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Geographical differences in seroprevalence of *Borrelia burgdorferi* antibodies in Norway, 2011–2013

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ABSTRACT

Detection of specific antibodies against *Borrelia burgdorferi* sensu lato is a useful aid for the diagnosis of Lyme borreliosis. However, antibodies are present in the general population. The seroprevalence increase with age, and varies according to the prevalence of infected ticks. We performed a seroprevalence study of IgM and IgG antibody reactivity against *B. burgdorferi* sensu lato in Norway by age-groups and geography, in order to provide a reference set of seroprevalence to inform the interpretation of positive test results.

We used two commercially available enzyme immuno assays (EIA) and a multiplexed bead assay to detect *Borrelia* IgG antibodies in a convenience sample of 3057 sera collected from clinical chemistry laboratories in 10 of 19 counties in Norway between December 2011 and January 2013. We estimated seroprevalence by age and county by a logistic regression model. IgM antibodies were detected by two commercially available EIAs and a multiplexed bead assay.

The overall seroprevalence of *Borrelia* IgG was 4.0% (95% CI: 2.4–6.6%) and 4.2% (2.6–6.8%) by the two EIAs, respectively. The seroprevalence increased by age, and by geography from north to south. The IgG assays showed a good agreement for positive test results. All sera positive for IgG in the multiplexed bead assay reacted with the VlsE antigen, and also had high antibody levels by EIA.

The *Borrelia* seroprevalence varied by geography and increased by age. The results indicate regional differences in pre-test probabilities for positive test results, and can inform the interpretation of laboratory results.

1. Introduction

Lyme borreliosis (LB) is the most common tick borne disease in Europe. LB is caused by infection with the spirochaete *Borrelia burdorferi* sensu lato, transmitted by *Ixodes ricinus* ticks. In Europe, the most common genospecies of *B. burdorferi sensu* lato are *Borrelia garinii*, *Borrelia afzelii* and *Borrelia burgdorferi* sensu stricto (Stanek et al., 2012).

The incidence of LB in Europe is highest in central Europe, and decrease to the North and to the West (EUCALB, 2009). However, LB incidence rates from population-based surveillance are dependent on case-definition and notification criteria, awareness of infection, and availability of diagnostic testing and the diagnostic methods used (Ertel et al., 2012), thus comparison across surveillance sites is difficult. In Norway, only disseminated LB, and not erythema migrans, is notifiable to the Surveillance System for Communicable Diseases (MSIS) since 1995. In the period 2004–2013 the incidence rate of LB in Norway ranged between 5.0 and 7.3 cases per 100,000 inhabitants, with the highest annual incidence in coastal areas in the south of Norway (Folkehelseinstituttet, 2015).

The seroprevalence of *Borrelia* IgG in the general population has been reported to range between 4% and 20% in Europe (Carlsson et al., 1998; Dehnert et al., 2012; Hristea et al., 2001; Tomao et al., 2005; Wilking et al., 2015). The seroprevalence increase with age, and by risk of infection, and may reach levels of 54% (Cetin et al., 2006; Dehnert et al., 2012; Wilking et al., 2015). In Norway, seroprevalences of 9.6% and up to 18% have been described in healthy adult blood donors from western and southern parts of the country, respectively (Hjetland et al., 2014; Mygland et al., 2006).

A two-tier testing algorithm consisting of screening with a sensitive enzyme immuno assay (EIA) and confirmation by immunoblot is recommended for diagnosis of LB the United States and in central Europe (Brouqui et al., 2004; EUCALB, 2009; Stanek et al., 2011; Wilske et al., 2007; Wormser et al., 2006). However, a single-step

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testing with EIA is currently recommended for diagnosis of LB in Scandinavia due to the high specificity of commercially available EIAs (Dessau et al., 2011; Grude et al., 2012).

We performed a seroprevalence study of IgG and IgM antibody reactivity against *B. burgdorferi* sensu lato in Norway by age-groups and geography to give an indication of regional differences in pre-test probabilities for positive test result. We compared results from two commercially available diagnostic kits and a multiplexed bead assay.

