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### Tick-host-Borrelia interaction

*Implications for host immunity and vaccination strategies*

Wagemakers, A.

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# Chapter 5

## *Borrelia miyamotoi*: a widespread relapsing fever spirochete

A. Wagemakers<sup>1</sup>, P.J. Staarink<sup>1</sup>, H. Sprong<sup>2</sup>, J.W.R. Hovius<sup>1,3,4</sup>

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1. Center for Experimental and Molecular Medicine, Academic Medical Center, Meibergdreef 9, 1105AZ Amsterdam, The Netherlands.
2. Laboratory for Zoonoses and Environmental Microbiology, National Institute for Public Health and Environment (RIVM), Antonie van Leeuwenhoeklaan 9, PO Box 1, Bilthoven, The Netherlands
3. Department of Internal Medicine, Division of Infectious Diseases, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands;
4. Amsterdam Multidisciplinary Lyme Center, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.

## ABSTRACT

*Borrelia miyamotoi* is a relapsing fever spirochete that has only recently been identified as a human pathogen. *Borrelia miyamotoi* is genetically and ecologically distinct from *Borrelia burgdorferi* sensu lato, while both are present in *Ixodes* ticks. Over 50 patients with an acute febrile illness have been described with a *B. miyamotoi* infection, and two infected immunocompromised patients developed a meningoencephalitis. Seroprevalence studies indicate exposure in the general population and in specific risk groups, such as patients initially suspected of having human granulocytic anaplasmosis. Here, we review the available literature on *B. miyamotoi*, describing its presence in ticks, reservoir hosts, and humans, and discussing its potential impact on public health.

### **Highlights:**

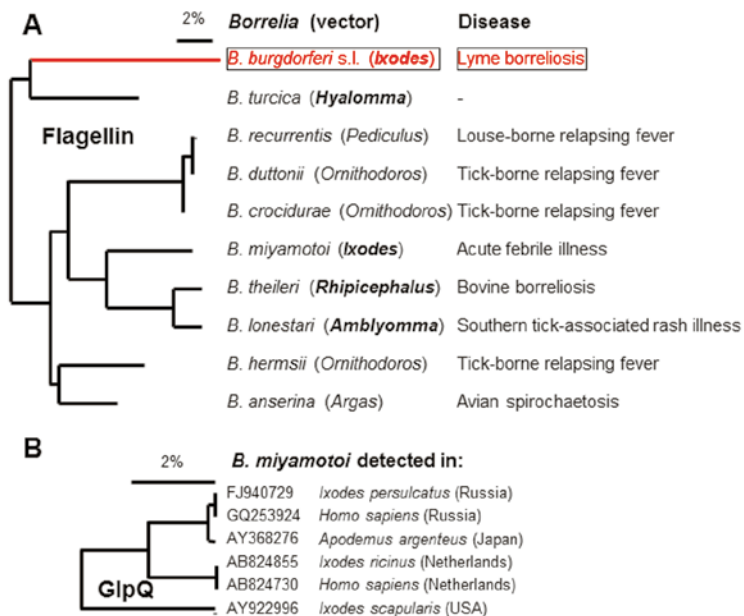
- *Borrelia miyamotoi* is a relapsing fever spirochete in *Ixodes* ticks and several reservoir hosts.
- *Borrelia miyamotoi* disease presents as an acute nonspecific febrile illness after a tick bite.
- Central nervous system involvement has been described in two immunocompromised patients.
- More information on the public health burden and validated diagnostic tools are required.

**I** xodes species are hard-bodied ticks that transmit various pathogens, such as *Borrelia burgdorferi* sensu lato (s.l.; see **Glossary**), tick-borne encephalitis virus (TBEV), *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*, and *Babesia* spp., and that carry numerous other microorganisms with unknown pathogenic potential. A history of a tick bite is not always present because they can go unnoticed, emphasizing the importance of physicians' knowledge of geographical areas endemic for specific tick-borne diseases, and awareness of their presenting clinical symptoms<sup>1,2</sup>.

Soft ticks belonging to the genus *Ornithodoros* have a different life cycle, habitat, pathogen range, and transmission dynamics compared with hard ticks, and are vectors for relapsing fever spirochetes<sup>3</sup>. These spirochetes can cause TBRF and occur in various parts of the world: in the western USA, 450 cases of TBRF (mostly *Borrelia hermsii*) were described between 1987 and 2000; in northwestern Morocco, *Borrelia hispanica* infection accounted for more than 20% of unexplained fever; and a study in Senegal showed that *Borrelia crocidurae* infection had an incidence at the community level of 14 per 100 person years, the highest of any bacterial infection reported in Africa<sup>4-6</sup>. *Borrelia miyamotoi* is currently the only known relapsing fever spirochete present in *Ixodes* ticks and, although it was identified almost 20 years ago, it has only recently gained attention because it was identified as a human pathogen<sup>7</sup>. Given the lack of awareness of the pathogenic potential of *B. miyamotoi* until 2011, the disease incidence is currently unknown. Greater awareness of the clinical manifestations of *B. miyamotoi* and the development of novel diagnostic tools could help distinguish *B. miyamotoi* infection from other tick-borne infections in patients with a nonspecific febrile illness following a tick bite. Here, we discuss the available literature on *B. miyamotoi* to assess the importance of this novel spirochete to the field of tick-borne pathogens.

