Blood donor *Borrelia burgdorferi* sensu lato seroprevalence and history of tick bites at a northern limit of the vector distribution

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In order to study the antibody seroprevalence of the causal agent of Lyme borreliosis, *Borrelia burgdorferi* sensu lato (s.l.), and the history of tick bites at a geographical distribution limit of *Ixodes ricinus*, we compared healthy blood donors in geographically extreme regions: the borreliosis-endemic Vestfold County (59° N) and the region of northern Norway. Blood samples were screened using IgG/VlsE ELISA, and positive/borderline samples were confirmed using C6 ELISA and immunoblot assays. Also, donors completed a questionnaire consisting of several items including the places they have lived, and whether they owned any pets. The seroprevalence was 0.48% (5/1048) in northern Norway and 9.25% (48/519) in Vestfold County. Seven donors (of 1048) had experienced a single tick bite in the southern part of Nordland County (65° N) in northern Norway. This first study on *B. burgdorferi* s.l. antibody seroprevalence and tick bites on humans and pets in northern Norway showed that the seroprevalence of *B. burgdorferi* s.l. infection and the risk of tick bite in northern Norway are insignificant; the fact that only five positive IgG samples were detected underscores the very low background seroprevalence. These results suggest that so far *I. ricinus* has not expanded north of the previously established geographical distribution limit.

Key words: *Ixodes ricinus*; VlsE protein; *Borrelia burgdorferi*; surveys and questionnaires; Arctic regions; tick bites.

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*Borrelia burgdorferi* sensu lato (s.l.), the agent of Lyme borreliosis, is transmitted in Europe by the common tick, *Ixodes ricinus*. The tick is found throughout the region (1), from the southern Europe to Scandinavia except Iceland (2). Although the exact tick distribution limit in northwestern Europe has not been precisely established, it is well known that *I. ricinus* occurs in the northern region of Norway (3, 4). Hvidsten et al. (5) showed that northwards from the district neighbouring Brønnøy (65° 28′ N) in Nordland County, there was a negative gradient of the number of *I. ricinus* collected from pets. Furthermore, the prevalence of *B. burgdorferi* s.l. in ticks was much higher in Brønnøy compared with the regions north of Brønnøy: 29% and 4%, respectively. In addition to *I. ricinus*, two other ixodid tick species undergo a full life cycle in northern Norway: the seabird tick, *Ixodes uriae*, which is infected with *B. burgdorferi* s.l. (6), and the rodent tick, *Ixodes trianguliceps*, which very rarely bites humans and is not known to transmit *B. burgdorferi* s.l. (7).

In Norway, all manifestations of Lyme borreliosis, except *erythema migrans*, are reported to the...
MATERIALS AND METHODS

The study consisted of two parts: (i) the analysis of blood donor serum samples for anti-
Borrelia IgG antibodies (supplemented by two different, confirmatory tests), and (ii) a questionnaire. As a part of routine blood donation, donors in 14 blood banks in the four counties were asked to participate in the study (Fig. 1; Table 1). The number of participants in each county was chosen to find a difference with acceptable statistical power between positive donors in Vestfold County and northern Norway.

Blood samples

A quantity of 5 mL of blood was drawn from each donor and transferred to the departments of medical microbiology in Tromsø, Bodø and Tønsberg. After centrifugation, sera were divided into three tubes of approximately 1 mL each and stored in –20 °C freezers. Sera in northern Norway were transferred in frozen condition to the accredited microbiological department at the Vestfold Hospital Trust, Tønsberg, where all Borrelia antibody analyses were performed.

