Detection of *Bartonella quintana* in African Body and Head Lice

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Abstract. Currently, the body louse is the only recognized vector of *Bartonella quintana*, an organism that causes trench fever. In this work, we investigated the prevalence of this bacterium in human lice in different African countries. We tested 616 head lice and 424 body lice from nine African countries using real-time polymerase chain reaction targeting *intergenic spacer region 2* and specific *B. quintana* genes. Overall, *B. quintana* DNA was found in 54% and 2% of body and head lice, respectively. Our results also show that there are more body lice positive for *B. quintana* in poor countries, which was determined by the gross domestic product, than in wealthy areas (228/403 versus 0/21, $P < 0.001$). A similar finding was obtained for head lice (8/226 versus 2/390, $P = 0.007$). Our findings suggest that head lice in Africa may be infected by *B. quintana* when patients live in poor economic conditions and are also exposed to body lice.

INTRODUCTION

Sucking lice (Anoplura) are hematophagous wingless insects that can infest birds and mammals.¹ Among these hosts, humans constitute the preferred host for only two species: *Pediculus humanus* and *Phthirus pubis* (pubic lice). *P. humanus* includes two morphotypes: *P. humanus* morphotype *capitis* (head lice) and *P. humanus* morphotype *humanus* (body lice).² Each louse has a specific ecotype; head lice live and lay eggs in the hair and are prevalent in all countries and all levels of society, whereas body lice live in clothing and multiply when cold, promiscuity, and lack of hygiene are present.³ Louse coloration was described at the beginning of 20th century. The variability in louse color on a single host may be affected by not only the color of the skin but also, the color of the hair and clothing.^{4,5}

Many genetic studies have been conducted on human lice—first based on 18S ribosomal RNA^{6,7} and then, the mitochondrial genes (cytochrome oxidase subunit 1 [*CoxI*] and cytochrome b [*Cytb*]). These studies allowed scientists to classify lice into three different clades: Clade A, the most common clade found worldwide and comprised of both head and body lice; Clade B, composed of only head lice and found in Central and North America, Europe, and Australia⁸; and Clade C, including black head lice found in Nepal,⁹ Ethiopia,¹⁰ and Senegal.¹¹

The body louse is linked to poverty. Its transmission occurs in crowded environments, such as homeless shelters, refugee camps, and jails, especially when hygienic standards are lacking.¹² The body louse is the main vector of three pathogenic bacteria: *Bartonella quintana*, the agent of trench fever; *Borrelia recurrentis*, the agent of louse-borne relapsing fever; and *Rickettsia prowazekii*, the agent of epidemic typhus.^{13,14} *B. quintana* is a Gram-negative bacterium that causes trench fever, bacillary angiomatosis, endocarditis, chronic bacteremia, and chronic lymphadenopathy.¹⁵ It has typically been trans-

mitted by body lice, but recently, DNA of *B. quintana* has also been found in head lice collected from homeless individuals in Nepal,⁹ the United States,¹⁶ France,¹⁷ Senegal,¹¹ and Ethiopia.^{10,18}

The detection of *B. quintana* in African lice remains limited to only a small number of countries. Therefore, the objectives of this study were to investigate the presence of *B. quintana* in head and body lice in different areas of African countries suffering from poverty, social instability, or war and identify the relationship between louse phenotypes and genotypes.

MATERIALS AND METHODS

Ethics statement. Lice from African countries were obtained from the private frozen collection of our laboratory (The Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes [URMITE]/World Health Organization [WHO] Collaborative Research Center). The lice in that collection were required for various epidemiological and entomological studies or to perform diagnoses abroad, and they were sent to our laboratory as a WHO reference facility. Body lice were collected from clothing and head lice were removed from hair with the verbal consent of the infested individuals. Written consent was not obtainable in the majority of cases, because most of the subjects were illiterate. However, in most instances, the investigator, local authorities/Institutional Review Boards (IRBs), and/or village/family chief approved and were present when collection was performed (with individual consent).

