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Research Article

Prevalence and Genetic Diversity of *Rickettsia* Species in Ixodid Ticks Collected from Cattle in Republic of Guinea in 2024

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Abstract

Spotted fever group rickettsioses, caused by several *Rickettsia* species, are important tick-borne infections in Africa. For evaluation spreading of *Rickettsia* spp. in Republic of Guinea, a panel of 919 adult ticks were collected from cattle in the Kankan and Nzerekore regions in 2024. Six tick species were identified: *Rhipicephalus decoloratus*, *Rh. microplus*, *Rh. annulatus*, *Rh. senegalensis, Amblyomma variegatum*, and *Hyalomma truncatum.* The PCR with sequencing of *gltA* gene in individual ticks were used for detection *Rickettsia* spp. The average infection rate for *Rickettsiae* spp. in ticks collected from cattle was 2.7%. The genetic material of *R. africae, R. massiliae*, and *R. aeschlimannii* was detected in studied ticks and *R. aeschlimannii* DNA was detected for the first time in ticks sampled in these regions of Guinea. Phylogenetic analysis demonstrated that all detected *Rickettsia* spp clustered to other typical representatives of the tick-borne rickettsia belonging to the spotted fever group infections. Thus, genetic material of *R. africae, R. massiliae*, and *R. aeschlimannii* was detected in ixodid ticks collected from cattle in Guinea in 2024.

Keywords: Rickettsia; *R. africae*; *R. massiliae*; *R. aeschlimannii*; Tick; Guinea

Introduction

Febrile illness caused by *Rickettsia* spp. belonging to the Spotted Fever Group (SFG) are the second most common cause of infections among travelers to sub-Saharan Africa, after malaria [1]. In this region, the incidence of SFG infections in travelers may be approximately 5.6%. A high seroprevalence level to SFG rickettsioses is also recorded in the local population.

Previously, *R. conorii* was considered the only causative agent of SGF infections in Africa [2]. Later, *R. africae* was discovered and isolated in 1992 from the blood of a patient with fever in Zimba-

bwe who developed an erythematous lesion at the tick bite site [3]. In 1996, *R. africae* was recognized as the etiologic agent of African Tick-Bite Fever (ATBF). The clinical course of ATBF includes classic signs of tick-borne rickettsiosis, such as fever, eschar at the tick bite site, rash, and nonspecific symptoms, including headache, myalgia, regional lymphadenopathy, and others. The main vectors are *Amblyomma variegatum* and *Am. hebraeum* ticks, which are the dominant and most aggressive tick species in Africa [4]. Furthermore, it is now known that *R. africae* DNA has also been detected in ticks belonging to *Rhipicephalus* spp., *Hyalomma* spp., and *Haemaphysalis* spp. [5]. Most cases of ATBF are recorded in South Africa (more than 80% of cases), but molecular genetic analysis has shown that *R. africae* is widespread throughout the continent and is found in most countries of South Africa, Kenya, Chad, Burundi, Mali, Niger, and Sudan. Also, *R. africae* has been detected in *Amblyomma* spp ticks and in humans living rural regions from more than 30 countries in sub-Saharan Africa [6-8]. Nevertheless, data on tick species diversity, their presence, prevalence, distribution, and the pathogens they carry are much less available in West African countries [9].

The *R. massiliae*, which was early discovered in the suburbs of Marseille, France [9] and this infection was later detected in the Central African Republic in *Rh. muhsamae, Rh. lunulatus*, and *Rh. sulcatus* ticks in 1994. Then, in 2001, *R. massiliae* DNA was detected in *Rh. muhsamae* ticks collected from cattle in Mali, and in northern Algeria in *Rh. turanicus* ticks [11,12]. Later, *R. monacensis, R. rhipicephali, R. aeschlimannii,* and *R. sibirica mongolitimonae* are also recorded in sub-Saharan Africa. Thus, the number of new rickettsia species is constantly increasing as a result of their active study and the using of molecular genetic methods. The discovery of rickettsioses is also an important reason for study of molecular epidemiology these infections.

It is worth noting that data on the distribution of *Rickettsia* spp. in Guinea are fragmented and highly uneven across different regions. Nevertheless, the serological studies indicate that antibody levels to SFG infections in the local population are approximately 10.5%, but in some regions can reach 25.5% (Faranah, Upper). Recent studies have shown the circulation of *R. africae, R. massiliae* and one unidentified rickettsia isolate in Guinea [13]. The *R. africae* was associated with *Am. variegatum*, *Rh. decoloratus, Hy. marginatum, Ha. paraleachi* ticks and *R. massiliae* with *Rh. senegalensis* ticks.

