Review Article: Relapsing Fever Borreliae in Africa

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Abstract. The study of relapsing fever borreliae in Africa has long suffered from the use of non-specific laboratory tools for the direct detection of these spirochetes in clinical and vector specimens. Accordingly, Borrelia hispanica, Borrelia crocidurae, Borrelia duttonii, and Borrelia recurrentis have traditionally been distinguished on the basis of geography and vector and the unproven hypothesis that each species was exclusive to one vector. The recent sequencing of three relapsing fever Borrelia genomes in our laboratory prompted the development of more specific tools and a reappraisal of the epidemiology in Africa. Five additional potential species still need to be cultured from clinical and vector sources in East Africa to further assess their uniqueness. Here, we review the molecular evidence of relapsing fever borreliae in hosts and ectoparasites in Africa and explore the diversity, geographical distribution, and vector association of these pathogens for Africans and travelers to Africa.

INTRODUCTION

Relapsing fever borreliae are a group of ectoparasite-borne, fastidious bacteria responsible for various febrile presentations, most commonly malaria-like symptoms.1 In Africa, Arnould, a French Army doctor, first clinically described relapsing fever among prisoners near Constantine, Algeria in March 1866.2 Two years later, borreliae were observed microscopically by Obermeier in the blood of relapsing fever patients.3 This observation was confirmed by a subsequent study4 and borreliae were further detected in Ornithodoros moubata ticks.5 The role of lice in the transmission of what is currently called louse-borne relapsing fever was hypothesized6 and experimentally demonstrated7 in the early 20th century. Because relapsing fever borreliae remained uncultured, they were classified on the basis of their vectors and virulence in animal models.8 Indeed, Borrelia hispanica remained uncultured in axenic medium until 1976,9 Borrelia recurrentis until 1994,10 and Borrelia duttonii until 199911, Borrelia crocidurae, first described in musk shrew blood in Senegal in 191712 was only cultured in axenic medium in 1999.13 Other relapsing fever borreliae have been identified along the eastern border of Africa in Eurasia, chiefly Borrelia persica in Israel and the Palestinian territories,14 Iran15 and Uzbekistan and Tajikistan,16 and Borrelia microti in Iran.17 Because these species have not been reported in the African continent itself, they will not be discussed in this review.

In 2008–2011, our laboratory sequenced the genomes of three of the four known cultured Borrelia from Africa, i.e., B. duttonii, B. recurrentis,18 and B. crocidurae.19 Recent efforts to culture and sequence relapsing fever borreliae have provided new information for a reassessment of the diversity of these bacteria. In this review, we consider the molecular evidence of relapsing fever borreliae in hosts and ectoparasites in Africa and explore the diversity, geographical distribution, and vector association of these pathogens for Africa and travelers to Africa.

CLINICAL FEATURES

The main symptom characteristic of relapsing fever borreliosis is recurrent febrile episodes interrupted by afebrile periods. For the louse-borne B. recurrentis, fever is accompanied in more than 90% of patients with tachycardia, headache, myalgia, and arthralgia and is less frequently accompanied by hepatosplenomegaly, epistaxis, petechial rash, and jaundice. For the tick-borne B. crocidurae, the disease is characterized by a fever, asthenia, and vomiting in some patients. Most infected patients experience 1 to 2 relapses; however, up to eight relapses have been observed. The clinical signs and density of Borrelia are not affected by the age or sex of the patient. Immunity after infection is not permanent and patients may be newly infected as soon as 6 months after recovery. However, no deaths from borreliae were recorded in Senegal over a period of 14 years.20 In this country, 0.9% of 1,340 children were smear positive,21 and real-time PCR for the 16S rRNA Borrelia gene detected borreliae in 27 (13%) of 206 samples from febrile patients in rural Senegal.22 The clinical features of B. duttonii infection have been well studied in Tanzania, in which the total mortality rate of the disease is ~2.3%.23 Symptoms of this pathogen include fever, which is accompanied in more than 90% of cases with tachycardia, headache, myalgia, arthralgia, conjunctivitis, hepatosplenomegaly, and splenomegaly, along with orange urine in a few cases.24 In Tanzania, investigators found a perinatal mortality caused by B. duttonii of 436/1,000 births.25 In North Africa, B. hispanica causes 20.5% of unexplained fever cases in Northwestern Morocco.26

