Excretion of Living *Borrelia recurrentis* in Feces of Infected Human Body Lice

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Louse-borne relapsing fever (LBRF), caused by *Borrelia recurrentis*, is 1 of the most dangerous arthropod-borne diseases. Infection is thought to occur through louse crushing. Lice feces have not been shown to contain living borreliae. We infected 800 body lice by feeding them on a rabbit made spirochetemic by the injection of $2 \times 10^6$ borreliae. The life span of infected lice was not shortened. Once infected, lice remained infected for life but did not transmit borreliae to their progeny or to nurse rabbits. *B. recurrentis* infection was observed throughout lice and spread into hemolymph on day 5 after infection. We describe 2 unprecedented phenomena. In hemolymph, *B. recurrentis* formed clumps of aggregated borreliae. Using immunofluorescence assay, transmission electron microscopy, and culture, we detected borreliae excreted in lice feces beginning on day 14 after infection. We conclude that, similar to epidemic typhus and trench fever, transmission of LBRF may be caused by lice feces.

*Borrelia recurrentis*, a slender microaerophilic spirochete, is the etiologic agent of louse-borne relapsing fever (LBRF; also known as “epidemic relapsing fever”). This disease can cause large outbreaks under certain conditions. In the 20th century, at least 7 major epidemics occurred. The latest large outbreak (~10 million cases) occurred during and immediately after World War II [1]. After World War II, the incidence of LBRF decreased dramatically, disappearing in all areas except the Andean foothills and the highlands of central and eastern Africa—mainly Ethiopia [2–4], southern Sudan [2, 3, 5, 6], and Rwanda [7]. Nonetheless, louse-borne infections remain prevalent and occasionally reemerge, often in association with a decline in social and hygienic conditions provoked by civil unrest, economic instability, or war [8]. In developed countries, homeless populations are especially at risk [9]. The mortality rate of LBRF varies widely and may reach 80% in untreated cases. With appropriate treatment, the mortality rate is 5% [1]. Tetracyclines and penicillins are very effective but may, within 2 h, induce a severe Jarisch-Herxheimer reaction, which can be fatal [10, 11]. A major virulence factor of *B. recurrentis* is the organism’s ability to undergo antigenic variation. This occurs as a result of a genetic rearrangement whereby silent genes are moved to an expression locus [12]. Thus, as the immune response of the host develops against 1 antigenic type, another antigenic type appears. This effectively impairs the host’s ability to clear the infection, and the recurring relapses of fever correspond to the emergence of new antigenic types [13]. LBRF is characterized clinically by recurrent episodes of fever and spirochetemia [1, 14–16], both of which favor the body-louse infection with *B. recurrentis*.

Mackie, in 1907 [17], was the first to identify the human body louse, *Pediculus humanus humanus*, as the natural vector of LBRF. At present, it is believed that, when lice feed on infected blood, *B. recurrentis* passes from the gut to the hemolymph, where it multiplies [18]. The louse remains infected throughout its life span but cannot transmit borreliae to its progeny [8, 18, 19]. It has been reported that *B. recurrentis* is neither ex-
creted in lice feces nor present in their saliva [18, 20]. Therefore, it was believed that the only mode of human infection was autoinoculation by scratching into the skin the contents of a crushed, infected louse [1, 21]. These observations have been subject to debate. Mackie [17] reported systemic infection of the louse, whereas Nicolle et al. [20, 21] reported the infection of hemolymph only, the presence of negative phases when borreliae were not detectable, and the occurrence of vertical (or transovarial) transmission [21]. These studies were performed at a time when B. recurrentis could not be grown in axenic medium.

Our objective was to investigate the localization of B. recurrentis infection in the body louse by use of Barbour-Stoenner-Kelly (BSK-H) complete culture medium, which was used by Cutler et al. in 1994 [22] for the isolation of B. recurrentis. We used an experimental model of louse infection that reproduces the natural route of infection. This model has been used previously for Bartonella quintana [23] and Rickettsia prowazekii [24].