Borrelia serological testing

All samples were screened for anti-B. burgdorferi s.l. IgG antibodies with an enzyme-linked immunosorbent assay (ELISA), Enzygnost Lyme link IgG/VlsE (variable major protein-like sequence, expressed) (Siemens, Marburg, Germany), hereafter designated ‘IgG’. This assay consists of a mixture of native sonicated whole-cell antigen from Borrelia afzelii strain PKo and recombinant VlsE for anti-
B. burgdorferi s.l. IgG antibodies from three human pathogenic genospecies of B. burgdorferi s.l.: B. afzelii, Borrelia garinii and B. burgdorferi sensu stricto (s.s.). The kit insert states that the specificity is 98–100%, and the sensitivity is 100% (in ‘borreliosis stage III’). Only samples with positive or borderline results were examined with C6; the C6 Lyme ELISA kit (Immunetics, Boston, MA, USA) is based on a synthetic peptide antigen, C6 peptide, derived from the non-variable region 6 on the VlsE surface protein of the three genospecies of B. burgdorferi s.l. (as above). The assay does not distinguish between anti-Borrelia IgG and IgM antibodies. The specificity is 99%, according to the kit insert; the sensitivity is 100% (if the Western blot IgG confirmatory test was positive).

Only the borderline and positive IgG samples were retested in duplicate with C6, and, according to the algorithm (Fig. 2), analysed further with immunoblot, recomLine Borrelia IgG (Mikrogen Diagnostik, Neuried, Germany). This is a qualitative immunoblot for detection of IgG antibodies against B. burgdorferi s.s., B. afzelii, B. garinii, Borrelia bavariensis and Borrelia spielmani. According to the kit insert, the specificity is 100%, and the sensitivity is 96–100% (‘borreliosis stage III’). Results of all three assays were interpreted following the manufacturers’ instructions (see algorithm Fig. 2.). The standard deviation and coefficient of variation (CV%) of positive controls were 0.31 (3.8%) and 0.31 (5.5%) for IgG and C6, respectively.

Questionnaire

The blood donors completed a questionnaire consisting of several items including basic data (age in years, gender, postal code and profession), one question asked for the Norwegian municipalities or any foreign countries where the donor had lived. One question (Have you ever experienced a tick bite?) was about tick bites (single or multiple) in donors and/or in their pets – if the donors were or had been pet owners.

We anticipate that tick bites on pets represent a more sensitive expression of tick presence in a geographical area than tick bites on humans. A statement of the probable geographical origin of tick bites was essential for the study. The questionnaire examined whether the donors were familiar with tick encounters, particular those living in northern Norway. The donors were asked about tick bite prophylaxis and knowledge of common symptoms and signs of borreliosis. Lastly, we asked for permission to obtain additional information, if deemed necessary.

In general, many seroprevalence studies in blood donors have been performed outside the summer season. But this study focused on the long-term prevalence of tick

Norwegian Surveillance System for Communicable Diseases (MSIS) (8). Shortly before the present study, in the decade 2003–12, the yearly average incidence of Lyme borreliosis in Norway was 5.4 per 100 000 population. This number contrasts with the average yearly incidence in the region of northern Norway which was only 0.8 per 100 000 population. During the last 25 years (1991–2015), only 58 cases have been reported in the counties of northern Norway, Nordland, Troms and Finnmark, defined as the region of northern Norway. This region, in which the Arctic Circle is located, comprises the northernmost and easternmost part of the country.

The purpose of this study was to study the prevalence of anti-Borrelia IgG antibodies in healthy blood donors and history of tick bites in humans and pets. In the light of climate changes, we anticipated that the consequences of the potential changes in the tick’s prevalence would be manifested first at a distribution limit of I. ricinus (9). No such study has been conducted in northern Norway before, so no earlier study on the history of tick bites exists for comparison, in contrast to a Swedish study (10). Completion of questionnaire by donors in northern Norway provided data on the burden of ticks in the region, which was compared with the situation in a county with an established tick population. Vestfold County (59° N, located at the western coast of the Oslofjord) is such a county, and it reports the sixth highest incidence of Lyme borreliosis in the country, 10 times higher than that found in northern Norway. Therefore, blood donors from this region participated in the study.
It was not considered necessary to consider recall bias because there is no reason to think that this would differ in the two study populations.

