RAPID COMMUNICATION

Detection of Bartonella quintana from Body Lice (Anoplura: Pediculidae) Infesting Homeless People in Tokyo by Molecular Technique

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ABSTRACT We report detection of Bartonella quintana Brenner, the pathogenic agent of trench fever, from body lice, Pediculus humanus L., infesting homeless people in Tokyo by polymerase chain reaction. Two of 12 (16.7%) homeless were infested with Bartonella-positive body lice. From the current status of the recent increase of homeless people in many large cities of the developed countries, a medical examination of homeless people should be carefully performed in the consideration of trench fever. Sampling of body lice from clothing of homeless people is recommended for quick and accurate diagnosis of trench fever through the detection of B. quintana DNA.

KEY WORDS Bartonella quintana, body lice, polymerase chain reaction, trench fever, detection

Bartonella quintana Brenner, a small gram-negative bacteria, is the causative agent of trench fever and is transmitted by the body louse, Pediculus humanus L. The organism was first identified as an important pathogen during World War I and affected an estimated 1 million troops, including civilians in Europe (Maguina and Gotuzzo 2000). This infectious disease is characterized by fever, rash, bone pain, and splenomegaly ranging from a mild flu-like illness to a more severe endocarditis. Recently, outbreaks of trench fever have been reported among homeless people in Marseilles, France (Brouqui et al. 1996, 1999); in Seattle, United States (Jackson and Spach 1996); in Moscow, Russia (Gagua et al. 1999); and rural Andean Communities in Peru (Raoult et al. 1999). In this article, we report the second finding of B. quintana infection among homeless people in a metropolitan city of an industrialized country, Tokyo Metropolitan Government. A recent increase of homeless in Tokyo suggests that epidemiological studies of louse-borne diseases in Japan should be carried out.

Materials and Methods

Between May 1999 and May 2000, lice-infested underclothing from homeless people were obtained at a local health center in Tokyo. Body lice were collected from underclothing and kept in a freezer at −80°C until used. The center reported in their guidelines of prevention and control of body louse infestation that 6% of the homeless were infested with body lice (Makigami and Yaguchi 1999). The number of body lice from discarded clothing ranged from 6 to 643, including both nymphs and adults. The body lice were collected individually from underclothing from 12 homeless persons. DNA of each louse was prepared from the crushed louse by using ISOGEN (Wako Pure Chemical, Osaka, Japan) according to the manufacturer’s instructions. The extraction effectiveness and the absence of polymerase chain reaction (PCR) inhibitors were assessed by PCRs incorporating broad-range 18S rRNA gene primers as described previously (Roux and Raoult 1999). PCR amplifications were carried out by Bartonella genus-specific primers described previously (Roux and Raoult 1999) using a Peltier Thermal Cycler PTC-150 (MJ Research, Incline Village, NV) under the following conditions: an initial 3-min denaturation step at 95°C was followed by 40 cycles of denaturation at 95°C (30 s), annealing at 50°C (30 s), and extension at 72°C (1 min). In the positive cases of amplification, the PCR products were purified by using QIAEXII (Qiagen, Tokyo, Japan) according to the manufacturer’s instructions, and sequencing was carried out in an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Tokyo, Japan). The nucleotide sequences were tabulated with the Sequence software package of the Genetics Computer Group (Madison, WI). The reference database consisted of 1,196,123 sequences retrieved and was combined from GenBank and EMBL.

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Results and Discussion

As shown in Fig. 1, Bartonella genus DNA was detected in two body lice (lanes 6 and 11) on the partial citrate synthase gene (gltA) and from a body louse in lane 6 on the 16S-23S rRNA intergenic spacer region 1 (ITS 1). In these PCR conditions, the amplification efficiency of gltA may be a higher degree than that of ITS 1. Sequencing of DNA fragments amplified by PCR confirmed the identities of B. quintana by BLAST (Table 1). The two gltA products (481 bp) amplified with PCR in lanes 6 and 11 were closely related (99.2% DNA identity) to that of the strain fuller of B. quintana. The gltA amplified with PCR in lane 6 showed identity to that from lane 11 (GenBank accession number AY035823). In this article, the presence of B. quintana DNA was clearly shown in body lice collected from clothing of homeless people (2 positive clothing/12 lice-infested clothing: 16.7%) in Tokyo by molecular tools. The frequencies of B. quintana-infection in Zimbabwe and Russia were 16.7 and 12.3%, respectively (Roux and Raoult 1999). Those percentages were almost equal to that in Tokyo. Current increases in the number of homeless in some large cities in Japan suggest that physicians of local health centers, homeless care centers, and emergency hospitals should perform minute medical examinations in consideration of the possibility of B. quintana infection in homeless persons. Detection of specific antibodies, IgM or IgG, to B. quintana from the serum of homeless is also recommended if a blood sample can be easily obtained. Recently, Roux and Raoult (1999) reported that three pathogens, Rickettsia prowazekii da Rocha-Lima, B. quintana, and Borrelia recurrentis Lebert, were detected from body lice collected from homeless persons in African and European countries and persons in South America by using molecular approaches, and that these tools are convenient for the detection and identification of bacterial DNA in body lice and for the epidemiological study of louse-borne diseases.

<table>
<thead>
<tr>
<th>Lane no.</th>
<th>Bacterial species</th>
<th>Sequence identity percentages</th>
</tr>
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<tbody>
<tr>
<td>6, 11</td>
<td>B. quintana (strain fuller)</td>
<td>99.2</td>
</tr>
<tr>
<td></td>
<td>B. koehlerae</td>
<td>90.7</td>
</tr>
<tr>
<td></td>
<td>B. henselae</td>
<td>90.4</td>
</tr>
</tbody>
</table>

In our research, homeless people did not show remarkable clinical signs such as quintan fever, rash, bone pain, and splenomegaly. In an outbreak of urban trench fever among homeless people in Marseilles, B. quintana infection was associated with the infestation of body lice in patients with nonspecific symptoms or no symptoms (Brouqui et al. 1999). It is speculated that Bartonellosis of homeless patients in Tokyo became chronic, which is the same as in homeless patients in Marseilles.
In separate experiments we confirmed the insecticide susceptibility of the body louse from homeless people. Currently, development of resistance to phenothrin, which is the only insecticide registered officially for the treatment of the head and body lice in Japan, has not been observed in Tokyo (Tomita et al. 2000). We also are requiring health care professionals to survey for the presence of Rickettsia prowazekii and Borrelia recurrentis in body lice collected from homeless people, because interepidemic survival of R. prowazekii causes outbreaks of Brill-Zinsser disease (Raoult and Roux 1999). It is likely that factors such as overcrowding, malnutrition condition, and inadequate access to medical care affect the transmission and spread of louse-borne diseases among homeless. As with other reemerging infectious diseases, further efforts of prophylaxis, diagnosis, and treatment for B. quintana infection among homeless people should be intensified and the coordinated efforts of physicians, laboratory staffs of local health centers, epidemiologists, and medical entomologists should be required.

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