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The prevalence of *Borrelia burgdorferi* sensu lato in questing *Ixodes ricinus* ticks in Norway

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Norway represents the northern limit in the geographical distribution of *Ixodes ricinus* ticks in Europe. During the last decade an expansion of the range of this tick further north and at higher altitudes has been detected. This could affect the spread of *Borrelia burgdorferi* sensu lato pathogens in new territories and increase the risk of human infection in the country. The aim of the present study was to determine the prevalence of *B. burgdorferi* s.l. in *I. ricinus* ticks in different localities of Norway. Ticks were collected in 24 locations from northwest to southeast Norway during spring–summer 2004 and 2006–2008. We used *fla* gene as a target for amplification of spirochete DNA. Multiplex PCR based on *ospA* gene of *B. burgdorferi* s.l. was used for species identification. The heterogeneity of *B. garinii* strains was investigated using *ospA*-based sequencing analysis. *B. burgdorferi* s.l. was detected in 171 (7.2%) out of 2360 *I. ricinus* ticks analyzed. The infection rate varied from zero to 32% in the investigated locations. Infection rate in adults (11.9%) was significantly higher than in nymphs (4.7%). The overall infection rate in ticks from western Norway (4.0%) was significantly lower than in those from southern Norway (10%). Four *B. burgdorferi* s.l. genospecies were identified, and the most common was *B. afzelii* (71.9.0%), followed by *B. garinii* (12.9%), *B. burgdorferi* s.s. (7%) and *B. valaisiana* (1.2%). A total of eight (4.8%) of the infected ticks harboured *mixed infections.* Phylogenetic relationship between *B. garinii* strains and their correspondence to OspA serotype types was compared with the sequences registered in GenBank database. Five genotypes of *B. garinii* derived from ticks were identified. The most frequent was OspA type 6.

Key words: *Borrelia burgdorferi* s.l., *Ixodes ricinus*, Lyme borreliosis, OspA type, Norway

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INTRODUCTION

Lyme borreliosis (LB) is the most common tick-borne multisystemic disease of humans caused by *Borrelia burgdorferi* s.l. spirochete in temperate climates of the northern hemisphere. *B. burgdorferi* s.l. is adapted mainly in wild animals with the nature cycle involving ixodic ticks as vectors. The main European vector for *B. burgdorferi* s.l. is the sheep tick *Ixodes ricinus*. During the last decades, distribution and abundance of *I. ricinus* ticks have increased in Europe due to climatic changes. Norway represents the northern limit in geographical distribution of this species in Europe, and a recent study has indicated an expansion of the range of *I. ricinus* further north and at higher altitudes (Jore et al., 2011). This could affect the spread of *B. burgdorferi* s.l pathogens in new territories and increase the risk of human infection in the country. In Norway LB is a common disease, however, only disseminated and chronic forms of LB are notifiable, which lead to an underestimation of the total incidence. The annual number of cases during 2003–2013 varied from 149 in 2003 with a top in 2008 with 346 cases as shown in MSIS annually report (www.msis.no).

At present it is known that seven genospecies *B. afzelii*, *B. garinii*, *B. valaisiana*, *B. burgdorferi* s.s., *B. lusitaniae*, *B. bissetii*, *B. spielmanii,* and in addition novel *B. bavariensis* (the rodent-associated ecotype of *B. garinii*, OspA serotype 4 strains) are present in Europe (Stanek and Reiter, 2011). Additionally, Hasle et al. (2011) found *B. turdi* in an *I. ricinus* tick infested on a migrating bird in Norway. However, only three of them, namely *B. afzelii*, *B. garinii*, and *B. burgdorferi* s.s are known to be clearly pathogenic to humans. They cause different clinical manifestations (*B. afzelii* is associated with dermatoborreliosis, *B. garinii* with neuroborreliosis and *B. burgdorferi* s.s. with arthritis) (Ružić-Sabljić et al., 2008). Other species of *B. burgdorferi* s.l. complex *B. bissetii*, *B. spielmanii, B. valaisiana*, and *B. lusitaniae* have been detected in patients, but their pathogenicity remains unclear and still discussed (Stanek and Reiter, 2011). It is known that three human pathogenic species (*B. afzelii, B. garinii* and *B. burgdorferi* s.s.) comprise at least seven OspA serotypes in Europe (Wilske et al., 1993; Michel et al., 2003; Wilske et al., 2007). Earlier studies showed that OspA strains of different pathogenicity have been detected with different prevalence in different tissue of humans and animals (Marconi et al., 1999; Wiske et al., 1993; Wiske et al., 2007). It was discovered that skin isolates primarily consist of *B. afzelii* (OspA type 2), while isolates from cerebrospinal fluid (CSF) and ticks are heterogeneous with a predominance of *B. garinii* (OspA type 3–7).