2. Materials and methods

A sample of 3057 residual sera was collected from clinical chemistry laboratories in 10 of 19 counties in Norway; Akershus, Oslo, Telemark, Vest-Agder, Hordaland, Sogn og Fjordane, Sør-Trøndelag, Hedmark, Nordland and Troms. The sera were drawn for purposes of clinical chemistry analyses between 01.12.2011 and 17.01.2013. We defined age-groups as 2–4 years ($n = 412$), 5–9 years ($n = 655$), 10–19 years ($n = 1213$), 20–39 years ($n = 460$) and ≥50 years ($n = 317$). The sample was de-identified, and no information on clinical status was available. The sample was originally collected for a *Bordetella pertussis* seroprevalence study, and the age groups under 18 years were overrepresented in the sample.

IgG antibodies to *B. burgdorferi* sensu lato were identified using two commercially available EIAs; assay A (Enzygnost Lyme link VlsE, Siemens, Marburg, Germany) and assay B (recomWell *Borrelia* IgG, Mikrogen, Neurid, Germany). IgM antibodies were analysed by two assays; assay C (Enzygnost Borreliosis IgM, Siemens, Marburg, Germany) and assay D (recomWell Borrelia IgM, Mikrogen, Neurid, Germany). Assays A and C (Enzygnost) consisted of deactivated *B. burgdorferi* antigens, supplemented with recombinant VlsE in the IgG assay (assay A). Assays B and D (Mikrogen) consisted of recombinant antigens (IgM: OspC, p41, VlsE; IgG: p100, OspC, VlsE, p18). The specificity for both IgG assays was reported to be 98–99% by the manufacturers. The sensitivity for neuroborreliosis was reported to be 87.9% by Assay A, and 99% by Assay B. Analyses were run manually according to manufacturers' instructions. Absorbance was red with a BioTec EL 808 (Winooski, VT) microplate reader and the Gen5 software. The results were interpreted according to instructions provided in the test kits.

We used a multiplexed bead assay (recomBead *Borrelia* IgG 2.0, Mikrogen, Neurid, Germany) to identify specific IgG antibodies to 13 antigens (p100, VlsE, p58, p39, OspA, OspC *Borrelia burdrorferi* sensu stricto, OspC *B. afzelii*, OspC *B. garinii*, p18 *B. burdrorferi* sensu stricto, p18 *B. afzelii*, p18 *Borrelia bavariensis*, p18 *B. garinii*, p18 *Borrelia spielmanii*). A subsample of 1483 sera were analysed for specific IgM antibodies by the multiplex bead assay (recomBead Borrelia IgM 2.0, Mikrogen, Neurid, Germany). Sera were analysed using BioPlex (Bio-Rad Laboratories AB, Oslo, Norway), and interpreted as positive when two or more antigens scored positive, according to the kit specific software algorithm provided by the manufacturer.

Seroprevalence was defined as the percentage of IgG positive sera in the sample by each IgG assay. Equivocal or grey-zone results were coded as negative. We estimated seroprevalence by age-group and county with 95% confidence interval (CI) from a mixed-effects logistic regression model with random intercepts for county and a restricted cubic spline for the age term. The restricted cubic spline was chosen to model the non-linear association between seroprevalence and age. The random intercepts for each county were used to provide a parsimonious way to model the differences between counties.

We described IgG-levels of positive sera by region using the value for % of cut-off from assay A.

We estimated concordance of results for in the EIAs for IgG and IgM, and between each EIA and the overall conclusion in the multiplex assay.

Data analyses were performed in Excel and Stata.

Ethical clearance was obtained from the regional committee for research ethic (REK 2012/429-8).

3. Results

The overall *Borrelia* IgG seroprevalence was estimated to be 4.0% (95% CI: 2.4–6.6%) and 4.2% (2.6–6.8%) by assay A and assay B, respectively (Table 1). The results from assay B gave a higher seroprevalence (difference not statistically significant) than that using assay A for all age-groups except for the ≥50 years group (Table 1). With assay A the seroprevalence increased by age, finding a 6.3% (95% CI: 3.6–10.7%) seroprevalence in the ≥50 years group, as compared to 1.8% (95% CI: 1.1–2.9%) in children aged 2–4 years (Table 1, Fig. 1). Overall, the seroprevalence among children and adolescents aged <20 years was 2.2% (95% CI: 1.3–3.5%) and 3.0% (95% CI: 2.0–4.3%) by assay A and B, respectively (Table 1). Among adults aged ≥20 years the overall seroprevalence was 4.6% (95% CI: 2.8– 7.6%) and 4.7% (95% CI: 3.0–7.3%) by assay A and B, respectively (Table 1).

