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Borrelia miyamotoi: an emerging tick-borne pathogen with growing public health significance in northern latitudes

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Table of contents

Abbreviations

ALT:	Alanine aminotransferase
APTT:	Activated partial thromboplastin
AST:	Aspartate aminotransferase
DNA:	Desoxyribonucleic acid
GLpQ:	Glycerophosphodiester phosphodiesterase, periplasmic
HGA:	Human granulocytic anaplasmosis
MKP:	Modified Kelly-Pettenkofer
PCR:	Polymerase chain reaction
RNA:	Ribonucleic acid
RT-PCR:	Real time – polymerase chain reaction
SCID:	Severe combined immunodeficiency
TBD:	Tick-borne disease
TBEV:	Tick-borne encephalitis virus
TBP:	Tick-borne pathogen
TLR:	Toll-like receptor

1. Introduction

Borrelia miyamotoi is a spirochete transmitted by hematophagous arthropod vectors that circulates in a wide range of reservoir species and causes relapsing fever in humans (Siński, et al., 2016).

Borrelia miyamotoi was first discovered in Japan in 1995, where it was isolated from *Ixodes persulcatus* ticks (Siński, et al., 2016). It was later found to be transmitted by the same hard tick species that transmit lyme borreliosis: the *Ixodes ricinus - persulcatus* complex, and maintained largely by natural rodent or bird populations (Szekeres, et al., 2017). Despite being only recently discovered it is thought to have been circulating in certain areas for much longer, while being continuously misdiagnosed as the closely related *Borrelia burgdorferi* sensu lato (s.l.) species (Siński, et al., 2016).

There are three known genotypes of *Borrelia miyamotoi*: European, American, Asian/Siberian. The European type is transmitted by *I. ricinus*, the American type by *Ixodes scapularis* and *Ixodes pacificus*, and the Asian/Siberian type by *I. persulcatus* (Crowder, et al., 2014). The first human case of disease was reported in Russia in 2011. Whereas before this it was thought to be non-pathogenic, all three strains are now considered pathogenic to humans (Cochez, et al., 2015).

There are three major groups of *Borrelia* species: the Lyme Borreliosis group, the Relapsing Fever group, and the Reptile-associated Borrelia group. *Borrelia miyamotoi* belongs to the Relapsing Fever group and causes a relapsing non-specific fever with other signs and the potential to cause meningoencephalitis in immunocompromised patients (Siński, et al., 2016). Complex interactions between host, pathogen and tick are continuously at play, with *B. miyamotoi's* strategies of adaptation in both vector and host aiding its survival and ultimate disease-causing abilities in humans (Hovius, et al., 2007).

There is thought to be a large range of potential reservoir hosts for *B. miyamotoi* as shown in Table 1 (Hamer, et al., 2012; Siński, et al., 2016; Szekeres, 2017). Natural rodent populations play a significant role in transmission, as do certain bird species and larger vertebrate hosts such as roe deer (Szekeres, et al., 2017). The variation of reservoir and maintenance host species largely varies with geographical location. In Northern European countries with cooler climate roe deer have been shown to have a greater importance as maintenance hosts, while countries with warmer climates show an increased significance of bird and small mammal species in transmission of *B. miyamotoi*.

Table 1: Known reservoir and maintenance hosts of *B. miyamotoi* (Hamer, et al., 2012; Siński, et al., 2016; Szekeres, 2017)

Reservoir host species	Location	Reference
Small rodent		
- *Apodemus flavicollis*	Europe, Japan	(Siński, et al., 2016; Szekeres, 2017)
- *Apodemus argentus*		
- *Apodemus speciosus*		
- *Myodes glareolus*	Europe	(Siński, et al., 2016) (Szekeres, 2017)
- *Myodes rutilus*		
- *Myodes rufocanus*		
- *Peromyscus leucopus*	USA	(Siński, et al., 2016)
Bird		
- *Turdus merula*	Europe	(Siński, et al., 2016; Hamer, et al., 2012)
- *Cardinalis cardinalis*	USA + South America	(Hamer, et al., 2012)
Suspected maintenance hosts		
- *Capreolus capreolus*	Europe	(Siński, et al., 2016)
- *Odocoileus virginianus*	USA	(Siński, et al., 2016)
- *Sus scrofa*	Europe	(Siński, et al., 2016)

Shifting trends in density, emergence, and seasonality of *Borrelia miyamotoi* and other tick-borne pathogens show a strong correlation to the warming effects of climate change and several other environmental changes (Mysterud, et al., 2017).

Due to key genetic similarities, it is thought that *Borrelia miyamotoi* has in the past been commonly misdiagnosed or misdetected as *B. burgdorferi* s.l. Partly due to the great improvements in detection capabilities we are seeing in increasing trend in *B. miyamotoi* prevalence in various countries. This is an area that has a lot of unanswered questions and requires further research.

The fairly recent discovery and emergence of relapsing fever caused by *B. miyamotoi,* combined with its similarities to other tick-borne diseases, mean that there is a lack of public awareness of this disease. A vaccine against relapsing fever is a long way off, however there are large improvements to be made in the preventative methods of tick control in high risk areas and individuals (Aenishaenslin, et al., 2017).

2.1 Transmission

Transmission of the spirochete *Borrelia miyamotoi* involves a complex set of factors and cycles involving vectors, reservoir, maintenance and final hosts. Hematophagous arthropod vectors such as hard ticks are responsible for its transmission to reservoir and maintenance hosts.

As previously stated there are 3 major groups of *Borrelia* species (Wagemakers, et al., 2015). The Lyme Borreliosis group consists of 20 species which are collectively known as *B. burgdorferi* s.l. They are transmitted by hard ticks (Szekeres, 2017). There is much more research published about *B. burgdorferi* s.l. than *B. miyamotoi*, however much can be drawn on this research as they hold many key similarities in their transmission and pathogen cycles.

Borrelia species belonging to the Relapsing Fever group are largely transmitted by soft ticks (Argasid ticks of the *Ornithodoros* genus) and lice, for example, *Borrelia recurrentis* is transmitted by the human body louse (*Pediculus humanus corporis*). *Borrelia miyamotoi* is the exception in that it is transmitted by hard ticks, with *Ixodes* species, rodents and birds serving as the main reservoir hosts (Szekeres, 2017). This is the same *Ixodes ricinus - persulcatus* complex responsible for the transmission of the causative agents of lyme borreliosis. *Borrelia miyamotoi* is the only known agent causing relapsing fever that is transmitted by hard ticks (Wagemakers, et al., 2015).

There are several key factors influencing the impact of hard ticks in the transmission of pathogens, including where they quest, moult and lay their eggs, their host range, how many host species their lifecycle depends on, and how they find their hosts (Szekeres, 2017). Ticks belonging to the *I. ricinus-persulcatus* complex are exophilic ticks. They are opportunistic feeders with a non-specific host range and often act as bridging vectors between humans and small mammals. They have a 3-host life cycle which is outlined in Figure 1.

Within the Relapsing Fever group, there is some debate around the most appropriate phylogenic classification, with some researchers believing it should be classified into groups or clusters based on geographical differences, while others believe vector-based classification to be more relevant. This is a potential area for future research (Wagemakers, et al., 2015).

