Detection and Genetic Characterization of Relapsing Fever Spirochete Borrelia miyamotoi in Estonian Ticks

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Abstract

During the years 2008–2010 I. ricinus and I. persulcatus ticks were collected from 64 sites in mainland Estonia and on the island Saaremaa. Presence of B. miyamotoi was found in 0.9% (23/2622) of ticks. The prevalence in I. persulcatus and I. ricinus ticks differed significantly, 2.7% (15/561) and 0.4% (8/2061), respectively. The highest prevalence rates were in found South-Eastern Estonia in an area of I. persulcatus and I. ricinus sympatry and varied from 1.4% (1/73) to 2.8% (5/178). Co-infections with B. burgdorferi s.l. group spirochetes and tick-borne encephalitis virus were also revealed. Genetic characterization of partial 16S rRNA, p66 and glpQ genes demonstrated that Estonian sequences belong to two types of B. miyamotoi and cluster with sequences from Europe and the European part of Russia, as well as with sequences from Siberia, Asia and Japan, here designated as European and Asian types, respectively. Estonian sequences of the European type were obtained from I. ricinus ticks only, whereas the Asian type of B. miyamotoi was shown for both tick species in the sympatric regions.

Introduction

The Borrelia genus consists of two groups of species [1]. The Lyme borreliosis (LB) group of spirochetes include agents that cause disease (LB) in humans as well as some species not associated with human disease. The LB group organisms are widely spread in Europe and North America and transmitted between vertebrates by hard (ixodid) ticks [2]. The relapsing fever (RF) group spirochetes mainly use soft (argasid) ticks as vectors [3] but some of them are transmitted also by hard tick vectors. This group includes B. theileni, which is vectored by Rhipicephalus ticks and causes infections in large livestock, B. lonestari, which is transmitted by Amblyomma americanum and causes infections in deer [4], as well as B. miyamotoi, which is transmitted by Ixodes ticks and is found in a small percentage of ticks in Eurasia and North America [5,6,7,8,9]. B. miyamotoi was isolated for the first time in Japan in 1995 from I. persulcatus ticks as well as from blood of Apodemus argentus mice [7,10]. DNA of closely related spirochetes was subsequently detected in I. scapularis [11] and I. pacificus [12] in the USA. In Europe, B. miyamotoi was detected in Ixodes ticks in Sweden [6] and Germany [9]. In European and Asian regions of Russia DNA of B. miyamotoi was detected in Ixodes ticks [13] and Ixodes as well as in human blood [8]. In addition to A. argenteus, it has been shown that white-footed mice (Peromyscus leucopus) may serve as host reservoirs for B. miyamotoi [11] and detection of B. miyamotoi from wild turkeys (Meleagris gallopavo) was also recently reported [14]. Unlike LB spirochetes, B. miyamotoi and other relapsing fever spirochetes are vertically transmittable from a female adult tick to her offspring [15,16,17]. Also transmission of spirochetes by co-feeding from nymph to larva and horizontal transmission from infected mice to ticks was experimentally shown [4,12], although at a lower rate compared to B. burgdorferi s.l. [11].

Over the last decade B. miyamotoi has been detected in Ixodes ticks in the USA [11,12], Sweden [18], Czech Republic [16], France, and Germany [9] as well as in Russia [13,19,20]. Human disease caused by this RF group spirochete has not been well characterized, but recently probable cases of B. miyamotoi infection in RF-patients were reported from Russia [8,21,22].

Our aim was to investigate the presence and the prevalence of B. miyamotoi in different areas of Estonia.

Materials and Methods

Ethics Statement

According to Estonian legislation no specific permits were required for the described field studies. None of the locations described in the study were situated on the private land, in the National parks nor protected area. The described field studies did not involve endangered or protected species.

Collection of Ticks

Ticks were collected from the vegetation by flagging from April to November during 2008–2010 at 64 sites in mainland Estonia and on Saaremaa island (Figure 1, Table 1). Tick species were...
independently identified by morphological criteria by two entomologists, washed in 70% ethanol, rinse twice with sterile PBS and individually stored at −70°C.

Extraction of DNA

Ticks were homogenized in 300 μl of PBS by TissueLyser (Retsch, Haan, Germany). Two hundred microliters of suspensions were used for DNA extraction. DNA was extracted by the guanidinium thiocyanate-phenolchloroform method using the TriPure isolation system (Roche Diagnostics, Lewes, UK) according to the manufacturer’s recommendations. Sterile water was included as a negative control for every DNA preparation set.

