JOURNAL OF CLINICAL MICROBIOLOGY, May 2001, p. 1707–1709 Vol. 39, No. 5
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.5.1707–1709.2001

Detection and Culture of *Bartonella quintana, Serratia marcescens*, and *Acinetobacter* spp. from Decontaminated Human Body Lice

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Received 13 October 2000/Returned for modification 29 December 2000/Accepted 20 February 2001

As part of a survey for trench fever among homeless people in Marseilles, France, we attempted isolation of *Bartonella quintana* from body lice. A decontamination protocol of immersion in 70% ethanol with 0.2% iodine was devised and was tested with a laboratory colony of body lice. Lice which had been experimentally contaminated with either *Escherichia coli, Staphylococcus epidermidis*, or *Acinetobacter* spp. were successfully decontaminated, and this process did not prevent the culture of *B. quintana* from these lice. One hundred sixty-one lice obtained from homeless patients were studied by the protocol. *B. quintana* was isolated on axenic medium from 15 of 161 body lice and was detected in 41 of 161 lice by PCR. *Acinetobacter* spp. and *Serratia marcescens* were also isolated from body lice. The sensitivities of PCR and culture of *B. quintana* were 98 and 36%, respectively. These procedures may be useful for epidemiologic studies of trench fever and for the recovery of strains for characterization and comparison.

Human infections due to *Bartonella* species are widely considered emerging diseases, although they also include long-recognized syndromes such as Carrion's disease due to *Bartonella bacilliformis*, trench fever due to *Bartonella quintana*, and cat scratch disease due to *Bartonella henselae* and possibly *Bartonella clarridgeiae* (7, 8, 13). Newer clinical manifestations (such as bacillary angiomatosis caused by both *B. henselae* and *B. quintana*; peliosis hepatitis due to *B. henselae*; chronic lymphadenopathy due to *B. quintana*; and endocarditis due to *B. henselae, B. quintana*, and in one case each *Bartonella elizabethae, Bartonella vinsonii* subsp. *berkfofii*, and, possibly, *Bartonella vinsonii* subsp. *arupensis*) have recently been identified (1, 13, 15, 20, 26). Trench fever, the first reported clinical manifestation of infection with *B. quintana*, was reported during World War I, when it was estimated to have affected more than 1 million people (15). Body lice were shown to be the vectors, and improvements in hygienic conditions have prevented large outbreaks since then. A reemergence of trench fever has been observed in Marseilles, France (2); Seattle, Wash., and Baltimore, Md. (4, 6, 24); Peru (17); Russia (23); and Burundi (18). Homeless people and alcoholics are most at risk for *B. quintana* infections in developed countries. We have demonstrated that body lice play a role in the transmission of urban trench fever, as previously observed under wartime conditions (2, 3). However, direct isolation of *B. quintana* from its vector has never been achieved; previous studies demonstrating the association of *B. quintana* with body lice were based on morphologic data, with identification of rickettsia-like bacteria within the gut of lice following nonspecific staining (9) or on the inoculation of louse feces into humans, uninfected lice (by intrarectal inoculation), an egg yolk sac, and blood agar plates (9, 25). Recent studies have been performed by PCR-based methods with infected body lice (2, 18).

When not infected by *B. quintana, Borrelia recurrentis*, or *Rickettsia prowazekii*, body louse midguts are sterile (lice feed only on sterile human blood) (19). However, as lice live on the skin and in the clothes of humans (usually under poor hygienic conditions), their external surface is highly contaminated. Thus, our work consisted of, first, the design of a protocol for the surface decontamination of laboratory colony body lice that still permitted isolation of *B. quintana* directly from living lice and, second, application of the protocol to lice in the context of a survey of trench fever among the homeless population in Marseilles, France.

MATERIALS AND METHODS

Source of lice. Body lice were obtained during a study performed in January 2000 in two homeless shelters that involved examination of 250 homeless people. Informed consent was provided by all participants. On presentation everyone was given a full physical examination and body lice were sought. Body lice were transferred to sterile plastic tubes and were processed for culture within 7 h.