**MATERIALS AND METHODS**

**B. recurrentis isolate.** B. recurrentis strain A1 (ATCC 700241), which was isolated from the blood of an Ethiopian patient, was subcultured in vitro at 32°C in 100 mL of BSK-H medium (Sigma) [22]. The concentration of spirochetes was determined visually by counting the number of borreliae present in 10 μL of culture suspension under a dark-field microscope (Nikon). Numbers were assessed on day 7 after inoculation and then daily until day 10, when the optimal concentration of 1 × 10^7 borreliae/mL was reached. Thirty milliliters of BSK-H medium was centrifuged at 5283 g for 10 min, and the resulting pellet was re-suspended in PBS to obtain a final titer of 10^8 borreliae/mL.

**Infection of body louse with B. recurrentis.** A group of 400 15-day-old human body lice (P. humanus humanus, strain Orlando) [24] were infected by feeding on a specific pathogen–free (SPF) rabbit (R1) made bacteremic by an auricular intravenous injection infused over the course of 15 min that consisted of 20 mL of PBS containing 2 × 10^6 borreliae/mL. The day of infection was referred to as day 0. On subsequent days, infected lice were fed once on another SPF rabbit (R2). To determine whether B. recurrentis would influence lice mortality, 400 15-day-old B. recurrentis–free lice were used as negative controls and were fed daily on a third SPF rabbit (R3). Each lice population was maintained in a separate plastic container at 29°C and 70%–90% humidity [25]. The experiment was repeated once with fresh lice and rabbits. Each rabbit was maintained in an individual cage. The animal study was approved by the Animal Ethics Committee, Marseille School of Medicine, Marseille, France.

**Study of rabbit infection.** For each experiment, 200 μL of blood was drawn from the infected rabbit (R1) immediately, 30 min, and 1, 4, 22, 27, 43, and 68 h after infection and then was assayed for borreliae via polymerase chain reaction (PCR). PCR was performed by use of the B. recurrentis–specific primers glpQF (5′-TCACCTAGGCTTATGGA-3′) and glpQR (5′-TAGACCATCTACTTATTGCT-3′) that amplify a 605-bp fragment of the glpQ gene, which encodes the glycerolphosphodiester phosphodiesterase enzyme. Primers were determined from the glpQ gene sequences of B. recurrentis (GenBank accession nos. AF247152, AF247153, AF247154, and AF247155) [6]. PCRs were performed for 44 cycles with a Peltier model PTC-200 thermocycler (MJ Research). Each cycle consisted of denaturation at 95°C for 30 s, annealing at 52°C for 30 s, and extension at 68°C for 90 s. After 44 cycles, an additional 7-min extension was done at 68°C. DNA was extracted by use of the MagNA Pure LC DNA Isolation Kit III (for bacteria and fungi; Roche), in accordance with the manufacturer’s instructions. DNA of B. recurrentis in our inoculum was used as a positive control for PCR amplification, and DNA extracted from uninfected rabbit blood was used as a negative control. PCR by use of β-globin–specific primers was used to monitor the efficiency of DNA extraction and PCR, as described elsewhere [26]. Weekly, 4 drops of blood were collected onto blotting paper (Fisher Scientific) from each of the 6 rabbits by auricular puncture; this was used to detect antibodies to B. recurrentis by immunofluorescence assay (IFA), as described elsewhere [27].

**Study of lice infection.** Daily, the number of surviving and dead lice in both the infected and uninfected populations was noted, and 1 surviving louse from each population was killed immediately after feeding, for use in IFA (table 1). Each louse was fixed in absolute ethanol at 4°C for 3 weeks, to increase the fixation efficiency, and embedded in paraffin. IFA was performed on 5-μm-thick sections by use of 30 μL of mouse anti–B. recurrentis strain A1 polyclonal antibody (PAb) diluted to a concentration of 1:250 in PBS with 3% (wt/vol) nonfat dry milk. Sections of uninfected lice were used as negative controls.

