# Tick-borne infections in Sogn og Fjordane, western Norway

Seroprevalence, risk factors and subjective health complaints in blood donors

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# Scientific environment

This study was initiated and for the most part planned and performed at the Department of Microbiology, Medical Clinic, Helse Førde.


Collaboration partners both in practical laboratory work and as co-authors of the publications were the Department of Virology, National Institute of Public Health, Oslo, and Department of Clinical Microbiology, Ryhov County Hospital, Jönköping, Sweden.

In addition, there were collaborating co-authors from the Antibiotic Centre for Primary Care at the University of Oslo, the Norwegian University of Life Sciences, Ås, and Department of Microbiology at Vestfold Hospital Trust, and Department of Microbiology and Centre for Clinical Research, Haukeland University Hospital, Bergen.

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Tick-borne diseases has been engaged in the study from the start, as has professor Morten Lindbæk, Head of the Antibiotic Centre for Primary Care at the University of Oslo. Knut Eirik Eliassen, general practitioner and researcher at the same centre has been an enthusiastic co-worker. Kirsti Vainio and Susanne Dudman at the Department of Virology at National Institute of Public Health kindly helped with analyses for tick borne encephalitis virus and writing paper III. The only foreign co-author, Anna J. Henningsson, from Jönköping, Sweden, also helped analysing antibodies to *Anaplasma phagocytophilum*, and has been a pleasant and valuable addition to all the Norwegians.

Finally, I am deeply grateful to my wife Karina and our children Ingebjørg, Kristian and Gunnhild for all their love, support and patience throughout the years.

# Abstract

**Background:**

The tick *Ixodes ricinus* is involved in the transmission of a large variety of pathogens of medical and veterinary importance in Norway. The most prevalent human tick-borne disease in the country is Lyme borreliosis, caused by the bacterium *Borrelia burgdorferi* sensu lato (s.l.). Granulocytic anaplasmosis, caused by the bacterium *Anaplasma phagocytophilum*, is prevalent in livestock, but only a few human cases have been published. The viral infection tick-borne encephalitis (TBE), caused by the TBE-virus (TBEV), is endemic in the southernmost parts of the country.

**Aims:**

The aims of the present thesis were to assess the frequency and risk factors of tick bites, and the seroprevalence of antibodies to *B. burgdorferi* s.l., *A. phagocytophilum* and TBEV in Sogn og Fjordane county on the western coast of Norway. In addition, we wanted to assess any association between tick bites or seropositivity for *B. burgdorferi* s.l. and common subjective health complaints. Finally, we wanted to compare different laboratory methods for detection of antibodies to *B. burgdorferi* s.l.

**Methods:**

During the first half of 2010, serum samples and questionnaires were collected from 1,213 blood donors at the four blood banks in the county.

The questionnaire included questions about demographics, various life style factors, data on tick bites, and a set of questions designed to measure common and prevalent health complaints in the general population.

Antibodies to *B. burgdorferi* s.l. were tested in Enzygnost Lyme link VlsE/IgG, Enzygnost Borreliosis IgM and Immunetics C6 Lyme ELISA kit. Sera showing positive or grey-zone reactivities in any of these tests were further tested in Euroimmun Borrelia-EUROLine-RN-AT IgG and Borrelia-EUROLine-RN-AT IgM.

A random subgroup of 301 sera was examined for IgG-antibodies to *A. phagocytophilum* by an indirect immunofluorescence assay (IFA).

All 1,213 sera were analysed for IgG-antibodies to TBEV in Serion ELISA *classic* TBE IgG.

**Results:**

Among the participants, 65.7% had experienced tick bites during their lifetime, and 30% had experienced tick bites during the last 12 months. Donors from the eastern-most blood bank in Lærdal reported the lowest occurrence of ticks in their living area as well as the lowest number of tick bites. In the younger age-groups, males reported more bites than females. This was reversed in subjects older than 50 years of age, with females reporting more tick bites than males. Tick bites were more common among participants with the highest educational level, increased outdoor activity and among hunters and owners of domestic animals.

Using the laboratory's routine tests for detecting antibodies to *B. burgdorferi* s.l., Enzygnost IgG and IgM, 9.6% were positive for IgG and 8.2% for IgM. There was a positive association of IgG-seropositivity with age, and more males than females were positive for IgG (13.0% and 5.5%, respectively). IgG prevalence was higher in persons spending more time outdoors. There was a delayed age-related rise in seroprevalence in women compared to men. Subjects from the blood bank in Lærdal had the lowest prevalence of IgG.

We found a substantial agreement between Enzygnost IgG and Immunetics C6 ELISA, most discrepancies were found in weakly reactive sera. IgM only was seen in 55 subjects (4.5%), of which more than half had a positive immunoblot for IgM. This pattern was seen more often in women and younger age-groups.

Among the 301 blood donors tested for IgG-antibodies to *A. phagocytophilum,* 49 (16.2%) were positive with a titer ≥80 (range 80-1280).

Among the 1,213 sera tested, six (0.5%) gave positive or grey-zone results in the ELISA test for TBEV IgG. Five of these were from persons having received vaccines that might give positive reactions in the TBE ELISA, and the last was further examined by neutralising antibodies to TBEV, with negative result.

We found no association between the number of tick bites or antibodies to *B. burgdorferi* s.l. and subjective health complaints, reduced general function or reduced physical fitness. The number of tick bites was positively associated with good physical fitness.

**Conclusions:**

The results provide insight into the epidemiology of tick bites and tick-borne diseases in western Norway, and confirm the endemicity of Lyme borreliosis in the region. There were no indications that TBE is established as a human disease in the area, but there were serological indications that human granulocytic anaplasmosis should be considered in patients with compatible symptoms after a tick bite. The results also give insight into strengths and weaknesses of serological methods in diagnosing Lyme borreliosis, and may help to establish prudent test algorithms for this disease. There were no indications of adverse chronic health effects of tick bites or *B. burgdorferi* s.l. infection in this overall healthy population.

# List of publications

This thesis is based on the following papers, referred to in the text by their roman numerals:

I Hjetland, R., Eliassen, K.E., Lindbaek, M., Nilsen, R.M., Grude, N., Ulvestad, E., 2013. Tick bites in healthy adults from western Norway: Occurrence, risk factors, and outcomes. Ticks Tick Borne Dis 4, 304-310.

II Hjetland, R., Nilsen, R.M., Grude, N., Ulvestad, E., 2014. Seroprevalence of antibodies to Borrelia burgdorferi sensu lato in healthy adults from western Norway: risk factors and methodological aspects. APMIS 122, 1114-1124.

III Hjetland, R., Henningsson, A.J., Vainio, K., Dudman, S.G., Grude, N., Ulvestad, E., 2015a. Seroprevalence of antibodies to tick-borne encephalitis virus and Anaplasma phagocytophilum in healthy adults from western Norway. Infect Dis (Lond) 47, 52-56.

IV Hjetland, R., Reiso, H., Ihlebaek, C., Nilsen, R.M., Grude, N., Ulvestad, E., 2015b. Subjective health complaints are not associated with tick bites or antibodies to Borrelia burgdorferi sensu lato in blood donors in western Norway: a cross-sectional study. BMC Public Health 15, 657.

# Contents

# Abbreviations

| | |
|---|---|
| A. | Anaplasma |
| ACA | Acrodermatitis chronica atrophicans |
| ATP | Adenosine triphosphate |
| B. | Borrelia |
| BBK32 | Borrelia burgdorferi k32 |
| Bbsl | Borrelia burgdorferi sensu lato |
| Bbss | Borrelia burgdorferi sensu stricto |
| Bmp | Borrelia membrane protein |
| BSK medium | Barbour-Stoenner-Kelly medium |
| CFH | Factor H |
| CFHR | Factor H-related protein |
| CI | Confidence interval |
| CRASPs | Complement regulator-aquiring surface proteins |
| Dbp | Decorin binding protein |
| EIA | Enzyme immunoassay |
| ELISA | Enzyme-linked immunosorbent assay |
| FHL1 | Factor H-like protein 1 |
| Fla | Flagellar antigen |
| HAI | Haemagglutination inhibition |
| HGA | Human granulocytic anaplasmosis |
| I. | Ixodes |
| IFA | Immunofluorescence assay |
| Ig | Immunoglobulin |
| LB | Lyme borreliosis |
| LNB | Lyme neuroborreliosis |
| MSIS | Meldingssystem for smittsomme sykdommer (Norwegian Surveillance System for Communicable Diseases) |
| ORF | Open reading frame |
| Osp | Outer surface protein |
| PCR | Polymerase chain reaction |
| RF | Relapsing fever |
| s.l. | Sensu lato |
| s.s. | Sensu stricto |
| SDS-PAGE | Sodium dodecyl sulfate-polyacrylamide gel electrophoresis |
| SF | Spotted fever |
| SF-36 | Medical Outcomes Study 36-Item Short-Form Health Survey |
| SHC | Subjective health complaints |
| TBE | Tick-borne encephalitis |
| TBEV | Tick-borne encephalitis virus |
| vlsE | Variable major protein-like-sequence expressed |
| VMP | Variable major protein |

# 1 Introduction

The many different microorganisms that cause tick-borne diseases in humans are transferred from animal to animal by tick vectors. Such serial transfers are illustrated in Figure 1 for the bacterium *Borrelia burgdorferi* sensu lato and the tick *Ixodes ricinus.* As indicated, humans are outside the enzootic cycle and are thus not able to propagate the bacterium. They are therefore denoted incidental or dead-end hosts.

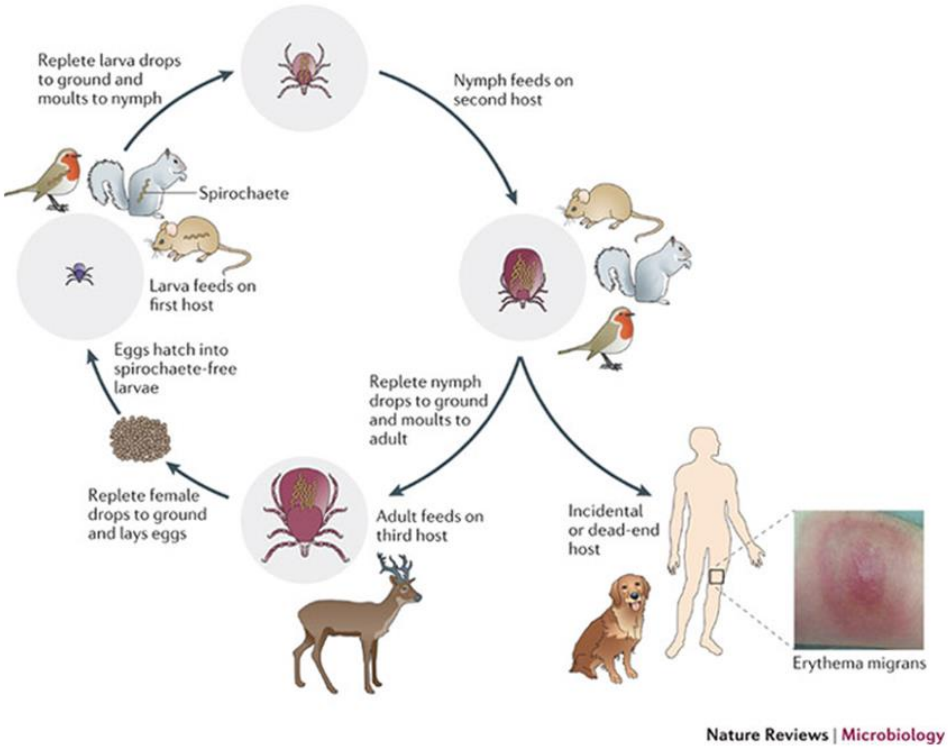


**Figure 1. The enzootic cycle of *Borrelia burgdorferi*** (Radolf et al., 2012).
Reprinted by permission from Macmillan Publishers Ltd: Nat Rev Microbiol

In the following introduction, the different aspects of this cycle are reviewed; the ticks, the animal hosts, the microorganisms, and the diseases in humans, including clinical presentation and diagnosis. Special emphasis is given to the bacterium *Borrelia burgdorferi* sensu lato (s.l.) and its associated human disease, Lyme borreliosis.

# 1.1 Ticks

## 1.1.1 Tick species globally and in Norway

About 900 tick species exist in the world. These are broadly divided into two families; the hard ticks (*Ixodidae*), and the soft ticks (*Argasidae*) (Table 1). The hard ticks – some of which are relevant in this thesis – comprise members of five subfamilies and six genera. The most important of these are various *Ixodes, Dermacentor, Amblyomma, Haemaphysalis, Hyalomma,* and *Rhipicephalus* species (Dennis and Piesman, 2005). The hard ticks are so called because of the presence of a hard plate (scutum), covering the dorsal body surface (dorsum). The soft ticks lack a scutum, and instead have a cuticle, with a soft leathery appearance.

**Table 1. Grouping of principal tick vectors of disease to humans**
Modified from (Dennis and Piesman, 2005). 

| Family | Subfamily (subgroup) | Genus (genera) |
|---|---|---|
| *Ixodidae* | *Ixodinae* | *Ixodes* |
| | *Amblyomminae* | *Amblyomma* |
| | *Haemaphysalinae* | *Haemaphysalis* |
| | *Hyalomminae* | *Hyalomma* |
| | *Rhipicephalinae* | *Dermacentor, Rhipicephalus* |
| *Argasidae* | *Argasinae* | *Ornithodoros* |

Of these 900 tick species, only 26 are found in north-west Europe, and barely 10 in Norway (Table 2).

**Table 2. Ticks found in Norway**
Modified from (Mehl, 1983; Kjelland et al., 2014), with permission

| Species | Norwegian name | English name | Common host animals |
|---|---|---|---|
| **Common Norwegian species** | | | |
| *Ixodes ricinus* | Skogflått | Sheep tick | Many mammals and birds |
| *Ixodes uriae* | Fuglefjellflått | Seabird tick | Birds (seabirds in colonies) |
| *Ixodes frontalis* | | Passerine tick | Birds |
| *Ixodes lividus* | Sandsvaleflått | Sand martin tick | Birds (sand martins) |
| *Ixodes hexagonus* | Piggsvinflått | Hedgehog tick | Hedgehogs |
| *Ixodes trianguliceps* | Museflått | Vole tick, shrew tick | Bank vole, mouse, rat |
| *Ixodes caledonicus* | | Northern bird tick | Birds (normally found in nests on cliffs or buildings) |
| *Ixodes arboricola* | | Tree-hole tick | Birds |
| *Argas vespertilionis* | Flaggermusflått | Bat tick | Bats |
| **Sporadically found tick species** | | | |
| *Rhipicephalus sanguinea* | Husflått | Brown dog tick | Dogs |
| *Hyalomma spp.* | | | Migratory birds |
| *Dermacentor spp.* | | | Migratory birds |

In male ixodid ticks, the scutum covers most of the dorsum, whereas in the females, it is usually restricted to the anterior third to half of the dorsum. Several characteristics are utilized for classification of ixodid ticks to the genus level, including the length of the mouthparts, presence or absence of eyes, presence or absence of festoons, colour or markings on the scutum, and shape and orientation of the anal groove (Mathison and Pritt, 2014) (Figure 2). The mouthparts of the tick includes two chelicerae (cutting tools), two palps (limbs with sensory organs), and one hypostome, a barbed tube that anchors the tick to the host, and through which blood is drawn up into the tick gut.

### 1.1.2 Life cycle

The life cycle of ticks includes the four stages of egg, larva, nymph, and adult. Ixodid ticks have only one nymphal stage, whereas argasids have two or more. All ticks feed

on blood during some or all stages of the life cycle. The larvae have six feet, while the nymphs and adults have eight.

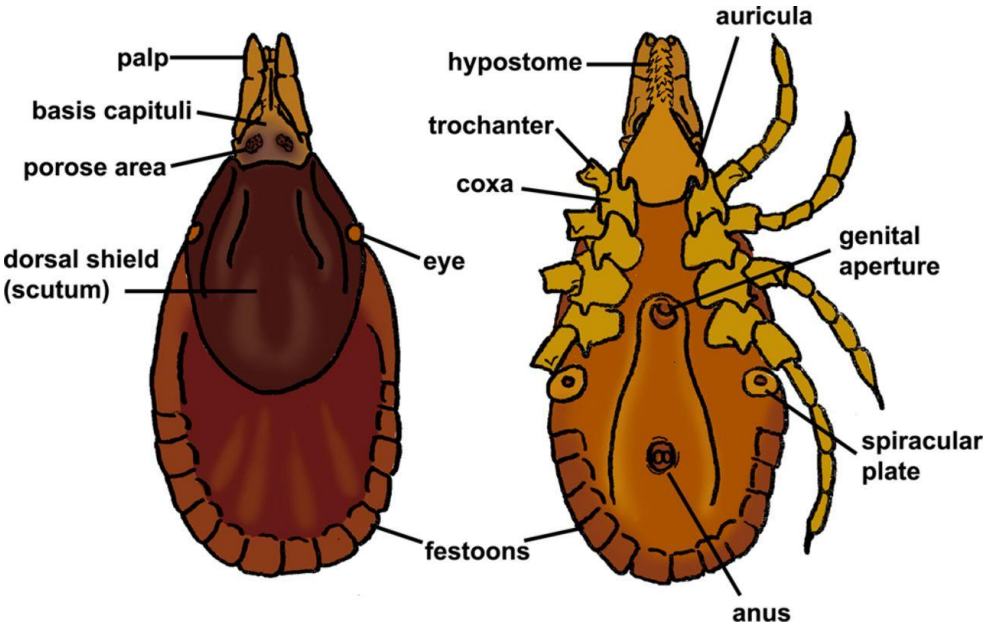


**Figure 2. Anatomy of a female hard tick (*Ixodidae*)**
(Mathison and Pritt, 2014) ©2014 American Society for Microbiology. Used with permission.

*Ixodes ricinus*, the major vector for enzootic transmission of disease-inducing microbes in Norway, has a three-host cycle, i.e., feeding occurs in each of the three parasitic stages, and on three different host animals (Figure 3). Larvae seek hosts, attach, feed, detach, and develop in sheltered microenvironments where they molt to nymphs. Following the same pattern, nymphs molt to adults. The adults again seek hosts, feed and mate. Mating in *I. ricinus* can occur on or off the host. After having completed her feeding, the mated female drops off and after varying time deposits her thousands of eggs (oviposition), whereupon the female dies. The development of *I.*

*ricinus* typically spans over a period of 2-3 years. During winter in arctic or temperate climates, the ticks go into diapause (Dennis and Piesman, 2005; Sonenshine, 2005).

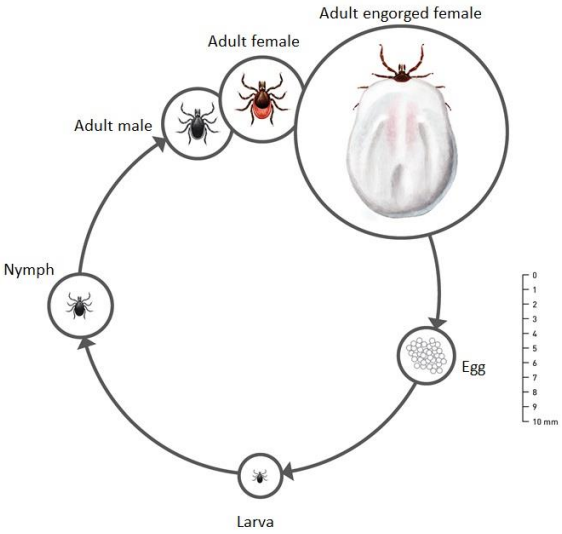


**Figure 3. Life stages of *Ixodes ricinus***
Modified from Norwegian Institute of Public Health (2016), with permission

### 1.1.3 Distribution of the *Ixodes ricinus* species complex globally

The primary tick vectors for *B. burgdorferi* s.l. are members of the *I. ricinus* complex, a group of closely related hard ticks (Xu et al., 2003). *I. ricinus* is the main vector in Europe, while *I. persulcatus* is most important in Asia. *I. scapularis* is the main vector in north-eastern and upper mid-western USA, as is *I. pacificus* in western USA, see Figure 4 (Stanek et al., 2012).

### 1.1.4 Distribution of *Ixodes ricinus* in Norway

*I. ricinus* is the predominating tick species in Norway, and three estimates of the distribution of ticks in Norway have been published (Tambs-Lyche, 1943; Mehl, 1983; Jore et al., 2011).

In 1943, the veterinarian Hans Tambs-Lyche published a distribution of *I. ricinus* based on collected ticks as well as information of bovine babesiosis from veterinarians

around the country (Tambs-Lyche, 1943). According to his findings, *I. ricinus* was found at altitudes lower than 150-160 meters above sea level in the south-eastern part of Norway, below 350-500 meters in the south-western part, and below 100-150 meters in the northern parts as far north as 66 $^{o}$N (Figure 5).

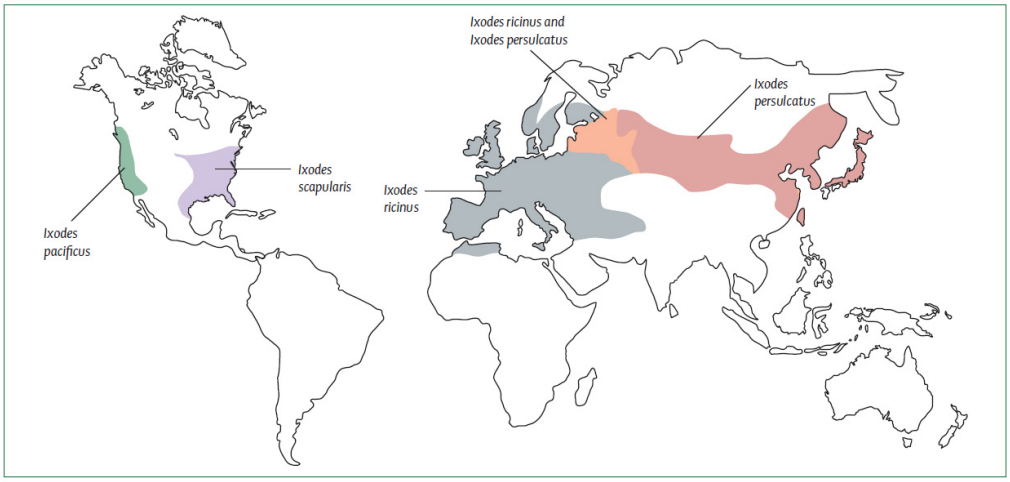


**Figure 4. Global distribution of the *Ixodes ricinus* species complex**
(Stanek et al., 2012) Reprinted from The Lancet, 379, Stanek, G., Wormser, G.P., Gray, J., Strle, F. Lyme borreliosis, 461-73, Copyright 2012, with permission from Elsevier.

A new estimate of the distribution of different tick species was published in 1983 by Reidar Mehl on the basis of extensive field surveys (Mehl, 1983). His findings on *I. ricinus* corresponded to those of Tambs-Lyche (Figure 6).

A third publication on the distribution of *I. ricinus* was published in 2011 by Solveig Jore and co-workers (Jore et al., 2011) (Figure 7). The study was based on five inputs; the incidence data for Lyme borreliosis reported to the National surveillance system for infectious diseases (MSIS) 1991-2008, the incidence of bovine babesiosis reported to Norwegian School of Veterinary Science 1996-2008, cervid hunters' webpage

registration in 2007 on http://www.flattogflue.no of tick infestation on roe deer, red deer and moose, a newspaper webpage registration of tick observations in 2009, and a web-based questionnaire to Norwegian veterinarians in 2009. The authors concluded that *I. ricinus* at the time was found further north (to approximately 69 $^{o}$N) and at higher altitudes (up to 583 meters above sea level) than previously reported. Recently, Hvidsten and co-workers studied the distribution of ticks near the northern distribution limit in Norway, and found that tick occurrence is high in the Brønnøy district (65°28′ N), but low further north across the Arctic Circle (Hvidsten et al., 2014; Hvidsten et al., 2015).


**Figure 5. The distribution of *Ixodes ricinus* in Norway according to Tambs-Lyche (1943)**


**Figure 6. The distribution of *Ixodes ricinus* in Norway according to Mehl (1983)**

With permission

*Solid circles* = localities where ticks have been collected and identified. *Open circles* = localities where hosts for the individual tick species were examined without finding ticks. *Triangles* = observations which originate from localities outside of the species "normal" range. Such observations are ascribed to transportation with birds or domestic animals.

In Sogn og Fjordane county, the distribution maps (Figures 5-7) clearly demonstrate that the density of ticks is lower in the eastern, more inland-like parts of the county, compared to the western regions nearer to the coast.

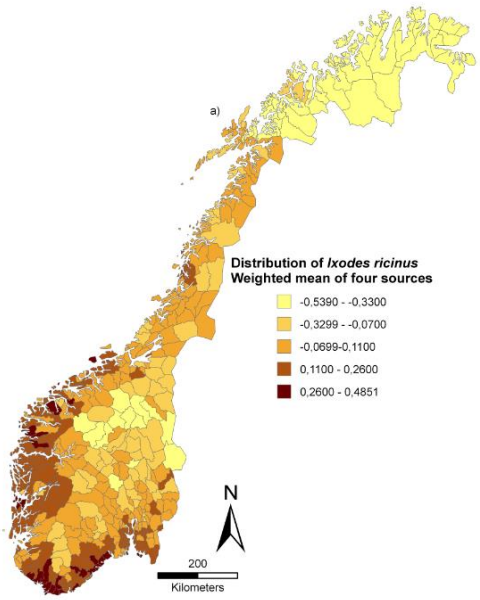


**Figure 7. The distribution of *Ixodes ricinus* in Norway according to Jore et al. (2011)**

The present distribution of *Ixodes ricinus* in Norway depicted by the weighted mean obtained by the first principal component (PC1) of a PCA-analysis of four different sources, see reference for further explanation.

### 1.1.5 Factors affecting the distribution of *I. ricinus*

*I. ricinus* requires a humidity of the microenvironment of at least 80%, as uptake of water occurs by direct sorption from the atmosphere (Sonenshine, 2005; Stanek et al., 2012).

The temperature is also critical for maintaining the life cycle of ticks. Tambs-Lyche stated in 1943 that the year isotherm of 5 $^{o}$C was the best predictor of *I. ricinus* occurrence in Norway. In addition, he stated that the tick needed an oceanic climate (Tambs-Lyche, 1943). Later, Ottesen (2010) argued that the occurrence in Norway is better correlated to the vegetation period, i.e., the mean number of days with a mean temperature of at least 5 $^{o}$C, exceeding 170-180 days. A similar conclusion has also been reached in Swedish studies (Jaenson et al., 2009).

The effects of climate on the occurrence of ticks as well as the epidemiology of different tick-borne diseases have been discussed in several publications (Lindgren and Jaenson, 2006; Gray et al., 2009; Randolph, 2010; Mannelli et al., 2012). Table 3 summarises thresholds for temperature and humidity for *I. ricinus*.

**Table 3. Climate factors linked to tick vector survival and activity**
(Lindgren and Jaenson, 2006) Reprinted with permission from WHO Regional Office for Europe

| *I. ricinus* life-stages | Temperature thresholds | | | | Humidity |
|---|---|---|---|---|---|
| | Minimum survival | Activity threshold | | Optimum | |
| | | Air | Soil | | |
| Larvae | -5– -7°C[a,1] | No data | | 15–27°C [2] | 80–85% [5,6] |
| Nymph | No data | 4–5°C[4] | 4–5°C[4] | 10–22°C [2] | |
| Adult female | -20°C[a,1] | 7 °C [3] | 4–5°C [3] | 18–25°C [2] | |

[a]Ticks have been shown to resist very low temperatures; however the length of the cold exposure is important.
*Source*: [1] Dautel & Knülle, 1997; [2] Daniel & Dusabek, 1994; [3] Sonenshine, 1993; [4] Balashov, 1972; [5] Gray, 1991; [6] Kahl & Knülle, 1988.

The availability of host animals for *I. ricinus* is also of major importance for the occurrence and density of the tick, as the feeding of blood from vertebrates is necessary for survival.

More than 300 species of vertebrates have been recorded as hosts for *I. ricinus*. While larvae and nymphs can feed on almost any vertebrate, including birds, small mammals, and larger mammals like dogs, sheep, and humans, adult ticks restrict their host range to medium-sized or larger mammals, especially deer or other ungulates (e.g., sheep, cattle, goats and pigs) (Sonenshine, 2005). In Norway, the most important small mammal hosts are the common shrew (*Sorex araneus*), the wood mouse (*Apodemus sylvaticus*) and the bank vole (*Myodes glareolus*), while the most important medium-sized animal hosts are red fox (*Vulpes vulpes*), marten (*Martes martes*), mountain hare (*Lepus timidus*) and also domestic cats (*Felis catus*). Birds may be important hosts for *Ixodes* species, and are probably important for spreading ticks and associated microorganisms over greater distances (Hasle, 2010). Among large animals in Norway are moose (*Alces alces*), roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) the most important tick vectors (Mysterud et al., 2015).

## 1.2 Tick bites

During questing for vertebrates to find a blood meal, *I. ricinus* ticks exhibit a stationary and mostly passive “ambush” strategy. They climb onto grasses, bushes, or

other leafy vegetation to wait for passing host animals. Adult ticks usually climb higher on such vegetation than larvae and nymphs. They can remain there for hours, until they have to descend to regain their water balance. As a host animal brushes by, they extend their forelegs and cling to the hair or clothing of the animal. This behaviour is excited by different stimuli including odorants and physical factors.

The tick then seeks a protected area of the skin, e.g. under the hair coat or between adjoining body parts, such as the groin or axillae in humans.

The “tick bite” starts by the tick’s cutting through the skin into the dermis by using a pair of appendices on its head, the chelicerae. Thereafter the tick starts to secrete a cement substance, which anchors the tick to the wound site. After completion of this process, which can last for 1 to 2 days, the tick starts sucking blood. The tissue damage attracts the host’s leukocytes, thereby enlarging the feeding pool. Periods of blood sucking alternate with salivation. The saliva which is excreted into the wound, contains a variety of pharmacologically active substances including anticoagulants, antihistamine, apyrase (catalysing ATP hydrolysis), and other enzymes that facilitate successful bloodsucking activity. Excess water and salt from the blood meal is also excreted in the saliva. The blood meal allows the weight of an adult female ixodid tick to increase 100-120 fold, while larvae and nymphs increase their weight 10 to 20 fold. The increase in size following the blood meal necessitates growth of a new cuticle. The cycle from attachment to completed feeding lasts from 2-3 days for larvae, and as much as 13 days for adult females (Sonenshine, 2005; Richter et al., 2013).