The aim of the study was to investigate the genetic diversity of *Rickettsia* spp. detected in various species of ticks collected from cattle (*Bos taurus*) in the eastern and southeastern of the Guinea (Kankan and Nzerekore regions) in 2024.

Materials and Methods

Ticks collection

In this study, adult ticks were collected from cattle in the Kankan and Nzerekore regions, located in eastern and southeastern Guinea in 2024. Ticks were collected manually from cattle from predilection sites (dewlap, udder/scrotum, fore/hind legs, perineum, and tail) using blunt-nose forceps. The collected ticks were washed twice with 70% ethanol to remove external contaminants and external microflora, following which they were stored at a temperature of -40°C for subsequent analysis. The morphological classification of the collected ticks was carried out in accordance with published taxonomic criteria under a stereomicroscope based on keys as described [14].

DNA extraction

Adult ticks were homogenized using the Bioprep-24 Homogenizer (Hangzhou Allsheng, China) in 500 µl 0.9% saline solution. DNA from 100 µl tick suspensions was isolated with Ribo-Prep (AmpliSense, Russia) according to the manufacturer's protocols. Screening of the studied samples for the presence of rickettsia DNA was performed by real-time PCR on a CFX-96 instrument (BioRad, USA) in a volume of 25 µl of the reaction mixture using a reagent kit (Biolabmix, Russia) and a set of oligonucleotide primers [15].

PCR detection of genetic markers

To verify rickettsia DNA, a fragment of the *gltA* gene approximately 760 bp in length was amplified using a pair of oligonucleotide primers CS409d (CCTATGGCTATTATGCTTGC) and RP1258n (ATTGCAAAAAGTACAGTGAACA) at a final concentration of 0.4 µM each to amplify a fragment of the *gltA* gene 769 bp [16]. PCR was performed on a T-100 thermal cycler according to the following program: preliminary polymerase activation - 95ºC for 5 min.; 38 cycles: 95ºC - 15 sec., 56ºC - 20 sec., 72ºC - 45 sec.; final elongation at 72ºC for 5 min. Electrophoresis of PCR products was performed in 2% agarose gel in 1% TAE buffer (0.04 M Tris, 0.04 M CH3COOH, 2 mM EDTA) with the addition of 5 µl of a saturated ethidium bromide solution. Also, to confirm that the genetic markers of *Rick-*

ettsia spp. detected in the DNA extracts was that from these tick species, the *16S rRNA* gene of ticks were amplified as described previously [17].

The confidence interval (95% CI) for the proportion of PCR positive samples were calculated using the Wilson method [18]. at a confidence level of 0.95 using the Epitools Epidemiological Calculators online service (http://epitools.ausvet.com.au). Fisher's test was used to assess the significance of differences in the groups.

Sequencing and phylogenetic analysis

The nucleotide sequences of PCR fragments were determined using the BigDye™ v3.1 Terminator Cycle Sequencing Kit (ThermoFisher Scientific, USA) followed by analysis of the reaction products on a Nanofor 05 genetic analyzer (Synthol, Russia). The resulting chromatograms were analyzed using SeqMan software (DNAstar, Madison, WI, USA). Phylogenetic analysis was performed using the maximum likelihood method for the *gltA* gene fragment in MEGA 11 [19]. The statistical significance of the phylogenetic tree topology was assessed using Bootstrap analysis; calculations were performed for 1000 bootstrap replications. The sequences obtained during this study and used in phylogenetic analyses have been deposited to GenBank under the following accession numbers: PV261001-PV261025.

Results

Ticks were collected from cattle in the Kankan (409 ticks) and Nzerekore (510 ticks) regions (Figure 1). A total of 919 adult ticks were collected from cattle of both sexes and were identified as the following six species: *Rhipicephalus decoloratus* Koch, 1844; *Rh. microplus* Canestrini, 1888; *Rh. annulatus* Say, 1821; *Amblyomma variegatum* Fabricius, 1794*; Rh. senegalensis* Koch, 1844; *Hyalomma truncatum* Koch, 1844. Most of the collected ticks were *Rh. decoloratus* (363 ticks), *Rh. microplus* (222), *Rh. annulatus* (160). The genus *Amblyomma* was also widely represented by *Am. variegatum* (128). Small samples were represented by *Rh. senegalensis* (32) and *Hy. truncatum* (14) ticks.



Figure 1: Geographic distribution of the locations in Guinea where the rickettsia positive ticks were detected by PCR.

PCR detection of Rickettsia spp. markers

SFG rickettsia DNA was detected in all tick species by PCR with sequencing of *gltA* gene in individual ticks (Table 1). The highest detection rates for genetic markers of *Rickettsia* spp. were observed for *Rh. senegalensis* and *Hy. truncatum* (12.5 % and 14.3 %, respectively) however, it is worth noting that due to the small representation of these ticks the confidence interval is quite large. Overall, the average infection rate *Rickettsia* spp. in ticks collected from cattle was 1.8 and 3.9 % for Forest Guinea (Nzerekore) and Kankan, respectively (significant differences, significance level p = 0.047).