Although there are some data on the distribution of *B. burgdorferi* s.l. species in Norway (Jenkins et al., 2001; Paulauskas et al., 2008; Rosef et al., 2009; Kjelland et al., 2010a; Mysterud et al., 2013), no studies regarding the heterogeneity of OspA types are available. Since different species and OspA types of *B. burgdorferi* s.l. have different pathogenic potential, the information about their distribution in ticks is essential for local risk assessment. The aim of the present study was to define the prevalence of different *B. burgdorferi* s.l. species and OspA types in *I. ricinus* ticks in different localities of Norway.

MATERIALS AND METHODS

Tick collection

Unfed *I. ricinus* ticks were collected in spring–summer seasons during 2004 and 2006–2008 in 24 localities with different habitats spread from northwest to southeast of Norway (Fig. 1).

In the north-western part ticks were collected from Fjelløyvær, Strøm localities, in the western part – from Etne, Mundheim, Hermansverk, Bakke, Svanøy, Utvik, Hellesylt, Stranda, Surnadal, in the southern part – from Lista, Søgne, Odderøya,Tjore,Tromøy, Hinnebu, Tvedestrand, Risør, and in the southeastern part – from Jomfruland, Kjosvik, Løvøya, Hvasser and Mølen localities (Fig. 1).

Fig. 1. Map of sampling localities in Norway

1 – Fjelløyvær; 2 – Strøm; 3 – Surnadal; 4 – Stranda; 5 – Hellesylt; 6 – Utvik; 7 – Svanøy; 8 – Hermansverk; 9 – Bakke; 10 – Mundheim; 11 – Etne; 12 – Lista; 13 – Søgne; 14 – Odderøya; 15 – Tjore; 16 – Tromøy; 17 – Hinnebu; 18 – Tvedestrand; 19 – Risør; 20 – Jomfruland; 21 – Kjosvik; 22 – Løvøya; 23 – Hvasser; 24 – Mølen

I. ricinus ticks were collected using a standard 'flagging' method for collecting active ticks on vegetation (Hillyard, 1996). A cotton cloth of 1 m^2 was waved and inserted deep through low bushes, tall grass, shrubs, and low tree branches. The cloth was in contact with the surface and checked for ticks every 10 m. All the attached ticks were removed from the cloth, placed into sealed vials containing 70% ethanol, and stored until processed. Ticks were classified according to their stage and were identified by their morphological characteristics (Fillippova, 1977; Hillyard, 1996). To calculate the overall prevalence of *B. burgdorferi* s.l. in ticks and compare the infection rate and distribution of different *B. burgdorferi* s.l. genospecies in different regions, some data of our previous studies (Paulauskas et al., 2008; Rosef et al., 2009) were included in the analysis.

DNA extraction

All the ticks were analyzed individually. Extraction of the DNA from the ticks was carried out by lyses of tick in ammonium hydroxide (NH_4OH) (Rijpkema et al., 1996; Ambrasiene et al., 2004). Each nymph and adult samples were put in separate 0.5 ml microcentrifuge tubes, filled with 2.5% ammonia solution (80 µl for nymphs and 100 µl for adults), and then heated at 99 °C for 25 min in thermostat. Further on the tubes were opened and heated at 99 °C for evaporation of ammonia (approx. 15–20 min). The lysates were stored at –20 °C.

To validate the morphological identification of ticks and efficiency of the DNA extraction (subsequently avoid potential false-negative results due PCR inhibition) PCR analysis with species-specific primers IxriF and IxriR resulting in a 150 bp segment of the 5.8 srRNA gene amplification was performed (Fukunaga et al., 2000; Radzijevskaja et al., 2005). Only tick-DNA positive samples were further analyzed for the presence of *B. burgdorferi* s.l.

Screening for *Borrelia burgdorferi* sensu lato

Detection of *B. burgdorferi* s.l. infection was performed using direct PCR amplification of *B. burgdorferi* s.l. DNA from ticks. We used *fla* gene that is highly conserved among *Borrelia* species as a target for amplification of spirochete DNA (Picken, 1992). PCR was performed by using FL6 and FL7 primers as previously described in Paulauskas et al. (2008).