The risk of humans being bitten by ticks depends on the density of questing ticks in the local area and the degree to which humans expose themselves to the tick-infested areas. Exposure depends on whether people live in urban or rural settings, on the amount of time spent outdoors in tick infested areas during the tick season, and on whether they protect themselves by clothing, use of repellants, awareness of ticks, etc.

The literature on occurrence and risk factors for tick bites in the general population is scant. Two Dutch and one Belgian investigation demonstrated local geographical differences within these small countries (de Mik et al., 1997; den Boon et al., 2004;

Vanthomme et al., 2012). In Sweden, Stjernberg and Berglund (2002) found a 4% risk of being bitten by ticks after 10 hours spent outdoors, and another Swedish study found an increased risk of contracting tick bites for women more than 40 years of age (Bennet et al., 2007). In a study from the island of Åland in Finland, 85% of the general population older than 8 years of age reported having been bitten by ticks (Wahlberg, 1990). In Connecticut and Wisconsin, 4.1% of blood donors reported having been bitten by ticks during a 6 month period (Leiby et al., 2002). In a recent study of blood donors, 10.8% of donors in the three northernmost counties of Norway had been bitten by ticks at least once during their lifetime, as opposed to 70.6% in the county of Vestfold in the south-eastern part of the country (Dag Hvidsten, personal communication 2016).

## *1.3 Borrelia burgdorferi*

### 1.3.1 *Borrelia* species

Bacteria in the genus *Borrelia* belong to the order *Spirochaetales*, family *Spirochaetaceae*, of which two genera, *Borrelia* and *Treponema*, cause human disease. Among the *Treponema* spp., *T. pallidum* is the cause of syphilis. Based on genetic relatedness, the genus *Borrelia* is divided into the relapsing fever (RF) and Lyme borreliosis groups. The latter is called *B. burgdorferi* sensu lato *(*hereafter abbreviated Bbsl, sensu lato meaning "in a broad sense"). All these have blood-feeding arthropods as vectors, and most cause zoonotic infections, with humans being rare and dead-end hosts (Schriefer, 2015).

RF *Borrelia* species are transmitted by lice or ticks. Louse-borne RF *Borrelia* is endemic only in east Africa (Schriefer, 2015), but imported cases have recently been seen in refugees in Europe (Hoch et al., 2015). Tick-borne RF is caused by a variety of *Borrelia* spp., and occurs in focal areas throughout the world. Most tick-borne RF species have soft ticks of the genus *Ornithodoros* as vectors (Schriefer, 2015). However, a newly discovered species of *Borrelia* belonging to this group, *B. miyamotoi*, has hard ticks as vector (Platonov et al., 2011). In Norway, tick-borne RF is occasionally seen as imported cases (Norwegian Institute of Public Health, 2015b).

## *1.3.2 Borrelia burgdorferi* sensu lato

### *1.3.2.1 History*

The cause of Lyme borreliosis, *Borrelia burgdorferi*, was first described by Wilhelm Burgdorfer and co-workers in the early 1980s (Burgdorfer et al., 1982). This occurred after several years of search for the etiology behind an outbreak of what was initially thought to be juvenile rheumatoid arthritis in the Old Lyme district in Connecticut, USA (Steere et al., 1977). Clinical disease compatible with what we now recognise as manifestations of Lyme borreliosis was, however, described in Europe and Scandinavia at a much earlier time. The Swedish dermatologist Arvid Afzelius described the common skin manifestation seen in Lyme disease, erythema migrans in 1910 and 1921 (he named it erythema chronicum migrans), and connected this to the bite of an *Ixodes* tick (Afzelius, 1910, 1921). Radiating pain compatible with what we today would call neuroborreliosis was described by Bannwarth in Germany in 1941 (Bannwarth, 1941). In the early 1960s, Brennaas and Ræder (1962) described a disease corresponding to Lyme neuroborreliosis on the island of Stord in western Norway.

Bbsl has been divided into different genospecies. The term genospecies denotes a taxonomic category subordinate to a genus (or subgenus) and superior to the subspecies or variety. It is thus at the same taxonomic ranking as the species. The difference between the two terms relates to their characteristics of inclusion; a species is composed of individuals similar in certain morphologic and physiologic characteristics, while a genospecies can be identified only by its genotype, not by its phenotype (http://medical-dictionary.thefreedictionary.com/, accessed 02.11.2016). Significant differences between strains may also exist within the genospecies, e.g. as demonstrated by Cerar et al. regarding *B. burgdorferi* sensu stricto (s.s.) in USA and Europe (Cerar et al., 2016). In the literature on *B. burgdorferi*, the words species and genospecies are used interchangeably.

Studies published since 1992 have divided Bbsl into three prevalent, human-pathogenic genospecies - *B. burgdorferi* sensu stricto (hereafter abbreviated Bbss), *B. afzelii*, and *B. garinii* – and 12 other genospecies, some of which have been linked to human disease (Table 4). With few exceptions, Bbss is the only well-documented

human-pathogenic Lyme borreliosis genospecies found in North America; in contrast, all three genospecies have been isolated from humans in Europe. From central to eastern Asia, *B. garinii* and *B. afzelii* are the agents causing almost all human cases of Lyme borreliosis.

**Table 4. Species belonging to *Borrelia burdgdorferi* sensu lato**

Modified from (Schriefer, 2015). ©2015 American Society for Microbiology. Used with permission. No further reproduction or distribution is permitted without the prior written permission of American Society for Microbiology.

| ***Borrelia* species** | **Arthropod vector** | **Animal reservoir** | **Geographic distribution** | **Disease** |
|---|---|---|---|---|
| *B. burgdorferi* sensu stricto | *Ixodes scapularis* | Rodents | Eastern and north-central United States | Lyme borreliosis |
| | *Ixodes pacificus* | Rodents | Western United States | Lyme borreliosis |
| | *Ixodes ricinus* | Rodents | Europe | Lyme borreliosis |
| *B. garinii* | *Ixodes ricinus, Ixodes persulcatus* | Birds | Europe, Asia | Lyme borreliosis |
| | *Ixodes uriae* | Birds | Europe, Asia | ? |
| *B. afzelii* | *Ixodes ricinus, Ixodes persulcatus* | Rodents | Europe, Asia | Lyme borreliosis |
| *B. bavariensis* | *Ixodes ricinus, Ixodes persulcatus* | Rodents | Europe, Asia | Lyme borreliosis |
| *B. spielmanii* | *Ixodes ricinus* | Rodents | Europe | Lyme borreliosis (few cases) |
| *B. japonica* | *Ixodes ovatus* | Rodents | Japan | ? |
| *B. andersonii* | *Ixodes dentatus* | Rabbits | United States | ? |
| *B. bissettii* | *Ixodes scapularis, Ixodes pacificus* | Rodents | United States | Lyme borreliosis (few cases) |
| *B. tanukii* | *Ixodes tanukii, Ixodes ovatus* | Rodents | Japan | ? |
| *B. turdi* | *Ixodes turdus* | ? | Japan | ? |
| *B. sinica* | *Ixodes ovatus* | | China | ? |
| *B. valaisiana* | *Ixodes ricinus* | Birds | Europe, Asia | Lyme borreliosis (one case) |
| *B. lusitaniae* | *Ixodes ricinus* | Reptiles (birds?) | Europe, North Africa | Lyme borreliosis (few cases) |
| *B. californiensis* | *?* | Rodents | Western United States | ? |
| *B. carolinensis* | *Ixodes minor* | Rodents | Southeastern United States | ? |

A further division of *Borrelia* occurred in 2009 when Margos and co-workers suggested that OspA serotype 4 of *B. garinii* (rodent-associated) was sufficiently distinct from bird-associated *B. garinii* strains to deserve species status as *B. baviariensis* (Margos et al., 2009). A few studies have reported the detection of this and other *Borrelia* species (*B. valaisiana*, *B. spielmanii*, *B. bissettii*, and *B. lusitaniae*) in patient samples in Europe (Stanek et al., 2012).

#### *1.3.2.2 Structure*

*Borrelia* spp. are similar in length (8 to 30 μm) but wider (0.2 to 0.5 μm) than the two other human pathogenic spirochetes, *Treponema* and *Leptospira* spp. The cell wall of *Borrelia* consists of an outer membrane, flagellar filaments, peptidoglycan and an inner membrane (Figures 8 and 9). The outer membrane contains outer-surface lipoproteins (Osps) in high density and β-barrel outer-membrane-spanning proteins in lower density. The inner membrane is rich in integral membrane proteins, many of which are transporters (Schriefer, 2015).

*Borrelia* spp. are highly motile, with a corkscrew and oscillating motility that enables movement through highly viscous mediums. The flagella of spirochetes are endoflagella, in contrast to the exoflagella of most other bacteria. The endoflagella (7 to 20 per terminus) are localised beneath the outer membrane and insert subterminally at one end of the protoplasmic cylinder (Schriefer, 2015).

Cystic variants of Bbsl have been described (Mursic et al., 1996; Brorson and Brorson, 1997).

#### *1.3.2.3 Genome*

*Borrelia* spp., unlike most bacteria, have a small linear chromosome of approximately 1,000 kb and both linear and circular plasmids. Also unlike most other bacteria, *Borrelia* spp. have a low G+C content (approximately 30 mol%). The complete nucleotide sequences of the chromosome and the 21 plasmids (9 circular and 12 linear) have been published for the type strain Bbss B31 (originally recovered from an *I. scapularus* tick). The large linear plasmid lp54 encodes two major outer surface

proteins, OspA and OspB. Another major outer surface protein, OspC, is encoded on a circular plasmid (cp26).

Many genes in Bbss B31, of which several are plasmid borne, are differentially expressed at 23 °C and 35 °C. This demonstrates the potential importance of plasmid-borne genes in the adaptation of Bbsl to mammalian hosts and tick vectors (Schriefer, 2015).

There are no recognisable genes for toxins or other virulence factors in the Bbsl genome (Steere et al., 2005).

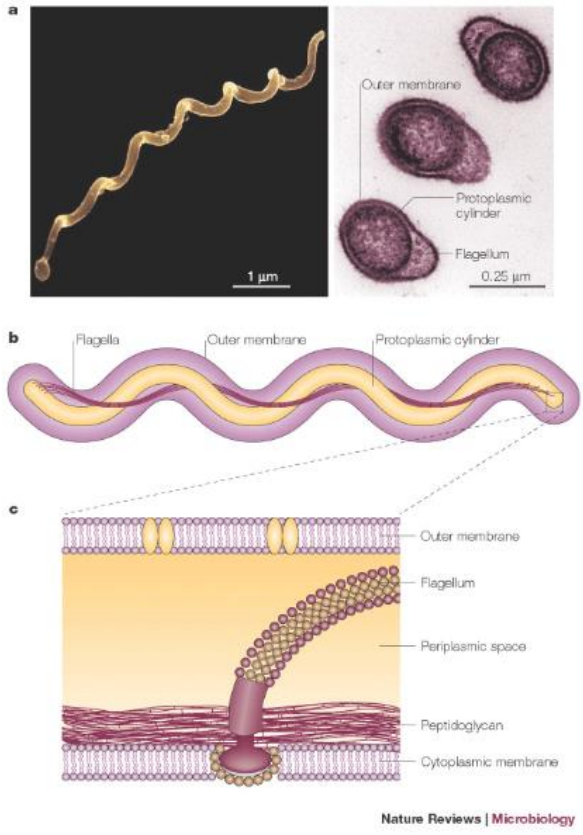


**Figure 8. Structure and morphology of *Borrelia burgdorferi***
(Rosa et al., 2005) Reprinted by permission from Macmillan Publishers Ltd: Nat Rev Microbiol

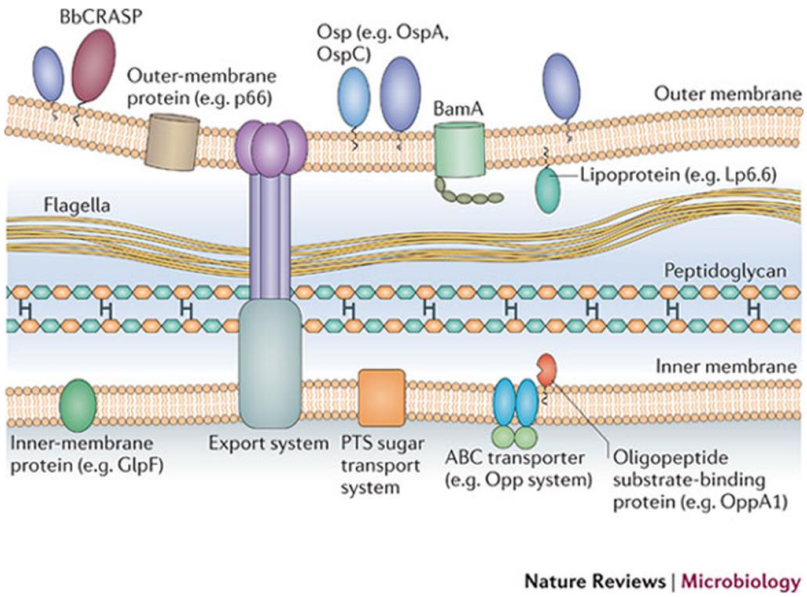


**Figure 9. The cell envelope of *Borrelia* spp.**
(Radolf et al., 2012) Reprinted by permission from Macmillan Publishers Ltd: Nat Rev Microbiol

This drawing of the borrelial cell envelope shows the outer membrane, flagellar filaments, peptidoglycan and inner membrane. The outer membrane contains outer-surface lipoproteins (Osps) in high density and β-barrel outer-membrane-spanning proteins such as BamA in low density. The inner membrane is rich in integral membrane proteins, many of which are transporters. BbCRASP, complement regulator-acquiring surface protein; OppA1, oligopeptide permease A1; PTS, phosphotransferase system

### *1.3.2.4 Immune evasion by Borrelia burgdorferi* s.l.

Bbsl has evolved several mechanisms for avoiding destruction by the host's immune system.

One important mechanism involves the inactivation of host complement attack through acquisition of human complement regulatory molecules, including factor H (CFH), factor H-like protein 1 (FHL1), factor H-related protein 1 (CFHR1), CFHR2, and/or CFHR5. Binding of these host proteins to the bacterium is primarily mediated by bacterial surface-exposed proteins that have been collectively referred to as complement regulator-acquiring surface proteins, or CRASPs (Kraiczy and Stevenson, 2013).

The bacterium also evades the immune system by antigenic variation of distinct surface antigens, thus making antibodies induced against one form of the antigen unable to recognise subsequent variants. In RF *Borrelia*, a Variable Major Protein (VMP) system was described in the 1980s (Barbour et al., 1982). In 1997, a similar antigenic variation system was identified in Bbss B31. Because of sequence similarity between this system and the VMP system, its genetic locus was referred to as vls (VMP-like sequence). Its expression site, called "vls Expressed" (vlsE), has several neighbouring vls silent cassettes (Figure 10). The vlsE locus undergoes remarkable sequence variation involving segmental gene conversion events from these silent cassettes. vls sequences have been identified in every Bbsl organism for which a genomic sequence is available, and the antigenic variation system is thus ubiquitous among all Lyme disease *Borrelia* spp. (Norris, 2014). Molecules encoded from the vlsE are strong inducers of antibody responses, predominantly directed against the conserved, non-variable regions of vlsE. The immune responses are particularly strong against the invariable region 6 (IR6, also called C6) within the vls cassette, but serologic reactivity has also been demonstrated against other regions outside this area. Either full-length VlsE protein or the C6 peptide have been incorporated into many serological diagnostic tests for Lyme borreliosis in humans (Norris, 2014).

#### *1.3.2.5 Epidemiology and transmission of Borrelia burgdorferi s.l.*

The prevalence of vector-competent ticks and their infection-permissive vertebrate hosts largely defines human risk and case number.

**Tick-host-pathogen interactions**

The bacterium is maintained in nature in an enzootic cycle that involves transmission from a tick vector to a vertebrate host and subsequent acquisition from a vertebrate host to a new tick vector, see Figure 1. There are several spirochetal factors that promote persistence, maintenance and dissemination of *B. burgdorferi* in the tick, some of which were recently reviewed (Caimano et al., 2016).

Bbsl has its natural habitat in the midgut of unfed ticks. The major lipoprotein OspA is upregulated during colonization of the gut, but during the blood meal, the spirochete

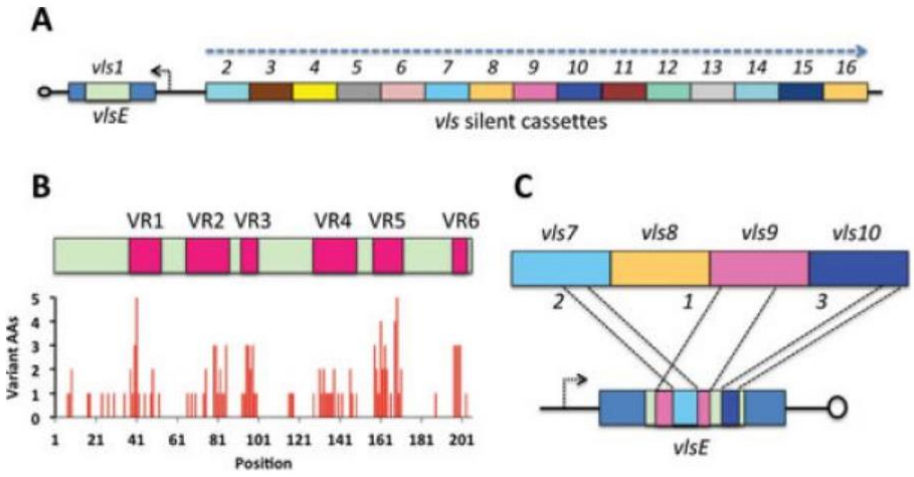


**Figure 10. Characteristics of the vls locus of *B. burgdorferi* B31**
(Norris, 2014) ©2014 American Society for Microbiology. Used with permission. No further reproduction or distribution is permitted without the prior written permission of American Society for Microbiology.

A. Arrangement of the vlsE expression site and the 15 silent cassettes near the telomere of the linear plasmid lp28-1. The promoter for vlsE is indicated by the short arrow and the orientation of the silent cassettes is shown by the large arrow. B. The cassette regions contain six variable regions (VR1 through VR6) separated by relatively invariant regions (IRs). The graph indicates the number of different amino acids encoded by the silent cassettes at each codon in the aligned sequences. C. Unidirectional, random, segmental recombination occurs sequentially during mammalian infection, as indicated by this hypothetical example of sequential recombination between vlsE and silent cassettes 9, 7 and 10.

downregulates expression of OspA and instead upregulates OspC. OspA probably mediates Bbsl adherence to gut epithelial cells, while OspC is involved with dissemination of the bacterium to the salivary glands. Osp C expression also correlates with acquisition of the bacterium by the mammalian host. Migration of spirochetes from the midgut of the feeding *I. ricinus* tick, via its salivary glands, to the skin of the animal host takes >17 hours (Munderloh et al., 2005; Schriefer, 2015). Inside the mammalian host, OspC is again downregulated, while vlsE expression is increased (Liang et al., 2004).

**Reservoir hosts for Bbsl**

A reservoir host is a vertebrate animal species that participates significantly in the circulation of the spirochete in nature. The spirochetes multiply and disseminate in the host and also persist for a considerable time, and ticks feeding on such an animal become infected (Gern et al., 1998). Ticks may also be infected by short term amplifying hosts, i.e., animals where the bacterium is present only for a shorter time, and by co-feeding transmission. This last mechanism of transmission from infected to uninfected ticks will be dealt with later (page 55-56). An overview of European reservoir hosts for Bbsl was given by Gern et al. (1998) and can also be found at www.eucalb.com.

Many mammalian species serve as reservoirs for *Borrelia*. The majority of these are rodents, the most important probably being mice (*Apodemus spp*.), voles (*Myodes* (formerly *Clethrionymus*) *spp*.) and squirrels (*Sciurus*). *B. afzelii* appears to be especially associated with these species, though *B. bavariensis* has also been found in *Apodemus* spp. Several insectivores are also involved including shrews (*Sorex, Neomys*) and hedgehogs (*Erinaceus*). Amongst the lagomorphs, hares (*Lepus*) have been shown to be reservoirs, but rabbits (*Oryctolagus cuniculi*) appear to have low reservoir capacity.

The role of carnivorous species is also probably limited. Both foxes (*Vulpes vulpes*) and dogs (*Canis familaris*) probably have small significance as reservoir hosts. The role of feral cats (*Felis catus*), which may be numerous in some areas, is uncertain.

Ungulates are hosts for a great number of ticks. However, most evidence suggest that ungulates do not transmit *Borrelia* to a high proportion of the ticks that feed on them, though they are very important in the epidemiology of borreliosis as maintenance hosts for the ticks (www.eucalb.com). They may in fact help “dilute” the occurrence of Borrelia in ticks and thus reduce the prevalence of *Borrelia* in ticks in the area (Mysterud et al., 2013).

Birds are often infested with ticks, and *B. valaisiana* seems to be transmitted exclusively from birds, especially members of the thrush family. Most strains of *B.*

*garinii* are also transmitted from birds, especially pheasants (*Phasianus clochicus*) and blackbirds (*Turdus merula*). Certain seabirds can transmit strains of *B. garinii* to *Ixodes urea*, but whether these *Borrelia* strains are transmitted to other hosts is uncertain. The role of birds as hosts for *Borrelia* and other pathogens in Norway has recently been studied by Gunnar Hasle. He found that of almost 10,000 passerine birds examined in southern Norway, 713 carried a total of 517 larvae and 1,440 nymphs. The highest prevalence of tick infestation was observed in the *Turdus* species and other ground-feeding birds. The predominant tick species was *Ixodes ricinus*. The prevalence of tick infestation and the number of ticks per bird varied with location, year and month. *Borrelia* spp. were found in 70/513 nymphs (19 *B. afzelii*, 38 B. *garinii*, 2 *B. turdi* and 11 *B. valaisiana*) and in 14/172 larvae (10 *B. garinii*, 1 *B. turdi* and 3 *B. valaisiana*) (Hasle et al., 2009; Hasle et al., 2011a; Hasle et al., 2011b; Hasle, 2013).

The association of different *B. burgdorferi* genospecies to specific reservoir hosts is likely due to different capacities of the bacteria to inactivate complement-mediated destruction through CRASPs (Kraiczy and Stevenson, 2013).

Humans are unable to transmit Bbsl back to bloodsucking ticks, and are thus dead ends for *Borrelia* propagation. This means that the bacterium will be unable to adapt to humans during a co-evolutionary cyclic race, and consequently that there should be little evolution of virulence characteristics in the *Borrelia*. In addition – since Bbsl is nontransmissible when locked within humans – antibiotic treatment will not select for increasing resistance patterns in the enzootic *B. burgdorferi* population. Long term treatment of the bacterium with antibiotics may nevertheless have an impact on the evolution of antibiotic resistance in other bacteria.

**The prevalence of Bbsl in ticks**

The prevalence of Bbsl in *Ixodes ricinus* in Norway has been examined in a number of studies (Jenkins et al., 2001; Rosef et al., 2009a; Kjelland et al., 2010b, a; Kjelland et al., 2011; Jenkins et al., 2012; Mysterud et al., 2013; Soleng and Kjelland, 2013; Tveten, 2013; Hvidsten et al., 2014). Generally, a prevalence of 5%-30% is found. The highest prevalence is found in adult female ticks, less in nymphs. The bacterium has

also been found in larvae. There seems to be a south-north gradient, with a lower prevalence in the north, although a high prevalence has been demonstrated in Brønnøysund. A distinct south-north gradient was also found in Sweden (Gustafson et al., 1995).

The bacterium was detected in host-seeking *I. ricinus* ticks in 22.1% – 31.3% of nymphs and adults in the southernmost part of Norway (Kjelland et al., 2010b). In Sogn og Fjordane county, the prevalence was 12% and 3.5% in ticks at two different locations (Rosef et al., 2009a), while a recent study from the county found a prevalence of 21.6% in adult female ticks, 11.5% in adult male ticks and 10.9% in nymphs (Mysterud et al., 2013).

Of interest is the low prevalence found in ticks from roe deer and moose in the southernmost counties of Norway, contrasted to the findings in questing ticks in that region (Kjelland et al., 2011).

### 1.3.3 Lyme borreliosis

#### *1.3.3.1 Epidemiology*

**Notification of clinical disease**

Countries around the world have different methods for surveillance of infectious diseases. A common method of surveillance is making selected important diseases notifiable to the health authorities. The actual diseases included, the case definitions, and the adherence to the notification system will vary. Of 30 countries in the EU/EFTA region, 17 have comprehensive surveillance systems for Lyme borreliosis. Twenty-one countries have surveillance systems which operate at a national level, others at a sub-national or regional level. Mandatory reporting operates in 16 countries and voluntary reporting in five. Heterogeneity of applied case definition and absence of a centralised reporting and surveillance system at an EU level make data acquisition and comparison challenging. Also, laboratory diagnosis in the EU is not standardised, leading to both under and over-reporting (European Centre for Disease Prevention and Control, 2011).

**Seroprevalence**

Seroprevalence studies are widely used to assess the occurrence of infectious diseases in a population. The prevalence of especially IgG antibodies to a pathogen reflects to a certain degree the extent to which a population has been exposed to the pathogen. It does not, however, reflect actual prevalence of clinical disease. IgG antibodies can be lost in some persons despite earlier exposition and infection, especially if the antigenic stimulus ceases. An additional level of uncertainty is added by the fact that different laboratory methods used for demonstration of the antibodies may give differing results.

Blood donors are often used for seroprevalence studies because of the ease with which sampling can be performed. Due to differences in laboratory methods, comparisons between populations in different regions should, however, be made cautiously. The direct comparison between prevalence numbers of 1.1% using Immunetics C6 ELISA in the USA (Wormser et al., 2013), and 30% in Dar es Salaam, Tanzania, using the DAKO flagellar ELISA (Mhalu and Matre, 1996), is – for example – not advisable. In Europe, prevalence numbers between 4% and 20% for IgG in different ELISAs have been published, as summarised by Tjernberg et al. (2007). Quite analogously, a report by Dessau et al. (2011) showed that seropositivity rates for blood donors in some Scandinavian laboratories were markedly different depending on the ELISA method used. For example, one Swedish laboratory using Immunetics 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.

**Epidemiology of LB in Norway**

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/). The notification system for LB has recently been evaluated, and a case is made for revising the criteria (MacDonald et al., 2016). In the period 2005-2014, the mean reported annual incidence in Sogn og Fjordane was 17.3 cases

per 100,000 inhabitants, compared to 24.5 in the southernmost county of Vest-Agder, and 6.2 nation-wide, see Table 5 (http://www.msis.no/).

The actual practice for notifying MSIS may vary from county to county, i.e., from laboratory to laboratory, as well as over the years. There is therefore some uncertainty in comparing the numbers both geographically as well as over time. In addition, different laboratories use different serological methods. Still, during the last 10 years these numbers should be comparable on a longitudinal basis within each county, as few major methodological changes have been introduced, and since the notification criteria have not changed.

As notified LB cases in Norway only represent a minor fraction of all LB cases, an estimate of this fraction would be of interest. As will be seen from section 1.3.3.3 on clinical disease, the number of notified cases should probably be multiplied with a number between 3 and 20. One recent survey found that almost 96% of cases of LB in Norwegian general practice were erythema migrans, and the authors estimated a national incidence of 148 EM/100,000 inhabitants/year (Eliassen et al., 2017). Using this result for the proportion of EM cases in relation to notified cases, the mean incidence of EM in Sogn og Fjordane county should be close to 400 EM/100,000 inhabitants/year.

In Norway, an IgG seropositivity rate of 18% was found in 247 blood donors from the county of Vest-Agder, using the Enzygnost ELISA (Mygland et al., 2006). This is the county with the highest incidence of notified Lyme borreliosis cases in Norway, see Table 5. Using the same method, Hvidsten and co-workers in a recent study of blood donors found a seroprevalence of 0.45% in the three northernmost counties (n=1048), as contrasted to 9.25% in the county of Vestfold (n=519) (Dag Hvidsten, personal communication 2016).

