging to the spotted fever group (SFG). In recent years, an increasing number of studies have been carried out, providing new insights into the zoonotic role and diversity of these agents [15]. These researches have also helped to clarify the geographical distribution of these agents indeed, some rickettsiae, previously considered limited to a specific geographical area have been detected on different continents [13]. Little information is available on Africa, as rickettsial agents often cause mild disease and are generally not diagnosed. *R. aeschlimannii* is characterized by a more heterogeneous geographical distribution and has been detected mainly in ticks of the genus *Hyalomma* from several continents [15]. *R. aeschlimannii* causes symptoms similar to

those of Mediterranean spotted fever, which have been reported so far in patients leaving Africa to European and American countries or, to a lesser extent, in African patients [13]. Despite the presence of *R. aeschlimannii* in cattle ticks [3, 5] and the exposure of target populations, people do not know much about the disease, and almost undiagnosed in our health centers. Consequently it is a neglected disease. The aim of this study was to improve the management of febrile diseases in Côte d'Ivoire.

## 2. Material and Methods

### *2.1. Study Areas and Designs*

This study was carried out 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).

### *2.2. Collection of Patient Blood Samples*

The blood samples of patients intended for the surveillance of arboviruses, in particular yellow fever and dengue, from the bank and belonging to the Department of Epidemic Viruses of the Institut Pasteur of Côte d'Ivoire were used. Concerning the choice of these patients, some inclusion and non-inclusion criteria were defined. Indeed, from the defined inclusion and non-inclusion criteria, a total of 150 blood samples were retained, thirty per zone.

### *2.3. Ethical Consideration*

It is imperative to point out that prior to the use of these samples the institutional approval of the study protocol was granted by the National Research Ethics Committee (CNER) of Côte d'Ivoire (N/Ref: 003/MSHP/CNER-km) concerning the use of patient samples.

### *2.4. 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 200 µl of the buffy coat was collected. Then the DNA was extracted with the previously mentioned kit and further stored at 80°C until amplification. Molecular detection was performed by real-time PCR targeting the *RaescSca1* gene of *R. aeschlimannii*. The Ambion kit with forward primer: AAAGAAATGGATTTCACGGCGAA, reverse primer: ACCAAGTAAACGTCTCGTAC and the specific FAM-TGGGGAAATATGCCGTATACGCAAGC-TAMRA [5, 9] 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.

### *2.5. Statistical Analysis*

The overall prevalence of *R. aeschlimannii* infestation in men was calculated and compared, with exact binomial 95% confidence intervals (95% CI). The analyses were performed using R software. For the cartographic figures, a Geographic Information System (GIS) software, in this case QGIS version 2.16 was used.