**Infection of lice hemolymph.** Another 5 surviving infected lice were killed daily immediately after feeding, for hemolymph extraction: 1 for IFA, 1 for scanning electron microscopy (SEM), and 3 for transmission electron microscopy (TEM) (table 1). Hemolymph was extracted by amputation of the distal segment of ≥1 leg under an enlarge-scope (Stemi 2000-C; Zeiss). Although hemolymph coagulates rapidly, we preferred to fix the slides for 5 min in absolute methanol after air-drying. Hemolymph extracted from 10 uninfected lice was used as a negative control. For SEM, glass coverslips containing extracted hemolymph were air-dried, fixed for 3 min in methanol and for 20 min in 2% glutaraldehyde, and washed in sterile water. The coverslips were coated with gold and examined with a QUANTA 200 electron microscope (FEI). Hemolymph from 2 uninfected lice was used as a negative control. TEM was per-
Table 1. Summary of the samples tested, no. of each sample tested by method, methods used, and results of the 2 louse populations for the 2 experiments performed.

<table>
<thead>
<tr>
<th>Sample, examination methods (sample no.: infected/uninfected)</th>
<th>First experiment</th>
<th>Second experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected population</td>
<td>Uninfected population</td>
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<tr>
<td>Lice</td>
<td></td>
<td></td>
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<tr>
<td>Survival rate</td>
<td>35 days</td>
<td>40 days</td>
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<tr>
<td>IFA (1/1 daily)</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Lice hemolymph</td>
<td>Days 0–4, negative; days 5–13, positive (days 5–10, individual borreliae; days 11–13, clumps of borreliae); days 14 and 15, negative; days 16–35, positive (days 16 and 17, individual borreliae; days 18–35, clumps of borreliae)</td>
<td>Negative</td>
</tr>
<tr>
<td>IFA (1 daily/10 whole experiment), SEM (1 daily/2 whole experiment), and TEM (3 daily/2 whole experiment)</td>
<td></td>
<td></td>
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<tr>
<td>Lice feces</td>
<td>Days 0–13, negative; days 14–22, positive; days 23–28, negative; and days 29–35, positive</td>
<td>Negative</td>
</tr>
<tr>
<td>IFA and TEM (daily)</td>
<td></td>
<td></td>
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<tr>
<td>Culture (daily)</td>
<td>Days 0–13, negative; days 14 and 15, positive; days 16–35, negative</td>
<td>Negative</td>
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<tr>
<td>Progeny</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eggs</td>
<td>6750</td>
<td>8778</td>
</tr>
<tr>
<td>glpQ PCR (1 daily)</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Culture (1 daily)</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Larvae</td>
<td></td>
<td></td>
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<tr>
<td>glpQ PCR (1 daily)</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Culture (1 daily)</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Feces IFA</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

NOTE. IFA, immunofluorescence assay; PCR, polymerase chain reaction; SEM, scanning electron microscopy; TEM, transmission electron microscopy.
formed as described elsewhere [24], with 1 min of centrifugation at 12,000 g after each step. However, ultracutmicrotomy (Reichert-Leica) was only possible for inclusion pellets on days 1, 5, 7, and 20 after infection.

Infection of lice progeny. The number of eggs produced by lice from both the infected and uninfected populations was recorded daily (table 1). Each day after infection, 2 eggs (starting from the laying of the first egg) and 2 larvae (starting from the hatching of the first egg) were sampled: 1 of each for PCR (performed as described above) and for culture. DNA extracted from uninfected eggs and larvae was used as a negative control. PCR with the 18S rRNA–derived primers 18Saidg and 18Sbi was used as a control for the efficiency of DNA extraction and PCR [24]. Before culture, the surface of the lice progeny was decontaminated, as described elsewhere [28], and used to inoculate 10 mL of BSK-H medium. Culture tubes were incubated under loose lids at 32°C and were monitored daily, under a dark-field microscope, on 40 μL of microaerophilic medium. Larvae feces were tested daily by use of IFA.