Climate of the study area

The climate of 10 cities in northern Norway is characterized as subarctic (Köppen–Geiger designation as Dfc) (11). The average yearly temperature in the northernmost city in the study, Hammerfest (70°40' N 23°41' E), is 1.7 °C. About 700 km further south, Sandnessjøen differs from the other cities in the region with its warm, humid, continental climate (Köppen–Geiger designation Cfc). In this city (66°01' N), the average temperature in all months is above −1.5 °C, and the yearly average is 5.7 °C. The cities of Vestfold County have a warm, humid, continental climate (Köppen–Geiger designation Cfb) with yearly average temperature of about 6.3 °C.

In the tick-abundant area of southern Nordland County, the annual average temperature and average spring temperature over the last 50 years reached lowest levels around 1980; thereafter, there has been a steady increase in temperature, in particular after the millennium change (12). The temperature data were obtained from the Norwegian Meteorological Institute.

Statistics and definitions

We defined seroprevalence as the percentage of positive samples to all samples analysed, with different assortments (e.g. northern Norway, Nordland, and Vestfold). Borderline results were calculated as positive, following the approach of Tjernberg (13) and Hjetland (14). Seroprevalence differences between the counties were analysed with the Chi square test; it was performed with Yates correction if any cell number was 5 or less. A p-value < 0.05

Fig. 1. Norway and neighbouring countries. Red dots = the blood banks in the study areas. From the latitude of 65°N and northwards, the three counties of Nordland (violet), Troms (brown) and Finnmark (blue) are defined as the region of northern Norway. In southern Norway: Vestfold County (59°N); and the counties wherein seroprevalence studies have been performed before: Vest-Agder County (green) (23) and Sogn og Fjordane County (ochre) (14). Brønnøysund is the city centre of Brønnøy municipality (12).
was considered significant. Mean age (and range) in years was given for both genders. Weighted kappa measure of agreement between IgG and the confirmatory tests, C6/blot, were analysed with Graphpad online (15); (weighted, i.e. negative/borderline/positive).

The analytical sensitivity of an assay is defined, according to Saah (16); the assay can detect a low concentration for a given substance (i.e. Borrelia antibodies) in a biological sample. The analytical specificity of IgG is defined as the assay’s ability to detect the Borrelia IgG antibodies but not cross-reactive antibodies (16). The specificity of IgG was calculated as,

Negative samples

Negatives + false positives + false borderline samples

The IgG-negative samples were assumed to be true negatives and not tested further.

RESULTS

We collected samples and questionnaires from 1567 blood donors: 404 (26%) in the middle 2 weeks of November 2012 and 1163 (74%) during 5 weeks in February and March 2013 (Table 2). We obtained consent from 1597 donors, but samples without acceptable questionnaires (n = 12; 0.8%) and questionnaires without samples (n = 18; 1.1%) were excluded. The mean age of the males was 45.2 years (n = 747; range 18 – 75) and that of the females was 44.0 years (n = 820; range 18 – 71). The male:female ratio was 0.48:0.52.

Borrelia burgdorferi s.l. seroprevalence

Fifty-three donors (3.38%) were B. burgdorferi s.l. IgG positive, of whom two in Vestfold County had borderline results. In Vestfold, the seroprevalence was 9.25% (48/519); in northern Norway, five donors (of 1048), all males, were IgG positive, giving a seroprevalence of 0.48% (Table 2). The seroprevalence in males was higher than that in females (5.1% and 1.8%, respectively; p < 0.001). In Vestfold County, the Borrelia seroprevalence in males and females was 13.0% (n = 253) and 5.6% (n = 266) (p < 0.05). However, there was no statistical difference in number of tick bites between the genders in Vestfold (nor in northern Norway). The highest IgG prevalence was found in the donors of 60 years of age or older (7.4%; 11/149; p < 0.001). The C6 verified the positive and borderline samples of IgG (n = 66; 4%), and the blot verified the negative and borderline samples of C6 (n = 13; 1%) (Fig. 2). As a result, the IgG screening test had an overall specificity of 99.1% (Table 2). Of the 66 IgG samples with positive or borderline result, five (of 53; 9%) samples in Vestfold and eight (of 13; 62%) samples in northern Norway were reclassified as false positive or false

