

Title Pages

Relapsing Fever *Borrelia*: A Global Review

Sally J. Cutler, PhD

School of Health, Sport & Bioscience, University of East London, Stratford, E15 4LZ, UK.

E-mail: s.cutler@uel.ac.uk

Disclosures: The author has no disclosures to declare or conflicts of interest.

Key words

Borrelia; tick-borne relapsing fever; louse-borne relapsing fever; Argasid (soft) ticks;

Pediculus humanus; clothing lice; epidemic relapsing fever; endemic relapsing fever.

Synopsis

Relapsing fever borreliae were notorious and feared infectious agents that earned their place in history through their devastating impact as causes of both epidemic and endemic infection. More recently they are considered more as an oddity and their burden of infection is largely overshadowed by other infections such as malaria, which presents in a similar clinical way. Despite this, they remain the most common bacterial infection in some developing countries. Transmitted by soft ticks or lice, these fascinating spirochaetes have evolved a myriad of mechanisms to survive within their diverse environments.

Key Points

- Most relapsing fever borreliae are transmitted by soft ticks belonging to the *Argasidae* genera. These are rapid-feeding ticks and their bites may go unnoticed.
- The epidemic member of this group, *Borrelia recurrentis*, is transmitted by the human clothing louse, *Pediculus humanus*.
- Most relapsing fever *Borrelia* are zoonotic, with the exception of *Borrelia duttonii* and *Borrelia recurrentis*.
- Consider a relapsing fever borreliosis among individuals with a relapsing febrile illness and travel history to an endemic region, particularly when malaria is the differential the diagnosis.
- Conventional diagnostic microbiological approaches are unlikely to detect relapsing fever spirochaetes.
- Most infections are successfully managed with penicillin, tetracycline or doxycycline. The Jarisch-Herxheimer reaction can complicate treatment.

Self-Test Questions

What is the time required for argasid ticks to feed to repletion?

- a) 5 minutes to 2 hours
- b) >48 hours
- c) 1-10 seconds
- d) >7 days

Which of the following statements regarding relapsing fever borreliosis is incorrect?

- a) These infections are acquired from arthropods including lice and ticks
- b) This infection is a strictly tropical disease
- c) Reinfection can occur
- d) Infection can be treated with antibiotics such as penicillin or doxycycline

Which of the following relapsing fever borreliae are not considered to be zoonotic?

- a) *Borrelia persica* and *Borrelia microti*
- b) *Borrelia crocidurae* and *Borrelia hispanica*
- c) *Borrelia hermsii* and *Borrelia turicatae*

d) Borrelia recurrentis and Borrelia duttonii

Borrelia recurrentis is believed to have evolved from which of the tick-borne relapsing fever?

- a) *Borrelia hermsii*
- b) *Borrelia duttonii*
- c) *Borrelia persica*
- d) *Borrelia hispanica*

Which vertebrate species is believed to have an important role in the ecology of relapsing fever in California?

- a) Flying squirrel
- b) Rat
- c) Bats
- d) Chipmunk

Historical Background

The term 'relapsing fever' was first coined following an outbreak of relapsing febrile illness in Edinburgh, UK. Whilst Otto Obermeier revealed the infectious aetiology of relapsing fever in 1868, fulfilment of Koch's postulates proved challenging due to the predilection of this spirochaete for its human host ¹. This limitation prevented publication of his findings until 1873, when sufficient additional evidence was generated to substantiate a causative role for the spirochaete. Mackie subsequently disclosed the role of the human clothing louse, *Pediculus humanus*, as the vector responsible for transmission of this infection in 1907 ². During these times, epidemic louse-borne relapsing fever (LBRF) resulted in substantial mortality, particularly during situations of overcrowding and poverty that favoured rapid spread of the organism, facilitated primarily by the clothing louse vector. Massive outbreaks resulted in millions of cases throughout Africa and globally during World Wars I and II ³.

Livingstone described another variant of relapsing fever in 1857, this time associated with soft tick vectors ⁴. Both Ross and Milne and Dutton and Todd independently established the role of ticks in transmitting this form of relapsing fever in 1904, with Dutton and Todd both becoming accidentally infected themselves whilst undertaking their research ^{5,6}. Dutton kept a temperature chart of his relapsing fever until he succumbed to the illness, with the infectious agent being named after him to reflect his contribution to our understanding of this infection. Interestingly, these researchers drew the parallel between this agent and its louse-borne variant and their observations were recently substantiated by full genomic sequencing of both infectious organisms.

Subsequently, other *Ornithodoros* soft ticks have been identified as vectors for different species of relapsing fever borreliae. The majority of these species appear to

have adapted to a particular tick species, and consequently, many are named after their tick vectors (Table 1).

Classification

Classification of members within the borreliae was initially based upon the type of tick species that serves as their vectors, with the *Borrelia burgdorferi* sensu lato complex transmitted by *Ixodes* species ticks (hard ticks), whilst the relapsing fever borreliae are transmitted by ticks belonging to the *Argasidae* genera (soft ticks; Figure 1). More recently, this rather simplistic division has been challenged with the finding that *Borrelia miyamotoi* (see Chapter 10) and *Borrelia lonestari* cluster phylogenetically amongst the relapsing fever *Borrelia*, yet are transmitted by hard ticks. Currently, there are 23 validated relapsing fever *Borrelia* species, though others are awaiting sufficient data to achieve such status, and many of these agents show a distinct preference for transmission by a specific tick vector species. *Borrelia recurrentis* is the notable exception being transmitted by clothing lice (*P. humanus*). Table 1 lists the majority of currently accepted species, though several novel species have recently been described, including “*B. mvumii*” in ticks from Tanzania ⁷, *B. microti* and other species from Iran ^{8,9}, *B. turicatae*-like *Borrelia* in bat ticks from the USA ¹⁰, and as of yet unnamed species from penguins in South Africa ¹¹, though the species status and potential virulence of this agent for humans remains to be established.

The taxonomic position of relapsing fever spirochaetes is a matter of controversy. Application of discriminatory typing tools (see section on diagnosis and typing) has revealed clades within species such as *B. hermsii*. Others have reported different sub-populations within *B. duttonii*, with all of the cultivable isolates falling into just one of four sub-types ¹². Conversely, 16S rRNA gene sequencing has underscored the similarity between some species ¹³. These similarities have been corroborated by

whole genomic sequencing, which suggests that the LBRF, *B. recurrentis*, actually represents a degraded sub-set of *B. duttonii* ¹⁴. Whilst sequencing of the closely related zoonotic *B. crocidurae* that predominates in west Africa, has further highlighted how conserved these three African species are, despite their profound differences in host preferences, severity and arthropod vectors ¹⁵. Based upon their conserved genomic make-up, yet diverse ecology, the above three relapsing fever *Borrelia* may best be considered as ecotypes of a single species.