By geography, the seroprevalence increased from the north to the south, and from inland to coast (Fig. 2A and B). The highest seroprevalence was found in the southernmost county (Vest-Agder) by both assays (Table 2); the seroprevalence in the ≥ 50 years group in this county was 13.7% (8.1–22.2%) and 13.0% (8.1–20.3%) by assay A and assay B, respectively.

The IgG level of seropositive sera, as measured by the median of % of cut-off-values by assay A, was highest in the southernmost county (Vest-Agder) (Table 3). Four counties had a median IgG level higher than the median of the overall sample of positive sera (Telemark, Vest-Agder, Sogn og Fjordane and Sør-Trøndelag).

In the sample, 123 sera (4.0%) were positive for IgG by either assay A or assay B; 65 (2.1%) sera were positive by both assays. A total of 2934 were negative or equivocal by both assays. We calculated the kappa coefficient for the agreement of positive and negative/equivocal test results between assay A and assay B. The kappa value was 0.69 (95% CI: 0.61–0.76), indicating good agreement.

Table 1
Number and percentage of *Borrelia* IgG positive sera, and estimated seroprevalence in Norway 2011–2013 by age-group and assay.

Age-group	Number of sera	% (number) of positive sera		Estimated seroprevalence (%)	
		Assay A	Assay B	Assay A	Assay B
2–4 years	412	1.7 (7)	3.6 (15)	1.8 (1.1, 3.0)	2.6 (1.7, 3.9)
5–9 years	655	3.7 (24)	4.1 (27)	2.0 (1.2, 3.2)	2.8 (1.9, 4.1)
10–19 years	1213	1.5 (18)	2.1 (26)	2.4 (1.5, 3.7)	3.1 (2.2, 4.5)
20–39 years	460	3.5 (16)	3.7 (17)	3.4 (2.1, 5.2)	3.9 (2.6, 5.6)
≥50 years	317	6.0 (19)	6.0 (19)	6.3 (3.6, 10.7)	5.9 (3.6, 9.6)
Overall 2–19 years	2280	2.1 (49)	3.0 (68)	2.2 (1.3, 3.5)	3.0 (2.0, 4.3)
Overall ≥20 years	777	4.5 (35)	4.6 (36)	4.6 (2.8, 7.6)	4.7 (3.0, 7.3)
Overall	3057	2.7 (84)	3.4 (104)	4.0 (2.4, 6.6)	4.2 (2.6, 6.8)

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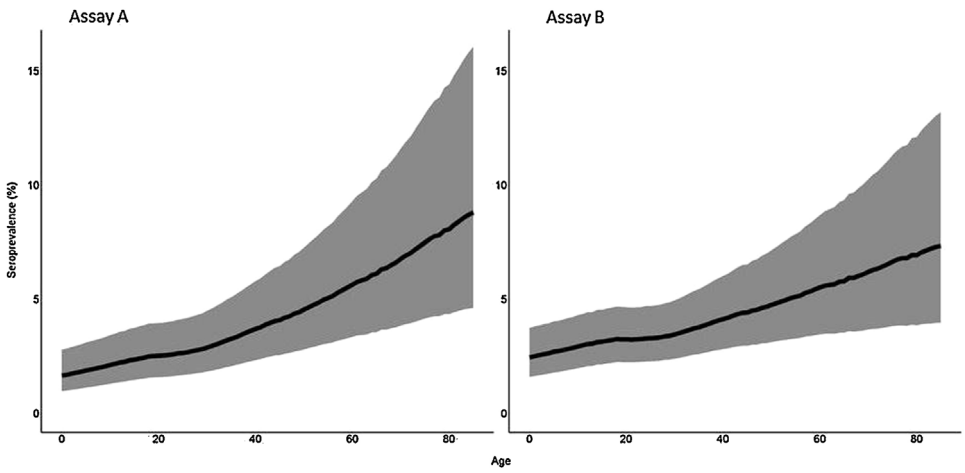

Fig. 1. Seroprevalence of *Borrelia* IgG by age in Norway, estimated by a logistic regression model using laboratory results from Assay A and Assay B. The shaded areas indicate the 95% confidence interval.

