EXPERIMENTAL INFECTION OF HUMAN BODY LICE WITH ACINETOBACTER BAUMANNII

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Abstract. The human body louse is currently recognized as a vector of Rickettsia prowazekii, Borrelia recurrentis, and Bartonella quintana. Previous studies have reported the isolation of Acinetobacter baumannii from the body lice of homeless patients. To study how the body louse acquires A. baumannii, we infected a rabbit by infusing 2 × 10^9 colony-forming units of the louse strain of A. baumannii. Two hundred body lice were infected by feeding on the bacteremic rabbit and compared with 200 uninfected lice and two groups of 200 lice feeding on rabbits infected either with another strain of A. baumannii or A. lwofii. Each louse group received maintenance feedings once a day on another seronegative rabbit. Body lice that fed on rabbits infused with each Acinetobacter species demonstrated a generalized infection. The body lice did not transmit their infection to the nurse rabbit by bite while feeding or to their progeny (eggs and larvae). The lice excreted living Acinetobacter species within their feces. Only the louse strain of A. baumannii was pathogenic for the body louse. An increased mortality rate was observed between the second and third days post-infection; however, they remained infected for their lifespan.

INTRODUCTION

Among the 3,000 louse species described, three are strictly human ectoparasites, and only the human body or clothing louse, Pediculus humanus corporis (Linne, 1758), is known to transmit human diseases. In nature, body lice transmit three pathogenic bacteria: Rickettsia prowazekii (the agent of epidemic typhus), Borrelia recurrentis (the agent of louse-borne relapsing fever), and Bartonella quintana (the agent of trench fever, bacillary angiomatosis, chronic bacteremia, endocarditis, and chronic lymphadenopathy).1 Our laboratory has previously reported the isolation of other human pathogens (Acinetobacter spp.,2 subsequently identified as A. baumannii,3 and Serratia marcescens4) from body lice collected from homeless people. Acinetobacter spp. are widespread in nature (water, soil, mud, living organisms,5 vegetables,6 and the skin of patients or healthy subjects7). Although many species of Acinetobacter can cause infection, A. baumannii is the most frequently encountered species in the clinical laboratory,8 most notably in intensive care units.9,10 It has been linked to many severe hospital-acquired infections,9,11 including those of skin,10 wounds,11,12 the urinary tract,11,13 pneumonia,4,7,11–13 and meningitis.3,12,14 It exhibits a high level of resistance to antimicrobial agents and is capable of persisting in hostile environments (humidity or dryness and in the presence of some antibiotics).8 The presence of A. baumannii in human body lice2,3 is likely a naturally occurring global phenomena, as suggested by its detection in 21% of body lice3 collected worldwide.15 Although the louse ingests only blood from humans, its midgut is sterile and the presence of A. baumannii is due mainly to ingestion of infective blood meals from patients with ongoing bacteremia,2 or likely during the passage through the human skin while feeding.6

Our objective was to evaluate the relationship between the human body louse and A. baumannii. For this purpose, we used our experimental model of louse infection, which reproduces the natural infection, as demonstrated previously with B. quintana16 and R. prowazekii.17

MATERIALS AND METHODS

Acinetobacter species. Three species of Acinetobacter were used in our study: a strain of A. baumannii from the Unité des Rickettsies (UR 10.72) isolated from a louse collected from a homeless patient,2 a strain of A. baumannii obtained from the Collection de l’Institut Pasteur (CIP 53.79) isolated from a whitlow in 1951, and strain of A. lwofii (ATCC 15309 = CIP 64.10). For each species, 100 µL of 10-fold serial dilutions (from undiluted to 10^-12) in phosphate-buffered saline (PBS) (bioMérieux, Marcy l’Etoile, France) were streaked onto Columbia sheep blood agar (bioMérieux) and incubated at 37°C. The number of colony-forming units (CFU)/mL in each dilution was determined on the third day following inoculation.

