An Experimental Model of Human Body Louse Infection with *Rickettsia prowazekii*

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*Rickettsia prowazekii* is transmitted to humans by the body louse. A new experimental model of body louse infection with *R. prowazekii* is reported here. Eight hundred human lice were infected by feeding on a rabbit that was made bacteremic by injecting $2 \times 10^6$ plaque-forming units of *R. prowazekii*. The bacterium invaded the stomach cells and was released in feces, in which it was detected 5 days after infection. At day 6 after infection, as a result of the cell burst and the spread of erythrocytes in the hemolymph, the louse became bright red and died within 4 h. The life span of infected lice was shortened by 20–23 days, compared with that of uninfected control lice. Infected lice did not transmit *R. prowazekii* to their progeny. Through cell culture, rickettsiae were cultivated from fecal samples up to 10 days after their emission. The administration of doxycycline to the rabbit during louse feeding did not cure lice from *R. prowazekii* infection.

Of the 3000 louse species described, 3 are strictly human parasites: the clothing or body louse, *Pediculus humanus*; the head louse, *Pediculus humanus capitis*; and the crab or pubic louse, *Pthirius pubis*. Only body lice are known to transmit human diseases. They transmit 3 pathogenic bacteria: *Borrelia recurrentis*, the agent of relapsing fever; *Bartonella quintana*, the agent of bacillary angiomatosis, chronic bacteremia, trench fever, endocarditis, and chronic lymphadenopathy; and *Rickettsia prowazekii*, the agent of epidemic typhus [1]. A recent study revealed a possible association with 2 other bacteria, *Serratia marcescens* and *Acinetobacter* species [2].

*R. prowazekii* is a short, gram-negative intracellular rod that retains basic fuchsin when stained with the Gimenez method [3]. It has a genome of 1,111,523 bp [4] and belongs to the alpha subgroup of *Proteobacteria*. Humans, as well as the eastern flying squirrel *Glaucomys volans* and the west flying squirrel *Glaucomys volans volans* in the United States, are the known reservoir of *R. prowazekii* [5, 6]. Epidemic typhus is associated with cold weather and lack of hygiene [7–9] and has reemerged as sporadic cases in louse-infested populations in industrial countries [10]. Recent cases have been reported in Russia [11, 12], United States [13], Peru [14], and northern Africa [15], and some imported cases and cases of Brill-Zinsser disease, a recurrent and milder form of typhus, also were found [16–19]. A huge outbreak also was reported in central Africa [20, 21]. *R. prowazekii* is transmitted to humans by contamination of the bite site with louse feces containing rickettsiae [22] or by contamination of conjunctivae or mucus membranes with the crushed bodies or feces of infected lice [1]. A single oral dose of 200 mg of doxycycline can save patients suffering from typhus [20, 23], but they may retain some rickettsiae for the rest of their lives and, under certain stressful conditions, may relapse and develop Brill-Zinsser disease [16, 17, 19].

Several experimental models of body louse infestation have been described in the literature, either based on natural feeding on infected humans [24, 25] or on various infected animals [22, 26], or artificially by using membrane (cadaver [27] or chick skin [28–30]) feeding, bleb feeding on rabbit skin [27], intravenous injection of rabbit [27], or by louse intrarectal injection [31, 32]. These experiments have shown that the body louse acquires *R. prowazekii* after feeding on an infected host as part of a blood meal [26] and that this bacterium infects the epithelial cells of the louse’s upper gut [32]. Because of its excessive growth, infected epithelial cells enlarge and eventually burst, releasing the rickettsiae into the gut lumen. After the rupture of the digestive epithelium, the blood passes into the hemolymph, and the louse becomes red [24]. Massive quantities of rickettsiae are discharged in the feces where they remain infectious [22, 24]. Our objective was to develop an efficient experimental model of body louse infection with *R. prowazekii*, to study, using modern tools, the relationships between *R. prowazekii* and its vector, and to examine the effect of a single dose of 200 mg of doxycycline administered to the host after louse infection. We adapted the experimental model of body louse infestation with *Bartonella quintana*, which was developed in our laboratory [33], to *R. prowazekii*. 

