Body Lice as Tools for Diagnosis and Surveillance of Reemerging Diseases

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Body lice are vectors of three bacteria which cause human disease: *Rickettsia prowazekii*, the agent of epidemic typhus; *Bartonella quintana*, the agent of trench fever; and *Borrelia recurrentis*, the agent of relapsing fever. A recrudescence of body lice is being observed as the numbers of individuals living under social conditions which predispose individuals to infestation have increased. Because this phenomenon may lead to the reemergence of infections transmitted by body lice, we aimed to assess the occurrence and prevalence of the three agents described above in more than 600 body lice collected from infested individuals in the African countries of Congo, Zimbabwe, and Burundi, in France, in Russia, and in Peru. The presence of the three bacteria in each louse was determined by specific PCR amplification, and the identities of the organisms detected were confirmed by determination of the nucleotide base sequences of the amplification products. Using this approach, we were able to confirm the presence of *R. prowazekii* in lice collected from refugees in Burundi, among whom typhus was epidemic, and the presence of *B. quintana* in lice collected from all locations except the Congo. *B. recurrentis* was never found. Molecular approaches are convenient tools for the detection and identification of bacterial DNA in body lice and for the epidemiological study of louse-borne bacteria from countries where no medical and biological laboratory facilities are available.

Lice are highly successful ectoparasites, and most species of mammals and birds are infested by at least one louse species (2). Body lice have long been recognized as human parasites and have been associated with disease for many years (48). Although pediculosis is typically highly prevalent among members of rural communities in upland areas of countries close to the equator, it is now being increasingly encountered in developed, temperate countries in association with the dramatic rise in the numbers of homeless or inner-city, economically deprived populations. Human body lice have not been shown to be capable of carrying or transmitting any type of virus, but they are recognized as the vectors of three different pathogenic bacteria: *Rickettsia prowazekii*, the agent of the epidemic typhus; *Bartonella quintana*, first described as the agent of trench fever; and *Borrelia recurrentis*, which causes relapsing fever (5). Typhus persists in cold climates and under circumstances in which people are crowded together under poor sanitary conditions which prevent the regular changing and washing of clothing. The principal geographical areas which remain affected are the upland countries of Central Africa and South America, although sporadic outbreaks occur elsewhere, possibly resulting from the recrudescence of latent infection known as Brill-Zinsser disease (20). In 1997, a huge outbreak of epidemic typhus occurred in Burundi among refugees displaced following the onset of civil war (29), and a small outbreak has been reported in Russia (43). Louse-borne relapsing fever has been epidemic in Africa throughout the 20th century. Although cases of louse-borne relapsing fever were first recognized in Ethiopia, cases have also been encountered in neighboring countries such Sudan, Uganda, Burundi, and Rwanda during the 1980s (25). The foci of relapsing fever persist in the highlands of Ethiopia and the Andean foothills (9, 41). Trench fever was epidemic during both world wars, and sporadic cases

have been reported in Africa, Europe, Japan, China (21), and, more recently, Mexico (44, 45). The reemergence of *B. quintana* as an organism of medical importance has also been noted recently. It has been described as the agent responsible for trench fever (39), bacillary angiomatosis (17, 18, 22, 32), bacteremia (38), endocarditis (11, 28, 37), and chronic lymphadenopathy (27) in immunocompromised patients and in homeless people in Europe and North America.

Because body lice and their associated pathologies are generally encountered in areas where medical and biological assistance is limited, local assessment of their roles as sources of infection is difficult. Lice are easy to collect and to transport to reference laboratories where suitable molecular biological approaches can be used. Over the last year we have demonstrated the usefulness of this approach in numerous situations, and as a result, we propose a standard protocol for its use.

MATERIALS AND METHODS

Body lice were collected by local investigators or medical staff from our laboratory in the following countries: Congo, Zimbabwe, Burundi, France, Russia, and Peru. The body lice were transported or sent to Marseille, France, in plastic boxes without specific temperature or hydrometric precautions. The sources of the body lice used in this study are presented in Table 1. Lice were stored without regard to their storage conditions. The delay between the time of collection of the lice and the time of analysis was from 1 day to 4 months.

Each louse was placed in an Eppendorf tube and was then crushed with a sterile pipette tip. DNAs were prepared from the crushed lice by the using reagents in the QIAamp Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions, except that incubation in lysis buffer was performed overnight at 37°C. The extraction effectiveness and the absence of PCR inhibitors was assessed by PCRs incorporating broad-range 18S rRNA gene primers (18sai and 18sbi5.0) as described previously (10).

