Seroprevalence of antibodies to *Borrelia burgdorferi* sensu lato in healthy adults from western Norway: risk factors and methodological aspects

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The aim of this study was to assess the seroprevalence of antibodies to *Borrelia burgdorferi* sensu lato in a healthy adult population from Sogn and Fjordane county in western Norway by different assays. Sera from 1213 blood donors at four different blood banks were analysed in Enzygnost Lyme link VlsE/IgG (IgG), Enzygnost Borreliosis IgM (IgM), and Immunetics C6 Lyme ELISA kit (C6). Sera showing positive or grey-zone reactivities were further examined with Borrelia-EUROLine-RN-AT IgG blot and Borrelia-EUROLine-RN-AT IgM blot. The seroprevalences were 9.6%, 8.2%, 8.4%, 6.4% and 5.7%, respectively. The seroprevalence for IgG was lower in the eastern part of the county and in owners of pet animals. It was higher in men, and increased with age and number of tick bites. C6 and IgG gave comparable results. IgM only was found in 4.5%, more often in women, did not increase with age, and showed no relationship with geography, and 56.4% were positive in IgM blot. In conclusion, antibodies to *B. burgdorferi* s.l. are common in blood donors in western Norway. The results may be used for evaluation of predictive values of test results in patients, as well as a basis for test algorithms in the laboratory.

Key words: *Borrelia burgdorferi*; seroprevalence; Norway; blood donors.

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Sogn and Fjordane county, located at the western coast of Norway, encompasses coastal, fjord and mountainous areas at 61–62° N and 5–7° E. The climate is temperate, with a high yearly rainfall in the western and middle areas, but with a more inland-like climate in the eastern part.

*Ixodes ricinus* is the predominating tick species in Norway, and is present along the coast as far north as 69° N. Its latitudinal and altitudinal distribution limits seem to be expanding (1). It is more abundant along the southernmost coastline. In Sogn and Fjordane county, there are more ticks in the western, coastal regions than in the eastern regions (1–3).

Among the blood donors included in the present study, 65.7% reported having been bitten by ticks at least once in their lifetime, and 30.0% reported tick bites during the last 12 months (4). Fewer tick bites were reported from the donors in the eastern than in the western part of the county.

Lyme borreliosis is the most prevalent human tick-borne disease in Norway, with a distribution corresponding to that of *I. ricinus*. In the Norwegian Surveillance System for Communicable Diseases (MSIS), only cases of systemic disease and chronic manifestations of Lyme borreliosis are notifiable, while the most prevalent manifestation, erythema migrans, is not (http://www.msis.no/). In the period 2001–2010, the mean reported annual incidence in Sogn and Fjordane was 14.1 cases per 100 000 inhabitants, compared to 26.1 in the southernmost county of Vest-Agder, and 5.3 nation-wide (http://www.msis.no/).

Published figures of the seroprevalence of antibodies to *B. burgdorferi* sensu lato in blood...
donors are not easy to compare. As far as we know, there is no standardized way to report this; different laboratory methods and algorithms are used, and the methods have changed over time. The numbers are ranging from 1.1%, using C6 ELISA in the USA (5), to 30% in Dar es Salaam, Tanzania, using the DAKO flagellar ELISA (6). In Europe, numbers between 4% and 20% for IgG in different ELISAs have been published, as summarized by Tjernberg et al. (7). In a report by Dessau et al., seropositivity rates for blood donors in some Scandinavian laboratories were presented (8), showing a marked difference in prevalence depending on the ELISA method used. One Swedish laboratory using C6 ELISA reported a positivity rate of 16.0%, whereas three laboratories using the IDEIA flagellar ELISA found 1.1–3.0%. Two Swedish laboratories using Liaison assays had IgG rates of 7.0% and 8.0%, and IgM of 3.0% and 0%, respectively. In Norway, a seropositivity rate for IgG of 18% was found in 247 blood donors from the county of Vest-Agder, using the Enzygnost ELISA (9). This is the county with the highest incidence of notified cases of Lyme borreliosis in Norway.

The mainstay of serological diagnosis is the enzyme immunoassay (EIA), of which several commercial variants exist in parallel. They differ in antigen composition, from single antigen assays (e.g., flagellum protein p41 and C6) to complex antigen mixtures based on extracts from cultivated B. burgdorferi s.l. and/or synthesized antigens. In the USA and central Europe, screening with an EIA test is recommended to be complemented with an immunoblot for confirmation, known as two-tiered testing (10, 11). There are also several different immunoblot assays, with variation in antigen composition and preparation. As has been demonstrated, the choice of which specific EIA and immunoblot test to use strongly influences the resulting conclusions (12).

