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Brief report

Borrelia miyamotoi: Should this pathogen be considered for the diagnosis of tick-borne infectious diseases in Spain?



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ABSTRACT

Introduction: *Borrelia miyamotoi* is a tick-borne pathogen belonging to the relapsing fever group. It had not been reported from Spain, but its wide distribution and the presence of the tick-vector (*Ixodes ricinus*) made us suspect its circulation. The aim of this study was to investigate the presence of *Borrelia* spp. in *I. ricinus* in Spain.

Methods: A total of 652 *I. ricinus* nymphs collected in northern Spain were processed. The DNA was extracted using incubations with ammonium hydroxide. *Borrelia* spp. DNA was amplified using *Borrelia*-specific PCR assays (*glpQ*, 16S rRNA and flagellin genes).

Results: *B. miyamotoi* was amplified in 4 specimens, and *Borrelia burgdorferi* sensu lato in 27 (8 *Borrelia afzelii*, 7 *Borrelia garinii*, 8 *Borrelia lusitaniae*, 3 *Borrelia valaisiana* and 1 *B. burgdorferi* sensu stricto).

Conclusion: *B. miyamotoi* should be considered in the differential diagnoses of patients with confirmed or suspected tick-bite in Spanish endemic areas for Lyme disease.

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Borrelia miyamotoi: ¿un patógeno a tener en cuenta en el diagnóstico de enfermedades transmitidas por garrapatas en España?

RESUMEN

Introducción: *Borrelia miyamotoi*, patógeno del grupo de las fiebres recurrentes transmitido por garrapatas, no había sido encontrado en España. Su amplia distribución y la presencia del vector (*Ixodes ricinus*) nos hizo sospechar su circulación. El objeto del estudio fue investigar la presencia de *Borrelia* spp. en *I. ricinus* de España.

Métodos: Se procesaron 652 ninfas recogidas en el norte de España. El ADN se extrajo mediante incubaciones de amoníaco. Se llevaron a cabo PCR específicas para la amplificación de ADN de *Borrelia* spp. (genes *glpQ*, ARNr 16S y flagelina).

Resultados: Se amplificó *B. miyamotoi* en 4 ejemplares y *Borrelia burgdorferi* sensu lato en 27 (8 *Borrelia afzelii*, 7 *Borrelia garinii*, 8 *Borrelia lusitaniae*, 3 *Borrelia valaisiana* y 1 *Borrelia burgdorferi* sensu stricto).

Conclusión: *B. miyamotoi* debe ser considerado en el diagnóstico diferencial de pacientes con picadura o posible picadura de garrapata en zonas endémicas de enfermedad de Lyme.

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Introduction

The genus *Borrelia* (Spirochaetes; Spirochaetales; Borreliaceae) includes two clades, *Borrelia burgdorferi* sensu lato and the relapsing fever complex. *Borrelia miyamotoi* is a tick-borne pathogen belonging to the latter group that has been recently associated with human clinical cases¹. *B. miyamotoi* infections have been reported in North America, Asia and Europe, with human cases in The Netherlands and Germany.^{1–3} This spirochete is transmitted by *Ixodes* ticks, including *Ixodes ricinus* (Europe), *Ixodes persulcatus* (Asia) and *Ixodes scapularis* (North America). The microorganism has been amplified from ticks, with higher prevalence in nymphal stages, and from vertebrates in several countries.¹ To date, no reports of *B. miyamotoi* presence have been published from Spain, but the occurrence of high populations of its vector (*I. ricinus*) made us suspect on its circulation. *I. ricinus* is the most prevalent species in the North of Spain and it is responsible for most tick-bites.⁴ This area is endemic for Lyme disease (LD), the most common tick-borne illness in temperate regions of the northern hemisphere with an increasing occurrence in many regions.^{5,6} Thus, our aim was to investigate the presence of *B. miyamotoi* and *B. burgdorferi* s.l. in *I. ricinus* nymphs from the North of Spain.

Materials and methods

Ticks were selected from the collection of the Center of Rickettsiosis and Arthropod-Borne Diseases (Logroño, Spain), and chosen according to this criterion: questing *I. ricinus* nymphs from La Rioja and neighboring areas. Nymphs were selected because nymphal stage is the one that most frequently bites humans in the studied area⁴ and their highest abundance overlaps with most LD cases.⁵ Moreover, the highest prevalences for *B. miyamotoi* infection have been reported in nymphs.¹ A total of 652 nymphs collected from La Rioja ($n=372$), Álava ($n=139$), Cantabria ($n=17$) and Navarra ($n=124$) were studied (Table 1). DNA was individually extracted using two incubations of 20 min each with 100 μ L of ammonium hydroxide 0.7 M at 100 and 90 °C, respectively. Conventional PCR assays were performed using KAPA Taq PCR Kit (Kapa Biosystems (Pty) Ltd, SA), following manufacturer's instructions. PCRs targeting 16S rRNA mitochondrial genome of ticks were performed for all samples to validate the DNA extraction procedure. *Borrelia* spp. screening was performed using PCR assays for 16S rRNA and flagellin (single-run) genes. In addition, PCRs for the partial glycerophosphodiester phosphodiesterase (*glpQ*) gene were carried out. This gene is present in the relapsing fever complex but not in the *B. burgdorferi* complex. Lastly, nested PCR assays targeting a species-specific flagellin region for the *B. burgdorferi* s.l. detection were used (Table 2). Negative and positive controls (DNA from *I. ricinus*, *B. miyamotoi* or *Borrelia spielmanii*) were included in all assays. The amplicons of interest were purified with Illustra™ ExoProStar™ Kit (GE Healthcare, UK). Sequencing reactions were performed using BigDye Terminator Kit (Applied Biosystems, Inc., CA) with sequence product resolution (ABI Prism 3130). Nucleotide sequences were compared with those available in NCBI database using BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Results