Microbiology

Members of this group have a characteristic Gram-negative helical structure with 3 to 10 coils and a length of 10-30µm and a width of 0.2-0.5µm ¹⁶. Typical of this genus, they have up to 30 flagellae residing within the periplasmic space between the outer membrane and protoplasmic cylinder. These endow a rapid gyrating motility to these spirochaetes that can be seen using dark-field or phase microscopy of freshly collected specimens (see diagnostic methods below).

Porin proteins are found spanning the outer cell membrane and serve as a conduit for diffusion of low-molecular weight compounds. The first of these described among relapsing fever spirochaetes was p66 ¹⁷. Others have subsequently been described, such as Oms38 which appeared to be conserved between *B. recurrentis*, *B. duttonii*, *B. hermsii* and *B. turicatae* ¹⁸ that shows homology with the DipA porin found among Lyme-associated borreliae ¹⁹. Elucidation of such features will provide insights into the physiological characteristics of these spirochaetes and might additionally reveal potential targets for vaccine development.

The outer membrane lipid bilayer contains both lipidated and non-lipidated trans-membrane proteins. Much attention has been focussed upon the relatively

abundant variable membrane proteins (Vmp) as these appear to be pivotal for rapidly switching between and surviving within the diverse environments of the arthropod vector and the mammalian host (see section on pathogenesis below). Furthermore, these highly antigenic proteins are thought to contribute to the rapid switching from arthropod to host environment, binding of host factors, adhesion and even tissue tropism^{20,21}. Vmps have been extensively studied as they are subject to antigenic variation with an estimated recombination frequency of 10^{-4} to 10^{-3} per cell generations, providing a mechanism for evasion of the vertebrate host immune system. Such persistence mechanisms are likely to maximise the chances of transmission to uninfected arthropod vectors from infected vertebrates, thus providing a vital means of sustainability. They can generally be divided into one of two groups, small Vmps (also called vsp) with molecular weight ranging from 20-24kDa or large Vmps (also called vlp) that typically range from 35-45kDa²². The small Vmps have been likened to OspC of the Lyme disease spirochaete, *Borrelia burgdorferi*. This has a key role in the transmission from arthropod to vertebrate host and establishment of early infection. Furthermore, expression of different small Vmps within *B. turicatae* has been demonstrated to result in either central nervous infection (vspA) or blood-borne disease (vspB) with associated arthritis and myocarditis in a mouse infection model (see pathogenesis section below). Expression of one small Vmp (vtp or vsp33) has been proposed as a tick adapted variant. The large Vmps can be further sub-divided into one of four groups (α , β , γ , δ) base upon their sequence homologies. In the author's personal experience, human clinical isolates of *B. recurrentis* and *B. duttonii* expressed either small or large Vmps with no evidence of correlation.

The physiological requirements of these microbes is poorly understood, however their fastidious nature probably arises from their limited metabolic capabilities. This

restricted biosynthetic repertoire necessitates the supply of amino acids, fatty acids, nucleotides and enzyme co-factors from their surrounding environment, whether this is in their arthropod vector, the mammalian host or complex growth medium (see section on diagnostic methods). Whilst within the vertebrate host, the phenomenon of “rosetting” has been noted and it is hypothesised that the borreliae are “grazing” to harvest essential nutrients such as purines from red blood cells ^{23,24}. Indeed, unlike *B. burgdorferi*, the relapsing fever spirochaetes contains a full set of purine salvage genes and demonstrate efficient acquisition and incorporation of hypoxanthine, the purine catabolic product found within red blood cells, that might explain in part why these borreliae achieve higher blood densities compared to the Lyme disease spirochaetes.

The genomic organisation of these spirochaetes differs from conventional bacterial dogma, as these microbes containing linear chromosomes with covalently closed telomeres and a combination of both circular and linear plasmids that comprise approximately 10% of the full genetic complement of these microorganisms ²⁵. The majority of essential “house-keeping” genes reside on the chromosome, whilst the lipoprotein genes tend to locate to the plasmids ²⁶.

Whole genome sequencing has been undertaken for six relapsing fever spirochaetes including *B. hermsii*, *B. parkeri*, *B. turicatae*, *B. crocidurae*, *B. duttonii* and *B. recurrentis* (for further information see “BorreliaBase” ²⁷) and will provide a valuable resource to gain greater insight into the underpinning microbiological features of these organisms ^{28,29}. A striking observation arising from the project thus far has been the lack of any unique virulence features for *B. recurrentis* when compared to the closely related *B. duttonii*. As indicated above, this has led to the conclusion that rather than being a distinctive species, *B. recurrentis* is instead a degraded subspecies of *B. duttonii* that has

become louse-transmitted, though taxonomically it is still recognised as a separate species.

Ecology and transmission

Most of the relapsing fever spirochaetes are zoonoses with vertebrate reservoirs (Table 1). In the majority of cases, these reservoirs are rodents, however bats, birds and reptiles may also play a role in the environmental maintenance of these organisms³⁰⁻³³. Chipmunks have for example been noted as a significant reservoir species for *B. hermsii* in the Sierra Nevada mountains of California. Notably, for *B. recurrentis* and *B. duttonii* humans are the exclusive reservoir. Many consider the tick vector to also serve as a reservoir of infection for tick borne relapsing fever (TBRF). This is facilitated by transstadial, and for some species, transovarial transmission of the spirochaete between generations. Additionally, these ticks have an impressive longevity during periods of starvation, allowing them to survive for many years while harbouring the infectious spirochaetes. The typical argasid tick life cycle is depicted in Figure 2.

Some species that fall within the relapsing fever group have not been associated with human infection, but instead cause febrile infection among food-producing livestock. These zoonotic agents include *B. anserina*, the cause of avian borreliosis transmitted by *Argas persicus* ticks, and *B. theileri*, the agent of bovine borreliosis transmitted by *Rhipicephalus* tick species, *B. coriaceae* transmitted to deer and cattle by *O. hermsi* ticks among others³⁴⁻³⁷. It is highly probable that other livestock-related species exist^{37,38} and that a plethora of other relapsing fever-related *Borrelia* persist amongst other as of yet undisclosed wildlife reservoirs^{11,39-41}. Study of these borrelial species remains largely neglected.