In Scandinavia, the two-tier principle has never been systematically adopted, as it is thought to reduce sensitivity and only give a marginal add to specificity (13). Thus, most laboratories either perform only EIA-testing, or perform additional testing in certain circumstances (8). In Norway, optimal testing strategies are currently being discussed, as published in some documents available only in Norwegian (14, 15). Different alternatives to or variants of the two-tier testing are currently discussed worldwide, e.g., using C6 ELISA as the only test, or using an EIA also for second-tier testing, different from the one used for screening (5, 7, 16–19).

In the present study, we wanted to assess the seroprevalence of antibodies to B. burgdorferi s.l. in healthy blood donors in Sogn and Fjordane county, western Norway. In addition, we wanted to relate seropositivity to tick bites, demographics and other risk factors. By using two different ELISAs as well as immunoblot, we also wanted to compare different test strategies.

**MATERIALS AND METHODS**

**Study population**

During the period 13th January to 15th June 2010, blood donors at the four blood banks in Sogn and Fjordane, Norway, were asked to participate in the Tick-borne Infection Study in Sogn and Fjordane. A total of 1213 blood donors participated, resulting in a response rate of 76%. Characteristics of the participants are presented previously (4); mean age was 45.8 (range: 19–69) years and 55.2% were men. Informed consent was obtained from each participant, and the study was approved by the Regional Committee for Medical Research Ethics.

**Questionnaire**

All study participants filled in and returned a questionnaire on the day of blood donation. They were asked to record the number of tick bites ever experienced and tick bites experienced during the last 12 months. The responses for both these questions were given in the categories ‘none’, ‘one’, ‘2–5’, ‘6–20’ and ‘more than 20’. In addition, participants provided information on gender, age, marital status, education, household income and occupation, pet animals, farm animals, hours spent outdoors during summertime, hunting, orienteering, smoking, symptoms and treatment after tick bites, as well as on a number of subjective health complaints.

**Laboratory methods**

Blood samples were collected in serum separator tubes with gel, and after centrifugation, sera were frozen in aliquots at −70 °C until testing.

Antibodies to B. burgdorferi s.l., were tested in Enzygnost Lyme link VlsE/IgG, Enzygnost Borreliosis IgM (DADE Behring, Marburg, Germany) and Immunece C6 Lyme ELISA kit (Immunece, Cambridge, MA, USA). Sera showing positive or grey-zone reactivities in any of these tests were further tested in Borrelia-EUROLine-RN-AT IgG and Borrelia-EUROLine-RN-AT IgM (Euroimmun AG, Lübeck, Germany).

The Enzygnost Lyme link VlsE/IgG is based on a mixture of native Borrelia antigens from B. afzelii strain PKo and recombinant VlsE from the three genospecies B. burgdorferi sensu stricto, B. garinii and B. afzelii. Enzygnost Borreliosis IgM assay is based on a detergent extract from B. afzelii strain PKo. For both assays, sera were absorbed with antigens from Treponema phagedenis, and for the IgM assay they were treated with anti-IgG for removal of rheumatoid factor. The Enzygnost assays were processed by automated instrumentation (Behring BEP 2000 Advance), and the results were interpreted following the manufacturer’s instructions, including retesting of grey-zone results.
The cut-off value referred to in this paper is the one distinguishing grey-zone and positive results.

The Immunetics C6 Lyme ELISA kit uses the conserved synthetic peptide (C6 peptide) derived from the VlsE protein as antigen, and both IgG and IgM antibodies are detected. The analyses were performed semi-manually, using an automatic washer and spectrophotometer. Grey-zone results were repeated according to the manufacturer’s instructions.

The EUROLINE-RN-AT IgG and IgM test kits are qualitative immunoblot assays for antibodies of the IgG and IgM class against *Borrelia* antigens. The IgG and IgM assays differ in antigen composition. The assay is a combination of the classical Western blot and a line blot, in that some of the antigens are applied directly in lines to membranes, while some are removed from a classical Western blot and placed onto the test strip. Recombinantly produced and purified VlsE antigen from the three dominating *B. burgdorferi* s.l. genospecies are included in the IgG assay, and OspC from the three species in the IgM assay. The resulting blots were scanned using the EuroBlot Scanner, and interpreted according to the manufacturer’s instructions using the EuroLineScan software (Euroimmun AG).

Statistical analysis

We used IBM SPSS Statistics version 20 (SPSS Inc., Chicago, IL, USA) for statistical analyses. All p values were two-sided and values below 0.05 were considered statistically significant. All data were categorical and described as frequencies and percentages, age was categorized into age groups. ELISA results were categorized as positive or negative, while blot results were categorized as positive or negative, with grey-zone results included as positives; while blot results were categorized as positive or negative, with grey-zone results included as negatives.

The three ELISAs were compared using the kappa statistic to determine ‘consistency among raters’ where kappa values < 0 were interpreted as poor, 0.0–0.20 as slight, 0.21–0.40 as fair, 0.41–0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1.00 as almost perfect agreement (20).