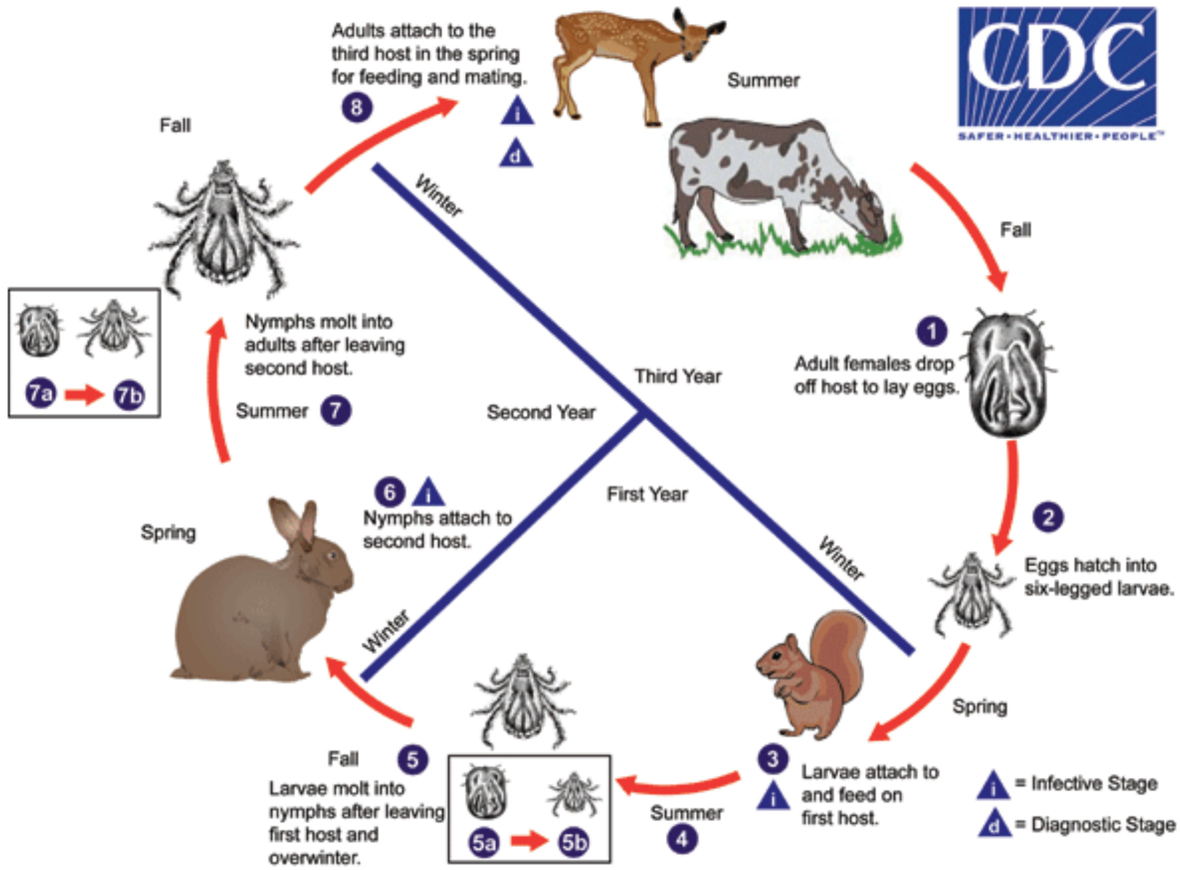

Figure 1. Three-host Ixodid tick life cycle (cdc.gov, 2017)

There are three known genotypes of *Borrelia miyamotoi*: American, European and Siberian/Asian, however it is currently unclear as to the main cause of these genetic differences (Crowder, et al., 2014). More research is needed to determine if these differences can be purely or partially explained by geographical location or if they are connected to differences in pathogenicity, host range and vector competence (Wagemakers, et al., 2015).

The three genotypes have been found to be transmitted by different *Ixodes* species, which are largely found in the northern hemisphere (Wagemakers, et al., 2015). The American type is found in *I. scapularis* and *I. pacificus*, the European type is found in *I. ricinus* and the Siberian/Asian type is found in *I. persulcatus* (Szekeres, 2017).

The spirochaete was first discovered in *I. persulcatus* and *Apodemus argenteus* mice in Japan in 1995 (Szekeres, 2017). Since then it has been found in *Ixodes* species all over the northern hemisphere. According to research carried out by Wagemakers et al in 2015 on questing ticks, *I. persulcatus* was found to carry the highest load of *B. miyamotoi*, with on average 3.6% of ticks being infected, compared to *I. scapularis* and *I. ricinus* (2.0% and 1.3% respectively). In areas of high tick and reservoir host density, these infection rates have been found to be significantly higher, showing that infection rates can vary greatly between and within different geographical regions, otherwise known as "spatial" variation. There is also temporal variation in infection rates of tick and host species with the bimodal/seasonal pattern of the tick lifecycle. These spatial and temporal variations will be further discussed in Chapter 2.2 (Crowder, et al., 2014).

The exact infectious cycle of *B. miyamotoi* is still not completely known and requires more research. However, the main route of transmission within the hard tick is thought to be transovarial, due to *B. miyamotoi* being found in all life stages of the tick (according to multiple PCR-based studies). Studies have shown that in many cases *B. miyamotoi* is the only spirochete present in unfed larvae. This suggests that previous findings indicating sole *B. burgdorferi* s.l. infections may have been misidentified as *B. miyamotoi* due to their genetic similarities and the limitations of previous investigative methods (Wagemakers, et al., 2015). Current PCR-based and mass spectrometry methods have higher specificity and are able to better identify these different species (Crowder, et al., 2014).

Although its presence in unfed larvae strongly suggests transovarial transmission is at play, it is currently unknown how significant this route is in transmission of *B. miyamotoi* to humans (Wagemakers, et al., 2015). As it stands, it is known that infection from nymphs and adult ticks pose the highest threat to humans. Under experimental conditions larvae were found to feed on humans at inconsistent levels and it remains unclear whether they are able to transmit *B. miyamotoi* during this feeding. In other host species however, such as wild rodents, larvae were found to transmit the spirochete, thereby suggesting an important role for larvae within the infectious cycle (Wagemakers, et al., 2015). More research is required to determine the significance of larvae as a source of infection and how this varies between different host species.

The tick-pathogen interface is a highly complex and interesting area in the transmission of *B. miyamotoi*. There is a lot more research needed into this spirochete specifically, however conclusions can be drawn from existing research concerning other tick-borne pathogens (TBPs), particularly those regarding *B. burgdorferi* s.l.

Research has been carried out into the mechanisms used by TBPs to manipulate their tick hosts at various levels in order to aid their own survival and transmission. The end result of most tick-pathogen interactions usually aids both tick and pathogen survival, multiplication and transmission. Not only is tick survival and amplification advantageous for the pathogen for the aforementioned reasons, but some TBPs will increase tick performance in certain environments, thereby promoting tolerance to pathogen infection as an advantageous trait (Cabezas-Cruz, et al., 2019). Indeed the response of a tick host to the manipulative effects of their pathogens is largely determined by their tolerance to these pathogens.

In 2007 Hovius et al published their research regarding tick-pathogen interactions between *B. burgdorferi* s.l. and *Ixodes* ticks. It is thought that *B. miyamotoi* behaves in a very similar way to *B. burgdorferi* s.l. in regard to its tick interactions, thereby allowing us to draw conclusions from this study regarding both spirochetes. Many different strategies of adaptation are required for *Borrelia* species to survive in their multi-host environments. One of the ways in which *B. burgdorferi* s.l. does this in *Ixodes* ticks is to use the tick's metabolism to change its physiological and cell processes in such a way to aid survival of both spirochete and tick. These metabolic effects are largely due to the preferential expression of various genes, which shows that this TBP has the ability to manipulate its host at a protein transcription level (Hovius, et al., 2007).

The publishing of the first tick genome sequence in 2016 (*I. scapularis*) has made it easier to study the genomic impact of tick-pathogen interactions (Cabezas-Cruz, et al., 2019). Since then other approaches have focused on the metabolic interactions between ticks and the pathogens they bear, further confirming that tick-borne pathogens can affect protein transcription (largely that of metabolic enzymes), in turn altering several metabolic pathways in ticks (Hovius, et al., 2007).

Two main physiological processes that are altered within ticks include cellular immunity and apoptosis. Regarding the tick's nutritional immunity and iron levels, regulation of iron uptake from a host's blood may benefit both tick and pathogen survival, as excess iron levels can lead to formation of a reactive molecule causing oxidative damage. Other metabolic processes that are altered to some degree within ticks include glycolysis, glyconeogenesis, lipid and amino acid metabolism, and redox pathways. Fatty acids provide a key energy source for both tick and pathogen, particularly during times of fasting between blood meals (Cabezas-Cruz, et al., 2019).

