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Short Report: Prevalence of *Bartonella quintana* in Patients with Fever and Head Lice from Rural Areas of Sine-Saloum, Senegal

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Abstract. Trench fever is poorly known by the staff of health facilities that manage febrile patients in Senegal. *Bartonella quintana* DNA was identified in 5 of 274 (2%) febrile patients from two rural dispensaries and 2 of 71 (3%) head lice specimens collected from the same villages.

INTRODUCTION

Bartonella quintana, a small facultative intracellular α-Proteobacterium, is the causative agent of trench fever. It was first identified as an important human pathogen during World War I, when it caused an epidemic of louse-borne trench fever among 1 million troops in Europe. Currently, this bacterium is regarded as a re-emerging pathogen in the homeless population in Europe, where it is responsible for asymptomatic chronic bacteremia, endocarditis, and bacillary angiomatosis.[1] The disease is transmitted to humans through the body louse *Pediculus humanus humanus*, which has also been implicated as a vector for other human diseases.[2] These body lice have been shown to be the vectors for the transmission of two other louse-borne diseases: epidemic typhus caused by *Rickettsia prowazekii* and relapsing fever caused by *Borrelia recurrentis*.[3] Both head and body lice are specific blood-sucking ectoparasites of humans,[4] and they are morphologically different from each other. Genetically, lice are classified into three clades, and body lice all over the world belong to clade A.[5] Boutellis and others[5] recently revealed the presence of clades A and C in the head lice from Senegal. Recent studies have shown that the head lice collected from rural populations in Ethiopia[6] and females in Senegal[5] were infected with *B. quintana*. A human case of endocarditis caused by *B. quintana* was reported in a patient living in Senegal[7] as well as Senegalese traveling to France[5] and Switzerland.[5] Our study aimed to investigate the role of *B. quintana* as the cause in the occurrence of acute febrile illness in rural Senegal, describe its epidemiology, and show that this often forgotten disease must be taken into account in the management of febrile patients.

MATERIALS AND METHODS

Dielmo (13°45′ N/16°25′ W), with 350 inhabitants, is located in the Fatick region of Sine-Saloum (280 km southeast of Dakar and approximately 15 km north of the Gambian border), and Ndiop (13°41′ N/16°23′ W; 400 inhabitants) is situated 8.5 km south of Dielmo (Figure 1). Inhabitants of these two villages participated in a long-term malaria surveillance program that has been described in detail elsewhere.[8] In each village, there is a functional dispensary. One nurse, two technicians, and three fieldworkers are present every day in the village. Since 2011, a point-of-care laboratory has been functioning in Dielmo,[9] where the molecular diagnostics of multiple bacterial pathogens are performed. All patients with fever (an axillary temperature > 37.5°C) from Dielmo and Ndiop treated at the dispensaries from June of 2010 until the end of 2011 were included in the study. Blood samples were taken from each individual and placed in a sample tube containing 20 μL 3.2% trisodium citrate; then, a questionnaire was completed.[9] A 200-μL sample of whole blood (three or four drops) was collected from each patient by a lancet stick of a fingertip, and this sample was used for DNA extraction and subsequent molecular tests.

In March of 2011, head lice were collected from female individuals from both villages and preserved dry in sterile conditions at room temperature. The general sanitary and hygienic conditions were poor. The samples were then sent to our Unit of Rickettsioses in Marseille, France for DNA extraction and molecular studies.

DNA was extracted from blood samples of febrile patients using the QIAGEN EZ-1 Kit (QIAGEN, Courtaboeuf, France) according to the manufacturer's protocol.[9] All samples were first subjected to quantitative polymerase chain reaction (qPCR) specific for *Bartonella* spp. (Table 1). When the qPCR was positive for *Bartonella* spp., the sample was tested using a second genus-specific qPCR and a qPCR specific for *B. quintana*, which targeted the *yopP* gene.[5,10] A sample was considered positive when the qPCR reaction was positive for at least one *Bartonella* genus-specific qPCR and the *B. quintana*-specific qPCR had a cycle number (Ct) lower than 40; this value was considered to be the threshold level for log-based fluorescence. DNA extraction from the blood samples collected between June and November 11, 2010 was performed in the Dielmo village dispensary using the QIAamp Kit (QIAGEN, Hilden, Germany), and the columns with the bound and dry DNA were stored at 4°C until the final elution in Marseille, France.[11] In contrast, from November 12, 2010 to the end of December of 2011, DNA extracted from the blood samples was tested in the point-of-care laboratory based in Dielmo[9] before being sent to our Unit of Rickettsioses in Marseille, France.

