

in Kingston's study. This implies that the antimicrobial effect is dominant over immunomodulatory effects in macrolide therapy, at least as far as duration of fever in *M. pneumoniae* infection is concerned. On the other hand, we did not assess the duration of other symptoms, such as malaise, sore throat, and cough, and it is possible that the immunomodulatory effects of macrolides can shorten these symptoms even in MR *M. pneumoniae* infection.

A difference of three febrile days in MR patients might not have a great impact in the management of *M. pneumoniae* infection, because it is often a mild and self-limiting disease, and the fever resolved even when the initially prescribed macrolide was not changed. However, it is reasonable to consider the use of alternative antimicrobial agents, such as minocycline, when macrolides are less effective than expected in patients more than 8 years old with possible *M. pneumoniae* infection.

The criteria for *M. pneumoniae* infection used in this study were stringent enough to confirm acute *M. pneumoniae* infection. This was a retrospective study based on a review of medical records, and patients with incomplete records were excluded. In general, clinical records of patients showing mild illness with *M. pneumoniae* infection were incomplete, and their clinical evaluation was excluded from this study.

In conclusion, we compared clinical outcomes in 11 MR patients and 26 MS patients given macrolide therapy. The MR patients showed more febrile days (by a median of 2 days) during the initial macrolide therapy than MS patients. On the other hand, no apparent treatment failure or serious illness was reported for MR patients. The influence of the emergence of MR *M. pneumoniae* on the treatment for *M. pneumoniae* infection deserves further study.

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Original Article

## Epidemiological Studies on *Bartonella quintana* Infections among Homeless People in Tokyo, Japan

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**SUMMARY:** In an epidemiological investigation of trench fever in Japan, we compared the seroprevalence of *Bartonella quintana* in homeless people and in the general population. In homeless rescue outreach programs held in Tokyo from May 2001 to March 2003, 151 blood samples were taken from non-hospitalized homeless people. The prevalence of IgM and IgG antibodies against *B. quintana* in these people was compared with that in 200 healthy blood donors using a commercially available indirect fluorescent antibody test. Although IgG titers of  $> \text{or} = 1:128$  were found in 57% (86/151) of homeless people and 51% (101/200) of blood donors, high titers of  $> \text{or} = 1:1,024$  were encountered only in homeless people (11%, 16/151). Attempts to isolate *B. quintana* from the blood of homeless people were unsuccessful, but polymerase chain reaction based detection, using *Bartonella* genus specific primers, demonstrated the presence of *B. quintana* DNA in the blood of 10 homeless people. Our data suggest that urban trench fever is endemic among the Japanese homeless population.

### INTRODUCTION

The bacterium *Bartonella quintana* is the agent of trench fever, otherwise known as five-day fever, quitan fever and Wolhynia fever (27). Humans are the only known reservoir of *B. quintana*, and transmission among people occurs via the body louse (*Pediculus humanus*). The genus *Bartonella* now comprises 20 species or subspecies (15), several of which are recognised pathogens, including *B. henselae*, the agent of cat-scratch disease (CSD) (1), which is transmitted by cat fleas (*Ctenocephalides felis*) and dog fleas (*C. canis*) (30). Although trench fever has been recognised for almost a century, our perception of its medical importance has significantly changed in the last decade as new syndromes associated with the pathogen and new susceptible populations have been identified worldwide. Recently, there have been scientific reports of *B. quintana*-induced bacillary angiomatosis in patients from South Africa (4) and Zimbabwe (5), as well as widespread trench fever among Burundian refugees (21). In Europe and the United States, a broadening spectrum of *B. quintana* infections, ranging from life-threatening bacillary endocarditis to asymptomatic bacteraemia, are now being identified, particularly in homeless people (9), AIDS patients (2,12) and chronic alcoholics (16).