Laboratory-raised lice (*Pediculus humanus corporis* strain Orlando) were kindly provided by D. Richard-Lenoble (Laboratoire de Parasitologie, Faculté de Médecine, Tours, France). A colony of these lice was maintained by daily feeding on the shaved abdomens of specific-pathogen-free (SPF) New Zealand white rabbits in a controlled, arthropod-free facility, the University Animal Facility. Lice were regularly monitored for *B. quintana* by periodic PCR amplification of material derived from their guts and feces. Body lice experimentally infected with *B. quintana* were obtained as follows. One SPF rabbit was intravenously injected with 20 ml of a suspension of 10^6 CFU *B. quintana* per ml in saline. *B. quintana* strain Oklahoma (ATCC 49793) was obtained from the American Type Culture Collection (Rockville, Md.). Fifteen minutes following the injection of bacteria, 15-day-old lice were allowed to feed on the rabbit and were then kept at 30°C with 70% humidity before being processed for inoculation onto agar plates. Laboratory lice were also superficially contaminated with a high-concentration bacterial inoculum. Three bacterial suspensions at a concentration of 10^7 CFU/ml, containing *Staphylococcus epidermidis, Escherichia coli*, or *Acinetobacter anitratus* were prepared on the day of the experiment. The *E. coli* and *S. epidermidis* strains used in this study were isolated from clinical samples in our laboratory. The *A. anitratus* strain was isolated from a wild body louse. Bacteria were grown at 37°C with 5% CO_2 on Columbia sheep blood agar (Biomérieux, Marcy l'Etoile, France). Fifteen-day-old laboratory body lice were allowed to feed on an SPF rabbit. They were then immersed for 15 min in one of the bacterial suspensions and put onto absorbent paper in order to remove excess fluid.

Decontamination procedure. Each louse was 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. This procedure was used to decontaminate 161 wild

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human body lice, 10 lice experimentally infected with *B. quintana*, 25 lice superficially contaminated with *S. epidermidis*, 25 lice superficially contaminated with *E. coli*, and 25 lice superficially contaminated with *A. anitratus*.

In order to evaluate the efficiency of the decontamination procedure, 25 lice superficially contaminated with either *S. epidermidis, E. coli*, or *A. anitratus* were immersed in sterile distilled water instead of iodinated ethanol.

Isolation procedure. After the decontamination procedure, lice were allowed to dry and were cut in half longitudinally with a sterile surgical blade in a sterile petri dish. One half was put in a sterile tube and frozen for later use in a PCR amplification-based study. The other half was cut into small pieces with the same sterile surgical blade and then crushed on the bottom of the petri dish with a sterile cotton swab moistened with 2 drops of sterile water. The crushed louse was then removed from the petri dish by using the swab used for crushing and was inoculated onto a sheep blood agar plate. All plates were placed in transparent polyethylene bags and were incubated at 37°C in 5% CO_2 (Genbag CO_2 system; Biomérieux). The plates were examined weekly for evidence of growth for up to 3 months. Examination of plates was made without opening the bags in order to avoid desiccation of the agar. When *Bartonella*-like colonies were observed, confirmation of their identity was achieved by the methods outlined below.

Identification of *B. quintana* isolates. Presumptive identification of isolates from wild lice was made by determination of oxidase and catalase reactions and microscopic examination after Gram and Gimenez staining. Definitive identification was performed by microimmunofluorescence assay with a *B. quintana*-specific monoclonal antibody. Briefly, plate-grown bacteria were harvested and suspended in phosphate-buffered saline. Bacteria were then placed on a 24-well microscope slide with a pen nib. A suspension of *B. henselae* (strain Houston 1) was used as a negative control, and a suspension of *B. quintana* (strain Oklahoma) was used as a positive control. The microimmunofluorescence assay was performed by using Bq73H4, a *B. quintana*-specific monoclonal antibody, as described previously (12).