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Materials and Methods

Bacteria. R. prowazekii, strain Breinl (ATCC VR-142) [34], was propagated on mouse fibroblast monolayers, strain L 929 (European Collection of Cell Cultures; cell culture no. 85011425) in MEM supplemented with 2% fetal bovine serum and 2% L-glutamine (M22 medium; Life Technologies). Ten flasks of 150 cm² (Falcon, Becton Dickinson) were infected and incubated overnight at 37°C with 5% CO₂ and then were incubated for 6 days at 35°C without CO₂. Infection of L929 cells was monitored by means of Gimenez staining [3]. Day 7 after inoculation, R. prowazekii was harvested by use of sterile glass beads. L929 cells were sonicated, and their fragments were eliminated by means of centrifugation at 94 g for 10 min. The supernatant from the 10 flasks was centrifuged for 10 min at 5283 g, and the pellet containing R. prowazekii was resuspended in 20 mL of K36 buffer. This suspension was immediately used to infect an uninfected rabbit (total of 4 uninfected rabbits infected; referred to as group R1). The final titer was estimated to be 10⁷ pfu/mL of R. prowazekii, as described elsewhere [35].

Human body lice. Body lice (P. h. corporis, strain Orlando) were kindly provided by Richard-Lenoble (Laboratoire de Parasitologie, Faculté de Médecine, Tours, France). A colony of lice from the parent colony, which was collected from healthy individuals in Orlando, Florida, in 1942 and reared since that date by feeding on humans [36] until 1945 and then on rabbits [37, 38], was established on rabbits in our laboratory. Lice were shown to be free from R. prowazekii by use of polymerase chain reaction (PCR) amplification, using the citrate synthase (gltA)-derived primers CS877F and CS1258R, as described elsewhere [39], and the extraction effectiveness was assessed by use of PCR incorporating the 18S rRNA-derived primers 18Saidg [2] and 18Sbi [40]. Lice fed once daily on the shaved abdomen of 2 specific pathogen-free New Zealand white rabbits that were alternately used in a controlled, arthropod-free University Animal Facility. Before louse feeding, rabbits were anesthetized with an intramuscular injection of 17 mg of chlorpromazine and 67 mg of ketamine chlorhydrate. Once fed, lice were kept on a tissue piece in an incubator at 29°C with 70%–90% humidity [41].

Experimental model of body louse infection with R. prowazekii. One uninfected rabbit in group R1 was infected by an intraveneous auralcian injection with 20 mL of a suspension of 10⁷ pfu/mL of R. prowazekii for 15 min by means of an electric syringe, to obtain a persistent artificial bacteremia. A series of 200 15-day-old lice were fed at the same time on the abdomen of this rabbit until 10 min after the end of the injection. The day of infection was referred to as day 0. In the following days, infected lice were fed every day for 25 min on 2 alternately used uninfected rabbits (2 from group R2 and 2 from group R3). A series of 200 15-day-old R. prowazekii-free lice were used as controls and were alternately fed for 25 min on 2 other uninfected rabbits (2 from group R4 and 2 from group R5). In total, 400 lice were used for this experiment. To estimate the effect of 200 mg of doxycycline on louse infection, 55 of the 200 infected lice, sampled 3 days after infection, were fed for 25 min on the abdomen of another uninfected rabbit (from group R6). Five minutes after the beginning of louse feeding, this rabbit received an intravenous auralcian administration of 1 mL of doxycycline (200 mg/mL) for 15 min. The number and color of these lice were noted daily. The whole experiment was repeated 4 times in a biosafety level 3 laboratory. For each experiment, different lice and rabbits were used, and each rabbit was kept in an individual cage. In total, 24 rabbits, 800 uninfected control lice, and 800 infected lice, from which 220 were used in the 4 doxycycline experiments, were used.

Sampling strategy: In each of the 4 experiments, we noted daily the number and the color of live and dead lice in both the infected and uninfected groups. One live and one dead lice from both populations were sampled daily by use of PCR amplification. On day 6 after infection, 5 red lice were sampled: 3 were tested by shell vial culture, and 2 were tested by immunofluorescence. To estimate the evolution of digestive cell infection, 2 live lice from both populations were sampled at days 0, 5, and 6 after infection, one by immunohistochemistry and the other by electron microscopy. The number of eggs in both groups was noted daily. Every day, 2 eggs (beginning from the laying of the first egg) and 2 larvae (beginning from the hatching of the first egg) were sampled, one of each by PCR amplification and the other by cell culture. To evaluate the kinetics of excretion of R. prowazekii in louse feces, ~0.4 mg of feces was sampled daily from day 0 until the last infected louse died, with a sterile cotton swab moisturized with sterile water; half was used for PCR testing, and the other half was used for immunofluorescence staining. In addition, to estimate the survival of R. prowazekii in feces, we collected on day 6 after infection the feces from infected lice and stored them at 29°C. We tested daily 0.2 mg of these stored feces by means of shell vial culture.