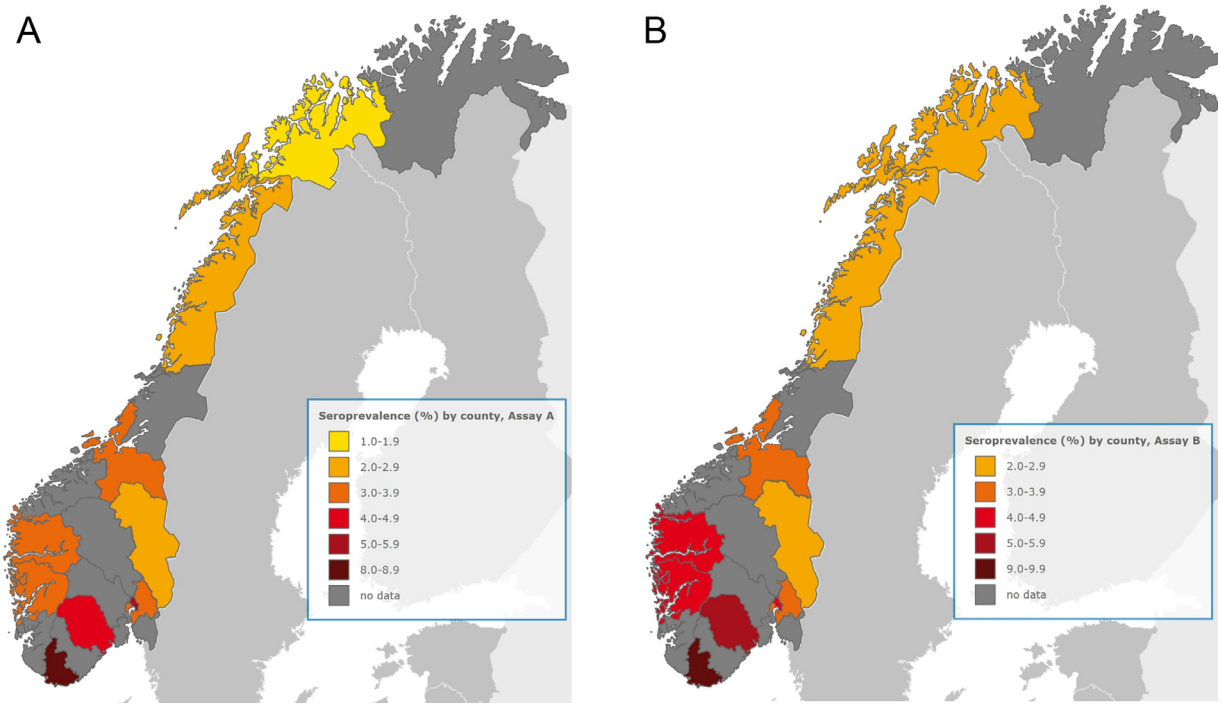

Fig. 2. Heat maps showing seroprevalence of *Borrelia* IgG by county in Norway, estimated by a logistic regression model using laboratory results from (A) Assay A, and (B) Assay B.

Table 2
Number and percentage of *Borrelia* IgG positive sera, and estimated seroprevalence in Norway 2011–2013 by county and assay.

County	Number of sera	% (number) of positive sera		Estimated seroprevalence	
		Assay A	Assay B	Assay A	Assay B
Akershus	601	2.0 (12)	1.8 (17)	3.1 (1.9, 5.2)	3.8 (2.4, 5.8)
Oslo	547	4.0 (21)	3.5 (19)	5.4 (3.3, 8.8)	4.3 (2.8, 6.7)
Telemark	178	3.9 (7)	4.5 (8)	4.8 (2.9, 7.9)	5.0 (3.2, 7.6)
Vest-Agder	198	7.6 (15)	9.6 (19)	8.8 (5.4, 14.0)	9.5 (6.3, 14.1)
Hordaland	499	2.4 (12)	3.8 (19)	3.6 (2.2, 6.0)	4.7 (3.1, 7.3)
Sogn og Fjordane	120	2.5 (3)	4.2 (5)	3.6 (2.1, 5.9)	4.6 (3.0, 7.1)
Sør-Trøndelag	301	2.7 (8)	2.7 (8)	3.9 (2.3, 6.4)	3.7 (2.4, 5.7)
Hedmark	194	1.5 (3)	1.0 (2)	2.8 (1.6, 4.6)	2.6 (1.7, 4.1)
Nordland	239	0.8 (2)	2.1 (5)	2.2 (1.3, 3.6)	3.2 (2.1, 5.0)
Troms	180	0 (0)	1.1 (2)	1.8 (1.0, 3.0)	2.7 (1.8, 4.3)
Overall	3057	2.7 (84)	3.4 (104)	4.0 (2.4, 6.6)	4.3 (2.8, 6.6)

