Tick-borne diseases under the radar in the North Sea Region

Quarsten, Hanne; Henningsson, Anna; Krogfelt, Karen; Strube, Christina; Wennerås, Christine; Mavin, Sally

Published in:
Ticks and Tick-Borne Diseases

DOI:
10.1016/j.ttbdis.2023.102185

Publication date:
2023

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Quarsten, H., Henningsson, A., Krogfelt, K., Strube, C., Wennerås, C., & Mavin, S. (2023). Tick-borne diseases under the radar in the North Sea Region. *Ticks and Tick-Borne Diseases*, *14*(4), Article 102185. https://doi.org/10.1016/j.ttbdis.2023.102185

Ticks and Tick-borne Diseases 14 (2023) 102185

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Review Article

Tick-borne diseases under the radar in the North Sea Region

Hanne Quarsten [a,*], Anna Henningsson [b,c], Karen A. Krogfelt [d], Christina Strube [e], Christine Wennerås [f], Sally Mavin [g]

[a] Department of Medical Microbiology, Sørlandet Hospital, Kristiansand 4615, Norway
[b] Department of Clinical Microbiology in Jönköping, County Hospital Ryhov, Jönköping 55185, Sweden
[c] Department of Biomedical and Clinical Sciences, Faculty of Medicine, Linköping University, Linköping 58183, Sweden
[d] Department of Science and Environment, University of Roskilde, Roskilde 4000, Denmark
[e] Institute for Parasitology, Centre for Infection Medicine, University of Veterinary Medicine Hannover, Hanover 30559, Germany
[f] Department of Clinical Microbiology, Sahlgrenska University Hospital, Gothenburg 413 46, Sweden
[g] Scottish Lyme Disease and Tick-Borne Infections Reference Laboratory, Raigmore Hospital, Inverness IV2 3BW, United Kingdom

ARTICLE INFO

Keywords:
Neoehrlichia mikurensis
Borrelia miyamotoi
Anaplasma phagocytophilum
Rickettsia
Babesia
Rituximab

ABSTRACT

The impact of tick-borne diseases caused by pathogens such as *Anaplasma phagocytophilum, Neoehrlichia mikurensis, Borrelia miyamotoi, Rickettsia helvetica* and *Babesia* species on public health is largely unknown.

Data on the prevalence of these pathogens in *Ixodes ricinus* ticks from seven countries within the North Sea Region in Europe as well as the types and availability of diagnostic tests and the main clinical features of their corresponding diseases is reported and discussed. Raised awareness is needed to discover cases of these under-recognized types of tick-borne disease, which should provide valuable insights into these diseases and their clinical significance.

1. Background

Tick-borne diseases present a growing health concern for humans worldwide. *Ixodes ricinus* ticks are the main vectors of pathogens causing human tick-borne infections in Europe. The most familiar infectious agents include *Borrelia burgdorferi* sensu lato and tick-borne encephalitis virus, which may cause Lyme borreliosis and tick-borne encephalitis, respectively, for which European centre for Disease Prevention and Control guidelines are available (ECDC, 2023). *I. ricinus* may also transmit lesser-known pathogens, such as *Anaplasma phagocytophilum, Neoehrlichia mikurensis, Borrelia miyamotoi, Rickettsia helvetica* and *Babesia* species, all of which can cause human disease rarely diagnosed in Europe (Sprong et al., 2018). The impact of these pathogens on public health is unclear. Clinicians' lack of experience on how these infections manifest and limited availability of proper diagnostic services jointly contribute to their likely underdiagnosis.

The goal of this work is to raise awareness of the under-recognized tick-borne diseases caused by *A. phagocytophilum* (anaplasmosis), *N. mikurensis* (neoehrlichiosis), *B. miyamotoi* (*B. miyamotoi* disease), *R. helvetica* (*R. helvetica* infection) and *Babesia* spp. (babesiosis) and the laboratory testing methods currently available, thereby contributing to improved patient management and recognition of the varied manifestations of these illnesses. This work was performed as part of NorthTick, a project co-funded by the European Union through the European Regional Development Fund and the North Sea Region Program. The geographic study area encompassed seven countries (Belgium, Denmark, Germany, Netherlands, Norway, Scotland and Sweden) participating in the project (Fig. 1).

2. Pathogen prevalence and infection risk

The probability of contracting a tick-borne disease from a tick bite depends on several factors, such as the risk of being bitten by a tick carrying a pathogen, the ability of the pathogen to be transmitted to the human host and its potency to cause symptoms and manifest as a disease (Sprong et al., 2018). The risk of developing disease versus an asymptomatic seroconversion also depends on the immune status of the host. Individuals with an impaired immunity, due to either primary immunodeficiency or immunosuppressive disease or treatment, are at higher risk of developing more severe symptoms than immunocompetent individuals.

Knowledge on how many individuals in Europe that have acquired a

* Corresponding author.
E-mail address: hanne.quarsten@sshf.no (H. Quarsten).

https://doi.org/10.1016/j.ttbdis.2023.102185
Received 3 March 2023; Received in revised form 18 April 2023; Accepted 18 April 2023
Available online 26 April 2023

Fig. 1. An overview of the countries within the North Sea Region, participating in the NorthTick project. Interreg North Sea Region gave the permission to use the map.

certain tick-borne disease is crucial for understanding the risk of infection and its impact on public health, but the number of infected individuals is generally not known due to insufficient investigation of cases and the lack of national surveillance and notification systems. Based on the scientific literature and authors' experiences, estimates of the total numbers of diagnosed European tick-borne diseases are indicated to be; ~300 anaplasmosis (Matei et al., 2019), >200 neoehrlichiosis (Höper et al., 2020), ~60 babesiosis (Hildebrandt et al., 2021), <10 *B. miyamotoi* disease (Hoornstra et al., 2022; Kubiak et al., 2021) and <10 *R. helvetica* infection cases (Nilsson, 2009; Nilsson et al., 1999a, 2010, 2011).

The infection rates of pathogens in *I. ricinus* ticks within defined geographical regions may also provide useful information on the risk of contracting a tick-borne diseases from a tick bite. Table 1 summarizes the prevalence rates of the less common tick pathogens in *I. ricinus* reported in the scientific literature, providing an overview of the pathogen distribution in the North Sea Region countries. It should be noted that the data is limited and potentially skewed due to factors as use of different methodologies for detection, small sample sizes (few ticks studied), and the reservoir, i.e., host animals for the various tick-borne pathogens in the areas where the ticks were collected (Sprong et al., 2018).

The overall prevalence rates of *A. phagocytophilum* and *B. miyamotoi* in *I. ricinus* seem to be quite similar in all seven North Sea Region countries. The rate of *A. phagocytophilum* differs greatly from site to site but the majority of studies report around 1–5% infected ticks (Blazejak et al., 2017; Coipan et al., 2013; Flattery et al., 2022; Franke et al., 2010; J. 2011; Galfsky et al., 2019; Gandy et al., 2022; Granquist et al., 2014; Guy et al., 1998; Hansford et al., 2015; Hartelt et al., 2004; Hauck et al., 2019; Henningsson et al., 2015; Heylen et al., 2016; Hildebrandt et al., 2010; A. 2011; Jahfari et al., 2014; Jensen et al., 2017; Karlsson and Andersson, 2016; Kjelland et al., 2018; Kjær et al., 2020; Klitgaard et al., 2019; Knoll et al., 2021; Lempereur et al., 2012; May and Strube, 2014; Michelet et al., 2014; Mysterud et al., 2013; Olsthoorn et al., 2021; Overzier et al., 2013a, 2013b; Quarsten et al., 2015; Rosef et al., 2009a, 2009b; Schicht et al., 2011; Schorn et al., 2011; Silaghi et al., 2008a, 2012a; Skarphédinsson et al., 2007; Soleng and Kjelland, 2013; Stigum et al., 2019; Takumi et al., 2021; Tappe and Strube, 2013; Tveten, 2014; Wallménius et al., 2012; Wielinga et al., 2006), whereas the rate of *B. miyamotoi* commonly is low and around 1% (Blazejak et al., 2018; Cochez et al., 2015; Cull et al., 2021; Eshoo et al., 2014; Fraenkel et al., 2002; Hansford et al., 2015; Heylen et al., 2016; Kjelland et al., 2015, 2018; Kjær et al., 2020; Klitgaard et al., 2019; Lambert et al., 2019; Layzell et al., 2018; Michelet et al., 2014; Olsthoorn et al., 2021; Page et al., 2018; Quarsten et al., 2015; Răileanu et al., 2020; Ruyts et al., 2018; Szekeres et al., 2017; Wagemakers et al., 2017). The tick infection rates seem to be more diverse for the other tick-borne pathogens. Most countries have a medium to high (up to 10–25%) prevalence of *N. mikurensis*, whereas a low or undetectable prevalence of *N. mikurensis* is reported in ticks from Belgium and United Kingdom (UK), respectively (Andersson et al., 2013; Coipan et al., 2013; Fertner et al., 2012; Galfsky et al., 2019; Hansford et al., 2015; Heylen et al., 2016; Jahfari et al., 2012; Jenkins et al., 2019; Kjelland et al., 2018; Kjær et al., 2020; Klitgaard et al., 2019; Larsson et al., 2018; Michelet et al., 2014; Olsthoorn et al., 2021; Pedersen et al., 2020; Richter and Matuschka, 2012; Silaghi et al., 2012b). The infection rate of *R. helvetica* appears to be very high (up to ≥ 25%) in Germany and the Netherlands and medium-high (10–25%) in Sweden, Denmark and Belgium, whereas in the UK it is below 10% and in Norway around 1% (Coipan et al., 2013; Eshoo et al., 2014; Franke et al., 2010; J. 2011; Galfsky et al., 2019; Hauck et al., 2019; Heylen et al., 2016; Hildebrandt et al., 2010; Hvidsten et al., 2020; Kantsø et al., 2010; Kjær et al., 2020; Klitgaard et al., 2019; Knoll et al., 2021; Lindblom et al., 2016; May and Strube, 2014; Michelet et al., 2014; Nilsson 1999b; Olsthoorn et al., 2021; Overzier 2013a; Quarsten et al., 2015; Schicht et al., 2011; Severinsson et al., 2010; Silaghi et al., 2008b, C. 2011; Skarphédinsson et al., 2007; Sprong et al., 2009; Svendsen et al., 2009; Tappe and Strube, 2013; Tijsse-Klasen et al., 2011; Wallménius et al., 2012). The percentage of ticks carrying *Babesia* spp. seems to be higher (5–10%) in Belgium and Germany than in Sweden and the Netherlands (up to 3–4.5%) and in Norway, Denmark and the UK with prevalence rates below 1% (Azagi et al., 2021; Coipan et al., 2013; Eshoo et al., 2014; Fertner et al., 2012; Franke et al., 2010; J. 2011; Galfsky et al., 2019; Hartelt et al., 2004; Hildebrandt et al., 2010; A. 2011; Jensen et al., 2017; Karlsson and Andersson, 2016; Kjær et al., 2020; Klitgaard et al., 2019; Lempereur et al., 2012; Michelet et al., 2014; Olsthoorn et al., 2021; Overzier 2013a; Sands et al., 2022;

Table 1
Reported pathogen prevalence in *Ixodes ricinus* ticks within the investigated North Sea Region countries.

