EXPERIMENTALLY INFECTED HUMAN BODY LICE (PEDICULUS HUMANUS HUMANUS) AS VECTORS OF RICKETTSIA RICKETTSII AND RICKETTSIA CONORII IN A RABBIT MODEL

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Abstract. The human body louse, the natural vector of Rickettsia prowazekii, is able to experimentally transmit the normally flea-borne rickettsia R. typhi, suggesting that the relationships between the body louse and rickettsiae are not specific. We used our specific infection model to test the ability of body lice to transmit two prevalent tick-borne rickettsiae. Each of two rabbits was made bacteremic by injecting intravenously 2 × 10^6 plaque-forming units of either R. rickettsii or R. conorii. Four hundred body lice were infected by feeding on the bacteremic rabbit and were compared with 400 uninfected lice. Each louse group was fed once a day on a separate seronegative rabbit. The survival of infected lice was not different from that of uninfected controls. Lice remained infected for their lifespan, excreted R. rickettsii and R. conorii in their feces, but did not transmit the infection to their progeny. The nurse rabbit of infected lice remained asymptomatic and seronegative. Those rabbits used to feed infected lice developed bacteremia and seroconverted. Although the body louse is not a known vector of spotted fevers, it was able in our study to acquire, maintain, and transmit both R. rickettsii and R. conorii.

INTRODUCTION

Spotted Fever (SF) rickettsioses constitute one of the most severe and dramatic acute infectious diseases. Rocky Mountain spotted fever (RMSF), caused by Rickettsia rickettsii, is the major SF rickettsiosis of the Western Hemisphere ^{1,2} and Mediterranean spotted fever (MSF), caused by R. conorii, is one of the major SF rickettsioses in the Eastern Hemisphere. ^{3-7} Rickettsia rickettsii and R. conorii are principally transmitted to humans by infected ticks, in addition to aerosol transmission ^{8} and blood transfusion. ^{9} The infection occurs when humans are bitten by ticks, usually via their saliva. During feeding, the ticks inject virulent rickettsiae contained in their salivary glands. ^{10} The chief vectors of R. rickettsii are the Rocky Mountain wood tick Dermacentor andersoni, and the common American dog tick D. variabilis. ^{10} The major vector of R. conorii in the Mediterranean area is the brown dog tick Rhipicephalus sanguineus. ^{11}

The human body louse, Pediculus humanus humanus, a strict blood sucking arthropod, is the natural vector of R. prowazekii, the agent of epidemic typhus. Together with R. rickettsii, R. prowazekii is among the most virulent of all rickettsiae. Rickettsia prowazekii belongs to the typhus group of rickettsiae which includes R. typhi, the agent of murine typhus, which is transmitted mainly by the oriental rat flea Xenopsylla cheopis. The body louse has been suggested as an alternate vector for R. typhi, ^{12,13} suggesting that the relationships between the body louse and the rickettsiae are not specific. In connection with the important role of the body louse as a vector of rickettsial disease, our objective was to evaluate whether the body louse could acquire a persistent infection with R. rickettsii, R. conorii, or both. We used our experimental model of body louse infection previously used with R. prowazekii, ^{14} R. typhi, ^{13} and Bartonella quintana. ^{15}

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MATERIALS AND METHODS

Experimental model of body louse infection with R. rickettsii (Sheila Smith strain [ATCC VR-149]) and R. conorii (Seven [Malish] strain [ATCC VR-613]). The experiment was performed in a biosafety level 3 laboratory. Each of two specific pathogen–free New Zealand white rabbits was infected by an auricular intravenous injection of 20 mL of K36 buffer containing 2 × 10^6 plaque-forming units of either R. rickettsii or R. conorii ^{16} infused over a 15-minute period to obtain a persistent artificial bacteremia. Two groups of 200 15-day-old uninfected human body lice (adults), strain Orlando, ^{14} were infected by feeding on the shaved rabbit abdomen during the artificial bacteremia. The day of infection was referred to as day 0. In the following days, each infected louse group was fed once a day on one of two other uninfected nurse rabbits. Two hundred 15-day-old uninfected lice were used as negative controls and were fed daily on another uninfected nurse rabbit. Each louse group was kept in a separate plastic container at 29°C with a humidity of 70–90%, and each rabbit was kept in an individual cage. The experiment was repeated once and lice and rabbit populations were renewed for each experiment. The animal study was reviewed and approved by the Animal Ethics Committee of the Marseille School of Medicine.