## TAXONOMIC POSITION OF *B. MIYAMOTOI*

The genus *Borrelia* is a group of helical-shaped, motile bacteria that form a monophyletic lineage within the phylum *Spirochetes*, and comprises two major clades<sup>8-11</sup>. The *B. burgdorferi* s.l. complex includes the causative agents of Lyme borreliosis and related species, and are found only in *Ixodes* ticks. The relapsing fever complex includes species that are mostly found in soft (argasid) ticks, several hard (ixodid) ticks, and in lice (*Borrelia recurrentis*) (Figure 1A). There is some discussion on the exact phylogeny within the relapsing fever complex and, while some have suggested phylogenetic clustering based on geographic differences (Old World versus New World), others have found relapsing fever spirochetes in hard ticks (including *B. miyamotoi*, *Borrelia theileri*, and *Borrelia lonestari*) to cluster together phylogenetically and have suggested this to be a separate group within the relapsing fever complex<sup>9,12-14</sup>. Several *Borrelia* species found in hard ticks and reptiles have recently been suggested to form a third major clade, although it shares ancestry with the relapsing fever complex<sup>15</sup>. More whole-genome based phylogenetic analyses and studies on the biology of relapsing fever spirochetes will elucidate whether classification based on geographic region or vector is appropriate. The relapsing fever spirochete *B. miyamotoi* was first discovered in *Ixodes persulatus* ticks in Hokkaido, and was named after Kenji Miyamoto, the entomologist who first isolated spirochetes from *Ixodes* ticks in Japan<sup>16</sup>. Since then, *B. miyamotoi* has been found in various *Ixodes* species across the Northern Hemisphere (see also Table S1 in the supplementary material online) and shares several vector tick species with *B. burgdorferi* s.l., namely *Ixodes ricinus*, *Ixodes scapularis*, *Ixodes pacificus*, and *Ixodes persulcatus*, all of which can bite humans and transmit *B. burgdorferi* s.l.<sup>17-19</sup>. Recent phylogenetic analysis revealed genetic differences between the Asian, American, and European *B. miyamotoi* isolates<sup>9,20</sup>. Whether these genetic differences correlate with differences in vector competence ( Figure 1B), host range, and pathogenicity, or only reflect geographic distances remains to be investigated ( Box 1).



**Figure 1.**

Molecular epidemiological relationships between *Borrelia* species. **(A)** Neighbor-joining tree based on 558 base pair (bp) flagellin gene fragments from different *Borrelia* species. The representative of the *Borrelia burgdorferi* sensu lato (s.l.) complex is shown in red, whereas all others are relapsing fever borreliae (black). Relapsing fever spirochetes are present in several families of soft and hard (in bold) ticks, whereas *B. burgdorferi* s.l. circulates in one family of hard ticks (*Ixodes*) only. The exception is *Borrelia recurrentis*, which is transmitted by lice. **(B)** Unweighted pair group method with arithmetic mean-based dendrogram produced using fragments of the *glpQ* gene (366 bp) from different *Borrelia miyamotoi* isolates. The genetic differences correspond to both tick species and geographic origin. All three variants are able to cause disease in humans. All sequences are derived from Genbank, and are available upon request.

## **B. MIYAMOTOI IN TICKS AND RESERVOIR HOSTS**

### *Prevalence in ticks*

We have assessed the available literature examining the presence of *B. miyamotoi* in *Ixodes* ticks by polymerase chain reaction (PCR) (see also Table S1 in the supplementary material online)<sup>7,12,14,21-46</sup>. In total, 1.8% of the described individual questing *Ixodes* ticks were infected, with the highest prevalence in *I. persulcatus* (3.6%), followed by *I. scapularis* (2.0%) and *I. ricinus* ticks (1.3%). Of all questing *Ixodes* ticks, 0.5% of larvae, 1.8% of nymphs, and 1.2% of adults were found to be positive for *B. miyamotoi*.