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Table 3
Level of *Borrelia* IgG-level in positive sera, as measured by % of cut-off in Assay A, by county.

	Number of positive sera	IgG level (% of cut-off)		
		Minimum	Maximum	Median
Akershus	12	189.9	1347.7	401.0
Oslo	21	164.5	1658.0	304.2
Telemark	7	165.8	1374.2	543.2
Vest-Agder	15	207.0	1443.0	935.6
Hordaland	12	162.8	1132.9	333.2
Sogn og Fjordane	3	778.1	1096.8	915.7
Sør-Trøndelag	8	155.3	1379.2	739.9
Hedmark	3	190.0	840.0	210.5
Nordland	2	300.9	529.9	415.4
Troms	0	0	0	0
Overall	83	155.3	1658.0	529.9

By the multiplexed bead assay, 50 sera were scored as *Borrelia* IgG positive by the interpretation rules provided by the manufacturer. Of these, 45 sera (90%) were positive by both assay A and assay B, while four (8.0%) were negative by both assays. All positive sera had a strong reaction for the VlsE antigen. The *B. afzelii* p18 antigen was positive in 37 (74%) of the positive sera, while the other species-specific antigens were sporadically positive.

The IgG level, measured by the median of % of cut-off-values by assay A, was higher in sera positive in both assay A and the multiplexed bead assay (median 935.6% of cut-off, range 259.7% to 1658.0%) compared to sera positive in assay A only (median 279.4% of cut-off, range 77.6% to 849.8%).

A total of 332 (10.9%) sera were positive for IgM in either Assay C or Assay D; 54 (1.8%) sera were positive by both assays, and 2532 (82.8%) sera were negative by both assays. The kappa coefficient for agreement of the assays was 0.23 (95% CI: 0.17–0.29), indicating fair agreement. Of the 1483 sera analysed by the multiplexed bead assay for IgM, 52 (3.5%) and 6 (0.4%) sera were scored as positive and equivocal, respectively. In the 52 sera positive by the mulitiplex assay, specific IgM reactions were most frequently found for OspC from *B. burgdorferi* ss, *B. garinii and B. afzelii*, in 46 (85%), 33 (61%) and 23 (43%) positive sera, respectively. Of the 52 sera positive by multiplex, 18 sera were concordantly positive by both EIA assays.

4. Discussion

The seroprevalence of *Borrelia* IgG in the healthy population is reflecting the force of infection, as demonstrated by a higher seroprevalence in endemic areas and population groups with more frequent exposure to ticks than in the general population (Cetin et al., 2006; Sonnleitner et al., 2015). We included children in the present study, and demonstrated that the seroprevalence among adults was higher than among children. By geography, the seroprevalence was found to be highest in the southernmost county. However, the overall seroprevalence among adults aged ≥20 years, 4.6% and 4.7% by assay A and B, respectively, is lower than what has previously been reported from healthy blood donors Norway, although the 13.7% (8.1–22.2%) seroprevalence found in the age-group ≥50 years in Vest-Agder by assay A is within the same range as the 18% seroprevalence described among blood donors in the same area by Mygland et al. (2006). Interestingly, the median IgG level was highest in the southernmost county, possibly reflecting repeated or more recent exposures.

Our study is limited by the convenience sample used. Clinical information was not available, and no associations between analytical result and exposure, diagnosis or other laboratory parameters could be made. Furthermore, the sample used in the present study was gathered from clinical chemistry laboratories, while in other studies of seroprevalence, sera from healthy blood donors or risk-groups such as hunters are commonly used (Cetin et al., 2006; Hjetland et al., 2014; Hristea et al., 2001). This could bias the results, as healthy blood donors might have a more active life-style with a higher risk of exposure to ticks and borrelia than persons who have blood drawn for purposes of clinical chemistry analyses. However, in a study performed in Åland, the proportion of seropositivity and the distribution of antibody titres were the same among blood donors and health service clients from whom serum had been drawn for routine diagnostic purposes (Carlsson et al., 1998).