Infection of human body louse with Acinetobacter spp. Each of three specific pathogen–free (SPF) New Zealand white female rabbits (weighing 6 kg and containing between 360 and 420 mL of blood) was infected by a 15-minute auricular intravenous infusion of 20 mL of PBS containing 10^9 CFU/mL of one of the three species of Acinetobacter to obtain a persistent artificial bacteremia. Thus, the number of bacteria given to the rabbit host was between 4.5 and 5.26 × 10^9 CFU/mL of rabbit blood. One rabbit (R1) was infected with the louse strain of A. baumannii, the second rabbit (R2) with A. baumannii (CIP 53.79), and the third rabbit (R3) with A. lwofii strain CIP 64.10. Each of three series of 200 fifteen-day-old uninfected human body lice (P. h. corporis, strain Orlando)17 were infected by feeding on the previously shaved abdomen of one of the three infected rabbits during the artificial bacteremia. The day of infection was referred to as day 0. Each infected louse group then received a maintenance feeding once a day on one of three other SPF rabbits. Lice infected with the louse strain of A. baumannii were nourished on rabbit R4, those infected with A. baumannii (CIP 53.79) on the rabbit R5, and those infected with A. lwofii on rabbit R6. Two hundred fifteen-day-old uninfected lice were used as negative controls and were nourished daily on a fourth SPF rabbit (R7). Each louse group was kept in a separate plastic container at 29°C at a relative humidity of 70–90%,18 and each rabbit was kept in an individual cage. The animal study was approved by the Animal Ethics Committee of the Marseille School of Medicine.

Study of rabbit infection. Two hundred microliters EDTA-blood mixture from each of the three infected rabbits (R1,
A. baumannii

The DNA, but not (louse strain) – A. est spp. was used as a positive control. The recA gene using the recA-specific primers recAF (CACATGACATTGCAAGCAATTTG) and recAR (ACCAATTTTCATACGAATCTGG) that amplify a 382-basepair fragment. This was used to detect A. baumannii DNA. A third PCR with recA gene using the recA-specific primers recAF (CACATGACATTGCAAGCAATTTG) and recAR (ACCAATTTTCATACGAATCTGG) that amplify a 382-basepair fragment. This was used to detect A. lwoffii DNA, but not A. baumannii DNA. A PCR with β-globin gene-derived primers was also used as a control of the efficiency of DNA extraction and the PCR. The PCRs were conducted in a Peltier model PTC-200 thermal cycler (MJ Research, Watertown, MA). Four drops of blood were collected every week by ear puncture from each of the seven rabbits onto blotting paper (Fisher Scientific, Elancourt, France) and used to detect antibodies by an immunofluorescence assay (IFA), as described elsewhere.

Study of lice infection. The number and the color of surviving and dead lice were noted daily in each of the four louse groups. The number of dead lice in each infected louse group was compared with that of uninfected lice using the chi-square in Epi-Info version 6.0 software (Centers for Disease Control and Prevention, Atlanta, GA). Four surviving and eventually four dead lice from each group were sampled daily. One of each group was assayed by the PCR, one by the IFA, and one by culture, and the hemolymp of the fourth one was extracted for culture and the IFA. A PCR was performed with the recA (both strains of A. baumannii) or the est (A. lwoffii) genes. A PCR with the 18S ribosomal RNA (rRNA)21-derived primers 18SaIdg2 and 18Sbi22 was used as a control for the efficiency of DNA extraction and the PCR. DNA extracted from uninfected lice was used as a negative control, and DNA of Acinetobacter spp. was used as a positive control. For the IFA, each louse was fixed in absolute ethanol at 4°C for 2–3 weeks to increase the fixation efficiency. Ethanol-fixed, 5 μm-thick paraffin-embedded sections were generated for the IFA using 30 μL of an anti-Acinetobacter spp. mouse polyclonal antibody diluted to 1:400 in PBS with 3% (w/v) non-fat dried milk. Uninfected lice sections were used as negative controls. For culture, each louse was surface-decontaminated as described elsewhere2 and crushed in 500 μL of PBS. One hundred microliters were used to inoculate Columbia sheep blood agar (bioMérieux). Bacterial identification was made by the oxidase reaction (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom), microscopic examination after Gram staining (bioMérieux), and with API 20 NE strips (bioMérieux) according to the manufacturer’s instructions. Hemolymph of surviving infected lice was extracted by amputation of one or more legs under an enlarged microscope (Stemi 2000-C; Zeiss, Jena, Germany). Extracted hemolymph was immediately suspended in 100 μL of PBS and 70 μL were used to inoculate blood agar (bioMérieux). The remaining 30 μL were air-dried, methanol-fixed for five minutes, and tested by IFA.