Cabezas-Cruz et al studied the metabolic interactions of TBPs largely using *Anaplasma phagocytophilum* as a basis in 2019. This was a valuable study, however more research is needed into the differences between TBPs and the specific mechanisms they use in order to manipulate their tick hosts. This information would help us to understand how we might in the future be able to manipulate these tick-pathogen relationships to reduce survival, replication and transmission of both tick and pathogen, thereby reducing incidences of tick-borne diseases.

The *Ixodes ricinus-persulcatus* complex has a large host range due to its "generalised feeding behaviour" (Radolf, et al., 2012). It is this opportunistic feeding that ultimately leads to incidental infection in humans. There are many factors determining which maintenance, reservoir or incidental hosts these ticks feed on, many of which rely on temporal and spatial determinants. The geographical and temporal distribution of ticks and their hosts in the northern hemisphere is discussed in detail in Chapter 2.2.

The identification of the reservoir hosts of *Ixodes* ticks has been carried out by several different methods. The first "classical" method is to compare infection rates in questing (unfed) larvae before and after a blood meal on specific host species. This works well with pathogens that do not have transovarial transmission, such as *B. burgdorferi* s.l., however as *B. miyamotoi* is transmitted from the adult tick to the larvae, this is not an effective method to identify its natural reservoir hosts. The second more reliable method is the use of "xenodiagnosis", whereby specific pathogen-free larvae are tested for pathogens after feeding on known-infected hosts and subsequent moulting (Burri, et al., 2014).

Known identified reservoir hosts of *Borrelia* species include small mammals (such as rodents), certain bird species, and larger vertebrates (such as deer species) (Radolf, et al., 2012; Szekeres, 2017). The natural reservoir hosts for *B. miyamotoi* have been identified as various rodents and bird species (Burri, et al., 2014; Wagemakers, et al., 2017). The reservoir hosts involved depend largely on the stage of the tick's lifecycle. Larvae and nymphs of the *Ixodes* species feed largely on small mammals and birds, whereas the adult ticks feed more often on larger vertebrates (Wagemakers, et al., 2015).

The interface between tick, host and pathogen occurs at the bite site on the skin of the host. Upon attachment of an infected tick, the spirochete is injected with the tick's saliva into the dermis of the host. Here it can later be acquired by a naive tick vector as it feeds from the same blood pool. Acquisition rates of *B. miyamotoi* are different from that of *B. burgdorferi* s.l. due to both differences in tissue tropism and in the transmission of the spirochete within the tick lifecycle (Wagemakers, et al., 2015).

Borrelia miyamotoi is found in lower levels in the skin compared to *B. burgdorferi* s.l. spirochetes. Whereas a common symptom of infection with *B. burgdorferi* s.l. in the human host is erythema migrans, this has not been recorded in infections with only *B. miyamotoi*, however it has been found in patients with co-infection from both *Borrelia* species (Wagemakers, et al., 2017). *Borrelia burgdorferi* s.l. can survive in the dermis for months without inflammation, while still disseminating throughout the body. It is not known if *B. miyamotoi* can also do this. Most skin studies focusing on the relationship between *Borrelia* species and their hosts have been carried out using mouse skin models, and therefore do not provide the full picture as to what could be happening within the skin of infected humans (Bernard, et al., 2020).

As previously mentioned, there is transovarial transmission of *Borrelia miyamotoi* from the adult female tick to its larvae. This means that larvae feeding for the first time may not be naive, leading to variation in acquisition rates of the spirochete from its hosts. There are also differences in acquisition between different life stages of the *Ixodes* tick of *B. burgdorferi* s.l. and *B. miyamotoi*. In the transmission of *B. burgdorferi* s.l. spirochetes nymphs pose the greatest risk as they are infected at the highest levels. This high infection rate combined with their small size and high abundance mean that nymphs pose the greatest risk of infecting other hosts with *B. burgdorferi* s.l. compared to larvae and adults (Bernard, et al., 2020). In contrast, nymphs are infected at lower levels with *B. miyamotoi* spirochetes, highlighting the importance of other life stages of the tick, such as larvae, in its transmission. There is also a faster transmission of the spirochete during tick feeding of *B. miyamotoi* (Tokarz, et al., 2020). These key differences between the *Borrelia* groups highlight areas of potential research needed into the lifecycle and transmission of relapsing fever spirochetes.

The mechanisms of spirochete survival in the tick have already been discussed. In the host there are several mechanisms at play to aid spirochete survival and amplification.

Overcoming a host's innate immune response at the bite site is the first barrier to spirochete survival. This is largely due to interactions between the tick and its host, however these complex interactions are regulated by the previously discussed alterations within the tick genome that occur as a result of spirochete modulation (Hovius, et al., 2007).

Tick saliva is injected into a host during feeding, which can last anywhere between 3 - 10 days (Bernard, et al., 2020). This saliva contains host factors enhancing spirochete survival (Radolf, et al., 2012). These include specific tick proteins affecting coagulation, pain, wound healing, and the innate and adaptive immunity of the host. After this initial evasion of the immune response spirochetes may be later phagocytosed by monocytes and macrophages. However some *Borrelia* species (notably *B. burgdorferi* s.l.*)* are very motile and therefore able to successfully evade these phagocytes. Toll-like receptor (TLR) - mediated cytokine and chemokine release by phagocytes attract more phagocytes to the site of initial infection. Hosts with repeated tick bites will have intensified inflammatory responses and are less likely to develop lyme borreliosis as these intensified responses combined with antibodies to tick saliva will affect the ability of the tick to feed completely (Bernard, et al., 2020).

After the initial interactions at the tick bite site, *Borrelia* species must disseminate and evade the host's defences further. The next barrier to overcome is the adaptive immune response. The recombination of amino-acid sequences within the spirochete genome are responsible for protecting them from destruction by host antibodies (Hovius, et al., 2007).

Ultimately the success of spirochete dissemination to their target organs will determine the outcome and clinical significance of the infection (Radolf, et al., 2012).

2.2 Eco-epidemiology, emergence, predictions

Borrelia miyamotoi was first isolated in 1995 from *Ixodes persulcatus* ticks in Japan. This is the first official record of the spirochete's existence, however it is thought to have been present but misdetected as *B. burgdorferi* s.l. prior to this (Siński, et al., 2016). Since its discovery there has been in increasing trend in detection of the spirochete, with expansion both at a temporal and spatial level (Li, et al., 2012). It's detection in multiple countries spanning multiple continents is shows pattern of emergence that classifies this spirochete as a key emerging tick-borne pathogen.

The emergence of *B. miyamotoi* over the past 25 years can be in part explained by the improvement in diagnostic tests and the ability to better differentiate it from *B. burgdorferi* s.l. spirochaetes using new PCR and mass spectrometry techniques (Crowder, et al., 2014). However, these trends are also greatly influenced by other key factors such as environmental and socio-economic factors which influence the spatial and temporal distribution of the pathogen, vector and hosts (Michelet, et al., 2014). These factors will be discussed throughout this chapter.

Borrelia miyamotoi, alongside *B. burgdorferi* s.l. is a spirochete currently found predominantly in the northern hemisphere. Cases of relapsing fever from *B. miyamotoi* have been increasingly diagnosed in the USA, Europe, and Asia since this spirochete was discovered to be pathogenic to humans in 2011 (Mysterud, et al., 2017). The detection and diagnostics of *B. miyamotoi* and its relapsing fever are further discussed in Chapter 2.4.

A complex chain of environmental factors is responsible for the expansion of many tick-borne pathogens (Bede-Fazekas & Trájer, 2019). Climate change, significant environmental changes, and the resulting variation in distribution of both tick vectors and reservoir hosts are among the key factors influencing this expansion. Others include certain socio-economic factors and increased global travel of humans and animals (Colwell, et al., 2011).