### *Transovarial transmission and the infectious cycle of Borrelia miyamotoi*

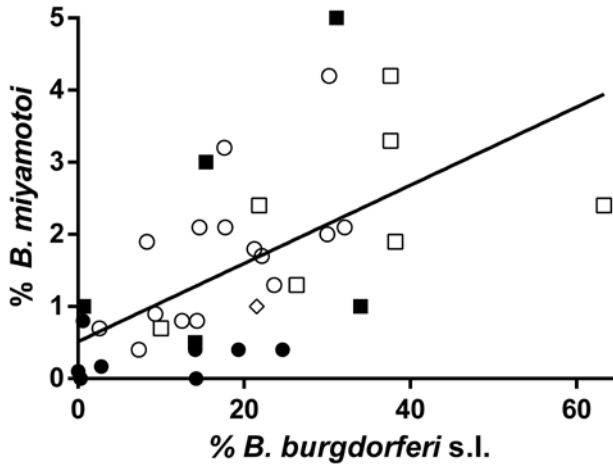
In nature, *B. miyamotoi* is found in all three tick life stages, and its presence in unfed larvae points towards transovarial transmission from the adult female tick to its offspring. Indeed, in an experimental setting, 6–73% of larvae originating from *B. miyamotoi*-infected female ticks were shown to be infected<sup>12</sup>. Other PCR-based studies identified *B. miyamotoi* as the sole spirochete in unfed larvae, and showed that 1.6–3.0% of wild female ticks hatched *B. miyamotoi*-infected larval pools, with an infection rate of individual larvae within infected pools of more than 90%<sup>30,47</sup>. Both studies suggest that earlier reports of transovarial transmission of *B. burgdorferi* s.l. have in fact identified *B. miyamotoi* infections. These spirochetes resemble each other in dark-field microscopy and immunofluorescence assays, while PCR sequencing can reliably distinguish between the two. Various studies have described the presence of spirochetes in *Ixodes* larvae upon direct fluorescence assays in up to 21% of questing ticks, and one study demonstrated that spirochete-infected larvae feeding on mice gave rise to a relapsing spirochetemia<sup>48-53</sup>. The fact that larvae can be infected with *B. miyamotoi* raises the question of whether larvae are able to transmit the pathogen to humans. Under optimal trial conditions, only 30–50% of larvae were found to feed on human test subjects<sup>54</sup>, and only a small proportion of identified ticks feeding on humans were larvae<sup>24, 27, 55</sup>. Moreover, when humans do get bitten by *B. miyamotoi* infected larvae, it is currently unknown whether transmission can occur. Studies in wild rodents have shown that spirochetemia in these animals was related to concurrent larval infestation, indirectly suggesting an important role for larval ticks in transmission of *B. miyamotoi* to reservoir hosts<sup>14,56</sup>. However, direct proof that *Ixodes* larvae are able to transmit *B. miyamotoi* has not yet been described. Although the exact infectious cycle of *B. miyamotoi* is currently unknown, based on the transovarial transmission described above, it is different from that of *B. burgdorferi* s.l. species. Given the apparently effective transovarial

transmission, it is surprising that the prevalence of *B. miyamotoi* in ticks is not higher, and that *B. miyamotoi* can sustain its widespread presence with only small numbers of ticks being infected. A possible explanation for the relatively low infection rate could be a detrimental effect of *B. miyamotoi* infection on tick survival; however, this requires further investigation. Another explanation could be inefficient acquisition of *B. miyamotoi* from infected reservoir hosts by ticks, which might be caused by the difference in tissue tropism and infection dynamics between *B. miyamotoi* and *B. burgdorferi* s.l.: a study performed in wild mice identified *B. miyamotoi* in only 2% of skin biopsies, while 76% of skin biopsies were positive for *B. burgdorferi*, and a PCR on blood showed *B. miyamotoi* in 6% and *B. burgdorferi* in 12% of mice. Loads of *B. miyamotoi* were lower in infected skin biopsies and higher in infected blood samples compared with *B. burgdorferi* s.l.<sup>14</sup>. Another study suggested there to be relatively low acquisition of *B. miyamotoi* by ticks because *B. miyamotoi*, in contrast to *B. burgdorferi* s.l., does not seem to cause persistent infections in rodents<sup>56</sup>. By contrast, several studies show higher infection rates in adult ticks compared with nymphs within the same area, which suggests that nymphs are still (to a certain extent) able to acquire *B. miyamotoi* in nature<sup>30, 37, 44</sup>.

### *Correlation between the prevalence of Borrelia miyamotoi and other Borrelia species in tick populations*

Lyme borreliosis is caused by at least eight *B. burgdorferi* s.l. genospecies, which are transmitted by various *Ixodes* ticks<sup>57</sup>. The geographical distribution of *Ixodes* ticks is largely dependent on climatologic as well as habitat and host factors. However, the determinants for the prevalence of various *B. burgdorferi* s.l. species in different *Ixodes* tick populations and their interrelatedness are still under debate<sup>57,58</sup>. Various studies have described the prevalence of *B. miyamotoi* and *B. burgdorferi* s.l. in the same *Ixodes* tick populations. One study reported that *B. miyamotoi* and *B. burgdorferi* sensu stricto did not co-infect individual ticks at a higher or lower frequency than would be expected based on their prevalence in the tick population, indicating the different spirochetes do not enhance or impair the survival of the other within individual ticks<sup>14</sup>. Interestingly, however, there appears to be a correlation between the infection rate of *B. miyamotoi* and the infection rate of *B. burgdorferi* s.l. at a tick population level (Figure 2). Future studies should elucidate their ecological relation and to what extent *B. miyamotoi* and *B. burgdorferi* s.l. have the same range of reservoir hosts.





**Figure 2. Prevalence of *Borrelia miyamotoi* in ticks versus the prevalence of *Borrelia burgdorferi sensu lato* (s.l.) complex.**

Studies were included in which a polymerase chain reaction (PCR) for *B. burgdorferi* s.l. as well as a *B. miyamotoi*-specific analysis was performed on questing ticks. Prevalences are given for each geographic region within a study, defined as a country or a US state, where >200 nymphal or adult Ixodes ticks were individually tested. Sites and studies were excluded if a PCR was performed only in culture and/or direct fluorescence assay-positive ticks, and if accurate establishment of prevalence was impossible due to a bias (i.e., only some of the *B. burgdorferi* s.l.- positive ticks were tested for *B. miyamotoi*) or lack of clarification (i.e., the total sampled tick population was unknown or no distinction was made between host-derived and questing ticks). Data were collected from [12,14,21–25,27,29–35,37,38,40,42–45]. One study [38] tested ticks from multiple states but did not clarify prevalences per state. All other samples were subdivided per country or state within the study. Squares signify adult ticks, circles signify nymphs, and one study [31] in which the prevalence in nymphs and adults could not be separated is represented by a diamond. Host-derived and questing ticks are represented by black symbols and white symbols with black outline, respectively. Linear regression of all tick populations is indicated by a black line:  $r^2 = 0.34$ , slope deviation from zero  $P = 0.0002$ . Spearman  $r = 0.67$  [95% confidence interval (CI) 0.42–0.82,  $P < 0.0001$ ].