RESULTS

The various combinations of results are given in Table 1. Using the laboratory’s routine method, Enzygnost IgG and IgM, 117 (9.6%) of the 1213 sera were positive in IgG and 99 (8.2%) in IgM, totalling 172 subjects (14.2%), of which 78 (45.3%) were positive in the IgG blot, and 66 (38.4%) in IgM blot. In the C6 assay, 102 sera (8.4%) were positive, of which 70 (68.6%) were positive in IgG blot, and 28 (27.5%) in IgM blot.

The reported number of tick bites and seropositivity to *B. burgdorferi* s.l. showed a close correlation both for IgG and IgM (Table 2). Among the 409 subject reporting not to have experienced any tick bite, 20 (4.9%) were positive in IgG and 22 (5.4%) were positive in IgM. The relationship between tick bites, gender and seropositivity for IgG is further elucidated in Fig. 1, showing a close relation between tick bites and seropositivity in men. This relationship is less obvious in women.

The results of IgG, IgM and C6 according to geographical location of the blood banks are presented in Table 3. Compared with the other blood banks, there were fewer IgG positives in Lærdal (p = 0.021), and there were more C6 positives in Førde (p = 0.004).

### Table 1. Seropositivity to *Borrelia burgdorferi* sensu lato in the Tick-borne Infection Study in Sogn and Fjordane, Norway, 2010 (n = 1213)

| Total, n (%) | Blot IgG, n (%) | Blot IgM, n (%) |
|-------------|----------------|----------------|
|             | –/–+           | –/–+           |
| IgG+         | 117 (9.6)      | 74 (63.2)      | 71 (60.7) |
| IgM+         | 99 (8.2)       | 34 (34.3)      | 27 (27.3) |
| C6+          | 102 (8.4)      | 70 (68.6)      | 66 (64.7) |
| IgG and/or IgM and/or C6+ | 198 (16.3) | 109 (55.1) | 69 (34.8) |
| IgG+, IgM+, C6+ | 31 (2.6) | 29 (93.3) | 6 (19.4) |
| IgG+, IgM+, C6– | 13 (1.1) | 29 (23.1) | 5 (38.5) |
| IgG+–, IgM+, C6+ | 44 (3.6) | 39 (88.6) | 38 (86.4) |
| IgG+–, IgM–, C6+ | 29 (2.4) | 3 (10.3) | 22 (75.9) |
| IgG–, IgM+, C6+ | 1 (0.1) | 0 (0.0) | 0 (0.0) |
| IgG–, IgM–, C6+ | 54 (4.5) | 2 (3.7) | 16 (29.6) |
| IgG–, IgM–, C6– | 26 (2.1) | 5 (19.2) | 22 (84.6) |
| IgG–, IgM–, C6– | 1015 (83.7) | – | – |

IgG, Enzygnost Lyme link VlsE/IgG; IgM, Enzygnost Borreliosis IgM; C6, Immunetics C6 Lyme ELISA kit; Blot IgG, EUROLINE-RN-AT IgG; Blot IgM, EUROLINE-RN-AT IgM.

1Percentages of the total population of 1213.

2Percentages within this group.
There was a positive association of IgG-seropositivity with age, and more males than females were positive to IgG (Table 4). The results are further elucidated in Fig. 2, showing a delayed age-related rise in seroprevalence in women compared with men.

The relation of different risk factors to seropositivity for IgG and IgM are presented in Table 4. Cat or dog owners had a significantly lower seropositivity rate for IgG. There were, however, no statistical differences regarding educational level, household yearly gross income, daily smoking, outdoor hours per week during summertime, hunting during the preceding 12 months, orienteering, or ownership of domestic animals.

Comparing the qualitative results (positive or negative), the two EIA-methods showing the highest agreement were Enzygnost IgG and C6, with a kappa value of 0.654 (CI 0.578–0.730), indicating a substantial agreement between these two assays. Comparing the combined seropositivity in Enzygnost ELISA (IgG and/or IgM) with C6, the kappa value was 0.502 (CI 0.428–0.576), indicating moderate agreement. There was poor agreement between C6 and isolated IgM, with a kappa value of 0.049 (CI 0.077 to 0.021).

In Fig. 3, we see that the concordance of positivity for IgG and C6 was good at strong reactions in both assays, but there were more discrepancies between the two tests in the lower ranges of reactivity. Thus, all IgG stronger than 260% of the cut-off value, corresponding to 36 units/mL in the manufacturer’s unit, were also positive in C6. Similarly, we found that IgG reactions stronger than 252% of the cut off (34 U/mL) were positive in IgM blot, and for C6 EIA, the corresponding limit was 504% (Fig. 4). Among the 60 sera with a positive IgG weaker than 260% of the cut off, 18 (30.0%) were positive also in C6. Of these, 11 (61.1%) were positive in IgM blot, while among the 42 C6 negatives, only 6 (14.3%) were positive.

