SHORT COMMUNICATION

Infection of *Ixodes ricinus* (Acari: Ixodidae) by *Borrelia burgdorferi* sensu lato in North Africa

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ABSTRACT Free-living adult *Ixodes ricinus* L. were collected in Amdoun, situated in the Kroumiry mountains in northwestern Tunisia (North Africa). Using direct fluorescence antibody assay, the infection rate of field-collected *I. ricinus* by *Borrelia burgdorferi* sensu lato was 30.5% ($n = 72$). No difference in infection rate was observed between male and female ticks. Spirochetes that had been isolated from *I. ricinus* from Ain Drahim (Kroumiry Mountains) in 1988 were identified as *Borrelia lusitaniae* (formerly genospecies PotiB2). This is the first identification of a genospecies of *Borrelia burgdorferi* sensu lato from the continent of Africa.

KEY WORDS *Ixodes ricinus*, *Borrelia burgdorferi*, *Borrelia lusitaniae*, Tunisia

IN EUROPE, *Ixodes ricinus* L. is the principal vector of *Borrelia burgdorferi*, the etiologic agent of Lyme borreliosis (Aeschlimann et al. 1986). *Borrelia burgdorferi* sensu lato is now known to include at least 5 genospecies associated with *I. ricinus: Borrelia burgdorferi* sensu stricto, *Borrelia garinii*, *Borrelia afzelii*, *Borrelia valaisiana* (formerly genospecies VS116), and *Borrelia lusitaniae* (formerly genospecies PotiB2) (Baranton et al. 1992; Canica et al. 1993; Postic et al. 1994, 1997; Le Flèche et al. 1997; Wang et al. 1997). The geographical distribution of *I. ricinus* covers southern Scandinavia, the British Isles, Central Europe, France, Spain, Portugal, Italy, the Balkans, East Europe, northern Iran, and North Africa (Perez and Rodhain 1977, Stanek et al. 1988). Infection of *I. ricinus* by *B. burgdorferi* sensu lato has been reported from many European countries. In Africa, however, primarily serological and clinical results have been reported (Stanek et al. 1986, Ginsburg et al. 1991, Zahaf et al. 1994, Mhalu and Matre 1996), and few entomological studies have been performed (Zhioua et al. 1989). The objectives of this study were to determine the infection rate of *I. ricinus* by *B. burgdorferi* sensu lato at a sample site in Northern Africa (Tunisia), and to identify the genospecies of *Borrelia* isolated from *I. ricinus* from Tunisia.

Materials and Methods

Study Site. In Tunisia, *I. ricinus* is localized in the northwest, mainly in the mountains of Kroumiry (Atlas mountains), where it is abundant in oak forests (Zhioua et al. 1989, Bouattour and Darghouth 1996). These forests have rich undergrowth and harbor small mammals, deer, and wild boar (Gharaibeh 1997). The activity peak of adult *I. ricinus* in this area is in February–March (Senevet and Rossi, 1924, Sergent and Poncet 1937, Yousfi-Monod and Aeschlimann 1986), so we sampled ticks in March 1996. Unfed adult ticks were collected by flagging or by direct collection from the grass in the region of Amdoun (Kroumiry).

Spirochete Detection. Adults were surface-sterilized and dissected to remove the gut. Midguts were chilled, placed on glass slides, and examined for the presence of spirochetes using direct fluorescent antibody assay (DFA) (Zhioua et al. 1994). Antigens were dried for at least 2 h at 34°C and subsequently fixed with acetone for 10 min. Preparations were stained directly with high-titered fluorescein isothiocyanate conjugated anti-*B. burgdorferi* goat antibody (KPL, Gaithersburg, MD).

In 1988, spirochetes were isolated from field-collected *I. ricinus* from the region of Ain Drahim (Kroumiry, Tunisia) (Zhioua et al. 1989). Unfortunately, identification of these spirochetes was not possible in 1988 because the cultures were contaminated (Zhioua et al. 1989), and the isolates were stored at −70°C. Technical advances now allow characterization of these isolates. *Borrelia* isolates were identified in 1996 by *rrf* (5S)-*rrf* (23S) spacer restriction fragment-length polymorphism (RFLP) analysis as described by Postic et al. (1994).