To perform the PCR amplifications, we chose genus-specific primers. In the course of our investigations we used different pairs of genus-specific primers, but we describe for the protocol presented here those with the best sensitivities and specificities. We tested the ability of each primer pair to amplify DNAs of all members of the target genus. The specificities of the primers were also assessed by incorporating DNAs derived from a wide range of the bacteria into amplification reactions, including DNAs from the following strains: *Rickettsia rickettsii* R (ATCC VR-891), *Ehrlichia chaffeensis* 91HE17, *Bartonella elizabethae* F9251 (ATCC 49927), *Borrelia burgdorferi* B31 (ATCC 35210), and *Coxiella burnetii* Nine Mile. The following bacteria are clinical strains obtained from La Timone Hospital in Marseille: *Brucella melitensis*, *Escherichia coli*, *Salmonella typhi-*

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TABLE 1. Sources of body lice and PCR results

Country	Population sampled or time of sampling	No. of samples	No. of samples with the following result:		
			R. prowazekii positive	*B. quintana* positive	*B. recurrentis* positive
Congo	Inhabitants of refugee camps	7	0	0	0
Zimbabwe	Homeless people in Harare	12	0	2	0
Burundi	During typhus outbreak				
	Burundi jail	10	2	0	0
	Burundi refugee camp	63	22	6	0
	After typhus outbreak (refugee cap)	91	0	13	0
France	Homeless people in Marseille	75	0	3	0
Russia	Homeless people in Moscow	268	0	33	0
Peru	Rural communities	73	0	1	0

murium, *Proteus mirabilis*, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, *Campylobacter jejuni*, *Chlamydia trachomatis*, *Staphylococcus aureus*, *Clostridium perfringens*, *Enterococcus faecalis*, and *Listeria monocytogenes*. Sensitivity was determined as follows. The bacteria *B. elizabethae*, *Borrelia burgdorferi*, and *R. rickettsii* were diluted, deposited on a slide, and colored by Gimenez or Giemsa staining. At the appropriate dilution, the bacteria were counted, and the initial concentrations of the bacterial suspensions were determined. The DNAs were extracted, and PCRs were done with genus-specific primers. The DNAs were diluted, and the last dilution which allowed a positive PCR result was determined. With knowledge of the concentrations of the initial suspensions, the sensitivity of the PCR was determined for each pair of primers.

Specific PCR amplifications were performed with primers CS-877 and CS-1273 or primers 120-M59 and 120-797 obtained from the gene coding for *Rickettsia* citrate synthase (*gltA*) (31, 34) and the rickettsial protein rOmpB (30) (the numbers correspond to the 5′ ends of the primers determined with the *R. prowazekii* sequence [GenBank accession no. M37647]), respectively; primers CSBAR-218 and CSBAR-698 or primers QHVE1 and QHVE3, which amplified a part of the *Bartonella gltA* gene (the numbers correspond to the 5′ ends of the primers determined with the *B. henselae* sequence [GenBank accession no. L38987]) and a part of the ITS1 (33), respectively; and primers Bf1 and Br1, which amplify the *Borrelia* 16S rRNA-encoding gene (29). All primers used in the study are listed in Table 2. *R. rickettsii*, *B. elizabethae*, and *B. burgdorferi* were used as respective positive controls for the PCR amplifications. These controls were chosen because *R. rickettsii* has been described only in New World ticks, *B. elizabethae* has been described only once in a patient suffering from endocarditis, and *Borrelia burgdorferi* has never been encountered in lice; thus, any cross-contamination by the positive control should be easy to detect. Negative controls consisted of DNA extracts prepared from *Pediculus humanus corporis* (*vestimentis*) lice handled concurrently with the study lice. These negative control lice were provided by the Gamaleya Research Institute and were rested on pathogen-free laboratory rabbits. One negative control louse was used for every 10 test samples.

A total of 2.5 μl of the extracted DNA was amplified in a 25-μl reaction mixture containing 12.5 pmol of each primer, dATP, dCTP, dGTP, and dTTP each at a concentration of 200 μM, and 1 U of *Taq* DNA polymerase in 1× PCR buffer with 0.8 μl of 25 mM MgCl$_2$ (Gibco BRL, Cergy Pontoise, France). PCRs were carried out in a Peltier Thermal Cycler PTC-200 (MJ Research, Inc., Watertown, Mass.) under the following conditions: an initial 3-min denaturation step at 95°C was followed by 40 cycles of denaturation at 95°C (30 s), annealing at 50°C (30 s), and extension at 72°C (1 min); for the Bf1-Br1 primers, however, annealing was carried out at 55°C. The cycle was finished with 7 min at 72°C to allow complete product extension. If the amplification was positive, the PCR products were purified with Qiagen columns (QIAquick Spin PCR purification kit; QIAGEN), and sequencing reactions were carried out.

