

Borrelia miyamotoi Infections in Humans and Ticks, Northeastern China

Technical Appendix

Sequence Amplification of *Borrelia miyamotoi* Infection

Primer Brm1/Brm2 was used for real-time PCR screening of *B. miyamotoi* (353-bp) infection, the positive representative melting temperature peaks in $86 \pm 0.5^\circ\text{C}$. All the positive outcomes (353-bp) were sequenced. For every positive specimens, primer Bf1 (or rrs1F)/rrs4R was used as nested-PCR outer primers, Bf1 (or rrs1F)/BmR and BmF/rrs4R were used as inner primers for amplification front and back fragments of *rrs* gene respectively, splicing with middle fragments (Brm1/Brm2, 353-bp), eventually a 1499-bp or 1523-bp fragments for *rrs* gene was acquired based on overlapping sequence. Primer FLA120F/FLA920R was used as nested-PCR outer primers, FLA120F/flab625R as inner primers for amplification *fla* gene partial sequences (506-bp). Primer glpQ67F/glpQ789R was used as nested-PCR outer primers, glpQ67F (or glpQ86F) /glpQ546R as inner primers for amplification *glpQ* gene partial sequences (480-bp or 461-bp).

Technical Appendix Table 1. Nucleotide sequences of the primers used in the study

Target gene	Primer	Sequence (5'-3')	Position*	Objective	Reference
<i>rrs</i>	Brm1	CGCTGTAAACGATGCACACTTGGTGTTAATC	807F	Screening of <i>B.</i>	(1)
	Brm2	CGGCAGTCTCGTCTGAGTCCCATCT	1159R	<i>miyamotoi</i> (353-bp)	(1)
	Bf1	GCTGGCAGTGCCTTAAGC	38F	Amplification and	(1)
	rrs1F	GTTTGATCCTGGCTTAGAAC	14F	splicing <i>rrs</i> complete	(2)
	rrs4R	GAGGTGATCCAGCCACACTTT	1536R	sequence	(2)
	BmF	AGATGGGGACTCAGACGAGACTGCCG	1134F		This study
	BmR	GATTAACACCAAGTGTGCATCGTTTACAGCG	837R		This study
<i>fla</i>	FLA120F	AGAATTAATMGHGCWTCTGATGATG	103F	Amplification <i>fla</i> partial	(1)
	FLA920R	TGCYACAAYHTCATCTGTCATT	912R	sequence (506-bp)	(1)
	Flab625R	CTGGAGCTGCTTGAGCACCTTCT	622R		This study
<i>glpQ</i>	glpQ67F	ATGGGTTCAAACAAAAGTCACC	67F	Amplification <i>glpQ</i>	This study
	glpQ86F	CACCATTRATYATAGCTCACAG	86F	partial sequence (461-	This study
	glpQ546R	GATGTCTTTACCTGTTGTTTATGCCA	546R	bp)	This study
	glpQ789R	CCAGGGTCCAATTCCATCAGAATATT	789R		This study

*F, forward direction; R, reverse direction. Primers position are referring to the corresponding sequence of *rrs*, *fla*, and *glpQ* of *B. miyamotoi*.

Technical Appendix Table 2. *Borrelia miyamotoi* infection prevalence in ticks

Tick species	<i>No. B. miyamotoi</i> -positive ticks (%)		
	Male	Female	Total
<i>I. persulcatus</i>	13/343 (3.8)	6/284 (2.1)	19/627 (3.0)
<i>H. concinna</i>	1/12 (8.3)	0/24 (0)	1/36 (2.8)
<i>D. silvarum</i>	0/13 (0)	0/16 (0)	0/29 (0)
Total	14/368 (3.8)	6/324 (1.9)	20/692 (2.9)

A total of 692 adult ticks including 627 (90.6%) *I. persulcatus*, 36 (5.2%) *H. concinna*, and 29 (4.2%) *D. silvarum*.

Technical Appendix Table 3. *Borrelia miyamotoi* gene amplification in ticks

No.	Year	Tick ID	Collection site	Tick species	Tick sex	<i>rrs</i>		<i>fla</i>	<i>gfpQ</i>
						<i>rrs</i> partial	complete	partial	partial
1	2013	101	E44°55', N129°30'	<i>I. persulcatus</i>	Female	353 bp	NA.	NA	NA
2	2013	105	E44°55', N129°30'	<i>I. persulcatus</i>	Female	353 bp	NA.	NA	NA
3	2013	119	E44°55', N129°30'	<i>I. persulcatus</i>	Male	353 bp	NA.	NA	NA
4	2013	176	E44°55', N129°30'	<i>H. concinna</i>	Male	353 bp	NA.	NA	NA
5	2013	237	E44°55', N129°30'	<i>I. persulcatus</i>	Female	353 bp	NA.	NA	NA
6	2013	350	E44°58', N129°13'	<i>I. persulcatus</i>	Female	353 bp	1499 bp,	506 bp	461 bp
7	2013	392	E44°58', N129°13'	<i>I. persulcatus</i>	Male	353 bp	1499 bp	506 bp	461 bp
8	2013	421	E44°58', N129°13'	<i>I. persulcatus</i>	Male	353 bp	NA.	NA	NA
9	2013	428	E44°58', N129°13'	<i>I. persulcatus</i>	Male	353 bp	1499 bp	506 bp	461 bp
10	2013	544	E44°58', N129°13'	<i>I. persulcatus</i>	Male	353 bp	1499 bp	506 bp	461 bp
11	2013	606	E44°55', N129°30'	<i>I. persulcatus</i>	Male	353 bp	1499 bp	NA	461 bp
12	2013	609	E44°55', N129°30'	<i>I. persulcatus</i>	Male	353 bp	NA.	NA	NA
13	2013	622	E44°55', N129°30'	<i>I. persulcatus</i>	Male	353 bp	NA.	NA	NA
14	2014	1	E44°58', N129°13'	<i>I. persulcatus</i>	Male	353 bp	NA.	NA	NA
15	2014	52	E44°58', N129°13'	<i>I. persulcatus</i>	Female	353 bp	1499 bp	506 bp	461 bp
16	2014	114	E44°55', N129°30'	<i>I. persulcatus</i>	Male	353 bp	1499 bp	506 bp	461 bp
17	2014	121	E44°55', N129°30'	<i>I. persulcatus</i>	Male	353 bp	NA.	NA	NA
18	2014	124	E44°55', N129°30'	<i>I. persulcatus</i>	Male	353 bp	NA.	NA	NA
19	2014	126	E44°55', N129°30'	<i>I. persulcatus</i>	Male	353 bp	NA.	NA	NA
20	2014	150	E44°55', N129°30'	<i>I. persulcatus</i>	Female	353 bp	NA.	506 bp	

References

- Hovius JW, de Wever B, Sohne M, Brouwer MC, Coumou J, Wagemakers A, et al. A case of meningoencephalitis by the relapsing fever spirochaete *Borrelia miyamotoi* in Europe. Lancet. 2013;382:658. PubMed [http://dx.doi.org/10.1016/S0140-6736\(13\)61644-X](http://dx.doi.org/10.1016/S0140-6736(13)61644-X)
- Potkonjak A, Kleinerman G, Gutiérrez R, Savić S, Vračar V, Nachum-Biala Y, et al. Occurrence of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks with first identification of *Borrelia miyamotoi* in Vojvodina, Serbia. Vector Borne Zoonotic Dis. 2016;16:631–5. PubMed <http://dx.doi.org/10.1089/vbz.2016.2008>