Vice versa, among the 60 sera with a positive C6 weaker than 465% of the cut-off, 33 (55.0%) were positive also in IgG. Of these, 26 (78.8%) were positive in IgM blot, while among the 27 C6 negatives, only 2 (7.4%) were positive.

A comparison of IgM accompanying a positive IgG with isolated IgM (i.e. without a concomitant

### Table 2. Prevalence of *Borrelia* IgG and IgM (Enzygnost) according to reported tick bites ever and tick bites latest 12 months in the Tick-borne Infection Study in Sogn og Fjordane, Norway, 2010 (n = 1213)

|                | IgG (%) | Odds ratio\(^2\) (95% CI) | Adjusted odds ratio\(^3\) (95% CI) | IgM (%) | Odds ratio\(^2\) (95% CI) | Adjusted odds ratio\(^3\) (95% CI) |
|----------------|---------|---------------------------|------------------------------------|---------|---------------------------|------------------------------------|
| **Tick bites ever** |         |                           |                                    |         |                           |                                    |
| None           | 409 (34.3) | 4.9                       | 1                                 | 5.4     | 1                         |                                    |
| One            | 215 (18.0) | 9.3                       | 2.0 (1.0–3.8)                     | 1.9     | 1.0 (0.7–2.6)             | 1.3 (0.6–2.6)                      |
| 2–5            | 284 (23.8) | 11.3                      | 2.5 (1.4–4.4)                     | 2.2     | 1.6 (0.9–3.0)             | 1.5 (0.8–2.7)                      |
| 6–20           | 176 (14.8) | 12.5                      | 2.8 (1.5–5.2)                     | 2.4     | 2.4 (1.3–4.5)             | 2.3 (1.2–4.3)                      |
| >20            | 110 (9.2)  | 18.2                      | 4.3 (2.2–8.4)                     | 3.3     | 3.3 (1.7–6.6)             | 2.7 (1.3–5.2)                      |
| **p trend**    |         |                           |                                    |         |                           |                                    |
| None           | 836 (70.0) | 7.7                       | 1                                 | 6.2     | 1                         |                                    |
| One            | 177 (14.8) | 14.1                      | 2.0 (1.2–3.3)                     | 1.9     | 1.7 (0.9–3.2)             | 1.8 (0.9–3.3)                      |
| 2–5            | 138 (11.6) | 10.9                      | 1.5 (0.8–2.7)                     | 1.6     | 1.0 (0.7–1.6)             | 1.0 (0.7–1.6)                      |
| 6–20           | 33 (2.8)   | 21.2                      | 3.2 (1.4–7.8)                     | 3.0     | 2.1 (0.7–6.1)             | 2.0 (0.7–6.0)                      |
| >20            | 10 (0.8)   | 30.0                      | 5.2 (1.3–20.5)                    | 5.6     | 3.8 (0.8–18.2)            | 3.6 (0.7–17.6)                     |
| **p trend**    |         |                           |                                    |         |                           |                                    |

1Numbers do not total 1213 because of missing data.
2Odds ratio was estimated using binary logistic regression.
3Adjusted for gender, age group and blood bank.

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**Borrelia Antibodies in Western Norway**
positive IgG) in relation to age and gender is shown in Table 3 (geography) and Fig. 5 (age and gender), irrespective of C6 result. IgM only was seen in 55 subjects (4.5%), whereas IgM concomitant with IgG was seen in 44 subjects (3.6%). Immunoblot for IgM was positive for 31 (56.4%) and 28 (63.6%) of these, respectively. The IgM alone compared to IgM accompanying IgG had some distinctions: More women than men had IgM alone (81.4% vs 35.7%, \( p < 0.001 \), Fisher’s exact test), the mean age of the subjects with IgM alone was lower (44.5 vs 53.4 years, \( p < 0.001 \), Student’s \( T \)-test), and IgM alone had no statistically significant correlation with the number of tick bites, contrary to IgM accompanying IgG (binary logistic regression, data not shown).

**DISCUSSION**

The main findings in this study were that 9.6% of healthy blood donors in Sogn and Fjordane were positive in Enzygnost Lyme link VlsE/IgG ELISA, 8.2% in Enzygnost Borreliosis IgM ELISA, and 8.6% in the Immuneetics C6 Lyme ELISA kit. The IgG and C6 results were comparable.

Blood donors are not fully representative of the population in Sogn and Fjordane county, as they are healthy, not all municipalities are equally represented, and there are no children or individuals more than 70 years of age. There was, however, a fair distribution in age and gender (4).

