





Research Article

Serological Evidence of *Rickettsia* Exposure among Patients with Unknown Fever Origin in Angola, 2016-2017

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Spotted fever group *Rickettsia* (SFGR) is one among the aetiologies that cause fever of unknown origin in Angola. Despite their occurrence, there is little information about its magnitude in this country either because it is misdiagnosed or due to the lack of diagnostic resources. For this purpose, eighty-seven selected malaria- and yellow fever-negative serum specimens collected between February 2016 and March 2017 as part of the National Laboratory of Febrile Syndromes, from patients with fever ($\geq 37.5^\circ\text{C}$) for at least 4 days and of unknown origin, were screened for *Rickettsia* antibodies through an immunofluorescence assay (IFA). Serological results were interpreted according to the 2017 guidelines for the detection of *Rickettsia* spp. Three seroreactive patients had detectable IgM antibodies to *Rickettsia* with an endpoint titre of 32 and IgG antibodies with endpoint titres of 128 and 256. These findings supported a diagnosis of *Rickettsia* exposure amongst these patients and highlight that rickettsioses may be among the cause of unknown febrile syndromes in Angola. Therefore, physicians must be aware of this reality and must include this vector-borne disease as part of aetiologies that should be considered and systematically tested in order to delineate appropriate strategies of diagnostic and control of *Rickettsia* in Angola.

1. Introduction

Rickettsioses are vector-borne diseases of medical importance, particularly in African countries where an increasing number of cases have been reported amongst residents and

tourists [1]. Despite its public health importance, the epidemiological characteristics linked to rickettsial diseases are poorly defined in the African continent [2]. *Rickettsia* species are strictly intracellular, Gram-negative bacteria from the order Rickettsiales comprising 30 recognized

species and numerous uncharacterized strains [3]. Ticks are vectors and reservoirs for several rickettsial agents, but some *Rickettsia* spp. are transmitted by fleas, lice, and mites [4]. These bacteria present several antigenically distinct groups, with those belonging to the spotted fever group (SFG) remaining an important cause of human and animal diseases, characterized by vascular invasion and tissue necrosis [5]. The classical triad of clinical manifestations of SFG *Rickettsia* infection includes fever, eschar, and rash [6]; however, these vary depending on the rickettsial species involved.

In Angola, a high percentage of the population lives in suburban neighbourhoods, characterized by adobe and cement constructed houses with limited access to public basic resources such as potable water, energy supply, health, and education. These highly unhealthy living conditions associated with domestic animals in close proximity increase the exposure to ectoparasites and to the pathogens that they might harbour.

Many studies report rickettsioses acquired by travellers, but the majority refers to sub-Saharan Africa tourists who develop African tick-bite fever (ATBF) [1]. In African countries, fevers of unknown origin can have different aetiologies including rickettsial infection but, due to the overlapping symptomatology with other endemic diseases (e.g., malaria, dengue, HIV, and brucellosis) that also cause fever, as well as the lack of available diagnostic tests and laboratory resources [7], rickettsioses are often under-diagnosed [2].

The aim of this study was to perform the laboratory diagnosis of *Rickettsia* spp. exposure among febrile patients from Angola with malaria and yellow fever already clinically and laboratory discarded.

2. Methods

2.1. Sample Collection. Between February 2016 and March 2017, a total of 87 serum specimens were obtained from public hospitals, as part of the Febrile Syndrome Surveillance Programme of Angola. These serum specimens were collected from patients from different cities (Benguela, Cabinda, Huambo, Luanda, and Malanje) and provinces (Huila, Kwanza Sul, Kwanza Norte, Lunda Norte, and Zaire) of Angola and were selected for this study if belonging to individuals presenting fever for at least four days ($\geq 37.5^{\circ}\text{C}$) and with at least one of the following inclusion criteria: malaise, myalgia, arthralgia, nausea, vomiting, and rash. These selected serum specimens were also malaria and yellow fever negative, previously tested through peripheral blood smear, malaria antigen detection test (SD BIOLINE), and RT-PCR, respectively.

A questionnaire including patient demographic (age and gender) and epidemiological data (province and municipality of origin, type of residence, household characteristics, season of specimen collection, access to potable water, contact with animals, and clinical manifestations) was filled for each patient by the health care professionals.