All samples tested positive for the partial 16S rRNA gene of ticks. *Borrelia* spp. were detected in 4.6% (30/652) of ticks. The *glpQ* nucleotide sequences corresponding to 4 *I. ricinus* from La Rioja were homologous to *B. miyamotoi* sequences from GenBank. Moreover, *B. burgdorferi* s.l. was amplified in 27 samples using a nested-flagellin-PCR. Sequences showed presence of *Borrelia afzelii* ($n=8$), *Borrelia garinii* ($n=5$), *Borrelia lusitaniae* ($n=3$), *Borrelia valaisiana* ($n=1$) and *B. burgdorferi* sensu stricto ($n=1$) in ticks from

La Rioja. In addition, *B. garinii* ($n=2$) and *B. lusitaniae* ($n=5$) were detected in Álava and *B. valaisiana* ($n=2$) in Navarra. One tick from La Rioja showed coinfection with *B. miyamotoi* and *B. afzelii*. Only one amplicon corresponding to *B. garinii* was obtained with the *Borrelia*-specific 16S rRNA PCRs (Table 1). No samples yielded positive PCR results using a single-run flagellin PCR assay.

The sequences corresponding to *B. miyamotoi* found in Spain were submitted to GenBank under accession number MG136725. Sequences corresponding to *B. garinii* that differed from those previously included in the database were submitted to GenBank under accession numbers MG136723–24. Remaining sequences are available upon request to the authors.

Discussion

This study demonstrates the presence of *B. miyamotoi* in the North of Spain, and it corroborates the circulation of *B. burgdorferi* genospecies (*B. afzelii*, *B. garinii*, *B. lusitaniae*, *B. valaisiana* and *B. burgdorferi* s.s.), all agents of LD.^{5,6}

The complete spectrum of *B. miyamotoi* infection is being written. Non-European patients commonly presented a nonspecific flu-like febrile syndrome, and some of them showed an erythema migrans.¹ Accordingly, we have found co-infection with *B. miyamotoi* and *B. afzelii*.

Most *B. miyamotoi*-related human cases have been reported from Russia, with a clinical picture of fever and malaise in almost 100% cases, followed by myalgia, chills, nausea and arthralgia.¹ Patients from USA and Japan presented similar clinical characteristics. Only one immunocompromised patient from USA suffered from neurological involvement.¹ Two cases have been reported in Europe, both in non-febrile immunocompromised patients with a tick-bite history. In the meningoencephalitis case reported from The Netherlands, an immunocompromised 70-year-old-man affected by a diffuse large B-cell lymphoma developed a slow cognitive process, memory deficits and disturbed gait. He had pleocytosis with raised protein values in cerebrospinal fluid (CSF) and serological assays for *B. burgdorferi* were inconclusive (positive C6 assay and, negative and indeterminate IgM and IgG western-blot). Treated like a Lyme neuroborreliosis, the patient recovered with 2-week-course of ceftriaxone (2 g IV). *B. miyamotoi* infection was retrospectively confirmed using darkfield microscopy and PCRs on blood and CSF.³ In the German case, a 74-year-old-woman with a previous non-Hodgkin lymphoma developed a syndrome resembling Lyme neuroborreliosis (dizziness, vomiting and headache) with pleocytosis and raised proteins on CSF. Serological assays against *B. burgdorferi* were negative for serum and CSF. The infection was diagnosed by *B. miyamotoi*-DNA amplification on CSF. The patient recovered under prescription of ceftriaxone (2 g IV; 3 weeks).²

Since there are not commercial tests available for *B. miyamotoi*, and serological tests for diagnosing LD are equivocal or negative, we must have a high level of suspicion for diagnosing *B. miyamotoi* infection. Spirochetes can be observed by darkfield microscopy or stain during the acute phase although, to date, only positive PCRs on blood or other sterile fluids like CSF can demonstrate *B. miyamotoi* infection. Doxycycline and ceftriaxone have been successfully prescribed.¹

Herein, we have found 3 *B. burgdorferi* recognized pathogenic genospecies (*B. afzelii*, *B. garinii*, *B. burgdorferi* s.s.). The remaining genospecies amplified, *B. valaisiana* and *B. lusitaniae*, have been occasionally implicated in clinical cases, although their pathogenicity is still uncertain.⁴ All these genospecies had been previously reported from Spain.^{12–14} In this country, most LD cases are diagnosed in the North, where *B. garinii* was hypothesized as the main pathogen.⁶ Nevertheless, the high prevalence of other recognized

Table 1*Ixodes ricinus* nymphs processed in this study and *Borrelia* species detected.