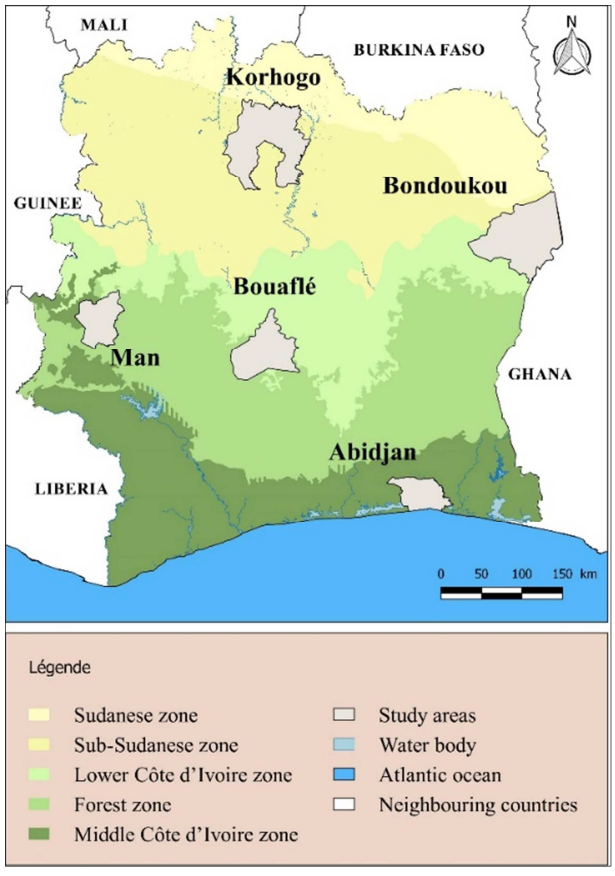



***Figure 1.*** *The geographical positioning of the five study areas.*

## 3. Results

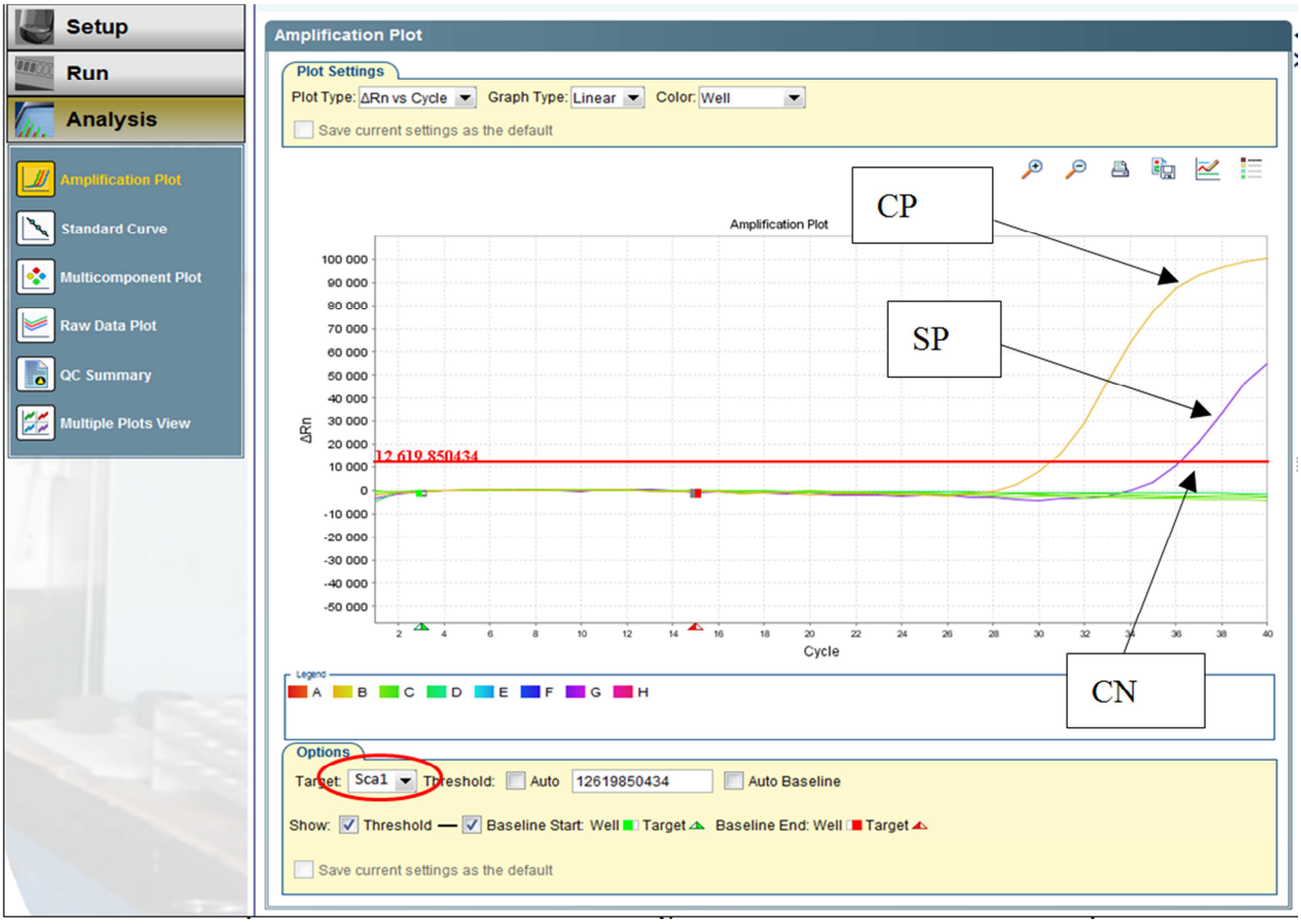
### *3.1. Overall Prevalence of Humans Infected with Rickettsia Aeschlimannii*