Table 1. Geographical, demographical and Lyme borreliosis surveillance data in six counties in Norway

<table>
<thead>
<tr>
<th>Residence</th>
<th>Blood banks, n</th>
<th>Population, n1</th>
<th>% of population</th>
<th>LB2 incidence per 100 0003</th>
<th>% of mean LB incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finnmark</td>
<td>2</td>
<td>74 534</td>
<td>1.5</td>
<td>0.3</td>
<td>5</td>
</tr>
<tr>
<td>Troms</td>
<td>2</td>
<td>160 418</td>
<td>3.2</td>
<td>0.6</td>
<td>10</td>
</tr>
<tr>
<td>Nordland</td>
<td>7</td>
<td>239 611</td>
<td>4.7</td>
<td>1.1</td>
<td>21</td>
</tr>
<tr>
<td>Northern Norway</td>
<td>11</td>
<td>474 563</td>
<td>9.4</td>
<td>0.8</td>
<td>15</td>
</tr>
<tr>
<td>Vestfold</td>
<td>3</td>
<td>238 748</td>
<td>4.7</td>
<td>8.0</td>
<td>148</td>
</tr>
<tr>
<td>Study area</td>
<td>14</td>
<td>713 311</td>
<td>14.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sogn og Fjordanea</td>
<td>4</td>
<td>108 700</td>
<td>2.2</td>
<td>15.1</td>
<td>279</td>
</tr>
<tr>
<td>Vest-Agder5</td>
<td>1</td>
<td>176 353</td>
<td>3.5</td>
<td>23.7</td>
<td>437</td>
</tr>
<tr>
<td>Norway</td>
<td>5 051 275</td>
<td>100.0</td>
<td>5.4</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

2LB, Lyme borreliosis.
3Data from the Norwegian Surveillance System for Communicable Diseases, MSIS (8), in the years 2003–2012.
4For comparison, surveillance data and blood donor seroprevalence of anti-Borrelia burgdorferi sensu lato antibodies are shown for two counties: Sogn og Fjordane County (13) and Vest-Agder County (20).

Fig. 2. Algorithm of the analyses and the results of anti-Borrelia burgdorferi sensu lato antibodies in samples from 1567 healthy blood donors in the region of northern Norway and Vestfold County.
borderline. All 13 samples analysed using blot were negative. Weighted kappa value between IgG and C6/blot was 0.90, designated as very good according to Altman (17).

**Tick bites**

Among the donors in northern Norway, 51 (5%) subjects had been bitten once, and 42 (4%) had experienced multiple bites (Table 3). Seven (0.7%) out of 1048 had been bitten by tick in northern Norway, and six of them identified the geographical origin of the tick bite as the coastal area in the southern part of Nordland County (data not shown). During scientific work, a blood donor in Tromsø reported several tick bites of *I. uriae* on two seabird islands off the coast of Finnmark County. These eight tick-bitten donors were seronegative. Of all seropositive donors in the study, 71% (32/45; no response, n = 8) of the subjects were aware of the tick bite.

Seroprevalence in the subjects with pets (3.5%; 34/984) and without pets (3.3%; 19/577) was similar. The donors in northern Norway pointed out that their pets had been bitten once (6%; 37/641) or multiple (8%; 52/641) times in northern Norway, with decreasing risk northward from the southern part of Nordland County to the middle of Troms County (Fig. 1), while the percentages of the pets bitten in Vestfold County were 3% (10/343) and 79% (271/343) (Vestfold vs northern Norway, p < 0.0001), respectively.