In Norway, more than 60,000 sera are analysed annually for specific antibodies to *Borrelia* by EIA (MacDonald E., manuscript submitted), corresponding to at least 1200 tests per 100,000 inhabitants. With suboptimal specificity, this testing frequency will lead to a high number of misdiagnoses; a test with 99.0% specificity would result in at least 600 false-positives. The two EIAs used in our study have specificities of 98.0% to 99.0%, as stated by the manufacturer. In Norwegian guidelines, it is recommended to restrict laboratory testing to cases with clinically suspected LB other than erythema migrans in order to limit the number of false positives (Grude et al., 2012). This way of improving the pre-test probability can be achieved by clinicians who selectively request serological analysis for LB, as sera are rarely rejected by the laboratory due to incomplete or irrelevant clinical information (MacDonald E., manuscript submitted). As pre-selection is rarely done in the laboratory, the proportion of IgG positivity among sera requested for borrelia diagnostics at medical microbiology laboratories has been shown to be comparable to the seroprevalence in the healthy population (Coumou et al., 2014; Dessau et al., 2010). Knowledge about the background seroprevalence is needed to interpret the clinical relevance of the laboratory result. As the analytical specificity appears to improve when the levels of specific antibody increase, a quantitative or semi-quantitative evaluation of the antibody level could provide useful information for the overall diagnostic interpretation of a positive test result.

The two IgG assays provided comparable results for seroprevalence. The present study demonstrates a substantial concordance between EIA assays, and an even higher concordance of sera positive in both EIA assays and the multiplexed bead assay. Sera with strong antibody reactions have a higher inter-assay concordance, as has been shown by Hjetland et al. (2014), and it has been suggested to add a second EIA or immunoblot analysis for testing of sera with low-grade positive reactions. An EIA for an internal peptide of VlsE, C6, has been proposed as a confirmatory test in these instances in order to improve the analytical specificity (Grude et al., 2012; Hjetland et al., 2014).

Results of the multiplex assay can be analysed and scored automatically, providing an alternative to the more labour intensive and subjective immunoblots used in two-tier testing algorithms. The utility of the multiplex assay in a Scandinavian setting has been discussed and found to improve by using adapted algorithms of interpretation (Dessau et al., 2015). In the present study, only 40% of EIA-positive specimens were confirmed by multiplex analysis. In a seroprevalence study from Germany, Wilking et al. (2015) reported 68% confirmation of EIA results by line-blot. Dessau et al. (2015) described differences in specificity of the multiplex assay between Sweden and Denmark. The reason for these discrepancies is not evident. However, the sensitivity for EIA appears to be higher than for multiplex assays. Furthermore, the main reactivity in the multiplex assay was for the VlsE antigen, and the utility of multiplex assays and C6-EIA assays should be compared in further studies of clinical information available.

The two EIA assays used for detection of IgM had a different antigen composition; assay C was composed of extracted and inactivated antigens, while assay D was composed of recombinant antigens. This might explain the limited agreement between EIA

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assays for borrelia IgM. However, the low concordance between EIA and multiplex assay for IgM, might also be reflecting a limited analytical specificity in these assays.

The seroprevalence varied by geography and increased by age. Although we report a lower overall seroprevalence than in previous studies from Norway, the seroprevalence is within the same range, and a distinct geographical distribution is evident, corresponding to the prevalence of ticks and the incidence of LB in Norway. The results indicated regional differences in pre-test probabilities for positive test results, and could inform the interpretation of laboratory results.

The diagnostic specificity of the recommended one-step testing algorithm in Norway should be further investigated in a different study setting, in order to improve the use of serology as a tool for the diagnosis of LB. In the Norwegian setting with high testing activity and a one-step testing algorithm, the recommendation of only testing sera from cases with clinically suspected disseminated disease should be reinforced. Alternative strategies for improved specificity that could be explored include adding a second test for low-grade positives, or by increasing the cut-off for positivity.

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