As in other industrialized countries, the size of the urban homeless population in Japan has increased markedly in recent years. In 2003, the Japanese Ministry of Health, Labour

and Welfare estimated that the national homeless population had surpassed 25,000 (online only; available from: <http://www.mhlw.go.jp/shingi/2003/12/s1216-5u.html>) (18), and in the same year, official reports by local managers of parks, roads, and river basins in Tokyo's 23 cities indicated the presence of over 5,600 homeless people (29). Although *B. quintana* infections have yet to be documented in this population, the presence of *B. quintana* DNA in the body lice collected from several homeless volunteers provides strong support for their existence (23). In the study reported herein, we used serological and PCR-based methods to diagnose *B. quintana* in blood samples collected from homeless and normal populations in an attempt to clarify the epidemiological status of these infections in urban areas of Japan.

### MATERIALS AND METHODS

**Collection of blood samples:** A prospective study was carried out in P city, an area inhabited by 250,000 people in the northwest of Tokyo that is characterized as a typical downtown area containing entertainment and business districts. An estimated 200 homeless people live around railroad stations and in the small parks scattered throughout the central downtown area. Since 1988, the P city municipal government has supported rescue outreach programs in which municipal officers provide occupational and medical consultations for these homeless people. The programs take place two to four times each year, and typically involve chest X-ray checks for pulmonary tuberculosis, provision of clothing and food, a haircut and a shower. Public health office doctors, nurses and sanitary inspectors provide infection control expertise and advice, specifically on tuberculosis and louse-borne diseases.

The homeless people participating in these outreach programs were systematically approached for inclusion in the study, and the aims were clearly explained to each person approached. Prior to their inclusion in the study, informed

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consent was obtained from all participants. The consenting participants underwent a brief clinical examination and were asked about their current health condition and any recent disease manifestations that they could remember. Blood samples were collected from an upper limb vein after iodine dressing. All participants were also asked if they had body louse or cat flea infestations. Public health nurses specifically checked participants for any clinical presentations associated with *B. quintana* infection.

**Serological testing:** For detection of specific antibodies to *B. quintana*, we used a commercially available indirect fluorescent antibody (IFA) kit (Focus Technologies, Cypress, Calif., USA). Control sera were chosen from healthy people who donated blood for the blood bank at the National Institute of Infectious Diseases, Tokyo. These controls were all males aged 30-60, and each group consisted of 50 samples.

**Isolation and identification of *B. quintana* from blood samples:** Approximately 3 ml of blood were inoculated into a 30-ml Bactec™ PEDS PLUSTM blood culture bottle (Dickinson & Company, Sparks, Md., USA) and incubated at 35°C for 7 days. An aliquot of each blood culture was then sub-cultured onto Columbia sheep blood agar plates and kept in a 5% CO<sub>2</sub> incubator for 3 weeks. A 500-μl aliquot of each blood culture was also prepared for use as template for polymerase chain reaction (PCR) by centrifugation at 15,000 rpm for 20 min, then DNA extraction by using the Sepa Gene kit (Sanko Chemicals, Tokyo, Japan). These extracts were incorporated into a *Bartonella*-specific nested PCR targeting a fragment of the 16S/23S rRNA intergenic spacer region incorporating the previously described primers QHVE-1/QHVE-3 (20) and our established *Bartonella*-specific nested primers QHVE-12 (5'-CCGGAGGGCTTGT AGCTCAG-3') and QHVE-14 (5'-CACAAATTTCAATAGAAC-3'). The conditions used for both rounds of amplification were the same, and were comprised of one cycle of 94°C for 5 min, then 30 cycles of 94°C for 45 s, 55°C for 45 s, 72°C for 45 s, then one cycle of 72°C for 5 min. The PCR products were subjected to electrophoresis in 1% agarose gels, then visualized under UV illumination following ethidium bromide staining. These primers were also reacted with *B. henselae* DNA. The PCR products were then sequenced to identify *B. quintana* or *B. henselae*.

**Statistical analysis:** Statistical analysis was performed using statistical package SPSS for Windows, Release 10.0J (standard version: SPSS, Inc., Chicago, Ill., USA).

