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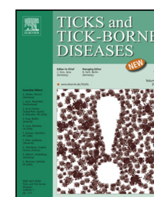

# Identification of rickettsial pathogens in ixodid ticks in northern Senegal

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ABSTRACT

The spotted fevers, caused by the *Rickettsia* bacteria, are a group of emerging diseases that are responsible for significant human morbidity. In Africa, the distribution of different species of *Rickettsia* in their tick vectors is poorly studied. We have collected 1169 hard ticks from 5 different species in the northern Senegal, close to the Saharan border. In a far northern collection site, corresponding to the *Rickettsia africae* distribution area, we collected three *Amblyomma variegatum* ticks infected by *R. africae*. *Rickettsia africae* was also identified in a *Hyalomma marginatum rufipes* tick, which may represent the secondary host for the pathogen. *Rickettsia aeschlimannii* was identified in *H. m. rufipes*, *Rhipicephalus evertsi evertsi*, and *Hyalomma impeltatum* ticks.

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## Introduction

Rickettsia are small Gram-negative obligate intracellular Alphaproteobacteria. These bacteria often have a close relationship with arthropod vectors that may transmit the organism to mammalian hosts (Dworkin et al., 2006). The family Rickettsiaceae consists of 2 genera: *Rickettsia* and *Orientia*. The genus *Rickettsia* is further divided into 4 groups: the spotted fever group (SFG) *Rickettsia*, the typhus group (TG) *Rickettsia*, the *Rickettsia bellii* group, and the *Rickettsia canadensis* group (Merhej and Raoult, 2011). The SFG *Rickettsia* includes the following species: *R. akari*, *R. australis*, *R. africae*, *R. conorii*, *R. honei*, *R. japonica*, *R. sibirica*, *R. helvetica*, *R. slovaca*, *R. massiliae*, *R. rhipicephali*, *R. aeschlimannii*, *R. montanensis*, and *R. parkeri*, among others (Bouyer et al., 2001; Roux and Raoult, 2000; Zhang et al., 2000). Most of the species are pathogenic, cause spotted fevers in humans and are transmitted by arthropods, mainly ticks. For some, the arthropod hosts (ticks, mites, fleas, and lice) are both a reservoir and a vector. Each rickettsiosis has a particular geographic distribution determined by its natural history with an arthropod host and, in some cases, a zoonotic vertebrate host (Dworkin et al., 2006).

The geographic distribution of rickettsiae in Africa is now fairly documented (Parola et al., 2013). Most of the available data are concerned with the detection of *R. conorii* and *R. africae*. *Rickettsia conorii* is the causative agent of Mediterranean spotted fever (MSF) and is transmitted by the brown dog tick *Rhipicephalus sanguineus*. MSF is widely distributed in northern Africa (Mouffok et al., 2009; Parola et al., 2013; Sarih et al., 2008), and in Sub-Saharan Africa, few cases have been reported, and the distribution has been established mainly through detection of *R. conorii* in ticks. *Rickettsia conorii* was detected in *Rhipicephalus mushamae* from cattle in the Central Africa Republic (Parola et al., 2005), in *Rh. evertsi evertsi* from a horse in rural Senegal (Mediannikov et al., 2010a), and in *Haemaphysalis punctaleachi* in Uganda (Socolovschi et al., 2007).

*R. africae* is the etiologic agent of African tick-bite fever (ATBF) and is transmitted by *Amblyomma variegatum* and *A. hebraeum* in Sub-Saharan Africa (Parola et al., 2005). The distribution area of ATBF is generally considered to correspond to the distribution of these 2 species of ticks. *Rickettsia africae* has also been detected in *A. lepidum* in Djibouti (Socolovschi et al., 2007); *Rh. annulatus*

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in Guinea, Senegal, and Nigeria (Mediannikov et al., 2010a, 2010b, 2012a; Reye et al., 2012); *Rh. evertsi evertsi* in Senegal and Nigeria (Mediannikov et al., 2010a; Reye et al., 2012); *Rh. decoloratus* in Nigeria and Botswana (Ogo et al., 2012; Portillo et al., 2007); *Rh. sanguineus* and *Hyalomma impeltatum* in Nigeria (Ogo et al., 2012); *Rh. geigyi* in Liberia (Mediannikov et al., 2012a), and *A. compressum* in the Democratic Republic of the Congo and Liberia (Mediannikov et al., 2012a, 2012b).

*Rickettsia aeschlimannii* is the agent of tick-borne spotted fever. Several cases have been reported in Africa, including Algeria (Mokrani et al., 2012) and South Africa (Parola et al., 2005). Ticks from the genus *Hyalomma* appear to be both the reservoirs and vectors of *R. aeschlimannii*. This bacterium was detected in *H. marginatum rufipes* and *H. truncatum* collected from domestic animals in Senegal (Mediannikov et al., 2010a). *Rickettsia aeschlimannii* was also detected in *Rh. evertsi evertsi* from Senegal and Nigeria (Mediannikov et al., 2010a; Reye et al., 2012) and from *Rh. annulatus* and *A. variegatum* from Nigeria (Reye et al., 2012) (Fig. 1).

Northern Senegal covers a semi-arid area representing the transition between sub-Saharan Africa and northern Africa. The objective of our study was to detect pathogenic *Rickettsia* species in ticks collected from domestic animals in northern Senegal and to compare the data with those from southern Senegal and northern Africa.

