Short Report: *Bartonella quintana* in *Cimex hemipterus*, Rwanda

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The common bed bug species *Cimex lectularius*, which has a worldwide distribution, and *C. hemipterus*, which has tropical climate distribution, are hematophagous arthropods.¹ The bugs are attracted to the human host by warmth and carbon dioxide, and bed bug infestations are rapidly increasing worldwide. Moreover, bed bugs are likely to be more problematic in the future because of travel, immigration, and insecticide resistance. Reactions to bites vary, and some patients present only asymptomatic macules, whereas others develop indurated, bulbous lesions that resemble erythema multiforme.² Although no case of bed bug-borne infection has been reported yet, bed bug infestations can have an adverse effect on health and quality of life in the general population, particularly among homeless persons living in shelters.^{1,3} The transmission of more than 45 human diseases has been attributed to bed bugs; however, there is little evidence that they are vectors of communicable disease.¹ The objective of this study was to analyze with molecular assays a large number of bed bugs from Rwanda, where epidemiological and clinical studies on zoonosis are scarce.

Bed bugs were collected from two prisons in Rwanda (Miyove and Muhanga prisons) (Figure 1) from May to January

of 2011 and taken to a local laboratory. Bed bugs were preserved in sterile conditions at room temperature and then sent to our reference center in Marseille to test for lice-borne infections. Before DNA isolation, each bug was rinsed two times in sterile water for 15 minutes. Overall, 100 bed bugs were tested. DNA was extracted from each bug using a QIAamp tissue kit (Qiagen, Hilden, Germany) and then used as template in a real-time polymerase chain reaction (PCR) assay targeting a portion of the *Bartonella* 16S–23S intergenic spacer region (ITS) as described previously.⁴ Two specific *B. quintana* genes (fabF3 and yopP) encode for 3-oxoacyl-[acyl-carrier-protein] synthase and a hypothetical intracellular effector, respectively.⁵ All three sets were positive by real-time PCR for one bed bug. Amplicons were then purified using the QIAquick Spin PCR Purification Kit (Qiagen, Courtaboeuf, France) and sequenced on an ABI 3100 Automated Sequencer (Perkin Elmer, Courtaboeuf, France) using the dRhodamine Terminator Cycle-Sequencing Ready Reaction Kit (PE Applied Biosystems, Les Ulis, France) according to the manufacturer's instructions. Sequences obtained after sequencing of the ITS gene shared 100% similarity to the corresponding ITS fragment of the genome of *B. quintana* (Toulouse strain;

FIGURE 1. The two areas in Rwanda where the bed bugs were collected.

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GenBank accession number BX897700). *B. quintana*-positive bugs were further studied by PCR amplification and DNA sequencing of the mitochondrial (16S DNA) gene as previous described.⁶ Sequences obtained shared 99.7% similarity to the corresponding 16S DNA fragment of the genome of *C. hemipterus* (GenBank accession number GU985560.1). Moreover, *B. quintana*-positive bugs were triturated in brain-heart medium before inoculation onto Columbia 5% sheep blood agar plates (BioMerieux, Marcy l'Etoile, France). Plates were placed in polyethylene bags and incubated at 37°C in 5% CO₂ (Genbag CO₂ system; BioMerieux). The agar plates were examined weekly. After 1 month, no evidence of growth was observed.

In this study, we identified the presence of *B. quintana* in *C. hemipterus* collected from a prison in Rwanda. *B. quintana* and *Rickettsia prowazekii* have been previously identified in body lice collected from a similar jail in Rwanda,⁷ and recently, *B. quintana* was found in head and body lice in Ethiopia.⁸ To avoid the potential of misinterpretation of the PCR data caused by laboratory contamination, three different genes were assessed, and all of them were successfully detected. However, we were not able to cultivate *B. quintana* from this bug. This result suggests that living microorganisms may be dead before testing or that only DNA but no living organisms were present in the bug. *C. hemipterus* might be contaminated when they feed on the blood of prisoners infected with *B. quintana*. Unfortunately, a human blood sample was not collected at the time of sampling the bugs. There are few data to support bed bugs as vectors for transmission of human disease agents, and older studies consist of only logical and not evidence-based postulates.⁹ Recently, Delaunay and others¹ found no published evidence for completion of all the necessary steps leading to the conclusion that bed bugs transmit a pathogen. It is possible that the bed bug might only play the role of vector in pathogen transmission, and consequently, it may be involved in human disease in special circumstances not yet discovered.¹ Nonetheless, the role of the bed bugs in the maintenance and transmission of *B. quintana*

remains to be determined, and it is currently being tested in our laboratory.

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