Human Pathogens in Body and Head Lice

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Using polymerase chain reaction and sequencing, we investigated the prevalence of Rickettsia prowazekii, Bartonella quintana, and Borrelia recurrentis in 841 body lice collected from various countries. We detected R. prowazekii in body lice from Burundi in 1997 and in lice from Burundi and Rwanda in 2001; B. quintana infections of body lice were widespread. We did not detect B. recurrentis in any lice.

The body louse, Pediculus humanus corporis, is the vector of three human pathogens: Rickettsia prowazekii, the agent of epidemic typhus; Borrelia recurrentis, the agent of relapsing fever; and Bartonella quintana, the agent of trench fever, bacillary angiomatosis, endocarditis, chronic bacteremia, and chronic lymphadenopathy (1). Louse-borne diseases can be associated with high incidence of disease and death, especially epidemic typhus and relapsing fever, which can be fatal in up to 40% of patients (2). The diseases are most prevalent in people living in poverty and overcrowded conditions, for example, homeless people and those involved in war situations (2).

Epidemic typhus, trench fever, and relapsing fever have been the subject of many studies, most of which were conducted between World War I and the 1960s. However, medical interest in the diseases and lice waned for almost 30 years. Since 1995 louse-borne diseases have had a dramatic resurgence, and trench fever has been diagnosed in many countries including the USA (3), Peru (4), France (5), Russia (6), and Burundi (7). In 1997 the largest outbreak of epidemic typhus since World War II occurred in Burundi among refugees displaced by civil war (7). A small outbreak also occurred in Russia (8), and evidence of R. prowazekii infection in Algeria was provided (9).

At the Unité des Rickettsies, we developed a polymerase chain reaction (PCR) assay to survey for human pathogens transmitted by the parasites; the assay can detect as few as 1–20 copies of the DNA of R. prowazekii, B. quintana, and Borrelia recurrentis in body lice (10). In 1995, we found R. prowazekii–positive lice in inmates of a Burundi jail (11), which was the source of a major outbreak of epidemic typhus in the country in 1996 (12). In 1997, we investigated an outbreak of pediculosis in refugee camps in Burundi. We identified R. prowazekii and B. recurrentis in body lice and epidemic typhus and trench fever in refugees (7,10). From April 1997 to December 1998, after our reports, a new strategy was designed to control typhus and trench fever. Health workers treated any patient with fever >38.5°C with a single dose of doxycycline (200 mg), a drug highly effective in the treatment of typhus (7). The program proved extremely successful, and in a follow-up in 1998 (10) we did not detect R. prowazekii in body lice collected in refugee camps in the country (Table 1).

Since 1998, we have continued our efforts and have collected 841 body lice obtained by medical staff from our laboratory or local investigators in Burundi, Rwanda, France, Tunisia, Algeria, Russia, Peru, China, Thailand, Australia, Zimbabwe, and the Netherlands (Table 1). In Burundi, lice were collected during the outbreak of epidemic typhus and on three occasions (1998, 2000, and 2001) after the outbreak had been controlled. Lice found on any part of the body, except the head and pubis, were regarded as body lice. The lice were transported to France in sealed, preservative-free, plastic tubes at room temperature. Delays between collection and analysis ranged from 1 day to 6 months. As negative controls, we used specific pathogen-free laboratory-raised body lice (Pediculus humanus corporis strain Orlando). To prevent contamination problems, as positive controls we used DNA from R. rickettsii R (ATCC VR-891), Bartonella elizabethae F9251 (ATCC 49927), and Borrelia burgdorferi B31 (ATCC 35210), which would react with the primer pairs we used in our PCRs but give sequences distinct from the organisms under investigation. To prevent false-positive reactions from surface contaminants, each louse was immersed for 5 min in a solution of 70% ethanol–0.2% iodine before DNA extraction and then washed for 5 min in sterile distilled water. After each louse was crushed individually in a sterile Eppendorf tube with the tip of a sterile pipette, DNA was extracted by using the QiAamp Tissue Kit (Qiagen, Hilden, Germany), according to the manufacturer’s instructions. This kit was also used to extract DNA from the organisms cultivated in our laboratory under standard conditions to be used as positive controls. The effectiveness of the DNA extraction procedure and the absence of PCR inhibitors were determined by PCR with broad-range 18S rDNA-derived primers (10).