Undoubtedly, poverty is a main contributory factor associated with increased risk for acquisition of a relapsing fever infection. Figures 3 and 4 portray some of these risk factors, including poor housing conditions and street beggars, which are at particular risk for relapsing fever. Both LBRF and TBRF have their greatest burden among those living in extreme poverty, who are often unaware of or unable to undertake the appropriate precautionary measures to reduce the risk of infection³. Those living in close proximity to ticks or vertebrate reservoirs are at additional risk for acquiring TBRF^{9,42}. With increasing industrialisation, urban homeless populations have seen an upsurge in clothing lice (Figures 5 and 6), which could provide new clusters of disease if infected lice are introduced into permissive regions⁴³. Another intriguing possibility for transmission and spread beyond the confines of clothing lice arises from the similarity between head and clothing lice exemplified by the overlapping phylogeny of clothing lice with cytochrome B clade A of head lice, "*Pediculus humanus var capitis*"^{44,45}. Specifically, this raises the question as to whether head lice could potentially serve as alternative vectors for *B. recurrentis*? Individuals co-infested with both types of lice have revealed the presence of *B. recurrentis* in head lice, however role of head lice in transmission requires further investigation⁴⁶. This is further supported by the finding of other clothing louse-borne infections (*Bartonella quintana*) in head lice from individuals with no evidence of co-infestation with both ecotypes of *P. humanus*⁴⁷⁻⁴⁹.

Occupational contact with tick-infested environments has resulted in clusters of TBRF infection. For example, among military personnel in Israel who used caves during training activities, approximately 6.4 cases of TBRF occurred per 100,000 individuals⁵⁰. Similarly, environmental conservation workers in endemic areas are also at risk of infection. Imported cases have been encountered through migration and tourism where clinical suspicion in non-endemic regions may not be as heightened to "exotic"

infections⁵¹⁻⁵⁴. Several clusters and sporadic infections have also been traced back to vacation destinations in rural regions where intermittently used accommodations provide refuge for reservoir hosts and their associated tick vectors⁵⁵.

Epidemiology

The relapsing fever spirochaetes have historically been divided into Old and New World species, however with improving phylogenetic tools, this division now appears rather artificial. The prevalence of tick borne strains does show correlation with clearly demarked regions, as is particularly evident for African TBRF, and probably resulted from climatic conditions conducive for the specific tick vector⁵⁶. This has not been the case for the louse-borne *B. recurrentis*, which was formerly worldwide, but is now restricted to areas where clothing lice persist^{3,57}.

What is becoming increasingly apparent, however, is the burden of relapsing fever infections occurring in endemic regions, many of which go undiagnosed or misdiagnosed as malaria^{4,58-60}. Recent reports from Senegal have suggested that relapsing fever borreliae are the cause of approximately 13% of fevers presenting at local dispensaries, representing an alarming 11 to 25 cases per 100 person years⁶¹. Studies of febrile patients in Morocco have suggested that 20.5% were due to TBRF⁶². Although not at such high levels, TBRF cases are more frequently being detected in the USA^{63,64}. Given such data, despite consideration of relapsing fever is a neglected disease, it certainly should not be forgotten.

The epidemiology of LBRF has changed drastically over recent years, with the demise of this once worldwide infection correlated directly with the reduced level of infestation with clothing lice⁶⁵. LBRF remains endemic in areas of extreme poverty such as in Ethiopia, at times spreading into adjacent regions, such as Sudan.

Pathogenesis

The pathogenesis of relapsing fever spirochaetes is poorly understood, though the release of host cytokines is thought to play a major role. Production of IL-10 by the host can have a huge influence on clinical outcome demonstrating protection against microvascular injury and apoptosis of innate immune mediators, but conversely can slow antibody-mediated clearance of spirochaetes ⁶⁶. The borreliae, including those associated with relapsing fever, are relatively neurotropic and their sequestration within the central nervous system provides an ideal refuge from which new antigenic variants can reseed the circulatory system ⁶⁷. Animal experimental studies reported survival of borreliae months to years post-infection underscoring the ability of these spirochaetes to reside within the brain.

Finally, the expressed surface Vmps play a pivotal role in pathogenesis. Some animal studies have shown correlation of particular clinical manifestations, for example high blood counts versus neurological infection with the expression of different Vmps of the same *B. turicatae* relapsing fever spirochaete (see microbiology section) ⁶⁸⁻⁷¹. Vmp proteins play a major role in the ability of these spirochaetes to maintain high blood densities in their vertebrate host sometimes reaching levels of 10^7 organisms per ml of blood. This is augmented through a gene conversion mechanism of antigenic variation that generate serotype switching and thus an intricate means of immunological evasion ⁷². This is further aided by the ability of borreliae to find serum factors such as factor H and factor H-like proteins ^{73,74}.

Clinical features

Transmission of tick-borne relapsing fever follows the bite of an infected tick whilst louse-borne infection ensues following inoculation of crushed lice or their faeces

into breaks in the skin such as through scratching. Unlike the transmission of Lyme disease borreliae that require attachment of their tick vector for an excess of 48 hours, transmission of tick-borne relapsing fever borreliae is rapid and has been demonstrated in murine models to be possible in just 15 seconds using transmission of *B. turicatae* by its *O. turicata* tick vector. This is largely a result of effective spirochaetal colonisation of the lumen of saliva-producing acini and possibly also the excretory ducts with spirochaetes facilitating rapid and effective delivery of borreliae upon initiation of tick feeding. Typically argasid ticks feed nocturnally with attachment times of 5 minutes to 2 hours being reported (average feeding time of approximately 20 minutes) and the tick taking up 2-6 times its original body weight in blood.

Following an incubation period of 3 to 10 days, patients typically develop an abrupt onset of fever and chills. The duration of febrile episodes may vary, but will generally subside in 3-5 days. This is followed by an afebrile period that lengthens as the disease progresses interspersed by further febrile episodes. Individuals with LBRF may have 3-5 febrile episodes, whilst those with TBRF may have up to 13 recurrences of fever if left untreated. Each febrile episode correlates with a change in the surface Vmp antigens of the spirochaete, whilst clearance is associated with development of a specific immunological IgM response to the preceding borrelial serotype. The severity of these febrile periods generally reduces with time, however organ involvement can complicate clinical recovery.

The most severe cases of disease are generally attributed to the human-adapted species, *B. recurrentis* and *B. duttonii*. Cases of LBRF typically present with fever, headache, hepatosplenomegaly, joint and body pains, and are often accompanied by abdominal tenderness, jaundice and epistaxis⁷⁵⁻⁷⁷. Thrombocytopenia and renal impairment are common features following infection with *B. recurrentis*. Major organ

involvement of the brain, liver, lungs and spleen result in a poor prognosis, with death associated with hepatic damage, cardiac failure, lobar pneumonia, subarachnoid haemorrhage or splenic rupture. The factors that predispose to poor clinical outcomes are poorly resolved, but are likely to involve the complex interplay between both host and microbial factors. The clinical manifestation of TBRF infections is largely dependent on the infecting *Borrelia* species and can range from a severe febrile disease similar to that described for LBRF as seen with *B. duttonii* infection, to a fairly mild febrile illness without associated mortality as seen for infection with *B. crocidurae*. Some species are associated with particular clinical correlates, such as *B. duttonii* and its adverse pregnancy outcomes whilst epistaxis and jaundice are typically clinical features of *B. recurrentis* infection. Historically this clinical variability and differential severity has been used as a means of speciation through use of animal infection models (see diagnostic section), but the biological basis remains poorly resolved.