Identification of non-*B. quintana* isolates. Presumptive Identification was made by determination of oxidase and catalase reactions, microscopic examination after Gram staining, and use of API 20E and API 20 NE strips (Biomérieux) according to the manufacturer's recommendations. Definitive identification of these isolates was performed by amplification and sequencing of the 16S rRNA gene followed by comparison of the sequence with sequences in the GenBank DNA sequence database with the program BLAST (version 2.0; National Center for Biotechnology Information) as described previously (10).

Detection of *B. quintana* in lice. Each half louse was separately crushed in sterile water. DNA extracts were prepared from crushed lice with the QIAmp tissue kit (Qiagen) according to the manufacturer's instructions. Detection of *Bartonella* spp. was performed by amplification and sequencing of the intergenic spacer region as described previously (11). The sequences were compared with sequences in DNA sequence databases by using the program BLAST (version 2.0; National Center for Biotechnology Information). Negative controls were 10 unifected lice, and the positive control was a suspension of *Bartonella elizabethae*. As a control for PCR amplification we used the 18Saidg-18Sbi primer pair, which allows amplification of a 18S rRNA gene fragment of arthropods. Consensus forward primer 18Saidg (5′-TCTGGTTGATCCTGCCAGTA-3′) was determined after alignment of the 18S rRNA gene sequences of *Drosophila melanogaster* (GenBank accession number M21017) and *Aedes aegypti* (GenBank accession number M95126). The consensus reverse primer 18Sbi primer was that described by DeSalle et al. (5).

RESULTS

The decontamination procedure was efficient. Only 6 of 25 of the cultures performed with lice that had been experimentally contaminated with *S. epidermidis* and then infected with iodinated alcohol were positive, and in those 6 cultures, three or fewer colonies appeared on each plate. Only 7 of 25 of the cultures performed with lice experimentally infected with *E. coli* were positive (positive cultures yielded less than eight colonies per plate). None of the cultures performed with lice experimentally infected with *A. anitratus* and then decontaminated were positive after decontamination. All the cultures performed with contaminated lice that had not been subjected to decontamination yielded more than 1,000 colonies per plate, indicating that the decontamination process led to a 1,000-fold decrease in the number of viable bacteria associated with the surfaces of the body lice.

TABLE 1. Results of *B. quintana* detection in 161 body lice tested by the procedure used for detection

PCR result	Culture result		Total
	+	−	
+	14[a]	27	41
−	1	119	120
Total	15	146	161

[a] Values are number of body lice.

B. quintana was detected by culture and PCR in the 10 experimentally *B. quintana*-infected lice. Although two agar plates were contaminated by *Staphylococcus* spsp. (one colony and three colonies, respectively), this did not prevent detection of *B. quintana* colonies.

One hundred sixty one-lice were collected from the 35 homeless patients, with 1 to 11 lice collected per patient. *B. quintana* was isolated from 15 of 161 (9.3%) of the lice obtained from eight different homeless people (Table 1). PCR amplification of an 18S rRNA gene fragment was positive for all 161 lice, demonstrating the lack of PCR inhibitors in lice. PCR amplification of the intergenic spacer region was negative with the 10 negative control lice and positive with the suspension of *B. elizabethae. B. quintana* was detected by PCR in 41 of 161 (25.5%) lice obtained from 14 homeless patients. Only one culture-positive louse was PCR negative. In total, 14 of 35 (40%) homeless patients harbored *B. quintana*-infected lice. Among the cultures attempted with the 161 wild human body lice, non-*B. quintana* colonies were detected in 10, of which 4 were *Serratia marcescens*, 4 were *A. anitratus*, and 2 were *A. baumanii*. One of the isolates of *A. anitratus* was later used to test our decontamination protocol. In all cases more that 1,000 colonies were detected per plate. All isolates of non-*B. quintana* bacteria were obtained from lice that tested negative by the *B. quintana*-specific PCR. The six *Acinetobacter* sp. strains and four *S. marcescens* strains were isolated from seven different patients. Thus, as 42 lice were positive for *B. quintana* by a combination of culture and PCR, the sensitivity of culture was estimated to be 36% (15 of 42 lice) and the sensitivity of PCR was estimated to be 98% (41 of 42 lice) ($P < 10^{-5}$ by the chi-square test).