***B. burgdorferi* s.l. genospecies identification**

Borrelia positive ticks were involved in further analysis in order to determine *Borrelia* genospecies. The primers used in the study for species identification are shown in Table 1. Three pairs of oligonucleotide primers based on *B. burgdorferi* s.l. *ospA* gene sequence have been used to type *B. afzelii, B. garinii,* and *B. burgdorferi* s.s. genospecies (Demaerschalck

Table 1. PCR primers used for identification of *B. burgdorferi* s.l genospecies

Target species	Sequences of specific primers	Target gene	Length (bp) of PCR products
B. burgdorferi s.s.	GI-L: 5'-AACAAAGACGGCAAGTACGATCTAATT-3' GI-R: 5-TTACAGTAATTGTTAAAGTTGAAGTGCC-3'	*ospA*	544
B. garinii	GII-L: 5'-TGATAAAAAC AACGGTTCTG GAAC-3' GII-R: 5'-GTAACTTTCAATGTTGTTTTGCCG-3'	*ospA*	345
B. afzelii	GIII-L: 5'-TAAAGACAAAACATCAACAGATGAAATG-3' GIII-R: 5'-TTCCAATGTTACTTTATCATTAGCTACTT-3'	*ospA*	189
B. valaisiana	OspA F 5′GCA AGT CAA ACG GGA TGT AGT′3 OspA R 3′GTA TTT TAT GCA TAG ACT TTA TAT G′5	*16S rRNA*	549
*B. lusitania*e	rpoB –F 5'AGA GCT TCT TGC TAA TAT ATA TA'3 rpoB –R 3'GCC TGG GGG ACT TTC AAG A'5	*rpoB*	250

et al., 1995; Paulauskas et al., 2008). The target region and primers for identification of *B. valaisiana* and *B. lusitaniae* (16S rRNR and *rpoB* gene, respectively) were chosen according to studies of Liebisch et al. (1998), Lee et al. (2000) and Vennestrøm et al. (2008). The PCR amplification products were separated by electrophoresis on 2% agarose gel and visualized under UV light.

Sequencing

To confirm the results of PCR amplification and investigate genetic variation among isolated strains thirteen PCR products of 345 bp *ospA* gene were extracted from the agarose gel and purified with Nucleospin extract II purification Kit (Macherey-Nagel, GmbH & Co). PCR products were bidirectional sequenced using the ABI PRISM 3130XL Genetic Analyzer (Applied BioSystems, USA). The obtained sequences were edited and analysed using the Mega 5.2 package programs. A phylogenetic tree was constructed using the neighbor-joining (NJ) method with bootstrap analysis of 500 replicates.

The partial sequences of the *B. garinii ospA* gene were submitted to the GenBank under the following accession numbers: HM623296 (5.3), HM623297 (5.26), HM623298 (9.36), HM623299 (11.25), HM623300 (12.5), HM623301 (12.6), HM623302 (12.10), HM623303 (15.29), HM623304 (16.8), HM623305 (H41), HM623306 (H68), HM623308 (St9) and KJ526129 (Tj46).

Statistical analysis

Differences in the prevalence of *B. burgdorferi* s.l. in different stages (nymphs versus adults) and sampling sites (western, south and south-eastern parts of the country) of *I. ricinus* were evaluated statistically using the two-tailed χ^2-test. Statistical analysis was performed using the statistical software package STATISTICA for Windows 5.5.

RESULTS

A total of 2 360 (697 female, 669 male and 994 nymphs) ticks collected from 24 locations during the period 2004 and 2006–2008 were analysed (Table 2). *B. burgdorferi* s.l. DNA was found in 7.2% (171 out of 2 360) of ticks. The overall infection rate varied from zero to 32% in different localities as shown in Table 2. Altogether, nymphs had a significantly lower infection rate (4.7%, 47/994) than adults (9.1%, 124/1366) (χ^2 = 16.19; df = 1; p = 0.0001) (Table 2). The mean infection rate in female *I. ricinus* ticks was significantly higher – 11.9% (83/697) than in males – 6.3% (42/669) (χ^2 = 13.02; df = 1; p = 0.003) and nymphs – 4.7% (47/994) (χ^2 = 29.76; df = 1; p = 0.0000). Infected ticks were found in 19 sampling sites (Table 2). The overall prevalence of infection in locations from western Norway (locations 1–12 in Table 2) – 4.0%; 43 out of 1 082 was significantly lower than in those from

Table 2. Prevalence and distribution of *Borrelia burgdorferi* sensu lato genospecies in questing *Ixodes ricinus* ticks in Norway