Statistics

Fisher’s exact and Poisson probability tests were used to assess differences in B. miyamotoi prevalence in I. ricinus and I. persulcatus ticks.

Detection of B. miyamotoi, B. burgdorferi s.l. and Tick-borne Encephalitis Virus in Ticks

The presence of Borrelia species was detected by amplification of 1256 bp product of 16S rRNA partial gene as described [7] with external primers 16S-Bor-S1F and 16S-Bor-S2R under the following conditions: 35 cycles, 94°C-10 sec, 60°C-1 min, 72°C-90 sec. Nested PCR was performed with primer pair 16S-Bor-S4F and 16S-Bor-S3R [13] and cycling conditions included 35 cycles of initial denaturation at 94°C for 10 sec, annealing at 65°C-1 min, elongation at 72°C-90 sec.

To distinguish B. miyamotoi from B. burgdorferi s.l. among all 16S PCR-positive samples primers targeted B. miyamotoi p66 gene were chosen. The amplification of p66 partial gene was performed as described previously [13] with external primers pair M1F and M2R at the following conditions: 35 cycles, 94°C-5 sec, 50°C-10 sec, 72°C-30 sec. A 532 bp product was generated using inner primers M3F and M4R. The annealing time was increased to 15 sec and elongation time to 45 sec.

All p66-positive samples were further used for glpQ partial gene amplification as described by Fomenko et al [13]. Primers Q1F and Q2R were used for the first round of PCR, and cycling conditions included 35 cycles, 94°C-10 sec, 50°C-15 sec, 72°C-35 sec. Inner primers Q3F and Q4R were used in a nested PCR for generation of a 379 bp product at the following cycling conditions: 35 cycles, 94°C-5 sec, 52°C-10 sec, 72°C-30 sec.

To reveal co-infections of ticks with B. miyamotoi and B. burgdorferi s.l., samples positive for B. miyamotoi were amplified by nested PCR for B. burgdorferi s.l.-group specific 5S-23S rRNA intergenic spacer (IGS) region as described previously [23,24] with a modified touch-down program. The first amplification round included 35 cycles, 94°C–1 min, 58°C–1 min and 72°C–2 min, and in the nested PCR, the annealing temperature was decreased to 52°C and amplification was performed for 30 cycles.

Tick-borne encephalitis virus (TBEV) detection was performed by PCR amplification and further sequencing of partial E gene as described earlier [25] with outer primers 283F1 and 827R1 used for the cDNA synthesis and inner primers 349F2 and 814R2 for the second round of PCR amplification.

To confirm the morphological tick species definition, B. miyamotoi positive samples were analyzed for mitochondrial 16S...
**Table 1.** *Borrelia miyamotoi* detection in ticks and estimated prevalence (%).

| Place of collection | L. ricinus | L. persulcatus | Total no. ticks infected/tested (%) |
|---------------------|------------|----------------|-----------------------------------|
|                     | No. adults infected/tested (%) | No. nymphs infected/tested (%) | Total no. ticks infected/tested (%) | No. adults infected/tested (%) | No. nymphs infected/tested (%) | Total no. ticks infected/tested (%) |
| Ida-Virumaa         | 0/43       | 0/19           | 0/62                              | 0/92                            | 0/9                           | 0/101                            | 0/163 |
| Viiljandimaa        | 0/2        | –              | 0/2                               | 0/44                            | –                             | 0/44                             | 0/46  |
| Tartumaa            | 0/226      | 2/66 (3.0%)    | 2/292 (0.7%)                      | 5/187 (2.7%)                    | 4/94 (4.3%)                   | 9/281 (3.2%)                     | 11/573 (1.9%) |
| Valgamaa            | 0/57       | –              | 0/57                              | 5/121 (4.1%)                    | –                             | 5/121 (4.1%)                     | 5/178 (2.8%) |
| Võrumaa             | 0/64       | –              | 0/64                              | 1/9 (11.1%)                     | –                             | 1/9 (11.1%)                      | 1/73 (1.4%) |
| Pärnumaa            | 0/180      | 0/106          | 0/286                             | 0/4                             | 0/1                           | 0/5                             | 0/291 |
| Liäänerma           | 0/97       | 1/10 (10%)     | 1/107 (0.9%)                      | –                               | –                             | –                               | 1/107 (0.9%) |
| Harjumaa            | 0/217      | 0/77           | 0/294                             | –                               | –                             | –                               | 0/294 |
| Saaremaa            | 4/508 (0.8%) | 1/389 (0.3%) | 5/897 (0.6%)                      | –                               | –                             | –                               | 5/897 (0.6%) |
| Total               | 4/1394 (0.3%) | 4/667 (0.6%) | 8/2061 (0.4%)                    | 11/456 (2.4%)                  | 4/104 (3.8%)                  | 15/561 (2.7%)                   | 23/2622 (0.9%) |

