First Record of Borrelia spielmani in Turkey

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Summary

The almost worldwide distribution of Borrelia burgdorferi sl spirochaetes promotes the association with different tick species and thus different epidemiological pattern can be observed. Seven B. burgdorferi genospecies are currently recognized in Europe. Two of them (B. bissettii and B. spielmani) are still poorly characterized and most details about distribution are ignored. This paper reports the PCR detection of B. spielmani in a tick in the city of Istanbul (Turkey) collected while biting a human. This genospecies was previously known from The Netherlands, Germany, France, Hungary and Slovenia. This finding contributes to the further knowledge of the distribution of the different genospecies of B. burgdorferi sl.

Keywords: B. burgdorferi sl, Tick, Turkey

Türkçe'de İlk Borrelia spielmani Bulgusu

Özet


Anahtar sözcükler: B. burgdorferi sl., Kene, Türkiye

INTRODUCTION

Lyme borreliosis (LB), a tick-transmitted, systemic disease produced by Borrelia burgdorferi sensu lato (sl) exists as a zoonosis in Europe, North America, Asia and North Africa. In Europe, the presence of the agent has been reported in at least 26 countries 1. Up to now, 12 Borrelia genospecies have been described under the broader name B. burgdorferi sl. Among them 3 are recognized as pathogenic for humans: B. burgdorferi sensu stricto (ss), B. afzelii and B. garinii. It appears that these three species cause different clinical manifestations in humans 2. In North America, three Borrelia species have been reported, B. burgdorferi ss, B. andersonii and B. bissetti. In Asia, B. burgdorferi ss seems to be absent but B. garinii, B. afzelii, B. valaisiana, B. japonica, B. turkic, B. turdii and B. sinica have been isolated. The distribution of the last four species is restricted to Asia. In North Africa, B. lusitaniae, B. garinii and B. burgdorferi ss have been described in Ixodes ricinus 2. In Europe, 5

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species of *B. burgdorferi* s.l have been reported in ticks: *B. burgdorferi* ss, *B. garinii*, *B. afzelii*, *B. valaisiana* and *B. lusitaniae*. In addition, two other genospecies have been obtained from patient tissues. *B. bissettii*, a species present in North America, has been also isolated from patients in Slovenia. A novel *B. burgdorferi* s.l genospecies (A14S) was cultured from patients with Erythema Migrants in The Netherlands, Hungary, Germany, and Slovenia and from ticks in Ukraine, Germany and France. *B. spielmani* was proposed as name for this spirochete. Further species have been described from Spain and Turkey. The former has not yet been characterized. The later (named *B. turcica*) was isolated from *Hyalomma aegyptium*, a hard tick infesting tortoises, and formed a different cluster within the relapsing-fever spirochaetes. For that last species, further microbiological, clinical and epidemiological studies are necessary to determine implications for human health. However, the high incidence of the *H. aegyptium* biting humans in the same area where *B. turcica* was detected has been already reported.

**CASE HISTORY**

Ticks were passively surveyed in Istanbul since 2006 from human patients applying hospitals and reporting tick bites. After adequate determination of the ticks, pathogen results were determined in these samples. A 30 years-old female who claimed to be bitten in Belgrad forest picnic area (30 km north of Istanbul) applied to the hospital as soon as she noticed the tick in her leg on May, 2007. For detection of *B. burgdorferi* s.l. species, DNA was extracted from the tick using a commercial DNA extraction kit (Nucleospin tissue kit, Macherey Nagel, Germany). Primers (OSPA FW1: ttg gga ata ggt cta ata tta gc, OSPA FW2: atg yaa gca aaa tgt tag c, BOR R: act aat gtt ttv cca tct tc) amplifying a 247 bp long part of outer surface protein A (osPA) of *B. burgdorferi* s.l were used with a nested protocol. The reactions were performed in a final volume of 50 μl, comprising 0.5 μM each primer, 10 mM Tris-HCl, 1.5 mM MgCl2, 50 mM KCl (pH 8.3), 0.2 mM each deoxynucleoside triphosphate (Fermentas®, Lithuania), and 1.25 U of Taq DNA polymerase (Fermentas®, Lithuania) and 10 μl of DNA template. Mixture was subjected to an initial denaturation at 94°C for 2 min followed by 50 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min and extension at 72°C for 2 min. Final extension was performed at 72°C for 10 min. For the second round amplification, 3 μl of the first round product was added to the 47 μl reaction mixture with the same concentrations and thermal cycling conditions of the first round mixture. The amplification products were visualized on 1.5% agarose gel electrophoresis under UV-light. After purification with a commercial PCR product purification kit (Roche®, Germany), the second round PCR products were subjected to the cycle sequencing using big-dye terminator kit (ABI®, USA). Following the cleaning-up procedure through sephadex-G50 fine columns the cycle sequencing products were run on an automated sequencer (ABI®, 310). The obtained sequence was edited and aligned using lasergene (DNA Star®) and Bioedit software packages and was compared against data available in GenBank. The detected pathogen had a 99.59% of identity with the ospA sequences of *B. spielmani* (AY 995900, AF 102057) according to the molecular study results. The sequence was deposited in the GenBank under the accession number EU545183.

**DISCUSSION**

In endemic areas in Europe, 6 *B. burgdorferi* s.l genospecies may circulate between vertebrate hosts and ticks. Interestingly, in North Africa, *B. lusitaniae* is very frequent and greatly exceeds the other genospecies in ticks, whereas *B. lusitaniae* is only sporadically reported in ticks from other areas in Europe. The fact that *B. lusitaniae* is by far the dominant species in *I. ricinus* ticks in Portugal, Morocco and Tunisia indicates that the genospecies diversity of *B. burgdorferi* s.l decreases towards the southern margin of its European distribution. *B. spielmani* has till now been detected in The Netherlands, Germany, France, Hungary, Ukraine and Slovenia. Previous reports of *B. burgdorferi* s.l in western Turkey including the city of Istanbul referred to *B. valaisiana, B. afzelii, B. garinii, B. lusitaniae* and *B. burgdorferi* s.l. Thus, the record of *B. spielmani* in the region considerably expands the geographical range of that spirochaete.

**REFERENCES**

6. Maraspin V, Ruzic-Sabljic E, Strle F: Lyme Borreliosis and...