Relapsing fever borrelioses are easily treatable by penicillin, doxycycline, and tetracycline27 but the Jarisch-Herxheimer reaction, characterized by an increased respiratory rate and drop in blood pressure, is a major side effect of antibiotic treatment, causing a mortality rate of up to 5%.28,29 This reaction is associated with the release of cytokines during the clearance of borreliae from the blood.30 A few studies indicated that the administration of antibodies against tumor necrosis factor-alpha does prevent Jarisch-Herxheimer reaction during relapsing fever.31,32 however such expensive medications are not currently available in African countries where relapsing fevers are endemic. Because field experience in Africa has indicated that relapsing fevers are readily cured by antibiotics, there has been no need for the in vitro susceptibility testing of antibiotics. Nevertheless, ceftriaxone clears B. duttonii in a mouse model of infection.33

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Phenotypic analyses. The relapsing fever borreliae species were initially distinguished on the basis of geography and

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vector; this classification was based on a cospeciation hypothesis that postulated that only one relapsing fever Borrelia species could be found in a particular host and vector in a given geographic area. However, recent demonstrations of the coexistence of B. duttonii and B. crocidurae in Togo and of B. crocidurae and B. hispanica in North Africa suggest that the previous geographical distribution studies were not comprehensive. Further microscopic observation of relapsing fever borreliae isolated from vectors and the blood of patients after Giemsa staining did not help to distinguish the species, nor did observations of characteristic morphology and motility by dark field microscopy, phase contrast microscopy, and electron microscopy. The various relapsing fever borreliae exhibit similar sizes and morphology. Further phenotypic characterization of relapsing fever borreliae isolated and cultured by xeniosolation in mice or in axenic, artificial culture medium is also unhelpful. In particular, matrix-assisted laser desorption ionization time-of-flight mass spectrometry identification has not been reported for relapsing fever borreliae, in contrast with other spirochetes. Phenotypic traits, therefore, do not discriminate between cultured African relapsing fever borreliae (Figure 1).

Genetic analyses. Relatively few genetic loci, such as the single-copy chromosomal 16S rRNA and flaB genes and the 16S-23S ribosomal RNA intergenic spacer (IGS), have been used to discriminate between the various African relapsing fever borreliae. Genetic comparisons indicate that the 16S rRNA gene exhibits two to six nucleotide differences among the four cultured species; these differences are scattered along the whole 16S rRNA gene sequence for each species, resulting in a high overall sequence similarity of 99.7–99.9% between the four species. Moreover, interrogation of GenBank database indicates that these signatures are not consistent over the 37 B. crocidurae 16S rRNA gene sequences recorded in GenBank, resulting in a < 1% 16S rRNA gene sequence intraspecies variation. The same observation is true for B. duttonii. For the 1,008-bp flaB gene, genetic comparison indicates that only two nucleotides differentiate B. duttonii from B. recurrentis, whereas 10 nucleotides differentiate B. crocidurae from B. duttonii and B. recurrentis. GenBank database consultation indicates that these flaB sequence signatures are not conserved across different strains. The flaB sequences in 18 B. duttonii strains contain 31 single nucleotide polymorphisms with 11 non-synonymous mutations. Five flaB sequences from B. crocidurae contain 11 single nucleotide polymorphisms with two non-synonymous mutations. For B. recurrentis the flaB sequences of 21 strains contain only one synonymous mutation. In addition several flaB sequences with 100% similarity are labeled with equal confidence as B. duttonii or B. recurrentis. For the 1,002-bp glpQ gene, genetic analysis indicates that 9 positions, located in a 200-bp hot spot, differentiate B. crocidurae from B. duttonii and B. recurrentis with a similarity level of 96%; only one position differentiates B. duttonii from B. recurrentis. The sequence signature differentiating B. crocidurae from B. duttonii and B. recurrentis is conserved across the glpQ sequences that have been deposited into GenBank.

Further discrimination between borreliae could be achieved by analyzing intergenic spacers. The IGS showed an interspecies sequence variability of 92–99%, making it a potential target to identify borreliae from vectors and from blood. The IGS sequence-based phylogenetic tree clustered B. crocidurae and B. hispanica into one clade and also generated a clade comprising B. duttonii and B. recurrentis. Indeed, IGS sequencing in Tanzania mixed four B. duttonii types with two B. recurrentis types. Because sequencing a single spacer may be inaccurate, we recently developed a multispacer sequence typing (MST) approach and showed that sequencing five appropriate intergenic spacers accurately identified cultured relapsing fever borreliae and revealed diversity among them.