	Location	Year	Total	Prevalence		female	male	nymph	Ba	Bg	Bss	Bv	Ba/Bss	Ba/Bg	B. spp.
			N	n	%	N(n)	N(n)	N(n)	n	n	n	n	n	n	n
1	Fjelløyvær	2006/7/8	204	0	0	34(0)	41(0)	129(0)							
2	Strøm	2006/7/8	237	5	2.1	50(1)	42(1)	145(3)	4	1					
3	Surnadal	2007	56	8	14.3	20(4)	21(3)	15(1)	3	4		1			
4	Stranda	2007	48	2	4.2	13(1)	16(1)	19(0)	1	1					
5	Hellesylt	2007	9	0	0	5(0)	3(0)	1(0)							
6	Utvik	2007	100	12	12.0	22(5)	26(4)	52(3)	11	1					
7	Svanøy	2004	60	5	8.3	10(0)	4(0)	46(5)	4	1					
8	Hermansverk	2007	86	3	3.5	33(3)	42(0)	11(0)	3						
9	Bakke	2004	5	4	=	2(2)	1(1)	2(1)	3	1					
10	Mundheim	2007	74	1	1.4	8(1)	5(0)	61(0)		1					
11	Etne	2007	74	0	0	11(0)	15(0)	48(0)							
12	Lista	2007	129	3	2.3	43(3)	32(0)	54(0)	3						
13	Søgne	2007	25	0	0	6(0)	5(0)	14(0)							
14	Odderøya	2007	164	3	1.8	57(1)	46(1)	61(1)	3						
15	Tjore	2006/7/8	162	17	10.5	47(4)	41(3)	74(10)	13	4					
16	Tromøy	2006/7	78	13	16.7	37(7)	30(2)	11(4)	9	1	2		1		
17	Hinnebu	2006/7/8	300	36	12.0	125(22)	130(10)	45(4)	34	2					
18	Tvedestrand	2006/7	107	10	9.3	36(8)	37(1)	34(1)	5	1	2	1			1
19	Risør	2006	59	7	11.9	19(2)	18(1)	22(4)	6				1		
20	Jomfruland	2006/7/8	243	13	5.3	67(6)	58(2)	118(5)	7		4		1		1
21	Kjosvik	2004	7	0	0	3(0)	4(0)								
22	Løvøya	2006	60	12	20.0	23(5)	23(4)	14(3)	8		2				2
23	Hvasser	2006	23	1	4.4	8(0)	4(0)	11(1)					1		
24	Mølen	2006	50	16	32.0	18(8)	25(7)	7(1)	6	4	2		1	3	
	Total		2360	171	7.2	697(83)	669(42)	994(47)	123	22	12	2	5	3	4

N – number of tested ticks; n – number of positive; Ba – *B. afzelii*, Bg - *B. garinii*, Bss – *B. burgdorferi* s.s., Bv – *B. valaisiana*

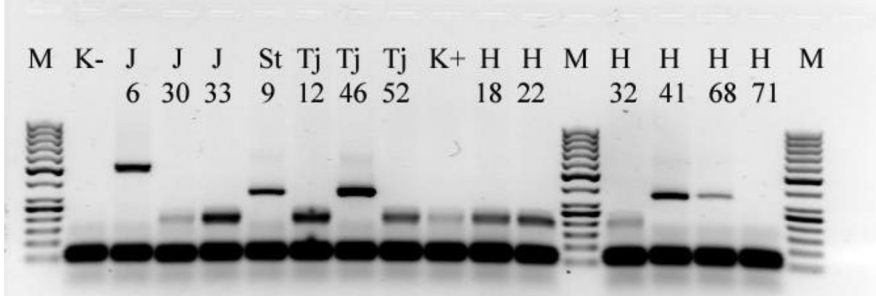

Fig. 2. Genotyping analysis of *B. burgdorferi* s.l. genospecies

Lanes M: 50-bp marker; lane K–: negative control; lane K+: positive control of *B. afzelii* (189 bp). The presence of DNA bands indicates samples infected with *B. burgdorferi* s.s. (544 bp) – sample J6; *B. garinii* (345 bp) – samples St9, Tj46, H41, H68; *B. afzelii* (189 bp) – samples J30, J33, Tj12, Tj52, H18, H22, H32. Sample H71 unspecified

southern Norway (location 13–24) – 10%; 128 out of 1 278 ($\chi^2 = 31.82$; df = 1; p = 0.0000).

Multiplex PCR analysis indicated the presence of *B. afzelii, B. garinii*, *B. burgdorferi* s.s., and *B. valaisiana* in ticks collected in Norway (Fig. 2; Table 2).

B. afzelii has been found as the dominant genospecies in *I. ricinus* and was detected in 17 from 24 investigated localities. No differences were observed between prevalence of *B. afzelii* in ticks from western and southern parts of Norway. Totally, among the 171 infected tick samples, 71.9% (123) contained *B. afzelii* DNA, 12.9% (22) contained *B. garinii* DNA, 7.0% (12) contained *B. burgdorferi* s.s. DNA and 1.1% (2) contained *B. valaisiana* DNA. Double infections were observed in 7% (12) of ticks (*B. afzelii* + *B. burgdorferi* s.s. and *B. afzelii* + *B. garinii*) (Table 2). In four samples (2.3%) of infected ticks, *Borrelia* infection was not identified to the species level by using the taken primers (Table 1).