**Table 5. Annual incidence of Lyme borreliosis notified to MSIS 2005-2014, cases per 100,000 inhabitants** (www.msis.no).

| County | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Østfold | 5.0 | 6.9 | 5.3 | 4.9 | 5.6 | 6.3 | 6.9 | 3.2 | 2.8 | 4.6 | 5.2 |
| Akershus | 1.6 | 2.6 | 2.2 | 5.0 | 3.0 | 1.3 | 2.6 | 3.2 | 3.2 | 2.6 | 2.7 |
| Oslo | 1.5 | 2.0 | 1.3 | 8.0 | 5.9 | 3.2 | 1.8 | 0.8 | 1.6 | 3.2 | 2.9 |
| Hedmark | 1.6 | 0.5 | 1.1 | 1.1 | 0.5 | 0.5 | 0.0 | 0.0 | 0.5 | 1.0 | 0.7 |
| Oppland | 2.2 | 0.0 | 1.6 | 1.6 | 1.6 | 1.6 | 0.0 | 0.5 | 2.1 | 1.6 | 1.3 |
| Buskerud | 3.3 | 2.4 | 4.4 | 2.4 | 2.7 | 1.6 | 1.5 | 4.1 | 4.1 | 1.8 | 2.8 |
| Vestfold | 10.9 | 6.3 | 8.5 | 4.4 | 13.1 | 10.8 | 9.4 | 5.5 | 8.0 | 11.6 | 8.8 |
| Telemark | 19.2 | 42.1 | 16.9 | 18.0 | 12.5 | 14.9 | 9.5 | 10.6 | 14.0 | 14.6 | 17.2 |
| Aust-Agder | 26.1 | 16.3 | 30.5 | 25.4 | 14.0 | 11.1 | 10.9 | 22.4 | 18.6 | 18.5 | 19.4 |
| Vest-Agder | 37.8 | 29.0 | 37.9 | 35.6 | 14.3 | 14.7 | 12.8 | 18.4 | 29.5 | 15.1 | 24.5 |
| Rogaland | 7.4 | 9.8 | 10.9 | 6.1 | 5.7 | 9.3 | 7.6 | 6.5 | 8.4 | 10.0 | 8.2 |
| Hordaland | 5.1 | 4.6 | 2.4 | 3.0 | 5.1 | 7.8 | 6.6 | 8.2 | 10.8 | 13.5 | 6.7 |
| Sogn og Fjordane | 14.0 | 23.4 | 26.4 | 17.9 | 22.5 | 13.1 | 12.1 | 10.2 | 15.6 | 17.4 | 17.3 |
| Møre og Romsdal | 6.1 | 8.6 | 14.7 | 17.0 | 10.5 | 18.7 | 10.6 | 10.1 | 11.2 | 8.0 | 11.6 |
| Sør-Trøndelag | 1.5 | 2.5 | 3.2 | 4.2 | 2.4 | 1.7 | 6.1 | 4.4 | 1.3 | 1.0 | 2.8 |
| Nord-Trøndelag | 2.3 | 2.3 | 3.9 | 3.1 | 0.0 | 3.0 | 1.5 | 2.2 | 3.0 | 3.7 | 2.5 |
| Nordland | 1.3 | 0.4 | 1.7 | 3.0 | 0.8 | 1.3 | 0.8 | 0.4 | 0.4 | 0.4 | 1.1 |
| Troms | 0.0 | 0.7 | 1.3 | 0.6 | 0.0 | 0.0 | 0.6 | 0.6 | 0.0 | 0.0 | 0.4 |
| Finnmark | 0.0 | 0.0 | 0.0 | 1.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 |
| Total | 6.1 | 6.8 | 7.0 | 7.3 | 5.7 | 5.9 | 5.0 | 5.1 | 6.2 | 6.3 | 6.2 |

There are also wide variations between regions within a county, as visualised for Sogn og Fjordane county, see Figure 11.

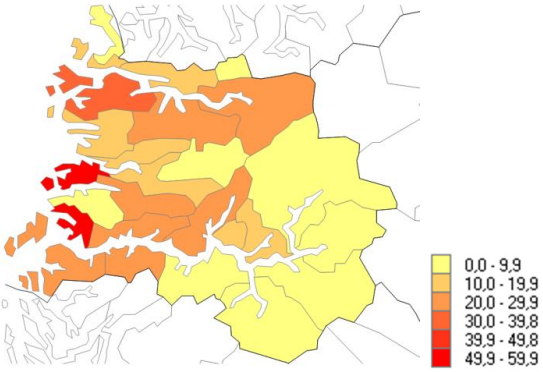


**Fig 11. Cases of Lyme borreliosis notified to MSIS in Sogn og Fjordane 2005-2014 by municipality. Mean yearly incidence per 100,000 inhabitants** (www.msis.no). Map source: Norwegian Map Authority

**Age and gender**

Most studies find that seroprevalence of *Borrelia* antibodies increases with age. Thus, a recent Norwegian study by Vestrheim and co-workers (2016) found that the seroprevalence among children 2-4 years old was about 1.8% using the Enzygnost Lyme link VlsE for *Borrelia* IgG antibodies, as opposed to 6.3% in persons 50 years or older. Children constitute a substantial part of notified cases of borreliosis in Norway, see Table 6. In Sweden, using the Dako ELISA test, Skogman and co-workers found a *Borrelia* IgG antibody seroprevalence of 3.2% in young Swedish children.

**Table 6. Cases of Lyme borreliosis notified to MSIS 2005-2014, according to age and gender** (www.msis.no).

| Age-group (years) | Females | Males | Total |
|---|---|---|---|
| 0 - 9 | 356 | 377 | 733 |
| 10 - 19 | 94 | 171 | 265 |
| 20 - 29 | 31 | 68 | 99 |
| 30 - 39 | 67 | 140 | 207 |
| 40 - 49 | 120 | 209 | 329 |
| 50 - 59 | 203 | 287 | 490 |
| 60 - 69 | 217 | 259 | 476 |
| 70 - 79 | 165 | 121 | 286 |
| 80+ | 36 | 50 | 86 |
| Total | 1289 | 1682 | 2971 |

In the USA, prevalence of IgG antibodies towards Bbsl in males slightly outnumbered the same prevalence in females during the period 1992-1998, especially among children and adolescents aged 5-19 years, but also in adults aged ≥ 60 years. In most European countries, e.g. Austria, Czech Republic, Germany, Slovakia, Slovenia, and Switzerland, there is a slight female preponderance (usual range of 54-60% females among recorded LB patients) (Hubalek, 2009). Similar numbers were found in south-eastern Sweden; among 3,442 EM cases reported in 1997-2003, 54.5% were females, and the predominance of females was especially marked in the age-group 50-74 years (60.1%) (Bennet et al., 2007). Reinfection with *Borrelia* was reported 6 times more frequently for females than for males in southern Sweden and nearly all reinfected women were older than 40 years and postmenopausal (Jarefors et al., 2006). Tick bites vary in their localization on the human body between genders; the predilection sites for ticks are the lower limbs and breast region in females, and the lower limbs and genital region in males (Berglund et al., 1995). In Slovenia, Strle and co-workers found a marked gender disparity in different clinical manifestations of LB, in that more women than men were

diagnosed with skin manifestations such as EM (59.3%) and ACA (68.6%), while men outnumbered women in Lyme neuroborreliosis (LNB) (39.2% females) and Lyme arthritis (25% females) (Strle et al., 2013). In Norwegian general practice, Eliassen and co-workers found a male dominance of EM-patients in the <40-year-old age-groups of 54.1%, in contrast to a female dominance in the >40-year-old age-groups of 55.6%. The difference could not be explained by the age and gender distribution in the population (Eliassen et al., 2017). Among notified cases of LB in Norway 2005-2014 males constitute 1,682 out of 2,971 cases, i.e., 56.6%, see Table 5. Pertaining to severity and complications of disease, Weitzner and co-workers (2016) found that males and females with culture-confirmed early Lyme disease had similar clinical features, rates of seropositivity, and long-term outcomes. Females were, however, significantly more likely than males to return for follow-up visits.

**Geography**

The global distribution of LB correlates closely with the range of ticks of the *I. ricinus* complex, see Figure 4. It thus occurs between 30 $^{o}$N and 55 $^{o}$N in North America, most of Europe, parts of North Africa, and northern Asia. In Europe in general, LB occurs between approximately 35 $^{o}$N and 60 $^{o}$N, although further north along the Norwegian coast line. Also the LB occurrence according to altitude corresponds to the occurrence of the vector tick, i.e., there are fewer cases at higher altitudes (Hubalek, 2009).

LB reveals a distinctly focal pattern of distribution, even within small countries and regions. This is mainly owing to the heterogeneous spatial distribution of the vector ticks.

**Time span**

Although some countries have reported no marked trends in the incidence of LB over time, many countries have reported a growing incidence of this disease during the last decades, among them Norway. Some of these increasing trends might be biased and owing to improved notification systems, greater awareness/vigilance, and better diagnostics for LB (Hubalek, 2009).

**Seasonality**
To a great extent, the annual distribution pattern of LB reflects the seasonal pattern of host-seeking (questing) tick activity, although delayed by the incubation period of the different manifestations of LB. Also, human behaviour and outdoor activity, i.e., the coincidence of maximum tick activity with summer-related leisure behaviour of people, enhances the risk of infection. For instance, vacation times overlapping with enhanced exposure to ticks during hiking and berry/mushroom picking in forests are usually June, July, and August in Europe. Therefore, LB incidence is low in winter and early spring and high during summer and autumn (Hubalek, 2009).

**Occupation**
Examples of population groups at risk are forestry workers, military field personnel, farmers, deer handlers, gamekeepers, hunters, rangers, and outdoor workers in general. For instance, seropositivity to Bbsl is high among forestry workers in most countries tested; France 22% (while only 4% in the general population), Austria 14-18%, Bulgaria 18% and Italy up to 27%. Farmers also often have a higher seropositivity rate, e.g. 15% in Bulgaria (Hubalek, 2009).

In most European countries, occupational exposure generally constitutes only 2% of LB cases (Gray, 1999); it is typically a recreation time disease, contracted during holidays and leisure time exposure, including sport in forested areas. In Switzerland, 8.1% of 558 orienteers seroconverted during one season, but only 0.8% of them revealed clinical symptoms of LB – the ratio of apparent to inapparent infection was therefore 1:9 (Fahrer et al., 1991).

#### *1.3.3.2 Pathogenesis*
Following subcutaeneous injection of Bbsl, the bacterium multiplies locally in the dermis, where it may reach a concentration of $10^5$ bacteria per gram tissue. Thereafter, the bacterium remains localised at the site of the bite for several days before it disseminates further, probably reflecting a process of adaptation to the new host. The organism then disseminates from the inoculation site by both a direct and an indirect haematogenic route. The spirochete adheres to host molecules, especially prominent in

connective tissue, by means of many different adhesins, and may migrate across the vascular epithelium by utilizing the endoflagellae. Unlike many other bacteria, Bbsl does not produce toxins or proteases that directly cause tissue damage. Debilitation of the host is rather caused by the multiple molecules that activate host responses and thus lead to localised and generalised inflammatory pathogenic responses (Moriarty et al., 2008; Samuels and Radolf, 2010; Weis, 2011).

#### *1.3.3.3 Clinical disease*

The spectrum of clinical disease in Lyme borreliosis may be divided into skin manifestations, nervous system manifestations, musculoskeletal manifestations, cardiac manifestations and ocular manifestations (Stanek et al., 2011). Division of the disease into stages I-III analogous to syphilis is often used, but is not always in agreement with clinical findings (Stanek et al., 2012). Interesting differences exist in clinical manifestations of the different genospecies of Bbsl. *B. afzelii* and *B. garinii* infections account for most Lyme borreliosis cases in Europe. *B. afzelii* is mostly associated with skin manifestations, *B. garinii* seems to be the most neurotropic, while Bbss seems to be the most arthritogenic (Stanek et al., 2012).

Infections are also divided into localised versus disseminated. Localised infection is typically manifested by an erythema migrans skin lesion. Early disseminated disease is usually characterised by two or more erythema migrans skin lesions or as Lyme neuroborreliosis or Lyme carditis. Late Lyme borreliosis usually manifests as arthritis or acrodermatitis chronica atrophicans, but can also include specific rare neurological manifestations (Stanek et al., 2012).

Erythema migrans (EM) usually occurs 2-30 days after a tick bite. The lesion starts from a macule or papule and expands over a period of days to weeks to form a red or bluish-red patch, with or without central clearing. The EM may be accompanied by fatigue, fever, headache, mild stiff neck, arthralgia and myalgia, but such symptoms are not indicative of LB if they occur in the absence of EM (Stanek et al., 2011).

Acrodermatitis chronica atrophicans (ACA) is almost exclusively seen in adults, predominantly women, though ACA-like lesions in children have been reported

occasionally. It is a long-lasting, usually progressive manifestation of LB, characterised by red or bluish-red lesions, usually on the extensor surfaces of the extremities. Initially there is a bluish-red discolouration, often with doughy swelling. Later on skin atrophy becomes more and more prominent (Stanek et al., 2011).

Borrelia lymphocytoma is a bluish-red nodule usually localised to an ear lobe, scrotum or nipple. The disease is seen more often in children than in adults (Stanek et al., 2011).

Lyme neuroborreliosis (LNB) is most often an acute disease that develops within a few weeks of infection. In adults, the disease typically presents as painful meningoradiculoneuritis (Garin-Bujadoux-Bannwarth syndrome) and unilateral or bilateral affection of the seventh cranial nerve, n. facialis (facial palsy). Less frequent manifestations include other cranial neuropathies involving the sixth cranial nerve (n. abducens), less frequently the fourth (n. trochlearis) or third (n. oculomotorius) and occasionally others. Isolated meningitis, myelitis, encephalitis and cerebral vasculitis presenting as stroke are other rare manifestations in adults. The most frequent symptoms and signs in children are headache due to meningitis, and facial palsy. *Borrelia* infection of the central nervous system lasting for at least six months (chronic borreliosis), including manifestations such as chronic meningitis, encephalomyelitis, and radiculomyelitis, are very rare (Øymar and Tveitnes, 2009; Stanek et al., 2011; Ljøstad and Mygland, 2012, 2013).

Lyme arthritis manifests as recurrent attacks or long-lasting objective joint swelling (synovitis), usually in one or a few large joints, most commonly the knee (Stanek et al., 2011; Haugeberg et al., 2014).

Cardiac manifestations in LB appear to be rare. They are most frequently observed during or shortly after an EM, but may also occur in association with neurological symptoms or arthritis. Varying degrees of atrioventricular conduction defects are typical manifestations. Other rhythm disturbances, endomyocarditis and pericarditis have also been reported (Stanek et al., 2011). A case of late Lyme carditis presumably

manifesting 17-18 years after the primary infection was published from Førde General Hospital in 1993 (Vegsundvåg et al., 1993).

Ophtalmic changes may present as conjunctivitis in the course of an early manifestation of LB. Uveitis (anterior, intermedia, posterior and panuveitis), papillitis, keratitis, and episcleritis may occur occasionally (Stanek et al., 2011). However, a recent Dutch study found that uveitis due to Borrelia is so seldom that there was no value for routine serologic screening for Bbsl in patients with uveitis in the Netherlands (Kazi et al., 2016).

Of the various objective clinical presentations of Lyme borreliosis in Europe, erythema migrans is the most common. In one case series of patients with Lyme borreliosis, 89% had erythema migrans only, 5% had arthritis, 3% had early neurological manifestations, 2% had borrelial lymphocytoma, 1% had acrodermatitis chronica atrophicans, and less than 1% had cardiac manifestations. None of the patients had late neurological Lyme borreliosis (Huppertz et al., 1999). Only 11% of these would have been notifiable in the Norwegian system (MSIS). A survey from southern Sweden found that the most frequent clinical manifestation was erythema migrans (seen in 77% of all cases), followed by neuroborreliosis (16%) and arthritis (7%). Carditis was rare (Berglund et al., 1995). In this series, 29% of the cases would have been notifiable in Norway.

#### *1.3.3.4 Questionable symptoms attributed to Lyme borreliosis*

There has been much controversy about the clinical manifestations of Lyme borreliosis, especially regarding chronic health consequences of treated and untreated disease (Cameron et al., 2004; Cairns and Godwin, 2005; Shapiro et al., 2005; Feder et al., 2007). Symptoms from many organ systems have been ascribed to “chronic Lyme borreliosis”, including fatigue, stiff neck, migrating arthralgias, myalgia, chest pain, palpitations, abdominal pain, nausea, back pain, headaches, etc. (Cameron et al., 2004). In addition, other agents transmitted by ticks, e.g. *Rickettsia* spp., are increasingly claimed to cause health problems (Berghoff, 2012).

In follow-up studies of patients with neuroborreliosis, several symptoms have been found to persist in some of the patients compared to controls, including malaise, fatigue, memory problems, concentration difficulties and paresthesias (Henningsson et al., 2010; Eikeland et al., 2011; Eikeland et al., 2012).

However, population based follow-up studies of patients treated for Lyme borreliosis in general have given discrepant results regarding long-term consequences. Some early American studies reported that Lyme borreliosis patients experience long-lasting symptoms (Shadick et al., 1994; Shadick et al., 1999), while more recent investigations found that symptoms at follow-up did not exceed that of a control population (Seltzer et al., 2000; Wormser et al., 2014; Wormser et al., 2015; Wills et al., 2016). Likewise, European studies have found that patients having suffered from early Lyme borreliosis do not have late manifestations that exceed the corresponding symptoms in matched controls (Cerar et al., 2010; Stupica et al., 2012).

In a recent Swedish study, patients bitten by *Borrelia*-infected ticks were compared to patients bitten by non-infected ticks (Fryland et al., 2011). The frequency of subjects reporting symptoms was higher in the group bitten by *Borrelia*-infected ticks compared to subjects bitten by uninfected ticks. There were, however, no differences between subjects bitten by a *Borrelia*-positive tick and subjects bitten by a *Borrelia*-negative tick when comparing the frequency of reported single symptoms, such as fatigue, myalgia/arthralgia, headache, and neck pain.

It thus seems that long-lasting symptoms after Lyme neuroborreliosis are frequent in adults; however, in properly controlled studies, lasting symptoms are rarely seen after early disease manifestations such as erythema migrans or after bites with *Borrelia*-infected ticks.

#### *1.3.3.5 Diagnostic testing*

In most patients with erythema migrans, clinical observation suffices to ascertain whether or not a patient is infected by Bbsl . All other cases are in need of verification by laboratory tests. A variety of different methods have been used, of which some are well established, some have failed, some are controversial, and some recently launched

tests remain to demonstrate how well they perform in real patient populations. *Borrelia* testing is sometimes fraught with interpretational difficulties.

**Direct detection methods**

*Microscopy*

Direct visualisation of *Borrelia* spp. in clinical samples is applicable only to cases of relapsing fever (Schriefer, 2015). During acute phases, spirochetaemia often reaches $10^6$ -$10^8$ organisms/ml, and motile spirochetes can be visualised by darkfield microscopy of wet preparations made from a drop of blood.

It is generally believed that there are too few organisms present in the blood to be seen by microscopy in Bbsl infections. A methodological paper, claiming to demonstrate *Borrelia* in blood samples, stirred some hope that microscopy would suffice (Mysterud and Laane, 2013). However, the investigation did not include alternative tests to verify whether the structures seen in the microscope were *Borrelia* or artefacts, and not unexpectedly the method was later refuted in a double blinded study (Aase et al., 2016; Dessau, 2016).

*Antigen detection*

Enzyme-linked immunosorbent assay (ELISA) and immunoblotting have been used for the detection of Bbsl antigens in body fluids, including CSF and urine. However, a commercial assay for antigen-detection in urine was shown to lack reproducibility, and its use is not recommended (Schriefer, 2015).

Recently, a test based on “nanotrap” technology was published, demonstrating OspA lipoprotein in urine specimens (Magni et al., 2015). Although the published results seem promising for identifying patients with current LB, further validation of the method in clinical settings will be necessary before its role can be consolidated. Likewise, a technique for demonstrating OspA in serum after high-speed centrifugation followed by targeted protein quantification by multiple reaction monitoring mass spectrometry (Cheung et al., 2015), also needs validation in clinical settings.

*Nucleic acid detection techniques*

The polymerase chain reaction (PCR) is widely used for detection of nucleic acids from Bbsl in clinical specimens. Tests for both chromosomal and plasmid targets have been developed, and an analytical sensitivity of 10-20 bacteria per test sample has been demonstrated. However, in a clinical setting, the sensitivity of the PCR has been disappointing for many specimen types. The best results have been achieved for synovial fluid and synovial biopsies in Lyme arthritis, with a sensitivity approaching 80%. PCR from skin biopsies in EM or ACA is moderately sensitive (25%-70%). Disappointing results have been found for spinal fluid, serum and urine, with sensitivities lower than 20%. False positive PCR results have also been reported (Aguero-Rosenfeld et al., 2005).

*Isolation procedures*

Bbsl may be cultured in artificial media. The method is time-consuming and has a low sensitivity, making it unpractical in clinical practice. Variants of liquid Barbour-Stoenner-Kelly medium (BSK-medium) are usually used. The culture is incubated at temperatures between 30 $^{o}$C and 34 $^{o}$C under microaerophilic conditions. Due to the slow generation time of 7-20 hours or longer, samples of Bbsl need to be incubated for 6-12 weeks. Detection of growth is accomplished by periodic examination of culture supernatant for the presence of spirochetes by dark-field or fluorescence microscopy. Identification of cultured *Borrelia* may be performed by molecular or serological techniques (Aguero-Rosenfeld et al., 2005).

The sensitivity of culturing is best for skin biopsies from EM and ACA lesions (40-88% and 22-60%, respectively), but only 10-17% for spinal fluid, and very low for blood (1.2-9% in European studies) (Aguero-Rosenfeld et al., 2005)

*Serological tests*

Borrelia antigens and the human humoral immune response

Bbsl possesses many antigens that elicit an immune response in humans. The various outer surface proteins (Osp A to OspF), tissue binding proteins, and parts of the flagellar apparatus are all strong inducers of immune responses. The antigens may differ from genospecies to genospecies as well as between strains. In addition, the

expression of antigens varies according to the bacterium's environment, whether it is inside the tick's midgut, in culture, or in the skin of a human patient. Some important antigens that are poorly expressed in culture, such as OspC and vlsE, are highly expressed during human infection.

The immune response of humans infected with Bbsl usually starts with an IgM response that subsequently develops into an IgG response.

The IgM response is often directed against OspC, the flagellar antigens p41 (FlaB) and p37 (FlaA), and p35 (BBK32, fibronectin binding protein), and is typically detectable within the first few weeks. Unlike the response towards many other microbes, the IgM response may by detectable for many months or years after the infection (Schriefer, 2015).

The early IgG response is typically against OspC, p35 (BBK32), p37 (FlaA), vlsE and p41 (FlaB). IgG-levels then increase and reactivity broadens during early-dissemination of the disease, and reactivity against Osp17 (DbpA (decorin binding protein A)), p39 (BmpA) and p58 often appears. The late-stage immune response is characterised by IgG antibodies to a wide variety of antigens (Aguero-Rosenfeld et al., 2005; Schriefer, 2015).

Laboratory methods for detecting antibodies to Bbsl

The most important method for detecting antibodies to Bbsl are the enzyme immunoassay (EIA), also called enzyme-linked immunosorbent assay (ELISA), and the immunoblot. Other methods, such as indirect immunofluorescence assay (indirect IFA) and indirect haemagglutination assay (IHA) are no longer used in diagnostic contexts in Europe.

ELISA

In the ELISA reaction, solubilized *Borrelia* antigens are first coupled to a solid phase. After incubation with patient serum, specific antibodies are bound to the corresponding antigens, and irrelevant antibodies are removed in a washing step. The nature of the bound antibodies are then ascertained by adding an enzyme-labelled antibody (called a conjugate) towards human IgG or IgM, which in turn will catalyse

the colour change in a chromogenic substrate, making detection possible (Figure 12). Depending on how the antigens are produced, ELISAs for Bbsl have been divided into first generation, using crude culture extracts as antigen, second generation, where the antigen preparation has been improved to limit cross-reactivity with other bacteria, and third generation, using synthetic peptides. Strains used as antigen source should express OspC, the immunodominant antigen of the IgM response, and DbpA, an immunodominant antigen for the IgG response. Recently, specific recombinant antigens (i.e., vlsE) or synthetic peptides (i.e., the C6 peptide derived from vlsE) have been introduced (Wilske et al., 2007). A review of the diagnostic accuracy of serological tests for Lyme borreliosis in Europe was recently presented by Leeflang and co-workers (Leeflang et al., 2016)

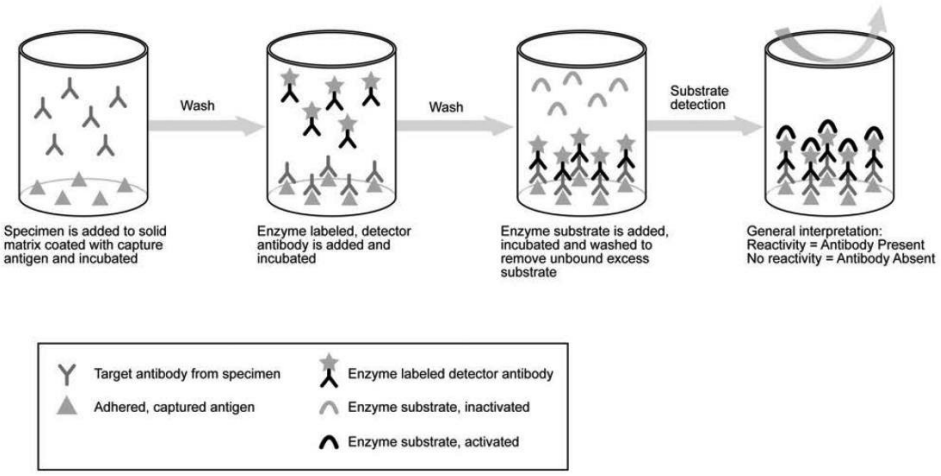


**Figure 12. Diagram of an EIA**

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### Western blot and immunoblot

Antigen preparations for Western immunoblotting include whole-cell lysates or recombinant protein antigen mixtures that are resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to blot

membranes (Figure 13). An alternative test format is the line immunoblot, in which purified recombinant or native *Borrelia* antigens are applied directly onto membranes (Schriefer, 2015). The membranes are cut into strips. In practice most laboratories purchase ready-made commercially available strips. The serum is incubated together with the strip, specific antibodies are bound to the antigens, and thereafter visualised by using a conjugate coupled to an enzyme causing a colour change of the strip where the antigen is located.

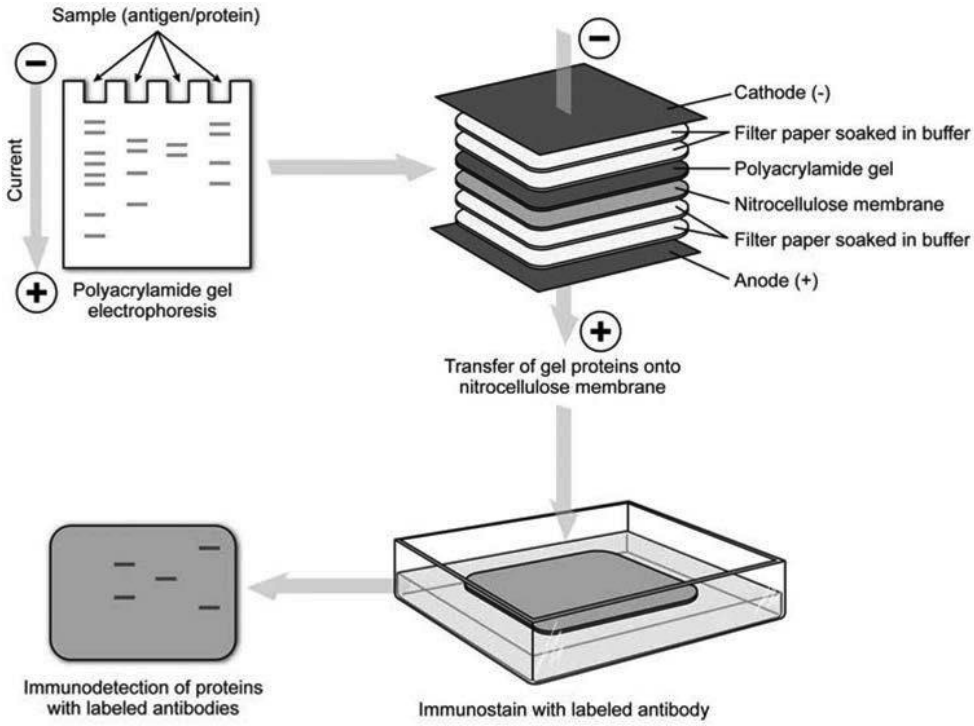


**Figure 13. Diagram of the Western blot procedure**
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Testing algorithms in serology

In the USA and central Europe, screening with an EIA test is recommended to be complemented with an immunoblot for confirmation of positve EIA results, known as two-tiered testing (Centers for Disease Control and Prevention, 1995; Brouqui et al., 2004). There are several different EIAs and immunoblot assays on the market, with varying antigenic compositions and performance characteristics. As a consequence, the laboratory's choice of EIA and immunoblot manufacturer influences the conclusions of the diagnostic procedure (Ang et al., 2011).

In Scandinavia, the two-tier principle has never been systematically adopted, as it is thought to reduce sensitivity and only gives a marginal add to specificity (Dessau et al., 2006). Thus, most laboratories either perform only EIA-testing, or perform additional testing in certain circumstances (Dessau et al., 2011). In Norway, optimal testing strategies are currently being discussed (Harbo, 2009; Grude et al., 2011). Different alternatives to or variants of the two-tier testing are currently discussed world-wide, e.g. using C6 ELISA as the only test (Tjernberg et al., 2007; Steere et al., 2008; Wormser et al., 2013), or using an ELISA different from the one used for screening for second-tier testing (Branda et al., 2011; Theel, 2016).

*Tests demonstrating cellular immune response to Borrelia burgdorferi* s.l.

In parallel with the humoral immune response, the patient also mounts a specific T-helper (CD4) and T-cytotoxic (CD8) cellular immune response towards antigens in the *Borrelia* bacterium. Assays designed to detect these reactions have not yet shown satisfactory sensitivity or specificity to be useful in the diagnosis of Lyme borreliosis (Dessau et al., 2014; Tveten et al., 2014).

Also, a low count of CD57 positive lymphocytes (a subset of natural killer cells (NK cells)) has been associated with chronic Bbsl infection. The scientific basis for this assumption has been questioned (Tveten et al., 2014).

Owing to their capability to detect an alternative immunologic response towards Bbsl, properly adjusted cellular tests have potentiality to become helpful tools in *Borrelia*-testing. But so far the assays have delivered meagre results.

**Other test principles**
Recently, a technique assessing small molecule metabolites in serum by liquid chromatography - mass spectrometry (LC-MS), has been reported to achieve significantly greater diagnostic sensitivity than current two-tier serology, while retaining high specificity (Molins et al., 2015). The usefulness and applicability of this principle has to be validated further.

**Conclusions on diagnostic methods**
Although serology is fraught with problems, it is still the most practical approach to laboratory testing of Lyme disease. In the case of arthritis, it may be supplemented with PCR of biopsies or joint fluid. Owing to the several shortcomings of contemporary *Borrelia* testing, new methods with better sensitivity and specificity for actual infection are needed.