Treatment and prognosis

Management of clinical cases is generally achieved with use of penicillin or doxycycline/tetracycline. Sometimes these are used in sequence with penicillin being followed by doxycycline as this might reduce the occurrence of potentially life-threatening Jarisch-Herxheimer reactions (JHR). This anecdotal observation has been substantiated by meta-analysis findings that showed more rapid clearance with tetracycline, but also a higher risk of JHRs, whilst mortality rates were similar between the two treatment regimens⁷⁸. The JHR is a “therapeutic shock” reaction that occurs within 24 hours of the start of therapy and is associated with a dramatic worsening of clinical symptoms in approximately 5% of cases. This is believed to occur through a pronounced release of pyrogenic cytokines with significant elevation of tumour

necrosis factor-alpha and interleukins IL-6 and IL-8⁷⁹. Indeed, some have reported benefit from use of anti-TNF-alpha⁸⁰. Although some studies have documented use of single-dose therapeutic regimes, more commonly antimicrobials are given for 7 to 14 days. Table 2 details some of the more commonly used treatment dosages and regimes.⁸¹

Mortality rates vary with the infecting agent, with most TBRF cases having less than 5% mortality. Mortality can be higher however with the East African species, *B. duttonii* and its louse-borne variant, *B. recurrentis*. Particularly high perinatal mortality rates reaching 475 cases/1000 pregnant women have been reported from Tanzania where *B. duttonii* is endemic. Notably, higher spirochaetal loads are reported among pregnant individuals compared to non-pregnant controls⁸². Life-long protection post-infection does not appear to be the norm with repeat infections being reported amongst individuals residing in endemic regions.

Diagnostic methods

Microscopy

Though refractory to visualisation by Gram-staining, these spirochaetes can be stained using Giemsa, Wright's stain or silver-staining methods (Figure 7). Darkfield or phase microscopy can be used for freshly collected blood or CSF if available (or to check results of cultivation or following animal inoculation), enabling observation of the highly motile spirochaetes. Assessment of ticks for infection has used salivary glands, haemolymph extracted post leg amputation or whole tick preparations. Whilst these methods can detect *Borrelia*, sensitivity is poor requiring in excess of 10⁵ organisms per millilitre for detection, particularly challenging for some species such as *B. crocidurae*, for which the blood burden is lower than for example *B. recurrentis*. Use of thick blood

films can improve sensitivity where numbers are low, but alternative methods such as molecular detection might provide more reliable data where sensitivity is a priority. This is further complicated by the need to collect blood during febrile episodes. An additional limitation of direct microscopy is the inability to differentiate the causative species.

Animal inoculation

Animal inoculation, particularly use of small rodents, was popular both for its ability to recover cultivable strains⁸³ and to identify these agents through comparative virulence studies (mice and guinea pigs)⁸. Although not now routinely undertaken, this technique is currently largely restricted to specialist institutes where it remains useful for recovery of primary isolates. On a cautionary note, it must be remembered that some species, such as *B. recurrentis*, are refractory to growth in rodent models, but can be isolated in primate models. Use of immunocompromised SCID mice to overcome this limitation was met with mixed success, with only low level infection being achieved⁸⁴.

Cultivation

Isolation of relapsing fever borreliae can also be achieved in culture by directly inoculating clinical samples into specialised liquid media (such as BSKII or MKP). However, the fastidious, slow-growing nature of these spirochaetes makes this technically demanding⁸⁵⁻⁸⁷. Not all batches of commercially-produced medium support growth equally well and consequently batch testing of media should be employed. In cases of culture contamination, inclusion of antimicrobials such as rifampicin, colistin sulphate, aminoglycosides or antifungals can be used to help purify isolates.

Alternatively, the slender *Borrelia* morphology enables purification of contaminated cultures through filtration.

On a cautionary note, not all species or strains appear uniformly cultivable¹². Until the 1990's, *B. recurrentis* and *B. duttonii* were deemed non-cultivable until isolates were successfully recovered using BSKII medium⁸⁸⁻⁹⁰. Despite the successful isolation of *B. duttonii*, comparison of genotypes recovered by cultivation and those detected by PCR/sequencing directly from ticks, revealed that all cultivable isolates belonged to only one of the four genotypic groups prevalent in the study area¹².

Serological detection

Specific antigens, including GlpQ or BipA, have been identified which are shared by all relapsing fever group spirochaetes, but are absent from *Borrelia* species associated with Lyme disease, thus precluding serologic cross-reactions between these borreliae^{91,92}. These antigens have been used successfully in enzyme-linked immunoassays to enable serological diagnosis or population screening. Such assays have not been produced commercially however as they would have little value in highly endemic regions, and the sporadic nature of imported cases makes commercialisation financially non-viable.

Molecular detection

Molecular detection and identification approaches offer distinct advantages over the recovery of these fastidious microbes and has become the mainstay for both detection and typing of relapsing fever *Borrelia*. Various conserved targets, such as 16S rRNA and flagellin (*flaB*) genes, have been used for diagnosis, but lack discriminatory power for typing^{37,93,94}. Availability of genomic sequence data has enabled development

of single nucleotide polymorphism (SNP) based multiplex identification methods and highly discriminatory multi-spacer typing methods^{95,96}. It must be remembered that these assays may not always detect newly described species. Use of 16S-23S ribosomal intragenic spacer region sequencing has provided a highly discriminatory means of delineating strains¹². Application of these high-resolution methods has enabled scrutiny of the genotypes circulating in specific enzoonotic regions and correlation with tick and vertebrate species⁴.

Proteomics

Although the application of proteomics for detection of relapsing fever borreliosis is in its infancy, this is an exciting potential application. Differences in proteomic profiles have been detected, differentiating tick haemolymph derived from *Borrelia*-infected ticks compared to their uninfected counterparts. This could provide a more cost-effective means of screening ticks for carriage of relapsing fever spirochaetes⁹⁷.

Control and Intervention

Relapsing fever spirochaetes currently remain exquisitely susceptible to antimicrobials including penicillin, tetracycline/doxycycline, chloramphenicol, ceftriaxone and erythromycin. Wide use of antimicrobials coupled with improvements in living conditions, particularly in areas where relapsing fever has its reservoir in humans, such as LBRF in Ethiopia, has been correlated with a declining incidence of infection⁶⁵.