Serologic analysis. Four drops of rabbit blood, which were obtained by puncture of the ear, were sampled weekly on blotting paper (Fischer Scientific) [42]. Immunofluorescence assay was used to detect antibodies to R. prowazekii in the rabbit blood, as described elsewhere [43].

PCR amplification and sequencing method. In each of the 4 experiments, 1 mL of blood was drawn immediately on EDTA from the infected rabbits and 2, 4, 6, 12, 24, 48, and 72 h after injection of R. prowazekii for DNA extraction. DNA was extracted from 200 µL of EDTA-blood, crushed lice, eggs, larvae, and feces by use of the QIAamp Tissue kit (Qiagen), according to the manufacturer’s directions. DNA was eluted in 100 µL of elution buffer AE. The gltA gene was amplified from all specimens and was sequenced by use of primers CS877F and CS1258R, as described elsewhere [39]. Obtained sequences were compared with DNA sequences in GenBank (accession no. U59715; NCBI). The extraction effectiveness and the absence of PCR inhibitors were assessed by use of PCR incorporating the 18S rRNA-derived primers 18Saidg [2] and 18Sbi [40]. The gltA amplicon was sequenced by GenBank. PCR sequencing was used to detect antibodies to R. prowazekii in the rabbit blood, as described elsewhere [43].

PCR inhibition role of louse feces was estimated by quantitative real-time PCR using the LightCycler thermocycler (Roche Diagnostics GmbH), which compared the number of R. prowazekii–DNA copies detected with or without uninfected louse feces. DNA amplification was performed by use of gltA-derived primers CS877F and CS1258R [39] and the LightCycler–FastStart DNA Master SYBER Green I kit (Roche Diagnostics GmbH), according to the manufacturer’s instructions. Real-time PCR was conducted on various amounts of R. prowazekii DNA (40, 80, 120, 200, 400, 1640 Houhamdi et al. JID 2002;186 (1 December)
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Electron microscopy. Because of the louse’s hard shell, it was impossible to cut the whole louse by using Ultracut microtome (Reichert-Leica). Therefore, louse gut was extracted manually after excision of the terminal abdominal segment; the abdominal contents were squeezed into 2.75% glutaraldehyde by immobilizing the thorax and compressing the abdomen from the thorax posteriorly with a needle under an stereo microscope (Zeiss, Stemi 2000-C; Jena). Fixed guts were postfixed for 1 h at room temperature in an acetone/epon mix (Euromedex), overnight in epon (Euromedex), and 3 days at 60°C and embedded in epon. Thin sections were cut from embedded blocks with the Ultracut microtome and were stained with uranyl acetate (Euromedex) and lead nitrate (Coger) with sodium citrate (Coger) in water. Samples were deposited on copper grids (Jose Delville) and were examined with JEOL 1220 electron microscope (Jeol).

Statistical analyses. The number of dead lice in the infected and control groups was compared by use of the Student’s t-test. Fisher’s exact test was used to compare the fertility of both louse populations. These 2 statistical tests were used by means of Epi Info software (version 6.0; Centers for Disease Control and Prevention).

Results

Effect of infection on rabbits. Blood drawn from the 4 infected rabbits (group R1) was positive for R. prowazekii by PCR the first 2 days after infection and negative on day 3. These rabbits did not present any abnormal symptom but developed an anti-R. prowazekii immunoglobulin response (IgG and IgM) with an antibody titer of 1:400 at day 15 after infection and 1:800 on day 22. Blood drawn from the other 20 rabbits (4 in group R2, 4 in group R3, 4 in group R4, 4 in group R5, and 4 in group R6 in each of the 4 experiments) was negative by PCR, and their serologic results remained negative.