Climate change is a key driver for the emergence of many vector-based diseases, in particular those caused by tick-borne pathogens (Bede-Fazekas & Trájer, 2019; Li, et al., 2016). There are many complex effects of climate change, with the main result on tick-borne pathogens being a change in their seasonality and ultimately in their expansion. This change in seasonality in seen at both the level of the tick vector and tick-borne pathogen, and at both temporal and spatial levels.

Global warming is one of the many consequences of climate change and has been linked to several key changes in the emergence of tick-borne diseases (Bede-Fazekas & Trájer, 2019). Studying increasing environmental temperatures alone provides the simplest model for investigation of the effects of climate change on the emergence of vector-based diseases.

The spatial expansion of these diseases has been shown by the emergence trends of tick vectors in northern latitudes, and the subsequent expansion of tick-borne disease incidence (Mysterud, et al., 2017). The overall effects of global warming are found to reduce with increasing altitude, meaning that the main consequences of climate change are described in tick populations in lower altitudes (Li, et al., 2016).

The temporal expansion of tick-borne diseases is described by longer activity periods of tick vectors and their pathogens. These longer activity periods caused by warmer climates mean that distribution patterns of tick-borne diseases are altered, with peak incidences of both ticks and their pathogens shifting (Bede-Fazekas & Trájer, 2019; Li, et al., 2016). Climate change has been found to increase vegetation seasons in certain areas, thereby lengthening the activity periods of different vector species and their pathogens. This was seen in Hungary in the 2000's where an elongated lyme borreliosis season was recorded. These findings suggest that the seasons of other pathogens spread by the *I. ricinus* tick, including *B. miyamotoi*, will also be elongated (Bede-Fazekas & Trájer, 2019).

The changing spatial and temporal distribution of these pathogens has significant public health impacts and must be monitored closely and continuously in order to predict shifting patterns in emerging tick-borne diseases.

Environmental changes come in many different forms and have several key effects on the seasonality and distribution of tick-borne diseases. Environmental catastrophes, urbanisation, and deforestation are some major, albeit rare, causes of changes to habitats that have significant dramatic and long-lasting effects on ecosystems (Colwell, et al., 2011). Other less extreme but more common changes which will be outlined here include landscape fragmentation and modified land usage (Li, et al., 2012; Michelet, et al., 2014).

In general, any habitat alteration will cause changes to different levels of its ecosystem. The variation in distribution of reservoir and maintenance hosts for *Borrelia* spirochetes is one of the most important consequences of habitat disturbance.

Changes in fauna abundance and diversity caused by habitat disturbance, such as landscape fragmentation or climate change, will consequently alter the numbers of small mammal hosts in these areas. Increasing numbers of small rodent reservoir hosts available for tick vectors to feed on will further amplify the transmission of their pathogens. This is particularly significant for *B. miyamotoi*, as small rodents account for a large proportion of this spirochete's reservoir hosts. Further research is needed to study the effects of environmental change on the activity and density levels of the migratory bird species involved in *B. miyamotoi* transmission, as it is thought that they may play a larger role than initially thought. (Li, et al., 2016).

Although deer themselves are thought to have a limited transmission capacity for *B. miyamotoi*, they are significant in the life cycle of the hard tick and its harbouring pathogens. Adult *Ixodes ricinus* ticks and small numbers of nymphs feed on larger vertebrates such as roe deer. The distribution and densities of deer present throughout various altitudes and latitudes are found to be directly correlated to tick densities and tick infestations of rodents in these areas.

Deer are found most commonly in northern latitudes, in particular in Northern Europe, and with rising environmental temperatures they are found increasingly in higher altitudes (Li, et al., 2016). Deer management practices in these countries have significant impacts on the distribution of these hosts and thereby impact the distribution of the *Ixodes* tick and its pathogens. Areas with more habitat disturbance from hunting, tourism, and management practices were found to have increased densities of ticks (Li, et al., 2014). This is discussed in more detail in Chapter 2.5 regarding potential prevention and control strategies of tick-borne diseases.

Habitat fragmentation is another environmental change with a significant impact on the transmission cycle of ticks and their pathogens. As previously stated, habitat disturbance of any kind can influence the densities and distribution of small mammals and deer in a particular area. Woodland fragmentation patterns were found to alter lyme disease risk in a study carried out by Li et al, in 2012. It was predicted that tick densities would be greater in highly fragmented woodlands, and that grasslands situated next to woodlands may help to reduce tick densities within these woodlands. A strong correlation was found between the level and type of woodland fragmentation and the spatial patterns of the local tick host populations present. This study focused on lyme disease risk, however the conclusions drawn are also valid for *B. miyamotoi* as it is harboured by the same tick species and its transmission cycle also involves the same small mammal and deer hosts (Li, et al., 2012).

It is also important when discussing the epidemiology and emergence of *B. miyamotoi* to consider the socio-economic factors at play which may influence the exposure rates of humans to infected ticks (Colwell, et al., 2011). Among those at highest risk of infection are forestry workers and people taking part in outdoor leisure activities in areas with high tick burdens. Forestry workers are among those at the highest risk of contracting tick-borne diseases due to working long-term in these areas. Warmer climates often mean there is an increase in people undertaking outdoor leisure activities, such as hiking, leading to increased exposure levels. However, it is important to remember that a high hazard does not always mean a high exposure rate, as the level to which people engage with their surrounding habitats must be taken into account (Li, et al., 2016). The exposure rates of forestry workers and other high-risk groups is further discussed in Chapter 2.5 when looking at surveillance and preventative control strategies.

The variable demographics in developing countries also highlights the influence of socio-economic status in the exposure and infection rates of people with vector-borne pathogens. This is seen when major environmental change occurs, such as environmental catastrophes or industrialisation, which in turn leads to the mass migration of people from rural to urban areas (Colwell, et al., 2011). This has been described in certain developing countries, where governments may be unable to carry out necessary surveillance and vector control programs due to economic limitations and significant shifts in population dynamics. Poverty itself was found to be strongly correlated to the rise in tick-borne encephalitis (TBE) cases in Eastern Europe after the fall of the Soviet Union (Colwell, et al., 2011). Ultimately it is thought that changed land usage, reduced pesticide use, increased unemployment and poverty may all result in the increased exposure levels of humans to vector-based diseases.

Studying the effects of climate and environmental change on the epidemiology of *B. miyamotoi* allows us to make predictions for the future of this emerging pathogen. It is predicted that there will be a further shift in distribution of *I. ricinus* and *I. scapularis* if their seasonality continues to be affected by climate and environmental changes (Bede-Fazekas & Trájer, 2019; Li, et al., 2016). Currently in the *Ixodes* tick – *Borrelia* transmission lifecycle there is bimodal tick activity seen but with a unimodal lyme borreliosis distribution. It is thought that lyme borreliosis activity will become bimodal in the future, with a long summer pause and a shifted spring maximum. Overall it is predicted that the lyme borreliosis season will increase in length and the bimodality of *I. ricinus* will be more pronounced (Bede-Fazekas & Trájer, 2019; Li, et al., 2016). Although there have been no direct predictions regarding the shifting seasonality of

B. miyamotoi, it is thought that its distribution will behave in a similar way to those *Borrelia* spirochetes causing lyme disease.

The predicted shifting of seasonality and increased duration of tick-pathogen activity is a cause for great concern regarding the transmission of all tick-borne pathogens and requires further research so we can be better prepared to tackle these increasing exposure risks.

2.3 Public health significance

The first human case of infection by *B. miyamotoi* was confirmed in Russia in 2011 (Platonov, et al., 2011). Prior to this it was assumed that this spirochete had no clinical significance (Krause, et al., 2015). Confirmed cases have now been described in Russia, the USA, the Netherlands, Germany and Japan. The precise geographical distribution of human infections with *B. miyamotoi* is not currently known, however it is assumed to be very similar to that of *B. burgdorferi* s.l. It must be remembered that the infection of tick vectors with these spirochetes will have a much broader distribution than that of confirmed cases of human infection (Krause, et al., 2015). It is thought that all three genotypes of *B. miyamotoi* are pathogenic to humans, however the full extent of its clinical significance is still largely unknown and requires further research (Siński, et al., 2016)

An acute infection with *B. miyamotoi* is thought to be more severe and with a greater number of clinical signs than an infection with *B. burgdorferi* s.l (Platonov, et al., 2011).