This is not so apparent for the tick-borne forms of disease that persist in their longer-lived tick vector/reservoirs and through the zoonotic vertebrate reservoirs. The

burden of TBRF among subsistence agro-pastoralist communities in developing nations remains substantial. Use of acaricides (including arsenicals, chlorinated hydrocarbons, organophosphates, carbamates and synthetic pyrethroids) has been met with some success, though costs are prohibitive in many areas that would benefit from use of such measures and the environmental consequences of their use should not be overlooked^{98,99}. Biological controls have been explored with entomopathic fungi showing some success against ixodid ticks, but control of argasid ticks has been largely neglected¹⁰⁰⁻¹⁰².

Prohibiting access to high-risk areas has successfully reduced disease incidence in areas endemic for *B. persica* in Israel where significant levels of infection occurred among military personnel whilst utilising tick-infested caves during training. If contact is unavoidable, doxycycline prophylaxis has been used for short-term prevention¹⁰³.

Given the limitations of acaricides to reduce the burden of infection, other options such as immunological controls have also been explored. These measures have been used to reduce relapsing fever and indeed other pathogens vectored by the same tick species, such as African swine fever virus, a haemorrhagic febrile infection of swine with a mortality rate approaching 100%. Control at the level of the vector is consequently an attractive prospect. Again, anti-tick vaccines against ixodid ticks have led the way, with commercial vaccines against *Rhipicephalus microplus* being marketed in Australia and Latin America⁹⁹. Analysis of both the gut transcriptome and proteome of argasid ticks is an essential prerequisite for the development of such vaccine candidates. Whether these potential vaccines would be directed primarily towards protection of livestock or companion animals from tick-borne infection, or whether a “one health” approach, whereby reduction of ticks through protection of their vertebrate hosts would indirectly reduce human infections, remains to be resolved.

Concluding remarks

The relapsing fever *Borrelia* have a long and notorious history, from being one of the earliest bacterial infectious diseases described associated with high mortality and morbidity, through to a period of neglect. This in part was a result of the demise of the clothing louse and hence also LBRF, through improved hygiene, living conditions and use of DDT. Similarly, improvements in housing have reduced contact between soft ticks and humans, except in areas of poverty. Despite these global reductions in relapsing fever, endemic regions persist and detection of cases is often sub-optimal. Clinical overlap with malaria and use of suboptimal diagnostic methods for relapsing fever agents, hamper the detection of this treatable infection.

Despite this neglect, application of improved methods for detection, typing and cultivation of these spirochaetes has allowed us to gain intriguing insights into the biology of these organisms. Newly described species, and deeper understanding of the previously established members of this group will help us dissect evolutionary and ecological relationships. Greater insights into the mechanisms of pathogenesis and strategies employed to evade the immune defences of the vertebrate host will provide future research goals.

References

1. Birkhaug K, Otto H, F. Obermeier. In: Moulton FR, ed. A symposium on relapsing fever in the Americas. Washington: American Association for the Advancement of Science. Section on Medical Sciences; 1942:7-9.
2. Bryceson ADM, Parry EHO, Perine PL, Warrell DA, Vukotich D, Leithead CS. Louse-borne relapsing fever: A Clinical and Laboratory Study of 62 Cases in Ethiopia and a Reconsideration of the Literature¹. *The Quarterly Journal of Medicine* 1970;39:129-70.
3. Cutler SJ, Abdissa A, Trape JF. New concepts for the old challenge of African relapsing fever borreliosis. *Clinical Microbiology and Infection* 2009;15:400-6.
4. Schwan TG, Anderson JM, Lopez JE, et al. Endemic foci of the tick-borne relapsing fever Spirochete *Borrelia crocidurae* in Mali, West Africa, and the potential for human infection. *PLoS Neglected Tropical Diseases* 2012;6.
5. Ross PH, Milne AD. Tick fever. *British Medical Journal* 1904;ii:1453-4.
6. Dutton JE, Todd JL. The nature of tick fever in the eastern part of the Congo Free State. *British Medical Journal* 1905;ii:1259-60.
7. Mitani H, Talbert A, Fukunaga M. New World relapsing fever *Borrelia* found in *Ornithodoros porcinus* ticks in central Tanzania. *Microbiol Immunol* 2004;48:501-5.
8. Naddaf SR, Ghazinezhad B, Bahramali G, Cutler SJ. Phylogenetic analysis of the spirochete *Borrelia microti*, a potential agent of relapsing fever in Iran. *Journal of Clinical Microbiology* 2012;50:2873-6.
9. Naddaf S, Ghazinezhad B, Sedaghat M, Asl H, Cutler S. Tickborne relapsing fever in southern Iran, 2011–2013. *Emerging Infectious Diseases* 2015;21:1078-80.
10. Schwan TG, Raffel SJ, Schrumph ME, Gill JS, Piesman J. Characterization of a novel relapsing fever spirochete in the midgut, coxal fluid, and salivary glands of the bat tick *Carios kelleyi*. *Vector-Borne and Zoonotic Diseases* 2009;9:643-7.
11. Yabsley MJ, Parsons NJ, Horne EC, Shock BC, Purdee M. Novel relapsing fever *Borrelia* detected in African penguins (*Spheniscus demersus*) admitted to two rehabilitation centers in South Africa. *Parasitology Research* 2012;110:1125-30.
12. Cutler SJ, Margarita Bonilla E, Singh RJ. Population structure of East African relapsing fever *Borrelia* spp. *Emerging Infectious Diseases* 2010;16:1076-80.
13. Ras NM, Lascola B, Postic D, et al. Phylogenesis of relapsing fever *Borrelia* spp. *Int J Syst Bacteriol* 1996;46:859-65.
14. Lescot M, Audic S, Robert C, et al. The genome of *Borrelia recurrentis*, the agent of deadly louse-borne relapsing fever, is a degraded subset of tick-borne *Borrelia duttonii*. *PLoS Genetics* 2008;4.
15. Elbir H, Gimenez G, Robert C, et al. Complete genome sequence of *Borrelia crocidurae*. *Journal of Bacteriology* 2012;194:3723-4.
16. Barbour AG, Hayes SF. Biology of *Borrelia* species. *Microbiol Rev* 1986;50:381-400.
17. Bárcena-Uribarri I, Thein M, Sacher A, et al. P66 porins are present in both Lyme disease and relapsing fever spirochetes: A comparison of the biophysical properties of P66 porins from six *Borrelia* species. *Biochimica et Biophysica Acta - Biomembranes* 2010;1798:1197-203.
18. Thein M, Bunikis I, Denker K, et al. Oms38 is the first identified pore-forming protein in the outer membrane of relapsing fever spirochetes. *Journal of Bacteriology* 2008;190:7035-42.

