MOLECULAR DETECTION OF BARTONELLA QUINTANA, B. ELIZABETHAE, B. KOEHLERAE, B. DOSHIAE, B. TAYLORII, AND RICKETTSIA FELIS IN RODENT FLEAS COLLECTED IN KABUL, AFGHANISTAN

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Abstract. The prevalences of Bartonella spp. and Rickettsia spp. were investigated using molecular methods in 77 rodent fleas collected in November 2002 by the French forces detachment in Kabul, Afghanistan. Overall, Bartonella DNA was detected in 15.5% of gerbil fleas and 40.5% of rat fleas, whereas Rickettsia felis was found in 9% of gerbil fleas. We described for the first time in this country Bartonella quintana, B. koehlerae, B. taylorii, and Rickettsia felis in fleas from the gerbil species Meriones lybicus, and B. elizabethae and B. doshiae in rat fleas. Of these, B. quintana, B. elizabethae, B. koehlerae, and R. felis are recognized human pathogens. These results emphasize the potential risk of flea-borne infections transmitted by rodents in this area, and suggest that preventive measures should be taken in the general framework of zoonoses management.

INTRODUCTION

Since the end of 2001, French forces have taken part in a multinational operation in Afghanistan that involves more than 20 different countries. In the French camp in Kabul, Afghanistan, high rate of rodent infestation was observed. Infections caused by Bartonella spp. and Rickettsia spp. occur worldwide and can cause severe human diseases. Most of these infections are transmitted from their animal reservoir to humans via arthropod ectoparasitic vectors such as ticks, fleas, and lice.1 Several studies have highlighted that Bartonella spp. are abundant in wild rodent populations2,3 and in rodent fleas.4 In Asia, pathogenic Bartonella spp. have been reported in rodents,2,5 and Rickettsia felis has previously been detected in mammal fleas.6 In addition, fleas have been shown to be competent vectors of at least some rodent Bartonella7 and Rickettsia8 species. Since few epidemiologic data are available in the international literature regarding zoonoses in Afghanistan, and given the potential risk of zoonosis transmission to military personnel, we investigated the prevalence of Rickettsia spp. and Bartonella spp. in rodent fleas.

MATERIALS AND METHODS

In November 2002, 55 rodents were captured in the French detachment near the Kabul airport. These rodents included 20 gerbils (Meriones lybicus), 10 rats (Rattus spp.), 23 mice (Mus spp.), and 2 hamsters (Cricetulus migratorius). The animals were caught in the same collection area in close proximity to the tents where the soldiers lived. Captures were made either using home-made traps, allowing rodents to be caught alive, or locally bought traps, which instantly killed them. Rodents were taxonomically identified by using morphologic criteria. We carefully searched for fleas on the rodents shortly after death or anesthesia conducted with ketamine. They were then conserved in sealed, preservative-free, plastic tubes, and were kept frozen at −20°C until further processing. Apart from fleas, no other arthropods were found on the rodents. For technical reasons, we only tested the rodent fleas, not the rodents themselves. Identification of the fleas was not carried out at the time of the study. To our knowledge, no description of flea species infesting rodents has been conducted in Afghanistan for more than 30 years. In Asia, Xenopsylla cheopis, the oriental rat flea, is often prevalent,9,10 but as many as 53 different flea species have been described among rodent populations in the same geographic area.11

Fleas were transported to Marseille, France and washed in phosphate-buffered saline for 72 hours at 4°C. They were subsequently rinsed in distilled water, dried on sterile filter paper, and crushed. The DNA was extracted from individual fleas by using the QIAamp tissue kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. Rickettsial DNA was detected by a polymerase chain reaction (PCR) using primers specific for the citrate synthase gene (gltA), a gene present in most Rickettsia species.12 The primers RpCS.877p and RpCS.1258 amplified a 396-basepair fragment of gltA.12 Bartonella spp. DNA was detected using the QHVE1 and QHVE3 primers, which amplify a fragment of variable size, depending on species, of the 16S-23S ribosomal RNA spacer (ITS).13 The PCR detection of Bartonella spp. and Rickettsia spp. was conducted separately, with each assay using genus-specific primers. The PCRs were carried out in a Peltier model PTC-200 thermal cycler (MJ Research, Inc., Watertown, MA). Each 25-μL reaction mixture contained 1 μL of a 1 pmol/μL solution of each primer, 0.5 μL of Elongase polymerase (GIBCO-BRL, Gaithersburg, MD), 2.5 μL of a solution containing 20 mM of each of dATP, dCTP, dGTP, and dTTP (Invitrogen, Carlsbad, CA), 1 μL of elongase buffer A, 4 μL of elongase buffer B, 7.5 μL of sterile distilled water, and 7.5 μL of DNA from fleas. Apart from primers, reaction mixtures were identical for both PCR assays. The following amplification program was used: an initial denaturation for three minutes at 94°C, followed by 44 cycles of denaturation at 94°C for 30 seconds, annealing at a temperature depending on the primers used for 30 seconds,12,13 and extension at 68°C for 90 seconds. At the end of the last cycle, extension was completed by incubation at 68°C for 7
minutes. Each PCR included negative (distilled water) and positive (DNA extracted from *R. montanensis* isolate 2-4-6 as described for *Rickettsia* amplification and from *B. elizabethae* for *Bartonella* amplification) controls. The PCR products were visualized after electrophoresis on a 1% agarose gel.