The figure 2 shows the amplifications of the *RaescSca1* gene of *R. aeschlimannii* in patients with febrile syndromes. The table 1 shows the overall prevalence of patients infected with *R. aeschlimannii* according to the different study areas,

sex, age group and occupation. Within the study areas, it is noted firstly in this table that only the people living in the Korhogo region had *R. aeschlimannii* infection. Secondly, the prevalence of this infection was low at 3.33% (95% CI: 0.08-17.22). The same observation was made gender issues, only the male sex was infected by *R. aeschlimannii*. Also the prevalence of this infection is not significant (1.09%, 95% CI: 0.03-5.91). Likewise, the 5 to 14-year-olds were infected with *R. aeschlimannii* with a low prevalence of infection (2.77%, 95% CI: 0.07-14.53). Finally, in terms of occupation, only the category of pupils/students were infected. However, this prevalence was not high (1.52%, 95% CI: 0.04-8.15).

### *3.2. Distribution of Patients Infested by R. aeschlimannii According to Sex, Age Group and Occupation*

The table 2 shows the distribution of patients infected with *R. aeschlimannii* according to sex, age group and occupation. In the Korhogo region, male patients were infected with a prevalence of 4.55%. The 5 to 14-year-olds infested patients infested patients were also all from Korhogo with a moderately low prevalence of 9.10%. Also, 5.88% of the category "Pupils-Students" from Korhogo region were infested with *R. aeschlimannii*. However, no infection was observed in patients living in Abidjan, Bondoukou, Bouaflé and Man.




CP: Positive control, CN: CN: Negative control, SP: Positive sample

***Figure 2.*** *Amplifications of the RaescSca1 gene in a patient by real-time PCR.*

***Table 1.*** *Overall prevalences of humans infected with R. Aeschlimannii.*

| | | N | n | % [CI 95%] |
|---|---|---|---|---|
| Study areas | | | | |
| | Abidjan | 30 | 0 | 0 [0-11,57] |
| | Bondoukou | 30 | 0 | 0 [0-11,57] |
| | Bouaflé | 30 | 0 | 0 [0-11,57] |
| | Korhogo | 30 | 1 | 3,33 [0,08-17,22] |
| | Man | 30 | 0 | 0 [0-11,57] |
| Sex | | | | |
| | F | 58 | 0 | 0 [0-6,16] |
| | M | 92 | 1 | 1,09 [0,03-5,91] |
| age group | | | | |
| | <5 years old | 41 | 0 | 0 [0-8,60] |

| | | N | n | % [CI 95%] |
|---|---|---|---|---|
| | 5-14 years old | 36 | 1 | 2,77 [0,07-14,53] |
| | 15-17 years old | 9 | 0 | 0 [0-33,63] |
| | ≥18 years old | 64 | 0 | 0 [0-5,60] |
| Occupation | | | | |
| | Other | 52 | 0 | 0 [0-6,85] |
| | Cultivators | 15 | 0 | 0 [0-21,80] |
| | Pupils/Students | 66 | 1 | 1,52 [0,04-8,15] |
| | Breeders | 2 | 0 | 0 [0-84,18] |
| | Officials | 15 | 0 | 0 [0-21,80] |

M: male; F: female; N: number of patients examined; n: number of positive patients;
%: prevalences; CI: confidence interval

***Table 2.*** *Prevalence of R. aeschlimannii infection according to sex, age class and occupation in the different study areas.*

| | Abidjan | | Bondoukou | | Bouaflé | | Korhogo | | Man | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | n (%) | N | n (%) | N | n (%) | N | n (%) | N | n (%) |
| M | 16 | 0 | 15 | 0 | 20 | 0 | 22 | 1 (4,55) | 19 | 0 |
| F | 14 | 0 | 15 | 0 | 10 | 0 | 8 | 0 | 11 | 0 |
| <5 years old | 3 | 0 | 9 | 0 | 11 | 0 | 9 | 0 | 9 | 0 |
| 5-14 years old | 4 | 0 | 7 | 0 | 6 | 0 | 11 | 1 (9,10) | 8 | 0 |
| 15-17 years old | 2 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 4 | 0 |
| ≥18 years old | 21 | 0 | 13 | 0 | 13 | 0 | 8 | 0 | 9 | 0 |
| Officials | 8 | 0 | 3 | 0 | 2 | 0 | 1 | 0 | 1 | 0 |
| Pupils/Students | 14 | 0 | 9 | 0 | 10 | 0 | 17 | 1 (5,88) | 16 | 0 |
| Cultivators | 0 | 0 | 2 | 0 | 2 | 0 | 10 | 0 | 1 | 0 |
| Breeders | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| Other | 8 | 0 | 16 | 0 | 16 | 0 | 0 | 0 | 12 | 0 |