19. Thein M, Bonde M, Bunikis I, et al. DipA, a pore-forming protein in the outer membrane of lyme disease spirochetes exhibits specificity for the permeation of dicarboxylates. *PLoS One* 2012;7.
20. Magoun L, Zückert WR, Robbins D, et al. Variable small protein (Vsp)-dependent and Vsp-independent pathways for glycosaminoglycan recognition by relapsing fever spirochaetes. *Molecular Microbiology* 2000;36:886-97.
21. Londoño D, Cadavid D. Bacterial lipoproteins can disseminate from the periphery to inflame the brain. *American Journal of Pathology* 2010;176:2848-57.
22. Barbour AG, Hayes SF. Biology of *Borrelia* species. *Microbiological Reviews* 1986;50:381-400.
23. Guo BP, Teneberg S, Munch R, et al. Relapsing fever *Borrelia* binds to neolacto glycans and mediates rosetting of human erythrocytes. *PNAS* 2009;106:19280-5.
24. Shamaei-Tousi A, Martin P, Bergh A, Burman N, Brannstrom T, Bergstrom S. Erythrocyte-aggregating relapsing fever spirochete *Borrelia crocidurae* induces formation of microemboli. *J Infect Dis* 1999;180:1929-38.
25. Miller SC, Porcella SF, Raffel SJ, Schwan TG, Barbour AG. Large linear plasmids of *Borrelia* species that cause relapsing fever. *Journal of Bacteriology* 2013;195:3629-39.
26. Restrepo BI, Kitten T, Carter CJ, Infante D, Barbour AG. Subtelomeric expression regions of *Borrelia hermsii* linear plasmids are highly polymorphic. *Molecular Microbiology* 1992;6:3299-311.
27. Di L, Pagan PE, Packer D, et al. *BorreliaBase*: A phylogeny-centered browser of *Borrelia* genomes. *BMC Bioinformatics* 2014;15.
28. Elbir H, Gimenez Gg, Robert C, et al. Complete Genome Sequence of *Borrelia crocidurae*. *Journal of Bacteriology* 2012;194:3723-4.
29. Lescot M, Audic S, Robert C, et al. The genome of *Borrelia recurrentis*, the agent of deadly louse-borne relapsing fever, is a degraded subset of tick-borne *Borrelia duttonii*. *PLoS Genet* 2008;4:e1000185.
30. Socolovschi C, Kernif T, Raoult D, Parola P. *Borrelia*, *Rickettsia*, and *Ehrlichia* species in bat ticks, France, 2010. *Emerging Infectious Diseases* 2012;18:1966-75.
31. Loftis A, Gill J, Schriefer M, et al. Detection of *Rickettsia*, *Borrelia*, and *Bartonella* in *Carios kelleyi* (Acari: Argasidae). *J Med Entomol* 2005;42:473-80.
32. Reeves W, Loftis A, Sanders F, et al. *Borrelia*, *Coxiella*, and *Rickettsia* in *Carios capensis* (Acari: Argasidae) from a brown pelican (*Pelecanus occidentalis*) rookery in South Carolina, USA. *Exp Appl Acarol* 2006;39:321-9.
33. Takano A, Sugimori C, Fujita H, et al. A novel relapsing fever *Borrelia* sp. infects the salivary glands of the molted hard tick, *Amblyomma geoemydae*. *Ticks and Tick-borne Diseases* 2012;3:259-61.
34. McCoy BN, Maïga O, Schwan TG. Detection of *Borrelia theileri* in *Rhipicephalus geigy* from Mali. *Ticks and Tick-borne Diseases* 2014;5:401-3.
35. Cutler S, Abdissa A, Adamu H, Tolosa T, Gashaw A. *Borrelia* in Ethiopian ticks. *Ticks and Tick-borne Diseases* 2012;3:14-7.
36. Yparraguirre LA, Machado-Ferreira E, Ullmann AJ, Piesman J, Zeidner NS, Soares CAG. A hard tick relapsing fever group spirochete in a Brazilian *Rhipicephalus* (*Boophilus*) *microplus*. *Vector-Borne and Zoonotic Diseases* 2007;7:717-21.
37. Kumsa B, Socolovschi C, Raoult D, Parola P. New *Borrelia* species detected in ixodid ticks in Oromia, Ethiopia. *Ticks and Tick-borne Diseases* 2015;6:401-7.

38. Mediannikov O, Abdissa A, Socolovschi C, Diatta G, Trape JF, Raoult D. Detection of a new *Borrelia* species in ticks taken from cattle in southwest Ethiopia. *Vector-Borne and Zoonotic Diseases* 2013;13:266-9.
39. Lane RS, Mun J, Parker JM, White M. Columbian black-tailed deer (*Odocoileus hemionus columbianus*) as hosts for *Borrelia* spp. in northern California. *Journal of Wildlife Diseases* 2005;41:115-25.
40. Thomas NJ, Bunikis J, Barbour AG, Wolcott MJ. Fatal spirochetosis due to a relapsing fever-like *Borrelia* sp. in a northern spotted owl. *Journal of Wildlife Diseases* 2002;38:187-93.
41. Evans N, Bown K, Timofte D, Simpson V, Birtles R. Fatal borreliosis in bat caused by relapsing fever spirochete, United Kingdom. *Emerg Infect Dis* 2009;15:1331.
42. Trape JF. Morbidity record in Africa for an unrecognized emergent disease. *Cahiers Sante* 2006;16:102.
43. Brouqui P, Stein A, Dupont H, et al. Ectoparasitism and vector-borne diseases in 930 homeless people from Marseilles. *Medicine (Baltimore)* 2005;84:61-8.
44. Li W, Ortiz G, Fournier P-E, et al. Genotyping of Human Lice Suggests Multiple Emergences of Body Lice from Local Head Louse Populations. *PLoS Negl Trop Dis* 2010;4:e641.
45. Light JE, Allen JM, Long LM, et al. Geographic distributions and origins of human head lice (*Pediculus humanus capitis*) based on mitochondrial data. *Journal of Parasitology* 2008;94:1275-81.
46. Boutellis A, Mediannikov O, Bilcha KD, et al. *Borrelia recurrentis* in head lice, Ethiopia. *Emerging Infectious Diseases* 2013;19:796-8.
47. Angelakis E, Rolain JM, Raoult D, Brouqui P. *Bartonella quintana* in head louse nits. *FEMS Immunology and Medical Microbiology* 2011;62:244-6.
48. Boutellis A, Veracx A, Angelakis E, et al. *Bartonella quintana* in head lice from Sénégal. *Vector-Borne and Zoonotic Diseases* 2012;12:564-7.
49. Cutler S, Abdissa A, Adamu H, Tolosa T, Gashaw A. *Bartonella quintana* in Ethiopian lice. *Comparative Immunology, Microbiology and Infectious Diseases* 2012;35:17-21.
50. Moran-Gilad J, Levine H, Schwartz E, et al. Postexposure prophylaxis of tick-borne relapsing fever: Lessons learned from recent outbreaks in Israel. *Vector-Borne and Zoonotic Diseases* 2013;13:791-7.
51. Colebunders R, De Serrano P, Van Gompel A, et al. Imported relapsing fever in European tourists. *Scandinavian Journal of Infectious Diseases* 1993;25:533-6.
52. Wyplosz B, Mihaila-Amrouche L, Baixench M-T, et al. Imported tickborne relapsing fever, France. *Emerging Infectious Diseases* 2005;11:1801-3.
53. Rummens JL, Louwagie A, Van Hoof A, Boelaert J, Gordts B, Van Landuyt HW. Relapsing fever imported into Belgium: A case report. *Acta Clinica Belgica* 1987;42:210-4.
54. Kutsuna S, Kawabata H, Kasahara K, Takano A, Mikasa K. Case report: The first case of imported relapsing fever in Japan. *American Journal of Tropical Medicine and Hygiene* 2013;89:460-1.
55. Schwan TG, Policastro PF, Miller Z, Thompson RL, Damrow T, Keirans JE. Tick-borne relapsing fever caused by *Borrelia hermsii*, Montana. *Emerging Infectious Diseases* 2003;9:1151-4.
56. Trape JF, Godeluck B, Diatta G, et al. The spread of tick-borne borreliosis in West Africa and its relationship to sub-Saharan drought. *American Journal of Tropical Medicine and Hygiene* 1996;54:289-93.