### *1.3.3.6 Treatment*
Bbsl is susceptible to tetracyclines, most penicillins, many second- and third-generation cephalosporins, and macrolides in-vitro (Schriefer, 2015). The bacteria are resistant to fluoroquinolones, rifampicin, and first-generation cephalosporins. Treatment is recommended in all cases of LB, usually for 2 to 4 weeks. Recommended treatment regimens in Norway include penicillin, amoxicillin, doxycycline, ceftriaxone or cefotaxime, depending on the actual manifestation of LB (Norwegian Directorate of Health, 2016; Norwegian Directorate of Health and Antibiotic Centre for Primary Care, 2016).

### *1.3.3.7 Prevention of LB*

**General precautions against tick bites**

*Reducing tick exposure*
Avoiding tick infested areas, which of course is a straight-forward approach, has the considerable drawback that it limits man's freedom to engage with nature.

As an alternative, wearing clothes covering naked skin and ideally using bright colours for easier detection of ticks has been advocated. The use of tick repellents on skin and clothing has some effect (Pages et al., 2014).

Reducing the number of questing ticks in human living areas may be achieved by the removal of leaf litter, by applying wood chips where lawns are adjacent to forests, by application of acaricides, and using fences to keep out deer.

*Removing attached ticks*
Having a shower or bath within 2 hours of tick exposure is shown to decrease the risk of LB.

Inspection of entire skin after exposure, especially hairy areas, may help removing ticks in a timely fashion, as the risk of infection with Bbsl increases with time of attachment.

Attached ticks should be removed as soon as possible by using forceps or tweezers and gently pulling it out (Stanek et al., 2012).

**Specific precautions against Bbsl**

*Antibiotic prophylaxis*
Chemoprophylaxis with the antibiotic doxycycline is shown to reduce the chance of developing LB after removal of *I. scapularis* or *I. persulcatus* ticks. In the USA, such prophylaxis with one dose of 200 mg doxycycline should be considered for individuals in highly endemic areas who are known to have been bitten by a nymphal or adult *I. scapularis* tick that has been attached for at least an estimated 36 hours. In Europe, only observation is recommended for *I. ricinus* tick bites, because the infection rate of ticks is lower than in the USA, and studies have not been done on the efficacy of antibiotic prophylaxis (Stanek et al., 2012).

*Vaccine*
In the USA, a vaccine against Bbsl was developed and licenced in 1998. The mechanism of action involved vaccinating humans against OspA with the subsequent development of circulating bactericidal antibodies that would be ingested by the tick during a blood meal. In turn, these antibodies were sufficient to bind and neutralise viable *Borrelia* spirochetes present in the tick gut, effectively preventing infection. The vaccine was withdrawn in 2002, on the background of low public demand as well

as safety issues of uncertain significance (Poland, 2011). There are currently no licenced Bbsl vaccines for human use on the market.

## *1.4 Anaplasma phagocytophilum*

*Ehrlichia* and *Anaplasma* spp. are related Gram-negative obligate intracellular bacteria that reside and propagate within membrane-lined vacuoles found in the cytoplasm of bone marrow-derived cells, such as granulocytes, monocytes, erythrocytes, and platelets (Reller and Dumler, 2015). The taxonomy of these bacteria has changed several times since their discovery, and *Anaplasma phagocytophilum* was earlier classified as *Ehrlichia phagocytophila* (Dumler et al., 2001). They form intracytoplasmic clusters of bacteria as small dense core forms (0.2 to 0.4 μm) and larger forms (0.8 to 1.5 μm) (Reller and Dumler, 2015). This clustered inclusion-like appearance of a microcolony in the host cell vacuole is called a morula, from the Latin word for mulberry (Dumler and Walker, 2015).

The major animal reservoirs for *A. phagocytophilum* are incompletely documented. Viable *A. phagocytophilum* organisms have been isolated from several hosts, such as cattle, sheep, goat, dog, horse, human, red deer, roe deer and white-tailed deer (Stuen et al., 2013). Another group of animals in which *A. phagocytophilum* is found in endemic countries is in small mammals such as rodents and insectivores. These animals also are major feeding hosts for ticks, especially for the developmental stages (Stuen et al., 2013). Tick bite is the most frequent route of transmission; however, there are others, including perinatal transmission, accidental inoculation of infected blood, and blood transfusion (Dumler and Walker, 2015).

Many strains of *A. phagocytophilum* have a restricted host range, and some strains appear to be apathogenic (Dumler and Walker, 2015).

*Ixodes* ticks are the primary vectors for *A. phagocytophilum*. In ticks, the bacterium is transmitted transstadially but not transovarially. Thus, nymphs and adults ticks may infect humans, but not larvae (Stuen et al., 2013). The organism was detected in 8.8% of *I. ricinus* in Sogn og Fjordane in a survey performed in 2011 (Mysterud et al.,

2013), while an earlier study performed in 2006-7 found a lower prevalence (1.4% and 0.0% in two different locations, respectively) (Rosef et al., 2009b).

Granulocytic anaplasmosis, caused by the bacterium *A. phagocytophilum*, is prevalent in livestock in Norway (Stuen and Bergström, 2008), and probably as much as 300, 000 lambs are infected yearly. The infection in domestic ruminants in Europe is called "tick-borne fever" (TBF). The Norwegian synonym of TBF is "sjodogg", and this name was used as early as year 1780 to describe a devastating illness in ruminants (Stuen et al., 2013).

After the verification of human infections in the United States in 1994 (Bakken, 1998), and from 1997 onwards also in Europe (Strle, 2004), two cases from 1998 were reported from southern Norway (Kristiansen et al., 2001). Serological evidence for human infection in the country has also been demonstrated (Bakken et al., 1996). In a recent Swedish study, seroconversion following bites by *Anaplasma*-infected ticks was estimated to be very low. After being bitten, none of 30 patients developed serological evidence of anaplasmosis, whereas one patient out of 60 bitten by ticks negative for *Anaplasma* seroconverted, probably due to another, unrecognised bite. The seroprevalence in this study population (90 patients) was 17% (Henningsson et al., 2015).

Human granulocytic anaplasmosis (HGA) has a median incubation period of 5 to 11 days after the bite of an infected tick. Patients most often present with high fever (93%), myalgias (68%), headache (62%), and malaise (93%). Gastrointestinal, respiratory, musculoskeletal, and central nervous system involvement occurs in fewer than half of the patients. Leukopenia with lymphopenia, thrombocytopenia, and increased serum aspartate transaminase activity is common early in the disease. The clinical course is worse for patients with cancer, diabetes, immunosuppression related to treatment, and functional or anatomic asplenia (Reller and Dumler, 2015).

Treatment of choice for HGA is doxycykline or tetracycline (Dumler and Walker, 2015). Vaccines against *A. phagocytophilum* are not yet available (Stuen et al., 2013).

The diagnosis of anaplasmosis is based on one or more of the following; microscopy of peripheral blood or buffy coat, antigen detection by immunohistology, analysis of blood specimens by nucleic acid detection techniques, cultivation in cell lines, or serologic tests. Among the serological methods, IFA with *A. phagocytophilum* propagated in cell lines is the preferred method. The typical serological response during acute infection is a rapid rise (within two weeks of onset) in antibody levels, reaching high titers (≥640) within the first month. In treated patients, antibody titers decline gradually over several months. About half of the patients have antibodies detectable by IFA one year after infection. False positive reactions may be observed in patients infected with *Rickettsia* spp., the Q fever agent *Coxiella burnetii*, and Epstein-Barr virus. Autoantibodies to platelets and other leukocyte components may also lead to a false-positive IFA test (Dumler and Walker, 2015).

## 1.5 Tick-borne encephalitis virus

Tick-borne encephalitis virus (TBEV) belongs to the genus Flavivirus within the family Flaviviridae. These are about 50 nm in diameter, icosahedral, and have a lipid envelope covered with surface projections consisting of M (membrane) and E (envelope) glycoproteins (Mansfield et al., 2009; Thomas et al., 2015). Included among the Flaviviruses are other arthropod-borne viruses such as Japanese encephalitis virus (JEV), dengue virus (DENV), yellow fever virus, (YFV) and West Nile virus (WNV). Three closely related subtypes of TBEV exist, whose names reflect the geographic areas that they principally affect; European, Siberian, and Far Eastern.

The enzootic cycle of TBEV involves ixodid ticks and wild mammalian hosts, particularly rodents. In the tick, the virus passes transovarially and transstadially, from egg to larva, nymph, and adult, so all stages of the tick and both male and female ticks transmit infections to animals and humans (Thomas et al., 2015). Rodents probably maintain a persistent infection with TBEV throughout the year. Larger animals are not thought to have an important role in virus transmission. The infected tick can transmit the virus to a vertebrate host during feeding and is also able to pass on the virus to a non-infected tick during so-called co-feeding at the same site on the host (Mansfield et al., 2009). This concept of transmission by co-feeding states that natural hosts having

neutralising antibodies to TBE virus (and no detectable viremia) still may support virus transmission between infected and uninfected ticks feeding closely together on the same animal. This is thought to occur via cellular infiltration of tick feeding sites and the migration of cells from these sites (Labuda et al., 1997). Evidence of co-feeding of nymphs and larvae was recently demonstrated in ticks in Sogn og Fjordane (Mysterud et al., 2015).

Infection by the virus leads to symptoms of TBE in as high as one in three persons. The time from tick bite to symptoms is 4-28 days (median 8 days). A small number of infections occur through consuming infected unpasteurised milk. The illness usually begins with nonspecific symptoms of fever, malaise, headache, nausea, vomiting, and myalgias. Within one week, these symptoms resolve spontaneously, and many patients do not experience further symptoms ("febrile form"). However, in some patients, after an asymptomatic period lasting 2–10 days, the disease then progresses to neurological involvement, when high fever, headache and vomiting resume. This second phase may be limited to a "meningeal form" with aseptic meningitis (common in children), or it may manifest as a "meningoencephalitis form", or a "poliomyelitic form" with a Guillain-Barré-like paralysis. Neurologic infections are usually benign in children, whereas severe disease occurs more often in elderly persons. Sequelae are reported in up to 40% to 60% of patients, most frequently consisting of psychological disturbances such as asthenia, headache, memory loss, etc. (Mansfield et al., 2009; Thomas et al., 2015).

Historically, the disease has been given a range of different names, including Central European encephalitis (CEE), Russian spring-summer encephalitis (RSSE), Far Eastern encephalitis, and biphasic milk fever. Descriptions of a disease compatible with TBE appeared in Austria and in the far eastern part of Russia in the early 1930s. In 1937 the virus was isolated from the blood of patients and from *Ixodes* spp. tick vectors (Thomas et al., 2015).

While TBE has been recognised throughout Europe, endemic transmission is most intense in Austria, areas of Sweden, Switzerland, Slovenia, Czech Republic, the Baltic

countries, and Russia. In these countries, yearly incidence rates in unvaccinated populations have approached 20 in 100,000 persons, but the risk is highly focal. In Austria, national vaccination programs have reduced the yearly incidence of disease to fewer than 1 in 1 million (Thomas et al., 2015).

In Norway, human cases of TBE acquired along the southern coastline have been diagnosed from 1998 onwards (Skarpaas et al., 2004; Blystad et al., 2009), and tick-borne encephalitis virus (TBEV) has been detected in ticks in this area (Andreassen et al., 2012). According to the Norwegian Surveillance System for Communicable Diseases (MSIS), 87 TBE cases were reported in Norway in the period 1994 to 2014. TBE cases have been reported from coastal areas in the counties of Vest-Agder, Aust-Agder, Telemark, and Vestfold (Norwegian Institute of Public Health, 2015a). In the county of Østfold, where no human cases of TBE have been notified so far, TBEV was recently detected in *I. ricinus* nymphs, and TBE IgG antibodies were detected in blood donors (Larsen et al., 2014).

So far, no human cases acquired in western Norway have been described. However, a case of TBEV-seropositivity in a red dear in the county of Møre og Romsdal, just north of Sogn og Fjordane, was recently reported (Ytrehus et al., 2013), and the virus has been demonstrated in ticks in Møre og Romsdal and Sør-Trøndelag (Paulsen et al., 2015). As far as we know, no recent survey of TBEV in ticks has been performed in Sogn og Fjordane county.

The laboratory diagnosis of TBE infection may be accomplished by several methods. In early disease, i.e., in the first viraemic phase, demonstration of TBEV nucleic acid in serum or spinal fluid may be successful. Later, the demonstration of specific IgM and IgG antibodies is the preferred method, for which an ELISA method is usually performed (Holzmann, 2003). Because of serological cross reactions to other flaviviruses, positive serology may have to be confirmed by other methods, e.g. a neutralisation assay (Hunsperger, 2015).

No specific antivirals are available for the treatment of TBE (Thomas et al., 2015). A vaccine against TBE is available, and this vaccine is currently recommended to

persons exposed to ticks in TBE-endemic areas in Norway, and to travellers at risk (www.fhi.no).

## 1.6 Other tick-borne infections

There are many tick-borne diseases other than those discussed above (Diaz, 2015). Such diseases are caused by bacteria, viruses, and protozoa. A number of these do not exist endemically in Norway, some have only recently been acknowledged, and some are of uncertain significance for human health. Many are transmitted by tick species not present in Norway or only in limited numbers.

Those that are established or probably established in Norway will be shortly mentioned in the following.

### *1.6.1 Borrelia miyamotoi*

The bacterium does not belong to the Bbsl complex, but is more related to the RF *Borrelia* spp. It was first demonstrated in Japan in 1995 in *I. persulcatus*, and thereafter in ixodid ticks including *I. ricinus* in parts of USA, in Russia and in Europe. Mean occurrence in ticks has been 1.9% (0-10.5%), i.e., about one third as prevalent as Bbsl. The bacterium has been found in *I. ricinus* in southern Norway (Kjelland et al., 2015; Quarsten et al., 2015). It is transmitted transovarially and transstadially. It has not yet been cultured on artificial media, and the mode of demonstration is by PCR. A series of 46 Russian cases were published in 2011 (Platonov et al., 2011), and 51 patients from USA in 2015 (Molloy et al., 2015). The symptoms include high fever, chills, marked headache, and myalgia or arthralgia (Platonov et al., 2011; Molloy et al., 2015). Human cases have so far not been demonstrated in Norway.

### *1.6.2 Rickettsia helvetica*

*Rickettsia* spp. is a large group of intracellular bacteria, whereof the tick-borne *Rickettsia* spp. give rise to spotted fevers, i.e., Rocky Mountain spotted fever, African tick bite fever, and boutounneuse fever. *R. helvetica* is the only *Rickettsia* demonstrated in ticks in Sweden, and has been found in *I. ricinus* in Norway (Quarsten et al., 2015; Toledo et al., 2015). A seroepidemiological study in Sweden found a seroprevalence of 2.6%; 4.4% in patients positive for *Borrelia* antibodies, 3.0% of

tick-bitten *Borrelia* serology negative patients, and 0.6% in healthy blood donors (Elfving et al., 2008). Bacteriaemia with this organism has been demonstrated in birds, which may represent an important reservoir (Hornok et al., 2014). The bacterium has been linked to human cases of neurologic disease including meningitis (Nilsson, 2009; Nilsson et al., 2010).

### *1.6.3 Candidatus Neoehrlichia mikurensis*

This *Ehrlichia*-like intracellular bacterium was first demonstrated in Japan in 2004, and has later been found in ixodid ticks in several European countries. In Sweden, it was found in 6% of ticks, and in Norway in 6-7% and 5.5% of ticks in two different studies (Jenkins and Kristiansen, 2013; Toledo et al., 2015). The reservoir is probably wild rodents (Andersson and Raberg, 2011). Since 2007, fourteen European human cases have been published, of these six in Sweden. Most patients have been immunocompromised, and the symptoms have been fever, cough, headache and arthralgia. The major complications have been aneurisms, thromboembolic events and bleeding (von Loewenich et al., 2010; Welinder-Olsson et al., 2010; Grankvist et al., 2015; Labbé Sandelin et al., 2015). However, asymptomatic infection in immunocompetent forestry workers in Poland has been described (Welc-Faleciak et al., 2014). Recently, one patient was diagnosed in Norway (Hanne Quarsten, personal communication 2016).

### *1.6.4 Francisella tularensis*

The bacterium *Francisella tularensis* is the cause of the zoonotic infection tularemia. The bacterium has two important subspecies; *F. tularensis* subsp. *tularensis* (type A), that gives serious human infections and occurs primarily in North America, and *F. tularensis* subsp. *holarctica* (type B), which occurs in Europe, Asia and North America and gives a milder disease. The bacterium occurs primarily in wild animals, especially hares and rodents, which themselves fall ill of this bacterium. Human cases occur after direct contact with, or bite from, ill or infectious animals, contaminated drinking water, by bites from mosquitos or ticks, or inhalation of contaminated dust. The clinical pictures are to a certain degree dependent upon the bacterium's site of entry into the body (Penn, 2015). In Norway, there are occasional regional outbreaks

of the disease (Norwegian Institute of Public Health, 2016b) . The most common clinical presentations are the oropharyngeal form after drinking contaminated water, and the ulceroglandular form after contact with animals or insect- or tick bites (Norwegian Institute of Public Health, 2016b). Some cases that are probably tick-borne have been published in Norway (Brantsæter et al., 1998).

### 1.6.5 *Babesia* spp.

*Babesia* spp. are intraerythrocytic protozoa. More than 100 *Babesia* species infect a wide array of wild and domestic animals, but only a few have been documented to infect humans. Babesiosis in cattle is prevalent in tick-endemic areas in Norway, and is called "blodpiss" due to the accompanying haematuria.

In USA, the majority of human cases are caused by *B. microti*. In Europe, most reported human cases have been attributed to *B. divergens* (Vannier and Krause, 2012). Moderate, mild, or asymptomatic infections generally occur in people who are immunocompetent. In contrast, severe *B. microti* is more common among patients who have undergone splenectomy and those with cancer, human immunodeficiency virus infection or other underlying diseases. In USA, more than 150 transfusion-related cases have been identified (Vannier and Krause, 2012). The clinical manifestations of babesiosis range from subclinical infection to fulminating disease resulting in death. There is one case report of babesiosis caused by *B. divergens* in Norway, in a splenectomised veterinarian (Mørch et al., 2015).

Different *Babesia* species have been demonstrated in ticks in Norway, whereof *B. venatorum* is the most prevalent (Øines et al., 2012). The clinical picture in humans infected with this species has been dominated by fever, fatigue, and headache (Jiang et al., 2015). Whether clinical cases of infection with *B. venatorum* exist in Norway is not known.

Of note is that a in a recent study of ticks recovered from domestic dogs in Denmark *Babesia* spp. was found in 8% of samples, of which *B. microti* constituted 82% (Stensvold et al., 2015). Likewise, in the Swedish STING study, *B. microti* constituted half of the findings of *Babesia* spp. in ticks that had bitten humans (Per-Eric Lindgren,

personal communication, 2017). In the Norwegian study by Øines et al. (2012), the authors state that the PCR was not optimised for detection of *B. microti*. It is thus possible that *B. microti* so far has been underestimated in Scandinavia.

# 2 Aims of the study

The general aim of this thesis was to increase the knowledge of tick-borne infections in Sogn og Fjordane county in a healthy adult population, including distribution, diagnosis and impact on health.

The specific aims for each paper were:

I. To assess the frequency of tick bites in a healthy adult population (blood donors) from Sogn og Fjordane county, western Norway, with regard to demographics and other risk factors. We also wanted to assess the frequency of symptoms following tick bites, visits to a medical doctor and antibiotic treatment, as well as to estimate whether tick occurrence leads to avoidance of outdoor activities in certain areas.

II. To describe the seroprevalence of antibodies to *B. burgdorferi* s.l. in these blood donors. 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.

III. To estimate the prevalence of antibodies to *A. phagocytophilum* and TBEV in blood donors, to obtain an indication of whether diseases caused by these agents currently should be considered in the evaluation of patients after tick bites in this region.

IV. To assess the association, if any, of general function, physical fitness and subjective health complaints with reported tick bites and antibodies to *B. burgdorferi* s.l. in this group of healthy adults.

# 3 Materials and methods

## 3.1 Study area

Located on the western coast of Norway at 61-62 $^{o}$N and 5-7 $^{o}$E, the county of Sogn og Fjordane encompasses coastal, fjord and mountainous areas. The fjords penetrate 100-200 km inland from the coast (Figure 14). In the coastal and middle areas, the climate is temperate, with a high yearly rainfall of 1,500 to 3,000 mm, with mean winter temperatures in the coldest month, February, of 1-2 $^{o}$C, while in summer, mean temperature in the warmest month (August) is 14 $^{o}$C. The eastern part has a more inland-like climate, with a yearly rainfall of less than 500 mm, and mean winter temperatures (January) of -3 to -6 $^{o}$C, and summer temperatures (July) of 15-17 $^{o}$C (Dannevig, 2009). The North Atlantic Drift of the Gulf Stream makes the temperature higher than the latitude would indicate.

The topography is rugged with summits reaching 1,000–1,500 m above sea level, just a few kilometers from the sea. The vegetation is within the so called boreonemoral vegetation zone. Forests are dominated by Scots pine (*Pinus sylvestris*) on marginal soils, with alder (*Alnus incana*) dominating in richer soils, and birch (*Betula* spp.) dominating at higher elevations. There are scattered stands of Norway spruce (*Picea abies*) planted by forestry mainly on better soils at low elevation (Mysterud et al., 2013).

The county spans an area of 18,623 km$^2$. As of January 1, 2010, the county had 107,080 inhabitants, i.e., 5.75 individuals per km$^2$. The society is predominantly rural, the two biggest towns had each about 10,000 inhabitants. The area is mostly agricultural, although there are some small industrial towns.

There are dense populations of red deer in the county. The number has been steadily increasing during the last two decades, and the number of harvested red deer in 2010 was 10,532, as opposed to 303 in 1993 (Hjorteviltregisteret, 2015). The red deer populations are partially migratory. There are few roe deer and moose. There are livestock in several areas, mainly free-ranging sheep (*Ovis aries*) during summer. Other available large hosts (>1 kg) are red fox, marten, mountain hare, and domestic

cats. Densities of these are not known, but the impression is that there are low densities of hare and marten, while red fox is more common (Mysterud et al., 2013). Information regarding densities of smaller hosts such as voles, mice and birds are unavailable.

## 3.2 Study population

The Helse Førde Hospital Trust serves the county of Sogn og Fjordane, and has four blood banks in the county (Figure 14). One is situated at the western coast (Florø), two by fjords somewhat further east (Førde and Nordfjordeid), and one is located in the easternmost part of the county (Lærdal), about 150 km from the coast. During the period January 13th to June 15th 2010, blood donors at the four blood banks were asked to participate in the Tick-borne Infection Study in Sogn og Fjordane. A total of 1,213 blood donors participated, a response rate of 76%. Characteristics of these are shown in Table 7. Owing to practical circumstances at the blood bank in Lærdal, with its several different small sites for donation, the response rate at that blood bank was only 39%.

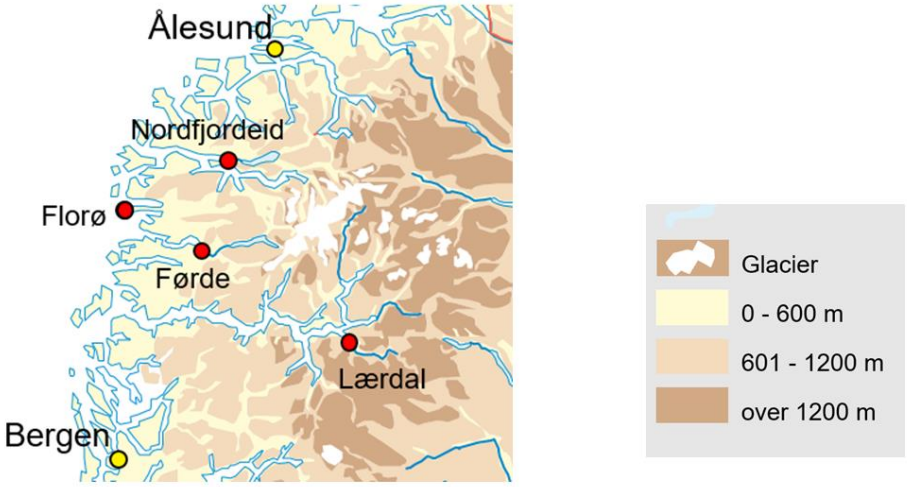


**Figure 14. The localisation of the four participating blood banks (red circles)**
Map source: Norwegian Map Authority.

**Table 7. Characteristics of the 1,213 participants**

| Characteristic | No. of subjects[1] | Percentage |
|---|---|---|
| Blood bank | | |
| Førde | 614 | 50.6 |
| Florø | 355 | 29.3 |
| Lærdal | 73 | 6.0 |
| Eid | 171 | 14.1 |
| Gender | | |
| Female | 544 | 44.8 |
| Male | 669 | 55.2 |
| Age | | |
| 19-29 | 80 | 6.8 |
| 30-39 | 235 | 19.9 |
| 40-49 | 414 | 35.0 |
| 50-59 | 344 | 29.1 |
| 60-69 | 110 | 9.3 |
| Marital status | | |
| Single | 189 | 15.7 |
| Married or cohabitants | 1,014 | 84.3 |
| Education | | |
| Primary school 9 years or less | 87 | 7.2 |
| Secondary school | 598 | 49.8 |
| University/college 1-4 years | 342 | 28.5 |
| University/college >4 years | 175 | 14.6 |
| Household yearly gross income (EUR) | | |
| <50,000 | 156 | 13.2 |
| 50-99,000 | 647 | 54.7 |
| 100-150,000 | 355 | 30.0 |
| >150,000 | 25 | 2.1 |
| Daily smoking | | |
| No | 935 | 81.9 |
| Yes | 207 | 18.1 |

[1]Because of missing data, not all numbers total the number of participants (n = 1,213).

## 3.3 Questionnaire

The questionnaire included questions about demographics such as gender, age, marital status, education, household income and occupation. Questions on pet animals, farm animals, hours spent outdoors during summertime, amount of ticks in their living area, hunting, orienteering, smoking, and symptoms and treatment after tick bites were also included. This part of the questionnaire was made specifically for this study.

The questionnaire included two questions on tick bites; tick bites ever experienced, and tick bites experienced during the last 12 months. The responses for both questions were given in the categories “none”, “one”, “2-5”, “6-20” and “more than 20”.

Another section of the questionnaire was constituted by the Subjective Health Complaints (SHC) Inventory, a set of questions designed to measure common and prevalent health complaints in the general population (Eriksen et al., 1999; Ihlebaek et al., 2002). The respondents were asked to report to what extent they had been affected by 29 different health disturbances during the last month. The response options were “Not at all” (score 0), “A little” (score 1), “Some” (score 2), and “Serious” (score 3).

The question related to general function was “How do you assess your ability to perform ordinary activity, your general function, is today?” The response options were “Good, as it usually is” (score 0), “Hardly reduced at all” (score 1), “Not much reduced” (score 2), “Moderately reduced” (score 3) and “Much reduced” (score 4), adapted from (Reiso et al., 2000).

Physical fitness was assessed by the question “«During the past 2 weeks: What was the hardest physical activity you could do for at least 2 minutes?», with the response alternatives «Very heavy (ex. run, at fast pace)» (score 0), « Heavy (ex. jog, at slow pace)» (score 1), « Moderate (ex. walk, at a fast pace)» (score 2), «Light (ex. walk, at a medium pace)» (score 3), and «Very light (ex. walk, at a slow pace or not able to walk)» (score 4). This question was taken from the COOP/WONCA charts (Weel et al., 1995).

The questionnaires were answered anonymously, and most were completed during the time spent at the blood bank for routine donation.

## 3.4 Laboratory methods

Blood samples were collected in serum separator tubes with gel, and after centrifugation, sera were frozen in aliquots at -70$^{o}$C until testing.

Testing for antibodies to Bbsl was performed at the Department of Microbiology, Helse Førde. The tests for anti-TBEV were performed at the Department of Virology, National Institute of Public Health, Oslo, while the testing for antibodies to *Anaplasma phagocytophilum* was performed at Department of Clinical Microbiology, Ryhov County Hospital, Jönköping, Sweden.

### 3.4.1 *Borrelia burgdorferi* s.l.

Antibodies to Bbsl were tested in Enzygnost Lyme link vlsE/IgG, Enzygnost Borreliosis IgM (DADE Behring, Marburg, Germany) and Immunetics C6 Lyme ELISA kit (Immunetics, 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 Bbss, *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 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 simultaneously. 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 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 derived from a classical Western blot. Recombinantly produced and purified vlsE antigen from the three dominating Bbsl 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, Lübeck, Germany).

### *3.4.2 Anaplasma phagocytophilum*

A random subgroup of 301 sera (every fourth serum) was examined for IgG to *A. phagocytophilum* by an indirect immunofluorescence assay (IFA) (Anaplasma phagocytophilum IFA IgG Kit, Focus Diagnostics, CA, USA). A screening analysis was performed at a serum dilution of 1:80, and sera with a positive reaction in this dilution were further examined in two-fold titrations.

### 3.4.3 Tick-borne encephalitis virus

All 1,213 sera were analysed for IgG-antibodies to TBEV in Serion ELISA *classic* TBE IgG (Institute Virion\Serion GmbH, Würzburg, Germany) according to the manufacturer's instructions. Grey-zone results were repeated. One serum positive in this test was further tested for neutralising antibodies to TBEV at the Swedish Institute for Communicable Disease Control (Vene et al., 1998).

## 3.5 Statistical methods

Data were analysed using the latest version of the SPSS statistical package (SPSS Inc., Chicago, IL, USA) (version 18 in paper I, version 20 in paper II and III, and version 21 in paper IV). In addition, Stata/SE version 13.1 for Windows (StataCorp LP, College Station, TX, USA) was used (paper IV).

All data were categorical and described as frequencies and percentages. Age was with few exceptions categorised into age-groups.

Chi-squared test and Fisher's exact test were used when comparing ratios (paper I and III), and Student's t-test was used for comparing age distributions (paper II).

Binary logistic regression was used for dichotomised outcomes, allowing for adjustment for gender, age-group and location of blood bank, when appropriate (paper II and IV).