Table 3. Genetic variation in the ospA nucleotide sequences of *B. garinii* derived from questing *Ixodes ricinus* ticks

Analyzed samples	Nucleotide position of variable sites											
	58	61	68	73	75	85	100	115	177	242	243	245
H41 I. r. Hinnebu	T	C	C	T	A	T	C	A	C	G	G	T
St9 I. r. Hitra(Straum)	.	.	.	.	.	.	.	.	.	.	.	.
9.36 I. r. Utvik	.	.	.	.	.	.	.	.	.	.	.	.
11.25 I. r. Stranda	.	.	.	.	.	.	.	.	.	.	.	.
5.3 I. r. Tjore	.	.	.	.	.	.	.	.	.	.	.	.
15.29 I. r. Tromøy	.	.	.	.	.	.	.	.	.	.	.	.
16.8 I. r. Tvedestrand	.	.	.	.	.	.	.	.	.	.	.	.
X80252_*B. garinii* TN	.	.	.	.	.	.	.	.	.	.	.	.
X80253 *B. garinii* PWudll	.	.	.	.	.	.	G	.	.	.	.	.
12.6 I. r. Surnadal	G	.	.	.	.	.	.	.	.	.	.	.
12.10 I. r. Surnadal	G	.	.	.	.	.	.	.	.	.	.	.
H68 I. r. Hinnebu	G	.	.	C	.	.	.	.	.	.	.	.
5.26 I. r. Tjore	G	.	.	C	C	.	.	.	.	.	.	.
12.5 I. r. Surnadal	G	T	A	C	C	.	.	.	T	.	.	.
Tj46 I. r. Tjore	G	T	A	C	C	.	.	.	T	.	.	.
X66065_*B. garinii* strain ZQ1	G	T	A	C	C	.	.	.	T	.	.	.
X85441 *B. garinii* WABSou	G	T	A	C	C	C		.	T	A	A	A
X80251 *B. garinii* PHei	G	T	A	C	C	C		G	T	A	A	A

Diversity of *B. burgdorferi* s.l. genospecies in ticks varied in western and southern localities. Three genospecies of *B. burgdorferi* s.l. were identified in ticks from western Norway *B. afzelii* (74.4%), *B. garinii* (23.3%) and *B. valaisiana* (2.3%). In southern Norway *B. afzelii* (71.1%), *B. garinii* (9.4%), *B. burgdorferi* s.s. (9.4%), *B. valaisiana* (0.8%), not identified *Borrelia* spp. (3.1%), and 9.4% of mixed infections were detected.

Sequence analysis

The sequence analysis of random selected positive samples confirmed *B. afzelii*, *B. garinii*, *B. burgdorferi* s.s., and *B. valaisiana* genospecies. In the present study we investigated the heterogeneity of *B. garinii* strains derived from thirteen positive tick samples. Sequencing of the partial (345 bp) *ospA* gene revealed 5 different sequence types (Table 3). The analyzed sequences had between one and six nucleotide differences at six positions and were 97.7–100% identical to each other, with 85.4–100% homology to *B. garinii* sequences obtained from GenBank.

To investigate the correspondence of strains to OspA serotype types the obtained sequences were compared with the strains of serotypes described for *B. garinii* (Wilske et al., 1993; Will et al., 1995; Wilske et al., 2007) from GenBank. Among the 13 *ospA* sequences obtained from ticks in this study, 5 different genotypes were identified. The relationship between *B. garinii* ospA sequences identified in this study (n = 13) and presented in GenBank (n = 10) are shown in Fig. 3.

