Prevalence Study of Antibody to Ratborne Pathogens and Other Agents among Patients Using a Free Clinic in Downtown Los Angeles

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Norway rats (Rattus norvegicus) are hosts for various microbes. Homeless people who have contact with rats may be at risk of infection by them. The Los Angeles County Department of Health Services initiated a seroepidemiologic study among patients who used a free clinic in downtown Los Angeles; 200 serum specimens obtained for other routine assays were tested for antibodies to ratborne pathogens and other agents. The seroprevalence of antibody to hepatitis E virus in this population was 13.6%; to Bartonella elizabethae, 12.5%; to B. quintana, 9.5%; to B. henselae, 3.5%; to Seoul virus, 0.5%; and to Rickettsia typhi, 0.0%. This study found that patients and locally trapped rats had antibodies to some of the same agents.

Methods

Specimen collection and submission. In June and August 2000, serum specimens obtained for other routine tests were obtained from patients who visited a free clinic in the “skid row” district of downtown Los Angeles. Most of these patients had been homeless during the previous year.

The serum samples were sent from the free clinic to the Los Angeles County Public Health Laboratory (LACPHL). At LACPHL, 3 aliquots were drawn from each specimen and stored at −80°C pending testing. An aliquot of each serum sample was sent to the Hepatitis Viruses Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH; Bethesda, MD) and to Focus Technologies (Cypress, CA) for HEV and hantavirus antibody testing, respectively. Focus Technologies used a pan-hantavirus screening test and sent serum samples that were positive for the virus to the Special Pathogens Branch, Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention (CDC; Atlanta) for SEOV-specific testing. The age and sex of the subjects were the only demographic data available. No clinical data were collected.

Laboratory methods. LACPHL tested the serum samples for BE, B. quintana (BQ), B. henselae (BH), and RT antibodies, using reagents provided by the Viral and Rickettsial Zoonoses Branch of the CDC. An indirect fluorescent antibody method [3] was used to screen for antibody production to these organisms. In brief, 4 antigens, RT, BE, BQ, and BH, were fixed to each slide, and serial dilutions of serum samples were applied to the antigens. The slides were incubated with the test samples, washed to remove unbound

Homeless people may be at risk for certain infectious and chronic diseases as a result of their lifestyle and of other social factors [1]. There have been few studies involving the homeless and their exposure to ratborne and other infectious diseases. Surveys conducted from 1996 through 1998 of Norway rats (Rattus norvegicus) in downtown Los Angeles revealed a 6.7% seroprevalence (8 seropositive rats of 120 rats tested) to Seoul virus (SEOV), a 25.9% seroprevalence (67 of 259) to Rickettsia typhi (RT), and a 73.1% seroprevalence (98 of 134) to hepatitis E virus (HEV) (M.P.R., unpublished data). SEOV and RE are known ratborne pathogens. In a recent study, 2 novel genotypes of Bartonella that were similar to Bartonella elizabethae (BE) were isolated in culture and identified in 45.2% of Norway rats trapped in downtown Los Angeles (19 of 42 rats) [2]. In response to these findings, the Los Angeles County Department of Health Services, Acute Communicable Disease Control Unit, initiated a study to determine the seroprevalence of antibody to ratborne pathogens and other agents among patients being treated at a free clinic in downtown Los Angeles. The clinic serves an area where many homeless people reside and where many of the rats were trapped.

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immunoglobulins, and allowed to dry. The slides were then overlaid with fluorescein isothiocyanate–labeled goat anti–human IgG, incubated, washed, and examined for fluorescence under UV illumination. The highest dilution at which distinct and specific fluorescence was seen was scored as the end-point titer for that serum sample. The serologic cutoff point for RT, BQ, and BH was established at 64, and the serologic cutoff point for a positive result for BE was set at 128.

The NIH tested the serum samples for HEV antibody. The putative capsid gene (open-reading frame 2) of HEV strain Sar-55 was cloned into a baculovirus expression vector, expressed in insect cells, and purified by ion-exchange and gel-filtration chromatography, as described elsewhere [4]. The resultant protein is a 56-kDa truncated form of the capsid protein that consists of aa 112–607 of the full-length protein. The protein was applied to the wells of microtiter plates, and specific antibodies were detected with horseradish peroxidase–labeled anti–human IgG, as described elsewhere [5]. The sensitivity and specificity of the assay were monitored with positive and negative controls that, in turn, were calibrated against a World Health Organization anti-HEV standard obtained from the National Institute for Biological Standards and Control (Hertfordshire, England). Serum samples were tested at a dilution of 100. All samples were tested in 2 separate tests, and serum samples with borderline results were tested at least once more.