Proportional odds models, i.e., logistic regression models with more than two ordinal categorical outcomes, were used when assessing the association of demographics and various life style factors with number of tick-bites (paper I), and when assessing the association between number of tick bites and general function and physical fitness (paper IV) (Armstrong and Sloan, 1989; Scott et al., 1997).

The kappa statistic was used to compare the different ELISA methods, 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 (Landis and Koch, 1977) (paper II).

A linear regression model with robust variance estimation was used to estimate differences in the total number of subjective health complaints (SHC) and the total SHC score between groups with and without the risk factors (paper IV).

All p-values were two-sided and values below 0.1 (paper I) and 0.5 (paper II-IV) were considered statistically significant.

## 3.6 Ethical issues

Informed consent was obtained from each participant before the study, and the study was approved by the Regional Committee for Medical Research Ethics (2009/950). In order to keep the blood specimens frozen for a long time for the possibility of follow-up projects, an approval of «general biobank» was granted (2009/2248).

Participants wanting information on their laboratory results, 834 in all, were informed by letter in 2014, together with a short explanation of the results.

# 4 Summary of results

## 4.1 Paper I

Among the 1,213 participants, 65.7% had experienced tick bites during their life time, and 30% had experienced tick bites during the last 12 months.

Donors from the blood bank in Lærdal reported the lowest occurrence of ticks in their living area as well as the lowest number of tick bites, both total and recent. The estimated mean total number of tick bites of 1.5 from this area contrasts with 5.6 – 7.2 from the other locations.

The number of tick bites increased with age, but there was no gender difference in overall analyses. In the younger age-groups, males reported more bites than females. In subjects older than 50 years old, this was reversed, with females reporting more tick bites than males.

Adjusted analyses further showed that both recent and total tick bites were more common among participants with the highest educational level, increased outdoor activity and among hunters and owners of domestic animals. Daily smokers reported fewer total tick bites.

Among simple medical outcomes of tick bites, the most common occurrence was a rash around the bite, reported by 22.7% of the bitten respondents. Joint pain or swollen joints was reported by 2.0%, followed by headache (1.0%), fever (0.5%) and palsy in the face or elsewhere (0.5%). A total of 12.7% of the bitten persons reported to have seen a doctor because of tick bite or consequence thereof, and 7.7% had received antibiotics. As most responders reported many bites, these percentages refer to the life-time risk of ever having experienced the symptoms after a tick bite, and do not reflect the risk after each single bite.

Avoidance of certain areas because of concern for tick bites was reported by 15.7% of 1,194 respondents in this study, 22.2% of women and 10.3% of men, respectively ($p<0.001$). There was no significant age difference.

## 4.2 Paper II

Using the laboratory's routine method, Enzygnost IgG and IgM, 117 (9.6%) of the 1,213 sera were positive for IgG and 99 (8.2%) for 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 further data refer to the results obtained by the Enzygnost ELISA.

The relationship between tick bites and seropositivity to Bbsl showed a close correlation both for IgG and IgM. Among the 409 subjects reporting not to have experienced any tick bite, 20 (4.9%) were positive for IgG and 22 (5.4%) were positive for IgM. There was a close relation between the number of tick bites and seropositivity in men. This relationship was less obvious in women.

Amongst the blood banks, there were fewer IgG positives in Lærdal ($p = 0.021$), and there were more C6 positives in Førde ($p = 0.004$).

There was a positive association of IgG-seropositivity with age, and more males than females were positive for IgG (13.0% and 5.5%, respectively). There was a delayed age-related rise in seroprevalence in women compared to men.

Cat or dog owners had a significantly lower seropositivity rate for IgG. There were 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 was 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 – -0.021).

Comparing the quantitative results (% of cut-off value), 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 results stronger than 260% of the cut-off value, corresponding to 36 Units/ml as categorised by the manufacturer, were also positive in C6, and conversely, all C6 results stronger than 465% of the cut-off were positive for IgG. Similarly, we found that IgG reactions stronger than 252% of the cut-off (34 U/ml) were positive in IgG blot, and for C6 EIA, the corresponding limit was 504%.

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 IgG 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 IgG blot, while among the 27 C6 negatives, only 2 (7.4%) were positive.

IgM only was seen in 55 subjects (4.5%), whereas IgM concomitant with IgG was seen in 44 subjects (3.6%). Of these, immunoblot for IgM was positive for 31 (56.4%) and 28 (63.6%), 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).

## 4.3 Paper III

### 4.3.1 Anaplasma phagocytophilum

A random selection of 301 blood donors were analysed for antibodies to *A. phagocytophilum*. Of these, 49 (16.2%) were positive with titer ≥80 (range 80-1280).

We found no association between seropositivity and gender, age, geography (location of blood bank), self-reported number of tick bites or presence of antibodies to Bbsl. In this subgroup, 192 (63.8%) reported to ever having been bitten by a tick. Among these, 23 (12.0%) had IgG antibodies to Bbsl, 32 (16.7%) to *A. phagocytophilum*, and 6 (3.1%) to both agents. The latter group was overrepresented among the 23 persons reporting to ever having consulted a doctor after a tick bite (p=0.024), and among the 12 persons having received antibiotic treatment after a tick bite (p=0.047, Fisher's exact test).

### 4.3.2 TBEV

Among the 1,213 sera tested, five (0.4%) gave positive and one (0.1%) gave grey-zone results in the ELISA. Among these six subjects, five reported having received vaccines that might give positive reactions in the TBE ELISA; two had received vaccines against yellow fever, one against Japanese encephalitis (in addition to yellow fever), and three against TBE. A positive serum from one subject denying having received any of these vaccines, and also denying symptoms of TBE, was further examined by neutralising antibodies to TBEV, with negative result.

## 4.4 Paper IV

In this study, there were three variables designated as risk factors; tick bites ever experienced, *Borrelia* IgG and *Borrelia* IgM, and three outcomes; general function, physical fitness and 29 different subjective health complaints.

The proportion reporting reduced general function did not differ significantly between subjects with and without the risk factors. The number of tick bites was positively associated with good physical fitness (adjusted p for trend < 0.001).

For the SHC-questions, we calculated the total number of complaints reported by each participant. In addition, a total SHC score was computed as the sum of the severity scores of all 29 items in the questionnaire. The proportion of subjects reporting any complaint within five subscales are reported as well; these included "musculoskeletal" complaints (headache, neck pain, shoulder pain, pain in arms, pain in upper back, low

back pain, leg pain, and pain in the feet during exercise), “pseudoneurological” complaints (extra heartbeats, heat flushes, sleep problems, tiredness, dizziness, anxiety and sadness/depression), “gastrointestinal” complaints (heartburn, stomach discomfort, ulcer/non-ulcer dyspepsia, stomach pain, bloating, diarrhoea and constipation), “allergic” complaints (asthma, breathing difficulties, eczema, allergies and chest pain) and “flu” (cold/flu and cough).

There were no significant associations between the sum score of “musculoskeletal” complaints or the individual related complaints with any of the risk factors.

For the sum score of “pseudoneurological” complaints, there were fewer complaints in the subjects with *Borrelia* IgG than in those without these antibodies (adjusted p = 0.010). None of the individual complaints in this group of questions were significantly associated with any of the risk factors; still, for all questions there were fewer in the group with IgG that reported symptoms than among the IgG negatives.

In the group “gastrointestinal” complaints, some more subjects with more than one tick bite reported ulcer- and non-ulcer dyspepsia , and there was a trend towards more IgM positives reporting constipation, but these did not reach statistical significance (adjusted p = 0.079 and 0.081, respectively).

There were no significant associations between the sum score of “allergy” or the individual related complaints with any of the risk factors.

The “flu”-related questions all showed a higher percentage of IgM positives reporting symptoms. These associations were weakened when adjusted for gender, age-group and blood bank location.

We found no significant associations between the total number of subjective health complaints or the total SHC score and any of the risk factors. There was a negative association between positive IgG and number of complaints (p = 0.031), that no longer was significant when tested separately for men (p = 0.575) and women (p = 0.140), as women as a group had more complaints, whereas more men had positive IgG.

# 5 General discussion

The topic of this thesis is tick-borne infections in the county of Sogn og Fjordane as manifested in a group of healthy adults, represented by blood donors. Methods employed included a questionnaire, different laboratory methods to detect antibodies to Bbsl, *A. phagocytophilum* and TBEV, and statistical tools. The results included data on tick bites, on laboratory results, and subjective health complaints.

## 5.1 Methodological considerations

### 5.1.1 Blood donors as representatives of the general population

Blood donors are not completely representative of the general population. They are healthy, and children and persons over 70 years are not represented. As seen in Table 7, there was a fair distribution regarding gender and age-groups amongst the blood donors. The area surrounding the innermost part of the Sognefjord was somewhat underrepresented, as were some municipalities distant from the blood banks. According to Norwegian blood bank regulations, persons that have been bitten by ticks should not donate blood within four weeks of the bite, and persons with suspected or verified Lyme borreliosis should not donate until six months after adequate treatment has been given. Donors with recent tick bites and/or Lyme borreliosis may therefore be underrepresented. Nevertheless, blood donors are widely used in medical literature because of the practicalities of obtaining blood specimens, informed consent, etc. Furthermore, scientific readers are aware of the shortcomings of blood donors as representatives of the general population.

### 5.1.2 The county of Sogn og Fjordane as representative of the country of Norway

Both the densitiy of the tick *I. ricinus* (Tambs-Lyche, 1943; Mehl, 1983; Jore et al., 2011), the number of borreliosis cases notified to MSIS (Table 6) as well as seroprevalence in blood donors in Norway – with 9.6% seropositivity for *Borrelia* IgG in the present study, as opposed to 18 % in the Agder counties (Mygland et al., 2006), 9.3% in Vestfold, and 0.5% in the three northern counties (Dag Hvidsten, personal communication 2016) – indicate that Sogn og Fjordane is representative of coastal

areas in southern Norway when it comes to ticks and borreliosis, with a prevalence between the southernmost counties and those further north. Also, the data are comparable to those from many areas in Sweden (Tjernberg et al., 2007; Dessau et al., 2011).

*Anaplasma phagocytophilum* is probably present wherever *I. ricinus* is endemic. There is reason to believe that the situation in Sogn og Fjordane is equivalent to other tick-endemic areas in Norway and Europe (Stuen and Bergström, 2008; Rosef et al., 2009a).

The epidemiology of TBE is, however, different from that of borreliosis, and our findings, together with data from MSIS, suggest that human TBE is not endemic in our county.

### 5.1.3 Questionnaire

The usefulness of questionnaires depends on their reliability and validity. Reliability (or consistency) refers to the stability of a measurement scale, i.e. to what extent it will give the same results on separate occasions, whereas validity is the degree to which a scale measures what it is intended to measure (Bannigan and Watson, 2009).

The first part of the questionnaire used in the present investigations, i.e. on demographics, should be without risk of major systematic errors. The questions on number of tick bites ever experienced and experienced during the latest 12 months is, however, subject to different sources of error. Firstly, whether or not everybody has the same notion of a tick bite is not self-evident. We cannot exclude the possibility that participants report ticks on the body that in fact did not bite, nor can we exclude the possibility that some individuals fail to recognise a tick. Adult ticks are large enough to be noticed, but they may have bitten in areas of the body not readily available for inspection, e.g. in hairy areas, or on the back, etc. The nymphs are very small, and may easily be overlooked. Thus, in several reports on clinically verified tick-borne infections, only a portion of the patients were able to recall a recent tick bite (Berglund et al., 1995; Strle et al., 1999) Thirdly, recollection error must be supposed to be present when asking an adult person on the number of tick bites ever

experienced. If we compare the number of tick bites reported during the last year and the number of tick bites ever experienced by the same person, we see that these number do not correspond (paper II). In our adult population, the estimated mean number of 1.2 tick bites the latest 12 months does not correspond to the mean estimated number of tick bites ever experienced, which was 5.7.

Also, the awareness of tick bites may vary between persons. Especially, our results may indicate that men fail to recognise and/or remember tick bites to a greater extent than women, as will be discussed later.

The questions on physical fitness and reduced general function are taken from other, validated questionnaires (Weel et al., 1995; Reiso et al., 2000).

The questions on the 29 different subjective health complaints, the SHC inventory, have shown good validity and reliability in other studies (Eriksen et al., 1999; Ihlebaek et al., 2002; Filipkowski et al., 2010).

Nevertheless, responses to several of the questions are based on the respondents' subjective estimations, and the reliability of data must therefore be interpreted with caution.

### 5.1.4 Statistics

Most of the data in this thesis are either categorical or ordinal. To make the most of the ordinal results, statistical methods for ordinal data were used wherever possible (Paper I, II and IV). Proportional odds models are logistic regression models with more than two ordinal categorical outcomes (Armstrong et al., 1989; Scott et al., 1997). By using this method in paper I we avoided dichotomising the outcome variable, number of tick bites, which possibly would have led to loss of information and decreased statistical power. The method, however, assumes that the odds ratio for a risk factor is homogenous for all possible cut-off points of the outcome variable (i.e. the proportional odds property). Given the correctness of this homogeneity presupposition, the odds ratio can be viewed as a constant and interpreted as the odds of being higher or lower on the outcome variable across the entire range of the variable. For some data sets with very few participants scoring in some categories,

this premise could not be substantiated, and the outcome data had to be dichotomised, e.g. for the SHC parameters in paper IV.

### 5.1.5 Laboratory methods

#### *5.1.5.1 Borrelia antibodies (paper II and IV)*

**General remarks**
Although serological testing is the primary tool for diagnosing Lyme borreliosis, several issues regarding sensitivity, specificity and predictive values are challenging. A recent meta-analysis of the diagnostic accuracy of serological tests for Lyme borreliosis in Europe by Leeflang and co-workers found that sensitivity was highly heterogeneous and varying with the clinical condition. Specificity was around 95 % in studies with healthy controls, but around 80 % in cross-sectional studies (Leeflang et al., 2016). This may be owing to the fact that different commercial EIA assays show differences in results for the detection of IgG and IgM-antibodies to Bbsl (Ang et al., 2015).

**Sensitivity**
Sensitivity is the proportion of patients with a defined disease that are positive in a test for that disease. The sensitivity of antibody testing for Lyme borreliosis is rather low in early disease, and increases in later phases. Not all cases of erythema migrans give rise to a measurable serologic response, and the development of antibodies may also be halted by early antibiotic treatment. On the other hand, almost all cases of longstanding infection, e.g. arhtritis and acrodermatititis chronica atrophicans (ACA) have positive IgG (Schriefer, 2015).

Though IgG antibodies may be present for many years after an infection, many patients will lose their IgG antibodies to Bbsl after some time. A seroprevalence study in healthy people may therefore underestimate the number of persons that have endured an infection.

Traditionally, for most agents, IgM antibodies are indicative of actual infection, and this is partly true also for Bbsl. Measuring IgM antibodies to Bbsl may be helpful in early stages of infection, especially in children, and combining IgG and IgM analysis

is therefore widely used to increase the sensitivity in early Lyme borreliosis, but is of little relevance in a seroprevalence study.

**Specificity**

*Specificity in general*

Specificity is defined as the proportion of individuals without a disease that are negative in a test for that disease. Thus, false positive reactions in a test lower the specificity. Since all donors in the present investigations were healthy, all positive tests should be regarded as false positives. There are a number of reasons for false positive test results.

Some of the antigenic properties of molecules in *B. burgdorferi* are quite similar to antigenic structures in other spirochetes, and this may give rise to false positive diagnostic tests for *Borrelia* owing to crossreacting antibodies, e.g. towards the syphilis spirochete *Treponema pallidum* and to apathogenic spirochetes that are part of the normal human microflora (Magnarelli et al., 1990). The first generation EIAs for Bbsl were prone to such cross reactions, but later test generations show higher specificities in this regard (Schriefer, 2015).

A variety of infectious agents may also trigger polyclonal activation of B-cells into producing antibodies to a variety of microbes other than the stimulating agent. Known inducers of such polyclonal stimulation include Epstein-Barr virus, cytomegalovirus, parvovirus B19, and *Mycoplasma pneumoniae* (Montes et al., 2007; Landry, 2016).

Some antibodies may also interact directly or indirectly with components in the test itself. One example is rheumatoid factor (RF), typically IgM antibodies directed against the Fc portion of IgG antibodies. RF binds to IgG that has bound its antigen or is present in immune complexes, but is not reactive with soluble IgG. If a serum that contains both RF and anti-*Borrelia* IgG is allowed to react with the solid phase of the EIA test, the *Borrelia*-specific IgG will bind to the antigens. Thereafter, the RF will bind to the IgG. If the test utilises an anti-IgM conjugate antibody to detect IgM, the conjugate will bind the RF and thus give a false positive signal. Therefore, in many EIA tests, steps are taken to avoid this interference. E.g. in the Enzygnost IgM assay

used in this study, the patient serum is pretreated with antibodies from sheep against human IgG to generate IgG-immune-complexes. This allows RF to bind to the complexes, and RF is thus neutralised and made non-reactive in the ELISA. Also heterophilic antibodies in the patient's serum – that is antibodies directed against immunoglobulins from other animal species – may interfere with the conjugate antibody of animal origin, see Figure 12.

As both *Borrelia* IgG and IgM antibodies may be detected for a long time after infection with Bbsl (Stanek et al., 2012), these antibodies should also be regarded as a source of "false positive" reactions in specificity measures.

*Specificity problems in tests for Borrelia IgM*

A positive IgM to Bbsl may indicate an early phase of Lyme borreliosis. On the other hand, false positive IgM reactions without relation to Bbsl infection are well known. The clinical significance of IgM-antibodies detected in the absence of IgG-antibodies in patients suspected of Lyme borreliosis thus represents a continuous dilemma (Ulvestad et al., 2001; Panelius et al., 2002).

In this study (Paper II), we chose to include measurement of IgM antibodies mostly to assess how common they are in healthy adults, not to use them as indicators of current or earlier infection.

IgM only was seen in 4.5% of these healthy subjects, whereas IgM concomitant with IgG was seen in 3.6%. More than half of both categories were positive in immunoblot for IgM.

As IgM alone, compared to IgM accompanying IgG, was seen more often in younger women, and was unrelated to geography and number of tick bites, we suspect that these IgM were false positives unrelated to actual or earlier Lyme borreliosis, and that they probably were due to cross-reacting antibodies or polyclonal IgM stimulation. The time of year for obtaining our specimens (January – June) also points to the same conclusion, as this is a period with a relatively low incidence of Lyme borreliosis, but with a high incidence of other infections.

Repeat specimens to look for the development of specific IgG is often suggested by the laboratory to resolve the dilemma of isolated *Borrelia* IgM. In the absence of development of specific IgG during e.g. 6 weeks, an IgM only is generally regarded as of little significance. American guidelines thus argue against using IgM blot in the second-tier testing when disease duration is longer than one month (Centers for Disease Control and Prevention, 1995). Our findings support this view.

*Increasing specificity*

Specificity may be increased by selecting a test giving fewer false positive results, or by adding a second test in cases of positivity in the first, the so-called two-tiered test algorithm. Western blot and immunoblot are traditionally recommended as this second test, but using another EIA than the one used for screening may be an alternative.

In paper II, we presented both the ELISA result as well as immunoblot results of the ELISA positive specimens. We did this to facilitate comparison with other Scandinavian laboratories, where immunoblot is seldom used, as well as for comparisons with studies presenting results after two-tier testing. In paper IV, we used the immunoblot verified results as the risk factors for subjective health complaints, in order to avoid the use of unspecific results.

The C6 ELISA has been reported to be more specific than EIAs with other antigens. Wormser et al. (2013) 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. 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 (Marangoni et al., 2005). High seropositivity rates in blood donors for C6 antibodies are also found in some Scandinavian laboratories, where 16% and 8% have been reported (Tjernberg et al., 2007; Dessau et al., 2011). In this study, we found a seroprevalence of 8.4% in the C6 assay, thus 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 the two assays, 63.2% and 68.6%, respectively.

The main difference seems to be that the C6 ELISA did not detect the isolated IgM's found in the Enzygnost assay, even though the C6 assay detects IgM as well as IgG antibodies.

It has been suggested that C6 antibodies tend to normalise after the clinical infection has resolved (Philipp et al., 2003; Philipp et al., 2005; Tjernberg et al., 2007), but others dispute this (Peltomaa et al., 2003; Fleming et al., 2004). Our findings of a relatively high prevalence of C6 antibodies in this healthy population support the latter view.

Immunoblots increase the specificity, but lower the sensitivity somewhat in early phases of infection (Aguero-Rosenfeld and Wormser, 2015). It is recognised that also immunoblot for IgM is prone to false positive reactions (Goossens et al., 1999; Seriburi et al., 2012). E.g. antibodies not specific for Bbsl may bind to the OspC antigen in the strip (Probst et al., 2012). The company Euroimmun AG has recently released a new immunoblot test, the "EUROLINE-RN-AT-adv" which includes a new formulation of the OspC antigen, and this test has been claimed to be 30% more specific than the one used in this study, the "EUROLINE-RN-AT".

**Comparing tests**

In 89 patients, among them 59 with suspected Lyme borreliosis, Ang et al. (Ang et al., 2011) found a kappa value of 0.86 when comparing the Enzygnost IgG and/or IgM with C6 ELISA. 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 these discrepancies are unknown, but may be related to differences in groups of individuals tested, as our study only included asymptomatic, healthy individuals.

In clinical practice, the magnitude of optical density values in ELISA tests are often used as a quantitative interpretation of results, in addition to the categorisations into positive versus negative. The rationale for this is partly that specificity is thought to be higher for strongly reactive specimens than for weakly reactive. In Figure 15, we

see that the concordance of positivity for Enzygnost IgG and C6 was good at strong reactions in both assays, and that there were more discrepancies between the two tests in the lower ranges of reactivity. These results indicate that strong IgG responses in Enzygnost do not have to be verified by C6 or blot, as one can presume that they are positive. Ang et al. (2011) 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.

Among sera with a positive Enzygnost IgG weaker than 300% of the cut-off, one third were positive also in C6. Most of these were positive in IgG blot, while among the C6 negatives, only a few were positive. Using C6 as a second-tier test in weak positive IgG thus might be an alternative to blot.

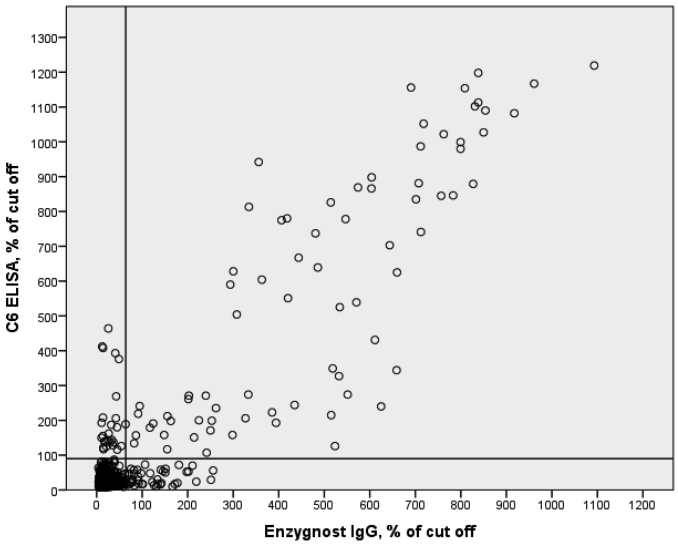


**Figure 15. Relationship between quantitative results of *Borrelia* antibodies in Enzygnost IgG and C6 ELISA (n = 1,213)**

The X-axis represents Enzygnost IgG reactivity, while the Y-axis represents C6 reactivity, both in percentage of cut-off. The vertical and horizontal lines mark the boundary between negative and grey-zone results for the two tests, at 64% and 90% of the cut-off value, respectively. Note the linear scale of both axes, in paper II the same figure is presented with logarithmic scales.

**Positive and negative predictive values**

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.

The positive predictive value (PPV) is defined as the probability that a positive test result reflects that a patient suffers from the disease in question. In addition to the test characteristics of sensitivity and specificity, the PPV also takes into consideration the prevalence of the disease in the patient group (Altman and Bland, 1994).

$$\mathrm{PPV} = \frac{\mathrm{sensitivity} \times \mathrm{prevalence}}{\mathrm{sensitivity} \times \mathrm{prevalence} + (1 - \mathrm{specificity}) \times (1 - \mathrm{prevalence})}$$

Vice versa; the negative predictive value (NPV) is the probability that a negative test result reflects that the patient does not suffer from the disease.

$$\mathrm{NPV} = \frac{\mathrm{specificity} \times (1 - \mathrm{prevalence})}{(1 - \mathrm{sensitivity}) \times \mathrm{prevalence} + \mathrm{specificity} \times (1 - \mathrm{prevalence})}$$

For a specific patient, the prevalence in these equations may be exchanged by the pre-test probability, i.e. the chance that the history and clinical picture at hand is caused by the actual disease. This pre-test probability will be high when there is a typical history of preceding tick bite and typical LB clinical manifestations. On the other hand, if the history and clinical picture is less typical, the pre-test probability is lower.

As depicted in Figure 17 (page 90), the seroprevalence was highest in the older age-groups in this healthy population. The test for IgG thus has a lower specificity in these groups, reducing its positive predictive value for elderly patients in our geographical area. The evaluation of the pre-test probability is therefore of utmost importance when applying the test in a clinical situation. E.g., the clinical information “chronic fatigue” and “musculoskeletal symptoms”, relatively common conditions in elderly and comparatively seldom caused by *Borrelia*, are frequently stated reasons for testing in our laboratory. Combined with low specificity, the resulting predictive value of the

test is very low. Thus, Dessau et al. (2010) in Denmark found that among sera submitted from persons with suspected Lyme arthritis, a relatively rare disease in Scandinavia, the rate of seropositivity did not exceed the background prevalence for Danish blood donors.

**Consequences for diagnostic algorithms in the laboratory**

A test algorithm that reduces 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 data is not possible. Nevertheless, our data do suggest that a two-tiered test strategy using only Enzygnost IgG or C6 as screening could eliminate the problem of probably 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. Immunoblot 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. 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.

**Conclusion – *Borrelia* antibodies**

Enzygnost IgG and C6 ELISA were comparable in this group of blood donors, and specimens strongly reactive in Enzygnost 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.

### *5.1.5.2 Antibodies to Anaplasma phagocytophilum (paper III)*

For epidemiological purposes like in this study, a cut-off in the IFA test of 1:64 or 1:80 is widely used. However, as discussed by several authors (Aguero-Rosenfeld et al., 2002; Walder et al., 2003), this may be set too low, as a significant proportion of adults and children without clinical evidence of human granulocytic anaplasmosis

(HGA) will test positive for *A. phagocytophilum* antibodies when these cut-offs are used. Thus, Walder and co-workers in Austria chose a cut-off of 1:128, the 98$^{th}$ percentile of a control population with low likelihood of having had HGA (Walder et al., 2003). In this study (paper III), we have therefore listed the titers of the positive reactions, in order to assess the seroprevalence critically.

#### *5.1.5.3 Antibodies to TBEV (paper III)*

For testing immunity after TBEV infection or after vaccination, most often the IgG ELISA is used. Due to the interference of flavivirus cross-reactive antibodies in ELISA (e.g. following vaccinations against yellow fever or Japanese encephalitis, or upon dengue virus infections), the performance of a neutralisation assay is necessary for assessing positive tests (Holzmann, 2003). Therefore, questions on vaccination data for TBE vaccine and other potentially cross-reacting vaccines were included in the questionnaire in this study, and this explained five of the six non-negative reactions in this cohort. The last specimen was negative in a neutralisation assay.

## 5.2 Epidemiological results– tick bites and *Borrelia* antibodies (paper I and II)

### 5.2.1 The relationship between tick bites and seropositivity for *Borrelia burgdorferi*

As expected, we found a close correlation between the number of tick bites and the seropositivity rate for both IgG (Figure 18) and IgM. The seropositivity rate was lower in women, and the relationship to the self-reported number of tick bites was less obvious in women than in men. This discrepancy will be discussed further below.

### 5.2.2 Geography (paper I and II)

#### *5.2.2.1 Europe and USA*

Comparing our results with studies from other countries, we found that a lower percentage of the population has experienced tick bites than in Åland (Wahlberg, 1990), 66% as contrasted to 85% . On the other hand, a survey in Alsace, France, found that only 16.5% of the general population had experienced one or more tick bites during the last year (Mitschler et al., 2004), which is lower than reported in our

study (30%). Also Leiby and co-workers' findings from Connecticut and Wisconsin indicated a lower frequency of tick bites in blood donors than we found (Leiby et al., 2002). Explanations for these differences may include variations in tick abundance, general awareness of ticks, and outdoor and recreational habits in the respective populations.

Studies from the Netherlands and Belgium showed an uneven geographical distribution of tick bites (de Mik et al., 1997; den Boon et al., 2004; Vanthomme et al., 2012). Difference in test populations (patients in general practice versus blood donors) precludes any direct comparison with our findings, other than stating that local geographical differences are important risk factors for tick bites.

#### *5.2.2.2 Norway and Sogn og Fjordane county*

A recent serological survey of *Borrelia* antibodies in 9 Norwegian counties found a seroprevalence of *Borrelia* IgG of 4.0% (95% CI: 2.4–6.6%) when using the Enzygnost Lyme link vlsE test on sera submitted for clinical chemistry (Vestrheim et al., 2016). The seroprevalence varied by geography and increased by age. For the 120 sera from Sogn og Fjordane the same study found that 3 (2.5%) were positive, giving an estimated seroprevalence of 3.6% (CI 2.1-5.9). This prevalence is lower than the 9.6% found in paper II, and Vestrheim et al. (2016) speculate that 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.

In accordance with current estimates of tick distribution as well as the variation in notified cases of systemic borreliosis within the county, blood donors from the easternmost blood bank of Lærdal reported a significantly lower number of tick bites than those from the other blood banks. Although entirely based on their subjective estimate, the respondents from this blood bank also reported a correspondingly low occurrence of ticks in their living area. As expected, we also found the seroprevalence of IgG antibodies to Bbsl to be lower in these donors.

The difference in IgM was, however, not significant (p = 0.268) between the different locations, and for isolated IgM especially, there was no difference whatsoever (p = 1.000). Why the donors from the blood bank in Førde had more positive reactions in C6 than the others is difficult to explain, as Nordfjordeid had the highest seropositivity rate for IgG.