**Tick-bite prophylaxis**

Fifty-four percent (280/518) of the donors in Vestfold had good knowledge of the most important symptoms and/or signs of *Borrelia* infection. Red ring (or blue ring or ring) was the response in 50% (261/519) of the subjects. In northern Norway, 10% (107/1031; no response, n = 17) gave the same answer (p < 0.0001). Nine (75%) of the physicians in the study wrote *erythema migrans*. This diagnostic term, or a more descriptive layman's version of it, was the predetermined optimal answer. Eighty-one percent (415/511) of the donors in Vestfold County routinely checked for ticks after a visit to tick-infested areas; in northern Norway, 49% (423/872) did so.

### Table 2. Seroprevalence of anti-*Borrelia burgdorferi* sensu lato antibodies in blood donors

<table>
<thead>
<tr>
<th>Residence</th>
<th>Samples, n</th>
<th>Samples per population¹, %</th>
<th><em>Borrelia</em>-positive samples, n</th>
<th>Seroprevalence², %</th>
<th>Seroprevalence (CI)³</th>
<th>IgG specificity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finnmark²</td>
<td>147</td>
<td>0.20</td>
<td>1</td>
<td>0.68</td>
<td>0.02–3.73</td>
<td>99.0</td>
</tr>
<tr>
<td>Troms⁶</td>
<td>344</td>
<td>0.21</td>
<td>1</td>
<td>0.29</td>
<td>0.01–1.61</td>
<td>99.5</td>
</tr>
<tr>
<td>Nordland⁷</td>
<td>557</td>
<td>0.23</td>
<td>3</td>
<td>0.54</td>
<td>0.11–1.57</td>
<td>99.5</td>
</tr>
<tr>
<td>Northern Norway</td>
<td>1048</td>
<td>0.22</td>
<td>5</td>
<td>0.48</td>
<td>0.16–1.11</td>
<td>99.2</td>
</tr>
<tr>
<td>Vestfold⁸</td>
<td>519</td>
<td>0.22</td>
<td>48⁹</td>
<td>9.25</td>
<td>6.90–12.08</td>
<td>98.9</td>
</tr>
<tr>
<td>In total</td>
<td>1567</td>
<td>0.21</td>
<td>53</td>
<td>3.38</td>
<td>2.54–4.40</td>
<td>99.1</td>
</tr>
</tbody>
</table>

²The difference in seroprevalence between the counties in northern Norway was not significant.
³Significant difference between the seroprevalence in northern Norway and Vestfold (p < 0.001).
⁴CI, confidence interval.
⁵Samples in Finnmark Hospital Trust: Clinic Kirkenes, n = 60 and Clinic Hammerfest, n = 87.
⁶Samples at the University Hospital of North Norway: Tromsø, n = 267 and Harstad, n = 77.
⁷Samples in Narvik Hospital, n = 22. Samples in Nordland Hospital Trust: Bodo, n = 214, Stokmarknes, n = 44 and Gravdal, n = 72. Samples in Helgeland Hospital Trust: Rana Hospital, n = 79, Mosjøen Hospital, n = 45 and Sandnessjøen Hospital, n = 81.
⁸The samples in the hospitals of Vestfold Hospital Trust: Tønsberg, Larvik and The Blood Bank of Sandefjord, n = 519.
⁹Two borderline samples were defined as positive.

The difference between any tick bites (i.e. single or multiple) and no tick bites in northern Norway and Vestfold County was significant (p < 0.0001).

**Table 3. Tick bites in blood donors**

<table>
<thead>
<tr>
<th>Residence</th>
<th>One tick bite¹</th>
<th>Multiple tick bites¹</th>
<th>No tick bites¹</th>
<th>No data</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Counties of Northern Norway², n</td>
<td>51</td>
<td>42</td>
<td>772</td>
<td>183</td>
<td>1048</td>
</tr>
<tr>
<td>Vestfold County, n</td>
<td>99</td>
<td>146</td>
<td>195</td>
<td>79</td>
<td>519</td>
</tr>
<tr>
<td>Anti-<em>Borrelia</em> IgG status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative, n (%)</td>
<td>143 (9)</td>
<td>163 (11)</td>
<td>954 (63)</td>
<td>254 (17)</td>
<td>1514</td>
</tr>
<tr>
<td>Positive, n (%)</td>
<td>7 (13)</td>
<td>25 (47)</td>
<td>13 (25)</td>
<td>8 (15)</td>
<td>53</td>
</tr>
<tr>
<td>Sum</td>
<td>150</td>
<td>188</td>
<td>967</td>
<td>262</td>
<td>1567</td>
</tr>
</tbody>
</table>