The serum samples were screened at Focus Technologies for the presence of IgG and IgM antibodies to hantavirus nucleoproteins, using 2 commercial hantavirus ELISA kits, one for IgM and the other for IgG, developed by Focus Technologies. The serum samples were diluted to 100 and incubated in a microtiter plate containing a cocktail of recombinant hantavirus nucleocapsid proteins. After the addition of goat anti–human IgG Fc or goat anti–human IgM Fc, an index value was obtained. Samples with IgG or IgM values >1.1 were considered to be positive, those with values <0.9 were considered to be negative, and those with values of 0.91–1.09 were considered to be equivocal. Because of the high cross-reactivity of antibody to hantavirus nucleoproteins, IgG or IgM antibodies to hantaviruses pathogenic for humans are typically detected. These ELISA tests do not discriminate among species-specific hantavirus antibodies. Therefore, all positive and equivocal specimens were sent to the Special Pathogens Branch, Division of Viral and Rickettsial Zoonoses, CDC, for confirmation, using methods described by Ksiazek et al. [6] and LeDuc et al. [7].

**Statistical analysis.** Epi Info version 6.0 software (CDC) was used to analyze the frequency of and association between variables. Mantel-Haenszel and Fisher’s exact tests (2-tailed) were used to assess the strength of association ($P < .05$ was considered to be significant) between variables. SAS software (SAS Institute) was used to compare, by $t$ test, the mean age of the patient with respect to presence of antibody.

**Results**

Samples from 200 patients were collected and tested. The ratio of men to women in the study was 3:1, and the median age was 41 years (range, 21–66 years). Most participants (85%) were between the ages of 25 and 54 years. Univariate analysis showed that sex, as a demographic factor, was not significantly associated with any agent ($P > .10$). Patients who had antibody to HEV were significantly older (mean age, 48 years; $P = .005$) than those who did not (mean age, 40 years). This association did not reach significance for the other agents ($P > .10$).

Overall, 31.5% of all specimens (63 of 200) were positive for antibody to at least 1 antigen. Seroprevalence for $\geq 1$ *Bartonella* species was 17.5% (35 of 200 specimens) (table 1). Twenty-seven specimens (13.5%) had antibody to HEV. Two of the specimens tested moderately positive against the hantavirus panel of antigens, and 2 specimens had equivocal results of testing. However, only 1 specimen (0.5%) was confirmed to have antibody to SEOV. None of the specimens had antibody to RT.

The seroprevalence was different for each *Bartonella* species. The rates of antibody were 3.5% (7 of 200 specimens), 9.5% (19 of 200), and 12.5% (25 of 200) for BH, BQ, and BE, respectively. Of those specimens seropositive for BE, 44% (11 of 25) were positive for BQ, and 20% (5 of 25) were positive for BH. Of the specimens that were positive for BQ, 58% (11 of 19) were positive for BE, and 21% (4 of 19) were positive for BH (table 1). In general, as measured by immunofluorescence, titers were low: 35 (68.6%) of the 51 samples that tested positive for any *Bartonella* species had titers that just exceeded the cutoff value, and only 4 samples with antibody to BQ had a titer that was $\geq$4-fold greater than the cutoff value.

The only significant association among different agents was found in specimens with *Bartonella* antibody. Antibody to BE antigen was significantly associated with antibody to BQ and BH antigen ($P < .001$). Specimens with HEV antibody were not significantly more likely to have antibody to any other antigens ($P > .10$), nor was the specimen with SEOV antibody ($P > .10$).