M: male; F: female; N: number of patients examined; n: number of positive patients; %: prevalences

# 4. Discussion

Despite the very low percentage of ticks infected with *R. aeschlimannii*, we managed to detect this *rickettsia* in the blood of a febrile patient who was suspected to have yellow fever. This infested patient came from the Korhogo region. Firstly, it should be noted that this infection overlaps with the tick infections in this study. Secondly, ticks considered as potential vectors of *R. aeschlimannii* are naturally only found in the northern part of Côte d'Ivoire. Thus, our results confirm the assertions of [2], according to which the epidemiological system of a tick-borne disease, like any other vector-borne disease, depends on a triad of "host, vector and pathogen", of which the "appointment" constitutes the risk factor. It is imperative to mention that although *R. aeschlimannii* DNA has already been detected in cattle ticks in the North-East transhumance zone by [5], no correlation between this bacterium in ticks and humans sharing the same territory has been established in Côte d'Ivoire. We therefore come to establish for the first time this "human-tick and *R. aeschlimannii*" correlation. The overall prevalence of *R. aeschlimannii* infection in the said region was low at 3.33%. As far as we are concerned, this low prevalence could be explained by the nature of the samples used (blood) and the type of patient concerned, as for [6], the yield of PCR on venous blood is lower than that obtained on dry cotton swabs of the eschar or on the inoculation eschar biopsy. In addition to this rationale, [8] Argues shepherds and farmers, are continuously exposed to ticks parasitizing their domestic animals. According to these same authors, the risk of contracting rickettsial infections such as *R. aeschlimannii* would be high in these populations. Consequently, Ivorian physicians should pay attention when diagnosing febrile illnesses or other clinical signs compatible with rickettsial diseases in their patients. Indeed, it seems appropriate to underline with [4] that *R. Aeschlimannii* was detected in a patient moving from Morocco to France with several clinical signs. These signs included fever, generalized maculopapular rash and a vascular lesion on the ankle that became necrotic and resembled the typical 'black spot' of Mediterranean spotted fever [14]. Similarly, two cases of infection by *R. Aeschlimannii* have been described, one in a patient bitten in Morocco and another in South Africa [12]. Further, three Algerian cases have been described [10]. Thus, many cases in Algeria that were clinically identified as FSM could, in fact, be due to *R. Aeschlimannii*, especially if the patient presented several pressure sores [1, 11]. In our study, we show that the patient infested with *R. Aeschlimannii*, is male and belongs to the age group 5-14 years with low prevalence, 1.09% and 2.77% respectively. Furthermore, he belonged to the category of pupils/students with an equally low prevalence of infestation (1.52%). In our opinion, these low prevalence are probably due to the nature of the samples and the type of patients used. Clearly, these prevalence would be high if pressure ulcer biopsy samples were used and the patients were at risk populations. Despite these low prevalence, our study provides new insights into the epidemiology of Mediterranean spotted fever caused by *R. Aeschlimannii* in Côte d'Ivoire. Regarding gender-based infestation, our result is shared by a previous study reporting the infestation of a male by *R. Aeschlimannii*, in Greece [7].

Regarding age, previous studies have associated older individuals with *R. Aeschlimannii* infestation [7, 14], which is contrary in our study. Finally, [7] reported in their study that the patient infested with *R. Aeschlimannii*, resided in an agricultural area where rabbits were raised and goats and sheep grazed near his residence. This could explain the man's infestation, since the incriminating ticks would have come from these animals.

# 5. Conclusion

The molecular method used allowed us to show the circulation of *R. aeschlimannii* in patients presenting febrile syndromes in Côte d'Ivoire. Other studies are necessary in populations at risk (herdsmen) to evaluate the real level of rickettsial infestation in Côte d'Ivoire, and determine the risk factors for rickettsial diseases in Côte d'Ivoire.

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