Effect of infection on lice. The first 5 days after infection, the mortality rate was low in both infected and control lice (figure 1). On day 6, 300 (37.5%) of the 800 infected lice (range, 73–77 infected lice [36.5%–38.5%], depending on the experiment) was shorter than that of mean of the 800 uninfected lice (mean, 13–19 days, de-14.5 days). Of these 300 red lice, 10 presented with rectorragia (figure 2). At this point, the death rate in the control group was zero. The last infected lice died between day 13 and day 19 after infection (figure 1); all dead infected lice were red. The life span of the 800 infected lice (mean ± SD) was shorter than that of the 800 uninfected lice: 14.5 ± 3 days (range, 13–19 days, depending on the experiment) for infected lice and 39 ± 1.7 days (range, 36–42 days, depending on the experiment) for control lice (P < .01). PCR amplification was conducted on 119 infected lice (63 live and 56 dead lice) and was positive for both tested genes for all these lice. As a negative control, PCR amplification conducted on 276 uninfected lice (161 live and 115 dead lice) was negative for gltA but positive for 18S rDNA. Shell vial culture from the 12 red lice sampled on day 6 after infection was positive on day 12 after inoculation.

Eggs, larvae, and fecal infection. Egg laying began on the same day for the infected and control louse populations (between day 2 and day 4 after infection, depending on the experiment). The total number of eggs laid by the 800 infected lice during their life span was 9120 eggs (range, 1920–2640 eggs, depending on the experiment) versus 28,020 eggs (range, 6580–7460 eggs) in that of the uninfected louse population. The average number of eggs laid daily per infected louse was 0.6 egg (range, 0.5–0.7 egg, depending on the experiment) versus...
0.9 egg (range, 0.84–0.95 egg) for the control group, but this difference was not significant (P = .1). For the 2 groups of lice, eggs hatched between day 12 and day 13 after they were laid. For the 4 experiments, no *R. prowazekii* growth or PCR amplification with *gltA*–encoding gene was obtained from the 175 eggs (51 from the infected group and 124 from the uninfected group) and 175 larvae (51 from the infected group and 124 from the uninfected group) examined by each test. PCR determined these samples to be positive by using 18S rDNA-derived primers showing the absence of inhibitors.

Feces sampled daily during the first 4 days after infection was determined to be negative by immunofluorescence staining. On day 5 after infection, one day before the first red louse was observed, *R. prowazekii* was detected in feces; from this day until the death of the last infected louse, fecal samples were always positive for *R. prowazekii*. When applying cell culture to the infected lice feces collected on day 6 after infection, we observed the survival of *R. prowazekii* in fecal samples inoculated up to 10 days after their emission. This culture was positive 22 days after inoculation. Two attempts of rickettsial culture from these fecal samples >10 days after their excretion resulted in contamination with *Aspergillus* species, despite the use of antibiotics in the culture medium (cotrimoxazole, gentamicin, and amphotericin B). PCR amplification of fecal samples was negative for any of the 3 tested primer pairs, whereas control *R. prowazekii* DNA was detected and quantified as expected. When mixed with DNA from uninfected fecal samples, detection of various concentrations of *R. prowazekii* DNA was lowered (from 40 to 1 DNA copies, from 80 to 1 DNA copies, from 120 to 1.5 DNA copies, from 200 to 1.7 DNA copies, from 400 to 1.7 DNA copies, from 800 to 9.6 DNA copies, from 2000 to 35 DNA copies, from 4000 to 61 DNA copies, and from 8000 to 61 DNA copies). Similar results were obtained among the 3 experiments.

**Morphological study of lice.** By use of confocal microscopy at 37°C and the P11A12 MAb on methanol-fixed louse gut, *R. prowazekii* was detected extracellularly in the red louse gut [47]. By use of the same technique on formalin-fixed sections of the 8 red lice sampled on day 6 after infection, the P11A12 MAb failed to detect *R. prowazekii*. Therefore, we used the P12H10 MAb, which detected *R. prowazekii* only in the stomach, the first part of the digestive tract of each of these 8 red lice (figure 3). Because of the autofluorescence of chitin, which we have described elsewhere [33], the external contour of infected and control lice also appeared fluorescent.
Discussion