Infection of lice feces. Daily until day 20 after infection, ~0.2 mg of feces was sampled for IFA, TEM, and culture (table 1). Starting on day 21 after infection, the excreted feces were divided equally into 3 parts for IFA, TEM, and culture. For IFA, lice feces were sampled with a sterile cotton swab moistened with sterile water and were resuspended in 100 μL of PBS. Then, 30 μL were air-dried at room temperature on the 18-well microscope slide and were used to perform IFA. For TEM and culture, lice feces were sampled with the point of a needle (18 gauge [1.2 mm]; Terumo). The fecal excretion of B. recurrentis was first tested by IFA by use of 30 μL of anti–B. recurrentis mouse PAb diluted to 1:250. IFA was also performed on uninfected lice feces as a negative control. TEM was performed as described elsewhere [24]. For culture, each sample was suspended in 4 mL of BSK-H medium. Two milliliters were used to inoculate 10 mL of BSK-H medium; however, coagulase-negative Staphylococcus contamination occurred in all cultures. In subsequent experiments, feces were decontaminated by filtration. The selectivity of the filtration method was demonstrated by recovering B. recurrentis after filtration of 2 mL of pure culture through 0.22-μm filters (Millipore). Later, 2 mL that contained ~0.1 mg of feces suspension was filtered through 0.22-μm filters. After inoculation, the cultures were monitored daily under a dark-field microscope.

Statistical analyses. The number of dead lice in the infected and uninfected populations and their fertility were compared by use of Student’s t test in Epi Info software (version 6.0; US Centers for Disease Control and Prevention).

RESULTS

Effect of infection on rabbits. In both experiments, glpQ PCR was positive on DNA extracted from blood sampled imme-

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Figure 1. Comparison of survival rate over time of lice infected with Borrelia recurrentis and uninfected lice. Day 0 is the day of infection. Values are given as percentages of nos. of surviving lice, not killed for the purposes of testing.
ate, 30 min, and 1 h after infection of infected rabbit R1. glpQ PCR was negative at 4, 22, 27, 43, and 68 h after infection. β-globin PCR was always positive, which demonstrates the efficiency of DNA extraction and PCR. The infected rabbit (R1) did not show any signs of illness but developed an anti–B. recurrentis immunoglobulin response (IgG plus IgM) with an antibody titer of 1:25 on day 14 after infection that, 6 months after infection, increased to 1:1600. The nurse rabbits (R2 and R3), which were used to feed infected and uninfected lice, respectively, remained asymptomatic and seronegative.

**Effect of infection on lice and their progeny.** From day 0 to 35 (the day of death of the last infected louse), the louse mortality rate was similarly low in both the infected and uninfected population (figure 1 and table 1). The survival of infected lice (35 days) was comparable to that of uninfected lice (40 days) (P = .19). Throughout the experiment, all surviving and dead lice in both populations were physically indistinguishable. On day 2 after infection, both infected and uninfected lice began laying eggs that hatched 11 days later. During their life span, the total number of eggs laid by infected and uninfected surviving lice, not killed for testing purposes, for each experiment was 6750 and 8778 eggs, respectively, for the first experiment and 7122 and 8480 eggs, respectively, for the second. For each experiment, the average number of eggs laid daily per louse was not statistically different between infected and uninfected lice (first experiment, 1.52 and 1.6 eggs/day/louse, respectively, P = .46; second experiment, 1.67 and 1.69 eggs/day/louse, respectively, P = .86).

**Detection of B. recurrentis in lice and their progeny.** By use of IFA (figure 2), B. recurrentis was detected daily throughout the body of each of the 68 surviving infected lice starting on day 1 after infection (table 1). glpQ PCR and culture were negative when the progeny of infected (66 eggs and 46 larvae) and uninfected (76 eggs and 56 larvae) lice were tested. 18S rRNA-PCR was always positive, which demonstrates the efficiency of the DNA extraction and PCR. IFA performed on the feces of larvae descended from infected lice was always negative.