	Pathogen prevalence (%)						
	Belgium	**Denmark**	**Germany**	**The Netherlands**	**Norway**	**UK**	**Sweden**
Anaplasma phagocytophilum	1.2–6.5	1–5 (0.4–24)	1–5 (0–25)	1–5 (0–11)	1–5 (0–19)	1–5 (0–20)	0.5–5 (0.5–15)
Neoehrlichia mikurensis	0.4–3	1–5 (0–13)	10–20 (8–27)	5–10 (0.4–16)	5–15 (0–25)	0	1–5 (0–11)
Borrelia miyamotoi	0.4–1.4	0–2.4	0–3.0	2.1–3.8	0.5–1.3	0–1	0–0.7
Rickettsia helvetica	17	5–10 (1–15)	5–20 (2–52)	5–30 (4.5–66)	1 (1–5)	0–6.5	5–10 (1.5–22)
***Babesia* spp**	8	0–1.5	0.4–11	0–4.5	<1	<1	0.2–3
Bab. divergens	Present*	Present	Present	Present	Present	Present	Present
Bab. venatorum	Present	Frequent	Present	Frequent	Present	Present	Frequent
Bab. microti	Present*	Not detected	Present	Present	Not detected	Present*	Sporadic

The range of the most commonly or all (when data is limited) reported pathogen prevalence rates in ticks are given, together with the spreading of all data (in parenthesis). Data was obtain by searching PubMed using combinations of the pathogen names and the country names together with *Ixodes ricinus*. Data on questing ticks was primarily included with a few exceptions (marked with *). *Only data from ticks collected from animals was available and presented (Abdullah et al., 2018; Lempereur et al., 2011). All other references are given in the main text.

Schorn et al., 2011; Silaghi et al., 2012b; Wielinga et al., 2009; Øines et al., 2012). *Babesia divergens* and *Babesia venatorum* are present in all countries with the latter species most frequently detected, whereas *Babesia microti* has not yet been detected in Denmark and Norway. However, there is no systematic tick-borne pathogen surveillance of ticks in the North Sea Region countries to date.

N. mikurensis and/or *R. helvetica* are the two pathogens that appear to infect the highest number of *I. ricinus* in many regions and if their transmission from tick to human is effective, there could be a high risk of being exposed to the bacteria when tick-bitten. The ability of both organisms to cause disease, however, seems to be very different since the numbers of diagnosed disease cases caused by *N. mikurensis* have increased successively since the first case reports were published in 2010, whereas published cases attributed to *R. helvetica* are scarce (Höper et al., 2020; Nilsson, 2009, 1999b, 2010, 2011; Wenneras, 2015).

3. Laboratory diagnosis of the under-recognized tick-borne diseases

Laboratory diagnosis of the under-recognized tick-borne diseases is a challenge. None of the pathogens involved is detected by the routine culture methods available in diagnostic microbiology laboratories. *A. phagocytophilum, N. mikurensis* and *R. helvetica* are intracellular bacteria that depend on cell lines for culture, and *B. miyamotoi* and the intraerythrocytic protozoan parasite *Babesia* are both fastidious and very difficult to culture without particular expertise. The three main categories of diagnostic methodologies are molecular (detection of pathogen DNA), serologic (detection of antibodies against the pathogen) or morphologic (microscopic examination of the patient's blood) analysis. The most common methods used for diagnosing the tick-borne diseases are listed in Table 2.

Direct detection of pathogen DNA by specific PCR or 16S (prokaryotic)/18S (eukaryotic)-based amplification and sequencing is generally useful in the early stage of the illness when higher amounts of the pathogen are present. PCR methods are established for all five pathogens, and they may be either species- or genus/group- (as for spotted fever group *Rickettsia*) specific. Commercially available kits exist for *A. phagocytophilum, B. miyamotoi, Rickettsia* spp. and *Babesia* spp., although many diagnostic laboratories use laboratory developed ("in-house") assays.

The 16S/18S-sequencing approach may be particularly advantageous in cases where the etiology is uncertain and there is no explicit suspicion of tick-borne diseases since the methods essentially will detect all types of prokaryotic/eukaryotic DNA. However, sensitivity is lower than for specific PCR. *A. phagocytophilum* (infecting neutrophilic granulocytes), *Babesia* (infecting red blood cells) together with *N. mikurensis* (infecting the endothelium lining the blood vessels) and *B. miyamotoi* (existing extracellularly) are pathogens normally detected in blood (Bakken et al., 1994; Kawahara et al., 2004; Krause et al., 2015; Rudzinska et al., 1976; Wass et al., 2019), whereas it is unknown if *R. helvetica* is present in blood in the acute phase of disease.

Molecular testing of whole blood is the method of choice for all symptomatic under-recognized tick-borne diseases except for the rickettsioses. Most rickettsioses diagnosed in the North Sea Region are infections imported from the southern part of Europe, such as the Mediterranean spotted fever caused by *R. conorii*, or elsewhere in the world, such as the African tick-bite fever caused by *R. africae* (Oteo and Portillo, 2012). The amount of rickettsial DNA in blood is generally low and the sensitivity of molecular testing in blood is suboptimal (Stewart and Stewart, 2021). A possible pitfall when diagnosing *B. miyamotoi* disease may be the relapsing nature of the disease, as *B. miyamotoi* is detectable for only around four days during a febrile episode (Karan et al., 2018). Thus, *B. miyamotoi* DNA may no longer be detectable in blood at the time the patient presents with an infection of the central nervous system (Boden et al., 2016). *B. miyamotoi* and rickettsial DNA can be detected in cerebrospinal fluid of patients with neurological symptoms, so molecular testing of cerebrospinal fluid should be considered (Hoornstra et al., 2022; Nilsson et al., 2010, 2011).

Serological assays for the detection of either species- or group-

Table 2
Methods currently available for diagnosing the under-recognized human tick-borne diseases.

Pathogen causing disease	Methods (listed in recommended order)	Patient material	Timing of test	Methodological limitations
Anaplasma phagocytophilum	Commercial or in-house PCR *16S rRNA* sequencing	Whole blood	Symptomatic phase	*16S rRNA* sequencing has commonly lower sensitivity than PCR
	Serology (primarily IFA, IgG)	Serum	2-4 weeks apart	Mainly demonstrates recent or past infection False positive reactions due to cross-reactive antibodies Weak/false negative reactions in immunosuppressed patients
	Blood smear	Whole blood	Symptomatic phase	Low sensitivity Lack of experienced test personnel
Neoehrlicha mikurensis	In-house PCR *16S rRNA* sequencing	Whole blood	Symptomatic phase	Only currently available diagnostic test *16S rRNA* sequencing has lower sensitivity than specific PCR
Borrelia miyamotoi	Commercial/in-house PCR *16S rRNA* sequencing	Whole blood Cerebrospinal fluid	Symptomatic phases	May be false negative between fever episodes *16S rRNA* sequencing has commonly low sensitivity than PCR
	Serology Relapsing fever group glpQ-assay (immunoblot)	Serum	2-4 weeks apart	Mainly demonstrates recent or past infection Only a few specialized laboratories in Europe Weak/False negative in immunosuppressed patients
***Rickettsia* spp** (including *Rickettsia helvetica*)	Serology (Spotted fever group IFA, IgM and IgG)	Serum	2-4 weeks apart	Mainly demonstrates recent or past infection Weak/false negative in immunosuppressed patients
	Commercial or in-house PCR 16S rRNA sequencing	(Whole blood) Skin biopsies Cerebrospinal fluid	Symptomatic phase	Sensitivity depends on testing of relevant patient material 16S rRNA sequencing has commonly lower sensitivity than PCR
***Babesia* spp**	Commercial or in-house PCR *18S rRNA* sequencing	Whole blood	Symptomatic phase	Sensitive, but less available and may delay diagnosis *18S rRNA* sequencing has commonly lower sensitivity than PCR
	Blood smear (Giemsa stained)	Whole blood	Symptomatic phase	Rapid but less sensitive than PCR Lack of experienced test personnel Findings may be misinterpreted as malaria
	Serology (IFA)	Serum	2-4 weeks apart	Mainly demonstrates recent or past infection Weak/false negative in immunosuppressed patients

IFA: Indirect fluorescence antibody test.

