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

Linda Houhamdi,1 Pierre-Edouard Fournier,1 Rong Fang,1 Hubert Lepidi,1,2 and Didier Raoult1

*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 humanus* or *Pediculus humanus corporis*; the head louse, *Pedicula 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 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 conjunctiva 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*.
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; 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 10⁶ 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. rickettsii until 1945 and then on rabbits [37, 38], was established on rabbits

Experimental model of body louse infection with R. prowazekii. One uninfected rabbit in group R1 was infected by an intravenous muscular injection of 17 mg of chloropromazine and 67 mg of ketamine chlorohydrate. Once fed, lice were kept on a tissue piece

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.

Sero logic 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 and 18Sb [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 intravenous injection of 17 mg of chloropromazine and 67 mg of ketamine chlorohydrate. Once fed, lice were kept on a tissue piece in an incubator at 29°C with 70%–90% humidity [41].

One uninfected rabbit in group R1 was infected by an intravenous auricular administration of 1 mL of doxycycline (200 mg/mL) for 15 min. The number and color of the number and 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.

Sero logic 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 (described above), which were carried out on lice, eggs, larvae and feces, and the β globin–derived primers, as described elsewhere [44], which were carried out on rabbit blood and louse feces. DNA of R. rickettsii was used as a positive control for PCR. Negative controls consisted of DNA extracts prepared at the same time from uninfected rabbit blood, lice, eggs, larvae, and feces.

The PCR inhibitory 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,
800, 2000, 4000, and 8000 DNA copies), either alone or mixed with 2 μL of DNA extracted from 16 mg of uninfected louse feces, and on DNA extracted from feces only. Because DNA from all specimens was extracted by use of the same extraction kit, we expected the rate of DNA recovery to be similar for all specimens. DNA from R. prowazekii and louse feces was extracted by means of the QiAamp Tissue kit (Qiagen). The DNA amount of the various samples was estimated by comparison with a sequence-specific standard curve generated by using DNA from 10-fold serial dilutions of a 10⁸ pfu of R. prowazekii suspension extracted using the QiAmp Tissue kit. This experiment was conducted 3 times.

**Shell vial culture of R. prowazekii.** All tested lice, eggs, and larvae were decontaminated by a 5-min immersion in a solution of 70% ethanol–0.2% iodine, followed by a 5-min immersion in sterile distilled water [2], and were cut into small pieces with a sterile surgical blade in 1 mL of M22 medium. To estimate the survival of R. prowazekii in louse feces, in each of the 4 experiments, 0.2 mg of feces, collected from infected lice on day 6 after infection, were resuspended daily in 1 mL of M22 medium, which contained 25 μL of cotrimoxazole (8 μg/mL), 25 μL of gentamicin (1 mg/mL), and 10 μL of amphotericin B (1 mg/mL). Four hundred microliters of the suspension of each sample was inoculated onto L929 cells by use of the centrifugation-shell vial technique [45]. Shell vial tubes were incubated at 37°C in a CO₂ atmosphere for 1 day and then at 35°C. Ricketsial infection was monitored 7 days after inoculation by cytospin centrifugation on 100 μL of culture supernatant, followed by Gimenez staining [3], and then every 5 days after, if the Gimenez staining remained negative.

**Immunofluorescence staining of lice.** Immunofluorescence study was carried out on 5-μm-thick paraffin-embedded sections of formalin-fixed lice and their methanol-fixed gut, as described elsewhere [46]. Louse legs were cut before fixation. For each section, a negative louse control was prepared. Slides were covered by 30 μL of the anti–R. prowazekii mouse monoclonal antibody (MAb) P11A12 directed against a 120-kDa protein [47] and diluted 1:400 in PBS with 3% (wt/vol) nonfat dried milk. The location of R. prowazekii in the digestive tract was determined by means of a laser scanning confocal fluorescence microscope (TCS-4D; Leica; original magnification, ×600). Because of louse dimensions, several photographs were taken, and the complete body was reconstituted by the computer.

**Immunohistological analysis.** Immunochemistry was performed on 5-μm-thick lice sections using the Zymed Histostain-Plus kit (Zymed Laboratories), according to the manufacturer’s directions. Each louse was tested with 2 mouse-produced MAbs: P11A12, described above [47], and P12H10 directed against a lipopolysaccharide-like epitope of R. prowazekii. Mayer’s hematoxylin was used to visualize the gut system. Uninfected lice were used as a negative control.

**Electron microscopy.** Because of the louse’s hard shell, it was impossible to cut the whole louse by using Ultracutmicrotome (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 Ultracutmicrotome 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).

**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) developed a bright red color and died within 4 h (figure 2). Of these 300 red lice, 10 presented with ractorrages (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.

**Figure 1.** Survival rate over time of lice infected with *Rickettsia prowazekii* (red) and uninfected control lice (blue). Day 0 is the day of infection. Data are percentages of mean no. of tested lice. SD for each number is shown.

**Figure 2.** *Rickettsia prowazekii*–infected (A) or –uninfected (B) dead *Pediculus humanus corporis*. The infected louse presents a red color and rectorragia.
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 $2 \times 10^8$ 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), 1 day after *R. prowazekii* appeared in feces. Infected lice died between day 6 and days 13–19 after infection, depending on the experiment, and all became red and died within 4 h. In contrast, Wolbach et al. [24] had observed that lice infected by feeding on a patient with typhus did not systematically acquire the red color. 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 infected 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 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 intraretinal injection on intraretally infected lice in Poland and in the former USSR by inoculating tetracyclines intraretally 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 increases 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