### *Borrelia miyamotoi* in reservoir hosts

The ability of ticks to feed on various hosts as well as the capacity of these hosts to serve as reservoirs for specific *Borrelia* species shows large variation<sup>59</sup>. However, as for many *Borrelia* species, mice and other small rodents appear to be reservoir hosts for *B. miyamotoi*, and *B. miyamotoi* has been found in the blood of mice (*Peromyscus leucopus*, *Apodemus argenteus*, and *Apodemus speciosus*) and voles (*Myodes rutilus*, *Myodes rufocanus*, and *Myodes glareolus*)<sup>14,16,56,60</sup>. Surprisingly, when wild turkeys (*Meleagris gallopavo*) in Tennessee were investigated for *B. miyamotoi*, as many as 35 out of 60 turkeys (58%) were infected with the relapsing fever spirochete<sup>36</sup>.

Rodents and specific birds are not alone in being capable of harboring *B. miyamotoi*. A recent study identified the hosts of different *Borrelia* species by linking the presence of pathogens in engorged *I. ricinus* ticks to the presence of host species DNA. In this study, *B. miyamotoi*-infected ticks contained DNA of wild boar (*Sus scrofa*), roe deer (*Capreolus capreolus*), and common blackbird (*Turdus merula*)<sup>21</sup>. However, it was not possible to ascertain whether these ticks had picked up *B. miyamotoi* from these animals or whether they were already infected with *B. miyamotoi* and subsequently fed on these animals. To conclude, *B. miyamotoi* has been identified in small rodents, while there are sporadic reports of infected birds and larger mammals; more studies are needed to establish the full range of reservoir hosts.

## **B. MIYAMOTOI-RELATED HUMAN DISEASE**

### *Human exposure to Borrelia miyamotoi*

Although the infection rate of *B. miyamotoi* in ticks is relatively low, a large and increasing number of humans are being bitten by ticks and, therefore, are at risk for exposure to *B. miyamotoi*<sup>61,62</sup>. In the case of Lyme borreliosis, between 0.3% and 5.2% of tick bites in endemic areas in Europe lead to an erythema migrans (EM), with seroconversion occurring in 3.5–8.2% after tick bites that do not lead to EM<sup>61,63–66</sup>. Assuming similar transmission dynamics, *B. miyamotoi* would lead to a lower risk of infection and seroconversion due to its lower prevalence in ticks. Indeed, in the Netherlands alone, each year an estimated 36 000 humans are bitten by ticks that were infected with *Borrelia miyamotoi*, compared with 183 000 estimated to be bitten by ticks infected with *B. burgdorferi* s.l.<sup>27</sup>. Although there is considerable exposure of humans to *B. miyamotoi*-infected ticks, it is unknown how often this exposure leads to infection, in part due to the lack of knowledge on *B. miyamotoi* transmission dynamics. Lyme borreliosis spirochetes are rarely transmitted <24 h after *Ixodes* tick attachment. They reside in the midgut of questing ticks and, after the tick bites, the spirochetes migrate from the midgut to the salivary glands and are transmitted with tick saliva into the host skin<sup>67,68</sup>. By contrast, some relapsing fever spirochetes are transmitted within seconds because they are already present in the salivary glands of questing (soft) ticks<sup>69</sup>. It is currently unknown whether *B. miyamotoi* resides in the midgut of questing *Ixodes* ticks or is already present in their salivary glands, and how rapidly it can be transmitted during a tick bite.

### Seroprevalence of anti-*Borrelia miyamotoi* antibodies in humans

Determining the seroprevalence of antibodies elicited against *B. miyamotoi* in the general population and high-risk populations provides information about the extent of past infections with this emerging pathogen. For Lyme borreliosis, it is known that antibodies can persist for many years after acute infection, leading to a variable seroprevalence of antibodies against *B. burgdorferi* s.l. in the general population in endemic areas (0.6–19.7%)<sup>70-76</sup>. Some studies have shown that standard serologic tests for Lyme borreliosis can fail to diagnose *B. miyamotoi* infections, and serology for *B. miyamotoi* is currently based on antibodies against the glycerophosphodiester phosphodiesterase (*glpQ*) gene, which is present in *B. miyamotoi*, but not in Lyme borreliosis spirochetes<sup>46, 77, 78</sup>. However, it is not specific for *B. miyamotoi* because homologs are present in other relapsing fever spirochetes<sup>78,79</sup>. Two studies in the USA analyzed the seroprevalence of *B. miyamotoi* using an enzyme-linked immunosorbent assay (ELISA) with confirmatory western blot to detect anti-GlpQ antibodies. In the healthy population, seroprevalence to *B. miyamotoi* was 0% in a nonendemic region and 1–3.9% in endemic regions ( Table 1)<sup>80,81</sup>.