2.2. Serological Testing. Sera were tested by an in-house immunofluorescence assay (IFA) using *R. africae* strain as antigen, prepared at the Portuguese National Institute of Health Dr. Ricardo Jorge, as previously reported [8]. Along with fever, *Rickettsia* exposure was defined when the sera presented both IgG titre ≥ 64 and IgM titre ≥ 32 , according to the previously published guidelines for the detection of *Rickettsia* spp. [9].

3. Results

A total of 87 patients from 10 different cities and provinces were analysed in this study (Figure 1). Out of the 87 patients, 27 (31%) were females and 60 (69%) were males. Patients' age ranged between 1 and 86 years, with 45% included in the 13–26 years interval. Eighty-three percent (72/87) of the participants lived in urban areas, while the remaining 17% (15/87) lived in rural areas. All the patients had contact with domestic animals such as dogs, cats, and chickens.

Of all sera from febrile patients of Angola analysed ($n=87$), three (3.5%; 95% CI: 1.2–9.7) clearly met the laboratory definition of *Rickettsia* exposure. One presented IgG antibody titre of 128 and IgM antibody titre of 32 and the other two seroreactive sera presented IgG antibody titre of 256 and IgM antibody titre of 32. Of the 3 seropositive individuals, two were females and one was a male, with ages ranging from 15 to 34 years, living in Luanda and Benguela cities.

4. Discussion

Rickettsia spp. is distributed worldwide, but the knowledge about their epidemiology and their health impact in Africa is scarce, with most serological studies focusing on IgG seroprevalence, namely, in South Africa [10, 11], Djibouti [12], Kenya [13, 14], Tunisia [15], Cameroon [16], Zimbabwe [17], Ivory Coast [18], Egypt [19], and Angola [20].

Rickettsioses are rarely considered when evaluating patients with undifferentiated febrile illnesses, and due to the overlapping symptomatology with other endemic diseases such as malaria, dengue, and yellow fever, diagnosis is difficult without confirmatory laboratory tests.

Our study aimed at ascertaining the association of *Rickettsia* exposure with fever of unknown origin by screening febrile patients from Angola that had been previously found to be negative for malaria and yellow fever.

The IFA is currently the gold standard test for serological diagnosis of *Rickettsia* [9, 21]. However, the cross-reactivity of this methodology does not allow the identification of the specific infecting *Rickettsia* species [22]. Several *Rickettsia* antigens should have been tested; however, due to serum sample volume constraints, as well as the availability of IFA slides coated only with *R. africae* antigen, the patient samples were only tested for this SFG species.

This study has detected three *Rickettsia* exposed patients among previously undiagnosed febrile patients (3.5%; 95% CI: 1.2–9.7).

Interestingly, these results are similar to a study reported by Botros and collaborators [19], in which only 1% of the

