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Bartonella quintana in Ethiopian lice

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ABSTRACT

Head and clothing lice from Jimma, Ethiopia were investigated for pathogenic bacteria. Genomic DNA from pools of lice was subjected to PCR analysis for *Bartonella* spp., *Borrelia* spp. *Coxiella burnetii*, *Rickettsia* spp. and *Yersinia pestis*. All 102 lice pools were negative for the afore mentioned pathogens, with the exception of *Bartonella* species found among 6 of 65 (9.2%) head lice pools and1 of 33 clothing lice pools. Identification was achieved by sequencing the ribosomal intragenic transcribed spacer region (ITS), revealing all to be *Bartonella quintana*. Although established as a clothing louse-borne infection, typically causing chronic bacteraemia, trench fever, bacillary angiomatosis and endocarditis, this has only been rarely reported among head lice. The higher numbers of infected head lice pools compared with clothing lice suggests their competence for maintaining this infection within Ethiopia.

1. Introduction

Human lice are often considered today more as a nuisance rather than vectors of disease, yet they have an impressive track record for their ability to result in epidemics of infection that have shaped the history of mankind. Indeed, it was likely that the demise of the great Napoleonic army was a result of epidemic typhus caused by the louse-borne *Rickettsia prowazekii* [1]. Similarly, extensive outbreaks of louse-borne relapsing fever caused by the spirochaete *Borrelia recurrentis*, were associated with high mortality and ravished the globe until the mid twentieth century [2]. Numerous troops during World War I and II were plagued by trench fever caused by *Bartonella quintana* [3]. More recently, the arthropod vector of bubonic plague has been questioned, with lice being proposed as a more likely means to explain the epidemic spread of this infection around the globe [4].

With increasing globalisation, displacement of populations through environmental or political catastrophes, and through the increasing numbers of homeless individuals seen in many urban cities, there is potential for resurgence of louse-borne disease [5,6]. The spread of infection has usually been associated with the clothing louse (*Pediculus humanus humanus*), with head lice (*Pediculus humanus capitis*) not incriminated as an infection threat. Phylogenetic analysis of louse populations has highlighted the close relationship of these lice [7], underscoring the possibility that head lice might be competent as vectors of louse-borne infection. We undertook a study of both head and clothing

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lice in Jimma, Ethiopia, an area where multiple infections are likely to persist and significant numbers of the human population remain infested with either or both head and clothing lice.

2. Materials and methods

2.1. Collections of lice

Poorer regions of Jimma in Ethiopia were targeted for collection of both head and clothing lice. Similarly, street beggars were used for louse collections. The sampling period was late July to early August 2010. Lice were processed as pools containing between 1 and 20 lice collected from the same individual, with head lice being separated from clothing lice. A total of 65 pools of head lice and 33 pools of clothing lice were processed.

2.2. Molecular screening for presence of pathogen DNA

Samples were processed using a glass bead-beater to homogenise louse pools, prior to digestion in lysis buffer containing proteinase K for an hour at 56 °C in a water bath (DNeasy, Qiagen). DNA extraction was then performed using the Qiacube Qiagen robot and DNeasy extraction kits according to the manufacturers protocol for tissues. A real-time PCR targeting the *Borrelia* flagellin of relapsing fever was used to screen for *Borrelia* using DNA from *Borrelia duttonii* as a positive control and water as negative control [8]. The insertion sequence *IS1111* was used in a real-time assay for *Coxiella burnetii* with DNA from the Nine Mile strain and water as positive and negative controls, respectively [9]. *Rickettsia* spp. were investigated using a real-time assay based upon the citrate synthase with a known positive sample containing *R. felis* and water used as a negative control [10]. A chromosomal marker real-time assay was selected to screen for *Yersinia pestis* given the potential lack of plasmid stability. Only negative controls were used in this assay [11]. Finally, a conventional assay was used to screen samples for *Bartonella* spp. targeting the transcribed intragenic spacer region (ITS) [12]. Amplicons were resolved using 2% agarose gel electrophoresis stained with safeview™ (NBS Biologicals). As positive controls were not available, DNA from kidney tissues from a series of 58 field caught UK rodents was tested together with water as a negative control.

2.3. Sequencing

Amplicons of approximately 319bp produced for ITS were submitted for sequence analysis at the Genome Centre Queen Mary's University of London using a ABI3700 (Applied Biosystems). The same primers used for detection were also used for sequencing. Data was aligned using ClustalW, trimmed and analysed using Mega5 [13].

2.4. Statistical significance

This was assessed using Fisher's exact test (two-tailed).

Table 1
PCR results of pathogen screening of Ethiopian louse pools.