**Table 1. Human *Borrelia miyamotoi* seroprevalence in several risk groups**

Healthy population in endemic areas	Patients evaluated for suspected Lyme borreliosis	Patients with Lyme borreliosis	Patients with viral-like illness in Spring-late Summer <sup>a</sup>	Forestry workers	Suspected HGA, serologically unconfirmed	Refs
6/584 (1.0%)	9/277 (3.2%)		3/14 (21%)			[80]
25/639 (3.9%)		Acute Lyme borreliosis: 19/194 (9.8%)	8/221 (3.6%)			[81]
3/150 (2%)		Lyme neuroborreliosis: 4/54 (7.4%)		12/120 (10.0%)	19/130 (14.6%);	[82]

a. Without signs of upper respiratory tract infection and/or gastroenteritis.

In these studies, 3.2% of patients suspected of Lyme borreliosis and up to almost 10% of patients with confirmed Lyme borreliosis had positive *B. miyamotoi* serology. Of note, 11% of *B. miyamotoi* seropositive sera were also positive for *B. burgdorferi*<sup>80,81</sup>. These findings were confirmed by a GlpQ-Luminex based study in the Netherlands, where a high seroprevalence to *B. miyamotoi* was found in forestry workers (10%) and patients with Lyme neuroborreliosis (7.4%) compared with 2% in blood donors<sup>82</sup>. Prospective studies should be performed to assess how often *B. miyamotoi* seropositivity in patients with Lyme borreliosis derives from co-infections, cross-reactivity, or previously experienced infections. In the Dutch study, 130 serum

samples that had been initially analyzed for human granulocytic anaplasmosis (HGA) serology, but turned out to be negative, were analyzed for *B. miyamotoi* antibodies. Interestingly, 19 of these patients (14.6%) were found to be seropositive for *B. miyamotoi*, suggesting that *B. miyamotoi* is a common cause of HGA-like disease<sup>82</sup>

### *Disease in immunocompetent patients*

Until 2011, *Borrelia miyamotoi* was believed to be a spirochete without clinical significance. This changed when 46 out of 302 Russian patients admitted with a suspected tick-borne infection were found to have PCR-confirmed *B. miyamotoi* infection<sup>7</sup>. Most of the patients with *B. miyamotoi* infection had a nonspecific febrile illness approximately 2 weeks after a tick bite, presenting with a high-grade fever (98%), fatigue (98%), headache (89%), myalgia (59%), chills (35%), and nausea (30%). Similar symptoms, designated by the author as a 'viral-like illness', have been described in several patients that seroconverted to the GlpQ antigen in a study in the USA and in a patient in Japan<sup>80,83</sup>. Only five out of 46 patients in Russia (11%) experienced a true 'relapsing fever' with a mean time of 9 days between relapses. Relapses were probably prevented in most cases by antibiotic treatment<sup>7</sup>. These relapses suggest that a mechanism of antigenic variation similar to that of other TBRF spirochetes exists and that the presence of *B. miyamotoi* in human blood is facilitated by its resistance to human complement (Box 2).

Although EM is the most common disease manifestation of Lyme borreliosis, presenting in approximately 80–90% of patients, it was reported in only 9% of patients with *B. miyamotoi* in Russia<sup>1,7,17</sup>. Although no *B. burgdorferi* s.l. or *B. miyamotoi* PCR was performed on these EM lesions, the authors hypothesized that the EM was caused by *B. burgdorferi* s.l. co-infection. Indeed, a recent USA-based study identified five patients with *B. miyamotoi* infection, four of whom developed EM and were co-infected with *B. burgdorferi*<sup>81</sup>. Two patients with *B. miyamotoi* infection in Japan also presented with EM and were positive on western blot for multiple *B. burgdorferi* s.l. antigens<sup>83</sup>. In Europe, acute Lyme borreliosis is infrequently accompanied by nonspecific febrile illness: of patients that presented with EM, few patients reported a fever (7%), headache (20%), fatigue (19%), or myalgia (10%). This is different in the USA, where as much as 33% of patients with acute Lyme borreliosis reported fever and where more patients described headache (36%), fatigue (47%), and myalgia (35%), possibly related to the difference between infecting *B. burgdorferi* s.l. genospecies<sup>1,84</sup>. Therefore, the presentation of *B. miyamotoi* infection, especially in Europe, has more in common with several other tick-borne diseases, such as TBEV and HGA, and with other *Babesia* spp. and several Rickettsiae (Table 2).