In immunocompetent patients an infection with *B. miyamotoi* causes an influenza-like illness with a fever of up to 40 °C (Krause, et al., 2015). It presents as a systemic illness with malaise and, if left untreated, with a relapsing fever (Hovius, et al., 2013). In one study up to 11% of patients were recorded to have a relapsing fever, however these patients were treated with anti-microbial drugs which may have prevented other cases of relapse. It is thought that infected patients left without therapeutic intervention would present with relapsing fever in a significantly higher proportion (Krause, et al., 2018). It is not yet clear if immunocompetent patients would develop neurological signs if left untreated.

Additional clinical signs include thrombocytopenia, leukopenia, and elevated transaminases (Wagemakers, et al., 2015). Erythema migrans has also been described in patients, with one study showing up to 9% of patients with this skin symptom. However, it is widely agreed that this is due to co-infection with *B. burgdorferi* s.l (Platonov, et al., 2011). Further research is needed to fully understand the similarities and differences in clinical signs between *B. miyamotoi* infection and other TBP infections. It has been found that there are differences in clinical signs seen in patients with *B. miyamotoi* infection in the USA and Europe (Wagemakers, et al., 2015). Patients in the USA were more commonly co-infected by both *B. miyamotoi* and *B. burgdorferi* spirochetes, with several overlapping clinical signs. In contrast, patients with *B. miyamotoi* infections in Europe commonly present with similar symptoms to infections by tick-borne encephalitis virus, human granulocytic anaplasmosis, babesiosis and infection from several Rickettsia species (Jahfari, et al., 2014; Krause, et al., 2015). This is outlined in Table 2.

Table 2: Tick-borne diseases that can be accompanied by fever (Wagemakers, et al., 2015).

Disease	**Pathogen**	**Tick vector**	**Global spread**	**Common presentation and laboratory findings**
Borrelia miyamotoi disease	*Borrelia miyamotoi*	*Ixodes* spp.	North America, Europe, Asia	(Relapsing) non-specific febrile illness, leukopenia, thrombocytopenia, elevated AST/ALT
TBRF (Tick-borne relapsing fever)	*Borrelia crocidurae, Borrelia duttonii, Borrelia hermsii, Borrelia persica, Borrelia parkeri, Borrelia turicatae*	*Ornithodoros* spp.	North America, Africa, Asia, Europe	(Relapsing) non-specific febrile illness, confusion, photophobia, eye pain, rash, abdominal pain, hepatosplenomegaly, jaundice, thrombocytopenia, anaemia
HGA (Human granulocytic anaplasmosis)	*Anaplasma phagocytophilum*	*Ixodes* spp.	North America, Europe, Asia	Non-specific febrile illness, leukopenia, thrombocytopenia, elevated AST/ALT
Lyme borreliosis	*Borrelia burgdorferi* s.l.	*Ixodes* spp.	North America, Europe, Asia	EM, non-specific symptoms with or without fever. Disseminated disease: multiple EM, arthritis, meningoradiculitis, myocarditis, ACA, *Borrelia* lymphocytoma. The presentation differs between Eurasia and the USA.
Rickettsioses	*Rickettsia conorii, Rickettsia rickettsii, Rickettsia africae, Rickettsia slovaca, etc.*	depends on *Rickettsia* species	Worldwide	depends on species. Mostly eschar, maculopapular rash, lymphadenopathy, non-specific febrile illness
Babesiosis	*Babesia microti, Babesia divergens*	*Ixodes scapularis, Ixodes ricinus*	North America, Europe	Non-specific febrile illness, hepato-/splenomegaly, jaundice, petechiae, ecchymosis, hemolytic anaemia, leukopenia, thrombocytopenia, elevated AST/ALT
Tick-borne encephalitis (TBE)	TBE virus (Flavivirus)	*I. ricinus, Ixodes persulcatus*	Europe, Asia	Non-specific febrile illness followed by meningo-encephalitis, myelitis, radiculitis

In rare cases immunocompromised patients infected with *B. miyamotoi* have been found to develop a form of neuroborreliosis similar to that caused by *B. burgdorferi* s.l. infection (Boden, et al., 2016). This presents as a degenerative disease with slow cognitive processing, memory loss and altered gait (Hovius, et al., 2013). Cases of meningoencephalitis have been recorded in the USA and Netherlands in patients previously undergoing chemotherapy for non-Hodgkin lymphoma (Krause, et al., 2018). These patients were treated with a combination of different drugs, one of which was "Rituximab". The growing indications for the use of this chemotherapeutic agent combined with the increasing infection rates of *Ixodes* ticks with *B. miyamotoi* mean that the significance of immunocompromised patients developing neurological signs is becoming greater (Boden, et al., 2016).

Regarding the public health significance of *B. miyamotoi* infection, the key question to be asked is "Who is at risk?". Growing numbers of people are bitten by ticks each year in the northern hemisphere and so this question is becoming increasingly important (Wagemakers, et al., 2015). As previously mentioned, those living in areas with high *Ixodes* tick densities are naturally at a higher risk of exposure to tick-borne pathogens. However, we must also take into account how people interact with their environment. Forestry workers are one such group that have a higher exposure rate to ticks than the general public, as was found in research carried out in the Netherlands by Wagemakers et al in 2017. Others at a higher risk of exposure include hunters, hikers, campers, and those taking part in other outdoor leisure activities in *B. miyamotoi* infected areas. As global temperatures rise, more people partake in these activities, further highlighting the immediate need for improved education of the general public regarding tick-bite prevention and surveillance. *B. miyamotoi* is found in very high rates in tick larvae, which due to their small size are hard to detect when they are feeding on the body (Wagemakers, et al., 2017). Tick bite awareness is discussed in Chapter 2.5 highlighting the importance of self-surveillance and attitudes towards prevention and control of tick bites.

Immunocompromised patients are among those at higher risk with *B. miyamotoi* infection (Krause, et al., 2018). Several studies have highlighted the specific risk of those treated for non-Hodgkin lymphoma with certain therapeutic agents as being at an increased risk of neuroborreliosis upon infection (Boden, et al., 2016). This increased risk of neuroborreliosis is also seen in immunocompromised patients infected with those *B. burgdorferi* s.l. spirochetes causing lyme disease. In 2016 Sinski et al published an article showing that several forestry workers in the Netherlands that were initially suspected of having human granulocytic anaplasmosis infection were in fact infected by *B. miyamotoi* instead. It was thought they were misdiagnosed due to the similarities in clinical signs between the two diseases as shown in Table 2.

These findings highlight the huge importance of up-to-date tick and TBP density awareness by physicians in endemic areas. They must have a good knowledge of the broad spectrum of presenting clinical signs from these infections and must be aware of their potential as differential diagnoses for unexplained neurological symptoms.

2.4 Detection, diagnosis and treatment methods

As previously discussed, *B. miyamotoi* was detected for the first time in 1995 in *I. persulcatus* ticks, however it was not thought to be clinically significant until 2011 when it was confirmed to cause infection in humans. Since its public health significance became more apparent there has been a marked increase in research aimed at creating simple tests to detect and diagnose infection by this spirochete.

As it currently stands, *B. miyamotoi* can be detected in a number of ways: microscopic examination of a blood smear can reveal spirochetemia, real time PCR (RT-PCR), serum antibody testing, in vitro cultivation, and isolation by inoculation and propagation into experimental animals.

In this chapter I will outline the timeline of research into the detection and diagnostic methods developed over the past 9 years as this pathogen has emerged in the northern hemisphere.