These data indicate that no single sequence accurately discriminates between relapsing fever Borrelia and that a combination of multiple sequencing is required. The MST can achieve this goal because sequencing both the glpQ gene and

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**Figure 1.** Geographical distribution of African relapsing fever of the Borrelia species.
the IGS is suitable for differentiating *B. crocidurae* and *B. hispanica* from *B. duttonii* and *B. recurrentis*, as illustrated in samples from Morocco and Tunisia.26–30 Conventional PCR with or without sequencing and real-time PCR targeting these genomic regions have been used to document borreliae in reservoirs, ectoparasites, and clinical specimens.43–46

**Relapsing fever borreliae with cultured representatives in Africa.** *Borrelia crocidurae* flagellin sequences have been detected in patients in Senegal and Mauritania.21 Accordingly, *B. crocidurae* has in turn been detected in travelers returning from Senegal in both France47,48 and Italy.49 In Tunisia, *B. crocidurae* was documented by 16S rRNA gene and ITS sequencing in 15% of *Ornithodoros erraticus* ticks but not in human patients.50 The *B. crocidurae* 16S rRNA gene sequence has also been identified in patients in Mali, Gambia, and Togo.13,35,41 The latter observation was unexpected because the tick vector has not been documented in Tunisia, and ITS sequencing in 15% of *O. erraticus* was documented by 16S rRNA gene and IGS sequencing, and *O. erraticus* is the known vector for this pathogen.50 An analysis of the unpublished *B. duttonii* 16S rRNA sequences in GenBank shows five sequences from Tanzania, two sequences from Zaire, one sequence from Rwanda, and three sequences without known geographical origin. A similar analysis for the *B. duttonii flaB* sequences shows 38 from Tanzania and 10 sequences without known origin. The 34 16S–23S sequences from this species were collected in Tanzania. *Borrelia recurrentis* has been confirmed in the East African countries of Ethiopia, Rwanda, Sudan, and Zaire.30,51 The disease was once distributed worldwide, however it has recently become less prevalent and more geographically restricted to African countries. Ethiopia and Sudan remain hotspot regions.

The genetic diversity is variable among relapsing fever species. The *Borrelia recurrentis* MST-based study showed that cultured strains collected over 10 years had two types; *B. duttonii* was more variable with four types; and *B. crocidurae* strains obtained in two closed rural villages in Senegal had more variability (8 MST) and exhibited geographical clustering.42

**Uncultured relapsing fever borreliae in Africa.** In addition to the classified species and the new species described in Tanzania,52 gene sequencing has provided evidence for three new yet uncultured *Borrelia* species in Africa. The *flaB* gene sequencing of spirochetes observed in the blood of two penguins (*Spheniscus demersus*) revealed the presence of a novel relapsing fever *Borrelia*.45 Indeed, this 327-bp *flaB* gene sequence shared 99% sequence similarity with that of *Borrelia* sp. K64, which was previously detected in *Carios sawaii* ticks removed from seabirds in Japan.53 Phylogenetic analyses included the penguin *Borrelia* sp. in a well-supported clade along with *Borrelia* sp. K64 and *Borrelia turicatae*. In Ethiopia, 16S rRNA real-time PCR screening of *Amblyomma cohaerens* ticks followed by *flaB* gene sequencing suggested the presence of a potentially new *Borrelia* species in three males, three females, and two nymphs (8 of 109; 7.3%). This 344-bp *flaB* gene sequence shared only 85–86% sequence similarity with *B. duttonii*, *B. recurrentis*, and *B. crocidurae*.54 Further phylogenetic analysis of a 297-bp portion of the sequence showed that the new *Borrelia* species formed a separate branch distinct from the Lyme disease and recurrent fever groups.54 Another potentially new species was described in *Rhipicephalus evertsi* ticks from Nigeria based on 16S rRNA gene sequencing.55

**PERSPECTIVES**

The data reviewed in this work indicate that at least 10 different relapsing fever borreliae have been documented in Africa, including five different borreliae in humans and five different borreliae in nonhuman hosts. The former include the pathogens classified as *B. hispanica*, *B. crocidurae*, *B. duttonii*, and *B. recurrentis*. Parallel to these results, the huge diversity of *B. crocidurae* strains has been illustrated using post-genomic MST genotyping.42 A few borreliae, however, have been identified from African sources, indicating the absolute necessity of effective laboratory tools for precisely identifying African relapsing fever borreliae. The sequencing of complete genomes should be extended to *B. hispanica* to allow the creation of such post-genomic tools after they have been validated on a set of well-documented strains, as has previously been conducted for the *recA* gene in *B. recurrentis*.56 These techniques may include repeat sequence-based tools with increased sensitivity and specificity. It will be important to develop these tests in a format that is compatible with the point-of-care approach.57 Such point-of-care diagnostic tests will allow for a better appraisal of the prevalence of different relapsing fevers, their associated clinical features, and their epidemiology by the documentation of hosts and vectors, aiding in the effective control of the pathogens.

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