**Table 2. Tick-borne diseases that can be accompanied by fever<sup>a,b</sup>**

Disease	Pathogen	Tick vector	Global spread	Common presentation and laboratory findings	Preferred treatment
<b>Bacterial</b>					
<i>Borrelia miyamotoi</i> disease	<i>Borrelia miyamotoi</i>	<i>Ixodes</i> spp.	North America, Europe, Asia	(Relapsing) nonspecific febrile illness, leukopenia, thrombocytopenia, AST/ALT↑	Doxycycline/ceftriaxone
TBRF	<i>Borrelia crocidurae</i> , <i>Borrelia duttonii</i> , <i>Borrelia hermsii</i> , <i>Borrelia persica</i> , <i>Borrelia parkeri</i> , <i>Borrelia turicatae</i>	<i>Ornithodoros</i> spp.	North America, Africa, Asia, Europe	(Relapsing) nonspecific febrile illness, confusion, photophobia, eye pain, rash, abdominal pain, hepatosplenomegaly, jaundice, thrombocytopenia, anemia	Doxycycline/ceftriaxone
HGA	A. <i>phagocytophilum</i>	<i>Ixodes</i> spp.	North America, Europe, Asia	Nonspecific febrile illness, leukopenia, thrombocytopenia, AST/ALT↑	Doxycycline
HME	<i>E. chaffeensis</i>	<i>Amblyomma americanum</i>	North America	Nonspecific febrile illness, rash, meningoenephalitis, leukopenia, thrombocytopenia, hyponatremia, AST/ALT↑	Doxycycline
Lyme borreliosis	<i>B. burgdorferi</i> s.l.	<i>Ixodes</i> spp.	North America, Europe, Asia	EM, nonspecific symptoms with or without (low-grade) fever. Disseminated disease: multiple EM, arthritis, meningoradiculitis, myocarditis, ACA, <i>Borrelia</i> lymphocytoma. The presentation differs between Eurasia and the USA	Doxycycline/ceftriaxone
Rickettsioses	<i>Rickettsia conorii</i> , <i>Rickettsia rickettsii</i> , <i>Rickettsia africae</i> , <i>Rickettsia slovaca</i> , etc.	Depends on <i>Rickettsia</i> species	Worldwide	Depends on species. Mostly eschar, maculopapular rash, lymphadenopathy, nonspecific febrile illness	Doxycycline
Tularemia	<i>Francisella tularensis</i>	<i>A. americanum</i> , <i>Dermacentor variabilis</i> , <i>Dermacentor andersonii</i> , <i>Ixodes ricinus</i>	North America, Europe, Asia	Ulcer, lymphadenopathy, pharyngitis, conjunctivitis, atypical pneumonia, nonspecific febrile illness	Streptomycin/gentamycin
<b>Parasitic</b>					
Babesiosis	<i>Babesia microti</i> , <i>Babesia divergens</i>	<i>Ixodes scapularis</i> , <i>I. ricinus</i>	North America, Europe	Nonspecific febrile illness, hepato-/splenomegaly, jaundice, petechiae, ecchymosis, hemolytic anemia, leukopenia, thrombocytopenia, AST/ALT↑	Atovaquone & azithromycin <sup>c</sup>
<b>Viral</b>					
Tick-borne encephalitis	TBEV (flavivirus)	<i>I. ricinus</i> , <i>Ixodes persulcatus</i>	Europe, Asia	Nonspecific febrile illness followed by meningo-/encephalitis, myelitis	Supportive
CCHF	CCHFV (bunyavirus)	<i>Hyalomma</i> spp.	Africa, Eurasia	Hypotension, conjunctivitis, nonspecific febrile illness, progressive hemorrhagic diathesis, DIC, anemia, leukopenia, thrombocytopenia, (A)PTT ↑, AST/ALT ↑	Supportive, potentially ribavirin

- a** The most relevant pathogens, ticks, geographic locations, clinical findings, and therapies are described. Other tick-borne diseases responsible for fever include Southern tick associated rash illness, Omsk hemorrhagic fever, Kyasanur Forest disease, Colorado tick fever, and severe fever with thrombocytopenia syndrome.
- b** Abbreviations: ACA: acrodermatitis chronica atrophicans; (A)PTT: (activated) partial thromboplastin time; AST: aspartate transaminase; ALT: alanine transaminase; DIC: diffuse intravascular coagulation; CCHF(V): Crimean Congo haemorrhagic fever (virus); HME: human monocytic ehrlichiosis; MOF, multiorgan failure.
- c** Alternatives (in more severe cases): clindamycin and quinine. Exchange transfusions should be considered.

Two patients in the USA were presumptively diagnosed with HGA but later tested positive for *B. miyamotoi*. Both patients presented with high-grade fever and various nonspecific symptoms in combination with elevated transaminases, thrombocytopenia, and leukopenia<sup>85</sup>. This clinical presentation seems common in the patients currently described to be infected with *B. miyamotoi* (Table 3).

