

NOTES

Experimental Model of Human Body Louse Infection Using Green Fluorescent Protein-Expressing *Bartonella quintana*

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A laboratory colony of human body lice was experimentally infected by feeding on rabbits made artificially bacteremic with a green fluorescent protein-expressing *Bartonella quintana*. *B. quintana* was detected in the gut and feces until death but not in the eggs. The life span of the lice was not modified. The rabbit model should provide valuable clues to the role of lice in the transmission of *B. quintana*.

Bartonella quintana is a fastidious gram-negative bacterium that is regarded as a reemerging human pathogen (1) and is responsible for various human diseases (12). Although trench fever, the first clinical manifestation of *B. quintana* infection to be recognized (13), affected thousands of soldiers during World Wars I and II, medical interest in trench fever waned for almost 30 years because the disease was only rarely encountered. In the 1990s, *B. quintana* was identified as an agent of bacillary angiomatosis in AIDS patients (17), endocarditis (5, 16, 18), chronic bacteremia (3, 19), and chronic lymphadenopathy (15). These diseases are associated with homelessness or cramped, unhygienic circumstances, together with cold weather and the presence of body lice. The role of lice had been observed as early as 1920 (4). Various experiments have demonstrated that the disease could be induced in human volunteers (20) and *Macacus rhesus* monkeys (14) by injection of *B. quintana* and that the bacterium multiplied in the gut lumen of naturally (21) or intrarectally (8) infected lice without interfering with viability and was excreted in their feces (4, 8). However, despite growing interest in louse-transmitted diseases, there is no currently available experimental model to describe the relationship between *B. quintana* and the louse.

GFP-expressing *B. quintana*. *B. quintana* strain Oklahoma (ATCC 49793) was obtained from the American Type Culture Collection (Rockville, Md.) and cultivated as previously described (10). Plasmid pJMBGFP, containing the *B. bacilliformis* flagellin promoter and a Rep origin of replication obtained from pBBR1-MCS2 (9) (Table 1), was provided by James M. Battisti (Division of Biological Sciences, University Montana, Missoula, Mont.). It was extracted and purified from *Escherichia coli* using a Midi Prep kit (Qiagen, Inc., Chatsworth, Calif.) and then adjusted to 0.5 µg/ml. Transformation proce-

dures were as previously described (6). The transformed bacteria were cultivated on 5% sheep blood agar containing 25 µg of kanamycin sulfate per ml and were further verified as *B. quintana* by PCR amplification of the 16S-23S rDNA intergenic spacer (*its*) followed by sequencing (6). The presence of plasmid DNA was checked as previously described (6).

Optimal transformation efficiencies were obtained at 6 ms for a field strength of 12.5 kV/cm and a plasmid DNA amount of 60 to 80 ng but were low, ranging from 3×10^{-5} to 7×10^{-5} . Colonies of green fluorescent protein (GFP)-expressing *B. quintana* began to appear at approximately 12 to 16 days following electroporation, which represents a growth lag time of approximately 8 to 12 days compared with the growth of untransformed bacteria on plate-to-plate passage. The colonies were typically smaller than those formed by untransformed *B. quintana* at the same stage of growth. When the colonies were subcultured on selective media, GFP expression was conserved after 15 passages, suggesting that plasmid maintenance was stable in the presence of antibiotic, but was lost after 5 passages in the absence of antibiotic. No growth was obtained on selective media with wild-type *B. quintana* prepared under the same conditions or *B. quintana* electroporated without DNA. Sequencing of PCR-products amplified from the *its* revealed 100% identity to known sequences of *B. quintana*, confirming that the transformants were *B. quintana*.