Province	Area (Coordinates)	Year	No. of ticks	No. of <i>Borrelia</i> positive samples (% of identity; GenBank accession number)					
				<i>B. miyamotoi</i> (<i>glpQ</i>)	<i>B. afzelii</i> (nested <i>flaB</i>)	<i>B. garinii</i>	<i>B. lusitaniae</i>	<i>B. valaisiana</i>	<i>B. burgdorferi</i> s.s.
Álava	Izki (42° 42' N; 2° 29' W)	2009	139			1 (99.7; EU401782) ^a 1 (99.7; HM345902) ^b	5 (100; LC035454)		
Cantabria	Coo (43° 16' N; 4° 6' W)	2009	17						
La Rioja	Almarza de Cameros (42° 14' N; 2° 35' W)	2009	1201 (100; KX885481)		1 (100; CP018262) 1 (100; KR782215)	2 (100; KT347456) ^c 1 (100; KT347455)	1 (100; DQ788618)	1 (100; CP009117)	1 (100; CP002312)
	Daroza (42° 22' N; 2° 35' W)	2010	117		1 (100; CP018262)	1 (100; AY342023)	2 (100; LC035454)		
	Lumbreras (42° 6' N; 2° 36' W)	2009	121 ^d (100; KX885481)		1 ^c (100; CP018262)				
		2010	43		2 (100; CP018262)	1 (100; AY342023)			
	Tobía (42° 17' N; 2° 50' W)	2016	742 (100; KX885481)		2 (100; KR782215)				
	Villalba de Rioja (42° 3' 7' N; 2° 53' W)	2009	6						
Navarra	Urbasa (42° 49' N; 2° 9' W)	2009	124					2 (100; CP009117)	
Total			6524 (0.6%)		8 (1.2%)	7 (1.1%)	8 (1.2%)	3 (0.5%)	1 (0.2%)

^a A single change: an 'A' in base number 219 compared to EU401782 sequence from GenBank.^b A single change: a 'G' in base number 272 compared to HM345902 sequence from GenBank.^c This sample was also positive with the *Borrelia*-specific 16S rRNA PCR assay, and the nucleotide sequences were homologous to *B. garinii* deposited on GenBank under the accession number CP007564.^d Same nymph specimen.**Table 2**

PCR primer pairs and conditions used in this study.

	Target gene	Primer sequence (5'→3')	Fragment size (bp)	Reference
Tick species	16S rRNA	CTGCTCAATGATTTTTTAAATTGCTGTGG CCGGTCTGAACCTCAGATCAAGT	456	7
<i>Borrelia</i> spp.	16S rRNA	GCTGGCAGTGCCTCTTAAGC GCTTCGGGTATCCTCAACTC	1350	8
	Flagellin	AGAATTAATMGHGCWTCTGATGATG TGCYACAAYHTCATCTGTCAAT	764	9
	<i>Borrelia miyamotoi</i> <i>glpQ</i> (seminested)	ATGGGTTCAACAAAAAGTCACC CATTACTGTGTCAGTAAATCTGTAAATATACCATCTAC	920	3
<i>Borrelia burgdorferi</i> s.l.		ATGGGTTCAACAAAAAGTCACC CCAGGGTCCAATTCCATCAGAAATATTGTGCAAC	700	10
	Flagellin (nested)	AARGAATTGGCAGTTCAATC GCATTTTCWATTTTAGCAAGTGATG	497	11
		ACATATTGATGACAGACAGAGGTTCTA GAAGGTGCTGTAGCAGGTGCTGGCTGT	389	

pathogens found in this study, e.g. *B. afzelii*, suggests that other *Borrelia* species could cause LD in Spain.

According to our data, the prevalence of *B. miyamotoi* in ticks is lower than the one of *B. burgdorferi* s.l. This tendency had been previously observed.^{1,8} Nevertheless, the dynamics of *B. miyamotoi* transmission remains unknown and human cases of *B. miyamotoi* infection could have been undetected or misdiagnosed.

The discrepancies between the results obtained using the nested and the single-run PCRs suggest that the latter have low sensitivity, although they have been reported as useful assays for *Borrelia* spp. detection.^{8,9}

In summary, we report the presence of *B. burgdorferi* genospecies in the vector, and the circulation in Spain of a non-previously reported tick-borne pathogen, *B. miyamotoi*, that must be considered in patients with neurological syndrome and/or fever after a confirmed or suspected tick-bite.

Addendum

While we were screening ticks for *B. miyamotoi* detection, Dr Pablo Díaz Fernández (Facultad de Veterinaria, Universidade de

Santiago de Compostela, Lugo, Spain) personally communicated the finding of *B. miyamotoi* in ticks from Galicia (unpublished data).

Conflict of interests

The authors declare that they have no conflict of interest.

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