Infection of lice feces. Approximately 0.1 mg of feces collected from each group was sampled three times a day and assayed by IFA, PCR, and culture. For the IFA, lice feces was sampled with a sterile cotton swab moistened with sterile water. For the PCR and culture, lice feces was sampled with the point of a needle (18 gauge, 1.2 mm; Terumo; Leuven, Belgium). Fecal excretion of Acinetobacter spp. was first assessed by an IFA using 30 μL of the anti-Acinetobacter spp. mouse polyclonal antibody diluted to 1:400. The IFA conducted on uninfected lice feces was used as a negative control. To detect Acinetobacter DNA from lice feces in the presence of PCR inhibitors,17 DNA was extracted using the high pure PCR template preparation kit (Roche Diagnostics GmbH, Mannheim, Germany), which contained an inhibitor blocker, as described elsewhere.23 In addition, 2 μL of bovine serum albumin (20 mg/mL) (Roche Diagnostics GmbH) was added to each PCR mixture to improve the PCR amplification. For culture, each sample was suspended in 500 μL of PBS and 100 μL were streaked onto agar plates and incubated at 37°C. Bacterial identification was performed as described earlier in this report.

Infection of lice progeny. The number of eggs produced by each louse group was recorded daily. Fisher’s exact test (Epi-Info version 6.0 software; Centers for Disease Control and Prevention) was used to compare the fertility of each of the four louse groups. Two eggs (starting from the laying of the first egg) and two larvae (starting from the hatching of the first egg) were sampled daily: one of each for PCR amplifications and the other for culture. The 18S rRNA PCR was used to control the efficiency of DNA extraction and the PCR. The recA-PCR was used to detect A. baumannii and the est-PCR was used to detect A. lwoffii. Before culture, each egg and larva was decontaminated as previously described.

RESULTS

Effect of infection on rabbits. The A. lwoffii-infected rabbit (R3) died on the first day post-infection, and rabbit R1, infected with the louse strain of A. baumannii, died on the second day post-infection. Only the rabbit infected with A. baumannii (CIP 53.79) (R2) remained asymptomatic throughout the experiment, although it developed an antibody response (IgG + IgM) to A. baumannii with a titer of 1:100 on the 7th and 14th days post-infection that increased to 1:200 on the 21st day post-infection and to 1:800 on the 28th and the 35th days post-infection. The β globin gene PCR always showed positive results with the 30 blood samples from the 3 infected rabbits (8 from R1, 16 from R2, and 6 from R3). This showed the efficiency of DNA extraction and the PCR. The A. baumannii (louse strain)–infected rabbit (R1) remained bacteremic until 40 days post-infection. Rabbit R2, which was infected with A. baumannii (CIP 53.79), remained bacteremic until 48 days post-infection, and the A. lwoffii (R3)–infected rabbit remained bacteremic until 23 days post-infection. The four nurse rabbits used to feed infected (R4, R5, and R6) and uninfected (R7) lice remained asymptomatic and seronegative throughout the experiment.
**Effect of the infection on lice.** Compared with the mean survival of uninfected lice (40 days), the survival of lice infected with *A. baumannii* (CIP 53.79) (38 days; \( P = 0.48 \)) and *A. lwofii* (40 days; \( P = 0.18 \)) was not significantly different (Figure 1). Only the survival of lice infected with the louse strain of *A. baumannii* (20 days) was significantly shorter than that of uninfected lice (odds ratio = \( \infty \), \( P < 10^{-2} \)). Interestingly, *A. baumannii* louse strain–infected lice showed a high mortality only on the second and third days post-infection with 33 (16.5%) lice dying on the second day post-infection and 47 (23.5%) lice dying on the third day post-infection. During these two days, only two lice infected with *A. baumannii* (CIP 53.79) died, but none of the uninfected and *A. lwofii*-infected lice died (Figure 1). During the first two days post-infection and starting from the fourth day post-infection until the death of the last infected louse, the mortality rate was low in all four louse groups (Figure 1). Throughout the experiment, all surviving and dead lice in the four louse groups were physically indistinguishable.