specific antibodies are established for all under-recognized tick-borne pathogens with the exception of *N. mikurensis*. Assays are generally commercially available, but only a few specialized clinical laboratories in Europe have tests validated for clinical use. Specific antibodies often take weeks to develop in response to infection, so serological tests may not be useful in the early phase of disease. A four-fold change in IgG titer in paired serum samples taken 2–4 weeks apart confirms a diagnosis. Furthermore, the presence of IgM may support the diagnosis, even after a single test result. A single positive antibody test should be interpreted carefully. Long-term persistence of antibodies from a previous pathogen exposure or infection may be mistaken as a current infection. In areas, highly endemic for tick-borne disease, seroprevalence rates against common tick-borne pathogens may be high, making it challenging to interpret serologic findings. Cross-reactive antibodies, especially of IgM class, may also be misleading. Antibodies produced against *N. mikurensis* are reported to cross-react in *A. phagocytophilum* antibody tests in such a way that a patient with neoehrlichiosis may be misdiagnosed as having anaplasmosis (Wass et al., 2018). Furthermore, *B. miyamotoi* infection may give rise to antibodies in cerebrospinal fluid that cross-react in *B. burgdorferi* C6 antibody tests (Koetsveld et al., 2020), a result that may be misinterpreted as Lyme neuroborreliosis (Hoornstra et al., 2018; Sudhindra et al., 2016). Another possible pitfall in serology with a particular impact on the under-recognized tick-borne diseases is that immunosuppressed individuals may have false negative tests due to impaired ability to produce antibodies when infected (Henningsson et al., 2019; Tavakolpour et al., 2019). Altogether, this underlines why molecular testing is the preferred diagnostic method and of particular importance when testing immunosuppressed patients for the under-recognized tick-borne diseases. In spite of that, antibody testing may be useful for the diagnosis of low-grade infections, such as between relapses of *B. miyamotoi* disease. The development of a serological method for neoehrlichiosis is also anticipated, which may enable the detection of the low-grade persistent type of infection observed among *N. mikurensis* exposed individuals (Grankvist et al., 2015; Quarsten et al., 2021; Welc-Faleciak et al., 2014). In addition, serology remains an important tool for seroprevalence surveys that may provide crucial information on the rate of pathogen exposure in a population.

Microscopy of Giemsa-stained blood smears is a well-established method for the identification of *Babesia*-infected red blood cells in endemic areas, whereas microscopic examination of stained acute phase blood smears for detection of intracytoplasmic morulae in anaplasmosis patients is less useful due to low diagnostic sensitivity for European *A. phagocytophilum* variants (Lotric-Furlan et al., 2006). Microscopic detection of *Babesia* in blood cells is technically easy to perform and may be done at local hospital laboratories, but it may be mistaken for *Plasmodium falciparum* malaria by inexperienced operators (Kukina et al., 2018). In practice, due to low awareness only a few specialized laboratories are experienced and use the technique.

4. Clinical features of the under-recognized tick-borne diseases

To understand when to suspect and test for one of the less established tick-borne diseases is demanding. Clinicians should remember to ask patients with unexplained illnesses about their history of tick bites. However, it is important to keep in mind that a previous tick bite may not always have been recognized by the patient since a large fraction of them pass unnoticed.

Most of the infections caused by the under-recognized tick-borne pathogens in immunocompetent individuals are probably asymptomatic or mild and self-limiting and do not require treatment. However, symptomatic cases occur and often but not always, manifest as a non-specific febrile illness with myalgia, and may warrant antibiotic treatment. In immunosuppressed patients, the symptoms can be more severe and even life-threatening making it crucial to identify such patients. Recognizing the clinical manifestations and biochemical laboratory findings indicating the lesser-known tick-borne diseases may, however, be challenging. Symptoms and disease features important for clinicians to be aware of are summarized in a diagnostic flowchart (Fig. 2) and briefly described. A more detailed description of the clinical and laboratory parameters associated with the various tick-borne diseases are beyond the scope of this work and should be pursued elsewhere.

Fever is a dominating symptom of most under-recognized tick-borne diseases. Immunosuppressed neoehrlichiosis patients often have episodes with daily fever (Wenneras, 2015). Patients with *B. miyamotoi* disease may have intermittent fever interspersed with afebrile episodes (recurrent fever) often totaling two or three relapse periods (Platonov et al., 2011; Wagemakers et al., 2015). Babesiosis may also present with relapsing fever episodes (Shuker et al., 2018). Febrile hemolytic disease, a clinical manifestation mimicking malaria, is an indication of babesiosis. Malaria, acquired during travels to endemic areas, is more common and should always be excluded as the cause of infection before testing for *Babesia*.

Increased liver enzymes are acknowledged to be common in anaplasmosis and babesiosis cases and could indicate testing for those particular infections, however, a certain fraction of all the under-recognized tick-borne disease patients will also express the same abnormality (Azagi et al., 2020; Dumic et al., 2022). A discriminatory finding that may be useful is blood platelet levels, which tend to be decreased in anaplasmosis and normal or even increased in neoehrlichiosis (Grankvist et al., 2014; Matei et al., 2019). Further, moderately increased white blood cells dominated by neutrophils is also a common feature of neoehrlichiosis (Wenneras, 2015).

Acute disease caused by *N. mikurensis, B. miyamotoi* or *Babesia* is most often, but not exclusively, diagnosed in immunocompromised patients. Rituximab (anti-CD20/B cell) treatment is a predominant risk factor for severe neoehrlichiosis and *B. miyamotoi* disease, whereas the majority of patients diagnosed with severe babesiosis together with a fraction of the neoehrlichiosis patients are asplenic (Hildebrandt et al., 2021; Wagemakers et al., 2015; Wenneras, 2015). On the contrary, many European anaplasmosis cases are diagnosed in immunocompetent individuals, although with milder manifestations than seen in the immunosuppressed (Azagi et al., 2020). The few reported cases of severe infection caused by *R. helvetica* are from individuals with intact immunity (Nilsson, 2009, 1999b, 2010, 2011).

Infected immunocompromised patients may often have a prolonged (for months) disease course as seen in several neoehrlichiosis and babesiosis patients as well as in some of the few reported cases of *B. miyamotoi*-meningitis (Henningsson et al., 2019; Wagemakers et al., 2015; Wenneras, 2015). Infections may be mistaken for non-infectious conditions in immunocompromised patients with underlying medical conditions, even when the burden of symptoms is severe, because routine microbiologic investigations remain negative (Grankvist et al., 2014). A hallmark of severe neoehrlichiosis, independent of immune status of the patient, is manifestation of vascular complications (Wenneras, 2015). Thromboembolic events in the venous circulation (thrombophlebitis, deep vein thrombosis, pulmonary embolism) seem to affect immunocompromised individuals whereas inflammation of medium-to large-sized arteries have only been seen in immunocompetent patients infected by *N. mikurensis* (Höper et al., 2020). Neoehrlichiosis is often diagnosed at the time the patient presents with atypical vascular and/or thromboembolic events with no obvious risk factors. More focus on testing for neoehrlichiosis in patients (in particular Rituximab treated) with fever and vascular events may prevent further potentially life-threatening complications that can be eradicated by antibiotic treatment (Sjöwall et al., 2021).

Neurological infection is solely a complication of severe disease caused by *B. miyamotoi* or *R. helvetica*, yet critical cases seem to be rare for both types of infections (Azagi et al., 2020; Cutler et al., 2019). Recurrent episodes of neurological symptoms with symptom-free intervals, may indicate relapsing *B. miyamotoi* meningitis (Henningsson et al., 2019).

The under-recognized tick-borne diseases are not treated with the

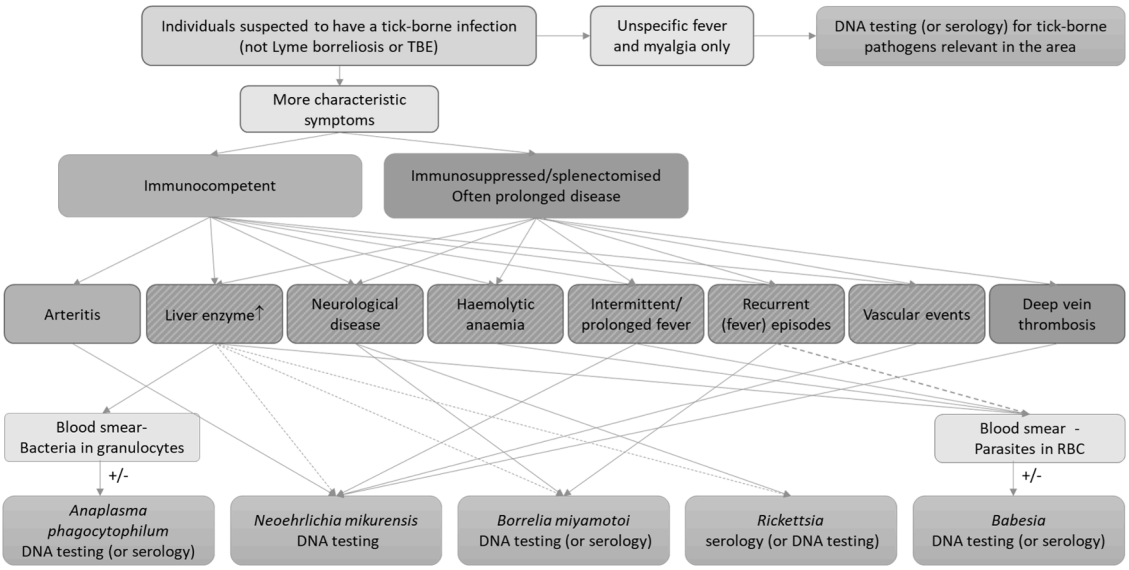

Fig. 2. Diagnostic flowchart for the under-recognized tick-borne diseases in the North Sea Region.

first-choice antibiotics commonly used for infections of unknown origin. Therefore, diagnosing the tick-borne diseases is of major importance for correct treatment and management of patients. Infections caused by the intracellular bacteria *A. phagocytophilum, N. mikurensis* and *R. helvetica* are primarily treated by high-dose doxycycline (Wass et al., 2018). Doxycycline is also the drug of choice for *B. miyamotoi* disease, including *B. miyamotoi* meningitis (Henningsson et al., 2019). Cases of babesiosis require treatment with antiparasitic drugs, e.g. atovaquone or quinine, given in combination with azithromycin or clindamycin. A missed diagnosis may delay or hinder correct treatment, putting the patient at risk of an extended infection period and severe complications.