Experimental body louse infection. Body lice (*Pediculus humanus corporis*, strain Orlando) were kindly provided by D. Richard-Lenoble (Laboratoire de Parasitologie, Faculté de Médecine, Tours, France). A colony of lice was established and nourished daily on the shaved abdomen of specific-pathogen-free (SPF) New Zealand White rabbits (Fig. 1a). The lice were shown to be free from *B. quintana* by periodic *its* PCR amplification of samples from their gut and feces. One SPF rabbit, designated R1, was first injected intravenously with 2.5 ml of a solution of 10 mg of kanamycin (Sigma, St. Louis, Mo.) per ml, and 15 min later was injected with 20 ml of a suspension of 10^6 CFU of pJMBGFP-expressing *B. quintana* per ml in saline,

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TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Genotype or relevant characteristics	Reference or source
Strains		
<i>E. coli</i> DH5	F ⁻ (Φ 80 <i>dlacZ</i> Δ <i>M15</i>) Δ(<i>lacZY AargF</i>) <i>U169 deoR recA1 endA1 hsdR17</i> (r _K ⁻ m _K ⁺), <i>supE44</i> λ- <i>thi-1 gyrA96 relA1</i> λ-CH616 ‘Oklahoma’	Gibco-BRL (7)
<i>B. quintana</i>		
Plasmids		
pBBR1-MCS2	Km ^r Mob Rep origin, <i>Bartonella</i> shuttle vector	Michael Kovach (7)
pFPV25	Source of promoterless GFP (<i>gfpmut3a</i>)	Raphael Valdivia
PJMBGFP	PBBR1-MCS2 with a 970-bp <i>Hind</i> III fragment containing <i>B. bacilliformis</i> flagellin promoter (<i>fla-pro</i>) upstream of <i>gfpmut3a</i>	James M. Battisti (2)

before 800 15-day-old lice, half of which were female, were allowed to feed on its abdomen. The lice were then kept at 30°C and 70% humidity. The day of infection was referred to as day 1. On the following days, lice were allowed to feed daily on a second SPF rabbit designated R2. Before the lice were allowed to feed each day, R2 was injected intravenously each

day with 2.5 ml of 10 mg of kanamycin sulfate (Sigma) per ml. To detect *B. quintana*, *its* amplification by PCR and culture on kanamycin-containing agar for up to 60 days at 37°C under a 5% CO₂ atmosphere were performed on blood drawn from the infected rabbit after the lice had fed then every day for 5 days, and then once a week for 2 months. Similar tests were per-