## RESULTS

**Demographics of the homeless population:** Between May 2001 and March 2003, 232 people participated in 3 outreach programs, of whom 183 (79%) agreed to participate in our study by informed consent. From these cases, we selected 151 individuals for the study, all of whom were male and under 70 years old. We selected only men because we had only 2 female samples. If a person had been enrolled into more than one outreach program during the survey period, only samples and information gathered during their first attendance were used. Among the 151 homeless people included in the study, the mean age was 54.7 years (SD ± 7.7) and mean length of time spent homeless was 32 months (range 1-300 months, 2 unknown). The infestation rate with body lice (including those who had noted previous body lice infestation) was 11% (16/151).

**Serological analysis:** The distribution of anti-*B. quintana*

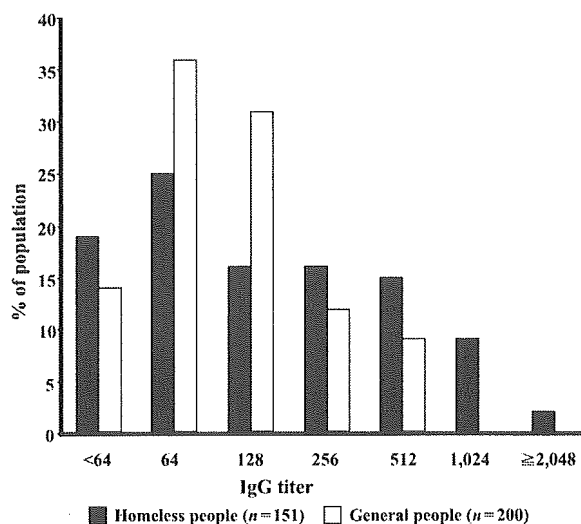


Fig. 1. Distribution of anti-*B. quintana* IgG antibody titers among surveyed homeless individuals and healthy blood donors.

IgG titers in the homeless people and blood donors is presented in Figure 1. Titers of  $\geq 1:128$  were detected in 86/151 (57%) of the homeless people but also in 101/200 (51%) of the blood donors. However, an IgG titer of 1:512 or greater was detected in 38/151 (25%) of the homeless people but in only 17/200 (9%) of the blood donors, and IgG titers of 1:1,024 or greater were detected only in the homeless people (16/151, 11%). The geometric mean IgG titers of the two groups were significantly different (the geometric mean in homeless and blood donors: 144 and 101, respectively,  $t = 3.47$ ,  $P < 0.001$ ). IgM titers below the cut-off titer should be negative, because significantly high levels of IgM antibodies were not detected (data not shown). Within the homeless population, variation in the prevalence of the serological evidence of *Bartonella* infection could only be correlated with the length of time spent homeless. Individuals who had been homeless for more than 1 year were significantly more likely to have serological evidence of infection than those who had been homeless for a shorter period (Wilcoxon Two-Sample test,  $P < 0.05$ ) (Table 1).

**Identification of *B. quintana* from blood samples:** No isolates of *B. quintana* were obtained. However, we obtained PCR-based evidence of *B. quintana* infections in 10 individuals (7%, 10/151) (Table 1). The size of amplicons obtained using the PCR is dependent on the source of DNA, with different *Bartonella* spp. yielding different size products. Thus, the fact that all 10 amplicons were of an indistinguishable size of about 500 base pairs (bp) indicated that all products were probably derived from strains of *B. quintana* (Fig. 2). Other human-associated *Bartonella* spp. either failed to yield an amplicon (*B. clarridgeiae*) or yield an amplicon that was markedly larger (e.g., *B. henselae* 568 bp, *B. elizabethae* 572 bp)(data not shown). To confirm this, four of the amplicons were sequenced, yielding unambiguous sequence data for the entire length of the amplicon when primers QHVE12 and QHVE14 were used. Alignment and comparison of the sequences obtained demonstrated them to be indistinguishable from one another and from the ISR fragment previously determined for the *B. quintana* type strain (Fig. 2). Collation of serological data for the 10 PCR positive individuals revealed that six were seropositive (IgG titers more than 1:128) but four were seronegative (IgG titers less than 1:64).