Each louse was rinsed two times in sterile water baths (Versylene Fresenius, Sèvres, France) for 15 minutes,[5,10] and DNA was extracted using the QIAamp Kit (QIAGEN, Hilden,

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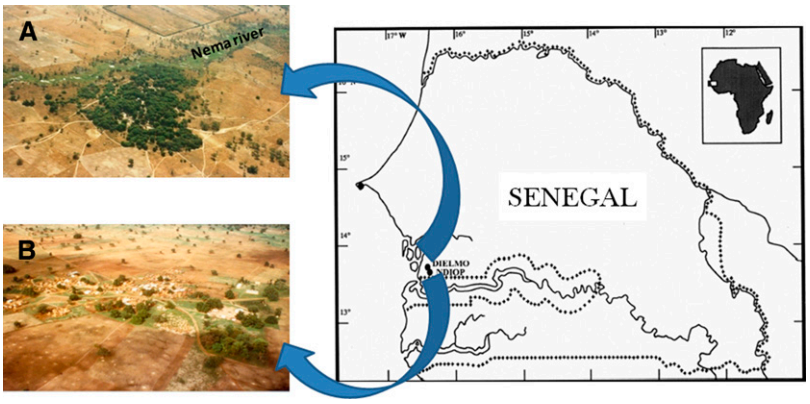

FIGURE 1. Location of the collection sites with blood samples and head lice studied between 2010 and 2011 in Sine-Saloum, Senegal. (**A**) Aerial view of the village of Dielmo, Sine-Saloum, Senegal. (**B**) Aerial view of the village of Ndiop, Sine-Saloum, Senegal.

Germany). PCR amplification was performed using the same primers and probes (Table 1) that were used for the blood samples; however, only one *Bartonella* genus-specific qPCR (internal transcribed spacer 2 [ITS2]) was performed followed by the *yopP*-based qPCR for *B. quintana* detection.[9] PCR results were assessed according to the appearance of a positive control and the negativity of the two negative controls.

RESULTS

***B. quintana* in febrile patients.** In total, 786 blood samples were collected from patients with fever. In five cases (0.6%), we identified DNA from *B. quintana* in the blood samples. All 5 of 247 (2%) infected samples came from Ndiop, whereas 0 of 539 remaining negative samples were collected in Dielmo. Among the infected patients found in Ndiop, four cases were from household 10, and one case was from household 19. One 4-year-old boy was infected, and four female patients (4, 10, 43, and 65 years old) were also infected. The only male case was detected in 2010, whereas three female cases with *B. quintana* infection were consecutively diagnosed in 2011.

***B. quintana* in head lice.** Overall, 19 girls and 1 adult woman were examined in Dielmo, and 17 girls and 5 adult women were examined in Ndiop. None of the five persons that had suffered from trench fever were found to have lice at the time of collection. Body lice were not found during the examination, and the elders of both villages stated that there had been no body lice in the villages for more than 30 years. All head lice that were collected were black, whereas body lice are described as grey transparent[12] (Figure 2); the collected lice belonged to the genotype A and/or C (data not shown). Of 148 head lice tested, 2 (1.3%) lice were infected with *B. quintana*. In Dielmo, 2 of 71 (3%) head lice were infected, whereas the remaining 0 of 77 tested in Ndiop were negative. All infected lice belonged to clade A (data not shown) as previously described.[5] The head lice that were found infected in Dielmo were collected from young girls ages 8 and 11 years old. These young girls did not visit the dispensaries for febrile disease for at least 6 months before or after the collection of the infected lice.