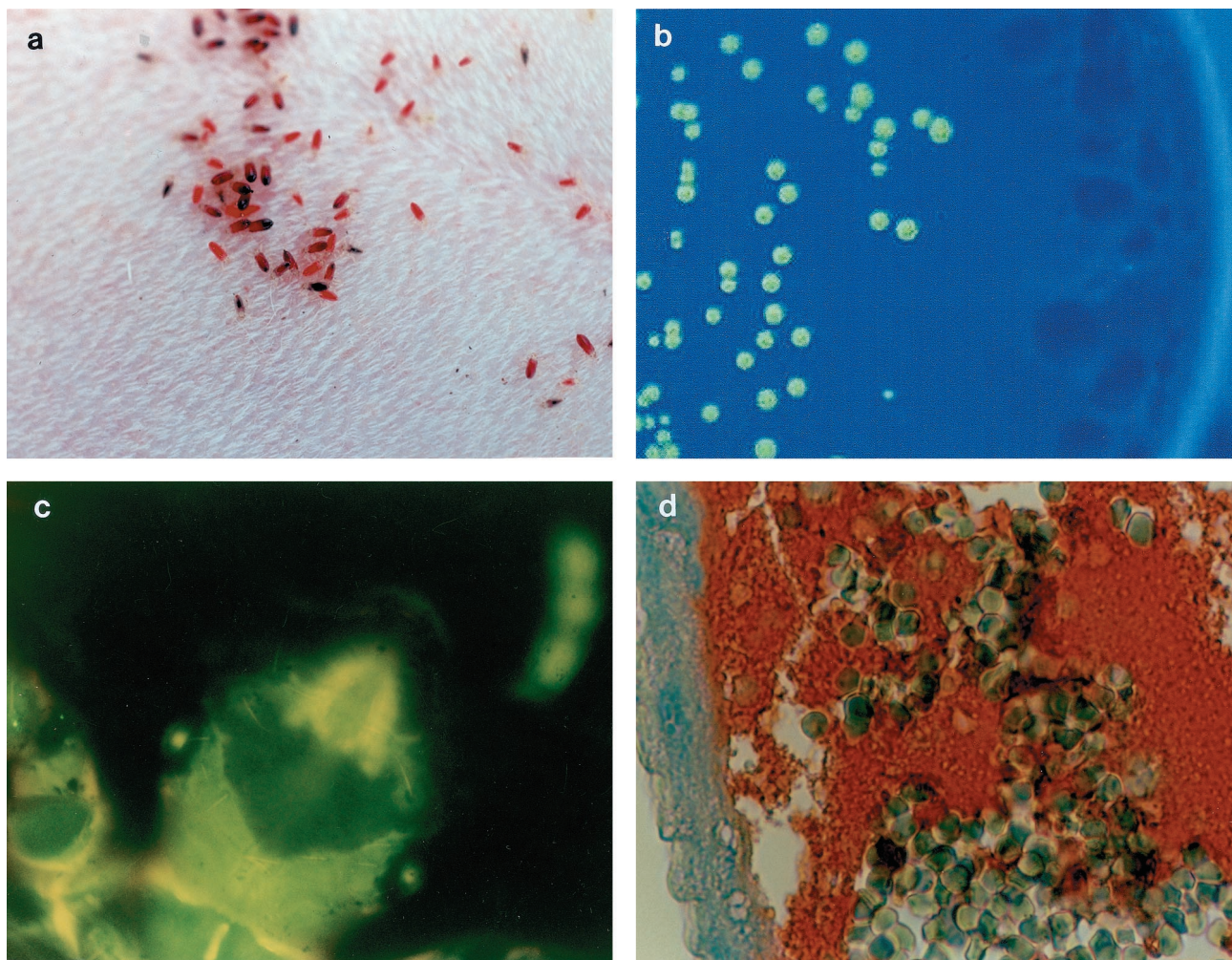


FIG. 1. Experimental model of body louse infection by GFP-expressing *B. quintana*. (a) *Pediculus humanus humanus* feeding on a rabbit; (b) fluorescent colony isolated on selective agar from an infected louse 10 days after the initial infection; (c) autofluorescence of body lice; (d) immunohistological detection of *B. quintana*. Note the numerous erythrocytes and the clusters of bartonellae in the intestine lumen (red clumps) and against the intestine wall (blue). Streptavidin-biotin-peroxidase method, polyclonal rabbit anti-*B. quintana* used at a dilution of 1:400, hemalun counterstain. Magnification, $\times 660$.

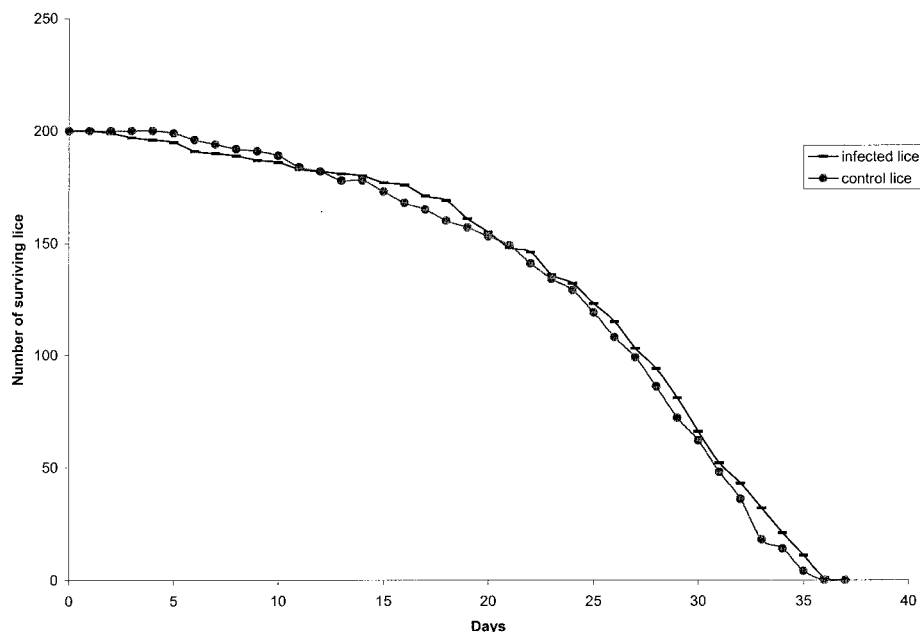


FIG. 2. Comparison of the survival rate over time of 200 lice infected with *B. quintana* and 200 control lice. Day 0, day of infection.