To detect louse-transmitted pathogens, we used each of the genus-specific primer pairs described in Table 2 in a separate assay. A total of 2.5 mL of the extracted DNA was used for DNA amplification as previously described (10). PCRs were carried out in a Peltier Thermal Cycler PTC-200 (MJ Research, Inc., Watertown, MA). PCR products were resolved by electrophoresis in 1% agarose gels. All lice yielded positive PCR products when amplified with the 18S rRNA-derived primers, demonstrating the absence of PCR inhibitors. Negative controls always failed to yield detectable PCR products, whereas positive controls always gave expected PCR products. PCR amplicons were purified by using the QiAquick Spin PCR purification kit (Qiagen) and sequenced using the dRhodamine Terminator cycle-sequencing ready reaction kit (PE Applied Biosystems, Les Ulis, France), according to the
manufacturer's recommendations. Sequences obtained were compared with those in the GenBank DNA database by using the program BLAST (14).

The sequences of the DNA amplicons we obtained were identical to those of \textit{R. prowazekii} and \textit{B. quintana} in GenBank. We detected \textit{R. prowazekii} in body lice collected in Burundi in 2001 but not in those collected in 1998 and 2000, although they were positive for \textit{B. quintana}. \textit{R. prowazekii} was also detected in 7\% of lice collected in Rwanda. We found \textit{B. quintana} in body lice collected in France, the Netherlands, Russia, Burundi, Rwanda, Zimbabwe, and Peru. No PCR products were obtained for any of the lice when primer pair BF1-Br1 was used, indicating lack of infections with \textit{Borrelia recurrentis}.

Our PCR may greatly facilitate the study of lice and louse-borne diseases as it can be used to survey lice for these organisms, detect infected patients, estimate the risk for outbreaks, follow the progress of epidemics, and justify the implementa-

### Table 1. Prevalences of infections in body lice collected in various areas of the world

| Country | Source, yr | Reference$^b$ | No. | Detection$^a$ of |
|---------|------------|---------------|-----|-----------------|
|         |            |               |     | \textit{Rickettsia prowazekii} (no., \%) | \textit{Bartonella quintana} (no., \%) |
| **Body lice** | | | |
| France  | Homeless in Marseille, 1998–2001 | PS$^c$ | 324 | 0 | 32 (9.9\%) |
| France  | Homeless shelter in Marseille, 2000 | (13) | 161 | 0 | 42 (26.1\%) |
| France  | Isolated homeless in Marseille, 1998 | (10) | 75 | 0 | 3 (4.0\%) |
| The Netherlands | Homeless in Utrecht, 2001 | PS | 25 | 0 | 9 (36.0\%) |
| Russia  | Homeless in Moscow, 1998 | (10) | 268 | 0 | 33 (12.3\%) |
| Tunisia | Homeless in Sousse, 2000 | PS | 3 | 0 | 0 |
| Algeria | Homeless in Batna, 2001 | PS | 33 | 0 | 0 |
| Congo   | Refugee camp, 1998 | (10) | 7 | 0 | 0 |
| Burundi | During typhus outbreak | | | | |
|         | Jail, 1997 | (10) | 10 | 2 (20\%) | 0 |
|         | Refugee camp, 1997 | (10) | 63 | 22 (35\%) | 6 (9.5\%) |
|         | After typhus outbreak | | | | |
|         | Refugee camp, 1998 | (10) | 91 | 0 | 13 (14.3\%) |
|         | Refugee camp, 1998 | PS | 38 | 0 | 8 (21.0\%) |
|         | Refugee camp, 2000 | PS | 111 | 0 | 100 (90\%) |
|         | Refugee camp, 2001 | PS | 33 | 7 (21\%) | 31 (93.9\%) |
| Rwanda  | Jail, 2001 | PS | 262 | 19 (7\%) | 6 (2.3\%) |
| Zimbabwe | Homeless in Harare, 1998 | (10) | 12 | 0 | 2 (16.7\%) |
| Australia | Homeless in , 2001 | PS | 2 | 0 | 0 |
| Peru    | Andean rural population | (10) | 73 | 0 | 1 (1.4\%) |
| Peru    | Andean rural population | PS | 10 | 0 | 0 |
| **Head lice** | | | | | |
| France  | Schoolchildren | PS | 20 | 0 | 0 |
| Portugal | Schoolchildren | PS | 20 | 0 | 0 |
| Russia  | Schoolchildren | PS | 10 | 0 | 0 |
| Algeria | Schoolchildren | PS | 18 | 0 | 0 |
| Burundi | Schoolchildren | PS | 20 | 0 | 0 |
| China   | Schoolchildren | PS | 23 | 0 | 0 |
| Thailand | Schoolchildren | PS | 29 | 0 | 0 |
| Australia | Schoolchildren | PS | 3 | 0 | 0 |