RESULTS

The sensitivities of the PCR assays with the primers CS-877 and CS-1273, CSBAR-218 and CSBAR-698, and Bf1 and Br1 were estimated to be from 1 to 10 copies of the gene (DNA). For the detection of *B. quintana*, PCR amplification was performed with the primers CSBAR-218 and CSBAR-698, and the positivity was confirmed with the primers QHVE1 and QHVE3. For the detection of *R. prowazekii*, PCR amplification was performed with the primers CS-877 and CS-1273, and the positivity was confirmed with the primers 120-M59 and 120-797. We did not encounter problems with either DNA extractions or PCR inhibitors; all samples tested yielded products when they were incorporated as templates into PCRs with the broad-range eukaryotic 18S RNA gene primers. Negative controls consistently failed to yield detectable products. As demonstrated in Table 1, *R. prowazekii* was detected only in lice collected during an outbreak of epidemic typhus in Burundi. However, 33% of the lice from that collection were found to be infected with the organism. *B. quintana* DNA was detected in lice from all sources except the Congo. No evidence of *Borrelia*

TABLE 2. Oligonucleotide primers used for PCR amplification and sequencing

Primer	Nucleotide sequence (5′→3′)	Organism or sequence used	Reference
CSBAR-218	CTATCGACCAATTGGCTGAAA	*Bartonella* species	Unpublished data
CSBAR-698	TTTTGTTCGTGATCTGCATG	*Bartonella* species	Unpublished data
QHVE1	TTCAGATGATGATCCCAAGC	*Bartonella* species	33
QHVE3	AACATGTCTGAATATATCTTC	*Bartonella* species	33
CS-877	GGGGGCCTGCTCACGGCGG	*Rickettsia* species	31
CS-1273	CATAACCAGTGTAAAGCTG	*Rickettsia* species	34
120-M59	CCGCAGGGTTGGTAACTGC	*Rickettsia* species	30
120-797	CCTTTTAGATTACCGCCTAA	*Rickettsia* species	Unpublished data
Bf1	GCTGGCAGTGCGTCTTAAGC	*Borrelia* species	29
Br1	GCTTCGGGTATCCTCAACTC	*Borrelia* species	29
18sai	CCTGAGAAACGGCTACCACATC	18S rRNA	10
18 sbi5.0	TAACCGCAACAACTTTAAT	18S rRNA	10

recurrentis infection was found in lice from any source. Sequencing of DNA fragments amplified by PCR confirmed the identities of the expected bacteria.

DISCUSSION

Although human louse-transmitted diseases are usually transmitted by body lice, it is theoretically possible for any species of human louse to act as a vector (20). Louse infestation always occurs in a blood meal, and the louse remains infected all its life, developing into an efficient epidemiological witness. The arthropod location, the effect of the location on louse survival, and microorganism transmission depend on the microorganism. After ingestion, *R. prowazekii* invades the midgut epithelium cells of the insect, in which they replicate, and a large number of infective organisms are released back into the gut. The organisms are then excreted with louse fecal matter (6) and are thus transmitted to humans when a skin wound is scratched or scraped; the louse will then die as a result of the *R. prowazekii* infection. *B. quintana* proliferates extracellularly in the lumen of the louse gut, and the bacterium neither invades the host tissues nor causes the louse serious detriment (42). The means of transmission to humans is the same as that for *R. prowazekii*. Spirochetes (*B. recurrentis*) pass through the louse gut after ingestion and enter the hemolymph, where they remain (8). Transmission to humans occurs by contamination of abraded skin with hemolymphs from crushed, infected lice (6).

Detection of bacteria in blood-sucking arthropods such as ticks, lice, mites, fleas, and mosquitoes has previously been achieved by nonspecific staining, immunodetection, and cell culture methodologies. Staining and cell culture approaches are difficult due to the presence of a complex microbial flora in arthropods. Serological methods are useful, but they require the production of antisera from cultured, purified antigens, and the results are prone to misinterpretation through cross-reactions. Although PCR-based methods do not necessarily indicate the viabilities of the detected pathogens, they offer the most sensitive and specific approaches to the detection and identification of arthropod-borne bacteria. Several groups have successfully used these methods with a variety of arthropods (12, 13, 46, 47), although lice have not previously been tested in this manner. A wide diversity of bacteria of medical and veterinary importance are transmitted or maintained in arthropods. The natural cycles of *Yersinia pestis*, *Rickettsia typhi*, *Rickettsia felis*, and *Bartonella henselae* involve fleas; those of the spotted fever group rickettsiae, *Borrelia* species, *Ehrlichia* species, *Anaplasma marginale*, *Cowdria ruminantium*, and *Francisella tularensis* involve ticks; and that of *Orientia tsutsugamushi* involves trombiculid mites. Several methods have been used to extract bacterial DNA from arthropods. The use of boiling of arthropod triturates in saline buffer (1, 3, 15, 16, 47) or in PCR extraction buffer (12, 16, 23) or boiled hemolymph (40) has been demonstrated to be effective. More sophisticated methods such as phenol-chloroform DNA purification (14, 26) or, more recently, those that use commercially available DNA-extraction kits (7, 24, 36) have also been described. The latter two approaches appear to be more efficient in the removal of substances which may potentially inhibit *Taq* polymerase activity or primer annealing. This study demonstrates that kit-based extraction procedures can be applied to lice and that these insects do not appear to possess particularly high levels of inhibitors.