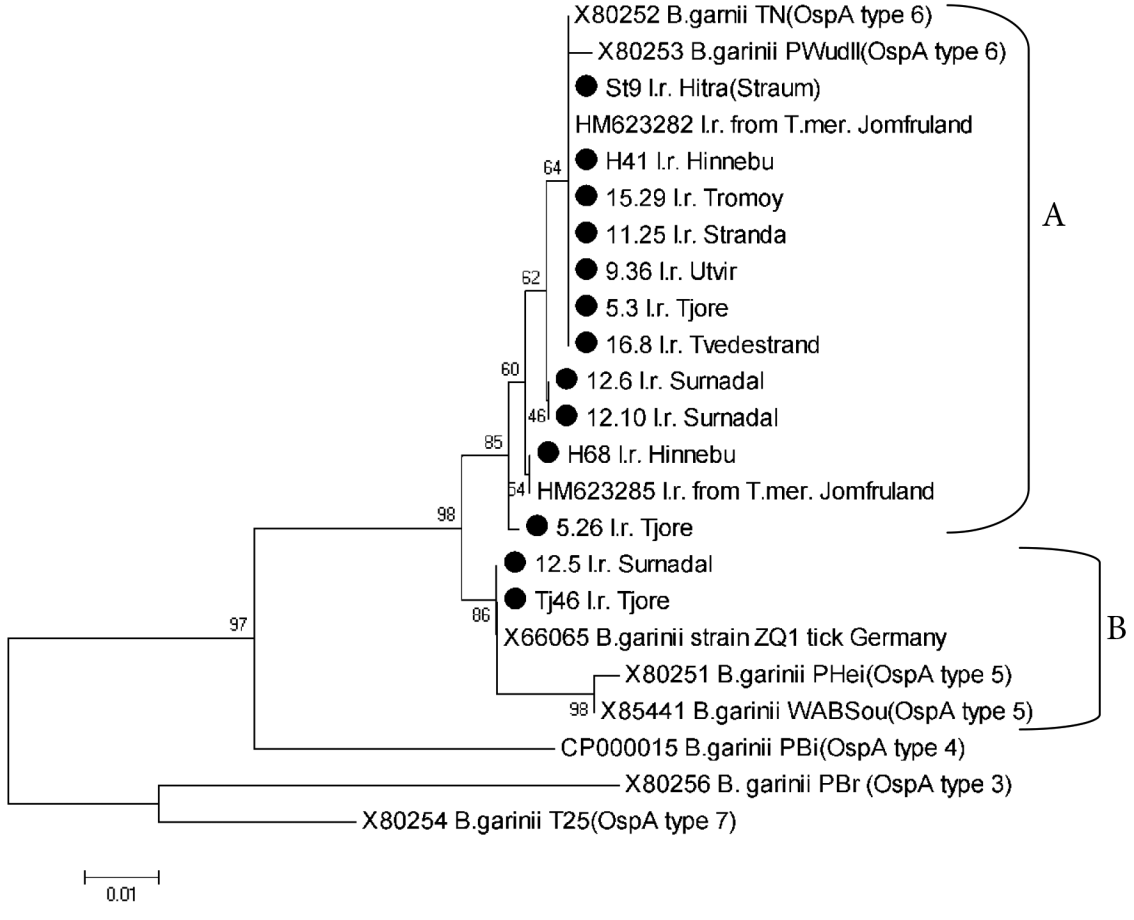

Fig. 3. Phylogenetic tree of the *ospA* gene sequences created using a neighbor-joining method and bootstrap analysis of 500 replicates. Strains with sequence accession numbers have been taken from GenBank for comparison. The scale bar indicates nucleotide substitutions per site. Abbreviations: I. r. – *Ixodes ricinus*; T. mer. – *Turdus merula*

Seven of analyzed *B. garinii* sequences were identical to *ospA* sequences of OspA 6 type (Fig. 3; Table 3). In the phylogenetic tree (Fig. 3) these sequences formed a separate cluster (A) with other four, closely related, sequences (pairwise distances range from 0.3 to 1.3%), which differed from each other at one to three nucleotide positions (Table 3). Cluster B on the phylogenetic tree represents *B. garinii* ospA sequences, which are similar (pairwise distances range from 0.3 to 3.6%) to OspA 5 type sequences (WABSou, PHei) and differ from the corresponding sequences at four to five nucleotide positions (Table 3; Fig. 3). Some of the sequences analyzed in this study were identical to those, previously obtained in engorged *I. ricinus* tick removed from birds in Norway (Paulauskas et al., unpublished). Five sequences (5.26; 12.6, 12.10; 12.5, Tj.46), representing three genotypes, were unique and have so far not been described (Table 3; Fig. 3). Separation of sequences within the phylogenetic tree seems to not depend on the geographic origin.

DISCUSSION

Previous studies in Europe have shown that the infection rate of *Borrelia* spirochetes in *I. ricinus* varies between geographical regions, and seems to be influenced by the nature of the habitat, in particular with tick hosts that are found in the area (Gern and Humair, 2002; Kurtenbach et al., 2002a; 2002b). Positive correlation between tick density and prevalence of *Borrelia* in ticks has been found (Tällerklint and Jaenson, 1996). Such correlation has been explained as a result of contact between vectors and the reservoir hosts. To better understand the disease risk in a particular region, knowledge about variation in pathogen prevalence in ticks and host is needed.