Tick species	Rickettsia species	Nzerekore region		Kankan region		Total	
		PCR positive/Total	Infection rate*	PCR positive/Total	Infection rate*	PCR positive/ Total	Infection rate*
Am. variegatum	*R. africae*	5/33	15.1 (6.6-30.9)	5/95	5.3 (2.3-11.7)	10/128	7.8 (4.3-13.8)
Hy. truncatum	*R. massiliae*	0/0		2/14	14.3 (4.0-39.9)	2/14	14.3 (4.0-39.9)
Rh. annulatus	*R. massiliae*	2/80	2.5 (0.7-8.6)	2/80	2.5 (0.7-8.6)	4/160	2.5 (0.9-6.2)
Rh. decoloratus	*R. massiliae*	0/219		2/144	1.4 (0.4-4.9)	2/363	0.6 (0.2-1.9)

Rh. senegalensis	*R. massiliae* *R. aeschlimannii***	0/1		4/31	12.9 (5.1-28.8)	4/32	12.5 (4.9-28.1)
Rh. microplus	*R. massiliae* *R. aeschlimannii****	2/177	1.1 (0.3-4.0)	1/45	2.2 (0.4-11.5)	3/222	1.4 (0.5-3.9)
Total		9/510	1.8 (0.9-3.3)	16/409	3.9 (2.4-6.3)	25/919	2.7 (1.2-4.7)

Table 1: PCR detection for *Rickettsia* spp markers and their genotype in the collected ticks. (Note: * denotes 95 % confidence intervals (CI) for the infection rates presented. ** detected only Kankan region *** detected only Nzerekore region).

Sequence and phylogenetic analysis

Sequencing fragments of the *gltA* gene allowed genotyping of PCR-positive samples from collected individual ticks. Their analysis revealed the presence of *R. africae, R. massiliae,* and *R. aeschlimannii* with a high genetic identity level. Among the 12 obtained *R. massiliae* isolates 11 were completely identical to previously detected variants. and one had a single synonymous C-T substitution characteristic of isolates from China (MF002497, MW802693) and Iran (ON001449). In 3 of the 10 analyzed *R. africae* isolates. a previously undescribed nonsynonymous T-A substitution leading to an S-T transion was found in the obtained *gltA* gene fragment. The identified isolates of *R. aeschlimannii* are completely identical to each other and to the prototype isolates according to the analyzed gene fragment. The *R. africae* was detected only in *Am. variegatum* ticks. while *R. massiliae* circulation was demonstrated in five tick species: *Rh. annulatus. Rh. decoloratus. Rh. senegalensis. Rh. microplus.* and *Hy. truncatum*. The *R. aeschlimannii* was only detected in *Rh. senegalensis* ticks collected in Kankan and *Rh. microplus* in Nzerekore.

Phylogenetic analysis demonstrated that the detected *Rickettsia* spp. clustered to other typical representatives of the SFG (Figure 2). Isolates of *R. massiliae* grouped with isolates from Portugal, Spain, China, Argentina, and United States. The first described in Guinea *R. aeschlimannii* also grouped with previously described isolates from Egypt, Iran, Turkey, Namibia, Mali, and China. The *R. africae* in addition to other African countries has also been grouped in Brazil and Madagascar isolates.

Thus, genetic material of *R. africae, R. massiliae,* and *R. aeschlimannii* genetically very similar to isolates from other world regions and these isolates were detected in six tick species collected from cattle in two regions of Guinea.