## Materials and methods

### *Ticks sample collection*

The study was performed in 4 villages situated in Keur Momar Sarr, Louga, Senegal: Ganket Balla (15°58′ N; 15°55′ W), Loboudou (15°57′ N; 15°59′ W), Ndour Roba (15°54′ N; 15°59′ W), and Ndimb (16°02′ N; 16°00′ W). Ixodid ticks were manually collected from domestic animals (cows, goats, sheep, horses, and donkeys) in the rural area of Louga region in July 2011. All ticks were kept alive in a flask with a relative humidity of 85–90%. To minimize contamination of the DNA, all of the collected ticks were washed in a soap solution. After washing, they were stored in 70% ethanol until morphological identification. Ixodid ticks were identified morphologically according to standard taxonomic indicators for adult ticks (Hoogstraal, 1956; Mathysse and Colbo, 1987; Walker et al., 2000, 2003)

### *DNA extraction*

To extract the DNA, the tick tissue was finely ground in 2% cetyl trimethyl ammonium bromide (CTAB) (Vroh Bi et al., 1996), with a disposable scalpel blade. The ground material was lysed by heating in a water bath at 65 °C for 5 min. Two hundred microliters of chloroform was added to the lysed material, and the supernatant was recovered after centrifugation at 12,000 rpm for 5 min. The nucleic acids were precipitated by 200 μl of isopropanol after 15 min of centrifugation at 12,000 rpm. The pellet was then dried in a speed vac for 3–4 min and resuspended in 200 μl DNase free water. The DNA solution was stored in the refrigerator between 2° and 8 °C.

### *DNA amplification*

We performed all PCR reactions in a Mastercycler thermal cycler (Eppendorff AG 22331 Hamburg, Germany). Each reaction was performed at a final volume of 25 μl, containing 2.5 μl of 10× PCR buffer (buffer), 2.5 μl of desoxyribonucleotides (dNTPs), 0.75 μl of magnesium chloride ($MgCl_2$), 0.5 μl of each primer, 0.1 μl of Taq polymerase, 15.15 μl of pure water, and 3.0 μl of DNA. A positive control (*Rickettsia conorii*) and a negative control (DNase free water) were included in each experiment. Rickettsial DNA was screened by PCR using the primers Rr190.70p and Rr190.701n, which amplify a 632-bp fragment of the gene *ompA* of *Rickettsia*, as described previously (Mediannikov et al., 2010a). Positive samples were subjected to the second PCR to amplify almost entire rickettsial *gltA* gene (Mediannikov et al., 2010a). PCR products were visualized by electrophoresis on a 1.5% agarose gel, stained with biotium (Cat 41003, GelRed Nucleic Acid Stain, 10,000× in water, USA), and examined using an ultraviolet transilluminator.

### *DNA sequencing*

Sequencing of the amplicons was performed using the BigDye Terminator Cycle Sequencing Kit (Perkin Elmer Applied Biosystems) with an ABI automated sequencer (Applied Biosystems). Obtained sequences were assembled (ChromasPro 1.49 beta, Technelysium Pty Ltd, Tewantin, Australia), edited by BioEdit Sequence alignment editor v. 7.0.9.0 and compared with those available in GenBank by NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

## Results

### *Ticks samples*

A total of 1169 ticks was collected including 5 species: *Amblyomma variegatum*, *Hyalomma impeltatum*, *Hyalomma marginatum rufipes*, *Rhipicephalus evertsi evertsi*, and *Rhipicephalus guilhoni* (Table 1). In Ganket and Loboudou, 240 and 268 ticks belonging to 3species were collected, respectively: *H. impeltatum*, *Rh. evertsi evertsi*, and *H. m. rufipes*. In Ndour Roba, 345 individuals belonging to 4 different species were collected: *H. impeltatum*, *H. marginatum rufipes*, *Rh. guilhoni* and *Rh. evertsi evertsi*. In the village of Ndimb, 316 individuals representing four species were collected: *H. impeltatum*, *A. variegatum*, *H. m. rufipes*, and *Rh. evertsi evertsi*. The majority of ticks in this region 557/1169 (47.6%) was represented by *H. impeltatum*. Only 3 *A. variegatum* ticks were collected, all of them from cows. Only one *Rhipicephalus guilhoni* was identified, which was collected from a sheep.

### *Detection of Rickettsia*

Out of 1169 ticks analyzed, 5.8% (68/1169) were infected with rickettsiae. The results of the amplification of *gltA* and *ompA* genes are shown in Table 2. *ompA* gene amplicon and additionally amplified from the positive ticks almost entire rickettsial *gltA* gene were sequenced. The analysis of the sequenced amplicons of the positive ticks showed 100% identity of both *ompA* and *gltA* genes among all ticks. BLAST search led to the identification of 2 species of the genus *Rickettsia*: *Rickettsia africae*, the agent of African tick-bite fever (*gltA* and *ompA*, 100% identity with *R. africae* ESF-5, NC_012633), and *Rickettsia aeschlimannii* recently recognized as an agent of the spotted fever group (*gltA* and *ompA*, 100% identity with *R. aeschlimannii* RH15, HM_050284). Among the 131 *H. marginatum rufipes* ticks collected, we identified in 0.8% (1/131) *R. africae* and in 27.5% (36/131) *R. aeschlimannii*. In *H. impeltatum*, only *R. aeschlimannii* (2.5%; 14/557) was identified. We tested also 3 specimens of *A. variegatum* and detected *R. africae* in each of them. We found that 3.6% of *Rh. evertsi evertsi* ticks collected in this region were infected with *R. aeschlimannii*. Only one *Rhipicephalus guilhoni* tick was collected, and it was negative when tested by rickettsial genus-specific PCR.

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