**Discussion**

In this study, we found that people using a free clinic had antibodies to organisms similar to those found in rats trapped in the same area and in a similar relative proportion, although the absolute prevalence in the rats was higher. Studies suggest that *R. norvegicus* is a reservoir host for BE and BE-like organisms [2]. How patients in our study acquired antibody to

**Table 1. Prevalence of antibody to Bartonella species among 200 patients who were treated at a free clinic in downtown Los Angeles.**

<table>
<thead>
<tr>
<th>Bartonella species</th>
<th>No. (%) of patients with antibody to indicated species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any</td>
<td>35 (17.5)</td>
</tr>
<tr>
<td>B. elizabethae only</td>
<td>13 (6.5)</td>
</tr>
<tr>
<td>B. quintana only</td>
<td>8 (4.0)</td>
</tr>
<tr>
<td>B. henselae only</td>
<td>2 (1.0)</td>
</tr>
<tr>
<td>B. elizabethae and B. quintana</td>
<td>7 (3.5)</td>
</tr>
<tr>
<td>B. elizabethae and B. henselae</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>B. quintana and B. henselae</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>B. elizabethae, B. quintana, and B. henselae</td>
<td>4 (2.0)</td>
</tr>
<tr>
<td>None</td>
<td>165 (82.5)</td>
</tr>
</tbody>
</table>

* The serologic cutoff point was a titer of 1:128.
* The serologic cutoff point was a titer of 1:64.
BE is not known, but if rats are reservoir hosts of BE, exposure through living or foraging in rat-infested areas may be a risk factor for humans.

Serum samples from the subjects in our study population were also tested for Bartonella species that are not associated with R. norvegicus. BQ is transmitted by the human body louse and is the etiologic agent of trench fever. Studies conducted in intravenous drug user and homeless populations have found that the seroprevalence to this agent is higher than that in blood donor populations (10%–20% vs. 0%–2%) [8, 9]. Although our study lacked a control group, the seroprevalence of 9.5% to BQ is consistent with the results of other studies. Our data also showed a seroprevalence to BH, the agent of cat scratch disease, similar to that in nonindigent populations [10].

HEV is transmitted through the fecal-oral route and has been implicated in epidemics in developing countries. The HEV seroprevalence we found was similar to the HEV seroprevalence in healthy blood donor populations in Sacramento (CA) and Baltimore, which ranged from 13.7% to 21.3% [11]. In a recent study, HEV-like viruses were recovered from wild rats trapped in Los Angeles and were transmitted via serum and feces to laboratory rats [12]. The zoonotic potential of HEV is under investigation.

Seroprevalence studies of SEOV have been conducted in Baltimore. Two of these studies found that 0.16% of intravenous drug users (1 of 635) and 0.25% of people visiting a sexually transmitted disease clinic (3 of 1180) had antibody to SEOV [13, 14]. The Los Angeles population had a seroprevalence that was slightly higher; however, the sample size of the present study was smaller than that of the Baltimore studies.

None of the patient specimens tested positive for antibody to RT. Cases of murine typhus associated with residence in downtown Los Angeles have been documented (M.P.R., unpublished data); however, the negative findings for RT in our study population suggest that, although evidence of infection in rodent reservoirs exists, the homeless human population is at low risk of exposure to infected fleas.

Sex was not a risk factor for seroprevalence in this population, which suggests that men and women are equally likely to be exposed to these antigens. Increasing age was found to be significantly associated with antibody to HEV antigen, a finding that has been documented in other studies [15, 16]. Neither HEV nor SEOV antibody was significantly associated with BE, BH, or BQ antibody, which may indicate that modes of transmission of these organisms differ. However, BE, BH, and BQ antibody were all significantly associated with each other, particularly BE with BQ and BH. Significant associations between BQ- and BH-positive specimens appeared to be confounded by BE antigen, because there were no specimens that tested positive for BQ and BH only. These associations could be the result of cross-reactivity of antibody to antigen or other factors.

The data presented here raise some questions about health among the homeless population. For example, does antibody to these agents represent clinical disease? Are these infections related to exposure to rats, or are they spread by other means? Do other populations in Los Angeles County have serologic evidence of exposure to these same agents? Additional studies may lead to effective public health interventions. These studies could include a seroprevalence survey of these agents among the nonhomeless population of Los Angeles, a prospective clinical study to look for disease in the homeless caused by these organisms, and interviews with homeless patients to ascertain risk factors for infection.

Understanding the dynamics of disease among homeless people is important. The homeless in an urban setting represent a subset of a larger population that may be less affected by certain diseases. However, the homeless population may serve as a predictor of disease in the general public, should the infrastructure of public health be undermined. Clinicians who treat homeless populations should be aware of the possibility of infection with atypical agents of disease.

Acknowledgments

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References