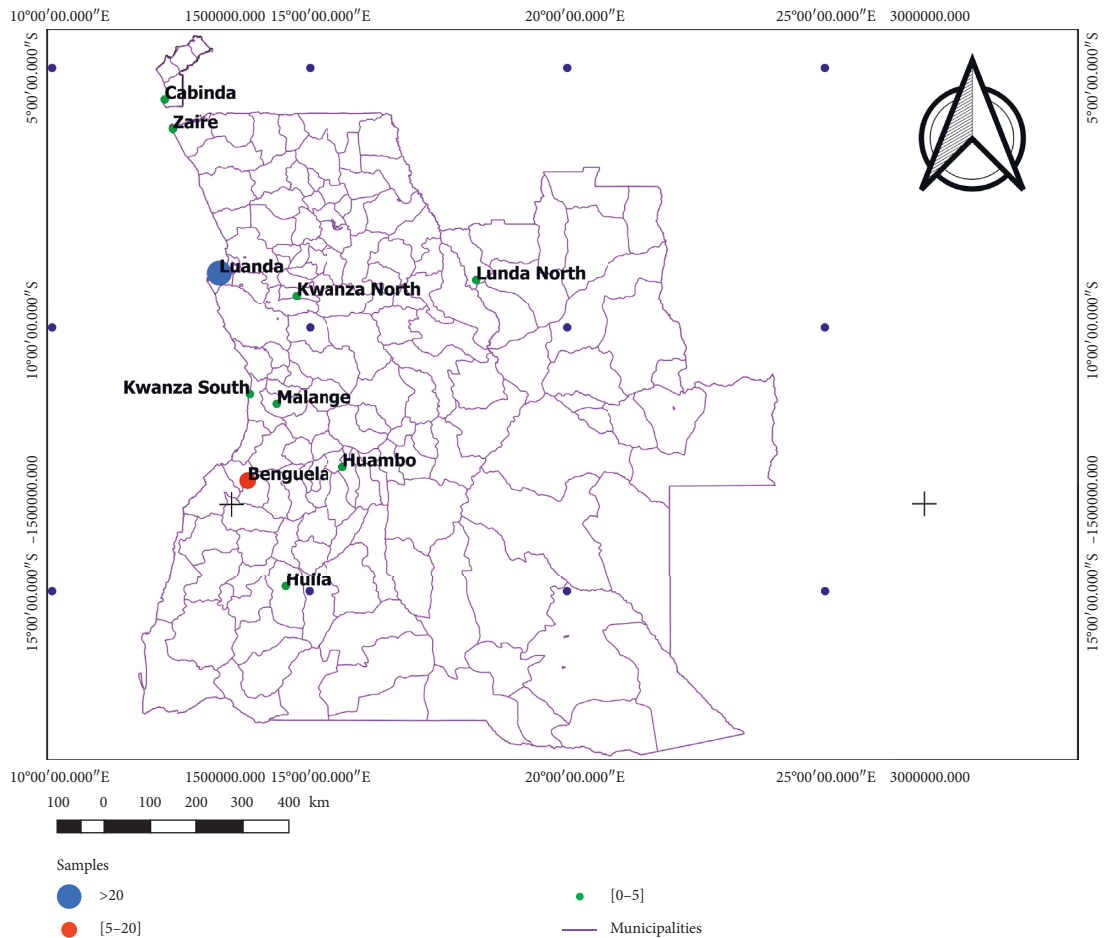


FIGURE 1: Study area with Angola sampling location cities (Benguela, Cabinda, Huambo, Luanda, and Malanje) and provinces (Huila, Kwanza Sul, Kwanza Norte, Lunda Norte, and Zaire).

Egyptian garbage collectors tested presented seroreactivity against *R. conorii*. Nevertheless, the herein study presents lower seroreactive serum samples when compared with other reports, demonstrating 17.68% of human *Rickettsia* exposure in Reunion Island, Southern Africa [23]; 21% in febrile patients from Mpumalanga, South Africa [10]; 24.1% in a pastoral HIV-endemic community of South Africa [11]; 16% in workers from a Djiboutian abattoir in East Africa [12]; 10% of febrile patients from Kenya; 22.4% of febrile children from western Kenya [14]; 66% of patients with fever of undetermined origin from Tunisia [15]; 32% of febrile patients from Cameroon [16]; and finally, 5.3% and 6.2% of a rural population of Sierra Leone and Ivory Coast, respectively [18].

Despite the important message that our results may arouse regarding a possible *Rickettsia* exposure, for several reasons, they should be carefully analysed and interpreted. According to Brouqui and collaborators [24], *Rickettsia* IgM and IgG antibodies are usually detected seven to 15 days after disease onset. The patients herein tested presented fever for at least 4 days. However, we cannot know exactly how long this clinical manifestation lasts, which makes it impossible to critically contextualize with the respective serological data. On the contrary, it is important to be aware that IgM cross

reactions with other pathogenic agents or false-positive IgM antibodies observed, for instance, when rheumatoid factor is present, may occur, as described in the guidelines for the detection of *Rickettsia* spp. [9]. Even though, our detected cases of IgM positive specimens were accompanied with positive titres of IgG (128 and 256) which, taken together, may reinforce the premise that rickettsiae are circulating in Angola. Nevertheless, and ideally, in order to confirm a current rickettsiae infection, these patients should be retested and checked for seroconversion or increased antibody titres in matched samples at 3-week intervals.

The transmission and dissemination of rickettsiae through vectors are a phenomenon of growing concern with the expanding human populations and increasing contact between humans and animals (domestic and wildlife) [25].

The rickettsiae-exposed patients who participated in our study lived in urban zones of Benguela and Luanda cities. One of them is a student, and the other two street vendors. A previous study done in pet dogs from Luanda [26] described a low *Rickettsia* seroprevalence in these animal species. Probably, ticks and fleas competent for *Rickettsia* appear with a low rate of parasitism in Angola.

Although in a low prevalence, this finding is relevant to the clinical management of patients with fever of unknown

origin and support the inclusion of this VBD in clinical diagnostic algorithms.