Sampling/country. Human lice samples were collected from patients in various regions of Africa (Figure 1). Nine countries were investigated. In total, 1,040 lice were collected: 616 head lice and 424 body lice. Exactly 381 head lice were collected in Senegal (2011), 75 head lice and 22 body lice were collected in Madagascar (2009, 2011, and 2012), 14 head and body lice were collected in Ethiopia (2011), 92 head lice were collected in Mali (2010), 37 body lice were collected in Kenya (1999), 166 body lice were collected in Rwanda (2011), 10 head and body lice were collected in Burundi (2008), 35 head lice and 154 body lice were collected in Congo (2010), and 9 head lice and 21 body lice were

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FIGURE 1. Map of Africa showing the prevalence of *B. quintana* in head and body lice collected in the various study areas during 1999–2012.

collected in Algeria (2000). In our laboratory, each sample was photographed on dorsal and ventral sides with a camera (Olympus DP71, Rungis, France). Lice were preserved in 70% alcohol, rinsed two times with sterile distilled water for 2 minutes, and dried with filter paper. Samples were then cut

in half on the longitudinal plane, and one-half was stored at -20°C for subsequent analysis.

Distribution of regions. We classified these countries into two regions: poor (with the gross domestic product [GDP] < \$1,844 per capita: Madagascar, Ethiopia, Mali, Kenya,

Figure 2-1 Phenotypes of head lice :

A= Senegal, B= Madagascar, C= Ethiopia.

Figure 2-2 Phenotypes of body lice :

D= Ethiopia, E= Madagascar, F= Kenya, G= Rwanda.

FIGURE 2. Phenotypes of head and body lice collected from African countries during 1999–2012 (Head lice: A, B and C; Body lice: D, E, F and G).

that encodes a hypothetical intracellular effector.¹⁷ Each real-time PCR assay was performed using a CFX96™ REAL-Time System C1000 Thermal Cycler (Bio-Rad Laboratories, Foster City, CA). For each PCR assay, two negative and positive controls were used. The identification of *B. quintana* was confirmed by amplification of both *ITS2* and *yopP* genes.

Genotypic status of lice. All samples that tested positive for *B. quintana* DNA were analyzed by multiplex real-time PCR that targeted a portion of the Phum PHUM540560 gene. This assay allowed for the discrimination of body lice from head lice, which has described previously.²⁰ As a positive control, we used a head louse and body louse with genotypic statuses that were known (VIC-positive for the head louse and FAM-positive for the body louse). Monoplex and multiplex real-time PCRs were performed in a CFX96 Thermal Cycler (Bio-Rad Laboratories, Foster City, CA).

Standard PCR and sequencing. Lice genotype was identified by the analysis of partial (347-bp) mitochondrial *Cytb* DNA. The primers used for the *Cytb* gene were CytbF1 (5'-GAGCGACTGTAATTACTAATC-3') and CytbR1 (5'-GGA CCCGATAATTTTGTG-3'). Overall, 10 lice belonging to each region were tested with a standard PCR assay targeting the *Cytb* gene as described previously.⁸ Each amplification reaction was performed on a PTC-200 thermocycler machine (MJ Research, Waltham, MA). The PCR reaction contained 9.9 µL water, 4 µL buffer 5 × Fusion HF Buffer, 0.4 µL 10 mM deoxynucleoside triphosphates (dNTPs), 1.25 µL each primer, 0.2 µL Phusion polymerase (Finnzymes, Thermo Scientific, Vantaa, Finland), and 3 µL DNA template to obtain a total volume of 20 µL. The cycling conditions were 98°C for 30 seconds, 35 cycles of 98°C for 5 seconds, 56°C for 30 seconds, and 72°C for 15 seconds, and a final extension time of 5 minutes at 72°C. The success of the PCR amplification was then detected by the migration of the PCR product on 2% agarose gel (Electrophoresis grade; Invitrogen, Carlsbad, CA) prepared with 0.5% Tris Borate ethylenediaminetetraacetic (EDTA) (TBE; Euromodex, Lake Placid, NY) and charged with a solution of 0.5% ethidium bromide (Invitrogen, Carlsbad, CA). Purification of PCR-amplified products was performed using distilled RNase-DNase free water on NucleoFast 96 PCR plates (Macherey-Nagel EURL, Hoerd, France). Sequencing of positive samples was performed using the ABI Prism Big Dye Terminator Cycle Sequencing Kit, version 1.1 (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions.