**Infection of lice.** The results of the 18S rRNA PCR done with 36 lice infected with the louse strain of *A. baumannii* (19 surviving and 17 dead), 60 lice infected with *A. baumannii* (CIP 53.79) (36 surviving and 24 dead), and 59 lice infected with *A. lwofii* (39 surviving and 20 dead) were always positive, showing the efficiency of DNA extraction and the PCR. The results of the *recA* PCR were always positive for the 36 lice infected with the louse strain of *A. baumannii* and the 60 lice infected with *A. baumannii* (CIP 53.79). The results of the *est* PCR performed on 59 lice infected with *A. lwofii* were always positive. The results of the *recA* and *est* PCRs for 53 uninfected lice (39 surviving and 14 dead) was always negative, but the results of the 18S rRNA PCR were always positive. The IFA (Figure 2) showed that the three *Acinetobacter* spp. exhibited similar localization within infected lice (surviving and dead), which demonstrated a diffuse infection. Uninfected lice always showed a negative result in the IFA. All cultures performed on 94 surviving infected lice (19 infected with the louse strain of *A. baumannii*, 36 with *A. baumannii* (CIP 53.79), and 39 with *A. lwofii*) and on 55 dead infected lice (16 infected with the louse strain of *A. baumannii*, 22 with *A. baumannii* [CIP 53.79], and 17 with *A. lwofii*) were always positive on the first day following inoculation. Only one colonial morphology, corresponding to *Acinetobacter* spp., was observed. The results of the cultures and IFA were always negative on the hemolymph extracted individually from 94 surviving infected lice (19 infected with the louse strain of *A. baumannii*, 36 with *A. baumannii* [CIP 53.79], and 37 with *A. lwofii*), and 55 dead infected lice (16 infected with the louse strain of *A. baumannii*, 20 with *A. baumannii* [CIP 53.79], and 16 with *A. lwofii*).

**Infection of lice feces.** Throughout the experiment, the 58 feces samples excreted by *A. baumannii*-infected lice with either strain (20 of *A. baumannii* [louse strain]–infected group and 38 of those infected with *A. baumannii* [CIP 53.79]) were always positive by the IFA (Figure 3A). The results of the cultures and IFA were always negative on the hemolymph extracted individually from 94 surviving infected lice (19 infected with the louse strain of *A. baumannii*, 36 with *A. baumannii* [CIP 53.79], and 37 with *A. lwofii*) and 52 dead infected lice (16 infected with the louse strain of *A. baumannii*, 20 with *A. baumannii* [CIP 53.79], and 16 with *A. lwofii*).

**Figure 1.** Survival rates over time of lice infected with *Acinetobacter baumannii* UR 10.72 (■), strain CIP 53.79 ( ), and *A. lwofii* strain CIP 64.101 ( ), and an uninfected control lice (△). Day 0 is the day of infection. Values are given as percentages of mean numbers of lice tested.

**Figure 2.** Detection of the louse strain of *Acinetobacter baumannii* by confocal microscopy in a 5 μm-thick section of an infected louse sampled on the second day postinfection (immunofluorescence stained, original magnification ×600). A high level of infection with *A. baumannii* is apparent throughout louse. Because of the louse dimensions, several photographs were taken and the body was reconstructed digitally. This figure appears in color at www.ajtmh.org.
of the fecal IFA were consistently positive. Uninfected lice feces always showed negative results in the IFA. The PCR (Figure 3B) used for detection of DNA of the *Acinetobacter* spp. in excreted feces exhibited the same kinetics reported for the IFA. The results of the *recA* PCR (detection of DNA of both *A. baumannii* strains) were consistently positive, and the results of the *est* PCR (detection of *A. lwoffii* DNA) were negative between day 0 and the 11th day post-infection, but remained positive between the 12th and 40th days post-infection. The same results were obtained by fecal culture. Cultures were positive one day following inoculation and only one colonial morphology was observed.

**Infection of lice progeny.** Lice of all four experimental groups began laying eggs on the fourth day post-infection that hatched 10 days later. The total number of eggs laid during the lifespan of surviving infected lice was 3,639 eggs by *A. baumannii* (louse strain)–infected lice, 6,212 eggs by those infected with *A. baumannii* (CIP 53.79), 6,391 eggs by *A. lwoffii*-infected lice, and 6,392 eggs laid by uninfected lice. The results of the PCR were consistently negative for the *recA* gene (17 eggs and 6 larvae from the *A. baumannii* [louse strain]–infected group, 35 eggs and 24 larvae from the *A. baumannii* [CIP 53.79]–infected group, and 37 eggs and 26 larvae from the uninfected group) and the *est* gene (37 eggs and 26 larvae from the *A. lwoffii*-infected group and 37 eggs and 26 larvae from the uninfected group). These samples were always positive by the 18S rRNA PCR, which showed the efficiency of DNA extraction and the PCR. All cultures of these samples were negative.