Lice collected during suspected outbreaks or during routine surveys can be easily transported or posted to laboratories equipped for analysis. Although sucking lice die within 24 h of their final blood meal, the infecting bacterial DNA will remain intact for extraction for several weeks if the samples are kept dry. Freezing is not necessary. Plastic tubes are good containers for the collection of lice; it is better to avoid glass tubes because they can be broken during transport. D. Raoult has noted that during epidemiological investigations, it may be difficult to obtain blood specimens because of the reluctance of volunteers to be sampled. It is a less traumatic process for them to provide lice. However, epidemiological field studies of body lice-transmitted diseases should optimally involve both louse and blood collection. To facilitate rapid sampling and provide the least discomfort for the volunteers, blood samples can be collected onto blotting paper by fingertip puncture (blotting paper test). Both of these approaches require only very simple and cheap materials.

On arrival in the laboratory, the lice can be processed very quickly and a diagnosis can be established rapidly (usually within 48 h), whereas several weeks are necessary to obtain bacterial culture and serological results, and those procedures do not always highlight the presence of bacteria. The diagnosis of epidemic typhus is traditionally done by microimmunofluorescence. However, because cross-reactivity exists between *R. prowazekii* and *R. typhi*, cross-absorption is often necessary and is a tedious and expensive procedure requiring large amounts of antigens and serum (which are not always available) (19). Although culture is the most valuable approach to the characterization of bacteria, all three louse-borne bacterial agents are fastidious. Nevertheless, it is the best technique for the characterization of *Bartonella* species in biological samples because cross-reactivity exists between *B. quintana* and *B. henselae*. Diagnosis of relapsing fever is mainly based on blood smears.

The usefulness of bacterial DNA detection in lice by PCR has been demonstrated by recent investigations. Sporadic cases of typhus have recently been reported in Rwanda, Burundi, Ethiopia, and Nigeria. Persons who contacted epidemic typhus previously represent a human reservoir and can develop recrudescent illness and become a source of a resurgent epidemic if louse infestation becomes prevalent. In 1995, *R. prowazekii* was identified in lice collected from patients in a Burundi jail (30). This observation predated the huge outbreak of epidemic typhus which erupted in refugee camps in Burundi in 1997. During an investigation of that outbreak, PCR-based methods were used to demonstrate that 33% of the collected lice were infected with *R. prowazekii* (29). Because infection of lice with this bacterium leads to the rapid death of the arthropod, detection of rickettsial DNA in lice is a useful tool for the detection of whether the illness is spreading as "red lice," which occurs as a result of the rupture of the gut due to the intracellular multiplication of the rickettsia and the mix of the blood from the gut with the hemolymph. In Burundi, 11.6% of the body lice were found to be infected with *B. quintana*, highlighting the presence of this bacterium on the African continent. The presence of *B. quintana* was later demonstrated in Zimbabwe in lice collected from homeless people, in Peru in lice infesting Andean peasants (26a), and in France in lice collected from homeless people (4). A study in Moscow, Russia, also demonstrated the presence of *B. quintana* in lice collected from homeless people (35). Apparently, a reservoir of the bacterium exists, at least in arthropods, highlighting the potential for an outbreak if auspicious conditions are combined. Although we have never detected *B. recurrentis* DNA in lice, we have never tested arthropods from Ethiopia, where the *B. recurrentis* bacterium is prevalent (41). Application of PCR-based methods to lice collected in Ethiopia may enhance our understanding of the epidemiology of the disease.

The durability of the louse as a sample and the ease with

which it can be collected and transported enhance its potential as a tool for the surveillance of louse-associated reemerging diseases. Investigators should consider the collection and transport of lice to a competent laboratory whenever a body louse outbreak occurs or when clinical manifestations suggestive of epidemic typhus, relapsing fever, or *B. quintana*-associated infections are encountered.

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