*Rickettsia* PCR products were sequenced using the primer pairs CS1d-CS409d and CS353d-RpCS.1258 as previously described. Bartonella spp. PCR products were sequenced using PCR primers. The PCR products were purified using the QiAquick PCR purification kit (Qiagen) in accordance with the manufacturer’s recommendations. Sequence reactions from PCR-positive products were carried out using the dRhodamine Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Warrington, United Kingdom) in an ABI 3100 PRISM (Applied Biosystems) automated sequencer. Sequences were identified by comparison with sequences present in GenBank using the BLAST software.

RESULTS

A total of 77 fleas were collected and tested (Table 1). Sixty-five percent (13 of 20) of the gerbils were infested with fleas, which included 100% (6 of 6) of the females and 50% (7 of 14) of the males. Seventy percent (7 of 10) of the rats were infested with fleas, which included 71% (5 of 7) of the males and 33% (1 of 3) of the females. The number of fleas collected on each individual animal ranged from 0 to 9. The mean number of fleas per infested rodent was 3.5 on gerbils and 4.6 on rats. In contrast, no fleas were found on the 23 mice and 2 hamsters. As determined by ITS gene sequencing, Bartonella spp. DNA was found in 26% (20 of 77) of the fleas, which included 15.5% (7 of 45) in gerbil fleas and 40.5% (13 of 22) in rat fleas (Table 1). In gerbil fleas, we identified *B. quintana* in 9% (4 of 45) of the fleas, *B. koehlerae* in 4.5% (2 of 45), and *B. taylorii* in 2% (1 of 45), whereas in rat fleas we found *B. elizabethae* in 19% (6 of 32) and *B. doshiae* in 22% (7 of 32) (Table 2). As determined by *gltA* gene sequencing, *Rickettsia felis* DNA was detected in 9% (4 of 45) of the gerbil fleas (Table 1).

*Bartonella quintana* was found in four fleas from three different gerbil hosts; 15% (3 of 20) of the gerbils and none of the rats harbored at least one *B. quintana*-infected flea. Similarly, 15% (3 of 20) of the gerbils and none of the rats had at least one *R. felis*-infected flea. Conversely, *B. elizabethae* was found in six fleas from five different rat hosts; 50% (5 of 10) of the rats and none of the gerbils had at least one *B. elizabethae*-infected flea. Similarly, *B. doshiae* was found in seven fleas from five different rat hosts; 50% (5 of 10) of the rats and none of the gerbils had at least one *B. doshiae*-infected flea. *Bartonella koehlerae* was found in two fleas from the same gerbil host, while *B. taylorii* was detected in one gerbil flea. In one gerbil, one flea was found positive for both *B. quintana* and *R. felis*, and a second flea was found positive for both *B. taylorii* and *R. felis*. All negative controls were PCR negative, whereas positive controls were amplified within each assay.

### Table 1

<table>
<thead>
<tr>
<th>Rodents</th>
<th>No. of specimens trapped</th>
<th>No. of specimens infected by fleas</th>
<th>No. of fleas</th>
<th>Bartonella-positive fleas</th>
<th>Rickettsia felis-positive fleas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. % No. %</td>
<td>No. % No. %</td>
<td>No. % No. %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gerbils</td>
<td>20 10</td>
<td>13 7</td>
<td>45 32</td>
<td>7 15.5 4 9</td>
<td></td>
</tr>
<tr>
<td>Rats</td>
<td>10</td>
<td>7</td>
<td>32</td>
<td>13 40.5 0 0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>20</td>
<td>77</td>
<td>20 26 4 5</td>
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</tbody>
</table>

DISCUSSION

Coinfection with Bartonella spp. and *Rickettsia* spp. has previously been shown in cat fleas in France. Apart from *B. doshiae* and *B. taylorii*, which cluster together, the five *Bartonella* species discovered in this study are scattered among different clades, according to a phylogenetic tree recently proposed. In the same geographic area, *Bartonella* spp. found in gerbil and rat fleas differ, but simultaneously different *Bartonella* spp. belonging to distinct phylogenetic groups have been found to infect fleas from the same rodent species. These findings are consistent with results from earlier studies. Indeed, experimental evidence of host specificity of *Bartonella* spp. infections in rodents, using parameters such as minimal infectious dose and duration and level of bacteremia, has been reported. A survey carried out in southern China demonstrated a strong specific bacteria-rodent relationship, since *Bartonella* spp. isolates obtained from different rodent species clustered separately, according to the genus of rodent host from which they were obtained. Nevertheless, host specificity is not absolute because the same *Bartonella* species can cross from one rodent species to another.