5. Availability of diagnostic services for the under-recognized tick-borne diseases

As part of the work of the NorthTick project, a brief survey on the availability of diagnostic services within the countries in the North Sea Region was conducted. It was demanding to get a reliable overview of all seven countries. Methods for the detection of the under-recognized tick-borne diseases validated for clinical use are available in only a few laboratories. Most countries seemed to have at least one laboratory offering serological and/or molecular diagnostic services for the tick-borne diseases caused by *A. phagocytophilum, B. miyamotoi, Rickettsia* and *Babesia.* The lack of an established laboratory method for detection of babesiosis in some countries may to a certain degree be compensated by using microscopic examination of blood in hospital laboratories. Some countries also have established international collaborations to fill in the methodological gaps. The most evident finding from the assessment was that the Scandinavian countries provide routine molecular testing for *N. mikurensis*, reflecting a higher awareness of neoehrlichiosis and a higher number of cases in those countries than in the non-Scandinavian countries of the North Sea Region.

6. Considerations on the diagnostic services of the under-recognized tick-borne diseases

As discussed earlier, *A. phagocytophilum, N. mikurensis, B. miyamotoi* and *Babesia* are carried by ticks in most North Sea Region countries, even if not always widespread. Although they apparently do not have a high potential to cause severe infections in immunocompetent individuals, evidence indicating a significant impact on infected immunocompromised individuals is growing (Hildebrandt et al., 2021; Hoornstra et al., 2022; Wenneras, 2015). The number of individuals at risk of contracting a severe infection and complications is continuously increasing due to the rising use of biological therapy for many common autoimmune and inflammatory diseases such as rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease, as well as for malignant B cell lymphomas.

The diagnostic services available should be harmonized with the anticipated need. Establishing a broad test repertoire in several laboratories in each country may be needless and costly. The authors suggest that the countries within the North Sea Region could consider consolidating diagnostic services to one or a few national reference centers. This would promote an increased level of competence and expertise at each center, facilitate research and make referral pathways clearer for testing and clinical advice. Closing significant diagnostic gaps in the national test repertoire could also be done by taking advantage of the diagnostics available in national reference or specialized laboratories in partner countries. This testing should be advertised and available for the clinicians upon request in the same manner as the nationally provided tests. Since there are no national surveillance systems for the more uncommon tick-borne diseases, a collected diagnostics competence will facilitate a better overview of diagnosed cases. However, national surveillance and notification services would be ideal especially since tick-borne diseases are anticipated to increase in the wake of climate change.

The national diagnostic services should primarily be molecular-based but should also include antibody testing and microscopy where appropriate and available. The symptoms of the under-recognized tick-borne diseases often resemble each other to a high degree, often featuring fever and myalgia, and clinicians rarely have experience on how to distinguish between these infections. Thus, choosing diagnostic strategies like multiplex PCR (or panel testing) to detect all relevant tick-borne pathogens will help increase the number of correctly diagnosed cases and contribute to improved knowledge. Clinicians suspecting an under-recognized tick-borne disease regularly request *A. phagocytophilum* testing only since anaplasmosis is the infection most familiar to them. However, in many regions, as seen in the southern part of Norway and Sweden, it is much more likely to contract neoehrlichiosis.

The need for specific tests for detection of *R. helvetica* is less clear as only a few severe cases of disease are known and a certain causality between tick exposure and disease is not established (Azagi et al., 2020). However, several countries are providing tests for detection of the rickettsial infections occasionally imported from the southern parts of

Europe or wider afield. These methods may be valuable for identification of infection caused by *R. helvetica* given that serological assays detecting particular *Rickettsia* spp. are frequently cross-reactive for other spotted fever group *Rickettsia* and molecular assays often detect a broad range of *Rickettsia* spp., including *R. helvetica*.

Closing remarks

The majority of infections caused by the under-recognized tick-borne diseases are thought to be asymptomatic or mild and self-limiting. However, their ability to cause severe disease, especially in the immunocompromised population, requires serious consideration. Due to the lack of characteristic symptoms, low awareness, absence of surveillance and limited use/availability of diagnostic tests for the under-recognized tick-borne diseases, most cases probably remain undiagnosed and the impact on public health remains unclear. Focus on raising the awareness of the infections among clinicians and clinical microbiologists and proper organization and evaluation of the diagnostic services is of high importance, and will together facilitate an increase in targeted testing and detection of more disease cases. Each new diagnosed tick-borne disease case will provide valuable epidemiological data on incidence and prevalence as well as increasing of important understanding of clinical manifestations. Rapid and accurate diagnosis is crucial to ensure adequate antimicrobial therapy when needed and reduce the number of patients with complications.

Funding

This work has been supported by the European Union through the European Regional Development Fund and the Interreg North Sea Region Program 2014–2020 as a part of the NorthTick project (reference number J-No. 38-2-7-19).

Declaration of Competing Interest

None

Data availability

No data was used for the research described in the article.

References

Abdullah, S., Helps, C., Tasker, S., Newbury, H., Wall, R., 2018. Prevalence and distribution of *Borrelia* and *Babesia* species in ticks feeding on dogs in the U.K. Med. Vet. Entomol. 32, 14–22. https://doi.org/10.1111/mve.12257.

Andersson, M., Bartkova, S., Lindestad, O., Råberg, L., 2013. Co-infection with '*Candidatus* Neoehrlichia Mikurensis' and *Borrelia afzelii* in *Ixodes ricinus* ticks in southern Sweden. Vector Borne Zoonot. Dis. 13, 438–442. https://doi.org/10.1089/vbz.2012.1118.

Azagi, T., Hoornstra, D., Kremer, K., Hovius, J.W.R., Sprong, H., 2020. Evaluation of disease causality of rare *Ixodes ricinus*-borne infections in Europe. Pathogens. 9 https://doi.org/10.3390/pathogens9020150.

Azagi, T., Jaarsma, R.I., Docters van Leeuwen, A., Fonville, M., Maas, M., Franssen, F.F. J., Kik, M., Rijks, J.M., Montizaan, M.G., Groenevelt, M., Hoyer, M., Esser, H.J., Krawczyk, A.I., Modrý, D., Sprong, H., Demir, S., 2021. Circulation of *Babesia* species and their exposure to humans through *Ixodes Ricinus*. Pathogens 10. https://doi.org/10.3390/pathogens10040386.

Bakken, J.S., Dumler, J.S., Chen, S.M., Eckman, M.R., Van Etta, L.L., Walker, D.H., 1994. Human granulocytic ehrlichiosis in the upper Midwest United States. A new species emerging? JAMA 272, 212–218.

Blazejak, K., Janecek, E., Strube, C., 2017. A 10-year surveillance of *Rickettsiales* (*Rickettsia* spp. and *Anaplasma phagocytophilum*) in the city of Hanover, Germany, reveals *Rickettsia* spp. as emerging pathogens in ticks. Parasites Vect. 10, 588. https://doi.org/10.1186/s13071-017-2537-2.

Blazejak, K., Raulf, M.K., Janecek, E., Jordan, D., Fingerle, V., Strube, C., 2018. Shifts in *Borrelia burgdorferi* (s.l.) geno-species infections in Ixodes ricinus over a 10-year surveillance period in the city of Hanover (Germany) and *Borrelia miyamotoi*-specific Reverse Line Blot detection. Parasites Vect. 11, 304. https://doi.org/10.1186/s13071-018-2882-9.

Boden, K., Lobenstein, S., Hermann, B., Margos, G., Fingerle, V., 2016. *Borrelia miyamotoi*-associated neuroborreliosis in immunocompromised person. Emerg. Infect. Dis. 22, 1617–1620. https://doi.org/10.3201/eid2209.152034.

Cochez, C., Heyman, P., Heylen, D., Fonville, M., Hengeveld, P., Takken, W., Simons, L., Sprong, H., 2015. The presence of *Borrelia miyamotoi*, a relapsing fever spirochaete, in questing *Ixodes ricinus* in Belgium and in the Netherlands. Zoonoses Public Health 62, 331–333. https://doi.org/10.1111/zph.12154.

Coipan, E.C., Jahfari, S., Fonville, M., Maassen, C.B., van der Giessen, J., Takken, W., Takumi, K., Sprong, H., 2013. Spatiotemporal dynamics of emerging pathogens in questing *Ixodes ricinus*. Front. Cell. Infect. Microbiol. 3, 36. https://doi.org/10.3389/fcimb.2013.00036.

Cull, B., Hansford, K.M., McGinley, L., Gillingham, E.L., Vaux, A.G.C., Smith, R., Medlock, J.M., 2021. A nationwide study on *Borrelia burgdorferi* s.l. infection rates in questing *Ixodes ricinus*: a six-year snapshot study in protected recreational areas in England and Wales. Med. Vet. Entomol. 35, 352–360. https://doi.org/10.1111/mve.12503.

Cutler, S., Vayssier-Taussat, M., Estrada-Pena, A., Potkonjak, A., Mihalca, A.D., Zeller, H., 2019. A new *Borrelia* on the block: *borrelia miyamotoi* - a human health risk? Euro. Surveill. 24 https://doi.org/10.2807/1560-7917.Es.2019.24.18.1800170.

Dumic, I., Jevtic, D., Veselinovic, M., Nordstrom, C.W., Jovanovic, M., Mogulla, V., Veselinovic, E.M., Hudson, A., Simeunovic, G., Petcu, E., Ramanan, P., 2022. Human granulocytic anaplasmosis-a systematic review of published cases. Microorganisms 10. https://doi.org/10.3390/microorganisms10071433.