*Bozarella* IgG and IgM may be positive for a long time after infection with *B. burgdorferi* s.l. (21). False-positive IgM reactions without relation to *B. burgdorferi* s.l. infection are well known (22, 23). Isolated positive IgMs without obvious relation to actual or earlier disease are a permanent problem in everyday clinical microbiology.

As tick bites are necessary for infection with *B. burgdorferi* s.l., a close correlation of the number of bites with seropositivity for specific IgG was expected. As seen in Table 2, this was also the case. Notably, persons who reported to never have been bitten had a seropositivity rate for IgG of 4.9%, supporting the well-known clinical observation that tick bites often go unnoticed (24, 25). The real proportion of these blood donors having experienced tick bites is therefore probably greater than the self-reported 65.7% (4). The occurrence of ticks is lower in the eastern, inland-like part of Sogn and Fjordane county than in the western, coastal areas, and the blood donors from this area (the blood bank in Lærdal) reported fewer tick bites than those from the other blood banks (1, 4). As expected, we found the seroprevalence of IgG antibodies to *B. burgdorferi* s.l. to be lower in donors from this area (\( p = 0.021 \)). The difference in IgM was, however, not significant (\( p = 0.268 \)), and for isolated IgM especially, there was no difference whatsoever (\( p = 1.000 \)). Why the donors from the blood bank in Forde had more positive reactions in C6 than the others (Table 3) is difficult to explain, as Nordfjordeid had the highest seropositivity rate for IgG.

There was a positive correlation between seroprevalence of IgG and age (Table 4). Looking at age and gender combined, we note that the prevalence in women rises at an older age than in men (Fig. 2). This may be in accordance with the age distribution of reported tick bites, as young men

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**Table 3. Prevalence of *Borrelia* IgG and IgM (Enzygnost) and C6 antibodies in the Tick-borne Infection Study in Sogn and Fjordane, Norway, 2010, according to location of blood bank (n = 1213)**

| Total | Blood bank | p<1 | p<2 |
|-------|------------|-----|-----|
| n (%) | 1213 (100.0) | 614 (50.6) | 355 (29.3) | 73 (6.0) | 171 (14.1) | 0.028 |
| IgG (%) | 9.6 | 10.7 | 7.6 | 2.7 | 12.9 | 0.111 |
| p<2 | 0.111 | 0.073 | 0.021 | 0.084 |
| IgM (%) | 8.2 | 8.8 | 8.2 | 4.1 | 7.6 | 0.239 |
| p<2 | 0.239 | 0.538 | 0.268 | 0.881 |
| IgM only (%) | 4.5 | 4.6 | 5.4 | 4.1 | 2.9 | 0.697 |
| p<2 | 4.6 | 0.000 | 0.169 | 0.222 |
| IgG only (%) | 6.0 | 6.5 | 4.8 | 3.7 | 8.2 | 0.280 |
| p<2 | 0.472 | 0.289 | 0.169 | 0.222 |
| IgG and IgM (%) | 3.6 | 4.2 | 2.8 | 0 | 4.7 | 0.183 |
| p<2 | 0.284 | 0.400 | 0.106 | 0.383 |
| IgG and/or IgM (%) | 14.2 | 15.3 | 13.0 | 6.8 | 15.8 | 0.187 |
| p<2 | 0.285 | 0.470 | 0.081 | 0.554 |
| C6 (%) | 8.4 | 10.7 | 5.9 | 2.7 | 7.6 | 0.004 |
| p<2 | 0.004 | 0.053 | 0.081 | 0.768 |

1p-value for association between blood bank and antibody prevalence by using chi-squared test.

2p-value for difference between actual blood bank and the total of the others by Fisher’s exact test.
Table 4. Prevalence of positive *Borrelia* IgG and IgM (Enzygnost) in the Tick-borne Infection Study in Sogn and Fjordane, Norway, 2010, according to patient characteristics (n = 1213)