Data analysis. Microsoft Excel was used for data management. Descriptive statistics, such as percentages and means, were computed to summarize the proportions of infestations

with lice. Statistical analysis was performed with Epi Info 6 (www.cdc.gov/epiinfo/Epi6/EI6dnjp.htm), and a *P* value of < 0.05 was considered significant.

Phylogenetic analysis. Each DNA sequence was aligned using multisequence alignment software (CLUSTALX, version 2.0.11). The ChromasPro program (Technelysium PTY, Australia) was used to analyze, assemble, and correct sequences. The sequence similarities were determined using MEGA 5, and phylogenetic trees were obtained using the maximum likelihood (ML) method with 100 bootstrap replicates.²¹ We compared our sequences with other sequences that are present in GenBank.

RESULTS

Morphological analysis. Phenotypical examination of the lice showed that all head lice from Senegal, Madagascar, and Ethiopia have a black color (Figure 2A–C). The color of all body lice in Madagascar and Rwanda is black; in Kenya, all body lice are brown, whereas in Ethiopia, all body lice are gray (Figure 2D–G). During our study, the phenotypes of lice from Congo, Burundi, Mali, and Algeria were not investigated, because they had been destroyed previously for other studies.

Phylogenetic analysis. On the basis of phylogenetic study of lice Clade A based on ITSs (ultispacer typing method [MST])²² and through the analysis of *Cytb* in this work (Figure 3), our results show that head lice from Algeria, Madagascar, Burundi, and Senegal belong to Clade A2. Clade C was found in head lice from Ethiopia, Mali, and Senegal. Clade B was found in head lice from Algeria. Based on *Cytb* analysis, all head and body lice that are positive for *B. quintana* DNA belong to Clade A2 (Table 1).

TABLE 1
Distribution of *B. quintana* DNA in head lice (*N* = 616) and body lice (*N* = 424) collected from African countries during 1999–2012

Country/area	Lice analyzed		Positive <i>B. quintana</i> DNA (%)		<i>Cytb</i>	
	Head lice	Body lice	Head lice	Body lice	Head lice	Body lice
Senegal						
Yeumbeul	101	0	0	–	Clade A2	–
Malika	69	0	0	–	Clade A2	–
Dielmo village	71	0	2 (2.8)	–	Clade A2	–
Ndiop village	77	0	0 (0)	–	Clade C	–
Keur Massar	63	0	0	–	Clade A2	–
Total	381	0	2 (0.52)			
Madagascar						
Borenty village	0	9	–	1 (11.1)	–	Clade A2
Tsiroanomandidy	21	13	0	0	Clade A2	Clade A2
Anjozorobe	39	0	2 (5.1)	–	Clade A2	–
Bedaro village	15	0	0	–	Clade A2	–
Total	75	22	2 (2.66)	1 (4.54)		
Ethiopia						
Gondar	14	14	0	0	Clade C	Clade A2
Mali						
Diankabou	92	0	0	–	Clade C	–
Kenya						
Nairobi	0	37	–	27 (72.9)	–	Clade A2
Rwanda						
Kigali	0	166	–	149 (89.7)	–	Clade A2
Burundi						
Bujumbura	10	10	0	1 (10)	Clade A2	Clade A
Congo						
RDC	35	154	6 (17.1)	50 (32.5)	Clade A2	Clade A2
Algeria						
Batna	9	21	0	0	Clades A and B	Clade A2
Total	616	424	10 (1.6)	228 (53.7)		

Molecular detection of *B. quintana* in lice. The DNA of *B. quintana* was detected in 10 of 616 (1.6%) head lice (the mean cycle thresholds [*ct*] value $\pm$ SD: 30.64 ± 6.34). Specifically, *B. quintana* DNA was found in 2 of 71 (2.8%) head lice from Senegal (Dielmo village), 2 of 39 (5.1%) head lice from Madagascar (Anjozorobe), and 6 of 35 (17.1%) head lice from Congo.²³ No *B. quintana* DNA was detected in Senegal (Yeumbeul, Malika, Ndiop village, and Keur Massar), Madagascar (Tsiroanomandidy and Bedaro village), Ethiopia, Mali, Burundi, or Algeria (Table 1).