DISCUSSION AND CONCLUSION

For the first time, our investigation showed the circulation of *B. quintana* in febrile patients visiting a health facility in rural Senegal. Additionally, the study provides more data[5] that suggests that head lice, similar to body lice, could transmit *B. quintana* to humans. The presence of *B. quintana* infections has been previously reported in homeless patients from Marseille, France in two studies,[13] with a prevalence of 14% (10 of 71) and 5.3% (50 of 930),[1,12] and the seroprevalence observed in homeless people in both the United States and Europe is high.[1,13] For a long time, it was thought that *B. quintana* was only transmitted by body lice.[12] The first possible involvement of head lice in the transmission of *B. quintana* was shown in homeless children in Nepal, where genotype C head lice are found.[5] Recent studies performed on head lice specimens collected in Ethiopia and Senegal (Dakar) highlighted the role of head lice as possible vectors

TABLE 1
The primers and probes used in the study

Organism	Gene	Type of oligonucleotide.	Primers (5′–3′) and probes (TAMRA)	Refs.
Screening samples				
Bartonella spp.	16S-23S ITS2	F	GGGGCCGTAGCTCAGCTG	10
		R	TGAATATATCTTCTCTTCACAATTTC	
		P	FAM-CGATCCCGTCCGGCTCCACCA-TAMRA	
Confirmation of positive sample				
Bartonella spp.	16S-23S ITS3	F	GATGCCGGGGAAGGTTTTC	10
		R	GCCTGGGAGGACTTGAACCT	
		P	FAM-GCGCGCGCTTGATAAGCGTG-TAMRA	
B. quintana	yopP	F	TAAACCTCGGGGGAAGCAGA	5,10
		R	TTTCGTCCTCAACCCCATCA	
		P	FAM-CGTTGCCGACAAGACGTCCTTGC-TAMRA	

F = forward; P = probe; R = reverse.

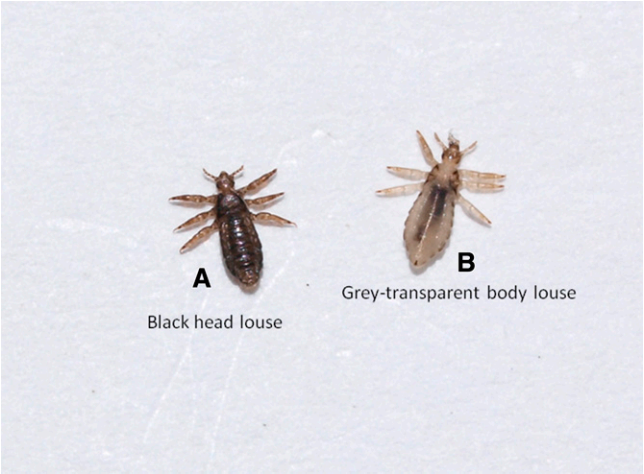

FIGURE 2. Difference in color between head and body lice collected from Senegal. (**A**) Black head louse collected from a young girl in Senegal. (**B**) Grey transparent body louse collected from a young boy talibé in Senegal.

in the transmission of *B. quintana* to humans.[5,6] The evidence of *B. quintana* DNA was also identified in the head louse nits collected from homeless individuals from Marseille, France,[10] indicating the possible vertical transmission of this pathogen. Molecular analyses of the head lice confirmed the existence of head lice from clades A and C in Senegal,[5] but only the head lice of clade A, the same clade that includes body lice, were found to be infected with *B. quintana*. Studies performed on lice collected in France, Russia, Peru, the United States, Tokyo, Rwanda, Burundi, Ethiopia, and Senegal[5,6,13] have shown that poor hygiene and poverty provide the ideal conditions to maintain the transmission cycle of *B. quintana* in a target population.

We believe that our report of simultaneous trench fever cases, discovery of *B. quintana* in the clade A head lice found in the same population, and absence of body lice presents additional evidence that *B. quintana* may be transmitted by clade A head lice. *B. quintana* infections in louse-infested populations, particularly in the homeless population, of rural or suburban areas or the large modern cities of developed countries could increase in the coming years if public authorities or non-governmental organizations do not take appropriate measures to fight the scourge of poverty that has been linked to poor hygiene.

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