57. Cutler SJ. Relapsing fever - A forgotten disease revealed. *Journal of Applied Microbiology* 2010;108:1115-22.
58. Nordstrand A, Bunikis I, Larsson C, et al. Tickborne relapsing fever diagnosis obscured by Malaria, Togo. *Emerging Infectious Diseases* 2007;13:117-23.
59. Diatta G, Souidi Y, Granjon L, et al. Epidemiology of Tick-Borne Borreliosis in Morocco. *PLoS Neglected Tropical Diseases* 2012;6.
60. Lundqvist J, Larsson C, Nelson M, Andersson M, Bergström S, Persson C. Concomitant infection decreases the malaria burden but escalates relapsing fever borreliosis. *Infection and Immunity* 2010;78:1924-30.
61. Parola P, Diatta G, Socolovschi C, et al. Tick-borne relapsing fever borreliosis, rural Senegal. *Emerging Infectious Diseases* 2011;17:883-5.
62. Sarih M, Garnier M, Boudebouch N, et al. *Borrelia hispanica* relapsing fever, Morocco. *Emerging Infectious Diseases* 2009;15:1626-9.
63. Schwan TG, Raffel SJ, Schrumpf ME, et al. Tick-borne relapsing fever and *Borrelia hermsii*, Los Angeles County, California, USA. *Emerging Infectious Diseases* 2009;15:1026-31.
64. Trevejo RT, Schriefer ME, Gage KL, et al. An interstate outbreak of tick-borne relapsing fever among vacationers at a Rocky Mountain cabin. *American Journal of Tropical Medicine and Hygiene* 1998;58:743-7.
65. Ramos JM, Malmierca E, Reyes F, Tesfamariam A. Results of a 10-year survey of louse-borne relapsing fever in southern Ethiopia: A decline in endemicity. *Annals of Tropical Medicine and Parasitology* 2008;102:467-9.
66. Cadavid D, Londoño D. Understanding tropism and immunopathological mechanisms of relapsing fever spirochaetes. *Clinical Microbiology and Infection* 2009;15:415-21.
67. Cadavid D, Sondey M, Garcia E, Lawson CL. Residual brain infection in relapsing-fever borreliosis. *Journal of Infectious Diseases* 2006;193:1451-8.
68. Mehra R, Londoño D, Sondey M, Lawson C, Cadavid D. Structure-function investigation of Vsp serotypes of the spirochete *Borrelia hermsii*. *PLoS ONE* 2009;4.
69. Cadavid D, Pennington PM, Kerentseva TA, Bergström S, Barbour AG. Immunologic and genetic analyses of VmpA of a neurotropic strain of *Borrelia turicatae*. *Infection and Immunity* 1997;65:3352-60.
70. Sethi N, Sondey M, Bai Y, Kim KS, Cadavid D. Interaction of a neurotropic strain of *Borrelia turicatae* with the cerebral microcirculation system. *Infection and Immunity* 2006;74:6408-18.
71. Larsson C, Andersson M, Pelkonen J, Guo BP, Nordstrand A, Bergström S. Persistent brain infection and disease reactivation in relapsing fever borreliosis. *Microbes and Infection* 2006;8:2213-9.
72. Dai Q, Restrepo BI, Porcella SF, Raffel SJ, Schwan TG, Barbour AG. Antigenic variation by *Borrelia hermsii* occurs through recombination between extragenic repetitive elements on linear plasmids. *Molecular Microbiology* 2006;60:1329-43.
73. Rossmann E, Kraiczy P, Herzberger P, et al. BhCRASP-1 of the relapsing fever spirochete *Borrelia hermsii* is a factor H- and plasminogen-binding protein. *International Journal of Medical Microbiology* 2008;298:272-83.
74. Grosskinsky S, Schott M, Brenner C, et al. *Borrelia recurrentis* employs a novel multifunctional surface protein with anti-complement, anti-opsonic and invasive potential to escape innate immunity. *PLoS ONE* 2009;4.