Effect of the infection on rabbits. Two hundred microliters of blood were sampled from R. rickettsii-infected rabbits immediately and 1, 5, 10, 21, and 24 hours post-infection and from R. conorii-infected rabbits immediately and 1, 2, 16, 19, 22, 24, 40, 48, and 64 hours post-infection. Blood was used in PCR assays with primers directed against the ompA gene, which encodes the rickettsial outer membrane protein A. ^{17} DNA was extracted using the QiAamp tissue kit (Qiagen, Hilden, Germany), according to the manufacturer’s directions. Rickettsia montanensis DNA was used as a positive control. A PCR with β globin-derived primers ^{18} was used as a control for the efficiency of DNA extraction and the PCR. All rabbits were bled weekly for the PCR (ompA and β globin) and four drops of blood were collected onto blotting paper (Fisher Scientific, Elancourt, France) by aural puncture for an immunofluorescence assay (IFA). ^{19}
Effect of the infection on lice. The number and color of surviving and dead lice were noted daily in each louse group. Two surviving, and eventually, two dead lice from each group were sampled daily: one of each for ompA-PCR, and the other decontaminated for cell culture. An 18S ribosomal RNA (rRNA) PCR using the primers 18SaIdg and 18Sbi was used as a control of the efficiency of DNA extraction and the PCR. Dead, infected lice were fixed with formalin and 5 μm-thick paraffin-embedded sections were prepared for the IFA using the RCS5A mouse monoclonal antibody (MAb), which was directed against the lipopolysaccharide-like epitope of SF group rickettsiae, diluted to 1:200. Uninfected louse sections were used as negative controls.

Assay of louse progeny. The number of eggs produced in each louse group was recorded daily. Fisher’s exact test (Epi-Info version 6.0 software; Centers for Disease Control and Prevention, Atlanta, GA) was used to compare the fertility of the infected and uninfected lice. Two eggs (starting from the hatching of the first egg) and two larvae (starting from the hatching of the first egg) were sampled daily: one of each for the PCR (ompA and 18S rRNA) and the other decontaminated for cell culture.

Assay of louse feces. Feces (0.1 mg) was sampled twice a day for the IFA using RCS5A MAb and for the PCR after removal of PCR-inhibitors present in louse feces using the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany).

RESULTS

Effect of the infection on rabbits. The β globin-positive control PCR result was uniformly positive. The ompA PCR results were consistently positive until day 21 post-infection on blood drawn from R. rickettsii-infected rabbits and until day 24 post-infection on blood drawn from R. conorii-infected rabbits. All rabbits remained asymptomatic throughout the experiment. Those infected with R. rickettsii seroconverted with an antibody titer of 1:100 on day 7, 1:200 on day 14, and 1:400 from days 21 to 42. Those infected with R. conorii exhibited identical antibody titers, although on day 21 the titer was 1:200. The results of the ompA PCR performed on blood of nurse rabbits upon which infected lice fed was positive on days 7 and 14 and negative from days 21 to 42. The results of the PCR performed on blood of nurse rabbits upon which infected lice fed were positive on days 7 and 14 and negative from days 21 to 42. The rabbits upon which infected lice fed remained seronegative.

Effect of the infection on lice. The mean survival of lice infected with R. rickettsii (41.5 days) or R. conorii (40.5 days) did not differ from that of uninfected controls (41.5 days) (Figure 1). All surviving and dead lice in all groups were uniformly positive for cell culture, but always positive by the 18S rRNA PCR. Dead, infected lice were fixed with formalin and 5 μm-thick paraffin-embedded sections were prepared for the IFA using the RC5A mouse monoclonal antibody (MAb), which was directed against the lipopolysaccharide-like epitope of SF group rickettsiae, diluted to 1:200. Uninfected louse sections were used as negative controls.

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Effect of the infection on lice. The mean survival of lice infected with R. rickettsii (41.5 days) or R. conorii (40.5 days) did not differ from that of uninfected controls (41.5 days) (Figure 1). All surviving and dead lice in all groups were physically indistinguishable, except on days 7 and 8 when 7.5% of R. rickettsii-infected lice and 8.5% of R. conorii-infected lice died while showing a diffuse reddening throughout the body. The results of the ompA and 18S rRNA PCRs performed on 147 R. rickettsii-infected lice (83 surviving and 64 dead) and 142 R. conorii-infected lice (81 surviving and 61 dead) were consistently positive. The results of the PCR performed on uninfected lice were always ompA negative and those of the 18S rRNA PCR were always positive. Cell culture was positive 12 days after the in vitro incubation of L929 cells with lice infected either with R. rickettsii (83 surviving and 44 dead lice) or R. conorii (81 surviving and 40 dead lice), and 6 days after inoculation with each of 8 red dead infected lice. Using confocal microscopy, we showed that visualization of R. conorii (Figure 2A) and R. rickettsii (Figure 2B) was limited to the anterior part of the digestive tract; however, the distribution of R. rickettsii in the anterior part was not uniform. Uninfected lice always showed negative results in the IFA.