Table 1. Comparison of demographic and diagnostic details of homeless individuals surveyed

|   | <64 | 64 | 128 | 256 | 512 | ≥1,024 | Total (%)   | P value  |
|---|-----|----|-----|-----|-----|--------|-------------|----------|
| Age (yr)                                  |     |    |     |     |     |        |             |          |
| 30s                                       | 0   | 2  | 4   | 0   | 0   | 0      | 6 ( 3.4)    |          |
| 40s                                       | 4   | 11 | 4   | 5   | 4   | 3      | 31 ( 20.5)  | n.s.     |
| 50s                                       | 14  | 18 | 13  | 8   | 7   | 7      | 67 ( 44.3)  |          |
| 60s                                       | 10  | 6  | 3   | 11  | 11  | 6      | 47 ( 31.1)  |          |
| mean ± S.D. = 54.72 ± 7.7                 |     |    |     |     |     |        | 151 (100.0) |          |
| Period of streetlife (mo)                 |     |    |     |     |     |        |             |          |
| <6m                                       | 14  | 17 | 8   | 5   | 6   | 3      | 53 ( 35.1)  |          |
| 6m-1y                                     | 3   | 6  | 3   | 4   | 1   | 1      | 18 ( 11.9)  | P < 0.01 |
| 1y-5y                                     | 8   | 10 | 8   | 8   | 11  | 8      | 53 ( 35.1)  |          |
| >5y                                       | 3   | 4  | 5   | 5   | 4   | 4      | 25 ( 16.6)  |          |
| unknown                                   | 0   | 0  | 0   | 2   | 0   | 0      | 2 ( 1.3)    |          |
| Mean ± S.D. = 32.56 ± 51.87 (range 1-300) |     |    |     |     |     |        | 151 (100.0) |          |
| PCR positive                              | 3   | 1  | 1   | 3   | 2   | 0      | 10 (6.62)   | n.s.     |

n.s. denotes not significant.

Table 2. Epidemiological and clinical findings associated with homeless individuals possessing high anti-*B. quintana* IgG titers

| Symptoms                         | IgG titer ≥128<br>n = 86 |        |      | IgG titer ≤64<br>n = 65 |        |       | OR   | 95% CI     |
|----------------------------------|--------------------------|--------|------|-------------------------|--------|-------|------|------------|
|                                  | n                        | (%)    |      | n                       | (%)    |       |      |            |
| General fatigue                  | 5                        | ( 5.8) |      | 6                       | ( 9.2) |       | 0.61 | 0.14-2.52  |
| Fever                            | 5                        | ( 5.8) |      | 2                       | ( 3.1) |       | 1.94 | 0.30-20.97 |
| Headache                         | 13                       | (15.0) |      | 7                       | (11.0) |       | 1.48 | 0.51-4.65  |
| Anorexia                         | 6                        | ( 7.0) |      | 4                       | ( 6.2) |       | 1.14 | 0.26-5.75  |
| Night sweat                      | 13                       | (15.0) |      | 6                       | ( 9.2) |       | 1.75 | 0.58-5.95  |
| Arthralgia                       | 23                       | (27.0) |      | 16                      | (25.0) |       | 1.12 | 0.50-2.53  |
| Lymphadenopathy                  | 2                        | ( 2.3) |      | 2                       | ( 3.1) |       | 0.75 | 0.05-10.63 |
| Bone pain                        | 7                        | ( 8.1) |      | 8                       | (12.0) |       | 0.63 | 0.18-2.13  |
| Eruption                         | 5                        | ( 5.8) |      | 5                       | ( 7.7) |       | 0.74 | 0.16-3.38  |
| History of body lice infestation | 11                       | (13.0) |      | 5                       | ( 7.7) |       | 1.76 | 0.53-6.80  |
| History of fleas infestation     | 4                        | ( 4.7) |      | 3                       | ( 4.6) |       | 1.01 | 0.16-7.13  |
|                                  | Mean                     | Range  | S.D. | Mean                    | Range  | S.D.  |      | P value    |
| Age (year)                       | 55.35                    | 34-68  | 7.76 | 53.89                   | 31-68  | 7.59  |      | 0.2        |
| Period of street life (month)*   | 40.75                    | 1-288  | 55.6 | 21.97                   | 1-300  | 44.79 |      | <0.01      |