DISCUSSION

We have demonstrated the efficiency of a 5-min immersion of lice in iodinated alcohol for superficial decontamination of the lice. We have previously used this protocol to isolate *Rickettsia* spp. from ticks (16) and cat fleas (unpublished data). This decontamination step is particularly useful for isolation of *Bartonella* spp., which are highly susceptible to most antibiotic agents, thus preventing the use of selective media (14). Even when the protocol led to incomplete decontamination, only a very few viable organisms remained, and it is likely that their presence would not have hampered isolation of *B. quintana*. Indeed, contamination by *Staphylococcus* spp. in two cases did not prevent isolation of *B. quintana*. Importantly, our protocol did not appear to have any detrimental effect on the isolation of *B. quintana*. All experimentally infected lice that were subjected to body surface decontamination yielded positive cultures. Isolations of very large numbers of *A. anitratus, Acineto-*

bacter haemolyticus, and *S. marcescens* were made from 10 of 161 wild body lice. We cannot determine if these organisms were derived from superficial or internal contamination. However, as our protocol proved to be efficient for surface decontamination of *A. anitratus*, the recovery of *Acinetobacter* spp. following decontamination suggests the presence of these bacteria within lice, possibly within the intestinal tract. As body lice ingest only blood, the sole route for colonization of the intestinal tract by *Acinetobacter* spp. or *S. marcescens* would be from the blood of patients with ongoing bacteremia. Moreover, since we found these bacteria in 10 lice collected from seven different patients, our findings may hint at the possible transmission of these bacteria by body lice.

Since we were able to obtain 10 isolates from all experimentally infected lice and 15 isolates from the 161 body lice collected from homeless people, our work demonstrates for the first time that *B. quintana* can be isolated directly from wild body lice. *B. quintana* infection is thought to be transmitted by infected feces from infected lice to human beings, as *B. quintana* survives well in louse feces, which remains infectious for up to 1 year (9). *B. quintana* multiplies in the louse intestine and can be recognized by staining of sections of the intestine (9, 25). Isolation of *B. quintana* on blood agar plates from the feces of lice that have been inoculated intrarectally with *B. quintana* or that have fed on trench fever patients has been reported (25). Nevertheless, collection of body lice from infested patients is easily performed, whereas collection of louse feces from such patients would be very difficult.

PCR detection of *B. quintana* in naturally infected lice appears to be a more sensitive technique than direct culture, thus underlining the potential of PCR for analysis of body lice (22). However, PCR and culture were equally and absolutely sensitive methods for detection of experimentally infected lice, whereas as the experimentally infected lice were all adults that had fed just before being processed, natural louse populations collected from homeless people contained a combination of adult, young, and unfed lice. One louse collected from a homeless patient was culture positive and PCR negative. As no PCR inhibitors were detected in this louse, we think that this discrepancy could be due to an incorrect dissection, with culture performed with the abdomen and PCR performed with the head.

Isolation of *B. quintana* from body lice allows assessment of the infectious status of a population that harbors body lice without performing blood sampling. Furthermore, isolation of strains allows epidemiologic studies for *B. quintana* within and between different populations. PCR-based procedures do not allow these epidemiologic studies to be performed; in addition, the most discriminatory tool for the differentiation of *B. quintana* strains is pulsed-field gel electrophoresis (21), for which the isolation of bacteria is prerequisite. As *B. quintana* may survive for long periods in louse feces (9), it is possible that laboratory processing of crushed lice may be successfully performed some time after sampling. Thus, when epidemiologic investigations are required in areas that lack suitable facilities, body lice may be sent by mail to specialized laboratories for culture and PCR. Because of its high degree of sensitivity, PCR detection will allow determination of the prevalence of disease in the population. Finally, the present study raises the possibility that *Acinetobacter* spp. and *S. marcescens* may be transmitted by lice, as their isolation from body lice is reported here for the first time.

ACKNOWLEDGMENT

We are indebted to Richard J. Birtles for reviewing the manuscript.

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