ECDC, 2023-2-31. https://www.ecdc.europa.eu/en/tick-borne-diseases.

Eshoo, M.W., Crowder, C.D., Carolan, H.E., Rounds, M.A., Ecker, D.J., Haag, H., Mothes, B., Nolte, O., 2014. Broad-range survey of tick-borne pathogens in Southern Germany reveals a high prevalence of *Babesia microti* and a diversity of other tick-borne pathogens. Vector Borne Zoonotic. Dis. 14, 584–591. https://doi.org/10.1089/vbz.2013.1498.

Fertner, M.E., Mølbak, L., Boye Pihl, T.P., Fomsgaard, A., Bødker, R., 2012. First detection of tick-borne "*Candidatus* Neoehrlichia mikurensis" in Denmark 2011. Euro Surveill. 17.

Flattery, A., McKiernan, F., Browne, J., Gray, J., Zaid, T., O'Connor, J., Zintl, A., 2022. The prevalence and distribution of *Anaplasma phagocytophilum* genotypes in *Ixodes ricinus* nymphs collected from farm- and woodland sites in Ireland. Ticks Tick Borne Dis 13, 101928. https://doi.org/10.1016/j.ttbdis.2022.101928.

Fraenkel, C.J., Garpmo, U., Berglund, J., 2002. Determination of novel *Borrelia* genospecies in Swedish *Ixodes ricinus* ticks. J. Clin. Microbiol. 40, 3308–3312. https://doi.org/10.1128/jcm.40.9.3308-3312.2002.

Franke, J., Fritzsch, J., Tomaso, H., Straube, E., Dorn, W., Hildebrandt, A., 2010. Coexistence of pathogens in host-seeking and feeding ticks within a single natural habitat in Central Germany. Appl. Environ. Microbiol. 76, 6829–6836. https://doi.org/10.1128/aem.01630-10.

Franke, J., Hildebrandt, A., Meier, F., Straube, E., Dorn, W., 2011. Prevalence of Lyme disease agents and several emerging pathogens in questing ticks from the German Baltic coast. J. Med. Entomol. 48, 441–444. https://doi.org/10.1603/me10182.

Galfsky, D., Król, N., Pfeffer, M., Obiegala, A., 2019. Long-term trends of tick-borne pathogens in regard to small mammal and tick populations from Saxony, Germany. Parasites Vect. 12, 131. https://doi.org/10.1186/s13071-019-3382-2.

Gandy, S., Hansford, K., McGinley, L., Cull, B., Smith, R., Semper, A., Brooks, T., Fonville, M., Sprong, H., Phipps, P., Johnson, N., Medlock, J.M., 2022. Prevalence of *Anaplasma phagocytophilum* in questing *Ixodes ricinus* nymphs across twenty recreational areas in England and Wales. Ticks Tick Borne Dis. 13, 101965 https://doi.org/10.1016/j.ttbdis.2022.101965.

Grankvist, A., Andersson, P.O., Mattsson, M., Sender, M., Vaht, K., Hoper, L., Sakiniene, E., Trysberg, E., Stenson, M., Fehr, J., Pekova, S., Bogdan, C., Bloemberg, G., Wenneras, C., 2014. Infections with the tick-borne bacterium "*Candidatus* Neoehrlichia mikurensis" mimic noninfectious conditions in patients with B cell malignancies or autoimmune diseases. Clin. Infect. Dis. 58, 1716–1722. https://doi.org/10.1093/cid/ciu189.

Grankvist, A., Sandelin, L.L., Andersson, J., Fryland, L., Wilhelmsson, P., Lindgren, P.E., Forsberg, P., Wenneras, C., 2015. Infections with *Candidatus* Neoehrlichia mikurensis and cytokine responses in 2 persons bitten by ticks. Sweden. Emerg. Infect. Dis. 21, 1462–1465. https://doi.org/10.3201/eid2108.150060.

Granquist, E.G., Kristiansson, M., Lindgren, P.E., Matussek, A., Nødtvedt, A., Okstad, W., Stuen, S., 2014. Evaluation of microbial communities and symbionts in *Ixodes ricinus* and ungulate hosts (*Cervus elaphus* and *Ovis aries*) from shared habitats on the west coast of Norway. Ticks Tick Borne Dis. 5, 780–784. https://doi.org/10.1016/j.ttbdis.2014.05.005.

Guy, E., Tasker, S., Joynson, D.H., 1998. Detection of the agent of human granulocytic ehrlichiosis (HGE) in UK ticks using polymerase chain reaction. Epidemiol. Infect. 121, 681–683. https://doi.org/10.1017/s0950268898001708.

Hansford, K.M., Fonville, M., Jahfari, S., Sprong, H., Medlock, J.M., 2015. *Borrelia miyamotoi* in host-seeking *Ixodes ricinus* ticks in England. Epidemiol. Infect. 143, 1079–1087. https://doi.org/10.1017/s0950268814001691.

Hartelt, K., Oehme, R., Frank, H., Brockmann, S.O., Hassler, D., Kimmig, P., 2004. Pathogens and symbionts in ticks: prevalence of *Anaplasma phagocytophilum* (*Ehrlichia* sp.), *Wolbachia* sp., *Rickettsia* sp., and *Babesia* sp. in Southern Germany. Int. J. Med. Microbiol. 293 Suppl 37, 86–92. https://doi.org/10.1016/s1433-1128(04)80013-5.

Hauck, D., Springer, A., Pachnicke, S., Schunack, B., Fingerle, V., Strube, C., 2019. *Ixodes inopinatus* in northern Germany: occurrence and potential vector role for *Borrelia* spp., *Rickettsia* spp., and *Anaplasma phagocytophilum* in comparison with *Ixodes ricinus*. Parasitol. Res. 118, 3205–3216. https://doi.org/10.1007/s00436-019-06506-4.

Henningsson, A.J., Hvidsten, D., Kristiansen, B.E., Matussek, A., Stuen, S., Jenkins, A., 2015. Detection of *Anaplasma phagocytophilum* in *Ixodes ricinus* ticks from Norway using a realtime PCR assay targeting the Anaplasma citrate synthase gene *gltA*. BMC Microbiol 15, 153. https://doi.org/10.1186/s12866-015-0486-5.

Henningsson, A.J., Asgeirsson, H., Hammas, B., Karlsson, E., Parke, A., Hoornstra, D., Wilhelmsson, P., Hovius, J.W., 2019. Two cases of *Borrelia miyamotoi* meningitis, Sweden, 2018. Emerging Infect. Dis. 25, 1965–1968. https://doi.org/10.3201/eid2510.190416.

Heylen, D., Fonville, M., van Leeuwen, A.D., Sprong, H., 2016. Co-infections and transmission dynamics in a tick-borne bacterium community exposed to songbirds. Environ. Microbiol. 18, 988–996. https://doi.org/10.1111/1462-2920.13164.

Hildebrandt, A., Krämer, A., Sachse, S., Straube, E., 2010. Detection of *Rickettsia* spp. and *Anaplasma phagocytophilum* in *Ixodes ricinus* ticks in a region of Middle Germany (Thuringia). Ticks Tick Borne Dis. 1, 52–56. https://doi.org/10.1016/j.ttbdis.2009.11.005.

Hildebrandt, A., Fritzsch, J., Franke, J., Sachse, S., Dorn, W., Straube, E., 2011. Co-circulation of emerging tick-borne pathogens in Middle Germany. Vector Borne Zoonotic Dis. 11, 533–537. https://doi.org/10.1089/vbz.2010.0048.

Hildebrandt, A., Zintl, A., Montero, E., Hunfeld, K.P., Gray, J., 2021. Human babesiosis in Europe. Pathogens 10 (9). https://doi.org/10.3390/pathogens10091165.

Hoornstra, D., Koetsveld, J., Sprong, H., Platonov, A.E., Hovius, J.W., 2018. *Borrelia miyamotoi* disease in an immunocompetent patient. Western Europe. Emerg. Infect. Dis. 24, 1770–1772. https://doi.org/10.3201/eid2409.180806.

Hoornstra, D., Azagi, T., van Eck, J.A., Wagemakers, A., Koetsveld, J., Spijker, R., Platonov, A.E., Sprong, H., Hovius, J.W., 2022. Prevalence and clinical manifestation of *Borrelia miyamotoi* in *Ixodes ticks* and humans in the northern hemisphere: a systematic review and meta-analysis. Lancet Microbe 3, e772–e786. https://doi.org/10.1016/s2666-5247(22)00157-4.

Hvidsten, D., Frafjord, K., Gray, J.S., Henningsson, A.J., Jenkins, A., Kristiansen, B.E., Lager, M., Rognerud, B., Slåtsve, A.M., Stordal, F., Stuen, S., Wilhelmsson, P., 2020. The distribution limit of the common tick, *Ixodes ricinus*, and some associated pathogens in north-western Europe. Ticks Tick Borne Dis. 11, 101388 https://doi.org/10.1016/j.ttbdis.2020.101388.

Höper, L., Skoog, E., Stenson, M., Grankvist, A., Wass, L., Olsen, B., Nilsson, K., Mårtensson, A., Söderlind, J., Sakinis, A., Wennerås, C., 2020. Vasculitis due to *Candidatus* Neoehrlichia mikurensis: a cohort study of 40 Swedish patients. Clin. Infect. Dis. 73, e2372–e2378. https://doi.org/10.1093/cid/ciaa1217.

Jahfari, S., Fonville, M., Hengeveld, P., Reusken, C., Scholte, E.J., Takken, W., Heyman, P., Medlock, J.M., Heylen, D., Kleve, J., Sprong, H., 2012. Prevalence of *Neoehrlichia mikurensis* in ticks and rodents from North-west Europe. Parasites Vect. 5, 74. https://doi.org/10.1186/1756-3305-5-74.