| Characteristic                      | n (%)   | IgG Positive (%) | Odds ratio | Adjusted odds ratio |
|-------------------------------------|---------|------------------|------------|---------------------|
|                                     |         | (95% CI)         |            | (95% CI)            |
| Gender                              |         |                  |            |                     |
| Female                              | 544 (44.8) | 5.5 | 1 | 1 | 7.9 | 1 | 1 |
| Male                                | 669 (55.2) | 13.0 | 2.6 (1.7–3.9) | 2.5 (1.6–3.8) | 8.4 | 1.1 (0.7–1.6) | 1.0 (0.7–1.6) |
| p difference                        |         | <0.001           |            | <0.001              |
| Age                                 |         |                  |            |                     |
| 19–29                               | 80 (6.8)    | 2.5         | 1         | 1 | 5.0 | 1 | 1 |
| 30–39                               | 235 (19.9)  | 3.8 | 1.6 (0.3–7.3) | 1.4 (0.3–6.5) | 6.4 | 1.3 (0.4–4.0) | 1.3 (0.4–4.0) |
| 40–49                               | 414 (35.0)  | 8.0 | 3.4 (0.8–14.4) | 2.9 (0.7–12.6) | 8.0 | 1.6 (0.6–4.8) | 1.6 (0.6–4.7) |
| 50–59                               | 344 (29.1)  | 12.5 | 5.6 (1.3–23.5) | 4.8 (1.1–20.3) | 8.4 | 1.7 (0.6–5.1) | 1.7 (0.6–5.1) |
| 60–69                               | 110 (9.3)    | 23.6 | 12.1 (2.8–52.5) | 9.8 (2.2–43.0) | 14.5 | 3.2 (1.0–10.1) | 3.2 (1.0–10.1) |
| p trend                             |         | <0.001           |            | <0.001              |
| Education                           |         |                  |            |                     |
| Primary school 9 years or less      | 87 (7.2)    | 16.1 | 1 | 1 | 10.3 | 1 | 1 |
| Secondary school                    | 598 (49.8)  | 9.4 | 0.5 (0.3–1.0) | 0.6 (0.3–1.2) | 8.2 | 0.8 (0.4–1.6) | 0.8 (0.4–1.8) |
| University/college 1–4 years        | 342 (28.5)  | 8.5 | 0.5 (0.2–1.0) | 0.6 (0.3–1.3) | 6.4 | 0.6 (0.3–1.3) | 0.7 (0.3–1.6) |
| University/college >4 years         | 175 (14.6)  | 9.1 | 0.5 (0.2–1.1) | 0.7 (0.3–1.5) | 10.3 | 1.0 (0.4–2.3) | 1.1 (0.5–2.6) |
| p trend                             |         | 0.198           | 0.594      | 0.967               | 0.855 |
| Household yearly gross income (EUR) |         |                  |            |                     |
| <50 000                             | 156 (13.2)  | 8.3 | 1 | 1 | 8.3 | 1 | 1 |
| 50–99 000                           | 647 (54.7)  | 10.5 | 1.3 (0.7–2.4) | 1.1 (0.6–2.1) | 8.0 | 1.0 (0.5–1.8) | 1.0 (0.5–1.9) |
| 100–150 000                         | 355 (30.0)  | 8.2 | 1.0 (0.5–1.9) | 0.9 (0.4–1.8) | 8.7 | 1.1 (0.5–2.1) | 1.1 (0.5–2.2) |
| >150 000                            | 25 (2.1)    | 8.0 | 1.0 (0.2–4.5) | 0.8 (0.2–3.9) | 8.0 | 1.0 (0.2–4.5) | 1.0 (0.2–4.7) |
| p trend                             |         | 0.606           | 0.378      | 0.833               | 0.837 |
| Daily smoking                       |         |                  |            |                     |
| No                                  | 935 (81.9)  | 9.4 | 1 | 1 | 8.0 | 1 | 1 |
| Yes                                 | 207 (18.1)  | 9.2 | 1.0 (0.6–1.6) | 1.1 (0.6–1.8) | 8.2 | 1.0 (0.6–1.8) | 1.0 (0.6–1.8) |
| p difference                        |         | 0.917           | 0.750      | 0.927               | 0.880 |
| Outdoor hours per week during summertime |         |                  |            |                     |
| ≤5                                  | 278 (23.1)  | 10.8 | 1 | 1 | 10.4 | 1 | 1 |
| 6–10                                | 412 (34.2)  | 8.0 | 0.7 (0.4–1.2) | 0.8 (0.4–1.3) | 7.3 | 0.7 (0.4–1.2) | 0.7 (0.4–1.1) |
| >10                                 | 515 (42.7)  | 9.9 | 0.9 (0.6–1.5) | 1.0 (0.6–1.6) | 7.6 | 0.7 (0.4–1.2) | 0.7 (0.4–1.2) |
| p trend                             |         | 0.859           | 0.982      | 0.215               | 0.209 |
| Hunting last 12 months              |         |                  |            |                     |
| No                                  | 964 (80.3)  | 9.4 | 1 | 1 | 8.3 | 1 | 1 |
| Yes                                 | 236 (19.7)  | 9.7 | 1.0 (0.6–1.7) | 0.9 (0.5–1.5) | 7.2 | 0.9 (0.5–1.5) | 0.9 (0.5–1.6) |
| p difference                        |         | 0.886           | 0.450      | 0.580               | 0.648 |
| Ever active orienteer               |         |                  |            |                     |
| No                                  | 1133 (94.6) | 9.4 | 1 | 1 | 8.1 | 1 | 1 |
| Yes                                 | 65 (5.4)    | 12.3 | 1.4 (0.6–2.9) | 1.0 (0.4–2.1) | 7.7 | 0.9 (0.4–2.4) | 0.9 (0.3–2.3) |
| p difference                        |         | 0.432           | 0.961      | 0.902               | 0.813 |
reported more bites than young women, while this was reversed in subjects older than 50 years of age (4). The steep rise in prevalence of IgM accompanying IgG in elderly women may also be in accordance with the self-reported increase in tick bites in this group (Fig. 5).