### 5.2.3 Age and gender (paper I and II)

As expected, we found a significant age related trend in reported total tick bites ever experienced. The somewhat lower reported number in the oldest group from 60-69 years may be related to sampling variation, a cohort effect in the oldest group reflecting a change in outdoor habits in the general population, or an increased density of ticks during the last decades. The same age trend was also present for recent tick bites (paper I). There was also a positive correlation between seroprevalence of IgG and age, see Figure 17 (Paper II).

For IgG, we found a significant difference in gender; men had a higher seroprevalence than women. This is not fully in accordance with self-reported tick bites, where no statistically significant difference between the genders across all age-groups was found. 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. The findings by Wilhelmsson and co-workers (2013), that the size of removed ticks was bigger in men than women, indicating that the tick had been feeding for a longer time, may support this view. Male dominance regarding *Borrelia* IgG antibodies has also been reported by others (Dehnert et al., 2012), but not universally (Hubalek, 2009) (paper II).

Looking at age and gender combined, we note that the seroprevalence in women rises at an older age than in men, see Figure 17 (Paper II). This may be in accordance with the age distribution of reported tick bites, as young men reported more bites than young women, while this was reversed in subjects older than 50 years of age (paper I). 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. Also, women reported more tick bites before seroconversion, see Figure 18.

In accordance with our findings, a number of studies have shown that women past the age of 40-50 years display an idiosyncratic *Borrelia* epidemiology. Bennett and co-workers (2007) found that women aged 40 years or older had a higher risk than men in the same age-group for contracting tick bites, and also higher than younger women. A similar tendency is also apparent in our study for women 50 years or older (Figure 16). A plausible hypothesis for the increased risk in middle-aged women could be the traditional but now declining custom of picking wild berries (e. g. blueberries, cloudberries, lingonberries) for the household, that shows a similar distribution in age and gender (Vaage, 2004). Unfortunately, questions on berry picking were not included in the questionnaire. Other hypotheses could be related to other behavioural or biological aspects of postmenopausal women, as discussed by Bennett and co-workers (2007).

Several studies have found gender differences in clinical manifestations of Lyme borreliosis, and there is some evidence that immunologic differences between men and women may be relevant in this disease (Jarefors et al., 2006; Bennet et al., 2007; Strle et al., 2013). Also, our finding of more isolated, probably nonspecific, *Borrelia* IgM antibodies in younger women (paper II), indicates differences in immunological characteristics.

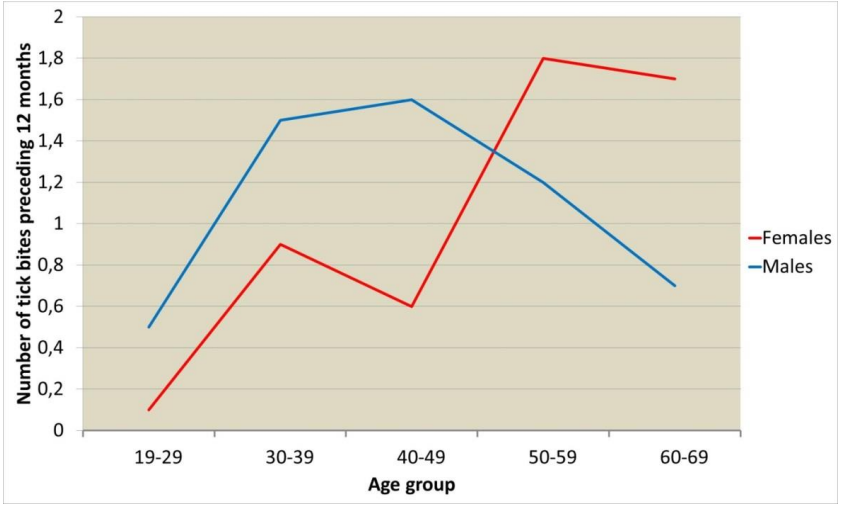


**Figure 16. Estimated number tick bites the preceding 12 months according to age and gender (n = 1,171)**

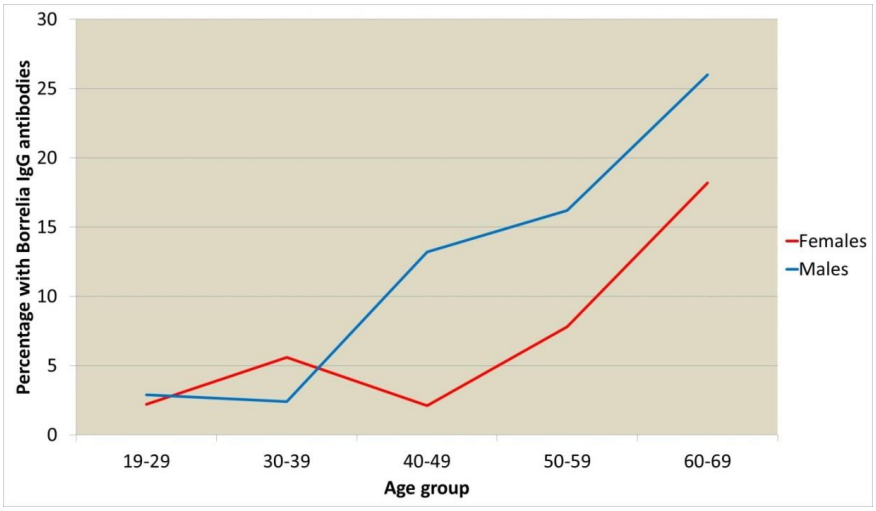


**Figure 17. Prevalence of *Borrelia* IgG (Enzygnost) according to gender and age group (n = 1,183)**

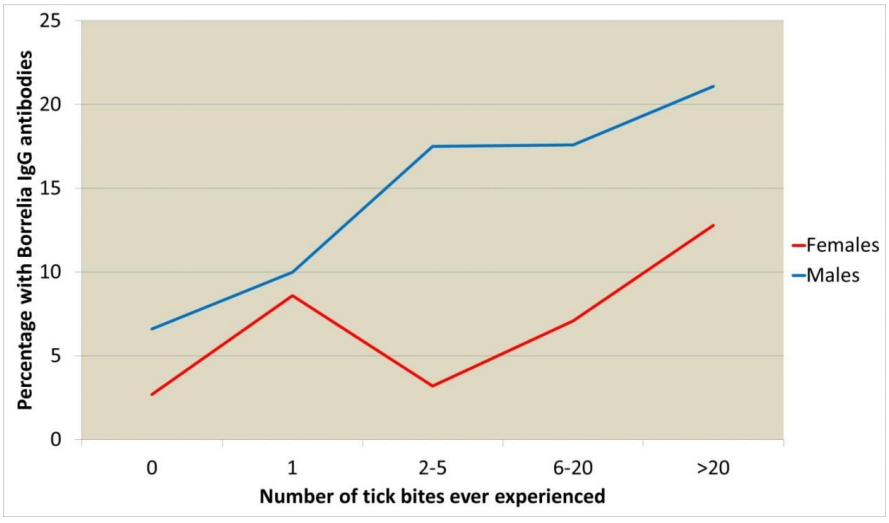


**Figure 18. Occurrence of *Borrelia* IgG-antibodies in relation to reported number of tick bites ever experienced (n = 1,171)**

### 5.2.4 Lifestyle factors (paper I and II)

We found that tick bites were more common among participants with the highest educational level (paper I). This is in accordance with observed life style differences in other studies (Vaage, 2004). We found no relation between risk of tick bites and household yearly gross income.

After adjustment for gender, age and blood bank, daily smoking seemed to protect against tick bites ($p = 0.007$), but after statistical correction also for outdoor hours per week, this association was no longer significant ($p = 0.017$) (paper I) according to the significance level chosen in this study.

As expected, the number of outdoor hours per week during summertime showed a significant relationship to the risk of tick bites. The estimated mean number of tick bites the preceding year increased from 0.5 in those spending less than 5 hours outdoors per week, to 1.7 in subjects spending more than 10 hours. This is in accordance with Stjernberg and Berglund's findings of a 4% risk of being tick-bitten per 10 hours spent outdoors in an endemic area in Sweden (Stjernberg and Berglund, 2002) (paper I).

Outdoor activities are in general regarded as positive for public health and quality of life, and it would be a concern if the population chose to avoid certain recreational outdoor areas in fear of tick bites. In our study, 15.7% reported such avoidance. Whether this is a sensible approach is difficult to ascertain. The marked gender difference, with avoidance-behaviour being twice as common in women as compared to men, is not easy to explain. It may reflect a general gender difference in risk avoidance (paper I).

The number of total and recent tick bites in this study population increased also with educational level, ownership of domestic animals, and hunting (paper I). 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 (paper II). Interestingly, we found a lower seroprevalence in owners of pet animals (cats or dogs), although this group reported some more tick bites. This

is in contrast to the findings of Dehnert et al. (2012), who in Germany found a higher seroprevalence in children and adolescents from households with cats.

Hunting, especially for deer, is a common leisure activity in Sogn og Fjordane, and 19.7% of the respondents had been hunting during the preceding 12 months. We found an increased risk of tick bites in this group, even after adjustment for "hours spent outdoors per week in summertime". This question does not discriminate between tick-infested and non-infested outdoor areas, and there is reason to believe that the increased risk in hunters reflects their activity in tick habitats.

Among our respondents, 65 (5.5%) reported to have been active orienteers. A Swiss study published in 1991 found that 78% of 995 orienteers had experienced at least one tick bite (Fahrer et al., 1991). In our material, that was the case for 92.3%. A Swedish study found that 53% of Swedish orienteers were bitten by ticks in one year (Gustafson et al., 1993), and we found a similar rate of 53.8%. We found that orienteers reported more tick bites than non-orienteers, but this association was not statistically significant at the chosen significance level of 0.01, possibly due to small numbers.

### 5.2.5 Disease (paper I, II and III)

This study was on healthy blood donors, and little emphasis has been given to disease. Some simple questions on symptoms after tick bites were included in the questionnaire, and these scores have been correlated to data on tick bites and laboratory results.

Several reports have investigated the risk for developing disease after a single tick bite, and most of them find the risk to be low (Shapiro et al., 1992; Maiwald et al., 1998; Nadelman et al., 2001; Stjernberg and Berglund, 2002; Nahimana et al., 2004; Jacobs et al., 2008; Fryland et al., 2011).

Wahlberg (Wahlberg, 1990) found that out of 441 bitten subjects, 14 reported ever having had erythema chronicum migrans and 73 had had other rashes around the tick bite, all together 19.7%. In our material, we found a similar frequency of "rash" of

22.7%. We did not try to discern between erythema migrans and other rashes, as we believe that to be an error-prone task in a retrospective questionnaire survey.

The overrepresentation of subjects with IgG antibodies both to *A. phagocytophilum* and Bbsl among those having seen a doctor after a tick bite and among those having received antibiotic treatment, is an interesting observation. Because of low numbers, this result should be interpreted with caution.

## 5.3 *Anaplasma phagocytophilum* (paper III)

Uncertainty of the proper cut-off as well as the possibility of serological cross-reactions may complicate the judgement of IFA results.

Surveys of antibodies to *A. phagocytophilum* among blood donors using the IFA have found seroprevalences of 11.3% (18/159) among *Borrelia* blot negative donors in Westchester county, New York, using a cut-off of ≥80, 0.5% (5/992) in Wisconsin and 3.5% (35/992) in Connecticut, USA (cut-off ≥64) (Leiby et al., 2002), and 9.0% (32/357) in Tyrol, Austria (cut-off ≥128) (Walder et al., 2003). In Denmark, Skarphedinsson and co-workers (2001) found that 2 out of 100 blood donors from Odense, Denmark and 5 out of 100 blood donors from Iceland were positive (cut-off ≥64).

In a survey of blood samples from patients with physician-diagnosed Lyme borreliosis in the county of Telemark in southern Norway, 10.2% (6/58) were positive in IFA (≥80, range 1:80 – 1:160) (Bakken et al., 1996). Dumler and co-workers (1997) found that 11.4% (21/185) of inhabitants at the island of Koster at the western coast of Sweden were positive (≥80), whereas Wittesjö and co-workers (2001) found a seroprevalence of up to 28% in inhabitants in Aspö island at the Baltic sea coast of Sweden. In Denmark, 21.0% (63/300) of sera from patients clinically suspected of having Lyme borreliosis were positive for antibodies to *A. phagocytophilum* (Skarphedinsson et al., 2001).

Compared to these studies, the seroprevalence of 16.2 % in our material was relatively high. As discussed above, this may represent an over-estimate, but the selected cut-off

allows for the comparison. To my knowledge, no clinical cases of HGA have been diagnosed in Sogn og Fjordane.

In conclusion, this study indicates that *A. phagocytophilum* is a potential pathogen for humans in Sogn og Fjordane, and should be taken into consideration when dealing with suspected tick-borne disease with compatible clinical picture.

## 5.4 Tick-borne encephalitis (paper III)

Traavik and co-workers found evidence of tick-borne encephalitis-like virus in the western counties of Sogn og Fjordane and Hordaland in studies of animals and humans in the 1970s (Traavik, 1973; Traavik et al., 1978; Traavik, 1979). However, there is evidence that these findings represented other flaviviruses (Gao et al., 1993; Haglund, 2002). The serological methods used at the time, haemagglutination inhibition (HAI) and gel diffusion, were probably not specific for TBEV, and the positive results probably reflect infection with one or more related viruses. Traavik and co-workers reported a seropositivity rate for humans in HAI of 19.6%. In contrast, in the present study, we found no true positive non-TBE-vaccinated cases, as all but one could be explained by vaccines giving cross-reacting antibodies, the only exception being negative in neutralisation test. Thus, current ELISA tests seem to be more specific than former tests, and the study results give no evidence for the existence of TBE in humans in Sogn og Fjordane.

Our negative findings are in accordance with the known current distribution of TBE in Norway (Blystad et al., 2009). In contrast, Skarpaas and co-workers found that 3 out of 126 (2.4%) inhabitants of Tromøy in Aust-Agder county at the southernmost coastline of Norway were seropositive (Skarpaas et al., 2002). A study from Sweden found a seropositivity rate of 4-22% in non-immunised participants, depending on the area investigated (Gustafson, 1994). The same study reported that in 362 orienteers from the county of Stockholm, 1% of the individuals were seropositive. Among the 65 subjects reporting to ever having been orienteers in our material, none were positive.

## 5.5 Subjective health complaints in relation to tick bites and Borrelia antibodies (paper IV)

The main findings in this study was the lack of association between the risk factors tick bites and *Borrelia* antibodies with the outcomes subjective health complaints, reduced general function and reduced physical fitness. On the contrary, we found a positive association between tick bites and good physical fitness, and between presence of *Borrelia* IgG and low occurrence of "pseudoneurological" complaints. For most complaints, we found no association.

The risk factor "number of tick bites" was included in this study to explore the possibility that seronegative LB or other tick-borne infectious agents not tested for, e.g. *Rickettsia* spp., etc., could give rise to chronic health problems of some magnitude.

IgG and IgM antibodies to Bbsl verified by blot were chosen as risk factors in this study in order to minimise the risk of including false positive ELISA results.

In several studies of symptoms after LB, the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) has been used (Shadick et al., 1994; Shadick et al., 1999; Seltzer et al., 2000; Eikeland et al., 2011). In addition, several questionnaires for assessing somatic symptoms in the general population exist (Zijlema et al., 2013). We are not aware of other studies using the SHC Inventory in blood donors in relation to LB.

Compared to other surveys using the SHC Inventory in the general population, summarised by Eriksen et al. (1999), and for a working population more recently by Ihlebæk and co-workers (2007), the proportion of subjects with any complaint were lower in our material for most complaints, reflecting the overall healthy status of the blood donors.

Comparing our results to those of other studies is not straight forward, for two main reasons. Firstly, the selection of study population differs between studies, e.g. blood donors, subjects having been bitten by ticks, patients having suffered from LB,

patients having suffered from LNB, etc. Secondly, the choice of questionnaire also varies.

According to alternative views on LB, there are many undiagnosed patients with nonspecific chronic symptoms attributable to the disease (Cameron et al., 2004). Among the listed symptoms, some are included in the SHC inventory. The prevalence of these was not significantly higher for any of the risk factors tick bites, IgG or IgM in our study. A number of symptoms are not explicitly included in the SHC inventory. If these symptoms were of any significance in our study population, it should be reflected in the score on "reduced general function" in subjects with the risk factors. This was, however, not the case.

The clear correlation between the number of tick bites and physical fitness is not surprising. Persons more exposed to ticks are presumably more involved in outdoors activities such as hiking and hunting, and are in a generally good physical condition.

The negative association of "pseudoneurological" complaints and Bbsl IgG antibodies, but not number of tick bites or IgM, is difficult to explain. Also here, a spurious relationship connected to confounding lifestyle factors can be suspected.

The strength of this study is the relatively large representation of healthy subjects from Sogn og Fjordane county, the good response rate, and the frequent occurrence of tick bites and seropositivity to Bbsl, as well as the scope of the questionnaire, covering a broad range of health complaints. Thus, major chronic health effects of tick bites and seropositivity to Bbsl should have been detected.

It is to be expected that persons with significant chronic health problems do not volunteer as blood donors, or will be excluded. Persons of this category are therefore likely underrepresented in this material. Still, given the commonness of these "soft" symptoms also in blood donors, one would expect that mild degrees of such complaints should be represented.

# 6 Conclusions

A majority of blood donors in the western part of Norway had experienced tick bites. Age, gender, outdoor activities, animal contact and geography affected the risk. Symptoms of disease were seldom reported.

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. Enzygnost IgG and C6 ELISA were comparable in this group of blood donors, and specimens strongly reactive in either of these tests were all positive in immunoblot for IgG. False positive IgM results, including immunoblot positive, seem to be a challenge. The findings may help laboratories to develop prudent testing algorithms and in assessing predictive values of testing.

We found no evidence of tick-borne encephalitis as an endemic disease in the county of Sogn og Fjordane. There was, however, serological evidence for the existence of human granulocytic anaplasmosis, indicating that clinicians should be aware of this condition in the diagnostic considerations after tick bites in this geographic area.

There was no association between number of tick bites or seropositivity to *B. burgdorferi* s.l. and more subjective health complaints, reduced general function or reduced physical fitness.

# 7 Future perspectives

Many unsolved questions remain in the field of tick-borne infections, both world-wide and locally in Norway and Sogn og Fjordane.

In view of the findings in this thesis, a re-examination of the same blood donors for *Borrelia* antibodies after some years would be interesting, to elucidate the normal timely development of the antibody response, including probably false positive IgMs. Likewise, one would get an estimate of newly seroconverted persons.

Also, it would be of interest to examine the specimens for tick-borne agents not performed in this thesis; e.g. *Babesia* spp., *B. myiamotoi*, *N. mikurensis*, and *R. helvetica*.

Likewise, testing of selected samples for potentially cross-reacting antibodies, especially for *Borrelia* IgM positive samples, would be of interest, to learn more about this diagnostic caveat. This could be autoantibodies of different kinds, as well as antibodies to different microorganisms, e.g., *Mycoplasma* antibodies.

Testing the blood donors for other possible immunological factors, e.g. blood group antigens or HLA types, could possibly help elucidate differences in immunological response to *B. burgdorferi*.

As TBEV seems not yet to be established in Sogn og Fjordane county, our results may serve as a reference for later seroepidemiological studies some further years ahead, as climate changes well might pave the way for the introduction of TBEV in the local fauna.

# 8 References

Aase, A., Hajdusek, O., Øines, Ø., Quarsten, H., Wilhelmsson, P., Herstad, T.K., Kjelland, V., Sima, R., Jalovecka, M., Lindgren, P.E., Aaberge, I.S., 2016. Validate or falsify: Lessons learned from a microscopy method claimed to be useful for detecting Borrelia and Babesia organisms in human blood. Infect Dis (Lond) 48, 411-419.

Afzelius, A., 1910. Verhandlungen der dermatorischen Gesellschaft zu Stockholm. Arch Derm Syphiligr 101, 404.

Afzelius, A., 1921. Erythema chronicum migrans. Acta Derm Venereol 2, 120-125.

Aguero-Rosenfeld, M.E., Donnarumma, L., Zentmaier, L., Jacob, J., Frey, M., Noto, R., Carbonaro, C.A., Wormser, G.P., 2002. Seroprevalence of antibodies that react with Anaplasma phagocytophila, the agent of human granulocytic ehrlichiosis, in different populations in Westchester County, New York. J Clin Microbiol 40, 2612-2615.

Aguero-Rosenfeld, M.E., Wang, G., Schwartz, I., Wormser, G.P., 2005. Diagnosis of Lyme borreliosis. Clin Microbiol Rev 18, 484-509.

Aguero-Rosenfeld, M.E., Wormser, G.P., 2015. Lyme disease: diagnostic issues and controversies. Expert Rev Mol Diagn 15, 1-4.

Altman, D.G., Bland, J.M., 1994. Diagnostic tests 2: Predictive values. BMJ 309, 102.

Andersson, M., Raberg, L., 2011. Wild rodents and novel human pathogen candidatus Neoehrlichia mikurensis, Southern Sweden. Emerg Infect Dis 17, 1716-1718.

Andreassen, A., Jore, S., Cuber, P., Dudman, S., Tengs, T., Isaksen, K., Hygen, H., Viljugrein, H., Ånestad, G., Ottesen, P., Vainio, K., 2012. Prevalence of tick borne encephalitis virus in tick nymphs in relation to climatic factors on the southern coast of Norway. Parasites & Vectors 5, 177.

Ang, C., Notermans, D., Hommes, M., Simoons-Smit, A., Herremans, T., 2011. Large differences between test strategies for the detection of anti-Borrelia antibodies are revealed by comparing eight ELISAs and five immunoblots. Eur J Clin Microbiol Infect Dis 30, 1027-1032.

Ang, C.W., Brandenburg, A.H., van Burgel, N.D., Bijlmer, H.A., Herremans, T., Stelma, F., Lunel, F.V., van Dam, A.P., Dutch Working group on diagnosis of Lyme borreliosis, 2015. A Dutch nationwide evaluation of serological assays for detection of Borrelia antibodies in clinically well-defined patients. Diagn Microbiol Infect Dis 83, 222-228.

Armstrong, B.G., Sloan, M., 1989. Ordinal regression models for epidemiologic data. Am J Epidemiol 129, 191-204.

Bakken, J.S., 1998. The discovery of human granulocytotropic ehrlichiosis. J Lab Clin Med 132, 175-180.

Bakken, J.S., Krueth, J., Tilden, R.L., Dumler, J.S., Kristiansen, B.E., 1996. Serological evidence of human granulocytic ehrlichiosis in Norway. Eur J Clin Microbiol Infect Dis 15, 829-832.

Bannigan, K., Watson, R., 2009. Reliability and validity in a nutshell. J Clin Nurs 18, 3237-3243.

Bannwarth, A., 1941. Chronische lymphocytäre Meningitis, entzündliche Polyneuritis und "Rheumatismus". Ein beitrag zum Problem "Allergie und Nervensystem". Arch Psychiatr Nervenkr 113, 284-376.

Barbour, A.G., Tessier, S.L., Stoenner, H.G., 1982. Variable major proteins of Borrelia hermsii. J Exp Med 156, 1312-1324.

Bennet, L., Stjernberg, L., Berglund, J., 2007. Effect of gender on clinical and epidemiologic features of Lyme borreliosis. Vector Borne Zoonotic Dis 7, 34-41.

Berghoff, W., 2012. Chronic Lyme disease and co-infections: Differential diagnosis. Open Neurol J 6, 158-178.

Berglund, J., Eitrem, R., Ornstein, K., Lindberg, A., Ringer, A., Elmrud, H., Carlsson, M., Runehagen, A., Svanborg, C., Norrby, R., 1995. An epidemiologic study of Lyme disease in southern Sweden. N Engl J Med 333, 1319-1327.

Blystad, H., Vold, L., Nygård, K., 2009. Tick-borne encephalitis in Norway. EpiNorth 10, 75-76.

Branda, J.A., Linskey, K., Kim, Y.A., Steere, A.C., Ferraro, M.J., 2011. Two-tiered antibody testing for Lyme disease with use of 2 enzyme immunoassays, a whole-cell sonicate enzyme immunoassay followed by a VlsE C6 peptide enzyme immunoassay. Clin Infect Dis 53, 541-547.

Brantsæter, A.B., Hoel, T., Kristianslund, T.I., Mæland, A., 1998. [Tularemia after tick bite in Vestfold]. Tidsskr Nor Laegeforen 118, 1191-1193.

Brennaas, O., Raeder, S., 1962. [Meningo-encephalomyeloradiculitis in Sunhordland. Endemic virus-caused disease transmitted by a bush tick?]. Tidsskr Nor Laegeforen 82, 739-743.

Brorson, Ø., Brorson, S.H., 1997. Transformation of cystic forms of Borrelia burgdorferi to normal, mobile spirochetes. Infection 25, 240-246.

Brouqui, P., Bacellar, F., Baranton, G., Birtles, R.J., Bjoersdorff, A., Blanco, J.R., Caruso, G., Cinco, M., Fournier, P.E., Francavilla, E., Jensenius, M., Kazar, J., Laferl, H., Lakos, A., Lotric Furlan, S., Maurin, M., Oteo, J.A., Parola, P., Perez-Eid, C., Peter, O., Postic, D., Raoult, D., Tellez, A., Tselentis, Y., Wilske, B., 2004. Guidelines for the diagnosis of tick-borne bacterial diseases in Europe. Clin Microbiol Infect 10, 1108-1132.

Burgdorfer, W., Barbour, A.G., Hayes, S.F., Benach, J.L., Grunwaldt, E., Davis, J.P., 1982. Lyme disease - a tick-borne spirochetosis? Science 216, 1317-1319.

Caimano, M.J., Drecktrah, D., Kung, F., Samuels, D.S., 2016. Interaction of the Lyme disease spirochete with its tick vector. Cell Microbiol 18, 919-927.

Cairns, V., Godwin, J., 2005. Post-Lyme borreliosis syndrome: a meta-analysis of reported symptoms. Int J Epidemiol 34, 1340-1345.

Cameron, D., Gaito, A., Harris, N., Bach, G., Bellovin, S., Bock, K., Bock, S., Burrascano, J., Dickey, C., Horowitz, R., Phillips, S., Meer-Scherrer, L., Raxlen, B., Sherr, V., Smith, H., Smith, P., Stricker, R., Ilads Working Group, 2004. Evidence-based guidelines for the management of Lyme disease. Expert Rev Anti Infect Ther 2, S1-13.

Centers for Disease Control and Prevention, 1995. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. JAMA 274, 937.

Cerar, D., Cerar, T., Ruzic-Sabljic, E., Wormser, G.P., Strle, F., 2010. Subjective symptoms after treatment of early Lyme disease. Am J Med 123, 79-86.

Cerar, T., Strle, F., Stupica, D., Ruzic-Sabljic, E., McHugh, G., Steere, A.C., Strle, K., 2016. Differences in genotype, clinical features, and inflammatory potential of Borrelia burgdorferi sensu stricto strains from Europe and the United States. Emerg Infect Dis 22, 818-827.

Cheung, C.S., Anderson, K.W., Benitez, K.Y., Soloski, M.J., Aucott, J.N., Phinney, K.W., Turko, I.V., 2015. Quantification of Borrelia burgdorferi membrane proteins in human serum: A new concept for detection of bacterial infection. Anal Chem 87, 11383-11388.

Dannevig, P., 2009. Sogn og Fjordane: klima, Store norske leksikon. Oslo: Foreningen SNL.

de Mik, E.L., van Pelt, W., Docters-van Leeuwen, B.D., van der Veen, A., Schellekens, J.F., Borgdorff, M.W., 1997. The geographical distribution of tick bites and erythema migrans in general practice in The Netherlands. Int J Epidemiol 26, 451-457.

Dehnert, M., Fingerle, V., Klier, C., Talaska, T., Schlaud, M., Krause, G., Wilking, H., Poggensee, G., 2012. Seropositivity of Lyme borreliosis and associated risk factors: a population-based study in children and adolescents in Germany (KiGGS). PLoS One 7, e41321.

den Boon, S., Schellekens, J.F., Schouls, L.M., Suijkerbuijk, A.W., Docters van Leeuwen, B., van Pelt, W., 2004. [Doubling of the number of cases of tick bites and lyme borreliosis seen by general practitioners in the Netherlands]. Ned Tijdschr Geneeskd 148, 665-670.

Dennis, D.T., Piesman, J., 2005. Overview of tick-borne infections of humans, in: Goodman, J.L., Dennis, D.T., Sonenshine, D.E. (Eds.), Tick-borne diseases of humans. Washington, DC: ASM Press.

Dessau, R., Eliasson, I., Skarpaas, T., Nyman, D., 2011. Testing for Lyme borreliosis in the Nordic countries - variations in strategies and rate of seropositivity. Report from a survey of laboratory methods used in Scandinavia.

Dessau, R.B., 2016. Microscopy of human blood for Borrelia burgdorferi and Babesia without clinical or scientific rationale. Infect Dis (Lond) 48, 420-421.

Dessau, R.B., Bangsborg, J.M., Ejlertsen, T., Skarphedinsson, S., Schønheyder, H.C., 2010. Utilization of serology for the diagnosis of suspected Lyme borreliosis in Denmark: survey of patients seen in general practice. BMC Infect Dis 10, 317.

Dessau, R.B., Bangsborg, J.M., Jensen, T.P., Hansen, K., Lebech, A.M., Andersen, C.O., 2006. [Laboratory diagnosis of infection caused by Borrelia burgdorferi]. Ugeskr Laeger 168, 2805-2807.

Dessau, R.B., Fingerle, V., Gray, J., Hunfeld, K.P., Jaulhac, B., Kahl, O., Kristoferitsch, W., Stanek, G., Strle, F., 2014. The lymphocyte transformation test for the diagnosis of Lyme borreliosis has currently not been shown to be clinically useful. Clin Microbiol Infect 20, O786-787.