Plantonov et al were responsible in 2011 for confirming the first human case of infection by *B. miyamotoi*. They did this using a set protocol to distinguish this spirochete from *B. garinii* and *B. burgdorferi* s.l. Diagnosis of *B. miyamotoi* infection required a confirmed tick bite, clinical signs from a set list and lab confirmation of the spirochete. This evidence was found using species-specific DNA amplification by PCR and by detection of serum anti-*B. miyamotoi* antibodies using ELISA. It was distinguished from *B. garinii*, which required evidence of a tick bite, erythema migrans or influenza-like symptoms and lab confirmation, and from *B. burgdorferi* s.l. which required evidence of Erythema migrans or influenza-like symptoms and a 4-fold increase in serum anti-*B. burgdorferi* s.l. antibodies (Platonov, et al., 2011).

In 2012 it was thought that many cases of *B. miyamotoi* were still being underreported due to ELISA cross-reactions with *B. burgdorferi* s.l. spirochetes. A study by Geller et al was the first to distinguish 2 different genotypes of *B. miyamotoi* from *I. persulcatus* and *I. ricinus* ticks found in Estonia. The European genotype was confirmed in *I. ricinus* ticks only, whereas the Asian genotype was confirmed in both *Ixodes* species. However, at this stage human disease from *B. miyamotoi* had only been reported from infection by the Asian genotype (Geller, et al., 2012).

Borrelia miyamotoi was cultivated for the first time in 2014 by Wagemakers et al. The spirochete was inoculated onto a specialised medium and used standard culture methods. DNA samples extracted from *I. scapularis* ticks in the USA were propagated by inoculation into

SCID (severe combined immuno-deficient) mice. Infected plasma from the mouse was then inoculated onto a modified Kelly-Pettenkofer (MKP) medium with 10% added fetal calf serum. This simple but effective culture method was the starting point from which in vitro research into *B. miyamotoi* could expand. It was also discovered that this spirochete showed serum resistance to human complement, which is a key factor when researching the pathogenesis of relapsing fever caused by this infection (Wagemakers, et al., 2014).

A new investigative tool allowing a high through-put screening of TBPs used for the first time in 2014 paved the way for more efficient monitoring of emerging diseases. This method allowed detection of a wide range of TBPs, including rarer ones such as *B. miyamotoi* and *Bartonella henslae*. DNA samples extracted from *Ixodes* nymphs in France, Denmark and the Netherlands were screened using a microfluidic system that performs RT-PCR on very low volumes of DNA. Samples of only a few microlitres are needed for this system which is a key advantage over traditional PCR methods as only very small volumes of DNA can be extracted from nymphs. Due to the larger quantity of DNA needed in conventional amplification-based assays, only a limited number of TBPs can be tested using traditional methods. This fast and efficient screening method marked a major improvement in epidemiological studies (Michelet, et al., 2014).

The importance of confirmation of an etiologic diagnosis of *B. miyamotoi* infection by laboratory evidence was emphasised in 2 studies by Wagemakers et al and Krause et al in 2015. They discussed the lack of a clinically validated test for this spirochete and the limitations of diagnosis from clinical signs alone. As previously mentioned, there are a wide range of clinical signs associated with relapsing fever from *B. miyamotoi* infection. Many of these symptoms also occur during infection with other pathogens carried by the same *Ixodes* species, such as lyme disease, babesiosis, HGA and TBEV. Therefore, clinical signs can only be used to support a diagnosis of *B. miyamotoi* that has been confirmed by other methods (Krause, et al., 2015; Wagemakers, et al., 2015).

Wagemakers et al also discussed the usefulness of assessing the seroprevalence of anti-*Borrelia miyamotoi* antibodies in humans to provide information about past infections. A high seroprevalence noted in forestry workers in the Netherlands and in humans initially suspected of HGA infection was found. A significant number of those who tested positive for these antibodies were found to be co-infected with *B. burgdorferi* s.l. (Wagemakers, et al., 2015). *Borrelia miyamotoi* and *B. burgdorferi* s.l. have many antigenic similarities which help to explain the cross-reactions between *B. miyamotoi* and *B. burgdorferi* s.l. that occur in serum antibody assays. These 2 spirochetes have been found to share 4 common antigens out of the 10 specified in standard Western Blot criteria used for lyme disease testing. Krause et al focused on methods to distinguish between *B. miyamotoi* and *B. burgdorferi* s.l. in their study in 2015. It was discovered that *B. miyamotoi* and other members of the relapsing fever group express a particular gene, the GLpQ (Glycerophosphodiester phosphodiesterase, periplasmic) gene, which is not expressed by the lyme borreliosis group. Antibodies against this GLpQ antigen are tested for and can be used to aid in the diagnosis of relapsing fever during acute and convalescent stages of infection. RT-PCR based on the same primers but different probes for the 16S ribosomal RNA gene was also carried out to distinguish relapsing fever spirochetes from *B. burgdorferi* s.l (Krause, et al., 2015).

The usefulness of cerebrospinal fluid (CSF) and serum samples for detection of relapsing fever spirochetes was discussed in 2016 by Boden et al. It was found that microscopy and PCR of these samples provides an easier detection method that is more sensitive for *B. miyamotoi* than for *B. burgdorferi* s.l spirochetes (Boden, et al., 2016).

In 2017 a Russian study highlighted the high levels of relapsing fever thought to be currently undiagnosed or misdiagnosed in humans. A lack of awareness by physicians in endemic areas combined with a lack of routine diagnostic tests and the antigenic similarities between *B. miyamotoi* and *B. burgdorferi* s.l. suggest that many cases of relapsing fever are likely misdiagnosed as lyme borreliosis. This study also discussed the relapses in clinical signs caused by *B. miyamotoi* infection and the role of inadequate antimicrobial therapy in these relapses (Wagemakers, et al., 2017).

The first successful isolation of *B. miyamotoi* from a human patient was carried out in 2017. Before this the spirochete had only been isolated *in vitro*. A centrifuged blood sample was used and the spirochete was cultured on MKP medium with added fetal calf serum. This method of detection takes much longer than PCR methods, however isolation of this spirochete directly from blood samples provides a useful tool for future research (Koetsveld, et al., 2017).

At this time a shift away from traditional PCR methods and towards serological testing was taking place. The sensitivity of culturing and PCR methods in detection of *B. miyamotoi* are limited by the level of spirochetemia present in the patient. A lack of or low level of spirochetes in the blood may lead to inconclusive or false negative results from these methods. Serum antibody testing is more reliable due to its lack of reliance on a patient's spirochetemia

(Koetsveld, et al., 2017). Fitting with this trend, in 2018 a study by Koetsveld et al aimed to bring serological diagnostic testing into the forefront of *B. miyamotoi* detection. It focused on the detection of anti-GLpQ antibodies and variable major proteins present in serum of infected patients. This anti-GLpQ antibody testing had previously been carried out at an experimental level but never significantly clinically validated. The outcome of this study was the confirmation of the strong potential of using these seromarkers in the future to create a quick serological diagnostic test to be used in clinical practice (Koetsveld, et al., 2018). A complication faced in this serodiagnostic testing is the multiple cross-reactions occurring between the different *Borrelia* species. In order for a future diagnostic test for *B. miyamotoi* to be clinically relevant it must be able to differentiate between all relapsing fever spirochetes (Krause, et al., 2018).

A widely used serodiagnostic test for *B. burgdorferi* s.l. uses an enzyme immunoassay based on the C6 peptide of a particular spirochete protein. A study by Koetsveld et al in 2019 looked at the potential cross-reactivity of *B. miyamotoi* spirochetes in this diagnostic test. It showed that cross-reactive antibodies are also produced against this C6 peptide during a *B. miyamotoi* infection. This further emphasised the importance of using a wide range of diagnostic tools during the detection of *Borrelia* spirochetes in order to avoid the complications of antigen cross-reactivity (Koetsveld, et al., 2019). In order to overcome these complications Tokarz et al published a study describing methods of identifying new linear targets for the serodiagnosis of *B. miyamotoi*. These targets consist of a panel of linear peptides, including those with the greatest potential for differential diagnosis of borrelial spirochetes. The creation of a "TBP-serochip" that is able to differentiate between the different borrelial spirochetes could be the answer to the longstanding diagnostic barrier created by their antigenic similarities (Tokarz, et al., 2020).