$^a$\textit{Borrelia recurrentis} could not be detected in any of the tested lice.

$^b$Data previously reported in the indicated reference.

$^c$PS, present study.
tion of controls to prevent the spread of infections. We have successfully applied the PCR assay to lice from homeless and economically deprived persons in inner cities of developed countries and found high prevalences of Bartonella quintana infections (3,5,6). Furthermore, we have emphasized the risk of R. prowazekii outbreaks in Europe, based on our findings of an outbreak of epidemic typhus in Russia, a case of Brill-Zins- 

Table 2. Oligonucleotide primers used for PCR amplification and sequencinga

| Primer (reference) | Nucleotide sequence | Organism or sequence used | Size of expected PCR product (bp) |
|-------------------|---------------------|---------------------------|----------------------------------|
| CS-877 (10)       | GGG GGC CTG CTC ACC GCG G | Rickettsia species         | 396                              |
| CS-1273 (10)      | ATT GCA AAA AGT ACA GTG AAC A | Rickettsia species         |                                   |
| QHVE1 (10)        | TTC AGA TGA TGA TCC CAA GC | Bartonella species         | 608                              |
| QHVE3 (10)        | AAC ATG TCT GAA TAT ATC TTC | Bartonella species         |                                   |
| Bf1 (10)          | GCT GGC AGT GGC TCT TAA GC | Borrelia species           | 1,356                            |
| Br1 (10)          | GCT TCG GGT ATC CTC AAC TC | Borrelia species           |                                   |
| 18saidg (10)      | TCT GGT TGA TCC TGC CAG TA | Arthropods                 | 1,526                            |
| 18sbi (10)        | GAG TCT CGT TCG TTA TCG GA | Arthropods                 |                                   |

aPCR, polymerase chain reaction.

Humans; the only way to eradicate the organism is to eliminate body lice. We were not able to detect Borrelia recurrentis in any of the lice, which indicates that infection rates with this organism are very low or the agent is restricted to specific geographic zones.

Our study has demonstrated the usefulness of PCR of body lice in ongoing surveillance of louse-associated infections. When faced with outbreaks of body lice or to follow-up outbreaks of louse-borne infections, investigators should consider using PCR for R. prowazekii, Bartonella quintana, and Borrelia recurrentis in body lice collected from the study area and shipped to their laboratories. Our results from Burundi highlight the necessity for using combinations of methods to control body lice and hence R. prowazekii infections.

Dr. Fournier is a physician in the French reference center for the diagnosis and study of rickettsial diseases. His research interests include the physiopathologic, epidemiologic, and clinical features of rickettsioses.

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