Jahfari, S., Coipan, E.C., Fonville, M., van Leeuwen, A.D., Hengeveld, P., Heylen, D., Heyman, P., van Maanen, C., Butler, C.M., Földvári, G., Szekeres, S., van Duijvendijk, G., Tack, W., Rijks, J.M., van der Giessen, J., Takken, W., van Wieren, S. E., Takumi, K., Sprong, H., 2014. Circulation of four *Anaplasma phagocytophilum* ecotypes in Europe. Parasites Vect. 7, 365. https://doi.org/10.1186/1756-3305-7-365.

Jenkins, A., Raasok, C., Pedersen, B.N., Jensen, K., Andreassen, Å., Soleng, A., Edgar, K. S., Lindstedt, H.H., Kjelland, V., Stuen, S., Hvidsten, D., Kristiansen, B.E., 2019. Detection of *Candidatus* Neoehrlichia mikurensis in Norway up to the northern limit of *Ixodes ricinus* distribution using a novel real time PCR test targeting the *groEL* gene. BMC Microbiol. 19, 199. https://doi.org/10.1186/s12866-019-1502-y.

Jensen, P.M., Christoffersen, C.S., Moutailler, S., Michelet, L., Klitgaard, K., Bødker, R., 2017. Transmission differentials for multiple pathogens as inferred from their prevalence in larva, nymph and adult of *Ixodes ricinus* (Acari: *ixodidae*). Exp. Appl. Acarol. 71, 171–182. https://doi.org/10.1007/s10493-017-0110-5.

Kantsø, B., Svendsen, C.B., Jensen, P.M., Vennestrøm, J., Krogfelt, K.A., 2010. Seasonal and habitat variation in the prevalence of *Rickettsia helvetica* in *Ixodes ricinus* ticks from Denmark. Ticks Tick Borne Dis. 1, 101–103. https://doi.org/10.1016/j.ttbdis.2010.01.004.

Karan, L., Makenov, M., Kolyasnikova, N., Stukolova, O., Toporkova, M., Olenkova, O., 2018. Dynamics of spirochetemia and early PCR detection of *Borrelia miyamotoi*. Emerging Infect. Dis. 24, 860–867. https://doi.org/10.3201/eid2405.170829.

Karlsson, M.E., Andersson, M.O., 2016. *Babesia* species in questing *Ixodes ricinus*, Sweden. Ticks Tick Borne Dis. 7, 10–12. https://doi.org/10.1016/j.ttbdis.2015.07.016.

Kawahara, M., Rikihisa, Y., Isogai, E., Takahashi, M., Misumi, H., Suto, C., Shibata, S., Zhang, C., Tsuji, M., 2004. Ultrastructure and phylogenetic analysis of '*Candidatus* Neoehrlichia mikurensis' in the family *Anaplasmataceae*, isolated from wild rats and found in *Ixodes ovatus* ticks. Int. J. Syst. Evol. Microbiol. 54, 1837–1843. https://doi.org/10.1099/ijs.0.63260-0.

Kjelland, V., Rollum, R., Korslund, L., Slettan, A., Tveitnes, D., 2015. *Borrelia miyamotoi* is widespread in *Ixodes ricinus* ticks in southern Norway. Ticks Tick Borne Dis. 6, 516–521. https://doi.org/10.1016/j.ttbdis.2015.04.004.

Kjelland, V., Paulsen, K.M., Rollum, R., Jenkins, A., Stuen, S., Soleng, A., Edgar, K.S., Lindstedt, H.H., Vaino, K., Gibory, M., Andreassen Å, K., 2018. Tick-borne encephalitis virus, *Borrelia burgdorferi* sensu lato, *Borrelia miyamotoi, Anaplasma phagocytophilum* and *Candidatus* Neoehrlichia mikurensis in *Ixodes ricinus* ticks collected from recreational islands in southern Norway. Ticks Tick Borne Dis. 9, 1098–1102. https://doi.org/10.1016/j.ttbdis.2018.04.005.

Kjær, L.J., Klitgaard, K., Soleng, A., Edgar, K.S., Lindstedt, H.E.H., Paulsen, K.M., Andreassen Å, K., Korslund, L., Kjelland, V., Slettan, A., Stuen, S., Kjellander, P., Christensson, M., Teräväinen, M., Baum, A., Jensen, L.M., Bødker, R., 2020. Spatial patterns of pathogen prevalence in questing *Ixodes ricinus* nymphs in southern Scandinavia, 2016. Sci. Rep. 10, 19376. https://doi.org/10.1038/s41598-020-76334-5.

Klitgaard, K., Kjær, L.J., Isbrand, A., Hansen, M.F., Bødker, R., 2019. Multiple infections in questing nymphs and adult female *Ixodes ricinus* ticks collected in a recreational forest in Denmark. Ticks Tick Borne Dis. 10, 1060–1065. https://doi.org/10.1016/j.ttbdis.2019.05.016.

Knoll, S., Springer, A., Hauck, D., Schunack, B., Pachnicke, S., Strube, C., 2021. Regional, seasonal, biennial and landscape-associated distribution of *Anaplasma phagocytophilum* and *Rickettsia* spp. infections in *Ixodes* ticks in northern Germany and implications for risk assessment at larger spatial scales. Ticks Tick Borne Dis. 12, 101657 https://doi.org/10.1016/j.ttbdis.2021.101657.

Koetsveld, J., Platonov, A.E., Kuleshov, K., Wagemakers, A., Hoornstra, D., Ang, W., Szekeres, S., van Duijvendijk, G.L.A., Fikrig, E., Embers, M.E., Sprong, H., Hovius, J. W., 2020. *Borrelia miyamotoi* infection leads to cross-reactive antibodies to the C6 peptide in mice and men. Clin. Microbiol. Infect. 26 https://doi.org/10.1016/j.cmi.2019.07.026, 513.e511-513.e516.

Krause, P.J., Fish, D., Narasimhan, S., Barbour, A.G., 2015. *Borrelia miyamotoi* infection in nature and in humans. Clin. Microbiol. Infect. 21, 631–639. https://doi.org/10.1016/j.cmi.2015.02.006.

Kubiak, K., Szczotko, M., Dmitryjuk, M., 2021. *Borrelia miyamotoi*-an emerging human tick-borne pathogen in Europe. Microorganisms. 9 https://doi.org/10.3390/microorganisms9010154.

Kukina, I.V., Guzeeva, T.M., Zelya, O.P., Ganushkina, L.A., 2018. Fatal human babesiosis caused by *Babesia divergens* in an asplenic host. IDCases 13, e00414. https://doi.org/10.1016/j.idcr.2018.e00414.

Lambert, J.S., Cook, M.J., Healy, J.E., Murtagh, R., Avramovic, G., Lee, S.H., 2019. Metagenomic *16S rRNA* gene sequencing survey of *Borrelia* species in Irish samples of *Ixodes ricinus* ticks. PLoS ONE 14, e0209881. https://doi.org/10.1371/journal.pone.0209881.

Larsson, C., Hvidsten, D., Stuen, S., Henningsson, A.J., Wilhelmsson, P., 2018. *Candidatus* Neoehrlichia mikurensis" in *Ixodes ricinus* ticks collected near the Arctic Circle in Norway. Parasites Vect. 11, 620. https://doi.org/10.1186/s13071-018-3168-y.

Layzell, S.J., Bailey, D., Peacey, M., Nuttall, P.A., 2018. Prevalence of *Borrelia burgdorferi* and *Borrelia miyamotoi* in questing *Ixodes ricinus* ticks from four sites in the UK. Ticks Tick Borne Dis. 9, 217–224. https://doi.org/10.1016/j.ttbdis.2017.09.007.

Lempereur, L., De Cat, A., Caron, Y., Madder, M., Claerebout, E., Saegerman, C., Losson, B., 2011. First molecular evidence of potentially zoonotic *Babesia microti* and *Babesia* sp. EU1 in *Ixodes ricinus* ticks in Belgium. Vector Borne Zoonotic Dis. 11, 125–130. https://doi.org/10.1089/vbz.2009.0189.

Lempereur, L., Lebrun, M., Cuvelier, P., Sépult, G., Caron, Y., Saegerman, C., Shiels, B., Losson, B., 2012. Longitudinal field study on bovine *Babesia* spp. and *Anaplasma* phagocytophilum infections during a grazing season in Belgium. Parasitol. Res. 110, 1525–1530. https://doi.org/10.1007/s00436-011-2657-0.

Lindblom, A., Wallménius, K., Sjöwall, J., Fryland, L., Wilhelmsson, P., Lindgren, P.E., Forsberg, P., Nilsson, K., 2016. Prevalence of *Rickettsia* spp. in ticks and serological and clinical outcomes in tick-bitten individuals in Sweden and on the Åland islands. PLoS ONE 11, e0166653. https://doi.org/10.1371/journal.pone.0166653.

Lotric-Furlan, S., Rojko, T., Petrovec, M., Avsic-Zupanc, T., Strle, F., 2006. Epidemiological, clinical and laboratory characteristics of patients with human granulocytic anaplasmosis in Slovenia. Wien. Klin. Wochenschr. 118, 708–713. https://doi.org/10.1007/s00508-006-0700-4.

Matei, I.A., Estrada-Peña, A., Cutler, S.J., Vayssier-Taussat, M., Varela-Castro, L., Potkonjak, A., Zeller, H., Mihalca, A.D., 2019. A review on the eco-epidemiology and clinical management of human granulocytic anaplasmosis and its agent in Europe. Parasites Vect. 12, 599. https://doi.org/10.1186/s13071-019-3852-6.

May, K., Strube, C., 2014. Prevalence of *Rickettsiales* (*Anaplasma phagocytophilum* and *Rickettsia* spp.) in hard ticks (*Ixodes ricinus*) in the city of Hamburg. Germany. Parasitol. Res. 113, 2169–2175. https://doi.org/10.1007/s00436-014-3869-x.