**Detection of B. recurrentis in lice hemolymph.** During the first 4 days after infection, extracted hemolymph was always negative by IFA (table 1). On days 5–13 after infection, IFA was positive. Only individual (nonaggregated) borreliae (figure 3A) were observed on days 5–10 after infection. Clumps of aggregated borreliae (figure 3B) and rare separate borreliae were observed on days 11–13 after infection. On days 14 and 15 after infection, we noted an eclipse, and IFA was negative. On days 16–35 after infection, IFA was positive. Individual borreliae were observed on days 16 and 17 after infection, and clumps were seen on days 18–35 after infection. When SEM was used, B. recurrentis was detected in hemolymph starting 5 days after infection that exhibited the same kinetics as those described for IFA. Only individual borreliae (figure 4A) were observed on days 5–10 and 16 and 17 after infection, and clumps were observed on days 11–13 and 18–35 after infection. No borreliae were observed during the first 4 days after infection and on days 14 and 15 after infection. When TEM was used, hemolymph extracted on day 1 after infection was negative. Only individual borreliae were observed on day 5 after infection (figure 4B). On day 20 after infection, clumps formed of several borreliae (3, 8, 9, 14, or 22 organisms) were observed in the same location without any cell-to-cell contact (figure 4C). No borreliae were detected within uninfected louse hemolymph by use of any of our methods.

**Detection of B. recurrentis in lice feces.** During the first 13 days after infection, infected lice feces were always negative by IFA (table 1). On days 14–22 and 29–35 after infection, B. recurrentis was detected in excreted feces (figure 5). On days 23–28 after infection, no borreliae were detected by IFA. Uninfected lice feces, which were used as a negative control, were always negative by IFA. When TEM was used, B. recurrentis was detected in all 16 IFA-positive feces samples, and no other cellular structure was observed. In both experiments, on days 14 and 15 after infection, an unknown number of motile B. recurrentis was detected 5 days after cultivation under dark-field microscope from lice feces after filtration on a 0.22-μm filter.
DISCUSSION

We validated our experimental infection model. Despite an inoculum of $2 \times 10^6$ borreliae, infected rabbits did not become sick. This confirmed previously reported studies that used rabbits and other laboratory animals (guinea pigs, rats, and hamsters) [21, 29]. Infected rabbits had spirochetemia for only 1 h after experimental infection and remained asymptomatic throughout the experiment but seroconverted, with an antibody titer of 1:1600, 6 months after infection. Both rabbits used to feed infected lice remained asymptomatic and seronegative. In our experiment, the mortality rate of infected lice was low (figure 1) and was not statistically different from that of control lice, which demonstrates that *B. recurrentis* was not pathogenic for its vector. A single infected blood meal was enough to infect lice for their entire life span. From the first days after infection, *B. recurrentis* was apparent throughout each louse (figure 2), as has been reported elsewhere [17, 30]. The fast spread of *B. recurrentis* may be explained by its motility [14, 31, 32]. Until
Figure 4. Scanning (A) and transmission (B and C) electron microscopy detection of *Borrelia recurrentis* (arrow) in hemolymph extracted from surviving infected lice. Only individual borreliae were observed on day 5 after infection (A and B). Clumps of aggregated borreliae were observed on day 20 after infection (C). For each micrograph, the magnification is indicated by the scale bar.

they died, infected lice fed regularly, mated, and continued to lay uninfected eggs, as has been reported elsewhere [18, 19]. The number of eggs laid by infected lice was 20% lower than that laid by uninfected lice. The difference in the daily sampling between the 2 louse populations (table 1) might explain the observed differences—either in the total number of the laid eggs or in the survival of lice—starting from day 11 after infection (figure 1). Although the difference in the number of eggs laid per day per louse was not significantly different, it remains difficult to conclude that there was no difference between the 2 louse populations, given that we did not know the exact number of females in each population.