The DNA of *B. quintana* was detected in 228 of 424 (54%) body lice (the mean *ct* value $\pm$ SD: 33.19 ± 3.79). Among 228 *B. Quintana*-positive body lice, 149 of 166 (89.7%) body lice

are from Rwanda, 27 of 37 (72.9%) body lice are from Kenya, 50 of 154 (32.5%) body lice are from the Democratic Republic of Congo,²³ 1 (11.1%) body louse is from Madagascar, and 1 (10%) body louse is from Burundi. No *B. quintana* DNA was detected in the Madagascar area of Tsiroanomandidy, Ethiopia, or Algeria. There are significantly more body lice with *B. quintana* DNA than head lice (54% versus 1.6%, $P < 0.001$) (Table 1).

Genotypic status of positive lice to *B. quintana* DNA. Among the African Clade A lice positive for *B. quintana* DNA, all head lice had the head louse genotype, and all body lice had the body louse genotype. No signal was detected in the negative controls.

TABLE 2
Prevalence of infections of *B. quintana* in body lice (this study and the literature)

Country/source	Year of collection	Percent (no. <i>B. quintana</i> /no. tested)	Source
Algeria			
Schoolchildren	2000	0 (0/21)	This study ³⁰
Homeless in Batna	2001	0 (0/33)	
Madagascar			
Local population	2009/2011/2012	4.5 (1/22)	This study
Ethiopia			
Bahir Dar	2011	0 (0/14)	This study ²⁹ 10
Poor regions (Jimma)	2010	3 (1/33)	
Poor regions (Jimma)	2010	18 (76/424)	
Kenya			
Local population	1999	72.9 (27/37)	This study
Rwanda			
Jail	2011	89.7 (149/166)	This study ³⁰
Jail	2001	2.3 (6/262)	
Burundi			
Refugee camp	2008	10 (1/10)	This study ³² 32 32 30 30 30 30 30 30 30
During typhus outbreak	1997	0 (0/10)	
Refugee camp	1997	9.5 (6/63)	
After outbreak in camp	1998	14.3 (13/91)	
During typhus outbreak	1997	0 (0/10)	
Refugee camp	1997	9.5 (6/63)	
After typhus outbreak	1998	14.3 (13/91)	
Refugee camp	1998	21 (8/38)	
Refugee camp	2000	90 (100/111)	
Refugee camp	2001	93.9 (31/33)	
Congo			
Local population	2010	32.5 (50/154)	This study ³² 30 23
Refugee camp	1998	0 (0/7)	
Refugee camp	1998	0 (0/7)	
Local population	2010	32.5 (50/154)	
France			
SDF (homeless)	1997	20 (3/15)	34
SDF (homeless)	1998	4 (3/75)	32
SDF (homeless)	2000	26 (42/161)	33
SDF (homeless)	1998–2001	9.8 (32/324)	30
Tunisia			
Homeless in Sousse	2000	0 (0/3)	30
Zimbabwe			
Homeless in Harare	1998	16.7 (2/12)	30–32
The Netherlands			
Homeless in Utrecht	2001	36 (9/25)	30
United States			
SDF (homeless)	2007–2008	33.3 (11/33)	16
Nepal			
Street children and children in slums	2002	20 (4/20)	9
Russia			
Homeless in Moscow	1998	12.3 (33/268)	32
Australia			
Homeless	2001	0 (0/2)	30
Peru			
Andean rural population	NA	1.4 (1/73)	32
Andean rural population	NA	0 (0/10)	30

NA = not available.

Relationship between *B. quintana* in body or head lice and socioeconomic level. We compared our results with the life conditions of the regions tested and found that the body lice that were *B. quintana*-positive were more likely to be found in poor countries than wealthy countries (228/403 versus 0/21, $P < 0.001$). The same finding was made for head lice (8/226 versus 2/390, $P = 0.007$).