*Bartonella quintana* is the historic etiologic agent of trench fever, a disease extensively reported during World War I, when approximately one million troops were affected in Europe. More recently, an outbreak of trench fever occurred in Burundi. During the last decade, *B. quintana* infections have re-emerged in both Europe and the United States, mainly among urban homeless populations, and have caused sporadic cases of endocarditis, bacillary angiomatosis, chronic bacteremia, and pericardial effusion. Currently, humans are the only identified reservoir host for *B. quintana*, and *Pediculus humanus humanus*, the human body louse, is its main vector. In addition, *B. quintana* has been found in cat fleas and ticks. Thus, there may be other arthropod vectors apart from lice, and other reservoir hosts apart from humans, also involved in the epidemiology of *B. quintana* infections.

*Bartonella elizabethae* is a recognized human pathogen that has been involved in at least one case of endocarditis and one case of neuroretinitis. This bacterium has also been detected in the blood of one sick dog, *Bartonella koehlerae*, which was previously isolated from domestic cats, has been detected by PCR in the valvular tissue of a patient with blood culture-negative endocarditis. *Bartonella doshiae* and *B. feli...
Taylorii were isolated from the blood of rodents, but have not yet been associated with human diseases.

Previously, Bartonella spp. have been detected in mammal fleas, and have even been found prevalent in rodent fleas. In Asia, pathogenic Bartonella spp., including *B. elizabethae*, have already been reported in rodents. Conversely, to date, *B. quintana*, *B. koehlerae*, and *B. taylorii* have not been reported in Asia, and their presence in Afghanistan is quite unexpected.

*Rickettsia felis*, the recently recognized agent of flea-borne spotted fever, appears to be widely distributed and has been identified wherever it has been investigated. This bacterium has also been detected in opossum fleas in the United States, in cat fleas in France, and in mammal fleas in Asia. It usually induces a non-specific febrile exanthema in humans, but neurologic involvement, with severe clinical course, has been reported. The cat flea is considered the main vector of this rickettsial agent.

Among potential zoonoses involving rodents in Afghanistan, a recent review mentioned leptospirosis, cutaneous leishmaniasis, sand fly fever, hantavirus syndrome, scrub typhus, and murine typhus. Up to now, few data involving *Bartonella* spp. and *Rickettsia* spp. and their flea vectors and rodent hosts were available for Afghanistan. Murine typhus caused by *R. typhi* is an example of a pathogenic *Rickettsia* species transmitted from its rodent reservoir to humans by a flea vector. Human contamination by rodent-associated *Bartonella* spp. has already been demonstrated. A recent study has highlighted the increasing association of *Bartonella* of rodent origin with human diseases. Fleas are very mobile and can escape from their usual host, in particular after the death of the host, and infest other mammals nearby, including humans. *Bartonella* spp. and *Rickettsia* spp. transmission could occur through contact between wounds or abraded skin, with feces from infected fleas, or through flea bites. Infestation of humans by rodent fleas is more likely to occur with commensal rodents, such as *Rattus* spp. or *Mus* spp., or when people live near rodent habitats, such as refuges in camps or soldiers on the ground. The stress due to military operations and the lack of adaptation of the immune system in a new microbiologic environment could represent factors that increase the susceptibility of foreign troops. Consequently, the emerging human pathogens discovered in this study led us to pursue and reinforce prevention against rodent-associated zoonoses. Rodent control included sanitary measures with adequate food storage and waste management, and also offensive measures with rodenticides (anti-clotting agents) and traps. Rodent bodies were eliminated as soon as possible with appropriate handling to prevent human infestation by fleas. In addition, the struggle against arthropod-transmitted diseases was carried out by regularly spraying insecticides in the environment and using skin repellents. Finally, soldiers were given medical information before leaving their country and during the operation whenever significant new epidemiologic information was obtained.

In summary, we describe for the first time the presence of five *Bartonella* species and one *Rickettsia* species in rodent fleas in Afghanistan. Our knowledge of the complex ecology and epidemiology of these bacteria is still incomplete, and additional studies are required, particularly to determine the relations between reservoir hosts, vectors, and microorganisms. Although the role of rodent fleas as competent vectors for human contamination by *B. quintana*, *B. elizabethae*, *B. doshiae*, and *R. felis* could not have been definitively demonstrated, a potential risk does exist for exposed persons belonging to local populations, foreign troops, and peacekeepers. This report is the first to describe *B. koehlerae* and *B. quintana* in rodent fleas. Consequently, other arthropod vectors, apart from lice, may be involved in the epidemiology of *B. quintana* infections. Our study reinforces the idea that both *B. quintana* and *R. felis* are distributed worldwide.

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