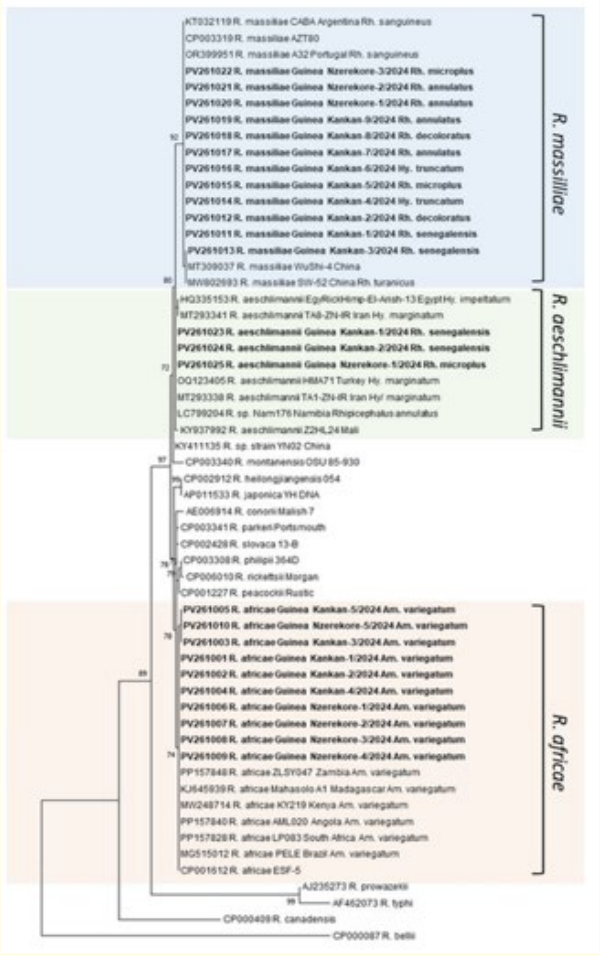


Figure 2: Phylogenetic tree based on 742 bp fragment of the *glt*A gene of *Rickettsia* spp., The GenBank accession numbers, *Rickettsia* species, and regions are indicated in the dendrogram. The genetic distance scale is below. The bold types were used for sequences obtained in this study.

Discussion

Early, in Guinea, the following tick species collected on cattle were identified: *Am. Variegatum, Rh. decoloratus, Rh. annulatus, Rh. geigy, Hy. truncatum, and Rh. senegalensis* with a dominance (near 90%) of *Am. variegatum, Rh. decoloratus,* and *Rh. annulatus* ticks [20]. Genetic markers for piroplasms (*Babesia caballi, Theileria mutans,* and *Th. velifera)* were also found in *Am. Variegatum, Rh. decoloratus, Rh. annulatus,* and *Rh. geigyi* ticks, and the total infection rate for these ticks was 4.2%. The highest infection rate was found in *Rh. annulatus* ticks (10.9%).

The circulation of two rickettsia species (*R. africae* and *R. massiliae*) was also previously demonstrated in *Am. Variegatum, Rh. geigyi, Rh. annulatus,* and *Rh. decoloratus* ticks collected from dogs, cows, and goats [13]. This study also confirmed the circulation of these rickettsia species in ticks collected from cows in 2024. The infection rate ranged from 1.8 to 3.9 % (average 2.7 %). The predominant ticks in this collection were *Rh. decoloratus, Rh. microplus,* and *Rh. annulatus* (near 81%). We also examined *Rh. microplus* ticks and firstly demonstrating circulation of *R. aeschlimannii* in Guinea. *R. aeschlimannii* was first isolated in 1992 from *Hy. marginatum* ticks in Morocco and they were later classified as SFG rickettsia [21]. The main carriers of this rickettsia are *Hyalomma* spp. ticks and this tick-borne pathogen was found in Zimbabwe, Niger, Mali, and Northern Algeria [3,12,22].

This study once again confirmed that tick-borne SFG infections are widespread in Guinea. In addition to previously described rickettsia species, the detection of *R. aeschlimannii* DNA was demonstrated for the first time. Nevertheless, the prevalence of most SFG rickettsioses occurring in different regions of Guinea remains poorly understood.

Conclusions

Rickettsia DNA was detected in 25 of 919 adult ixodid ticks collected from cattle in Kankan and Nzerekore, Guinea. Sequence analysis of the *gltA* fragment identified *R. africae* in *Amblyomma variegatum*, *R. massiliae* in five tick species, and *R. aeschlimannii* in *Rhipicephalus senegalensis* and *Rhipicephalus microplus*. These findings extend evidence of spotted fever group *Rickettsia* circulation in cattle-associated ticks in Guinea, including the first detection of *R. aeschlimannii* in the sampled regions. Broader, cluster-aware surveillance and multilocus characterization are needed to define distribution, host associations, and public-health significance.

Author Contributions

M.Yu.K.; Writing - original draft, visualization, investigation, formal analysis, data curation, A.S.Z.; investigation, data curation, E.V.N.; investigation. K.A.S.; investigation, formal analyses, K.S.Z.; investigation, field samples collection, tick identification, M.A.K.; investigation, M.B.B.; field samples collection, investigation, M.S.T.; resources, conceptualization, supervision, project administration, V.B.L.; writing, review, editing, conceptualization, supervision, resources.

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Institutional Review Board Statement

This article does not describe any studies involving humans as a test subject. The study protocol was approved by the decision of the National Ethics Committee of the Ministry of Health of the Republic of Guinea (Protocol No. 129/CNERS/16 of August 31. 2015).

Data Availability Statement

Data will be made available on request.

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Conflicts of Interest

The authors have no conflicts of interest associated with this study.

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