We correlated the GDP to the level of *B. quintana* infection. Our results indicate that the higher the GDP in a region, the less prevalent that *B. quintana* DNA was and *vice versa* (correlation $r = -0.178$).

DISCUSSION

This study of 1,040 human lice of the genus *Pediculus* collected from nine African countries has enabled us to better specify some characteristics of human lice. The phenotypic study allowed us to confirm several previously described observations, including the black phenotype of head lice in Senegal and Ethiopia.^{10,11,24} For the first time, we have established the phenotype of lice collected in Madagascar and Kenya. Body lice from Madagascar and Rwanda are black; body lice collected in Kenya are brown. The head lice from Madagascar had the same black color as body lice col-

lected in this country (Figure 2). In addition, it seems that the color of lice is more complex than indicated in the first findings provided in 1926 by Ewing,²⁵ and as suggested by a recent study,¹⁰ lice color may be independent of the host's skin color.

On the basis of phylogenetic study of lice Clade A, based on ITSs (MST)²² and through the analysis of *Cytb* in this work (Figure 3), we confirmed that head lice in Senegal are Clades A2 and C¹¹ and that head lice in Ethiopia are Clade C.¹⁰ In addition, we have found that head lice in Madagascar, Burundi, Congo, and Algeria belong to Clade A2 and that head lice in Mali are Clade C (Figure 3). The body lice of Algeria, Ethiopia, Madagascar, Kenya, Burundi, and Rwanda belong to Clade A2; it is the main clade in sub-Saharan Africa.²⁶ Therefore, the geographical distribution of lice seems to be complex and independent of the phenotype.

B. quintana is a re-emerging pathogen that is responsible for a range of clinical manifestations in humans.²⁷ It has long been established that trench fever can be transmitted by the body louse.²⁸ However, the role played by the head louse as a reservoir or vector of *B. quintana* remains unclear.²⁹ In total, *B. quintana* was found in 54% and 2% of body and head lice collected in Africa, respectively. Here, we find that there are more *B. quintana* in body lice than head lice. This finding could probably be explained through the role played by

TABLE 3
Prevalence of infections of *B. quintana* in head lice (this study and the literature)

Country/source	Year of collection	Percent (no. <i>B. quintana</i> /no. tested)	Source
Algeria			
Schoolchildren	2000	0 (0/9)	This study
Schoolchildren	NA	0 (0/18)	30
Burundi			
Schoolchildren	2008	0 (0/10)	This study
Schoolchildren	NA	0 (0/20)	30
Senegal			
Rural community	2011	0.52 (2/381)	This study
Rural community	NA	6.9 (19/274)	11
Ethiopia			
Poor region	2011	0 (0/14)	This study
Poor regions (Jimma)	2010	7 (19/271)	10
Poor regions (Jimma)	2010	9.2 (6/65)	29
Congo			
Local population	2010	17.1 (6/35)	This study
Local population	2010	17.1 (6/35)	23
Madagascar			
Local population	2010–2011	2.6 (2/75)	This study
Mali			
Schoolchildren	2010	0 (0/92)	This study
Portugal			
Schoolchildren	NA	0 (0/20)	30
China			
Schoolchildren	NA	0 (0/23)	30
Thailand			
Schoolchildren	NA	0 (0/29)	30
Australia			
Schoolchildren	NA	0 (0/3)	30
United States			
SDF (homeless)	2007–2008	25 (3/12)	16
Nepal			
Street children and children in slums	2002	9.5 (2/21)	9
Russia			
Schoolchildren	NA	0 (0/10)	30
France			
Schoolchildren	NA	0 (0/20)	30
Schoolchildren	2008–2009	0 (0/288)	31
SDF (homeless)	2008	100 (3/3)	17

NA = not available.