The current information available regarding treatment protocols for *B. miyamotoi* infection is based solely on case reports, as there are no therapeutic trials or experimental data published on antibiotic susceptibility. This means clinicians do not have data regarding optimal antibiotic choices, dosages or treatment durations for *B. miyamotoi* infection (Krause, et al., 2015). Therefore, current treatment protocols are largely the same as those used for other tick-borne diseases accompanied by fever, such as lyme borreliosis and tick-borne fever, as shown in Table 3 (Wagemakers, et al., 2015).

Russian patients first diagnosed and treated in 2011 were given one of two different treatment regimes. A single course of either doxycycline or ceftriaxone was found to clear the infection successfully (Platonov, et al., 2011). Since then, tetracyclines and beta-lactams have been confirmed as the best choice of antibiotic for treating relapsing fever from *B. miyamotoi*. A 2 week course of doxycycline has become the most commonly prescribed treatment regime for an uncomplicated *B. miyamotoi* infection. For young children, pregnant or nursing women to whom a tetracycline is not a safe option, amoxicillin or cefuroxime may be used. For patients with an infection complicated by meningoencephalitis, a 2 week course of ceftriaxone or a 4 week course of penicillin G is used. Other antibiotic groups such as macrolides or first generation cephalosporins may clear this infection but they are considered less effective than tetracyclines or beta-lactams. Due to the confirmed resistance of other borrelial species to fluoroquinolones and aminoglycosides, it is assumed that these antibiotics will also be ineffective in an infection by *B. miyamotoi* (Krause, et al., 2015).

Table 3. Tick-borne diseases that can be accompanied by fever (Wagemakers, et al., 2015)

Disease	**Pathogen**	**Preferred treatment**
Borrelia miyamotoi disease	*Borrelia miyamotoi*	doxycycline / ceftriaxone
TBRF[1]	*Borrelia crocidurae, B. duttonii, B. hermsii, B. persica, B. parkeri, B. turicatae*	doxycycline / ceftriaxone
HGA[2]	*A. phagocytophilum*	doxycycline
HME[3]	*Ehrlichia chaffeensis*	doxycycline
Lyme borreliosis	*B. burgdorferi* s.l. [4]	doxycycline / ceftriaxone

[1] tick-borne relapsing fever
[2] human granulocytic anaplasmosis
[3] human monocytic ehrlichiosis
[4] sensu lato

When treating infections by tick-borne pathogens, clinicians must be aware of the potential side effects of antimicrobial therapy. One such adverse reaction known as the “Jarisch-Herxheimer” reaction has been described in up to 15% of patients treated for *B. miyamotoi* infection and up to 50% of those treated for tick-borne fever. This reaction manifests as a set of mild to serious clinical signs, including a fever of >40 °C, diaphoresis, chills and in some cases a shock-like state with life-threatening hypotension. Patient monitoring and supportive therapy must be in place in order to mitigate the risk of serious side effects from antimicrobial therapy (Krause, et al., 2015).

2.5 Prevention and control strategies

When discussing the prevention and control of any tick-borne disease, there are 2 major strategies that must be followed. The first is the up-to-date epidemiological surveillance of the distribution and emergence of ticks and tick-borne pathogens. Colwell et al emphasised this in their 2011 study when they wrote “Awareness is the first step towards early diagnosis, better management, and more efficient prevention of TBDs”. The second strategy is the education of both physicians and the general public, in endemic and non-endemic areas, of the significance of tick-borne diseases and the personal protective measures that can be taken against them.

Continuous large-scale epidemiological surveillance of *Ixodes ricinus – persulcatus* ticks and their pathogens is crucial in the control and prevention of human infection with borrelial spirochetes. As discussed in the previous chapter, techniques for mass screening of TBPs are already available and should form an integral part of emerging disease prevention strategies carried out by national health services (Michelet, et al., 2014). It also is recommended that systematic entomological surveys should be carried out in vector-free areas that are at risk of introduction of ticks by humans and animals (Colwell, et al., 2011).

Education of both physicians and the general public is crucial in the prevention and early detection of tick bites and TBDs. Physicians in endemic areas must be continually aware of fluctuating trends in tick densities and their pathogens (Colwell, et al., 2011). They must also be able to differentiate the clinical signs of TBPs and be aware of the dangers and potential for misdiagnoses of TBDs (Hovius, et al., 2007). Clinicians in non-endemic areas must also be aware of these dangers and the risk faced when humans travel abroad (Boden, et al., 2016).

As the public health significance of TBDs has become more apparent in recent years, there have been increasing numbers of campaigns aiming to educate the public about the dangers they pose. However, it should be noted that knowledge of ticks and their association with danger is not a new phenomenon, but something that people have been aware of for thousands of years. A papyrus scroll found in the 16th Century BC contains a possible reference to a “tick fever,” and ticks were later described in writings by Aristotle. The prehistoric presence of ticks was uncovered in artefacts found dating back to 400-1300 AD and their association with danger was described again in the 12th Century AD in the form of a “tick paralysis” (Heyman, et al., 2010).

In recent years, as more research into TBPs and their diseases has been published, greater focus has been placed by health services on the education of members of the public who fall into high risk categories on effective prevention and control measures to avoid infection (Heyman, et al., 2010). Those falling into these high risk categories include forestry workers, hunters, hikers, campers, and people who are immuno-compromised (Boden, et al., 2016; Jahfari, et al., 2014; Szekeres, et al., 2015). These groups must be educated on the importance of using protective clothing when in environments with a high tick burden. The basis of this is the use of clothing or footwear that prevents excessive exposure of skin, such as long boots or gaiters (Heyman, et al., 2010).

Self-surveillance for tick bites is another key area where people can mitigate the risks of tick-borne diseases. Those in high risk categories should be aware of the need to check themselves thoroughly after being in environments with high tick burdens. This is especially important in the defence against relapsing fever from *B. miyamotoi* due to the larvae of the *Ixodes ricinus* – persulcatus complex often carrying the highest infective load (Wagemakers, et al., 2015). It is not yet completely clear how important the role of larvae is in transmission of the spirochete to humans, however they have not been ruled out as a source of infection. Larvae are very hard to detect when they are attached to the skin due to their small size thus highlighting the need for thorough checking after possible exposure. Proper removal of ticks is also an important factor to consider. If removed incorrectly fragments of the tick, such as the head, can remain in the skin potentially causing complications (Heyman, et al., 2010).

Members of the public in endemic areas should also be educated about the most common clinical signs of infection by TBPs. They should know what symptoms to look out for and when to seek medical advice as early medical intervention is an important factor in the prognosis of *B. miyamotoi* infections (Heyman, et al., 2010).

An awareness of the bimodality of ticks is also important, with the spring and autumn peaks requiring people to take more precautions when engaging with their environments (Crowder, et al., 2014). As previously discussed, the type of habitat also impacts tick densities, with high levels often associated with forested areas (Jahfari, et al., 2014). Tick burdens in deciduous and coniferous forests has been described in several northern European countries, such as Germany, Hungary, the Netherlands and Poland (Cochez, et al., 2015; Szekeres, et al., 2015; Wagemakers, et al., 2017). Exophilic ticks, such as *Ixodes* ticks, await their hosts on vegetation and due to their "generalist feeding behaviour" they have adapted to a large range of hosts found within these habitats (Radolf, et al., 2012). It should not be forgotten that ticks are also present in urban environments where they feed on small mammal reservoir hosts, as described by Szekeres in 2017. It is this adaptive feeding strategy that leads to humans becoming incidental hosts for

Ixodes ticks and leading to their infection by TBPs in both natural and urban environments (Radolf, et al., 2012).

As discussed in Chapter 2.2, environmental changes such as climate change, habitat fragmentation, and other forms of landscape modification impact the distribution and density of *B. miyamotoi*-infected ticks in northern latitudes (Bede-Fazekas & Trájer, 2019; Krause, et al., 2015). Continuous in-depth surveillance of environmental and climate changes is key to effectively monitoring the alterations in ecology and behaviour of ticks (Colwell, et al., 2011).