For IgG, we found a significant difference in gender; men had a higher seroprevalence than women, 13.0 vs 5.5%. This is not fully in accordance with self-reported tick bites, where no one in this group (Fig. 5).

| Characteristic | n (%) | IgG Positive | Odds ratio | Adjusted odds ratio | IgM Positive | Odds ratio | Adjusted odds ratio |
|----------------|-------|--------------|------------|---------------------|--------------|------------|---------------------|
| Cat or dog owner |       |              |            |                     |              |            |                     |
| No             | 639 (53.5) | 11.6 | 1 | 1.0 (95% CI) | 7.8 | 1 | 1.1 (95% CI) |
| Yes            | 555 (46.5) | 6.8 | 0.6 (95% CI) | 0.006 | 0.027 | 1 | 0.857 |
| Domestic animals |       |              |            |                     |              |            |                     |
| No             | 1030 (86.6) | 9.6 | 1 | 1.0 (95% CI) | 8.3 | 1 | 1.1 (95% CI) |
| Yes            | 159 (13.4) | 7.5 | 0.8 (95% CI) | 0.406 | 0.646 | 1 | 0.566 |

1Numbers do not add to 1213 due to missing data.
2Odds ratio was estimated using binary logistic regression.
3Adjusted for gender, age group and blood bank, as appropriate.
A statistically significant difference between the genders across all age groups was found, although men reported some more tick bites (4). Also, women reported more tick bites before seroconversion (Fig. 1). This lack of consistency may be caused by differences in subjective awareness of tick bites between the genders, leading both to reporting less bites and not taking precautions in timely removal of ticks in men. However, interesting differences between the genders in clinical manifestations of Lyme borreliosis have been reported (26–28), and an immunological basis for these differences cannot be ruled out. Male dominance regarding Borrelia IgG antibodies has also been reported by others (29), but not universally (30). The number of total and recent tick bites in this study population increased with hours spent outdoors during summertime, educational level, ownership of domestic animals, and hunting (4). Although we found an overall positive relationship of seropositivity of IgG to the number of tick bites, the other associations mentioned above were not reflected in IgG seroprevalence (Table 4). Interestingly, we found a lower seroprevalence in owners of pet animals (cats or dogs), although this group reported some more tick bites (4). This is in contrast to the findings of Dehnhert et al., who, in Germany, found a higher seroprevalence in children and adolescents from households with cats (29).

Discerning «clinical» from «biological» specificity might be fruitful when it comes to healthy populations like blood donors. Many studies of assays for antibodies to B. burgdorferi s.l. provide reactivity in blood donors as a measure of the specificity, and positives in this group are regarded as false-positives.
However, many of these positive reactions are in fact biologically specific in that they reflect earlier or actual, most often asymptomatic infections. However, some genuinely biologically false-positive results not bearing any relationship with *B. burgdorferi* s.l. at all are found as well. Immunoblot reactions may also be biologically false-positive, especially for IgM antibodies (23, 31), as well as clinically false-positive, i.e. reflecting earlier infection not relevant to the clinical picture at hand. In addition, immunoblot results are known to be less sensitive than EIAs in early disease (5, 16).

The C6 ELISA has been reported to be more specific than EIAs with other antigens. Wormser et al. reported a specificity of 99.2% for C6 ELISA in blood donors from non-endemic and 98.6% in blood donors from endemic areas for Lyme borreliosis in USA, contrasted to 95.9% and 96.5% for a whole cell sonicate ELISA for IgG/IgM, respectively (5). In endemic areas in Europe, the reported specificities for the C6 assay are not as high. Thus, in Italy, the specificity of this assay was 97.6% in 210 blood donors from a non-endemic area, and 87.5% in 24 donors from an endemic area (32). High seropositivity rates in blood donors for C6 antibodies are also found in some Scandinavian laboratories, where 16% and 8% has been reported (7, 8). In this study, we found a seroprevalence of 8.4% in C6, giving a specificity of 91.6%, not very different from that of Enzygnost IgG (90.4%). The proportions of IgG immunoblot positives were comparable between these two assays, 63.2% and 68.6%, respectively. The main difference seems to be that the C6 ELISA did not detect the isolated IgMs found in the Enzygnost assay, even though the C6 assay detects IgG as well as IgG antibodies. In conclusion, the C6 and IgG had comparable characteristics in our material.