Diaz, J.H., 2015. Ticks, including tick paralysis, in: Bennett, J.E., Dolin, R., Blaser, M.J. (Eds.), Mandell, Douglas, and Bennett's Principles and practice of infectious diseases, 8 ed. Philadelphia, PA: Elsevier, pp. 3266-3279.

Dumler, J.S., Barbet, A.F., Bekker, C.P., Dasch, G.A., Palmer, G.H., Ray, S.C., Rikihisa, Y., Rurangirwa, F.R., 2001. Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, descriptions of six new species combinations and designation of Ehrlichia equi and 'HGE agent' as subjective synonyms of Ehrlichia phagocytophila. Int J Syst Evol Microbiol 51, 2145-2165.

Dumler, J.S., Walker, D.H., 2015. Ehrlichia chaffeensis (human monocytotropic ehrlichiosis), Anaplasma phagocytophilum (human granulocytotropic anaplasmosis) and other Anaplasmataceae, in: Bennett, J.E., Dolin, R., Blaser, M.J. (Eds.), Mandell, Douglas, and Bennett's Principles and practic of infectious diseases, 8 ed. Philadelphia, PA: Elsesvier.

Eikeland, R., Ljøstad, U., Mygland, Å., Herlofson, K., Lohaugen, G.C., 2012. European neuroborreliosis: neuropsychological findings 30 months post-treatment. Eur J Neurol 19, 480-487.

Eikeland, R., Mygland, Å., Herlofson, K., Ljøstad, U., 2011. European neuroborreliosis: quality of life 30 months after treatment. Acta Neurol Scand 124, 349-354.

Elfving, K., Lindblom, A., Nilsson, K., 2008. Seroprevalence of Rickettsia spp. infection among tick-bitten patients and blood donors in Sweden. Scand J Infect Dis 40, 74-77.

Eliassen, K.E., Berild, D., Reiso, H., Grude, N., Christophersen, K.S., Finckenhagen, C., Lindbaek, M., 2017. Incidence and antibiotic treatment of erythema migrans in Norway 2005-2009. Ticks Tick Borne Dis 8, 1-8.

Eriksen, H.R., Ihlebaek, C., Ursin, H., 1999. A scoring system for subjective health complaints (SHC). Scand J Public Health 27, 63-72.

European Centre for Disease Prevention and Control, 2011. Second expert consultation of tick-borne diseases with emphasis on Lyme borreliosis and tick-borne encephalitis: European Centre for Disease Prevention and Control.

Fahrer, H., van der Linden, S.M., Sauvain, M.J., Gern, L., Zhioua, E., Aeschlimann, A., 1991. The prevalence and incidence of clinical and asymptomatic Lyme borreliosis in a population at risk. J Infect Dis 163, 305-310.

Feder, H.M., Jr., Johnson, B.J., O'Connell, S., Shapiro, E.D., Steere, A.C., Wormser, G.P., 2007. A critical appraisal of "chronic Lyme disease". N Engl J Med 357, 1422-1430.

Filipkowski, K.B., Smyth, J.M., Rutchick, A.M., Santuzzi, A.M., Adya, M., Petrie, K.J., Kaptein, A.A., 2010. Do healthy people worry? Modern health worries, subjective health complaints, perceived health, and health care utilization. Int J Behav Med 17, 182-188.

Fleming, R.V., Marques, A.R., Klempner, M.S., Schmid, C.H., Dally, L.G., Martin, D.S., Philipp, M.T., 2004. Pre-treatment and post-treatment assessment of the C(6) test in patients with persistent symptoms and a history of Lyme borreliosis. Eur J Clin Microbiol Infect Dis 23, 615-618.

Fryland, L., Wilhelmsson, P., Lindgren, P.E., Nyman, D., Ekerfelt, C., Forsberg, P., 2011. Low risk of developing Borrelia burgdorferi infection in the south-east of Sweden after being bitten by a Borrelia burgdorferi-infected tick. Int J Infect Dis 15, e174-181.

Gao, G.F., Jiang, W.R., Hussain, M.H., Venugopal, K., Gritsun, T.S., Reid, H.W., Gould, E.A., 1993. Sequencing and antigenic studies of a Norwegian virus isolated from encephalomyelitic sheep confirm the existence of louping ill virus outside Great Britain and Ireland. J Gen Virol 74 ( Pt 1), 109-114.

Gern, L., Estrada-Pena, A., Frandsen, F., Gray, J.S., Jaenson, T.G., Jongejan, F., Kahl, O., Korenberg, E., Mehl, R., Nuttall, P.A., 1998. European reservoir hosts of Borrelia burgdorferi sensu lato. Zentralbl Bakteriol 287, 196-204.

Goossens, H.A., Nohlmans, M.K., van den Bogaard, A.E., 1999. Epstein-Barr virus and cytomegalovirus infections cause false-positive results in IgM two-test protocol for early Lyme borreliosis. Infection 27, 231.

Grankvist, A., Sandelin, L.L., Andersson, J., Fryland, L., Wilhelmsson, P., Lindgren, P.E., Forsberg, P., Wenneras, C., 2015. Infections with candidatus Neoehrlichia mikurensis and cytokine responses in 2 persons bitten by ticks, Sweden. Emerg Infect Dis 21, 1462-1465.

Gray, J., 1999. Risk assessment in Lyme borreliosis. Wien Klin Wochenschr 111, 990-993.

Gray, J.S., Dautel, H., Estrada-Pena, A., Kahl, O., Lindgren, E., 2009. Effects of climate change on ticks and tick-borne diseases in Europe. Interdiscip Perspect Infect Dis 2009, 593232.

Grude, N., Tveten, Y., Stutzer, A., Hoddevik, G., Aaberge, I.S., 2011. Strategimøte 3. november 2011: Laboratoriediagnostikk ved borreliose. Oslo: Norwegian Institute of Public Health.

Gustafson, R., 1994. Epidemiological studies of Lyme borreliosis and tick-borne encephalitis. Scand J Infect Dis Suppl 92, 1-63.

Gustafson, R., Forsgren, M., Gardulf, A., Granström, M., Svenungsson, B., 1993. Antibody prevalence and clinical manifestations of Lyme borreliosis and tick-borne encephalitis in Swedish orienteers. Scand J Infect Dis 25, 605-611.

Gustafson, R., Jaenson, T.G., Gardulf, A., Mejlon, H., Svenungsson, B., 1995. Prevalence of Borrelia burgdorferi sensu lato infection in Ixodes ricinus in Sweden. Scand J Infect Dis 27, 597-601.

Haglund, M., 2002. Occurrence of TBE in areas previously considered being non-endemic: Scandinavian data generate an international study by the International Scientific Working Group for TBE (ISW-TBE). Int J Med Microbiol 291 Suppl 33, 50-54.

Harbo, S.O., 2009. Diagnostikk og behandling av Lyme borreliose. Rapport til Helsedirektoratet fra arbeidsgruppen. November 2009. Oslo: Norwegian Directorate of Health.

Hasle, G., 2010. Dispersal of ticks and tick-borne pathogens by birds: Dynamics of birds' transport of ticks to Norway, Dept. of Biology and Intistute for Health and Society, Faculty of Medicine. Oslo: University of Oslo.

Hasle, G., 2013. Transport of ixodid ticks and tick-borne pathogens by migratory birds. Front Cell Infect Microbiol 3, 48.

Hasle, G., Bjune, G., Edvardsen, E., Jakobsen, C., Linnehol, B., Roer, J.E., Mehl, R., Roed, K.H., Pedersen, J., Leinaas, H.P., 2009. Transport of ticks by migratory passerine birds to Norway. J Parasitol 95, 1342-1351.

Hasle, G., Bjune, G.A., Midthjell, L., Roed, K.H., Leinaas, H.P., 2011a. Transport of Ixodes ricinus infected with Borrelia species to Norway by northward-migrating passerine birds. Ticks Tick Borne Dis 2, 37-43.

Hasle, G., Leinaas, H.P., Roed, K.H., Øines, Ø., 2011b. Transport of Babesia venatorum-infected Ixodes ricinus to Norway by northward migrating passerine birds. Acta Vet Scand 53, 41.

Haugeberg, G., Hansen, I.J., Skarpaas, T., Noraas, S., Kjelland, V., 2014. Lyme arthritis in Southern Norway - an endemic area for Lyme Borreliosis. BMC Infect Dis 14, 185.

Henningsson, A.J., Malmvall, B.E., Ernerudh, J., Matussek, A., Forsberg, P., 2010. Neuroborreliosis--an epidemiological, clinical and healthcare cost study from an endemic area in the south-east of Sweden. Clin Microbiol Infect 16, 1245-1251.

Henningsson, A.J., Wilhelmsson, P., Gyllemark, P., Kozak, M., Matussek, A., Nyman, D., Ekerfelt, C., Lindgren, P.E., Forsberg, P., 2015. Low risk of seroconversion or clinical disease in humans after a bite by an Anaplasma phagocytophilum-infected tick. Ticks Tick Borne Dis 6, 787-792.

Hjorteviltregisteret, 2015. Tildelte og felte hjort. http://www.hjorteviltregisteret.no/Hjort/Jaktstatistikk/TildelteOgFelteDyr 2015, accessed Nov 24, 2015.

Hoch, M., Wieser, A., Loscher, T., Margos, G., Purner, F., Zuhl, J., Seilmaier, M., Balzer, L., Guggemos, W., Rack-Hoch, A., von Both, U., Hauptvogel, K., Schonberger, K., Hautmann, W., Sing, A., Fingerle, V., 2015. Louse-borne relapsing fever (Borrelia recurrentis) diagnosed in 15 refugees from northeast Africa: epidemiology and preventive control measures, Bavaria, Germany, July to October 2015. Euro Surveill 20.

Holzmann, H., 2003. Diagnosis of tick-borne encephalitis. Vaccine 21 Suppl 1, S36-40.

Hornok, S., Kovats, D., Csorgo, T., Meli, M.L., Gonczi, E., Hadnagy, Z., Takacs, N., Farkas, R., Hofmann-Lehmann, R., 2014. Birds as potential reservoirs of tick-borne pathogens: first evidence of bacteraemia with Rickettsia helvetica. Parasit Vectors 7, 128.

Hubalek, Z., 2009. Epidemiology of lyme borreliosis. Curr Probl Dermatol 37, 31-50.

Hunsperger, E.A., 2015. Arboviruses, in: Jorgensen, J.H., Pfaller, M.A. (Eds.), Manual of clinical microbiology, 11 ed. Washington, DC: ASM Press, pp. 1644-1659.

Huppertz, H.I., Böhme, M., Standaert, S.M., Karch, H., Plotkin, S.A., 1999. Incidence of Lyme borreliosis in the Wurzburg region of Germany. Eur J Clin Microbiol Infect Dis 18, 697-703.

Hvidsten, D., Stordal, F., Lager, M., Rognerud, B., Kristiansen, B.E., Matussek, A., Gray, J., Stuen, S., 2015. Borrelia burgdorferi sensu lato-infected Ixodes ricinus collected from vegetation near the Arctic Circle. Ticks Tick Borne Dis 6, 768-773.

Hvidsten, D., Stuen, S., Jenkins, A., Dienus, O., Olsen, R.S., Kristiansen, B.E., Mehl, R., Matussek, A., 2014. Ixodes ricinus and Borrelia prevalence at the Arctic Circle in Norway. Ticks Tick Borne Dis 5, 107-112.

Ihlebaek, C., Eriksen, H.R., Ursin, H., 2002. Prevalence of subjective health complaints (SHC) in Norway. Scand J Public Health 30, 20-29.

Ihlebæk, C., Brage, S., Eriksen, H.R., 2007. Health complaints and sickness absence in Norway, 1996-2003. Occup Med (Lond) 57, 43-49.

Jacobs, J.J., Noordhoek, G.T., Brouwers, J.M., Wielinga, P.R., Jacobs, J.P., Brandenburg, A.H., 2008. [Small risk of developing Lyme borreliosis following a tick bite on Ameland: research in a general practice]. Ned Tijdschr Geneeskd 152, 2022-2026.

Jaenson, T.G., Eisen, L., Comstedt, P., Mejlon, H.A., Lindgren, E., Bergstrom, S., Olsen, B., 2009. Risk indicators for the tick Ixodes ricinus and Borrelia burgdorferi sensu lato in Sweden. Med Vet Entomol 23, 226-237.

Jarefors, S., Bennet, L., You, E., Forsberg, P., Ekerfelt, C., Berglund, J., Ernerudh, J., 2006. Lyme borreliosis reinfection: might it be explained by a gender difference in immune response? Immunology 118, 224-232.

Jenkins, A., Hvidsten, D., Matussek, A., Lindgren, P.E., Stuen, S., Kristiansen, B.E., 2012. Borrelia burgdorferi sensu lato in Ixodes ricinus ticks from Norway: evaluation of a PCR test targeting the chromosomal flaB gene. Exp Appl Acarol 58, 431-439.

Jenkins, A., Kristiansen, B.E., 2013. Neoehrlichia--a new tick-borne bacterium. Tidsskr Nor Laegeforen 133, 1058-1059.

Jenkins, A., Kristiansen, B.E., Allum, A.G., Aakre, R.K., Strand, L., Kleveland, E.J., van de Pol, I., Schouls, L., 2001. Borrelia burgdorferi sensu lato and Ehrlichia spp. in Ixodes ticks from southern Norway. J Clin Microbiol 39, 3666-3671.

Jiang, J.-F., Zheng, Y.-C., Jiang, R.-R., Li, H., Huo, Q.-B., Jiang, B.-G., Sun, Y., Jia, N., Wang, Y.-W., Ma, L., Liu, H.-B., Chu, Y.-L., Ni, X.-B., Liu, K., Song, Y.-D., Yao, N.-N., Wang, H., Sun, T., Cao, W.-C., 2015. Epidemiological, clinical, and laboratory characteristics of 48 cases of “Babesia venatorum” infection in China: a descriptive study. Lancet Infect Dis 15, 196-203.

Jore, S., Viljugrein, H., Hofshagen, M., Brun-Hansen, H., Kristoffersen, A., Nygård, K., Brun, E., Ottesen, P., Sævik, B., Ytrehus, B., 2011. Multi-source analysis reveals latitudinal and altitudinal shifts in range of Ixodes ricinus at its northern distribution limit. Parasit Vectors 4, 84.

Kazi, H., de Groot-Mijnes, J.D., Ten Dam-van Loon, N.H., Ossewaarde-van Norel, J., Oosterheert, J.J., de Boer, J.H., 2016. No value for routine serologic screening for Borrelia burgdorferi in patients with uveitis in the Netherlands. Am J Ophthalmol 166, 189-193.

Kjelland, V., Korslund, L., Slettan, A., 2014. Fakta om flått. Oslo: Kagge Forlag.

Kjelland, V., Rollum, R., Korslund, L., Slettan, A., Tveitnes, D., 2015. Borrelia miyamotoi is widespread in Ixodes ricinus ticks in southern Norway. Ticks Tick Borne Dis 6, 516-521.

Kjelland, V., Stuen, S., Skarpaas, T., Slettan, A., 2010a. Borrelia burgdorferi sensu lato in Ixodes ricinus ticks collected from migratory birds in Southern Norway. Acta Vet Scand 52, 59.

Kjelland, V., Stuen, S., Skarpaas, T., Slettan, A., 2010b. Prevalence and genotypes of Borrelia burgdorferi sensu lato infection in Ixodes ricinus ticks in southern Norway. Scand J Infect Dis 42, 579-585.

Kjelland, V., Ytrehus, B., Stuen, S., Skarpaas, T., Slettan, A., 2011. Prevalence of Borrelia burgdorferi in Ixodes ricinus ticks collected from moose (Alces alces) and roe deer (Capreolus capreolus) in southern Norway. Ticks Tick Borne Dis 2, 99-103.

Kraiczy, P., Stevenson, B., 2013. Complement regulator-acquiring surface proteins of Borrelia burgdorferi: Structure, function and regulation of gene expression. Ticks Tick Borne Dis 4, 26-34.

Kristiansen, B.E., Jenkins, A., Tveten, Y., Karsten, B., Line, O., Bjoersdorff, A., 2001. [Human granulocytic ehrlichiosis in Norway]. Tidsskr Nor Laegeforen 121, 805-806.

Labbé Sandelin, L., Grankvist, A., Fryland, L., Wennerås, C., Lindgren, P.E., Forsberg, P., 2015. Subclinical neoehrlichiosis in two immunocompetent, tick-bitten Swedish women, ECCMID. Copenhagen.

Labuda, M., Kozuch, O., Zuffova, E., Eleckova, E., Hails, R.S., Nuttall, P.A., 1997. Tick-borne encephalitis virus transmission between ticks cofeeding on specific immune natural rodent hosts. Virology 235, 138-143.

Landis, J.R., Koch, G.G., 1977. The measurement of observer agreement for categorical data. Biometrics 33, 159-174.

Landry, M.L., 2016. Immunoglobulin M for acute infection: True or false? Clin Vaccine Immunol 23, 540-545.

Larsen, A.L., Kanestrøm, A., Bjørland, M., Andreassen, Å., Soleng, A., Vene, S., Dudman, S.G., 2014. Detection of specific IgG antibodies in blood donors and tick-borne encephalitis virus in ticks within a non-endemic area in southeast Norway. Scand J Infect Dis 46, 181-184.

Leeflang, M.M., Ang, C.W., Berkhout, J., Bijlmer, H.A., Van Bortel, W., Brandenburg, A.H., Van Burgel, N.D., Van Dam, A.P., Dessau, R.B., Fingerle, V., Hovius, J.W., Jaulhac, B., Meijer, B., Van Pelt, W., Schellekens, J.F., Spijker, R., Stelma, F.F., Stanek, G., Verduyn-Lunel, F., Zeller, H., Sprong, H., 2016. The diagnostic accuracy of serological tests for Lyme borreliosis in Europe: a systematic review and meta-analysis. BMC Infect Dis 16, 140.

Leiby, D.A., Chung, A.P., Cable, R.G., Trouern-Trend, J., McCullough, J., Homer, M.J., Reynolds, L.D., Houghton, R.L., Lodes, M.J., Persing, D.H., 2002. Relationship between tick bites and the seroprevalence of Babesia microti and Anaplasma phagocytophila (previously Ehrlichia sp.) in blood donors. Transfusion (Paris) 42, 1585-1591.

Liang, F.T., Yan, J., Mbow, M.L., Sviat, S.L., Gilmore, R.D., Mamula, M., Fikrig, E., 2004. Borrelia burgdorferi changes its surface antigenic expression in response to host immune responses. Infect Immun 72, 5759-5767.

Lindgren, E., Jaenson, T.G.T., 2006. Lyme borreliosis in Europe: influences of climate and climate change, epidemiology, ecology and adaptation measures. World Health Organization Regional Office for Europe http://www.euro.who.int/__data/assets/pdf_file/0006/96819/E89522.pdf.

Ljøstad, U., Mygland, Å., 2012. The phenomenon of 'chronic Lyme'; an observational study. Eur J Neurol 19, 1128-1135.

Ljøstad, U., Mygland, Å., 2013. Chronic Lyme; diagnostic and therapeutic challenges. Acta Neurol Scand Suppl, 38-47.

MacDonald, E., Vestrheim, D.F., White, R.A., Konsmo, K., Lange, H., Aase, A., Nygård, K., Stefanoff, P., Aaberge, I., Vold, L., 2016. Are the current notification criteria for Lyme borreliosis in Norway suitable? Results of an evaluation of Lyme borreliosis surveillance in Norway, 1995-2013. BMC Public Health 16, 729.

Magnarelli, L.A., Miller, J.N., Anderson, J.F., Riviere, G.R., 1990. Cross-reactivity of nonspecific treponemal antibody in serologic tests for Lyme disease. J Clin Microbiol 28, 1276-1279.

Magni, R., Espina, B.H., Shah, K., Lepene, B., Mayuga, C., Douglas, T.A., Espina, V., Rucker, S., Dunlap, R., Petricoin, E.F., Kilavos, M.F., Poretz, D.M., Irwin, G.R., Shor, S.M., Liotta, L.A., Luchini, A., 2015. Application of Nanotrap technology for high sensitivity measurement of urinary outer surface protein A carboxyl-terminus domain in early stage Lyme borreliosis. J Transl Med 13, 346.

Maiwald, M., Oehme, R., March, O., Petney, T.N., Kimmig, P., Naser, K., Zappe, H.A., Hassler, D., von Knebel Doeberitz, M., 1998. Transmission risk of Borrelia burgdorferi sensu lato from Ixodes ricinus ticks to humans in southwest Germany. Epidemiol Infect 121, 103-108.

Mannelli, A., Bertolotti, L., Gern, L., Gray, J., 2012. Ecology of Borrelia burgdorferi sensu lato in Europe: transmission dynamics in multi-host systems, influence of molecular processes and effects of climate change. FEMS Microbiol Rev 36, 837-861.

Mansfield, K.L., Johnson, N., Phipps, L.P., Stephenson, J.R., Fooks, A.R., Solomon, T., 2009. Tick-borne encephalitis virus – a review of an emerging zoonosis. J Gen Virol 90, 1781-1794.

Marangoni, A., Sparacino, M., Cavrini, F., Storni, E., Mondardini, V., Sambri, V., Cevenini, R., 2005. Comparative evaluation of three different ELISA methods for the diagnosis of early culture-confirmed Lyme disease in Italy. J Med Microbiol 54, 361-367.

Margos, G., Vollmer, S.A., Cornet, M., Garnier, M., Fingerle, V., Wilske, B., Bormane, A., Vitorino, L., Collares-Pereira, M., Drancourt, M., Kurtenbach, K., 2009. A new Borrelia species defined by multilocus sequence analysis of housekeeping genes. Appl Environ Microbiol 75, 5410-5416.

Mathison, B.A., Pritt, B.S., 2014. Laboratory Identification of Arthropod Ectoparasites. Clin Microbiol Rev 27, 48-67.

Mehl, R., 1983. The distribution and host relations of Norwegian ticks (Acari, Ixodides). . Fauna norvegica Series B 30, 46-51.

Mhalu, F.S., Matre, R., 1996. Serological evidence of Lyme borreliosis in Africa: results from studies in Dar es Salaam, Tanzania. East Afr Med J 73, 583-585.

Mitschler, A., Grange, F., Lipsker, D., Jaulhac, B., Piemont, Y., Belanger, P., Pagnon, X., Mayer, O., Guillaume, J.C., 2004. [Knowledge and prevention of tick-bite borreliosis: survey of the population in Alsace, an endemic area]. Ann Dermatol Venereol 131, 547-553.

Molins, C.R., Ashton, L.V., Wormser, G.P., Hess, A.M., Delorey, M.J., Mahapatra, S., Schriefer, M.E., Belisle, J.T., 2015. Development of a metabolic biosignature for detection of early Lyme disease. Clin Infect Dis 60, 1767-1775.

Molloy, P.J., Telford, S.R., 3rd, Chowdri, H.R., Lepore, T.J., Gugliotta, J.L., Weeks, K.E., Hewins, M.E., Goethert, H.K., Berardi, V.P., 2015. Borrelia miyamotoi disease in the northeastern United States: A case series. Ann Intern Med 163, 91-98.

Montes, C.L., Acosta-Rodríguez, E.V., Merino, M.C., Bermejo, D.A., Gruppi, A., 2007. Polyclonal B cell activation in infections: infectious agents' devilry or defense mechanism of the host? J Leukoc Biol 82, 1027-1032.

Moriarty, T.J., Norman, M.U., Colarusso, P., Bankhead, T., Kubes, P., Chaconas, G., 2008. Real-time high resolution 3D imaging of the lyme disease spirochete adhering to and escaping from the vasculature of a living host. PLoS Pathog 4, e1000090.

Munderloh, U.G., Jauron, S.D., Kurtti, T.J., 2005. The tick: a different kind of host for human pathogens, in: Goodman, J.L., Dennis, D.T., Sonenshine, D.E. (Eds.), Tick-borne diseases of humans. Washington, D.C.: ASM Press.

Mursic, V.P., Wanner, G., Reinhardt, S., Wilske, B., Busch, U., Marget, W., 1996. Formation and cultivation of Borrelia burgdorferi spheroplast-L-form variants. Infection 24, 218-226.

Mygland, Å., Skarpaas, T., Ljøstad, U., 2006. Chronic polyneuropathy and Lyme disease. Eur J Neurol 13, 1213-1215.

Mysterud, A., Byrkjeland, R., Qviller, L., Viljugrein, H., 2015. The generalist tick Ixodes ricinus and the specialist tick Ixodes trianguliceps on shrews and rodents in a northern forest ecosystem--a role of body size even among small hosts. Parasit Vectors 8, 639.

Mysterud, A., Easterday, W.R., Qviller, L., Viljugrein, H., Ytrehus, B., 2013. Spatial and seasonal variation in the prevalence of Anaplasma phagocytophilum and Borrelia burgdorferi sensu lato in questing Ixodes ricinus ticks in Norway. Parasit Vectors 6, 187.

Mysterud, I., Laane, M.M., 2013. A simple method for the detection of live Borrelia spirochaetes in human blood using classical microscopy techniques. Biological and biomedical reports 3, 15-28.

Mørch, K., Holmaas, G., Frolander, P.S., Kristoffersen, E.K., 2015. Severe human Babesia divergens infection in Norway. Int J Infect Dis 33, 37-38.

Nadelman, R.B., Nowakowski, J., Fish, D., Falco, R.C., Freeman, K., McKenna, D., Welch, P., Marcus, R., Aguero-Rosenfeld, M.E., Dennis, D.T., Wormser, G.P., Tick Bite Study Group, 2001. Prophylaxis with single-dose doxycycline for the prevention of Lyme disease after an Ixodes scapularis tick bite. N Engl J Med 345, 79-84.

Nahimana, I., Gern, L., Blanc, D.S., Praz, G., Francioli, P., Peter, O., 2004. Risk of Borrelia burgdorferi infection in western Switzerland following a tick bite. Eur J Clin Microbiol Infect Dis 23, 603-608.

Nilsson, K., 2009. Septicaemia with Rickettsia helvetica in a patient with acute febrile illness, rash and myasthenia. J Infect 58, 79-82.

Nilsson, K., Elfving, K., Pahlson, C., 2010. Rickettsia helvetica in patient with meningitis, Sweden, 2006. Emerg Infect Dis 16, 490-492.

Norris, S.J., 2014. The vls antigenic variation systems of Lyme disease Borrelia: eluding host immunity through both random, segmental gene conversion and framework heterogeneity. Microbiol Spectr 2.

Norwegian Directorate of Health, 2016. Antibiotika i sykehus. Nasjonal faglig retningslinje for bruk av antibiotika i sykehus. https://helsedirektoratet.no/retningslinjer/antibiotika-i-sykehus, accessed 9 Nov 2016.

Norwegian Directorate of Health, Antibiotic Centre for Primary Care, 2016. Antibiotikabruk i primærhelsetjenesten. http://www.antibiotikaiallmennpraksis.no/, accessed Jan 4, 2017.

Norwegian Institute of Public Health, 2015a. Skogflåttencefalitt (TBE-virusinfeksjoner) - veileder for helsepersonell. https://www.fhi.no/nettpub/smittevernveilederen/sykdommer-a-a/skogflattencefalitt-tbe-virusinfeks/, accessed Jan 29, 2017.

Norwegian Institute of Public Health, 2015b. Tilbakefallsfeber - veileder for helsepersonell. https://www.fhi.no/nettpub/smittevernveilederen/sykdommer-a-a/tilbakefallsfeber---veileder-for-he/, accessed Oct 10, 2016.

Norwegian Institute of Public Health, 2016a. Sheep ticks and tick bites. https://www.fhi.no/en/el/insects-and-pests/ticks-and-tick-borne-diseases/sheep-ticks-and-tick-bites/, accessed Jan 22, 2017.

Norwegian Institute of Public Health, 2016b. Tularemi - veileder for helsepersonell. https://www.fhi.no/nettpub/smittevernveilederen/sykdommer-a-a/tularemi---veileder-for-helseperson/, accessed Jan 29, 2017.

Ottesen, P., 2010. Helsekonsekvenser av klimaendringer i Norge. Bakgrunnsmateriale til NOU Klimatilpasning: Nasjonalt folkehelseinstitutt.

Pages, F., Dautel, H., Duvallet, G., Kahl, O., de Gentile, L., Boulanger, N., 2014. Tick repellents for human use: prevention of tick bites and tick-borne diseases. Vector Borne Zoonotic Dis 14, 85-93.

Panelius, J., Lahdenne, P., Heikkila, T., Peltomaa, M., Oksi, J., Seppala, I., 2002. Recombinant OspC from Borrelia burgdorferi sensu stricto, B. afzelii and B. garinii in the serodiagnosis of Lyme borreliosis. J Med Microbiol 51, 731-739.

Paulsen, K.M., Pedersen, B.N., Soleng, A., Okbaldet, Y.B., Pettersson, J.H., Dudman, S.G., Ottesen, P., Vik, I.S., Vainio, K., Andreassen, Å., 2015. Prevalence of tick-borne encephalitis virus in Ixodes ricinus ticks from three islands in north-western Norway. APMIS 123, 759-764.

Peltomaa, M., McHugh, G., Steere, A.C., 2003. Persistence of the antibody response to the VlsE sixth invariant region (IR6) peptide of Borrelia burgdorferi after successful antibiotic treatment of Lyme disease. J Infect Dis 187, 1178-1186.

Penn, R.L., 2015. Francisella tularensis (tularemia), in: Bennett, J.E., Dolin, R., Blaser, M.J. (Eds.), Mandell, Douglas, and Bennett's Principles and practice of infectious diseases, 8 ed. Philadelphia, PA: Elsevier, pp. 2590-2602.

Philipp, M.T., Marques, A.R., Fawcett, P.T., Dally, L.G., Martin, D.S., 2003. C6 test as an indicator of therapy outcome for patients with localized or disseminated lyme borreliosis. J Clin Microbiol 41, 4955-4960.

Philipp, M.T., Wormser, G.P., Marques, A.R., Bittker, S., Martin, D.S., Nowakowski, J., Dally, L.G., 2005. A decline in C6 antibody titer occurs in successfully treated patients with culture-confirmed early localized or early disseminated Lyme Borreliosis. Clin Diagn Lab Immunol 12, 1069-1074.