formed on 15 lice harvested after feeding on the infected rabbit, 15 lice harvested daily for 1 week and twice a week after that until no lice from the first generation remained, and, when present, eggs, larvae, and feces. Blood samples drawn from the infected rabbit (R1) were positive for *B. quintana* by culture and PCR on days 1 and 2, with 2×10^4 /ml and 7×10^2 CFU/ml, respectively, but were negative on day 3 and at all later times. On follow-up, the rabbit did not present with any abnormal symptoms but developed an anti-*B. quintana* immunoglobulin G response by day 15, with a titer of 1:400 as determined by indirect immunofluorescence. Blood drawn from rabbit R2 was negative by both culture and PCR, and they did not seroconvert. GFP-expressing *B. quintana* was grown and PCR amplified from all groups of lice. Between 7 and 20 CFU of *Bartonella* (mean \pm standard deviation = 16.3 ± 2.9) was obtained from each louse (Fig. 1b). The number of CFU did not decrease over time. *B. quintana* was also grown and PCR amplified from fecal samples harvested throughout the experiment. Qualitative but not quantitative analysis of the colonies grown from infected feces was performed. Only fluorescent colonies grew. The GFP marker was therefore very useful for directly identifying *B. quintana* colonies (Fig. 1B). None of the uninfected control lice were positive by culture or PCR analysis. The average daily number of eggs from the infected group was 682.8 ± 335.1 and was not significantly different from the 705.2 ± 357.9 observed in the control group ($P = 0.51$). No growth or PCR amplification of *B. quintana* was obtained from the 300 eggs and 100 larvae tested. Unfortunately, we observed that body lice were autofluorescent, and therefore detection of GFP-expressing *Bartonella* was not possible (Fig. 1c).

For histological examination, five lice collected on each of days 0, 3, 5, 7, and then once a week were fixed in 10% formalin overnight, paraffin embedded, and cut to 5- μ m thickness. Hematoxylin-eosin stain was used to visualize the gut system, and

Warthin-Starry stain was used to detect bartonellae. Immunohistochemistry using anti-*B. quintana* polyclonal antibodies (11) was performed on 5- μ m-thick formalin-fixed and paraffin-embedded gut sections. For each section, a negative control was prepared using gut sections from uninfected lice. The gut system was easy to recognize by its thin wall and large lumen. *Bartonella* were identified as dense clusters of bacteria in the intestinal lumen. Masses of bacteria occupied an extracellular location (Fig. 1d).

To determine whether *B. quintana* would influence louse mortality, 200 infected lice, half of which were female, were fed daily on rabbit R2 and were compared with 200 control lice, half of which were female, that fed only on an SPF rabbit (R3). The number of dead lice in both groups was compared using a Kaplan-Meier life table (GB-STAT version 6.5; Dynamic Systems Inc., Silver Springs, Md.), and the equality of survival between the two populations was estimated using the generalized Wilcoxon test. No significant difference in the mortality rate was observed between infected and control lice ($P = 0.49$) (Fig. 2).

In conclusion, we report the first experimental animal model of body louse infection by using a laboratory colony of lice feeding on a rabbit with a GFP-expressing *B. quintana* bacteremia. The lice maintained an asymptomatic extracellular infection inside their gut during their entire life but did not transmit *B. quintana* to the next generation, supporting the role of body lice as vectors, but not reservoirs, for *B. quintana*. This rabbit model of human body louse infection may be valuable for other louse-transmitted pathogens.

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