*Not collected.*

1P < 0.0001 Fisher’s exact and Poisson probability tests.

2P < 0.05 Fisher’s exact test; P < 0.001 Poisson probability test.

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rRNA partial gene PCR as described by Caporale et al [26] with further sequencing using primers 16Sα and 16Sβ. Cycling conditions included pre-PCR steps 95°C–1 min; 49°C–1 min, 72°C–2 min and 95°C–1 min; 47°C–1 min; 72°C–2 min and amplification 40 cycles, 95°C–30 sec; 45°C–1 min; 72°C–2 min.

PCR products were visualized by electrophoresis in 1% agarose gel stained with ethidium bromide. Deionized water was included in every PCR step as a negative control.

To minimize contamination, reaction mix preparation, sample addition step, amplification and gel electrophoresis were performed in three separate rooms with sterile techniques. Sample addition was performed in a laminar flow cabinet.

Phylogenetic Analysis

Analysis and alignment of sequences was performed using BioEdit 7.0.0 software. The Maximum Likelihood model was used for phylogenetic tree reconstruction of the partial 16S rRNA (1106 bp), p66 (349 bp and 355 bp for “I. persulcatus”-type and “I. ricinus”-type, respectively) and gplQ genes (379 bp), using the Tree Puzzle program. 10,000 puzzling steps were applied using the GTP model of substitution for the partial p66 gene and the Hasegawa-Kishino-Yano (HKY) model for the partial p66 gene.

Results

Tick Collections and Detection of B. miyamotoi DNA

In total, 2622 ticks (1851 adults and 771 nymphs) were collected in 64 sites of 9 Estonian counties, and among them 2061 (78.6%) were identified as I. ricinus and I. persulcatus, respectively (Table 1). DNA of B. miyamotoi was detected in 23 (0.9%) tick suspensions, 15 of which originated from I. persulcatus and 8 from I. ricinus ticks. Thus the overall prevalence of B. miyamotoi in I. persulcatus ticks was 2.7% (15/561) and 0.4% (8/2061) in I. ricinus ticks (P < 0.0001, Fisher’s exact and Poisson probability tests). The highest prevalence of B. miyamotoi in tick populations was detected in the South-Eastern Estonia, in Valgamaa (2.8%), Tartumaa (1.9%) and Võrumaa (1.4%) counties. This region is sympatric for both tick species and DNA of B. miyamotoi was found mainly in I. persulcatus. However, in other sympatric areas, Ida-Virumaa, Viljandimaa and Parnuma, B. miyamotoi was not detected. In regions where only I. ricinus circulates, the prevalence of B. miyamotoi was lower –0.9% and 0.6% in Läänemaa and on Saaremaa island, respectively. Comparison of the prevalence rates of B. miyamotoi in areas sympatric for both tick species and areas where only I. ricinus circulates demonstrated a statistically significant difference, 1.3% vs 0.5% (P < 0.05 Fisher’s exact test and P < 0.001 Poisson probability test).

In our study we did not find differences in B. miyamotoi prevalence between different tick stages, as B. miyamotoi DNA was detected in 1% of nymphal ticks (3 out of 771) and in 0.8% of adult ticks (15 out of 1515).

Co-infection with spirochetes belonging to Borrelia burgdorferi s.l. was demonstrated by amplification of 58-23S IGS, which is specific for the B. burgdorferi s.l. group. We showed that 5 ticks (21.7% from all positive ticks) were co-infected with B. afzelii, B. garinii or B. valaisiana (Table 2). Co-infection with another widely distributed tick-borne pathogen, TBEV, was found in adult I. ricinus on Saaremaa island. Genetic analysis of the partial E gene sequence revealed that this strain belonged to the European subtype of TBEV.