Results and Discussion

In total, 72 adult *I. ricinus* (41 females, 31 males) collected from Amdoun in 1996 were examined for the presence of *B. burgdorferi* sensu lato by DFA. The infection rate was 30.5% (22/72). No significant dif-

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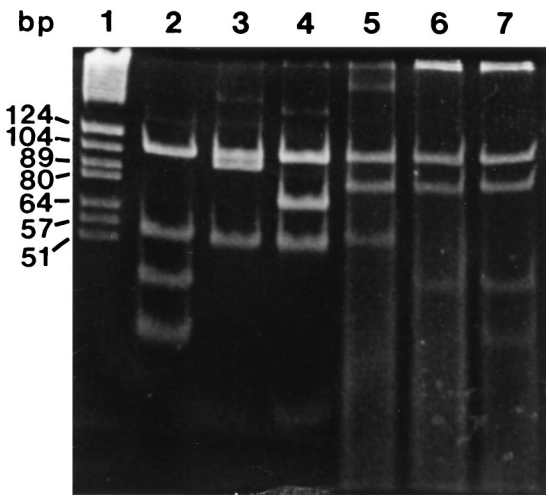

Fig. 1. MseI restriction patterns of *B. burgdorferi* sensu lato. Lane 1, DNA size standards; lane 2, *B. burgdorferi* sensu stricto; lane 3, *B. garinii;* lane 4, *B. afzelii;* lane 5, *B. lusitaniae;* lanes 6 and 7, Tunisian isolates.

ference was observed between the infection rates of females (36.6%, $n = 41$) and males (22.6%, $n = 31$) ($\chi^2 = 1.63$, df = 1, $P = 0.2$).

Identification of 2 strains of spirochetes isolated from Tunisia in 1988 showed 1 genospecies, *B. lusitaniae* (Fig. 1). *Borrelia burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, and *B. valaisiana* were not observed.

Here, we report the presence of *B. burgdorferi* sensu lato in *I. ricinus* on the African continent. On a subtropical island in the Atlantic, northwest of continental Africa, the infection rate of *I. ricinus* by *B. burgdorferi* was 1.3% (Matuschka et al. 1994). The 30.5% infection rate we observed in Tunisia is within the range reported from European countries—13.8% in Spain (Mareuez and Constan 1990), 9.6% in Belgium (Martin et al. 1990), 5–50% in Switzerland (Aeschlimann et al. 1986, Gern et al. 1992), 20% in the Netherlands (Rijpkema et al. 1994), 18% in Germany (Matuschka et al. 1992), 13% in Slovenia (Strle et al. 1995), 45% in Croatia (Rijpkema et al. 1996), and 12.4% in France (Zhioua et al. 1996). Additional studies are needed to determine the infection rates of immatures and to identify the reservoir hosts of *B. burgdorferi* sensu lato in Tunisia.

Borrelia burgdorferi sensu stricto is the only pathogenic genospecies currently known to be present in the United States (Postic et al. 1994). In Eurasia, *B. afzelii* and *B. garinii* are widely distributed (Fukunaga and Hamase 1995, Postic et al. 1997). In Europe, 3 genospecies associated with Lyme borreliosis have been recognized to date—*B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii*. These genospecies have been isolated from patients, rodents, birds, and *I. ricinus* from various areas in Europe (Humair et al. 1995, Olsen et al. 1995, Picken et al. 1996, Zhioua et al. 1996, Postic et al. 1997). Two additional genospecies have been observed in *I. ricinus* and identified as *B. valaisiana* and *B. lusitaniae* (formerly the genospecies VS116 and PotiB2, respectively) (Postic et al. 1994, 1997; Péter et al. 1995; Rijpkema et al. 1995; Le Flèche et al. 1997; Wang et al. 1997). To date, only a few isolates have been identified as *B. lusitaniae;* 3 from Portugal, 2 from Moldavia, 1 from Ukraine, 1 from Czech Republic (Postic et al. 1994, 1997), and now 1 from North Africa. The roles of *B. lusitaniae* and *B. valaisiana* in the etiology of Lyme borreliosis remain to be clarified.

In western, central, and northern Europe, *I. ricinus* is commonly infected with various *Borrelia* genospecies, but rarely with *B. lusitaniae*, which appears to be limited to the southern part of the northern Hemisphere. Additional isolates from *I. ricinus* are necessary to describe the heterogeneity of spirochetes and the distribution of the various genospecies in southern Europe and North Africa. In sub-Saharan Africa, the existence of *B. burgdorferi* sensu lato remains unclear, but seems to be improbable because of the absence of ticks of the *I. ricinus* species complex (Mason et al. 1994, Marjolet et al. 1995).

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