\*seropositive population = 84 (2 unrecorded)

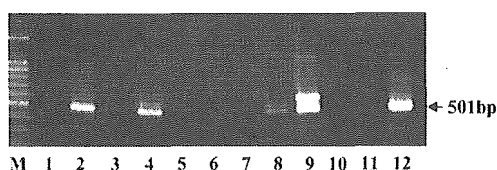


Fig. 2. Amplification products obtained from the cultured blood of Japanese homeless individuals by using a *Bartonella*-specific PCR targeting a fragment of the 16S-23S rRNA intergenic spacer region. M. marker. Lane 2, 4, 8, 9 show positive result. Lane 12 is Fuccer strain of *Bartonella quintana*.

None of the individuals with very high serological titers (>1,024) were found to be PCR positive.

**Clinical characteristics:** To evaluate if *B. quintana* seropositivity correlated with specific clinical features, we compared the results of clinical examinations of 65 seropositive homeless individuals with 86 seronegative individuals. Calculation of odds ratios (OR) and 95% confidence intervals (CI) indicated no significant differences between the two groups (Table 2).

## DISCUSSION

We found clear evidence of *B. quintana* infections in a homeless population in an urban area of Japan using both serological and molecular methods. Comparison of our estimates of ongoing infections in about 7%, and past exposure in over 10% of a non-hospitalized homeless population with other studies is difficult, as there have been only several similar studies, and most of these have focused on hospitalized homeless populations. The principle reason behind this dearth of information is unlikely to be that infections are rare, but rather that they go unrecognized. To our knowledge, to date only Brouqui and colleagues have reported surveillance of non-hospitalized French homeless people for evidence of *B. quintana* infections (2). In this report, only 2% of 221 individuals tested had serological evidence of infection. Surveys of homeless people attending clinics or hospitals are more numerous and have yielded evidence of higher infection rates. Jackson and colleagues (12) reported 39 (20%) of 192 homeless attendees of a downtown clinic in Seattle had serological evidence of *B. quintana* infections, whereas Brouqui and colleagues (2) found that of 43 hospitalized

homeless people, 16% had high antibody titers to *B. quintana*. Subsequently, Brouqui and colleagues (3) investigated *B. quintana* infections among all homeless people who presented to the emergency departments of hospitals in Marseille, France during 1997. A total of 71 people were included in the study, with 10 (14%) yielding *B. quintana* on blood culture and 21 (30%) possessing serological evidence of infection. Most recently, Smith and colleagues (25) reported a seroprevalence of 10% among 200 mainly homeless attendees of a free clinic in Los Angeles. Two other surveys have targeted inner city intravenous drug users, many of whom were homeless. In the earlier of these, 10% of 630 residents of Baltimore screened had serological evidence of *B. quintana* exposure (6), whereas in the latter study (7), involving 204 New York drug users, a seroprevalence of only 2% was recorded (although almost half had antibodies against other *Bartonella* spp.). From these data, our findings are not at all out of keeping with what has been observed previously elsewhere in the world.