Genetic and Phylogenetic Analysis of B. miyamotoi Sequences

Three genomic regions of B. miyamotoi, the partial p66 (532 bp), 16S rRNA (1256 bp) and gplQ (379 bp) genes, were sequenced for genetic characterization of Estonian samples. Fourteen tick suspensions were amplified for all three genes, 6 for two genes and 3 for one gene region. Analysis of nucleotide sequence similarity of the three genomic regions showed that Estonian samples were divided into two groups: the first with sequences identical to those amplified from I. ricinus in Sweden and the European part of Russia (European type) and the second with sequences identical to those found in I. persulcatus and human blood in the European part of Russia, Ural and Siberia (Asian type). Within each group, the sequences of the Estonian samples were identical for all three gene regions. Moreover, in the European type cluster, sequences of the partial 16S rRNA, p66 and gplQ genes amplified in the present study were identical to the B. miyamotoi sequences derived from GenBank and detected in Sweden, the European part of Russia, Poland, and France. In the Asian type cluster, the Estonian sequences were identical to those amplified from ticks from different parts of Russia (European part, Ural, Siberia) and also Japan, with the exception of D45192 and AF228023 for the partial 16S rRNA and p66 genes, respectively.

Nucleotide sequence identity between European type and Asian type groups were found 99.4–99.6% for the partial 16S rRNA gene, 90.5% for the partial gplQ gene and 91.6–93.3% for the partial p66 gene. Nucleotide sequences of the partial p66 gene were more diverse and insertion of 6 nucleotides was demonstrated for European type of B. miyamotoi when compared to the Asian type.

On the phylogenetic trees based of the partial p66, partial 16S rRNA and gplQ genes (Figure 2) sequences detected in the present study clustered with B. miyamotoi sequences detected in Siberia and Japan as well as with sequences from Europe and the European part of Russia. Estonian sequences together with previously reported sequences of B. miyamotoi formed well supported European type and Asian type clusters. Closely related sequences of B. miyamotoi amplified from I. scapularis in USA clustered together with the European type of sequences in the phylogenetic tree based on the partial p66 gene sequences, while on tree based on the partial 16S rRNA gene it formed its own lineage albeit with a low bootstrap support.

The two groups of B. miyamotoi correspond to the tick species from which sequences were amplified: European type sequences were amplified from I. ricinus in Europe and Asian type from I. persulcatus in Japan, Siberia and Ural, and additional sequences belonging to this type were detected in blood of patients in Siberia [21] and A. argenteus in Japan [7,10]. In the current study we found the Asian type of B. miyamotoi in two I. ricinus nymphs (E13489-2 and Est3115-1) in an area sympatric for both tick species (Tartumaa). Species identification of these nymphs as I. ricinus by morphological criteria was confirmed by sequencing of the partial mitochondrial 16S rRNA gene.

Discussion

In the current study, DNA of relapsing fever spirochetes of B. miyamotoi was for the first time detected in ticks in Estonia. We found statistically significant differences between the prevalence rates of B. miyamotoi DNA in I. persulcatus and I. ricinus ticks, 2.7% and 0.4%, respectively. Similar prevalence rates were reported from a sympatric region, Moscow province, at 1.5% in I. persulcatus and 0.6% in I. ricinus [8]. Previously published data of B. miyamotoi DNA detection in I. persulcatus demonstrated 2.3–4.5% prevalence
Table 2. *B. miyamotoi* infections in Estonian ticks.

| Place of collection | Species of tick | Type of *B. miyamotoi* | Co-infection with other TBP* |
|---------------------|-----------------|------------------------|-----------------------------|
| Est1868             | *I. persulcatus* | F Asian                |                             |
| Est1885             | *I. persulcatus* | F Asian                |                             |
| Est3943-4           | *I. persulcatus* | F Asian                |                             |
| Est1811             | *I. persulcatus* | N Asian                |                             |
| Est3466-4           | *I. persulcatus* | F Asian                |                             |
| Est3487-4           | *I. persulcatus* | N Asian                |                             |
| Est3696-2           | *I. persulcatus* | N Asian                |                             |
| Est722-2            | *I. persulcatus* | N Asian                |                             |
| Est1586             | *I. persulcatus* | N Asian                | *B. valaisiana*             |
| Est3115-1           | *I. ricinus*     | N Asian                |                             |
| Est3489-2           | *I. ricinus*     | F Asian                |                             |
| Est4318             | *I. persulcatus* | M Asian                |                             |
| Est4350             | *I. persulcatus* | F Asian                |                             |
| Est4372             | *I. persulcatus* | M Asian                | *B. afzelii* (VS461 group)  |
| Est4243             | *I. persulcatus* | F Asian                |                             |
| Est4412             | *I. persulcatus* | F Asian                | *B. garinii* (NT29 group)   |
| Est3633             | *I. persulcatus* | M Asian                | *B. afzelii* (VS461 group)  |
| Est2519             | *I. ricinus*     | F European             |                             |
| Est3849             | *I. ricinus*     | M European             |                             |
| Est2270             | *I. ricinus*     | M European             | TBEV-Eu subtype             |
| Est2409             | *I. ricinus*     | M European             | *B. garinii* (20047 group)  |
| Est2325-3           | *I. ricinus*     | N European             |                             |
| Est1129-4           | *I. ricinus*     | N European             |                             |