Organism	Head lice (%) $N = 65$	Clothing lice (%) $N = 33$
Bartonella spp.	6 (9.2)	1 (3)
Borrelia spp.	0	0
Coxiella burnetii	0	0
Rickettsia spp.	0	0
Yersinia pestis	0	0

3. Results

Of the 98 pools, 6 of the 65 (9.2%) of head lice and 1 of the 33 (3%) pools of clothing lice of lice yielded positive results for *Bartonella*. Although more head lice than clothing lice were positive, this was not a statistically significant difference ($p = 0.4$). The 58 UK rodent kidney tissues yielded 4 positive ITS amplicons. Four of the louse amplicons (3 from head lice and 1 from clothing lice) and four of the rodent amplicons were sequenced revealing their identity to be *B. quintana* and *B. taylorii*, respectively (see Table 1 and Fig. 3). These sequences have been deposited with GenBank under the numbers JN366399 to JN366402 and JN366403 to JN366406, respectively.

All of the louse pools were negative for DNA from *Borrelia*, *Coxiella*, *Rickettsia* spp. and *Yersinia pestis* (Table 1).

4. Discussion

Bartonella quintana has a long history of association with humans dating back over 4000 years [14]. This human-specific species is most notoriously known as the aetiological agent of trench fever. Infection was common during the World Wars I and II, and has been identified as an increasing problem among homeless people in developed countries [15].

B. quintana reside within red blood cells where they can also result in chronic bacteraemia, endocarditis and bacillary angiomatosis. Though classically transmitted by clothing lice, sporadic reports describe *B. quintana* in head lice of dually infested individuals [16]. More recently, this has also been described among head lice alone [17] and

Fig. 1. Louse infested clothing showing vast numbers of eggs cemented into the seams.

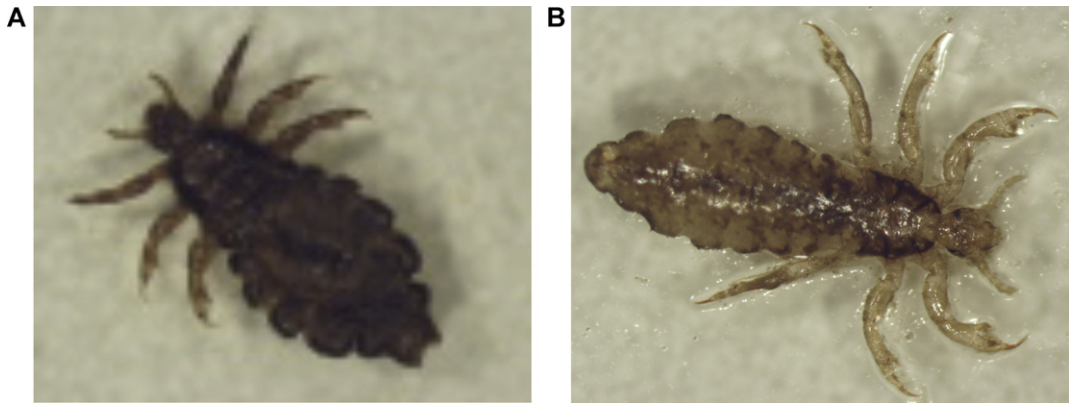

Fig. 2. Plate A shows head lice whist Plate B shows clothing lice from Ethiopians in Jimma.

among head louse nits [18] Our finding of over 9% of Ethiopian head lice positive for this pathogen is worrying. Furthermore, the severity of infection appears to be enhanced among HIV-infected individuals which is particularly concerning given the levels of infection among East African countries [19].

Our findings beg the question of whether other head louse lineages are equally competent for this pathogen that could facilitate re-emerging infection among individuals in Europe and beyond. It has been suggested that head lice might transform into clothing lice, with the morphological differences hypothesised as alteration in regulatory gene products (see Fig. 2) [7]. These authors also highlighted the diversity of louse lineages with at least three genomic clusters [20]. It is entirely possible that their competence as vectors may not be uniform. Indeed, the finding of *B. quintana* among head lice from Ethiopia and the previous reports of infected head lice from Nepal are particularly interesting [16]. A cluster of lice known as genotype C is prevalent in both Nepal and Ethiopia [20], thus the prior report of *B. quintana* among head lice from Nepal and our findings reported herein, might highlight the ability of this louse linage to serve as a disease vector rather than all head lice in general. This is supported by the failure to detect *B. quintana* among head lice collections from children elsewhere around the world, despite its presence in clothing lice from many of these locations [21]. Conversely, this lineage C of lice is unlikely to exist in San Francisco where *B.*