Contemporary urban trench fever was first reported less than 10 years ago, and thus medical and scientific analysis of the syndrome remains nascent (26). In developed countries, trench fever is predominantly encountered among the urban poor or the homeless. The susceptibility of these individuals is likely to result from their reduced immune status and frequent contact with body lice infected with *B. quintana*. As none of the homeless people included in our survey had been hospitalized during the preceding several months, we were not able to ascertain whether they had any immune suppressive infections (e.g., human immunodeficiency virus), but we were able to confirm that a significant proportion of our sample were exposed to body lice. Our estimate of prevalence of body lice infestation (11%) is similar to the figure of 7% obtained in an earlier sampling of a similar population in Tokyo (24). The Japanese homeless community has been growing since the economic downturn in the 1990s, and with no concerted intervention strategies for louse infestation being adopted, we predict that an increasing number of people will be exposed to body lice and the pathogens they transmit. To further emphasise that prolonged homelessness will also inevitably lead to pathogen exposure, we, like others, found a positive association between the length of time spent homeless and the risk of infection by *B. quintana*.

Confounding a wider recognition of trench fever by the medical community is not solely the nature of the most susceptible population. *B. quintana* infections are insidious, most often devoid of acute symptoms but inducing manifestations as a result of extreme chronicity. Brouqui and colleagues (2) reported that even an outbreak of trench fever cannot be readily clinically diagnosed, finding that among infected individuals, all symptoms except for a headache were not significantly associated with the presence of antibodies to *B. quintana*.

Laboratory diagnosis of *B. quintana* infections is also very problematic. As we observed, a broad-based adoption of recommended cut-off titers, even for commercially-available kits, is impractical, and, as previously suggested, researchers would be wiser to evaluate the study population and adjust cut-off titers accordingly (19). Unfortunately, we could not determine the cut-off titer for diagnosing trench fever, although the cut-off titer of  $\geq 256$  in IgG antibodies seems to be adequate to evaluate the seroprevalence of *B. quintana* in our surveillance. In previous serological studies, the specificity of assays has varied, typically between 70 and 99% (10,11, 22,32), such that cut-off titers for bartonella diagnosis were

encountered among 9% of healthy Greeks (28) and 16% of healthy Germans (22). One explanation for the apparent high seropositive rates in these and our healthy populations may be the lack of specificity of assays due to cross-reaction with other common pathogens. Cross-reaction between *Bartonella* spp. is well recognized (31), and cross reactivity between antigens of *Bartonella* and antigens derived from *Coxiella burnetii*, *Anaplasma phagocytophilum* and *Chlamydia* spp. has also been reported (8,13,17). Molecular diagnostic techniques should provide more clear-cut evidence of infections. However, evaluating the sensitivity of this approach is difficult given the unreliability of other diagnostic criteria. As others have done, we found no significant correlation between PCR results and serological titers, because the IgG response usually occurred 1-2 weeks after the infection of viral or bacterial infection. There is also a possibility that the detection of the *Bartonella* gene is derived from traces of previous infection. Our failure to isolate *B. quintana* from PCR-positive (or any other) individuals was disappointing, given that we adopted a protocol that has been used successfully elsewhere in the world (14). Why we failed is unclear, although it is interesting to note that isolation rates in some parts of the world (e.g., Australia) (10) are significantly higher than elsewhere. Geographic variation in reagent composition may occur, or there may be variation in the intrinsic "culturability" of the bartonellae themselves. From another point of view, targeted homeless people are non-hospitalized and almost none of the people showed typical symptoms of trench fever in our outreach program. Therefore, there is practically no possibility of encountering patients with bacteremia or trench fever in our surveillance.

Only very few physicians understand the current status of trench fever. However, the high IgG antibody titers to *B. quintana* and the presence of *B. quintana* DNA in the blood of the Japanese homeless population suggest that trench fever should be considered in the differential diagnosis of patients in emergency hospitals and even in small clinics, and a specific medical examination should be performed. Moreover, medical staff, sanitary inspectors, health workers and social workers working in health and welfare sections of municipal governments should be aware of the possibility of bartonellosis in the populations they attend to or care for. Further efforts to diagnose and control trench fever should be intensified in local public health offices of the larger cities in Japan.

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