Michelet, L., Delannoy, S., Devillers, E., Umhang, G., Aspan, A., Juremalm, M., Chirico, J., van der Wal, F.J., Sprong, H., Boye Pihl, T.P., Klitgaard, K., Bødker, R., Fach, P., Moutailler, S., 2014. High-throughput screening of tick-borne pathogens in Europe. Front. Cell. Infect. Microbiol. 4, 103. https://doi.org/10.3389/fcimb.2014.00103.

Mysterud, A., Easterday, W.R., Qviller, L., Viljugrein, H., Ytrehus, B., 2013. Spatial and seasonal variation in the prevalence of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sensu lato in questing *Ixodes ricinus* ticks in Norway. Parasites Vect. 6, 187. https://doi.org/10.1186/1756-3305-6-187.

Nilsson, K., 2009. Septicaemia with *Rickettsia helvetica* in a patient with acute febrile illness, rash and myasthenia. J. Infect. 58, 79–82. https://doi.org/10.1016/j.jinf.2008.06.005.

Nilsson, K., Lindquist, O., Pahlson, C., 1999a. Association of *Rickettsia helvetica* with chronic perimyocarditis in sudden cardiac death. Lancet 354, 1169–1173. https://doi.org/10.1016/s0140-6736(99)04093-3.

Nilsson, K., Lindquist, O., Liu, A.J., Jaenson, T.G., Friman, G., Påhlson, C., 1999b. *Rickettsia helvetica* in *Ixodes ricinus* ticks in Sweden. J. Clin. Microbiol. 37, 400–403. https://doi.org/10.1128/jcm.37.2.400-403.1999.

Nilsson, K., Elfving, K., Pahlson, C., 2010. *Rickettsia helvetica* in patient with meningitis, Sweden, 2006. Emerg. Infect. Dis. 16, 490–492. https://doi.org/10.3201/eid1603.090184.

Nilsson, K., Wallmenius, K., Pahlson, C., 2011. Coinfection with *Rickettsia helvetica* and Herpes Simplex Virus 2 in a Young Woman with Meningoencephalitis. Case Rep. Infect. Dis., 469194 https://doi.org/10.1155/2011/469194.

Olsthoorn, F., Sprong, H., Fonville, M., Rocchi, M., Medlock, J., Gilbert, L., Ghazoul, J., 2021. Occurrence of tick-borne pathogens in questing *Ixodes ricinus* ticks from Wester Ross, Northwest Scotland. Parasites Vect. 14, 430. https://doi.org/10.1186/s13071-021-04946-5.

Oteo, J.A., Portillo, A., 2012. Tick-borne rickettsioses in Europe. TicksTick Borne Dis. 3, 271–278. https://doi.org/10.1016/j.ttbdis.2012.10.035.

Overzier, E., Pfister, K., Herb, I., Mahling, M., Böck Jr., G., Silaghi, C., 2013a. Detection of tick-borne pathogens in roe deer (*Capreolus capreolus*), in questing ticks (*Ixodes ricinus*), and in ticks infesting roe deer in southern Germany. Ticks Tick Borne Dis. 4, 320–328. https://doi.org/10.1016/j.ttbdis.2013.01.004.

Overzier, E., Pfister, K., Thiel, C., Herb, I., Mahling, M., Silaghi, C., 2013b. *Anaplasma phagocytophilum* in questing *Ixodes ricinus* ticks: comparison of prevalences and partial *16S rRNA* gene variants in urban, pasture, and natural habitats. Appl. Environ. Microbiol. 79, 1730–1734. https://doi.org/10.1128/aem.03300-12.

Page, S., Daschkin, C., Anniko, S., Krey, V., Nicolaus, C., Maxeiner, H.G., 2018. First report of *Borrelia miyamotoi* in an *Ixodes ricinus* tick in Augsburg, Germany. Exp. Appl. Acarol. 74, 191–199. https://doi.org/10.1007/s10493-018-0220-8.

Pedersen, B.N., Jenkins, A., Paulsen, K.M., Okbaldet, Y.B., Edgar, K.S., Lamsal, A., Soleng, A., Andreassen Å, K., 2020. Distribution of *Neoehrlichia mikurensis* in *Ixodes ricinus* ticks along the coast of Norway: the western seaboard is a low-prevalence region. Zoonoses Public Health 67, 130–137. https://doi.org/10.1111/zph.12662.

Platonov, A.E., Karan, L.S., Kolyasnikova, N.M., Makhneva, N.A., Toporkova, M.G., Maleev, V.V., Fish, D., Krause, P.J., 2011. Humans infected with relapsing fever spirochete *Borrelia miyamotoi*. Russia. Emerging Infect. Dis. 17, 1816–1823. https://doi.org/10.3201/eid1710.101474.

Quarsten, H., Skarpaas, T., Fajs, L., Noraas, S., Kjelland, V., 2015. Tick-borne bacteria in *Ixodes ricinus* collected in southern Norway evaluated by a commercial kit and established real-time PCR protocols. Ticks Tick Borne Dis. 6, 538–544. https://doi.org/10.1016/j.ttbdis.2015.04.008.

Quarsten, H., Salte, T., Lorentzen Å, R., Hansen, I.J.W., Hamre, R., Forselv, K.J.N., Øines, Ø., Wennerås, C., Noraas, S., 2021. Tick-borne pathogens detected in the blood of immunosuppressed norwegian patients living in a tick-endemic area. Clin. Infect. Dis. 73, e2364–e2371. https://doi.org/10.1093/cid/ciaa971.

Răileanu, C., Tauchmann, O., Vasić, A., Wöhnke, E., Silaghi, C., 2020. *Borrelia miyamotoi* and *Borrelia burgdorferi* (sensu lato) identification and survey of tick-borne encephalitis virus in ticks from north-eastern Germany. Parasites Vect. 13, 106. https://doi.org/10.1186/s13071-020-3969-7.

Richter, D., Matuschka, F.R., 2012. "*Candidatus* Neoehrlichia mikurensis," *Anaplasma phagocytophilum*, and lyme disease spirochetes in questing european vector ticks and in feeding ticks removed from people. J. Clin. Microbiol. 50, 943–947. https://doi.org/10.1128/jcm.05802-11.

Rosef, O., Paulauskas, A., Radzijevskaja, J., 2009a. Prevalence of *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* in questing *Ixodes ricinus* ticks in relation to the density of wild cervids. Acta Vet. Scand. 51, 47. https://doi.org/10.1186/1751-0147-51-47.

Rosef, O., Radzijevskaja, J., Paulauskas, A., Haslekås, C., 2009b. The prevalence of *Anaplasma phagocytophilum* in host-seeking *Ixodes ricinus* ticks in Norway. Clin. Microbiol. Infect. 15 (Suppl 2), 43–45. https://doi.org/10.1111/j.1469-0691.2008.02169.x.

Rudzinska, M.A., Trager, W., Lewengrub, S.J., Gubert, E., 1976. An electron microscopic study of *Babesia microti* invading erythrocytes. Cell Tissue Res. 169, 323–334. https://doi.org/10.1007/bf00219605.

Ruyts, S.C., Tack, W., Ampoorter, E., Coipan, E.C., Matthysen, E., Heylen, D., Sprong, H., Verheyen, K., 2018. Year-to-year variation in the density of *Ixodes ricinus* ticks and the prevalence of the rodent-associated human pathogens *Borrelia afzelii* and *B. miyamotoi* in different forest types. Ticks Tick Borne Dis. 9, 141–145. https://doi.org/10.1016/j.ttbdis.2017.08.008.

Sands, B., Lihou, K., Lait, P., Wall, R., 2022. Prevalence of *Babesia* spp. pathogens in the ticks *Dermacentor reticulatus* and *Ixodes ricinus* in the UK. Acta Trop. 236, 106692 https://doi.org/10.1016/j.actatropica.2022.106692.

Schicht, S., Junge, S., Schnieder, T., Strube, C., 2011. Prevalence of *Anaplasma phagocytophilum* and coinfection with *Borrelia burgdorferi* sensu lato in the hard tick *Ixodes ricinus* in the city of Hanover (Germany). Vector Borne Zoonotic Dis. 11, 1595–1597. https://doi.org/10.1089/vbz.2011.0699.

Schorn, S., Pfister, K., Reulen, H., Mahling, M., Manitz, J., Thiel, C., Silaghi, C., 2011. Prevalence of *Anaplasma phagocytophilum* in *Ixodes ricinus* in Bavarian public parks, Germany. Ticks Tick Borne Dis. 2, 196–203. https://doi.org/10.1016/j.ttbdis.2011.09.009.

Severinsson, K., Jaenson, T.G., Pettersson, J., Falk, K., Nilsson, K., 2010. Detection and prevalence of *Anaplasma phagocytophilum* and *Rickettsia helvetica* in *Ixodes ricinus* ticks in seven study areas in Sweden. Parasites Vect. 3, 66. https://doi.org/10.1186/1756-3305-3-66.

Shuker, O., Subran, M., Hardie, R., Ghitan, M., Chapnick, E.K., Lin, Y.S., 2018. An 85-year-old man with recurrent fever and multiple splenic infarcts. Infect. Dis. Clin. Pract. (Baltim *Md*) 26, 300–302. https://doi.org/10.1097/ipc.0000000000000643.

Silaghi, C., Gilles, J., Höhle, M., Fingerle, V., Just, F.T., Pfister, K., 2008a. *Anaplasma phagocytophilum infection in Ixodes ricinus*, Bavaria, Germany. Emerg. Infect. Dis. 14, 972–974. https://doi.org/10.3201/eid1406.061513.

Silaghi, C., Gilles, J., Höhle, M., Pradel, I., Just, F.T., Fingerle, V., Küchenhoff, H., Pfister, K., 2008b. Prevalence of spotted fever group rickettsiae in *Ixodes ricinus* (Acari: *ixodidae*) in southern Germany. J. Med. Entomol. 45, 948–955. https://doi.org/10.1603/0022-2585(2008)45[948:*posfgr*]2.0.co;2.