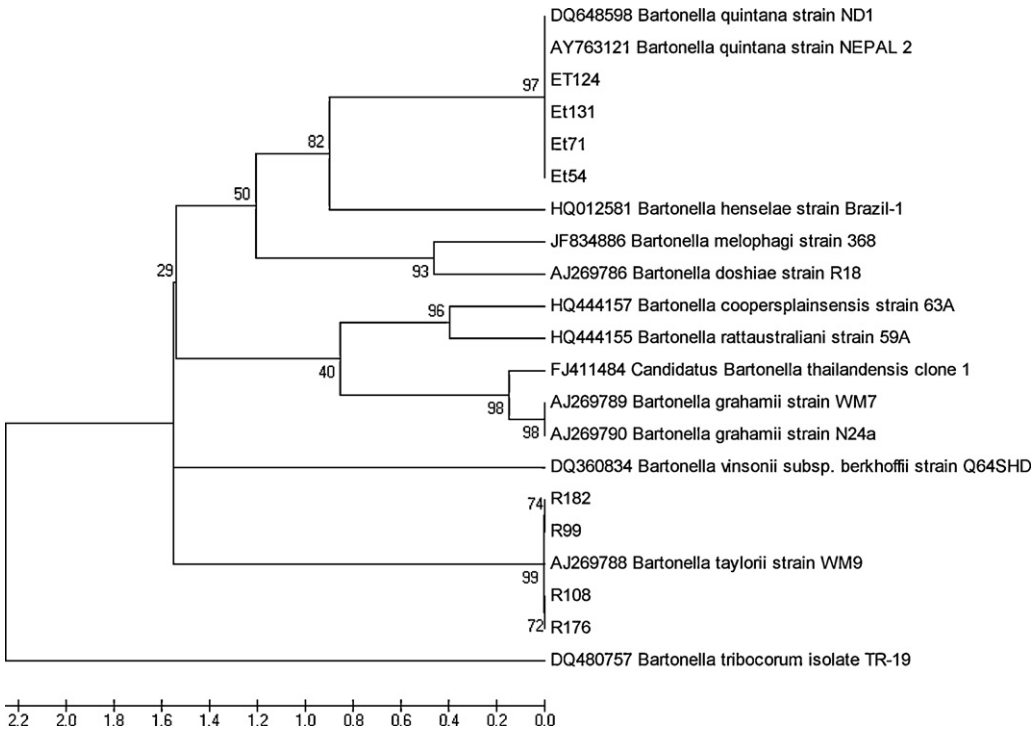

Fig. 3. Phylogenetic analysis (UPGMA with a bootstrap value of 1000 replicates) of *Bartonella* ITS amplicons demonstrating their homology with *B. quintana*. Sequences of *B. quintana* and *B. taylorii* have been deposited with GenBank under the numbers JN366399–JN366402 and JN366403–JN366406, respectively.

quintana has also been reported among head lice [17]. Supporting the alternative hypothesis that all head lice have the potential to serve as vectors for *B. quintana*. Given the burden of head lice globally, it is of paramount importance to address the competence of other louse lineages as potential disease vectors.

The region from which the lice were collected is adjacent to large coffee plantations, with seasonal influxes of migratory workers from vast distances descending upon Jimma during coffee harvest times. This has led to several outbreaks of louse-borne relapsing fever in this area [22]. Despite this local history, none of the lice tested yielded evidence of borrelial infection (see Figs. 1 and 2), supporting the recent demise of this infection in many areas of Ethiopia [23]. The lack of regular infection with this spirochaete, *Borrelia recurrentis*, has left the population naïve, thus open to the potentially devastating effects of this infection if reintroduced. The dwindling numbers of louse-borne relapsing fever are likely to be influenced by the declining numbers of people with clothing lice coupled with increasing extensive use of antimicrobials to treat or prevent other infections. The relapsing fever spirochaetes are remarkably susceptible to the effect of antimicrobials [24].

We also assessed our collected lice for the presence of *Coxiella burnetii*, the causative agent of Q fever. Little is known about the prevalence of this agent in Ethiopia with the exception of sporadic reports. Recent work from Senegal has shown this to be highly prevalent among largely pastoral farming communities [25]. Though not correlated with louse transmission, this pathogen shows a diverse multi-host range, thus it was not inconceivable that it could have been present among the lice collected. Our data failed to support any role for human lice in the epidemiology of this infection in Jimma and its surrounding area (Fig. 3).

Rickettsia species have been documented among various arthropods including fleas, ticks and lice. The louse-borne *Rickettsia prowazekii* is notorious through its ability to cause louse-borne epidemic typhus [26]. This has been described in clothing lice for both isolates of human epidemic typhus and *R. prowasekii* strains associated with flying squirrels [27], but not head lice. No evidence of this species, or indeed other *Rickettsia* was found among lice tested using a pan-genus assay based upon detection of citrate synthase.

It has recently been speculated that the historical outbreaks of plague across the globe would correlate better with the epidemiology of louse-borne infections, thus the possibility of lice to serve as vectors of this pathogen have been explored [4]. We failed to demonstrate presence of *Yersinia pestis* in any of the louse pools tested, thus are neither able to support or refute this hypothesis.

In conclusion, we tested both head lice and clothing lice collected from Jimma in Ethiopia for the presence of five pathogens. Only *B. quintana* was detected with the pools of lice negative for *Borrelia*, *Coxiella*, *Rickettsia* and *Yersinia.* Surprisingly, more pools of head lice were positive compared to the established clothing louse vectors. This raises the possibility that head lice might transmit *B. quintana* elsewhere around the world. The vector competence of different lineages of head lice should be explored for *B. quintana* together with surveillance of lice from different geographical regions.

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