75. Borgnolo G, Hailu B, Ciancarelli A, Almaviva M, Woldemariam T. Louse-borne relapsing fever. A clinical and an epidemiological study of 389 patients in Asella Hospital, Ethiopia. *Trop Geogr Med* 1993;45:66-9.
76. Borgnolo G, Denku B, Chiabrera F, Hailu B. Louse-borne relapsing fever in Ethiopian children: A clinical study. *Annals of Tropical Paediatrics* 1993;13:165-71.
77. Brown V, Larouze B, Desve G, et al. Clinical presentation of louse-born relapsing fever among Ethiopian refugees in northern Somalia. *Annals of Tropical Medicine and Parasitology* 1988;82:499-502.
78. Guerrier G, Doherty T. Comparison of antibiotic regimens for treating louse-borne relapsing fever: A meta-analysis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2011;105:483-90.
79. Negussie Y, Remick DG, DeForge LE, Kunkel SL, Eynon A, Griffin GE. Detection of plasma tumor necrosis factor, interleukins 6, and 8 during the Jarisch-Herxheimer reaction of relapsing fever. *Journal of Experimental Medicine* 1992;175:1207-12.
80. Fekade D, Knox K, Hussein K, et al. Prevention of Jarisch-Herxheimer reactions by treatment with antibodies against tumor necrosis factor α . *New England Journal of Medicine* 1996;335:311-5.
81. Belum GR, Belum VR, Chaitanya Arudra SK, Reddy BSN. The Jarisch–Herxheimer reaction: Revisited. *Travel Medicine and Infectious Disease*.
82. Jongen VH, van Roosmalen J, Tiems J, Van Holten J, Wetsteyn JC. Tick-borne relapsing fever and pregnancy outcome in rural Tanzania. *Acta Obstet Gynecol Scand* 1997;76:834-8.
83. Fritz CL, Bronson LR, Smith CR, Schriefer ME, Tucker JR, Schwan TG. Isolation and Characterization of *Borrelia hermsii* Associated with Two Foci of Tick-Borne Relapsing Fever in California. *Journal of Clinical Microbiology* 2004;42:1123-8.
84. Larsson C, Lundqvist J, van Rooijen N, Bergström S. A Novel Animal Model of *Borrelia recurrentis* Louse-Borne Relapsing Fever Borreliosis Using Immunodeficient Mice. *PLoS Negl Trop Dis* 2009;3:e522.
85. Barbour A. Isolation and cultivation of Lyme disease spirochetes. *Yale J Biol Med* 1984;57:521-5.
86. Wagemakers A, Oei A, Fikrig MM, Miellel WR, Hovius JW. The relapsing fever spirochete *Borrelia miyamotoi* is cultivable in a modified Kelly-Pettenkofer medium, and is resistant to human complement. *Parasites and Vectors* 2014;7.
87. Cutler SJ, Jones SE, Wright DJM, Zhang H. Cultivation of East African relapsing fever *Borrelia* and review of preceding events. *Journal of Spirochetal and Tick-borne Diseases* 2000;7:52-8.
88. Cutler SJ, Akintunde COK, Moss J, et al. Successful in vitro cultivation of *Borrelia duttonii* and its comparison with *Borrelia recurrentis*. *International Journal of Systematic Bacteriology* 1999;49:1793-9.
89. Cutler S, Moss J, Fukunaga M, Wright D, Fekade D, Warrell D. *Borrelia recurrentis* characterization and comparison with relapsing- fever, Lyme-associated, and other *Borrelia* spp. *Int J Syst Bacteriol* 1997;47:958-68.
90. Cutler SJ, Fekade D, Hussein K, et al. Successful in-vitro cultivation of *Borrelia recurrentis*. *Lancet* 1994;343:242.
91. Schwan TG, Schrupf ME, Hinnebusch BJ, Anderson Jr DE, Konkel ME. GlpQ: An antigen for serological discrimination between relapsing fever and Lyme borreliosis. *Journal of Clinical Microbiology* 1996;34:2483-92.

92. Lopez J, Schruppf M, Nagarajan V, Raffel S, McCoy B, Schwan T. A novel surface antigen of relapsing fever spirochetes can discriminate between relapsing fever and Lyme borreliosis. *Clin Vaccine Immunol* 2010;17:564-71.
93. Fukunaga M, Ushijima Y, Aoki L, Talbert A. Detection of *Borrelia duttonii*, a tick-borne relapsing fever agent in central Tanzania, within ticks by flagellin gene-based nested polymerase chain reaction. *Vector Borne Zoonotic Dis* 2001;1:331-8.
94. Assous MV, Wilamowski A, Bercovier H, Marva E. Molecular characterization of tickborne relapsing fever *Borrelia*, Israel. *Emerging Infectious Diseases* 2006;12:1740-3.
95. Elbir H, Henry M, Diatta G, et al. Multiplex Real-Time PCR Diagnostic of Relapsing Fevers in Africa. *PLoS Neglected Tropical Diseases* 2013;7.
96. Elbir H, Gimenez G, Sokhna C, et al. Multispacer sequence typing relapsing fever *Borreliae* in Africa. *PLoS neglected tropical diseases* 2012;6.
97. Fotso Fotso A, Mediannikov O, Diatta G, et al. MALDI-TOF Mass Spectrometry Detection of Pathogens in Vectors: The *Borrelia crocidurae*/*Ornithodoros sonrai* Paradigm. *PLoS Negl Trop Dis* 2014;8:e2984.
98. Talbert A, Nyange A, Molteni F. Spraying tick-infested houses with lambda-cyhalothrin reduces the incidence of tick-borne relapsing fever in children under five years old. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1998;92:251-3.
99. Díaz-Martín V, Manzano-Román R, Obolo-Mvoulouga P, Oleaga A, Pérez-Sánchez R. Development of vaccines against *Ornithodoros* soft ticks: An update. *Ticks and Tick-borne Diseases* 2015;6:211-20.
100. D'Alessandro WB, Humber RA, Luz C. Occurrence of pathogenic fungi to *Amblyomma cajennense* in a rural area of Central Brazil and their activities against vectors of Rocky Mountain spotted fever. *Veterinary Parasitology* 2012;188:156-9.
101. Greengarten PJ, Tuininga AR, Morath SU, Falco RC, Norelus H, Daniels TJ. Occurrence of soil- and tick-borne fungi and related virulence tests for pathogenicity to *Ixodes scapularis* (Acari: Ixodidae). *Journal of Medical Entomology* 2011;48:337-44.
102. Tavassoli M, Malekifard F, Soleimanzadeh A, Pourseyed SH, Bernousi I, Mardani K. Susceptibility of different life stages of *Ornithodoros lahorensis* to entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana*. *Parasitology Research* 2012;111:1779-83.
103. Assous MV, Wilamowski A. Relapsing fever borreliosis in Eurasia - Forgotten, but certainly not gone! *Clinical Microbiology and Infection* 2009;15:407-14.

Figure Legends

Figure 1: *Ornithodoros moubata* tick.

Figure 2: Life cycle of *Ornithodoros moubata* ticks, the vector of *B. duttonii*.

Figure 3: Ethiopian dwelling.

Figure 4: Street beggar in Ethiopia.

Figure 5: *Pediculus humanus* clothing lice showing adult and sub-adult stages.

Figure 6: *Pediculus humanus* clothing lice showing eggs cemented to clothing and recently hatched lice.

Figure 7: Blood film showing Giemsa-staining magnification x400.

Table 1: Relapsing fever group species, their vectors and geographical location.

Table 2: Treatment options commonly used for relapsing fever management.