On days 14–35 after infection, except on days 23–28, infected lice excreted borreliae in their feces, as demonstrated by IFA (figure 5) and TEM. Chung and Feng [18] reported the excretion of occasional dead borreliae in lice feces during the first 6 h after an infective meal. We have demonstrated by culture, for the first time, that *B. recurrentis* is viable in lice feces. It could therefore infect humans through a bite wound, abraded or intact skin [8], or conjunctiva or other mucosa through aerosolization, as is already known for the 2 other louse-transmitted human pathogens, *R. prowazekii* [24, 33] and *B. quintana* [34]. In the literature, many cases of LBRF reported in lice-free patients support this hypothesis. Mackie [17], in a study of an LBRF outbreak in Bombay, India, reported the infection of every casual visitor and attendant from outside who spent time in the wards of boys (who were heavily infested with lice). The 15 girls (who were completely free of lice at the outbreak’s beginning) who had contracted the disease had assisted as nurses or attendants in the boys’ wards [17]. Foley and Sergent (summarized in [21]) reported the infection of 2 people who used blankets borrowed from infested patients. In a study of an epidemic in 1912 in Tunis, Nicolle et al. [21] reported cases of LBRF in 2 laboratory contaminations, 2 bath employees, 2 nurses, 1 prison guard, and 1 laundry woman. Bousfield (summarized in [21]), who observed LBRF in Khar-

Figure 5. Detection of *Borrelia recurrentis* in infected lice feces sampled on day 14 after infection (immunofluorescence staining, confocal microscopy; original magnification, ×600).
toum, Sudan, reported 1 infection of an employee who handled patients’ linens. All of these data are in favor of LBRF transmission by lice feces. Moreover, infection from crushing lice may not explain the rapid and widespread contamination during epidemics, as was previously noted by Nicolle et al. [21]—according to the transmission mode assumed at present, a single louse can infect only a single person (because it is dead after being crushed). In our study, that the nurse rabbit of the infected lice did not seroconvert could be explained by the fact that there was no exposure to the lice feces—the rabbit’s abdomen was immediately cleaned by sterile water and alcohol after the lice feeding.

The ingested borreliae crossed the epithelium into the louse hemolymph on day 5 after infection (figures 3A, 4A, and 4B), and, except on days 14 and 15 after infection, *B. recurrentis* was present in hemolymph. The passage of *B. recurrentis* occurred without damage to the digestive epithelium. This explains the low mortality of infected lice. Heisch et al. [30] reported that fair numbers of *B. recurrentis* were first observed on day 4 after infection in the hemolymph of lice that had been infected by feeding on a patient with spirochetemia. Starting on day 11 after infection, clumps consisting of several (3–22) borreliae were observed (figures 3B and 4C). In the hemolymph of naturally infected lice, Heisch et al. [30] reported the presence of curious yeastlike organisms that did not interfere with the multiplication of *B. recurrentis*. Moreover, *B. recurrentis* has been reported to change shape, depending on its environment (i.e., lice hemolymph, human blood, or blood of newborn laboratory rats) [29]. In the hemolymph of intrarectally infected lice, it demonstrated regular multiplication; however, in the blood of patients or newborn rats, it was separate or formed cordonnets [29]. The formation of clumps may be either a survival mechanism of *B. recurrentis* to escape or overcome unfavorable conditions, which prevail during infection (e.g., louse digestive enzymes, tissue barriers, and immune response), or an ongoing process of spirochetal destruction. On day 14 after infection, borreliae, which were always present in the digestive tract, disappeared from hemolymph and appeared in excreted feces. The disappearance of *B. recurrentis* from the hemolymph might be the result of the louse immune response. On day 16 after infection, *B. recurrentis* reappeared in hemolymph, where it persisted, multiplied, and exhibited clumps starting 18 days after infection. The reappearance of *B. recurrentis* in the hemolymph could be due to antigenic variations [12].

In conclusion, we have demonstrated infected lice feces to be a potential source of human infection during LBRF epidemics, because they harbor viable *B. recurrentis*. Moreover, we show that, in louse hemolymph, *B. recurrentis* exhibits clumps and an eclipse of 2 days.

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**References**