¹The difference between any tick bites (i.e. single or multiple) and no tick bites in northern Norway and Vestfold County was significant (p < 0.0001).
²The difference between any tick bites and no tick bites in the Nordland County vs Troms County and Finnmark County was not significant.
870) followed the same procedure \( (p < 0.0001) \). Nearly all \( (97\%; 1487/1531) \) participants felt free to interact with nature without fear of ticks and tick-related diseases.

**DISCUSSION**

This is the first study to determine *B. burgdorferi* s.l. IgG seroprevalence in healthy blood donors in northern Norway; it was 0.48% and 19 times lower than that in Vestfold County. Together with the questionnaire results, these findings suggested that the risk of tick bites (in both humans and pets) in northern Norway was much lower than in Vestfold County and gradually decreased with increasing latitude in the region. The low number of seropositive samples in northern Norway therefore demonstrates the high specificity of the most commonly used anti-*B. burgdorferi* s.l. IgG assay in Norway.

The microbiological laboratories in Scandinavia apply different assays, and they report seroprevalences in blood donors between 2% and 25% \( (18) \). From the literature, we are aware of districts abroad with lower *Borrelia* seroprevalence in blood donors than seen in northern Norway \( (19) \). Low seroprevalence is usually ascribed to low tick abundance, but Robertson et al. \( (20) \) concluded that the reason for low prevalence in some tick-abundant districts in Ireland was because of the lack of appropriate *B. burgdorferi* s.l. reservoir hosts, and therefore uninfected ticks. In such areas, all tick life-cycle stages feed on large animals such as sheep and cattle, which are known to be inefficient hosts.

The seroprevalence in Vestfold County is statistically comparable with that of a study from Sogn og Fjordane County in western Norway which was 9.6% \( (14) \). The prevalences in both genders in Vestfold County were similar to that in Sogn og Fjordane. The difference in prevalence between genders is also seen in other studies \( (14, 21) \). In the southernmost county, Vest-Agder, the greatest known tick abundance in Norway \( (22) \), 18% of 247 blood donors were *Borrelia* IgG positive \( (23) \). These two studies, as well as ours, were performed with the same assay and are therefore comparable. They provide a solid overview of the difference in ‘background noise’ of anti-*Borrelia* IgG antibodies in four regions of the country. The seroprevalence in Vestfold County \( (i.e. \) southeastern) and Sogn og Fjordane County \( (i.e. \) western) is approximately 20 times higher than the prevalence in the northern region; the prevalence in the southern Vest-Agder County is nearly 40 times higher than in the north.

The specificity of the IgG/VlsE ELISA test in this study is high, as shown in other studies \( (14, 24) \), but cross-reactions, for example, due to treponemal antibodies, are not to be disregarded. In blood donors, however, testing such antibodies is obligatory before they are enrolled. Epstein–Barr virus infection produces polyclonal antibodies that can cross-react in *Borrelia* assays \( (13) \). However, such reactions seem unlikely in the present study because this infection mainly affects teenagers and young adults, who were seronegative for anti-*B. burgdorferi* s.l. IgG antibodies. It was therefore not considered necessary to test these possibly cross-reacting antibodies specifically, including rheumatoid factor.