In the present study we have investigated the prevalence of *B. burgdorferi* s.l. in questing ticks from 24 locations spread from northwest to southeast Norway. The study was conducted during the years 2004 and 2006–2008, and the data of prevalence of infection in different locations and different years was combined (Table 2). We found different prevalence at the localities ranging from zero to 32%. The locality with the highest prevalence (80%) represented only 5 ticks and was not included in calculation of prevalence at this site (Table 2). The overall infection rate in ticks from southern Norway (10.0%) was significantly higher than in those from western Norway (4%). A similar infection rate (11.5%) in southern Norway has been described by Jenkins et al. (2001). Kjelland et al. (2010a) recently conducted studies in southern Norway and detected 24.5% and 26.9% of infection prevalence in nymphs and in adult ticks, respectively. The higher prevalence obtained in Kjelland et al. (2010a) study compared to the present and Jenkins et al. (2001) studies could be caused by the sensitivity of the detection method (RT-PCR) used and the chosen target gene. In our study *ospA* gene located on a 54 linear plasmid was chosen as target for PCR. This gene previously has been successfully used as sensitive PCR target for detection of *B. burgdorferi* s.l. in patient and ticks (Rijpkema et al., 1997; Vasiliu et al., 1998; Michel et al., 2003). The heterogeneity of the causative strains is a challenge for the microbiological diagnosis of Lyme borreliosis in Europe. The heterogeneity observed among *ospA* sequences of European strains (especially regarding *B. garinii*), has been used for the development of diagnostic tools (Wilske et al., 1993; Will et al., 1995; Michel et al., 2003).

The present study provides data on the distribution of *B. garinii* OspA types in Norway using ospA-based sequencing analysis. Five genotypes of *B. garinii* strains from ticks were identified. Thirteen analyzed 345 bp *ospA* gene sequences showed similarity with sequences of OspA serotype 6 and OspA serotype 5 types. The most frequent *B. garinii* OspA type in the present study was OspA type 6. Eleven sequences derived from adult ticks, from which seven were identical and six closely related to OspA type 6 strains, comprised of four genotypes. Two from these genotypes have previously been found in nymphs feeding on migrating birds in Norway

(Fig. 3) (Radzijevskaja et al., 2011; Paulauskas et al., unpublished). Other strain, more similar to OspA type 5, was derived from two ticks (one female and one nymph). The obtained sequences differed from the corresponding OspA type 5 sequences at four to five nucleotide positions (Table 3; Fig. 3). As shown in the study of Lenčakova et al. (2006), the distribution of *B. garinii* OspA types in ticks could be related to development stages of ticks and specific transmission cycles of the pathogens. The authors found that *I. ricinus* nymphs harboured almost exclusively *B. burgdorferi* s.s. and *B. garinii* OspA type 4 (which are associated with rodents), while in adults a broad variety of *B. burgdorferi* s.l. types was present. According to previous investigations (Wilske et al., 1993; Wilske et al., 2007), OspA type 6 was rarely found in patients, and most frequently detected in the ticks and seems to have low pathogenicity. OspA serotype 5 strains, which according to Wilske et al. (1993) resulted from genetic recombination of serotype 4 and 6 *ospA* genes, have been detected in patients and rarely in ticks and rodents (Lenčakova et al., 2006; Radzijevskaja et al., 2011). *B. garinii* OspA serotype 4 strains, which are associated with rodents and found in ticks (Lenčakova et al., 2006), and an OspA type 7 strain (T25), previously isolated from ticks (Michel et al., 2003; Lenčakova et al., 2006), were not detected in the present study.

I. ricinus ticks acquire *Borrelia* primarily through blood meals and the risk of infection increases with the number of blood hosts. Consequently, a higher *Borrelia* infection is expected in adults compared to nymphs. We detected a significantly higher prevalence of *Borrelia* in adult ticks compared with nymphs and a higher infection rate among females than males (Table 2). Such differences were also registered in the study of Mysterud et al. (2013) conducted along the western coast of Norway in 2011.

Corresponding to previous European studies (Stanek and Reiter, 2011), four species of *B. burgdorferi* s.l., *B. afzelii*, *B. garinii*, *B. burgdorferi* s.s. and *B. valaisiana*, were detected in this study. The most frequently detected species was *B. afzelii* that is in accordance with other studies previously conducted in Norway (Jenkins et al., 2001; Paulauskas et al., 2008; Kjelland et al., 2010a).

Rodents are the main reservoir hosts for *B. afzelii* in Norway. As demonstrated in our previous studies in six locations of western, southern and south-western Norway, the prevalence of *B. afzelii* range from 5% in *Apodemus sylvaticus* to 6% in *A. flavicollis* mice (Paulauskas et al., 2008), and *B. afzelii* dominated in ticks collected from rodents (Radzijevskaja et al., 2013). Red squirrel was identified as host for *B. burgdorferi* s.s. and *B. afzelii* (Radzijevskaja et al., 2011).

Passerine bird species, like blackbirds and song thrushes, and a few seabird species have been shown to be reservoir competent for *B. garinii* and *B. valaisiana* (Kipp et al., 2006; Taragelòvá et al., 2008; Kjelland et al., 2010b; Hasle et al., 2011). We detected 85.5% prevalence of *B. garinii* in infected immature ticks collected from migratory birds in southern Norway (Paulauskas et al., unpublished).