To conclude, our findings suggest that rickettsioses are present in Angola and, therefore, should be taken into account in cases of febrile illness. The serological evidence of exposure with these bacteria raises attention for the need of appropriated public health interventions and diagnostic improvement. Forthcoming studies should include a higher specimen number, with the possibility of detecting the pathogen in acute infection phases, both acute and convalescent samples screening, antibodies testing against several antigens, and, if possible, application of molecular techniques in skin biopsy or swab samples from suspected cases with eschar. This will allow the identification of risk factors and the establishment of prevention and control disease strategies for *Rickettsia* spp. infection.

Abbreviations

ATBF: African tick-bite fever
 IFA: Immunofluorescence assay
 Ig: Immunoglobulin
 SFGR: Spotted fever group *Rickettsia*
 RTPCR: Reverse transcription polymerase chain reaction.

Data Availability

Data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

This study was approved by the ethics committee at the National Institute of Public Health, Ministry of Health, Angola, under the authorization number 38/2017 as part of the laboratory surveillance of febrile syndromes.

Consent

Adults and parents or legal guardians of children participating in this study authorized and provided written informed consent for this investigation and sample collection.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

- [1] C. Eldin and P. Parola, "Update on tick-borne bacterial diseases in travelers," *Current Infectious Disease Reports*, vol. 20, no. 7, p. 17, 2018.
- [2] S. Brah, M. Daou, L. Salissou et al., "Fever of unknown origin in Africa: the causes are often determined!" *Fever of Unknown Origin, Rickettsiosis, Borreliosis, Q Fever, Leptospirosis, Dengue, Chikungunya, Zika Virus, Africa*, vol. 16, 2015.
- [3] S. Shpynov, N. Pozdnichenko, and A. Gumenuk, "Approach for classification and taxonomy within family rickettsiaceae based on the formal order analysis," *Microbes and Infection*, vol. 17, no. 11-12, pp. 839–844, 2015.
- [4] A. Portillo, S. Santibáñez, L. García-Álvarez, A. M. Palomar, and J. A. Oteo, "Rickettsioses in Europe," *Microbes and Infection*, vol. 17, no. 11-12, pp. 834–838, 2015.
- [5] S. N. Shpynov, P.-E. Fournier, N. N. Pozdnichenko, A. S. Gumenuk, and A. A. Skiba, "New approaches in the systematics of rickettsiae," *New Microbes and New Infections*, vol. 23, pp. 93–102, 2018.
- [6] A. Portillo and J. A. Oteo, "Rickettsiosis as threat for the traveller," in *Current Topics in Tropical Medicine*, IntechOpen, London, UK, 2012.
- [7] M. J. Maze, Q. Bassat, N. A. Feasey, I. Mandomando, P. Musicha, and J. A. Crump, "The epidemiology of febrile illness in sub-Saharan Africa: implications for diagnosis and management," *Clinical Microbiology and Infection*, vol. 24, no. 8, pp. 808–814, 2018.
- [8] F. Bacellar, R. Sousa, A. Santos, M. Santos-Silva, and P. Parola, "Boutonneuse fever in Portugal: 1995-2000. data of a state laboratory," *European Journal of Epidemiology*, vol. 18, no. 3, pp. 275–277, 2002.
- [9] A. Portillo, R. de Sousa, S. Santibáñez et al., "Guidelines for the detection of *Rickettsiaspp.*," *Vector-Borne and Zoonotic Diseases*, vol. 17, no. 1, pp. 23–32, 2017.
- [10] A. M. Berrian, B. Martínez-López, V. Quan et al., "Risk factors for bacterial zoonotic pathogens in acutely febrile patients in Mpumalanga Province, South Africa," *Zoonoses and Public Health*, vol. 66, no. 5, pp. 458–469, 2019.
- [11] G. J. G. Simpson, V. Quan, J. Frean et al., "Prevalence of selected zoonotic diseases and risk factors at a human-wildlife-livestock interface in Mpumalanga province, South Africa," *Vector-Borne and Zoonotic Diseases*, vol. 18, no. 6, pp. 303–310, 2018.
- [12] K. C. Horton, A. Maina, E. Dueger et al., "Evidence of *Rickettsia* and *orientia* infections among abattoir workers in Djibouti," *The American Journal of Tropical Medicine and Hygiene*, vol. 95, no. 2, pp. 462–465, 2016.
- [13] A. N. Maina, C. M. Farris, A. Odhiambo et al., "Q fever, scrub typhus, and rickettsial diseases in children, Kenya, 2011-2012," *Emerging Infectious Diseases*, vol. 22, no. 5, pp. 883–886, 2016.
- [14] J. W. Thiga, B. K. Mutai, W. K. Eyako et al., "High seroprevalence of antibodies against spotted fever and scrub typhus bacteria in patients with febrile illness, Kenya," *Emerging Infectious Diseases*, vol. 21, no. 4, pp. 688–691, 2015.
- [15] N. Kaabia, J. M. Rolain, M. Khalifa et al., "Serologic study of rickettsioses among acute febrile patients in central Tunisia," *Annals of the New York Academy of Sciences*, vol. 1078, no. 1, pp. 176–179, 2006.