**Table 3. Clinical presentation of patients infected with *Borrelia miyamotoi*<sup>a</sup>**

Patient characteristics	Symptoms	Detection methods	Lab findings	Therapy	Refs
Five symptomatic patients with seroconversion, USA	Fever (5/5 patients); EM (4/5); headache, neck stiffness, fatigue, malaise, arthralgia, abdominal pain, cough, sore throat, inguinal lymphadenopathy (1/5)	GlpQ ELISA; GlpQ western blot	No information available	Doxycycline 7–14 days (four patients), amoxicillin/clavulanic acid (one patient)	[80, 81]
61-year-old man, USA	Fever, chills, headache, photophobia, myalgia, arthralgia, anorexia	PCR	Thrombocytopenia, leukopenia, elevated AST/ALT and CPK	Doxycycline 2 x 100 mg IV 4 days, 2 x 100 mg oral 2 weeks	[85]
87-year-old man, USA	Fever, chills, malaise, fatigue, dyspnea, anorexia, stiffness	PCR	Thrombocytopenia, leukopenia, anemia, elevated AST/ALT	Doxycycline 2 x 200 mg IV 2 days, 2 x 100 mg oral 2 weeks	[85]
46 patients suspected to have tick-borne disease (admitted to a hospital), Russia	Fever (45/46); fatigue (45/46); headache (41/46); myalgia (27/46); chills (16/46); nausea (14/46);	PCR	Proteinuria, elevated AST/ALT	Ceftriaxone 2 g IV 2 weeks (43/46), doxycycline 2 x 100 mg oral 2 weeks (3/46)	[7]
72-year-old woman, Japan	Fever, myalgia, anorexia, EM	PCR; GlpQ western blot	Elevated AST/ALT and CPK, leukopenia	Minocycline 100 mg 5 days	[83]
37-year-old man, Japan	Fever, EM	PCR	No information available	Ceftriaxone 1 g IV 7 days, followed by unknown antibiotic	[83]
70-year-old immunocompromised man, The Netherlands	Slow cognitive processing, memory deficits, disturbed gait	PCR (CSF and blood); dark-field microscopy (CSF)	CSF: pleocytosis, elevated protein	Ceftriaxone, 2 g IV 2 weeks	[46]
81-year-old immunocompromised woman, USA	Progressive decline in mental state, confusion, social withdrawal, disturbed gait, hearing difficulty, weight loss	PCR (CSF); (dark-field) microscopy (CSF)	CSF: pleocytosis, elevated protein	Ceftriaxone, 2 g IV, switch to penicillin IV 1 month	[86]

Abbreviations: AST, aspartate transaminase; ALT, alanine transaminase; CRP, C-reactive protein; IV, intravenous.

### *Disease in immunocompromised patients*

Two case reports, one from the USA and one from the Netherlands, reported *B. miyamotoi* infection in patients who were immunocompromised because of treatment for non-Hodgkin's lymphoma. Both patients underwent chemotherapy (cyclophosphamide, doxorubicin, and vincristine) and immunomodulatory therapy comprising prednisolone and the B cell depletory rituximab before showing signs of infection. The clinical symptoms differed from immunocompetent patients, with no evident signs of fever, but a chronic meningoencephalitis with declined mental status and disturbed gait developing over several months<sup>46,86</sup>. Both cases reported a marked pleocytosis in cerebrospinal fluid (CSF) and visible spirochetes by dark-field microscopy, but recovered after antibiotic therapy.

### *Diagnostic tools*

There is currently no clinically validated test available for *B. miyamotoi*. Until recently, for research purposes, *B. miyamotoi* was propagated by inoculation in SCID mice<sup>87</sup>. However, more recently, culture media based on modified Kelly-Pettenkofer medium were shown to successfully propagate the spirochete and might accelerate research into diagnostic modalities<sup>88,89</sup>.

Relapsing fever can be diagnosed by PCR on blood during acute infection<sup>7,85</sup>. A PCR on CSF was also positive in both cases of *B. miyamotoi*-induced meningoencephalitis<sup>46,86</sup>. Most PCRs performed on human or tick samples amplify and sequence the *glpQ* gene, flagellin gene, the 16S rRNA gene, or the 5S-16/23S intergenic spacer sequence. Another method to identify relapsing fever spirochetemia is the use of microscopy, for instance a thin smear with a Giemsa or Wright stain, which can directly demonstrate spirochetemia (Figure 3). However, this method seems to be less sensitive than PCR to diagnose relapsing fever<sup>90</sup>. When patients present after the initial fever has subsided and the sensitivity of PCR might further decrease, serology could be used to diagnose *B. miyamotoi* infection, although its diagnostic value in early infection is currently unknown.

### *Antibiotic therapy*

All patients that have currently been described in the literature recovered upon antibiotic treatment, which was mostly based on standard regimens used for Lyme borreliosis (Table 3). No *B. miyamotoi*-infected patients were described to have a relapse of fever after antimicrobial treatment. A Jarisch-Herxheimer-like reaction was found in 15% of the cases in Russia<sup>7</sup>. It is unclear whether chronic sequelae or atypical symptoms may develop or persist when *B. miyamotoi* infections remain undiagnosed, and whether infection can be cleared without therapeutic intervention.