The host for adult ticks is mainly large vertebrates. This may lead to high density of ticks in areas with wild cervids (moose, roe deer, red deer) and browsing livestock. It was suggested that the presence of deer has a diluting effect on boreal infections (Jaenson and Tällerklint, 1992; Rosef et al., 2009). As shown in our previous study on an island in Norway (Rosef et al., 2009) the wild cervids reduced the *Borrelia* in sucking adults and diluted the infection in questing ticks. As deer may eliminate infection in ticks, the difference in the population density of wild cervids is primarily responsible for the maintenance of the tick population and explains the lower prevalence in adult *I. ricinus* in some locations. Our study supports the hypothesis of the dilution effect in ticks according to the high density of red deer. We detected lower prevalence of *Borrelia* infection in questing ticks from western regions with high density of red deer compared to the southeast, that could be due dilution of *Borrelia* in feeding ticks. Another study recently

conducted in the west of Norway (Mysterud et al., 2013) confirmed the hypothesis of the dilution of *B. burgdorferi* s.l. in ticks in areas with high density of red deer. The migration of red deer, however, may play an important role in the dispersal to new locations of ticks and to keep a high abundance of ticks in certain areas (Qviller et al., 2013).

According to the MSIS annual report, the numbers of Lyme borreliosis are lower in the counties of Hedmark and Oppland (eastern and central parts of Norway) and in northern counties (Sør Trøndelag, Nord Trøndelag, Nordland, Troms and Finnmark) than in those along the coastline in the south. These locations represent climatic areas, which are not very suitable for ticks, and thereby the risk of infection is low. According to the recent investigations, *I. ricinus* is spread to new areas of the north of Norway, probably, because of the climate change (Jore et al., 2014). Soleng and Kjelland (2013), and Hvidsten et al. (2013) detected dominance of *B. afzelii* among infected ticks collected from northern localities and from dogs at the Arctic Circle, respectively.

Due to the climatic changes and expansion of *I. ricinus* distribution range, continued monitoring of Lyme borreliosis pathogens in ticks is essential for the assessment of disease risk in different regions of Norway.

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BORRELIA BURGDORFERI SENSU LATO PATOGENAIS UŽSIKRĖTUSIOS *IXODES RICINUS* ERKĖS NORVEGIJOJE

Santrauka

Ixodes ricinus erkių geografinio paplitimo arealo šiaurinė riba yra Norvegijoje. Pastaruoju dešimtmečiu pastebėtas šių erkių išplitimas toliau į šiaurę ir į kalnų aukštumas gali turėti įtakos *B. burgdorferi* s. l. patogenų plitimui į naujas teritorijas ir padidinti užkrato riziką žmonėms. Šio tyrimo tikslas – nustatyti *I. ricinus* užsikrėtimo *B. burgdorferi* s. l. lygį skirtingose Norvegijos vietovėse. Erkės buvo rinktos pavasarį ir vasarą 2004 ir 2006–2008 metais 24 vietovėse šiaurės vakarinėje, pietinėje ir pietrytinėje šalies dalyse. Bakterijų DNR aptikti buvo naudojama PGR, kurios metu buvo dauginamas flagelino (*fla*) genas. Rūšys buvo identifikuotos su PGR, padauginus specifinius kiekvienai rūšiai OspA geno fragmentus. *B. garinii* padermių heterogeniškumas buvo įvertintas atliekant OspA geno sekvenavimą. Vidutinis erkių užsikrėtimas *B. burgdorferi* s. l. sudarė 7,2 % (171 iš 2360 erkių). Užsikrėtimo lygis svyravo nuo 0 iki 32 % skirtingose vietovėse. Buvo nustatytas patikimas užsikrėtimo skirtumas tarp suaugėlių (11,9 % užsikrėtusių) ir nimfų (4,7 %), taip pat tarp užsikrėtusių erkių vakarinėse (4,0 %) ir pietinėse (10,0 %) Norvegijos vietovėse. Identifikuotos keturios borelijų rūšys: tarp užsikrėtusių erkių vyravo *B. afzelii* (sudarė 71,9 % visų užkrėstų erkių), *B. garinii* buvo identifikuota 12,9 %, *B. burgdorferi* s. s. – 7 %, *B. valaisiana* – 1,2 % ir 4,8 % užkrėstų erkių turėjo mišrią infekciją. Atlikus sekvenavimą identifikuoti penki skirtingi *B. garinii* genotipai. Filogenetiniai ryšiai tarp *B. garinii* padermių ir jų atitikimas OspA serotipus buvo įvertinti palyginus su sekomis, registruotomis genų duomenų banke. Nustatyta, kad dažniausiai erkėse buvo aptiktos OspA 6 tipo sekos.

Raktažodžiai: *Borrelia burgdorferi* s. l., *Ixodes ricinus*, Lyme borreliosis, OspA tipas, Norvegija