*Tick-borne pathogen.
*Nymph.*

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Figure 2. Phylogenetic trees based on the partial sequences of 16S rRNA, p66 and glpQ genes. The Maximum Likelihood model was used for phylogenetic tree reconstruction of the partial A) 16S rRNA (1106 bp), B) p66 (349 bp and 355 bp for “*I. persulcatus*”-type and “*I. ricinus*”-type, respectively) and C) glpQ genes (379 bp). Only quartet puzzling support values >70% are shown. Samples sequenced in the present study are underlined.
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in Siberia [13,19] and 0.9%-16% in Ural [8]. The reported prevalence of B. miyamotoi DNA in I. ricinus ticks fluctuated from 0.5% in the European part of Russia [9] to 3.5% in Germany [9]. In the USA, similar B. miyamotoi prevalence rates in ticks were reported and correspond with those found in Europe: 1.9-2.5% to 6% for I. scapularis [11] and 1.7% and 0.7% for I. pacificus nymphs and adults, respectively [12]. In all studies where B. miyamotoi and *Borrelia* species belonging to LB complex were simultaneously detected, the prevalence rates of *B. miyamotoi* were significantly lower than those of *B. burgdorferi* s.l. [4,9,13,19].

In the current study we found statistically significant differences of *B. miyamotoi* prevalence rates in ticks between the region of *I. ricinus* range (0.5%) and the region sympatric for both tick species (1.3%). This fact may be explained either by a higher tropism of *B. miyamotoi* to *I. persulcatus* ticks or by more favorable conditions for pathogen circulation (microclimate, abundance of small and big mammals and etc.) in sympatric area in Eastern Estonia. However, simultaneous detection of *B. miyamotoi* DNA in both species of ticks collected in the sympatric area, although less frequently in *I. ricinus* compared to *I. persulcatus*, allows us to suggest that the latter explanation is the more probable one. Our investigation of tick-borne encephalitis virus and *Borrelia burgdorferi* s.l. prevalence in ticks demonstrated similar results, with statistically significant differences of prevalence rates between Western and Northern Estonia (areas of *I. ricinus* circulation) and Eastern Estonia (sympatric area for *I. persulcatus* and *I. ricinus*). However, statistically significant differences were not found between the two tick species in the sympatric area (our unpublished data).

In the current study we did not find statistically significant difference in the prevalence of *B. miyamotoi* in adults (0.8%) and nymphs (1%); observations that correspond to findings in the American ticks *I. pacificus* and *I. scapularis* [4,12] as well as in European *I. ricinus* ticks [9]. Analysis of larval tick stages should be performed for accurate assessment of a cumulative risk of *B. miyamotoi* infection with each subsequent feeding.

In the present study, 21.7% of *B. miyamotoi* positive ticks were also co-infected with spirochetes of the *B. burgdorferi* s.l. genospecies, and in one case a co-infection with TBEV was found. Thus in Estonia *B. miyamotoi* and Lyme disease spirochetes may share hosts, which is in contrast to findings in Germany and France [9] and the USA [4,11]. Moreover, it has recently been reported that *B. burgdorferi* and *B. miyamotoi* circulate among a separate set of hosts and utilize different transmission loops: for *B. burgdorferi* it is exclusively transmission to susceptible larvae feeding on hosts previously infected by nymphs, while *B. miyamotoi* utilizes mix of vertical and horizontal transmission in the Midwest of the USA [15]. However, *B. miyamotoi* co-infections with *B. garinii* (2.8%) and *B. afzelii* (0.2%) have also been reported in *I. persulcatus* ticks in Siberia [19]. Thus we may suggest that co-feeding on the same host and consequently co-infections of relapsing fever and Lyme disease spirochetes depend on local climatic and environmental conditions and could occur in Estonia and Siberia.