The majority of Norwegian microbiological laboratories use the Enzygnost IgG/VlsE assay combined with IgM as a screening assay or as a single assay for *Borrelia* antibodies testing \( (18, 25) \). The assay’s package insert states that the sensitivity is 100% in long-standing Lyme borreliosis and the specificity is 98–100%. If the lower figure of 98% were accepted, we would expect at least 20 samples from northern Norway to be false positives. In order to decrease the number of false positive results, and since Borrelia serology lacks a ‘gold standard’, we used two assays to test and verify positive and borderline ELISA results (negative ones have not been retested) \( (Fig. 2) \).

Data from all analyses showed that northern Norway is a region of low *Borrelia* seroprevalence, with only five positive samples (false or true) out of more than a thousand samples; the specificity of the actual IgG assay was higher than 99% \( (Table 2) \). On the other hand, with only five positive samples in northern Norway, test sensitivity may be questioned. However, both the IgG and C6 assays contain VlsE recombinant antigens and antigens of three *Borrelia* genospecies, including *B. afzelii*. In northern Norway, *B. afzelii* is particularly abundant \( (60–95\% of the Borrelia-infected ticks) \) with *B. garinii* and/or *B. valaisiana* in the remaining proportion. Except for its presence in a single tick \( (5) \), *B. burgdorferi* s.s. has not been detected by PCR in northern Norway \( (5, 12, 26, 27) \).

Although there was very high level of agreement between the chosen assays of IgG, C6 and immunoblot in this study, as shown by others \( (14, 24) \), nearly 20% of the IgG borderline or positive samples were reclassified as negative after confirmatory testing. This may be partly due to the fact that the C6 assay is more useful in the verification of samples from acutely ill patients than in those from blood donors \( (28) \).

The low *Borrelia* seroprevalence in northern Norway together with the questionnaire results underscores the low burden of *Borrelia* infection in that region. The blood donors who had been bitten by ticks, indicated in the questionnaire which
municipality was the most likely origin of the tick, and coastal locations in the southernmost part of Nordland County were reported. Although *I. ricinus* is absent in most parts of northern Norway – except in the southern parts (3, 4) – it is surprising that only seven single tick bites have occurred among the thousand blood donors who have lived an average of 36 years in the region (data not shown). The donors described a few tick bites on pets in the middle and southern part of Troms County, but the majority of the bitten animals, like the humans, were bitten in the southern part of Nordland County. Unfortunately, we were unable to obtain seroprevalence or tick-bite data in the area with the highest tick prevalence in the region, Brønnøysund (12, 26, 27) (Fig. 1), because the city has no blood bank.

Although a thousand blood donors is only a small sample of the region’s population, the seroprevalence and questionnaire results indicate that the northern distribution limit of *I. ricinus* in Norway, in contrast to suggestions made for Sweden (10), has not extended much since the original articles by Tamb-Lyche (3) and Mehl (4) were published. No data on changes in diversity or abundance of important hosts, such as deer, are available in Norway to elucidate this situation. None of the five *Borrelia*-positive donors in northern Norway were able to indicate the regional origin of the tick bite. As ticks are not common in northern Norway, it was surprising that the bitten donors were convinced to the same extent as the donors in Vestfold County that they had been bitten by a tick.

**CONCLUSION**

In northern Norway, *B. burgdorferi* s.l. seroprevalence in blood donors is about 20–40 times lower than in the counties further south. The questionnaire results show that this is probably because of the very low tick challenge compared with southern Norway. However, contrary to observations made in Sweden, there is nothing in our data to suggest that the distribution of *I. ricinus* populations has extended northwards in Norway. Blood donor sera in the northern region may usefully serve as specificity controls for *B. burgdorferi* s.l. serological assays.

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**DISCLOSURE**

The IgG screening test applied in the study is the routine assay for anti-*Borrelia* antibody testing in the majority of Norwegian microbiological laboratories. In particular research studies, such as this, the manufacturer offers a 50% discount. Otherwise, the authors declare that they have no conflict of interest.

**ETHICAL APPROVAL**

The Regional Committee for Medical and Health Ethics approved the protocol (REC North no. 2011/2575). The donors received written information, and we obtained written consent from all. The blood samples and questionnaires were anonymous but not fully de-identified.

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