- [16] L. M. Ndip, D. H. Bouyer, A. P. A. T. Da Rosa, V. P. K. Titanji, R. B. Tesh, and D. H. Walker, "Acute spotted fever rickettsiosis among febrile patients, Cameroon," *Emerging Infectious Diseases*, vol. 10, no. 3, pp. 432–437, 2004.
- [17] P. J. Kelly and P. R. Mason, "Tick-bite fever in Zimbabwe. survey of antibodies to *Rickettsia conorii* in man and dogs, and of rickettsia-like organisms in dog ticks," *The South African Medical Journal*, vol. 80, no. 5, pp. 233–236, 1991.
- [18] M. A. Redus, R. A. Parker, and J. E. McDade, "Prevalence and distribution of spotted fever and typhus infections in Sierra Leone and Ivory Coast," *The International Journal of Zoonoses*, vol. 13, no. 2, pp. 104–111, 1986.
- [19] B. A. Botros, A. K. Soliman, M. Darwish, S. El Said, J. C. Morrill, and T. G. Ksiazek, "Seroprevalence of murine typhus and fièvre boutonneuse in certain human populations in Egypt," *Journal of Tropical Medicine and Hygiene*, vol. 92, no. 6, pp. 373–378, 1989.
- [20] H. T. Kazar, P. Brouqui, B. Faugere, and D. Raoult, "Prevalence of antibodies to *Coxiella burnetii*, *Rickettsia conorii*, and *Rickettsia typhi* in seven african countries," *Clinical Infectious Diseases*, vol. 21, no. 5, pp. 1126–1133, 1995.
- [21] M. C. Horta, M. B. Labruna, and L. A. Sangioni, "Prevalence of antibodies to spotted fever group rickettsiae in humans and domestic animals in a Brazilian spotted fever-endemic area in the state of São Paulo, Brazil: serologic evidence for infection by *Rickettsia rickettsii* and another spotted fever group *Rickettsia*," *The American Journal of Tropical Medicine and Hygiene*, vol. 71, no. 1, pp. 93–97, 2004.
- [22] N. M. Eisawi, D. A. Hassan, M. O. Hussien, A. B. Musa, and A. R. M. El Hussein, "Seroprevalence of spotted fever group (SFG) rickettsiae infection in domestic ruminants in Khartoum State, Sudan," *Veterinary Medicine and Science*, vol. 3, no. 2, pp. 91–98, 2017.
- [23] P. Gérardin, N. Zemali, M. Bactora et al., "Seroprevalence of typhus group and spotted fever group *Rickettsia* exposures on Reunion Island," *BMC Research Notes*, vol. 12, no. 1, p. 387, 2019.
- [24] P. Brouqui, F. Bacellar, G. Baranton et al., "Guidelines for the diagnosis of tick-borne bacterial diseases in Europe," *Clinical Microbiology and Infection*, vol. 10, no. 12, pp. 1108–1132, 2004.
- [25] J. Ehlers, A. Krüger, S. J. Rakotondranary et al., "Molecular detection of *Rickettsia* spp., *Borrelia* spp., *Bartonella* spp. and *Yersinia pestis* in ectoparasites of endemic and domestic animals in southwest Madagascar," *Acta Tropica*, vol. 205, Article ID 105339, 2020.
- [26] P. F. Barradas, H. Vilhena, A. C. Oliveira et al., "Serological and molecular detection of spotted fever group *Rickettsia* in a group of pet dogs from Luanda, Angola," *Parasites & Vectors*, vol. 10, no. 1, p. 271, 2017.