*R. prowazekii* has caused huge outbreaks of epidemic typhus in the past [48] and still constitutes a threat for populations in various areas of the world [12, 20, 49]. The role of body lice in the transmission of *R. prowazekii* to humans was first demonstrated by Charles Nicolle in 1909 [26, 50], who received the Nobel Prize in 1928 for his work on epidemic typhus [26]. Since being recognized as a vector of infectious diseases, the body louse has been used for the laboratory growth of *R. prowazekii* [50], the production of Weigl vaccine [32, 51], the estimation of antibiotic susceptibility of *R. prowazekii* [30, 52, 53], and diagnostic testing of *B. quintana* infection [54, 55]. In these studies, lice were infected either by blood feeding [24, 25, 27–30] or by intrarectal inoculation [31, 32]. We have used the intravenous injection of the rabbit model [27], which was used previously with *Bartonella quintana* in our laboratory [33]. Despite an inoculum of 2 × 10⁶ pfu of *R. prowazekii*, the infected rabbit did not develop any symptoms throughout the experiment, thus suggesting it was not susceptible to this bacterium, in contrast with the experiments of Snyder and Wheeler, in which infected rabbits died [27]. This discrepancy may be related to the composition of the inoculum. In our study, each of the 4 infected rabbits in group R1 remained bacteremic with *R. prowazekii* only for the first 2 days, and developed an anti–*R. prowazekii* immune response with an antibody titer of 1:800 on day 22. The 12 uninfected rabbits (4 in group R2, 4 in group R3, and 4 in group R6) on which the infected lice fed, and the 8 uninfected rabbits (4 in group R4 and 4 in group R5), on which the uninfected lice fed, remained seronegative. Thus, the infected lice did not transmit *R. prowazekii* through their bites while feeding on rabbit.

Epidemic typhus also has been named “red louse disease,” because of the color acquired by these arthropods before their death [24]. However, there is no picture of the red louse in the literature. In our experiment, we observed the appearance of the first red lice between day 6 and day 7 after infection (figure 2), in contrast with the experiments of Snyder and Wheeler, in which infected rabbits died [27]. This discrepancy may be related to the large inoculum dose used in our study; therefore, it is possible that the massive mortality observed on day 6 after infection also may be delayed during natural infection. Furthermore, we observed that some of the red lice presented with a rectorragia; to our knowledge, this has not yet been reported (figure 2). Compared with the uninfected lice, infected lice died 20–23 days earlier, thus confirming the harmful role of *R. prowazekii* for its vector, which thus cannot constitute the reservoir of the disease. Until they became red, lice fed regularly, mated, and continued to produce and deposit eggs. The daily number of eggs from infected lice was 33% lower than that of uninfected lice, but the difference was not significant.

Figure 3. Infection with *Rickettsia prowazekii* of the anterior part of the digestive tract of a red body louse dead on day 6 after infection. Peripheral fluorescence is related to the autofluorescence of chitin (immunofluorescence staining, confocal microscopy; original magnification, ×600).

After immunohistological examination at 37°C with the P11A12 MAb, *R. prowazekii* was not detected in any of the 12 tested lice, whereas, with the P12H10 MAb (figure 4), the bacterium was observed within the epithelial cells of the first part of the digestive tract of the 4 lice taken on day 5 after infection but not on those 4 collected on day 0. In the 4 red lice sampled on day 6 after infection, *R. prowazekii* was present in the gut as dense red clusters of bacteria, but epithelial cells were destroyed.

By means of electron microscopy (figure 4), no intracellular bacteria were observed in gut cells of the 4 lice obtained just after infection. In the gut of the 4 infected lice obtained on day 5, numerous rickettsiae were found within intact epithelial cells. In all 4 red lice sampled on day 6, all rickettsiae were extra-cellular, and no intact epithelial cell was observed.

Effect of doxycycline on lice. Among the 220 infected lice, which had fed on the abdomen of the 4 doxycycline-treated rabbits (group R6), 112 became red and died on day 6 after infection. The remaining 108 became progressively red and died; the last one died between days 18 and 19 after infection, depending on the experiment, on the same day as the last infected lice that did not receive doxycycline. No difference was found between the 2 louse populations.
Figure 4. Proposed model of infection kinetics of Pediculus humanus corporis with Rickettsia prowazekii. Immunochemistry (A) and electron microscopy (B) detection of R. prowazekii in lice sampled on days 0, 5, and 6 after infection are presented on the left. Immunochemistry detection of R. prowazekii was performed with the anti-lipopolysaccharide–like monoclonal antibody diluted to 1:400. R. prowazekii is colored in red and digestive cells in blue. Magnification is indicated by the scale bar. On day 0, the louse was infected by feeding with R. prowazekii–infected blood on a bacteremic rabbit. At that stage, the epithelial cells of the louse’s digestive tract were not infected. On day 5, bacteria have invaded and multiplied into stomach. Some infected cells start to burst, thus releasing R. prowazekii into the digestive tract lumen and then in feces. On day 6, the intense intracellular rickettsial multiplication (1) leads to the rupture of all infected cells. R. prowazekii is only found extracellularly (2) in the gut lumen. Erythrocytes invade the hemolymph through the intestine. Infected lice become red and die within 4 h.
Eggs and larvae were not infected, which allowed us to confirm that the transovarial transmission of *R. prowazekii* does not occur in lice. The effect of administering antibiotics on *R. prowazekii*-infected lice had been tested, either through their feeding on membrane containing 5 μg/mL of doxycycline or 5 μg/mL of rifampin, which showed that the replication of *R. prowazekii* was delayed only for the period of antibiotic administration, and the treated lice died from the same heavy of infection as untreated lice [30], or through intrarectal injection on intrarectally infected lice in Poland and in the former USSR by inoculating tetracyclines intrarectally to lice, which was shown to be efficient [52, 53]. In our study, the administration of a single-dose of doxycycline, as prescribed for patients with typhus [23], did not stop the diffusion of *R. prowazekii* in lice. However, we cannot extrapolate this result to the field situation.