Deer species play an important role in the transmission cycle of *B. miyamotoi*-infected ticks within several countries in the northern hemisphere. It must be remembered that deer do not act as simple reservoir hosts for *B. miyamotoi* but are in fact maintenance hosts. Therefore they are responsible for diluting the infection levels of *B. miyamotoi* within ticks in a certain area, while at the same time increasing the densities of ticks themselves (Heyman, et al., 2010).

Deer species are targeted by several direct management practices such as culling, the use of exclusive fencing, and translocation. A study by Li et al in 2014 looked at the effects of these practices on the spatial dynamics of the *I. ricinus* tick. It predicted that managing deer populations is not always an effective way to control tick populations. Four scenarios were chosen and studied that were considered relevant to current and future management practices in Europe. These scenarios were:

1. Reducing local deer densities by hunting;

2. Controlling grazing intensity of deer in grasslands by using exclusive fencing;

3. Translocation of deer by removing and then reintroducing them to new areas;

4. Controlling the migration of deer between woodland patches by limiting the effects of human disturbance (such as hunting, and tourism) (Li, et al., 2014).

The results of the study supported the hypothesis that tick populations cannot always be effectively controlled by managing deer populations. Hunting deer was found to be effective in some cases in reducing tick populations, but not in areas where woodland patches were well connected to each other, as deer were found to migrate between these areas. Exclusive fencing was found to reduce tick densities in the excluded grasslands but tick levels increased in the surrounding woodlands where deer were more concentrated. Local extinction of deer from certain areas was found to reduce tick densities, however it did not eliminate them entirely as these areas still harboured small mammals for the tick to feed on. Reduction of human disturbance meant that displacement of deer between woodland patches was lower, thereby reducing tick densities and avoiding specific areas with very high densities (Li, et al., 2014).

This study provides an important insight into the relationship between deer and tick densities, and how management practices can alter tick densities in ways we might not expect. This could be significant for future management strategies considered for controlling emerging tick-borne pathogens harboured by deer.

There is currently no vaccine available to protect against human diseases caused by borrelial spirochetes such as *B. miyamotoi*. A human vaccine against *B. burgdorferi* s.l. was created in 1998 but subsequently withdrawn in 2002 due to lack of efficacy as it was found that antibody titres did not persist in high enough levels after booster vaccinations (Heyman, et al., 2010). A canine vaccine against *B. burgdorferi* s.l. is currently available and recommended by some clinicians for dogs living in endemic areas, however there are questions to be answered regarding its safety and efficacy. A greater emphasis on tick bite prevention is thought to be a better management strategy for both humans and companion animals (Schuijt, et al., 2011). The greatest obstacle in the development of an effective vaccine is the limited range of antigenic targets available against borrelial spirochetes (Heyman, et al., 2010). There is the potential to use both borrelial and tick antigens as vaccine targets, however this is an area that requires a lot of future research (Schuijt, et al., 2011).

3. Conclusion

Borrelia miyamotoi is a relapsing fever spirochete prevalent in the northern hemisphere where it is carried by the same *Ixodes* ticks that transmit lyme borreliosis. These exophilic ticks have a wide host range due to their adaptive feeding strategies, with humans acting as incidental hosts in endemic areas. This spirochete is not as established in these ticks as *B. burgdorferi* s.l., however it poses a significant public health risk and its importance as an emerging tick-borne disease must not be overlooked.

Since its discovery in 1995 and its confirmed public health significance in 2011 *B. miyamotoi* has been increasingly detected in northern climates. It is largely agreed that misdetection and misdiagnosis of this spirochete has led to deceptively low cases of reported infections over the past 25 years. Improvements in diagnostic tests for *B. miyamotoi* have been a major focus of research over the past 9 years. However, as it stands there is still no simple diagnostic test available that can differentiate between the many different borrelial spirochetes.

As discussed in this literature review there are several factors influencing the distribution and density of *B. miyamotoi*-infected ticks in the northern hemisphere. Significant environmental changes such as climate change and habitat disturbance are some of the most important drivers of the emergence of tick-borne diseases. These shifting distribution and density patterns must be closely monitored by epidemiologists to allow predictions to be made regarding the spread of this spirochete.

There are many areas requiring further research to better understand the transmission cycle of *B. miyamotoi* and the preventative measures that can be taken to avoid human infection from this spirochete. An effective and safe vaccine against specific borrelial species is one such measure that scientists have so far failed to create. With the lack of vaccine available, tick-bite surveillance and education of the public remains at the forefront of the prevention of *B. miyamotoi* infection in humans. Exposure levels to *B. miyamotoi*-infected ticks are largely determined by the engagement of people with their environment in endemic areas. Therefore, public health campaigns focusing on the education of the high risk individuals are key to the prevention of tick bites and tick-borne diseases. The importance of this infection must also be highlighted to physicians in endemic areas, where it must be included in their differential diagnoses for cases of non-specific relapsing fever and neurological symptoms in high risk groups.

In this literature review I have discussed the main themes that research studies have focused on over the past 25 years into relapsing fever from *B. miyamotoi.* I have also highlighted the key areas that require further research so that the threat from this spirochete may be better understood and ultimately reduced.

4. Összefoglaló

Borrelia miyamotoi egy kullancsok által terjesztett spirohéta baktérium, amelynek növekvő jelentősége van az északi félgömbön. Az *Ixodes ricinus-persulcatus* fajkomplex kullancsai ennek a kórokozónak a terjesztői, amelyek a Lyme-kórt okozó *B. burgdorferi* sensu lato-nak a vektora is. Japánban 1995-ben *Ixodes persulcatus* kullancsokból mutatták ki a kórokozó. Úgy gondolják, hogy a *B. miyamotoi* fertőzés korábban tévesen Lyme-borreliózisként volt diagnosztizálva és kezelve is. Az első bizonyított humán fertőzés, amely 2011-ben volt, után a járványtani szakemberek felismerték a potenciális veszélyeket, amit ez a kórokozó jelenthet.

A járványtani vizsgálatokkal és a diagnosztikai módszerek fejlődésével az utóbbi 10 évben a fertőzések száma gyorsan növekedett. A változó környezeti feltételek, mint a klímaváltozás fontos szerepet játszik a kórokozó terjedésében a világon. Mint más kullancsok által terjesztett kórokozók esetében is a megelőző intézkedésekben a legnagyobb szerepe van a kullancscsípés megakadályozásának és annak, hogy a köztudatban legyen a fertőzés lehetősége, és hogy lehet elkerülni azt. A legfontosabb kérdés ezután is, hogy lehetne az antigén különbségeket a *B. miyamotoi* és a *B. burgdorferi* s.l. kórokozók között hasznosítani egy gyors egyszerű a két fajt elkülönítő diagnosztikai módszerben. Ezen túl kérdés még a különböző *Borrelia* kórokozók elleni, jövőbeni vakcina is.

Abstract

Borrelia miyamotoi is a tick-borne spirochete of increasing importance in the northern hemisphere. It is transmitted by the same *Ixodes ricinus-persulcatus* complex that is responsible for the transmission of *B. burgdorferi* sensu lato (s.l.). First discovered in 1995 in *Ixodes persulcatus* ticks in Japan, it is thought that it was commonly mis-detected as *B. burgdorferi* s.l. prior to this. However, it was not until the first confirmed human case of infection by *B. miyamotoi* in 2011, that epidemiologists became aware of the potential public health significance of this spirochete. Improvements in diagnostic testing and epidemiological surveillance methods have seen a rapid rise in infection rates over the past 10 years. The significance of environmental factors, such as climate change, are proving themselves key in the emergence of this tick-borne pathogen throughout the world. As with most other tick-borne diseases, the main preventative measures against infection remain focused on tick-bite prevention and increasing public awareness of the dangers tick-borne pathogens. Key questions still remain regarding the antigenic differences between *B. miyamotoi* and *B. burgdorferi* s.l. spirochetes and how this can be harnessed in the production of simple diagnostic tests able to differentiate between them. It also poses questions regarding future production of vaccines against borrelial spirochetes.

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