In 89 patients, among them 59 with suspected Lyme borreliosis, Ang et al. (12) found a kappa value of 0.86 when comparing the Enzygnost IgG and/or IgM with C6. This was a better agreement than the 0.502 found in our study. They also found a higher proportion of Enzygnost IgG and/or IgM and C6 positives being positive in blot (83–100% and 77–100%, respectively) than we did (60.6%, data not shown). The reasons for this discrepancy most probably are related to the patient mix, as our study only included asymptomatic, healthy individuals.

It has been suggested that C6 antibodies tend to normalize after the clinical infection is resolved (33, 34), but others dispute this (35, 36). Our findings of a relatively high prevalence of C6 antibodies in this healthy population support the latter view.

The concordance of positivity for IgG, C6 and IgG blot for strong reactivities indicates that strong responses in these ELISAs do not have to be verified by blot or the other ELISA, as one can presume that they are positive. Ang et al. (12) similarly found a good correspondence between the strength of the reactions in C6 and blot in patients, in that almost all samples stronger than 400% of the cut-off in C6 also were positive in blots. However, this correspondence was not as good for another EIA (Vidas), indicating that this relationship should be investigated for each assay separately. Using C6 as a second-tier test in weak positive IgG or vice versa might thus be an alternative to blot. However, as this study is performed on healthy blood donors, we do not know if these presumptions are valid for ill patients with Lyme borreliosis or other diseases.

The clinical significance of finding IgM antibodies only in the absence of IgG antibodies to *B. burgdorferi* s.l. in patients suspected of having Lyme borreliosis is a continuous dilemma. A positive IgM may indicate an early phase of Lyme borreliosis, and repeat specimens to look for the development of IgG are often suggested by the laboratory. In the absence of development of specific IgG during e.g., 6 weeks, the IgM only is generally regarded as of no consequence. American guidelines thus argue against using IgM blot in the second-tier testing when disease duration is longer than 1 month (10). As these specimens were obtained in the period January–June, there is little reason to presume that these IgM-only positives are indicative of early Lyme borreliosis. In spite of positive immunoblot for IgM in more than half of these sera, most of them being positive towards OspC, there is reason to believe that they are non-specific, also in the biological sense. The specificity of OspC-antibodies have been discussed in the literature (37), and the company Euroimmun AG has recently released a new immunoblot test, the ‘EUROLINE-RN-AT’, which includes a new formulation of OspC antigen, and is claimed to be 30% more specific than the one used in this study, the ‘EUROLINE-RN-AT’.

In endemic areas for Lyme borreliosis, the positive and negative predictive values of test results are important factors for the overall evaluation of patients suspected of suffering from an actual, symptomatic Lyme borreliosis. In this context, the positive results from this study represent ‘clinically false positives’. As shown in Table 4, the seroprevalence was lower in women and in the younger age groups. The positive predictive value of IgG thus will be higher in these groups in our geographical area. The evaluation of the pre-test probability of disease, the history and clinical picture, is therefore of utmost importance. Unfortunately, there is much
uncertainty among primary care physicians of the clinical manifestations of Lyme borreliosis. E.g., the clinical information ‘chronic fatigue’ and ‘musculoskeletal symptoms’ are frequent stated reasons for testing in our laboratory, resulting in very low positive predictive values of the tests. Thus, Dessau et al. in Denmark have shown that among sera submitted from persons with suspected Lyme arthritis, a rare disease in Scandinavia, the rate of seropositivity did not exceed the background prevalence for Danish blood donors (38).

A test algorithm reducing the positivity rate in normal individuals while still detecting a maximum of clinical cases would be preferable. As our data reflect normal individuals only, constructing an algorithm for clinical situations based on these is not possible. However, our data do suggest that a two-tiered test strategy using only Enzygnost IgG or C6 as screening could eliminate the problem of probable unspecific solitary IgM results. A positive screening test should be followed by testing for IgM, and for weak positives also the other ELISA (C6 or IgG as appropriate). Including IgM ELISA in the second-tier test would give some information on whether the infection is recent. Blot could be reserved for weak positive isolated C6 or IgG in clinically suspect cases. The algorithm would miss cases with isolated IgM as a single early finding. In our experience, this is seldom in adult patients suffering from Lyme borreliosis. An option could be to include IgM in the screening for selected patients with a short clinical history. Follow-up test after some weeks in case of negative screening test would be sensible if clinical suspicion is still present. In children, a screening including IgM seems prudent.

In conclusion, we have shown that seropositivity to B. burgdorferi s.l. was common in healthy blood donors in western Norway. The seroprevalence of IgG increased with age, male gender and number of tick bites. Owners of cats or dogs had a lower prevalence. IgG and C6 ELISA were comparable in this group of blood donors, and specimens strongly reactive in IgG and C6 were all positive in immunoblot for IgG. False-positive IgM results, including immunoblot positive, seem to be a challenge. The findings may help laboratories in developing prudent testing algorithms and in assessing predictive values of testing for these antibodies.

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