The multiplication of *R. prowazekii* occurred only in the stomach of infected lice (figure 3), as observed elsewhere by Weigl [32]. The preferential location of bacteria in the anterior part of the digestive tract may be explained by the priority invasion of the first cells encountered by *R. prowazekii*, which is not motile in cells and therefore cannot exit from infected cells and spread actively in its vector [57]. Then, the infection of the neighbor cells is possible only when the infected cells burst. Using the P11A12 MAb directed against a protein epitope, we could detect *R. prowazekii* at 37°C from lice and their feces fixed by methanol by means of immunofluorescence. However, this antibody tested at the same temperature by means of immunochemistry was unable to detect *R. prowazekii* in formalin-fixed lice, whereas the P12H10 MAb, directed against the lipopolysaccharide antigen, was efficient. This may be explained by the destruction of *R. prowazekii* proteic antigenic epitopes by formalin. When studying louse gut after death, we observed by immunofluorescence and electron microscopy that *R. prowazekii* was found only extracellularly, and that no intact intestinal cell structure persisted (figure 4). However, numerous *R. prowazekii* were detected by electron microscopy inside intact epithelial cells in gut of live lice collected on day 5 after infection (figure 4).

In our experiment, the fecal elimination of *R. prowazekii* began on day 5 after infection, 1 day before lice became sick. This early mortality demonstrated that lice themselves remain infective for humans only for a short time. However, *R. prowazekii* remains viable in emitted feces [22], thus constituting a persistent source of infection. Unfortunately, because of contamination of feces culture with fungus, we were able to demonstrate the survival of *R. prowazekii* in feces only until day 10 after their emission. We noted discrepancies among the techniques used for detection of *R. prowazekii* in feces. Although PCR analysis of *R. prowazekii*-infected feces was negative in all cases with the 3 tested genes, we were able to amplify the bacterium from crushed lice and from rabbit blood. In contrast, *R. prowazekii* was detected by means of culture and immunofluorescence in fecal samples. The presence of PCR inhibitors in human feces has been described in the literature [57]. We also have demonstrated the presence of PCR inhibitors in louse feces; indeed, the addition of their DNA decreases DNA detection of *R. prowazekii* by quantitative PCR.

Finally, we could summarize the natural infection kinetics of the body louse with *R. prowazekii* as follows (figure 4). (1) On the day 1 of infection, the louse was infected by feeding on a bacteremic rabbit. Because of its strictly intracellular character, *R. prowazekii* colonized the first cells encountered, the epithelial cells of the louse's stomach, where it began to multiply. (2) During days 1–4 after infection, *R. prowazekii* multiplied actively inside each individually infected epithelial cell. This bacterium, unable to polymerize actin [56], did not actively spread from cell to cell and did not infect the entire digestive tract. (3) On day 5 after infection, some infected cells burst, thus releasing *R. prowazekii* into the digestive tract lumen and subsequently in feces, where it remained viable and infectious for at least 10 days. (4) One day later (day 6), the intense intracellular rickettsial multiplication led to the rupture of all infected cells. *R. prowazekii* was found extracellularly in the gut lumen, and the ingested blood erythrocytes invaded the hemolymph through the intestine. Infected louse became red and died within 4 h.

In conclusion, we established an experimental model of body louse infection leading to the diminution of its life span and the elimination of live rickettsiae with feces. We consider that our model reproduces the natural infection and may be useful for further studies on the interactions of *R. prowazekii* with its vector. In addition, we report for the first time the isolation by cell culture of *R. prowazekii* from infected lice and from their feces.

References