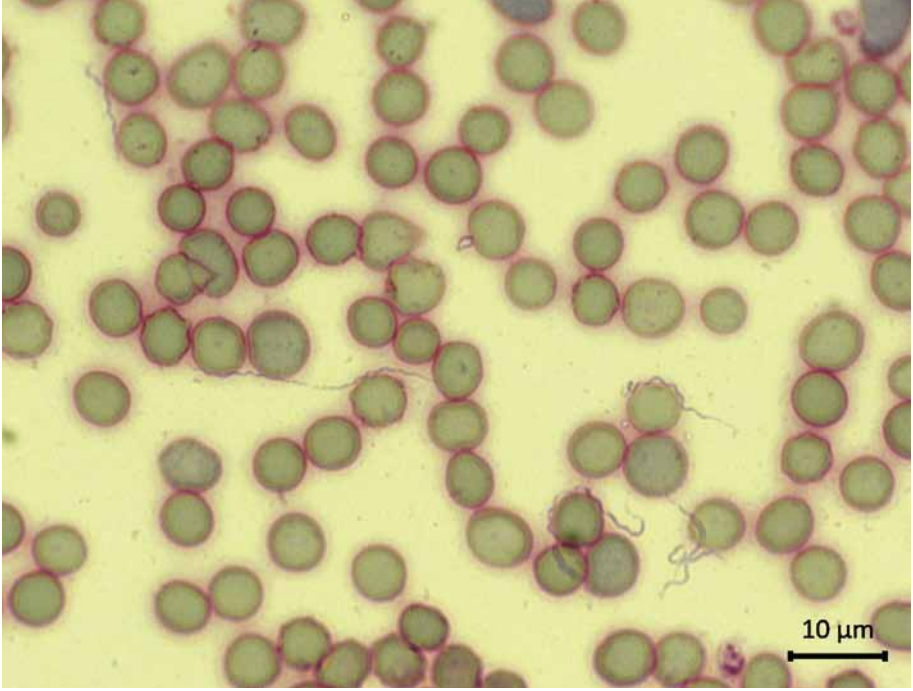


Figure 3. Giemsa-stained peripheral blood smear of a SCID mouse infected with *Borrelia miyamotoi* LB-2001.



## CONCLUDING REMARKS AND FUTURE DIRECTIONS

*Borrelia miyamotoi* is a relapsing fever *Borrelia* species that has recently been discovered to infect humans and is present in *Ixodes* ticks in Europe, Asia, and North America. *Borrelia miyamotoi* has been described to cause an acute nonspecific febrile illness in over 50 immunocompetent patients and more severe neurological disease in two immunocompromised patients. Antibodies against the spirochete are prevalent in individuals living in endemic areas. However, we are only just beginning to unravel its pathogenic potential, and it is only recently that *B. miyamotoi* was cultivated successfully *in vitro*, which is likely to accelerate research into diagnostic modalities. Further epidemiological studies should elucidate the incidence of *B. miyamotoi*-induced disease. We should be aware that *B. miyamotoi* is present in the same tick populations as *B. burgdorferi* s.l., and that *B. miyamotoi* infection can resemble other tick-borne diseases, including HGA. Therefore, *Borrelia miyamotoi* infection should be considered when patients in Lyme disease-endemic regions present with a nonspecific febrile illness after a tick bite, or with a tick bite-associated meningoencephalitis during immunosuppressive therapy. *Borrelia miyamotoi* infection appeared to present differently from Lyme borreliosis in the patients that have been described in literature and responded to the same antibiotic regimens. More studies are needed to improve the diagnosis and to increase knowledge of this potentially important relapsing fever spirochete.

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## SUPPLEMENTAL DATA

**Table S1. Prevalence of PCR-confirmed *Borrelia miyamotoi* infection in individually tested *Ixodes* ticks.**

All studies on PubMed and Web of Science describing the prevalence of *Borrelia miyamotoi* (by PCR) in *Ixodes* ticks published until June 2014 were included; however, studies or tick populations within studies were excluded in case of: 1. Bias in the prevalence of *B. miyamotoi* (only part of spirochete positive ticks were analyzed for *B. miyamotoi*) 2. insufficient description of tick populations and infection rates to include data for example, total number of tested ticks were not described) 3. Pooled ticks were tested 4. If only ticks positive by another method (DFA, culture) were subsequently tested by PCR. For each tested *Ixodes* species, the prevalence per tick developmental stage is presented. A distinction has been made between questing ticks and host-derived ticks. These data are available upon request. [S1-S28]

Tick species	Larvae	Nymphs	Adults	Unknown	Total
<b>Questing ticks</b>					
<i>I. ricinus</i>	1/185 (0.54%)	49/4239 (1.17%)	52/3397 (1.53%)	23/2202 (1.04%)	125/10023 (1.25%)
<i>I. scapularis</i>		215/10782 (1.99%)	29/1449 (2.00%)		244/12231 (1.99%)
<i>I. spinipalpis</i>		0/3 (0%)			0/3 (0%)
<i>I. pacificus</i>		4/261 (1.53%)	2/2674 (0.07%)		6/2935 (0.20%)
<i>I. persulcatus</i>		4/104 (3.85%)	11/456 (2.41%)	114/3017 (3.78%)	129/3577 (3.60%)
All <i>Ixodes</i>	1/185 (0.54%)	272/15389 (1.77%)	94/7976 (1.18%)	137/5219 (2.63%)	504/28769 (1.75%)
<b>Host-derived ticks</b>					
<i>I. ricinus</i>	3/741 (0.40%)	37/4134 (0.90%)	24/1523 (1.58%)		64/6398 (1.00%)
<i>I. scapularis</i>	0/4 (0%)	1/145 (0.69%)	23/5661 (0.41%)		24/5810 (0.41%)
<i>I. pacificus</i>		0/118 (0%)			0/118 (0%)
<i>I. spinipalpis</i>				0/17 (0%)	0/17 (0%)
<i>I. dentatus</i>		1/265 (0.38%)	0/121 (0%)	0/7 (0%)	1/393 (0.25%)
<i>I. brunneus</i>			0/1 (0%)		0/1 (0%)
All <i>Ixodes</i>	3/745 (0.40%)	39/4662 (0.84%)	47/7306 (0.64%)	0/24 (0%)	89/12737 (0.70%)



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