Silaghi, C., Hamel, D., Thiel, C., Pfister, K., Pfeffer, M., 2011. Spotted fever group *rickettsiae* in ticks. Germany. Emerg. Infect. Dis. 17, 890–892. https://doi.org/10.3201/eid1705.101445.

Silaghi, C., Woll, D., Hamel, D., Pfister, K., Mahling, M., Pfeffer, M., 2012a. *Babesia* spp. and *Anaplasma phagocytophilum* in questing ticks, ticks parasitizing rodents and the parasitized rodents–analyzing the host-pathogen-vector interface in a metropolitan area. Parasites Vect. 5, 191. https://doi.org/10.1186/1756-3305-5-191.

Silaghi, C., Woll, D., Mahling, M., Pfister, K., Pfeffer, M., 2012b. *Candidatus* Neoehrlichia mikurensis in rodents in an area with sympatric existence of the hard ticks *Ixodes ricinus* and *Dermacentor reticula*tus, Germany. Parasites Vect. 5, 285. https://doi.org/10.1186/1756-3305-5-285.

Sjöwall, J., Kling, K., Ochoa-Figueroa, M., Zachrisson, H., Wennerås, C., 2021. *Neoehrlichia mikurensis* causing thrombosis and relapsing fever in a lymphoma patient receiving rituximab. Microorganisms 9. https://doi.org/10.3390/microorganisms9102138.

Skarphédinsson, S., Lyholm, B.F., Ljungberg, M., Søgaard, P., Kolmos, H.J., Nielsen, L.P., 2007. Detection and identification of *Anaplasma phagocytophilum, Borrelia burgdorferi*, and *Rickettsia helvetica* in Danish *Ixodes ricinus* ticks. APMIS 115, 225–230. https://doi.org/10.1111/j.1600-0463.2007.apm_256.x.

Soleng, A., Kjelland, V., 2013. *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* in *Ixodes ricinus* ticks in Brønnøysund in northern Norway. Ticks Tick Borne Dis 4, 218–221. https://doi.org/10.1016/j.ttbdis.2012.11.006.

Sprong, H., Wielinga, P.R., Fonville, M., Reusken, C., Brandenburg, A.H., Borgsteede, F., Gaasenbeek, C., van der Giessen, J.W., 2009. *Ixodes ricinus* ticks are reservoir hosts for *Rickettsia helvetica* and potentially carry flea-borne *Rickettsia* species. Parasites Vectors 2, 41. https://doi.org/10.1186/1756-3305-2-41.

Sprong, H., Azagi, T., Hoornstra, D., Nijhof, A.M., Knorr, S., Baarsma, M.E., Hovius, J.W., 2018. Control of Lyme borreliosis and other *Ixodes ricinus*-borne diseases. Parasites Vect. 11, 145. https://doi.org/10.1186/s13071-018-2744-5.

Stewart, A.G., Stewart, A.G.A., 2021. An update on the laboratory diagnosis of *Rickettsia* spp. Infect. Pathog. 10 https://doi.org/10.3390/pathogens10101319.

Stigum, V.M., Jaarsma, R.I., Sprong, H., Rolandsen, C.M., Mysterud, A., 2019. Infection prevalence and ecotypes of *Anaplasma phagocytophilum* in moose *Alces alces*, red deer *Cervus elaphus*, roe deer *Capreolus capreolus* and *Ixodes ricinus* ticks from Norway. Parasites Vect. 12, 1. https://doi.org/10.1186/s13071-018-3256-z.

Sudhindra, P., Wang, G., Schriefer, M.E., McKenna, D., Zhuge, J., Krause, P.J., Marques, A.R., Wormser, G.P., 2016. Insights into *Borrelia miyamotoi* infection from an untreated case demonstrating relapsing fever, monocytosis and a positive C6 Lyme serology. Diagn. Microbiol. Infect. Dis. 86, 93–96. https://doi.org/10.1016/j.diagmicrobio.2016.06.015.

Svendsen, C.B., Krogfelt, K.A., Jensen, P.M., 2009. Detection of *Rickettsia* spp. in Danish ticks (Acari: *ixodes ricinus*) using real-time PCR. Scand. J. Infect. Dis. 41, 70–72. https://doi.org/10.1080/00365540802530653.

Szekeres, S., Lügner, J., Fingerle, V., Margos, G., Földvári, G., 2017. Prevalence of *Borrelia miyamotoi* and *Borrelia burgdorferi* sensu lato in questing ticks from a recreational coniferous forest of East Saxony, Germany. Ticks Tick Borne Dis. 8, 922–927. https://doi.org/10.1016/j.ttbdis.2017.08.002.

Takumi, K., Hofmeester, T.R., Sprong, H., 2021. Red and fallow deer determine the density of *Ixodes ricinus* nymphs containing *Anaplasma phagocytophilum*. Parasites Vect. 14, 59. https://doi.org/10.1186/s13071-020-04567-4.

Tappe, J., Strube, C., 2013. *Anaplasma phagocytophilum* and *Rickettsia* spp. infections in hard ticks (*Ixodes ricinus*) in the city of Hanover (Germany): revisited. Ticks Tick Borne Dis. 4, 432–438. https://doi.org/10.1016/j.ttbdis.2013.04.009.

Tavakolpour, S., Alesaeidi, S., Darvishi, M., GhasemiAdl, M., Darabi-Monadi, S., Akhlaghdoust, M., Elikaei Behjati, S., Jafarieh, A., 2019. A comprehensive review of rituximab therapy in rheumatoid arthritis patients. Clin. Rheumatol. 38, 2977–2994. https://doi.org/10.1007/s10067-019-04699-8.

Tijsse-Klasen, E., Jameson, L.J., Fonville, M., Leach, S., Sprong, H., Medlock, J.M., 2011. First detection of spotted fever group *rickettsiae* in *Ixodes ricinus* and *Dermacentor reticulatus* ticks in the UK. Epidemiol. Infect. 139, 524–529. https://doi.org/10.1017/s0950268810002608.

Tveten, A.K., 2014. Prevalence and diversity among *Anaplasma phagocytophilum* strains originating from *Ixodes ricinus* ticks from Northwest Norway. J. Pathog. 824897 https://doi.org/10.1155/2014/824897.

Wagemakers, A., Staarink, P.J., Sprong, H., Hovius, J.W., 2015. *Borrelia miyamotoi*: a widespread tick-borne relapsing fever spirochete. Trends Parasitol. 31, 260–269. https://doi.org/10.1016/j.pt.2015.03.008.

Wagemakers, A., Jahfari, S., de Wever, B., Spanjaard, L., Starink, M.V., de Vries, H.J.C., Sprong, H., Hovius, J.W., 2017. *Borrelia miyamotoi* in vectors and hosts in The Netherlands. Ticks Tick Borne Dis. 8, 370–374. https://doi.org/10.1016/j.ttbdis.2016.12.012.

Wallménius, K., Pettersson, J.H., Jaenson, T.G., Nilsson, K., 2012. Prevalence of *Rickettsia* spp., *Anaplasma phagocytophilum*, and *Coxiella burnetii* in adult *Ixodes ricinus* ticks from 29 study areas in central and southern Sweden. Ticks Tick Borne Dis. 3, 100–106. https://doi.org/10.1016/j.ttbdis.2011.11.003.

Wass, L., Grankvist, A., Mattsson, M., Gustafsson, H., Krogfelt, K., Olsen, B., Nilsson, K., Mårtensson, A., Quarsten, H., Henningsson, A.J., Wennerås, C., 2018. Serological reactivity to *Anaplasma phagocytophilum* in neoehrlichiosis patients. Euro. J. Clin. Microbiol. Infect. Dis. 37, 1673–1678. https://doi.org/10.1007/s10096-018-3298-3.

Wass, L., Grankvist, A., Bell-Sakyi, L., Bergström, M., Ulfhammer, E., Lingblom, C., Wennerås, C., 2019. Cultivation of the causative agent of human neoehrlichiosis from clinical isolates identifies vascular endothelium as a target of infection. Emerg. Microbes Infect. 8, 413–425. https://doi.org/10.1080/22221751.2019.1584017.

Welc-Faleciak, R., Sinski, E., Kowalec, M., Zajkowska, J., Pancewicz, S.A., 2014. Asymptomatic "*Candidatus* Neoehrlichia mikurensis" infections in immunocompetent humans. J. Clin. Microbiol. 52, 3072–3074. https://doi.org/10.1128/jcm.00741-14.

Wenneras, C., 2015. Infections with the tick-borne bacterium *Candidatus* Neoehrlichia mikurensis. Clin. Microbiol. Infect. 21, 621–630. https://doi.org/10.1016/j.cmi.2015.02.030.

Wielinga, P.R., Gaasenbeek, C., Fonville, M., de Boer, A., de Vries, A., Dimmers, W., Akkerhuis Op Jagers, G., Schouls, L.M., Borgsteede, F., van der Giessen, J.W., 2006. Longitudinal analysis of tick densities and *Borrelia, Anaplasma*, and *Ehrlichia*

infections of *Ixodes ricinus* ticks in different habitat areas in The Netherlands. Appl. Environ. Microbiol. 72, 7594–7601. https://doi.org/10.1128/aem.01851-06.

Wielinga, P.R., Fonville, M., Sprong, H., Gaasenbeek, C., Borgsteede, F., van der Giessen, J.W., 2009. Persistent detection of *Babesia* EU1 and *Babesia microti* in *Ixodes ricinus* in the Netherlands during a 5-year surveillance: 2003-2007. Vector Borne Zoonotic Dis. 9, 119–122. https://doi.org/10.1089/vbz.2008.0047.

Øines, Ø., Radzijevskaja, J., Paulauskas, A., Rosef, O., 2012. Prevalence and diversity of *Babesia* spp. in *questing Ixodes ricinus ticks from Norway*. Parasites Vect. 5, 156. https://doi.org/10.1186/1756-3305-5-156.