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**CASE REPORT**

# 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*

# Türkiye'de İlk *Borrelia spielmani* Bulgusu

## Özet

*Borrelia burgdorferi* sl. grubundaki spiroketlerin dünyadaki yayılışı farklı kene türleri ile ilişkilidir ve bu nedenle farklı epidemiyolojik özellikler gösterir. Avrupa ülkelerinde bugüne kadar yedi farklı *B. burgdorferi* genotipi bildirilmiştir. Bunlardan *B. bissettii* ve *B. spielmani* henüz yeterince tanımlanmamıştır ve yayılışları bilinmemektedir. Bu yazıda, İstanbul'da bir insanı tutmuş olan kene örneğinde PCR yöntemi ile saptanan *B. spielmani* bulgusu sunulmuştur. Bu genotip daha önce Hollanda, Almanya, Fransa, Macaristan ve Slovenya'dan bildirilmiştir. Bu bulgular, *B. burgdorferi* sl. genotipleri ve vektörlerinin yayılışları ile ilgili bilgilere katkıda bulunmaktadır.

**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. bissettii*. In Asia, *B. burgdorferi* ss seems to be absent but *B. garinii, B. afzelii, B. valaisiana, B. japonica, B. tanukii, 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* sl 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 [3]. A novel *B. burgdorferi* sl genospecies (A14S) was cultured from patients with Erythema Migrans in The Netherlands, Hungary, Germany, and Slovenia [4-7] and from ticks in Ukraine, Germany and France [8-10]. *B. spielmani* was proposed as name for this spirochete [10]. Further species have been described from Spain [11] and Turkey [12]. 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 [12,13]. For that last species, further microbiological, clinical and epidemiological studies are necessary to determine implications for human health. However, the high incidence of *H. aegyptium* biting humans in the same area where *B. turcica* was detected has been already reported [14].

## 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, pathogens 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* sl. 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) amplifiying a 247 bp long part of outer surface protein A (ospA) of *B. burgdorferi* sl 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 $MgCl_2$, 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 [15] 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 to the GenBank under the accession number EU545183.

## DISCUSSION

In endemic areas in Europe, 6 *B. burgdorferi* sl genospecies may circulate between vertebrate hosts and ticks. Interestingly, in North Africa [2], *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* sl 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 [2,4-6,8-10]. Previous reports of *B. burgdorferi* sl in western Turkey including the city of Istanbul referred to *B. valaisiana, B. afzelii, B. garinii, B. lusitaniae* and *B. burgdorferi* sl [13]. Thus, the record of *B. spielmani* in the region considerable expands the geographical range of that spirochaete.

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