the body louse in transmission of diseases. With the new method of multiplex real-time PCR assay with the Phum_PHUM540560 gene, all head lice were genotyped as head lice by the signal emitted by the VIC-labeled probe specific to head lice, and all body lice were genotyped as body lice by the signal emitted by the FAM-labeled probe specific to body lice. In our study, the infection rate was higher in Rwanda (149/166 versus 6/262, $P < 0.001$) (Table 2) and lower in Burundi (1/10 versus 158/346, $P = 0.025$) than the rate reported in 2002 by Fournier and others.³⁰ These differences could be explained by the living conditions in jail in Rwanda and probably, the sample size in this study in Burundi. We have confirmed the presence of *B. quintana* in body lice in Rwanda and Burundi.³⁰ *B. quintana* has also been detected in body lice from homeless people in Zimbabwe (16.7%, 2/12).³⁰ This bacterium has not been found in body lice in Tunisia.³⁰ We detected *B. quintana* for the first time in body lice collected in Madagascar (4.54%, 1/22) and Kenya (72.9%, 27/37). The high rate of *B. quintana* in body lice in some African countries (Kenya, Rwanda, and Congo) may reflect the low socioeconomic level of the study population. We tested this hypothesis by comparing the average GDPs of nine different countries with the level of *B. quintana* infection. We found that the higher the GDP increases, the lower the level of *B. quintana* decreases and *vice versa* (correlation $r = -0.178$). For example, for countries with high GDP, this hypothesis could be justified through the previous work by Fournier and others³⁰ in 2002, which reported 0% *B. quintana* in head lice in Portugal, China, Thailand, Australia, Algeria, and France.³⁰ This percentage of infection with *B. quintana* remains stable (0%) in body lice in Australia, Algeria, and Peru. In France, Bouvresse and others³¹ also found the same result (0%) in head lice. Roux and Raoult³² reported a rate of 1.4% in body lice in Peru. For countries with low GDP, this hypothesis could be confirmed by the work of Piarroux and others²³ in 2010, which reported 32.5% *B. quintana* in body lice in Congo, and the work of Fournier and others,³⁰ which found a higher prevalence of 93.9% *B. quintana* in body lice in Burundi (a refugee camp in 2001). Elsewhere, we compared the infection rate in *B. quintana* body lice in people in Africa with the infection rate in *B. quintana* body lice in homeless people in Marseille, France,^{30,32,33} and the result was statistically significant (228/424 versus 77/560, $P < 0.001$) (Table 2). In this study, we show a relationship between *B. quintana* presence in body lice and socioeconomic level, and we found that the body lice that were *B. quintana*-positive were more likely to be found in poor countries (GDP < \$1,844 per capita) than wealthy countries (GDP > \$1,844 per capita; 228/403 versus 0/21, $P < 0.001$).

Head lice can also be infected with *B. quintana*. In our study, we detected the DNA of *B. quintana* in 1.6% (10/616) of head lice collected: in Senegal, 0.52% (2/381); in Madagascar for the first time, 2.66% (2/75); in the Congo, 17.1% (6/35). *B. quintana* was detected in 7% (19/274) of head lice collected in Dakar, Senegal¹¹ and 9.2% (6/65) of head lice pools and 7% (19/271) of head lice on persons living in the poorest areas of Jimma, Ethiopia.^{10,29} No *B. quintana* DNA was detected in head lice from schoolchildren in Marseille, France,^{30,31} contrary to the work by Angelakis and others,¹⁰ which have reported this result in the homeless population. In Russia, Portugal, Algeria, Burundi, China, Thailand, and Australia, no *B. quintana* was found in schoolchildren³⁰ (Table 3). In this study, we also found the head lice that were

B. quintana-positive were more likely to be found in poor countries (GDP < \$1,844 per capita) than wealthy countries (GDP > \$1,844 per capita; 8/226 versus 2/390, $P < 0.007$). Head lice are prevalent around the world and in all levels of society. Thus, head lice may be infected with *B. quintana* when the host is coinfecting with body lice in a precarious environment in which proper hygiene is lacking.

In conclusion, it has been estimated that trench fever affected several million people, especially in Russia and on the Eastern, Central, and Western European fronts during World War II.^{12,15} Our study on the prevalence of *B. quintana* in lice from various geographical and socioecological situations associated with clinical information will help to evaluate the role of head lice in the transmission of this disease.

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