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 Jonkoping, County Hospital Ryhov, Jonkoping 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, Hannover 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...
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 (Blazekaj et al., 2017; Coipan et al., 2013; Flatterty 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; Lemperere 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; Rosell et al., 2009a, 2009b; Schicht et al., 2011; Schorn et al., 2011; Silaghi et al., 2008a, 2012a; Skarpédinson et al., 2007; Soleng and Kjelland, 2013; Stigum et al., 2019; Takumi et al., 2021; Tappe and Strube, 2013; Tveten, 2014; Wallménus et al., 2012; Wielinga et al., 2006), whereas the rate of B. miyamotoi commonly is low and around 1% (Blazekaj et al., 2018; Cochez et al., 2015; Cuill et al., 2021; Eshoo et al., 2014; Fraenkel et al., 2002; Hansford et al., 2015; Heylen et al., 2016; Jahfari et al., 2012; Jenkins et al., 2019; 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; Raileanu et al., 2020; Ruys 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., 2015, 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., 2016; Hvidsten et al., 2020; Kantso 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; Skarpédinson et al., 2007; Spong et al., 2009; Svendsen et al., 2009; Tappe and Strube, 2013; Tijssen-Klaseen et al., 2011; Wallménus 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; Lemperere et al., 2012; Michelet et al., 2014; Olsthoorn et al., 2021; Overzier 2013a; Sands et al., 2022; J. 2021).}

---

Table 1

<table>
<thead>
<tr>
<th>Pathogen prevalence (%)</th>
<th>Belgium</th>
<th>Denmark</th>
<th>Germany</th>
<th>The Netherlands</th>
<th>Norway</th>
<th>UK</th>
<th>Sweden</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anaplasma phagocytophilum</strong></td>
<td>1.2–6.5</td>
<td>1–5 (0.4–24)</td>
<td>1–5 (0–25)</td>
<td>1–5 (0–11)</td>
<td>1–3 (0–19)</td>
<td>1–5 (0–20)</td>
<td>0.5–5 (0.5–15)</td>
</tr>
<tr>
<td><strong>Neoehrlichia mikurensis</strong></td>
<td>0.4–3</td>
<td>1–5 (0–13)</td>
<td>10–20 (8–27)</td>
<td>5–10 (0.4–16)</td>
<td>5–15 (0.5–25)</td>
<td>0</td>
<td>1–5 (0–11)</td>
</tr>
<tr>
<td><strong>Borrelia miyamotoi</strong></td>
<td>0.4–1.4</td>
<td>0–2.4</td>
<td>0–3.0</td>
<td>2–3.8</td>
<td>0.5–1.3</td>
<td>0.1</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Rickettsia helvetica</strong></td>
<td>17</td>
<td>5–10 (1–15)</td>
<td>5–20 (2–52)</td>
<td>5–30 (4.5–66)</td>
<td>1 (1–5)</td>
<td>0–6.5</td>
<td>5–10 (1.5–22)</td>
</tr>
<tr>
<td><strong>Babesia spp</strong></td>
<td>8</td>
<td>0–1.5</td>
<td>0.4–11</td>
<td>0–4.5</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>0.2–3</td>
</tr>
<tr>
<td>Bab. divergens</td>
<td>Present*</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Bab. venatorum</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Bab. microti</td>
<td>Present</td>
<td>Not detected</td>
<td>Present</td>
<td>Present</td>
<td>Not detected</td>
<td>Present*</td>
<td>Sporadic</td>
</tr>
</tbody>
</table>

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 obtained 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; Lemperere 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.

<table>
<thead>
<tr>
<th>Pathogen causing disease</th>
<th>Methods (listed in recommended order)</th>
<th>Patient material</th>
<th>Timing of test</th>
<th>Methodological limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. phagocytophilum</em></td>
<td>Commercial or in-house PCR</td>
<td>Whole blood</td>
<td>Symptomatic phase</td>
<td>16S RNA sequencing has commonly lower sensitivity than PCR</td>
</tr>
<tr>
<td></td>
<td>16S RNA sequencing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serology (primarily IFA, IgG)</td>
<td>Serum</td>
<td>2-4 weeks apart</td>
<td></td>
<td>Mainly demonstrates recent or past infection; False positive reactions due to cross-reactive antibodies Weak/false negative reactions in immunosuppressed patients</td>
</tr>
<tr>
<td>Blood smear</td>
<td>Whole blood</td>
<td>Symptomatic phase</td>
<td></td>
<td>Low sensitivity; Lack of experienced test personnel</td>
</tr>
<tr>
<td><em>N. mikurensis</em></td>
<td>In-house PCR</td>
<td>Whole blood</td>
<td>Symptomatic phase</td>
<td>Only currently available diagnostic test; 16S RNA sequencing has lower sensitivity than specific PCR</td>
</tr>
<tr>
<td></td>
<td>16S RNA sequencing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. miyamotoi</em></td>
<td>Commercial/in-house PCR</td>
<td>Whole blood</td>
<td>Symptomatic phases</td>
<td>May be false negative between fever episodes; 16S RNA sequencing has commonly low sensitivity than PCR</td>
</tr>
<tr>
<td></td>
<td>16S RNA sequencing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serology</td>
<td>Relapsing fever group gpQ-assay</td>
<td>Serum</td>
<td>2-4 weeks apart</td>
<td>Mainly demonstrates recent or past infection; Only a few specialized laboratories in Europe Weak/false negative in immunosuppressed patients</td>
</tr>
<tr>
<td>(immunoblot)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rickettsia spp</em> (including <em>Rickettsia helvetica</em>)</td>
<td>Serology (as above)</td>
<td>Serum</td>
<td>2-4 weeks apart</td>
<td>Mainly demonstrates recent or past infection; Weak/false negative in immunosuppressed patients</td>
</tr>
<tr>
<td></td>
<td>Commercial or in-house PCR</td>
<td>Whole blood</td>
<td>Symptomatic phase</td>
<td>Sensitivity depends on testing of relevant patient material; 16S RNA sequencing has commonly lower sensitivity than PCR</td>
</tr>
<tr>
<td></td>
<td>16S RNA sequencing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood smear (Giemsa stained)</td>
<td>Whole blood</td>
<td>Symptomatic phase</td>
<td></td>
<td>Rapid but less sensitive than PCR; Lack of experienced test personnel; Findings may be misinterpreted as malaria</td>
</tr>
<tr>
<td>Serology (IFA)</td>
<td>Serum</td>
<td>2-4 weeks apart</td>
<td></td>
<td>Mainly demonstrates recent or past infection; Weak/false negative in immunosuppressed patients</td>
</tr>
</tbody>
</table>