Genetic and phylogenetic analysis of the three gene regions of *B. miyamotoi* revealed that the Estonian sequences divided into two groups, the European and Asian groups, respectively, and that within each group the sequences were identical or shared a high level of similarity in a very large geographical range from Northern Europe (Sweden, Estonia) to the European part of Russia for the first group, and from Estonia, the European part of Russia to Siberia and Japan for the second group. The European type of *B. miyamotoi* sequences have been detected in *I. ricinus* ticks while the Asian has been found in *I. persulcatus* ticks and human blood [5,6,7,13,27]. In the present study we found that the Asian type of *B. miyamotoi* may be exchanged between tick species in a sympatric area, although not at a very high rate: among 16 sequences of the Asian group, 14 were amplified from *I. persulcatus* and two from *I. ricinus*. Similar results we found for TBEV, sequences belonging to the Siberian subtype of TBEV (TBEV-Sib), which were detected not only in *I. persulcatus* (the natural vector of TBEV-Sib) but also in *I. ricinus* collected in the same sympatric area (our unpublished data).

Recently it has been reported that *B. miyamotoi* probably causes relapsing fever (RF) and Lyme disease-like symptoms in Ural [9] and Siberia [21], and all the reported sequences from patients belonged to the Asian group of *B. miyamotoi*. In Estonia and other parts of Europe human cases of RF caused by *B. miyamotoi* infection have to date not been reported. The reason remains unclear; it may be either underreporting of *B. miyamotoi* infection due to serological cross-reactions in ELISA with *B. burgdorferi* s.l. antigen or a different pathogenicity of the European lineage of *B. miyamotoi*. Further investigations need to be performed in order to understand the vector potential of *I. ricinus* ticks for the Asian lineage of *B. miyamotoi*, which may be useful for the prediction of a possible spread of this group of spirochetes in a westward direction into Europe.

**Author Contributions**

Conceived and designed the experiments: IG NF JG. Performed the experiments: JG LN OK IG. Analyzed the data: IG JG LJ NF OK. Contributed reagents/materials/analysis tools: IG LJ. Wrote the paper: IG JG OK LN LF LJ.

## References

1. Paster BJ, Dewhurst FE, Weisburg WG, Tordoff LA, Fraser GJ, et al. (1991) Phylogenetic analysis of the spirochetes. J Bacteriol 173: 6101-6109.
2. Steere AC.; Coburn JG, L. (2005) Lyme borreliosis. In: Goodman JD, D; ASM Press. 176–206.
3. Barbours A (2005) Relapsing fever. In: Goodman JD, D; Sonenhine, DE. editor. Tick-Borne Diseases of Humans. Washington, DC: ASM Press. 268–291.
4. Barbours AG, Buniko J, Travinisky B, Hoen AG, Dukk-Wasser MA, et al. (2009) Niche partitioning of *Borrelia burgdorferi* and *Borrelia miyamotoi* in the same tick vector and mammalian reservoir species. Am J Trop Med Hyg 81: 1120–1131.
5. Brunak J, Tsoo J, Garpmu T, Berglund J, Fish D, et al. (2004) Typing of *Borrelia* relapsing fever group strains. Emerg Infect Dis 10: 1661–1664.
6. Fraenkel CJ, Garpmu V, Berglund J (2002) Determination of novel *Borrelia* genospecies in Swedish *Ixodes ricinus* ticks. J Clin Microbiol 40: 3308–3312.
7. Fukumaga N, Takahashi Y, Tsuruta Y, Matsushita O, Ralph D, et al. (1995) Genetic and phenotypic analysis of *Borrelia miyamotoi* sp. nov., isolated from the wild tick *Ixodes persulcatus*, the vector for Lyme disease in Japan. Int J Syst Bacteriol 45: 804–810.
8. Platonov AE, Karon IS, Kolyasnikova NM, Makhneva NA, Toporkova MG, et al. (2011) Humans infected with relapsing fever spirochete *Borrelia miyamotoi*, Russia. Emerg Infect Dis 17: 1016–1023.
9. Richter D, Schlace DR, Matuschka FR (2003) Relapsing fever-like spirochetes infecting European vector tick of Lyme disease agent. Emerg Infect Dis 9: 697–701.
10. Fukumaga M, Koreki Y (1995) The flagellin gene of *Borrelia miyamotoi* sp. nov. and its phylogenetic relationship among *Borrelia* species. FEMS Microbiol Lett 134: 255–258.
11. Scoles GA, Papero M, Beal I, Fish D (2001) A relapsing fever group spirochete transmitted by *Ixodes scapularis* ticks. Vector Borne Zoonotic Dis 1: 21–34.
12. Min J, Eisen RJ, Eisen L, Lane RS (2006) Detection of a *Borrelia miyamotoi* sensu lato relapsing-fever group spirochete from *Ixodes pacificus* in California. J Med Entomol 43: 120–123.
13. Fomenko NV, Livanova NN, Borgnaikov V, Kozlova IV, Shulakina IV, et al. (2010) [Detection of *Borrelia miyamotoi* in ticks *Ixodes ricinus* from Russia]. Parazitologiya 44: 291–211.
14. Scott MC, Rosen ME, Hamer SA, Baker E, Edwards H, et al. (2010) High-prevalence *Borrelia miyamotoi* infection among [corrected] wild turkeys (*Meleagris gallopavo*) in Tennessee. J Med Entomol 47: 1238–1242.
15. Davis S, Bent SJ (2011) Loop analysis for pathogens: niche partitioning in the transmission graph for pathogens of the North American tick *Ixodes scapularis*. J Theor Biol 269: 96–103.