Assay of progeny. Infected and uninfected lice began laying eggs on day 4. Eggs hatched 11 days later. The average number of eggs laid by lice during their life span was 7,043 eggs in the R. rickettsii-infected group and 7,000 eggs in the R. conorii-infected group versus 7,263 eggs in uninfected lice. For each experiment, the average number of eggs laid daily per louse was not statistically different between (R. rickettsii- and R. conorii-) infected and uninfected lice (first experiment: 1.83, 1.81, and 1.87, respectively; P = 0.7; second experiment: 1.93, 1.9, and 1.95, respectively; P = 0.96). The progeny of lice infected with R. rickettsii (67 eggs and 57 larvae), R. conorii (75 eggs and 55 larvae) and uninfected lice (67 eggs and 57 larvae) were uniformly negative by the ompA PCR and cell culture, but always positive by the 18S rRNA PCR.

ASSAY OF FEACES. Rickettsia conorii were visualized by IFA in the feces of infected lice from day 1, and R. rickettsii was observed from day 3. Once IFA positive, the infected lice feces remained positive until the death of the last louse. Uninfected lice feces were always IFA negative. Similar results were obtained using the ompA PCR.

DISCUSSION

The human body louse is known to transmit R. prowazekii, the agent of epidemic typhus, which is also called “red louse disease” because of the color acquired by this arthropod before its death as a result of infected cell rupture and the passage of ingested blood into the louse hemolymph. Reddening of the arthropod is also observed with R. typhi-
In our study, red lice were only observed on days 7 and 8 following infection with either *R. rickettsii* or *R. conorii*. Although *R. rickettsii* and *R. conorii* are capable of intercellular spread by means of actin polymerization, the infection was limited to the anterior part of the louse digestive tract (Figure 2). Whereas *R. conorii* was evident throughout this anatomic region (Figure 2A), *R. rickettsii* infection was spottier (Figure 2B). The mechanism by which infection is limited to the body louse foregut is unknown.

The dynamics of louse infection with these two SF rickettsiae has been studied previously. Infection of lice with *R. conorii* strains (from South Africa, Kenya, and India) and the Brazil strain of *R. rickettsii*, unlike that observed in the North Africa strain of *R. conorii* and Bitterroot Valley (United States) and Mexico strains of *R. rickettsii*, was characterized by mild pathogenicity and limited multiplication inside only a few epithelial cells. In our study, lice remained infected for their lifespan, the length of which was comparable to that observed in uninfected lice (Figure 1). Both infected and uninfected lice mated regularly and continued to produce and lay uninfected eggs starting from day 4, which hatched 11 days later. Moreover, until the death of the last infected louse, lice began the fecal excretion of *R. conorii* on day 1 and *R. rickettsii* on day 3. Excretion of viable rickettsiae in feces is critical to the ability of the body louse’s ability to act as a vector for disease. Unfortunately, we were unable to perform cell culture on the feces excreted by either *R. rickettsii* or *R. conorii*-infected lice because of the numerous contaminants present in lice feces.

We demonstrated for the first time that the transmission of *R. rickettsii* and *R. conorii* by the body louse can occur during louse feeding. The nurse rabbits used to feed infected lice...
developed a bacteremia on days 7 and 14 and seroconverted with an antibody titer of 1:200 on day 42. However, these rabbits were infested by a huge number of lice, which is unusual in the field. The seroconverted nurse rabbits continued to serve throughout the experiment as feeders of infected lice. This may have contributed to the low mortality observed among infected lice because they would have imbibed the antibodies to rickettsia while feeding. The intravenously infected rabbits remained bacteremic for ≤ 24 hours post-infection, which was surprisingly shorter than what was observed in nurse rabbits. This may be due to consistent re-infection of these nurse rabbits with the infected lice during the daily feedings.

Winter is the season of body louse proliferation and the occurrence of typically louse-borne diseases. Although the majority of RMSF cases occur during the spring and summer27 and those of MSF in the summer,3,7 cases of RMSF28 and MSF29 during the winter have been reported. One fatal case of MSF has been reported in a homeless patient from Marseille, France.7 This population has been found to be heavily infested with body lice.30 It seems reasonable to conclude that in specific populations during the contemporaneous occurrence of body louse and RMSF or MSF cases, the body louse has the potential to transmit R. rickettsii or R. conorii. However, unlike epidemic typhus,31,32 relapses, which favor the body louse infection, do not occur with RMSF and MSF.

In conclusion, we showed for the first time that body lice infected with R. rickettsii or R. conorii are capable of acquiring, maintaining, and transmitting the organisms to naive rabbits during feeding. The nurse rabbits became bacteremic and seroconverted. Moreover, the infected lice excreted these rickettsial cells in their feces. Although the human body louse is not the natural vector of R. rickettsii and R. conorii and its presence does not perfectly coincide with the season of peak occurrence of RMSF or MSF, it may play a role, under favorable epidemiologic circumstances, in their transmission to humans.

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