IFA: Indirect fluorescence antibody test.
be done at local hospital laboratories, but it may be mistaken for the detection of A. phagocytophilum. Patients is less useful due to low diagnostic sensitivity for European and even life-threatening making it crucial to identify such patients. In immunosuppressed patients, the symptoms can be more severe and making it challenging to interpret serologic findings. Cross-reactive antibodies, especially of IgM class, may also be misleading. Antibodies produced against A. phagocytophilum 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 (Hoonstra 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; Wele-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; Dumin 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 (Henningson 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 (Sjowall 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
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
Azagi, T., Jaarsma, R.I., Docters van Leeuwen, A., Fonville, M., Maas, M., Franssen, F.F.
Gandy, S., Hansford, K., McGinley, L., Cull, B., Smith, R., Semper, A., Brooks, T.,
Surveill. 17.
The prevalence and distribution of Anaplasma phagocytophilum genotypes in
Ixodes ricinus nymphs collected from farm- and woodland sites in Ireland. Ticks Tick Borne
pathogens in host-seeking and host-finding ticks within a single natural
disease agents and several emerging pathogens in questing ticks from the German
disease in regard to small mammal and tick populations from Saxony, Germany.
Parasites Vect. 12, 131. https://doi.org/10.3390/pv12010013.
Gandy, S., Hansford, K., McGlinsey, L., Cull, B., Smith, R., Semper, A., Brooks, B.,
Anaplasma phagocytophilum in questing Ixodes ricinus nymphs across twenty
recreational areas in England and Wales. Ticks Tick Borne Dis 13, 101965. https://doi.
org/10.1002/ttbdis.2022101965.
Grankvist, A., Andersson, P.O., Mattsson, M., Sander, M., Vah, H., Hoper, L.,
Sakiniene, E., Trysberg, E., Stenson, M., Fehr, J., Pekova, S., Bogdan, C.,
Bloomberg, G., Wenneers, C., 2014. Infections with the tick-borne bacterium
" Candidatus Neoehrlichia mikurensis" mimic noninfectious conditions in patients
https://doi.org/10.1093/cid/ciu189.
Grankvist, A., Sandelin, L., Andersson, J., Fryland, L., Willemsho, P., Lindgren, P.E.,
Forssberg, P., Wenneers, C., 2015. Infections with Candidatus Neoehrlichia mikurensis
Granquist, E.G., Kristiansson, M., Lindgren, P.E., Matussek, A., Niedrig, A., Okstad, T.,
Stuen, S., 2014. Evaluation of microbial communities and symbionts in Ixodes ricinus
nymphs and ungulate hosts ( Cervus elaphus and Ovis aries) from shared habitats on the west
ttbdis.2014.05.005.
Ixodes Ricinus hosts ( Ixodes ricinus)–borne infections in Europe. Pathogens. 9
10.3390/pathogens10040386.
Havukainen, M., Niemi, K., Sandgren, J., Cervin, M., Kallio, S., Lindberg, T.,
Kettunen, P., Myllypuisto, J., Roine, R., 2006. Prevalence of Babesia spp. in
questing ticks in Finland and overwintering deer on the west
06506-4.
sp. in northern Germany: occurrence and potential vector role for Borrelia
sp., Rickettsia sp., and Anaplasma phagocytophilum in comparison with Ixodes
2082-9.
sp. in northern Germany: occurrence and potential vector role for Borrelia
sp., Rickettsia sp., and Anaplasma phagocytophilum in comparison with Ixodes
2082-9.
Havukainen, M., Niemi, K., Sandgren, J., Cervin, M., Kallio, S., Lindberg, T.,
Kettunen, P., Myllypuisto, J., Roine, R., 2006. Prevalence of Babesia spp. in
questing ticks in Finland and overwintering deer on the west
06506-4.
sp. in northern Germany: occurrence and potential vector role for Borrelia
sp., Rickettsia sp., and Anaplasma phagocytophilum in comparison with Ixodes
2082-9.
sp. in northern Germany: occurrence and potential vector role for Borrelia
sp., Rickettsia sp., and Anaplasma phagocytophilum in comparison with Ixodes
2082-9.
sp. in northern Germany: occurrence and potential vector role for Borrelia
sp., Rickettsia sp., and Anaplasma phagocytophilum in comparison with Ixodes
2082-9.
sp. in northern Germany: occurrence and potential vector role for Borrelia
sp., Rickettsia sp., and Anaplasma phagocytophilum in comparison with Ixodes
2082-9.
sp. in northern Germany: occurrence and potential vector role for Borrelia
sp., Rickettsia sp., and Anaplasma phagocytophilum in comparison with Ixodes
2082-9.