16. Richter D, Debski A, Hubalek Z, Matuschka FR (2012) Absence of Lyme disease spirochetes in larval *Ixodes ricinus* ticks. Vector Borne Zoonotic Dis 12: 21–27.

17. Schwan TG, Piesman J (2002) Vector interactions and molecular adaptations of Lyme disease and relapsing fever spirochetes associated with transmission by ticks. Emerg Infect Dis 8: 115–121.

18. Wilhelmsen P, Frylund I, Borjesson S, Nordgren J, Bergström S, et al. (2010) Prevalence and diversity of *Borrelia* species in ticks that have bitten humans in Sweden. J Clin Microbiol 48: 4169–4176.

19. Borjesiakov V, Fomenko NV, Panov VV, Chikova ED (2011) Study on the infection of taiga ticks with *Borrelia* in the territory of Novosibirsk Scientific Center SB PAS. Parazitologiya 44: 543–556.

20. Korotkov Y S, Kidenko GS, Burenkova LA, Rudnikova NA, Karan LS (2008) Spatial and temporal variability of *Ixodes ricinus* and *Ixodes persulcatus* infection with the Lyme disease agent in Moscow Region. Parazitologiya 42: 441–451.

21. Fomenko NV, Epikhina TI, Chernousova NY (2010) Detection of *Borrelia miyamotoi* in the blood of patients got disease in the spring-summer epidemiological period. Molekulyarnaya meditsina: 28–31.

22. Karan LS, Kollamikova NM, Toporkova MG, Makhneva MA, Nadezhdina MV, et al. (2010) Usage of real time polymerase chain reaction for diagnostics of different tick-borne infections. Zh Mikrobiol Epidemiol Immunobiol: 72–77.

23. Postic D, Assous MV, Grimont PA, Baranton G (1994) Diversity of *Borrelia burgdorferi* sensu lato evidenced by restriction fragment length polymorphism of rrf (5S)-rrl (23S) intergenic spacer amplicons. Int J Syst Bacteriol 44: 743–752.

24. Rap A, Fomenko NV, Dobrotvorsky AK, Livanceva NN, Rudakova SA, et al. (2005) Tickborne pathogen detection, Western Siberia, Russia. Emerg Infect Dis 11: 1708–1713.

25. Skarpaas T, Golovljova I, Vene S, Ljostad U, Sjursen H, et al. (2006) Tickborne encephalitis virus, Norway and Denmark. Emerg Infect Dis 12: 1136–1138.

26. Caporale DA, Rich SM, Spielman A, Telford 3rd, Kocher TD (1995) Discriminating between *Ixodes* ticks by means of mitochondrial DNA sequences. Mol Phylogenet Evol 4: 361–365.

27. Fomenko NV, Borjesiakov V, Panov VV (2011) Genetic features of *Borrelia miyamotoi* transmitted by *Ixodes persulcatus*. Mol Gen Microbiol Virusol: 12–17.