**DISCUSSION**

Our results demonstrate that the human body louse is able to acquire and maintain a persistent life-long infection with *A. baumannii* (both strains) and *A. lwoffii*. Until now, it was recognized that lice not infected with *R. prowazekii*, *Borrelia recurrentis*, or *Bartonella quintana*, as in our rearing, maintain a sterile midgut since lice feed only on sterile host blood. The infection of the human body louse with each of these three bacteria is mainly due to the fact that they induce relapsing bacteremia, rather than to a specific adaptation to the body louse. In addition to the isolation of *A. baumannii* from the body lice of homeless patients, La Scola and Raoult found that 21% of body lice collected worldwide were naturally
infected with *A. baumannii*. However, it is still unknown how all these body lice acquire their infections with *A. baumannii*. This infection can occur mainly after the ingestion of an infective blood meal from patients with ongoing bacteremia, or possibly by passage through the human skin while feeding. However, La Scola and Raoult reported that no common skin commensal agent has been isolated from body lice. In this study, the laboratory-induced infection of the body louse with *Acinetobacter* spp. occurred as a generalized septicemia throughout the infected louse (Figure 2). However, the hemolymph was found not to be infected. This discrepancy may be explained by the occurrence of a transitional passage, which we could not detect, of *Acinetobacter* spp. into the hemolymph following the burst of the most heavily infected cells, which resulted in the infection of all louse tissues. Only the louse strain of *A. baumannii* was pathogenic for body lice, which died 20 days earlier than the uninfected lice (*P* < 10^{-2}) (Figure 1). An increased mortality rate was observed between the second and third day post-infection, which could be explained by the intensive multiplication of the bacteria. However, we could not explain why 60% of the lice infected with the louse strain of *A. baumannii* had an infection for the remainder of their lifespan following the third day post-infection, or the difference in the behavior of the two strains of *A. baumannii* in the body louse, since the second strain was not pathogenic (*P* = 0.48). La Scola and Raoult have reported nucleotide variations in the recA gene and a difference in the ampicillin susceptibility among *A. baumannii* strains. Currently, only *R. prowazekii* is known to be pathogenic for the body louse, which develops a bright red color a few hours before death due to the spread of the ingested blood meal into the hemolymph following the rupture of infected cells and subsequent to the strict intracellular multiplication of *R. prowazekii*. However, in our experiment, *Acinetobacter* spp.–infected lice did not exhibit this red color.

Infected body lice began the fecal excretion of both strains of *A. baumannii* on the first day post-infection and of *A. lwoffii* on the 11th day post-infection. Moreover, *Acinetobacter* spp. excreted within feces were viable. In addition, *A. baumannii* and *A. lwoffii* have been reported to have the ability to survive for a long time on dry surfaces. Louse feces containing living bacteria are recognized to constitute a major source of infection to humans, in addition to contamination of their crushed bodies. Thus, the infested feces could transmit the *Acinetobacter* infection to the host (humans) either by contamination of the bite wound site, when scratched into the skin or via an aerosol. Until they died, the infected lice fed regularly, mated, and continued to produce and deposit eggs on the fourth day post-infection, which hatched 10 days later. Since eggs and larvae were not infected, we conclude that transovarial transmission does not occur in lice, as with the three louse-transmitted bacteria. This shows that *Acinetobacter* spp. is only acquired by lice through feeding on host (rabbit) and that its presence presupposes a bacteremia in the host.

The number of bacteria given in our study to the rabbit host (4.5–5.26 × 10^{10} CFU/mL of rabbit blood) was comparable to levels described during human infections with *Acinetobacter* species. It has been reported that the bacterial count in patients with ventilator-associated pneumonia due to *Acinetobacter* species was 10^{8} CFU/mL. Moreover, an inoculum of 5 × 10^{9} CFU of *A. baumannii* has been used in an experimen-