Platonov, A.E., Karan, L.S., Kolyasnikova, N.M., Makhneva, N.A., Toporkova, M.G., Maleev, V.V., Fish, D., Krause, P.J., 2011. Humans infected with relapsing fever spirochete Borrelia miyamotoi, Russia. Emerg Infect Dis 17, 1816-1823.

Poland, G.A., 2011. Vaccines against Lyme disease: What happened and what lessons can we learn? Clin Infect Dis 52 Suppl 3, s253-258.

Probst, C., Ott, A., Scheper, T., Meyer, W., Stocker, W., Komorowski, L., 2012. N-terminal disulfide-bridging of Borrelia outer surface protein C increases its diagnostic and vaccine potentials. Ticks Tick Borne Dis 3, 1-7.

Quarsten, H., Skarpaas, T., Fajs, L., Noraas, S., Kjelland, V., 2015. Tick-borne bacteria in Ixodes ricinus collected in southern Norway evaluated by a commercial kit and established real-time PCR protocols. Ticks Tick Borne Dis 6, 538-544.

Radolf, J.D., Caimano, M.J., Stevenson, B., Hu, L.T., 2012. Of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease spirochaetes. Nat Rev Microbiol 10, 87-99.

Randolph, S.E., 2010. To what extent has climate change contributed to the recent epidemiology of tick-borne diseases? Vet Parasitol 167, 92-94.

Reiso, H., Nygård, J.F., Brage, S., Gulbrandsen, P., Tellnes, G., 2000. Work ability assessed by patients and their GPs in new episodes of sickness certification. Fam Pract 17, 139-144.

Reller, M.E., Dumler, J.S., 2015. Ehrlicihia, Anaplasma, and related intracellular bacteria, in: Jorgensen, J.H., Pfaller, M.A. (Eds.), Manual of clinical microbiology, 11 ed. Washington, DC: ASM Press, pp. 1135-1149.

Richter, D., Matuschka, F.R., Spielman, A., Mahadevan, L., 2013. How ticks get under your skin: insertion mechanics of the feeding apparatus of Ixodes ricinus ticks. Proc Biol Sci 280, 20131758.

Rosa, P.A., Tilly, K., Stewart, P.E., 2005. The burgeoning molecular genetics of the Lyme disease spirochaete. Nat Rev Micro 3, 129-143.

Rosef, O., Paulauskas, A., Radzijevskaja, J., 2009a. Prevalence of Borrelia burgdorferi sensu lato and Anaplasma phagocytophilum in questing Ixodes ricinus ticks in relation to the density of wild cervids. Acta Vet Scand 51, 47.

Rosef, O., Radzijevskaja, J., Paulauskas, A., Haslekas, C., 2009b. The prevalence of Anaplasma phagocytophilum in host-seeking Ixodes ricinus ticks in Norway. Clin Microbiol Infect 15 Suppl 2, 43-45.

Samuels, D.S., Radolf, J.D., 2010. Borrelia. Molecular biology, host interaction and pathogenesis. Norfolk, UK: Caister Academic Press.

Schriefer, M.E., 2015. Borrelia, in: Jorgensen, J.H., Pfaller, M.A. (Eds.), Manual of clinical microbiology, 11 ed. Washington DC: ASM Press, pp. 1037-1054.

Scott, S.C., Goldberg, M.S., Mayo, N.E., 1997. Statistical assessment of ordinal outcomes in comparative studies. J Clin Epidemiol 50, 45-55.

Seltzer, E.G., Gerber, M.A., Cartter, M.L., Freudigman, K., Shapiro, E.D., 2000. Long-term outcomes of persons with Lyme disease. JAMA 283, 609-616.

Seriburi, V., Ndukwe, N., Chang, Z., Cox, M.E., Wormser, G.P., 2012. High frequency of false positive IgM immunoblots for Borrelia burgdorferi in Clinical Practice. Clin Microbiol Infect 18, 1236-1240.

Shadick, N.A., Phillips, C.B., Logigian, E.L., Steere, A.C., Kaplan, R.F., Berardi, V.P., Duray, P.H., Larson, M.G., Wright, E.A., Ginsburg, K.S., Katz, J.N., Liang, M.H., 1994. The long-term clinical outcomes of Lyme disease. A population-based retrospective cohort study. Ann Intern Med 121, 560-567.

Shadick, N.A., Phillips, C.B., Sangha, O., Logigian, E.L., Kaplan, R.F., Wright, E.A., Fossel, A.H., Fossel, K., Berardi, V., Lew, R.A., Liang, M.H., 1999. Musculoskeletal and neurologic outcomes in patients with previously treated Lyme disease. Ann Intern Med 131, 919-926.

Shapiro, E.D., Dattwyler, R., Nadelman, R.B., Wormser, G.P., 2005. Response to meta-analysis of Lyme borreliosis symptoms. Int J Epidemiol 34, 1437-1439; author reply 1440-1433.

Shapiro, E.D., Gerber, M.A., Holabird, N.B., Berg, A.T., Feder, H.M., Jr., Bell, G.L., Rys, P.N., Persing, D.H., 1992. A controlled trial of antimicrobial prophylaxis for Lyme disease after deer-tick bites. N Engl J Med 327, 1769-1773.

Skarpaas, T., Ljøstad, U., Sundøy, A., 2004. First human cases of tickborne encephalitis, Norway. Emerg Infect Dis 10, 2241-2243.

Skarpaas, T., Sundøy, A., Bruu, A.L., Vene, S., Pedersen, J., Eng, P.G., Csango, P.A., 2002. [Tick-borne encephalitis in Norway]. Tidsskr Nor Laegeforen 122, 30-32.

Skarphedinsson, S., Søgaard, P., Pedersen, C., 2001. Seroprevalence of human granulocytic ehrlichiosis in high-risk groups in Denmark. Scand J Infect Dis 33, 206-210.

Soleng, A., Kjelland, V., 2013. Borrelia burgdorferi sensu lato and Anaplasma phagocytophilum in Ixodes ricinus ticks in Brønnøysund in northern Norway. Ticks Tick Borne Dis 4, 218-221.

Sonenshine, D.E., 2005. The biology of tick vectors of human disease, in: Goodman, J.L., Dennis, D.T., Sonenshine, D.E. (Eds.), Tick-borne diseases of humans. Washington, DC: ASM Press.

Stanek, G., Fingerle, V., Hunfeld, K.P., Jaulhac, B., Kaiser, R., Krause, A., Kristoferitsch, W., O'Connell, S., Ornstein, K., Strle, F., Gray, J., 2011. Lyme borreliosis: clinical case definitions for diagnosis and management in Europe. Clin Microbiol Infect 17, 69-79.

Stanek, G., Wormser, G.P., Gray, J., Strle, F., 2012. Lyme borreliosis. Lancet 379, 461-473.

Steere, A.C., Coburn, J., Glickstein, L., 2005. Lyme borreliosis, in: Goodman, J.L., Dennis, D.T., Sonenshine, D.E. (Eds.), Tick-borne diseases of humans. Washington, D.C.: ASM Press.

Steere, A.C., Malawista, S.E., Snydman, D.R., Shope, R.E., Andiman, W.A., Ross, M.R., Steele, F.M., 1977. Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. Arthritis Rheum 20, 7-17.

Steere, A.C., McHugh, G., Damle, N., Sikand, V.K., 2008. Prospective study of serologic tests for Lyme disease. Clin Infect Dis 47, 188-195.

Stensvold, C.R., Al Marai, D., Andersen, L.O., Krogfelt, K.A., Jensen, J.S., Larsen, K.S., Nielsen, H.V., 2015. Babesia spp. and other pathogens in ticks recovered from domestic dogs in Denmark. Parasit Vectors 8, 262.

Stjernberg, L., Berglund, J., 2002. Risk of acquiring tick bites in south-eastern Sweden. Scand J Infect Dis 34, 840-844.

Strle, F., 2004. Human granulocytic ehrlichiosis in Europe. Int J Med Microbiol 293 Suppl 37, 27-35.

Strle, F., Nadelman, R.B., Cimperman, J., Nowakowski, J., Picken, R.N., Schwartz, I., Maraspin, V., Aguero-Rosenfeld, M.E., Varde, S., Lotric-Furlan, S., Wormser, G.P., 1999. Comparison of culture-confirmed erythema migrans caused by Borrelia burgdorferi sensu stricto in New York State and by Borrelia afzelii in Slovenia. Ann Intern Med 130, 32-36.

Strle, F., Wormser, G.P., Mead, P., Dhaduvai, K., Longo, M.V., Adenikinju, O., Soman, S., Tefera, Y., Maraspin, V., Lotric-Furlan, S., Ogrinc, K., Cimperman, J., Ruzic-Sabljic, E., Stupica, D., 2013. Gender disparity between cutaneous and non-cutaneous manifestations of lyme borreliosis. PLoS One 8, e64110.

Stuen, S., Bergström, K., 2008. [Human anaplasmosis--a hidden disease in Norway?]. Tidsskr Nor Laegeforen 128, 2579-2581.

Stuen, S., Granquist, E.G., Silaghi, C., 2013. Anaplasma phagocytophilum--a widespread multi-host pathogen with highly adaptive strategies. Front Cell Infect Microbiol 3, 31.

Stupica, D., Lusa, L., Ruzic-Sabljic, E., Cerar, T., Strle, F., 2012. Treatment of erythema migrans with doxycycline for 10 days versus 15 days. Clin Infect Dis 55, 343-350.

Tambs-Lyche, H., 1943. Ixodes ricinus og piroplasmosen i Norge (Meddelelse fra Bergens Museums zoologiske avdeling). Norsk veterinærtidsskrift 55, 337-542.

Theel, E.S., 2016. The past, present, and (possible) future of serologic testing for Lyme disease. J Clin Microbiol 54, 1191-1196.

Theel, E.S., Carpenter, A.B., Binnicker, M.J., 2015. Immunoassays for diagnosis of infectious diseases, in: Jorgensen, J.H., Pfaller, M.A. (Eds.), Manual of clinical microbiology, 11 ed. Washington, DC: ASM Press, pp. 91-105.

Thomas, S.J., Endy, T.P., Rothman, A.L., Barrett, A.D., 2015. Flaviviruses (Dengue, Yellow fever, Japanese encephalitis, West Nile encephalitis, St. Louis encephalitis, Tick-borne encephalitis, Kyasanur forest disease, Alkhurma hemorrhagic fever, Zika), in: Bennett, J.E., Dolin, R., Blaser, M.J. (Eds.), Mandell, Douglas, and Bennett's Principles and practice of infectious diseases, 8 ed. Philadelphia, PA: Elsevier.

Tjernberg, I., Krüger, G., Eliasson, I., 2007. C6 peptide ELISA test in the serodiagnosis of Lyme borreliosis in Sweden. Eur J Clin Microbiol Infect Dis 26, 37-42.

Toledo, Á., Espigares-Fuentes, B., Olmo, A., Stamberg, P., Csángó, P.A., 2015. Molecular detection of tick-borne bacteria in ticks in southern Norway, ECCMID. Copenhagen.

Traavik, T., 1973. Serological investigations indicating the existence of tick-borne encephalitis virus foci along the Norwegian coast. Acta Pathol Microbiol Scand B Microbiol Immunol 81, 138-142.

Traavik, T., 1979. Antibodies to tick-borne encephalitis virus in human sera from the western coast of Norway. Acta Pathol Microbiol Scand B 87B, 9-13.

Traavik, T., Mehl, R., Wiger, R., 1978. The first tick-borne encephalitis virus isolates from Norway. Acta Pathol Microbiol Scand B 86, 253-255.

Tveten, A.K., 2013. Prevalence of Borrelia burgdorferi sensu stricto, Borrelia afzelii, Borrelia garinii, and Borrelia valaisiana in Ixodes ricinus ticks from the northwest of Norway. Scand J Infect Dis 45, 681-687.

Tveten, Y., Noraas, S., Aase, A., 2014. [Cellular Borrelia tests]. Tidsskr Nor Laegeforen 134, 146-147.

Ulvestad, E., Kanestrøm, A., Sønsteby, L.J., Jureen, R., Omland, T., Edvardsen, B., Lundervik, J., Kristoffersen, E., van Dam, A.P., 2001. Diagnostic and biological significance of anti-p41 IgM antibodies against Borrelia burgdorferi. Scand J Immunol 53, 416-421.

Vaage, O.F., 2004. Training, exercise and outdoor activities, Rapporter 2004/13 Statistisk sentralbyrå.

Vannier, E., Krause, P.J., 2012. Human Babesiosis. N Engl J Med 366, 2397-2407.

Vanthomme, K., Bossuyt, N., Boffin, N., Van Casteren, V., 2012. Incidence and management of presumption of Lyme borreliosis in Belgium: recent data from the sentinel network of general practitioners. Eur J Clin Microbiol Infect Dis 31, 2385-2390.

Vegsundvåg, J., Nordeide, J., Reikvam, Å., Jenum, P., 1993. Late cardiac manifestation of infection with Borrelia burgdorferi (Lyme disease). BMJ 307, 173.

Vene, S., Haglund, M., Vapalahti, O., Lundkvist, A., 1998. A rapid fluorescent focus inhibition test for detection of neutralizing antibodies to tick-borne encephalitis virus. J Virol Methods 73, 71-75.

Vestrheim, D.F., White, R.A., Aaberge, I.S., Aase, A., 2016. Geographical differences in seroprevalence of Borrelia burgdorferi antibodies in Norway, 2011-2013. Ticks Tick Borne Dis 7, 698-702.

von Loewenich, F.D., Geissdorfer, W., Disque, C., Matten, J., Schett, G., Sakka, S.G., Bogdan, C., 2010. Detection of "Candidatus Neoehrlichia mikurensis" in two patients with severe febrile illnesses: evidence for a European sequence variant. J Clin Microbiol 48, 2630-2635.

Wahlberg, P., 1990. Incidence of tick-bite in man in Åland Islands: reference to the spread of Lyme borreliosis. Scand J Infect Dis 22, 59-62.

Walder, G., Tiwald, G., Dierich, M.P., Wurzner, R., 2003. Serological evidence for human granulocytic ehrlichiosis in Western Austria. Eur J Clin Microbiol Infect Dis 22, 543-547.

Weel, C.v., König-Zahn, C., Touw-Otten, F.W.M.M., Duijn, N.P.v., Meyboom-de Jong, B., 1995. Measuring functional health status with the COOP/WONCA Charts. A manual. Groningen: Northern Centre of Health Care Research.

Weis, J.J., 2011. Pathogenesis of Borrelia burgdorferi infection and disease, Critical needs and gaps in understanding prevention, amelioration, and resolution of Lyme and other tick-borne diseases: The short-term and long-term outcomes: Workshop report. Washington, DC: National Academies Press.

Weitzner, E., Visintainer, P., Wormser, G.P., 2016. Comparison of males versus females with culture-confirmed early Lyme disease at presentation and at 11-20 years after diagnosis. Diagn Microbiol Infect Dis 85, 493-495.

Welc-Faleciak, R., Sinski, E., Kowalec, M., Zajkowska, J., Pancewicz, S.A., 2014. Asymptomatic "Candidatus Neoehrlichia mikurensis" infections in immunocompetent humans. J Clin Microbiol 52, 3072-3074.

Welinder-Olsson, C., Kjellin, E., Vaht, K., Jacobsson, S., Wennerås, C., 2010. First case of human "Candidatus Neoehrlichia mikurensis" infection in a febrile patient with chronic lymphocytic leukemia. J Clin Microbiol 48, 1956-1959.

Wilhelmsson, P., Lindblom, P., Fryland, L., Nyman, D., Jaenson, T.G., Forsberg, P., Lindgren, P.E., 2013. Ixodes ricinus ticks removed from humans in Northern Europe: seasonal pattern of infestation, attachment sites and duration of feeding. Parasit Vectors 6, 362.

Wills, A.B., Spaulding, A.B., Adjemian, J., Prevots, D.R., Turk, S.P., Williams, C., Marques, A., 2016. Long-term follow-up of patients with Lyme disease: Longitudinal analysis of clinical and quality-of-life measures. Clin Infect Dis 62, 1546-1551.

Wilske, B., Fingerle, V., Schulte-Spechtel, U., 2007. Microbiological and serological diagnosis of Lyme borreliosis. FEMS Immunol Med Microbiol 49, 13-21.

Wittesjö, B., Bjoersdorff, A., Eliasson, I., Berglund, J., 2001. First long-term study of the seroresponse to the agent of human granulocytic ehrlichiosis among residents of a tick-endemic area of Sweden. Eur J Clin Microbiol Infect Dis 20, 173-178.

Wormser, G.P., Schriefer, M., Aguero-Rosenfeld, M.E., Levin, A., Steere, A.C., Nadelman, R.B., Nowakowski, J., Marques, A., Johnson, B.J., Dumler, J.S., 2013. Single-tier testing with the C6 peptide ELISA kit compared with two-tier testing for Lyme disease. Diagn Microbiol Infect Dis 75, 9-15.

Wormser, G.P., Weitzner, E., McKenna, D., Nadelman, R.B., Scavarda, C., Farber, S., Prakash, P., Ash, J., Nowakowski, J., 2014. Long-term assessment of fibromyalgia in patients with culture-confirmed Lyme disease. Arthritis Rheumatol 67, 837–839

Wormser, G.P., Weitzner, E., McKenna, D., Nadelman, R.B., Scavarda, C., Nowakowski, J., 2015. Long-term assessment of fatigue in patients with culture-confirmed Lyme disease. Am J Med 128, 181-184.

Xu, G., Fang, Q.Q., Keirans, J.E., Durden, L.A., 2003. Molecular phylogenetic analyses indicate that the Ixodes ricinus complex is a paraphyletic group. J Parasitol 89, 452-457.

Ytrehus, B., Vainio, K., Dudman, S.G., Gilray, J., Willoughby, K., 2013. Tick-borne encephalitis virus and louping-ill virus may co-circulate in Southern Norway. Vector Borne Zoonotic Dis 13, 762-768.

Zijlema, W.L., Stolk, R.P., Löwe, B., Rief, W., White, P.D., Rosmalen, J.G.M., 2013. How to assess common somatic symptoms in large-scale studies: A systematic review of questionnaires. J Psychosom Res 74, 459-468.

Øines, Ø., Radzijevskaja, J., Paulauskas, A., Rosef, O., 2012. Prevalence and diversity of Babesia spp. in questing Ixodes ricinus ticks from Norway. Parasit Vectors 5, 156.

Øymar, K., Tveitnes, D., 2009. Clinical characteristics of childhood Lyme neuroborreliosis in an endemic area of northern Europe. Scand J Infect Dis 41, 88-94.

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# Tick bites in healthy adults from western Norway: Occurrence, risk factors, and outcomes

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ABSTRACT

The frequency of tick bites, risk factors, as well as simple outcome measures after tick bites in a healthy adult population (blood donors) from Sogn and Fjordane county situated at the western coast of Norway, was assessed. The study was based on cross-sectional data from blood donors at 4 different blood banks in the county during the period January to June 2010. Data on tick bites and potential risk factors were collected from 1213 blood donors using a questionnaire. Among participants, 65.7% had experienced tick bites during their life time, whereas 30% reported recent tick bites (during the latest 12 months). There were fewer tick bites in the eastern, inland part of the county, where the tick *Ixodes ricinus* is less prevalent compared to the western, coastal regions. The number of total and recent tick bites increased with the respondent's age, hours spent outdoors during summertime, educational level, ownership of domestic animals, and hunting. Women older than 50 years reported more bites than similarly aged men and younger females.

Among bitten subjects, 22.7% reported ever having had a rash around a tick bite, whereas 12.7% had seen a medical doctor and 7.7% had received antibiotics owing to tick bite. Avoiding certain locations owing to a fear of tick bites was reported by 15.7% of all respondents, more women than men.

In conclusion, tick bites are common in the western part of Norway. The risk of being bitten varies with age and outdoor activities, animal contact, and geography. The consequences in terms of disease seem modest.



## Introduction

*Ixodes ricinus*, the predominating tick species in Norway, is present in coastal areas with its highest abundance along the southernmost coast. In earlier studies, its northern distribution limit was estimated to approximately 66° N (Tambs-Lyche, 1943; Mehl, 1983). Recent data indicate that the latitudinal and altitudinal borders for *I. ricinus* in Norway are expanding and that the tick now is present as far north as 69° N (Jore et al., 2011).

Human tick-borne diseases in Norway are dominated by Lyme borreliosis, with a distribution corresponding to that of *I. ricinus*, with the highest incidence along the southern coastline. Cases of systemic disease and chronic manifestations of Lyme borreliosis are notifiable to the Norwegian Surveillance System for Communicable Diseases (MSIS), i.e., erythema migrans alone is not reported. In the period of 2001–2010, the mean reported annual national incidence was 5.3 cases per 100,000 inhabitants, with 14.1 in Sogn and Fjordane, compared to 26.1 in the southernmost county of Vest-Agder (http://www.msis.no/). *Borrelia burgdorferi* sensu lato has been detected in host-seeking *I. ricinus* ticks in 22.1–31.3% of nymphs and adults in the southernmost part of Norway (Kjelland et al., 2010). In Sogn and Fjordane county, *B. burgdorferi* s.l. has been demonstrated in 12.0% and 3.5% of ticks at 2 different locations (Olav Rosef, personal communication). Tick-borne encephalitis has recently been detected along the southern coastline of Norway (Skarpaas et al., 2004; Andreassen et al., 2012). The incidence of human granulocytic anaplasmosis is not known, but 2 cases have been described so far (Kristiansen et al., 2001), and serological evidence for human infection has been demonstrated (Bakken et al., 1996).

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**Fig. 1.** Norway with the county of Sogn and Fjordane (shaded). The localization of the 4 blood banks are indicated by numbers. 1: Førde, 2: Florø, 3: Lærdal, 4: Nordfjordeid. Map source: Norwegian Map Authority.

Sogn and Fjordane county, located at the western coast of Norway, encompasses coastal, fjord, and mountainous areas at 61–62° N and 5–8° E; it stretches from the coast nearly 200 km eastwards (Fig. 1). The climate is temperate, with a high yearly rain fall in the western and middle areas, but with a more inland-like climate in the eastern part, with less precipitation and lower winter temperatures. Ticks are prevalent in the coastal areas and along the fjords and neighbouring valleys, up to about 400 m above sea level, but less so in the eastern parts (Jore et al., 2011). This distribution is reflected in the skewed incidence within the county of notified cases of disseminated Lyme borreliosis in humans (http://www.msis.no/).

The literature on occurrence and risk factors for tick bites in the general population is sparse. Two surveys from the Netherlands demonstrated marked geographical differences in the occurrence of tick bites, and the incidence seemed to double from 1994 to 2001 (de Mik et al., 1997; den Boon et al., 2004). A recent Belgian investigation likewise demonstrated local geographical differences (Vanthomme et al., 2012). In a study from the island of Åland in Finland, 85% of the general population more than 8 years old reported having been bitten by ticks (Wahlberg, 1990), and in Sweden, Stjernberg and Berglund (2002) found a 4% risk of being tick-bitten per 10 h spent outdoors. Another Swedish study found an increased risk of contracting tick bites in women more than 40 years old (Bennet et al., 2007). In Connecticut and Wisconsin, 4.1% of blood donors reported having been bitten by ticks during 6 months (Leiby et al., 2002).

In the present study, we wanted to assess the frequency of tick bites in a healthy adult population (blood donors) from Sogn and Fjordane with regard to demographics and other risk factors. We also wanted to assess the frequency of symptoms following tick bites, visits to a medical doctor and antibiotic treatment, as well as to estimate whether tick occurrence leads to avoidance of outdoor activities in certain areas.

## Materials and methods

The Helse Førde Hospital Trust has 4 blood banks in Sogn and Fjordane county (Fig. 1). One is situated at the western coast (Florø), 2 by fjords somewhat further east (Førde and Nordfjordeid), and one is located in the easternmost part of the county (Lærdal). During the period January 13th to June 15th 2010, blood donors at the 4 blood banks were asked to participate in the Tick-borne Infection Study in Sogn and Fjordane. A total of 1213 blood donors participated, a response rate of 76%. Owing to practical circumstances at the blood bank in Lærdal, with its several different small sites for donation, the response rate at that blood bank was only 39%. Informed consent was obtained from each participant before the study, and the study was approved by the Regional Committee for Medical Research Ethics. The study included a questionnaire as well as serum samples for antibodies to *Borrelia burgdorferi* sensu lato, tick-borne encephalitis virus, and *Anaplasma phagocytophilum*, the results of which are to be published separately.

### *Questionnaire*

The questionnaire included questions on demographics such as gender, age, marital status, education, household income, and occupation. Questions on pet animals, farm animals, hours spent outdoors during summertime, hunting, orienteering, smoking, and symptoms and treatment after tick bites were also included. A section of the questionnaire related to subjective health complaints, to be published separately.

The questionnaires were answered anonymously, and most were completed during the time spent at the blood bank for routine donation. The characteristics of the participants are presented in Table 1.

**Table 1**
Characteristics of the 1213 participants in the Tick-borne Infection Study in Sogn and Fjordane, 2010.

| Characteristic | No. of subjects[a] | Percentage |
|---|---|---|
| **Blood bank** | | |
| Førde | 614 | 50.6 |
| Florø | 355 | 29.3 |
| Lærdal | 73 | 6.0 |
| Eid | 171 | 14.1 |
| **Gender** | | |
| Female | 544 | 44.8 |
| Male | 669 | 55.2 |
| **Age** | | |
| 19–29 | 80 | 6.8 |
| 30–39 | 235 | 19.9 |
| 40–49 | 414 | 35.0 |
| 50–59 | 344 | 29.1 |
| 60–69 | 110 | 9.3 |
| **Marital status** | | |
| Single | 189 | 15.7 |
| Married or cohabitants | 1014 | 84.3 |
| **Education** | | |
| Primary school 9 years or less | 87 | 7.2 |
| Secondary school | 598 | 49.8 |
| University/college 1–4 years. | 342 | 28.5 |
| University/college >4 years | 175 | 14.6 |
| **Household yearly gross income (EUR)** | | |
| <50,000 | 156 | 13.2 |
| 50,000–99,000 | 647 | 54.7 |
| 100,000–150,000 | 355 | 30.0 |
| >150,000 | 25 | 2.1 |
| **Daily smoking** | | |
| No | 935 | 81.9 |
| Yes | 207 | 18.1 |

[a] Because of missing data, not all numbers total the number of participants ($n = 1213$).

*Outcome*

The main outcomes of this study are the reported number of tick bites ever experienced (referred to as "total"), and tick bites experienced during the preceding 12 months (referred to as "recent"). "Recent bites" are thus included in "total bites", and the responses for both questions were given in the categories "none", "one", "2–5", "6–20", and "more than 20". The estimated mean numbers of tick bites were calculated as the weighted averages of the midpoints of these categories, weighted by their relative frequencies. The category "more than 20" was assigned a value of 30. The resulting numbers are presented for ease of perception only; the original categorical data were used for statistical analyses.

*Risk factors*

Risk factors were reported as continuous (age) and categorical (educational level, gross household income, hours spent outdoor during summertime). Responses on questions on daily smoking, hunting, orienteering, pet animals, domestic animals, doctor visits, and avoidance of certain areas were given as yes or no.

*Statistical analysis*

We used PASW Statistic for Windows version 18 (SPSS Inc., Chicago, Illinois) for statistical analyses. Because of multiple testing, all $p$ values were two-sided and values below 0.01 were considered statistical significant. All data were categorical and described as frequencies and percentages. Age was categorized into age groups. To examine the association of the number of self-reported tick bites with blood bank locations, we used the chi-square test. To examine whether one blood bank deviated significantly from the others, tick bite frequencies from each blood blank were compared with the total frequencies of the others, using Fisher's exact test.

The association between self-reported tick bites and various risk factors was analyzed by using proportional odds models, i.e., logistic regression models with more than 2 ordinal categorical outcomes (Armstrong and Sloan, 1989; Scott et al., 1997). By using this method, we avoided dichotomising the tick bites variable which possibly would have led to a loss of information and decreased statistical power (Scott et al., 1997). The method, however, assumes that the odds ratio for a risk factor is homogenous for all possible cut-points of the tick bite variable (i.e., the proportional odds property). Given this homogeneity, the odds ratio can be viewed as a constant and interpreted as the odds of being higher or lower on the tick bites variable across the entire range of the variable. In PASW, the odds ratio was estimated by using the "plum" command. The proportional odds assumption was tested using the "tparallel" option.

The odds ratios for tick bites with 95% confidence intervals were estimated with and without adjustment for the participants' age, gender, and blood bank localization, and we used the method of listwise deletion to handle missing values. A significant deviation from the proportional odds assumption was only seen in the analyses of tick bites in relation to outdoor hours per week during summertime, suggesting that the results from this variable should be interpreted with some caution. $p$ value for trend was obtained by including the risk factor as a linear term in the regression models.

## Results

The self-reported numbers of total and recent tick bites are presented in Table 2. The table also includes the respondents' subjective estimation of the occurrence of ticks in his/her living area. Among participants, 65.7% had experienced tick bites during their life time, whereas 30% had experienced tick bites during the latest 12 months.

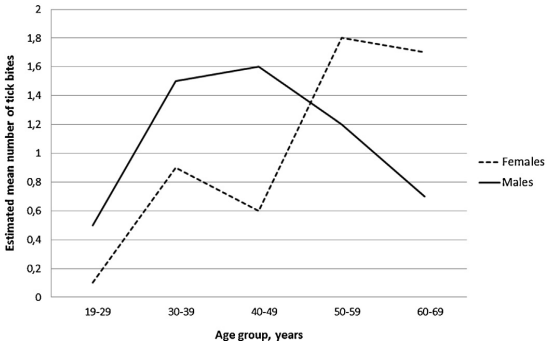


**Fig. 2.** Reported tick bites during the past 12 months according to age and gender in 1171 blood donors in Sogn and Fjordane, 2010. Number of tick bites was estimated by using midpoint averages as described in the methods.

*Ticks and tick bites in relation to geography*

Donors from the blood bank in Lærdal reported the lowest occurrence of ticks as well as the lowest number of tick bites, both total and recent. The estimated mean total number of tick bites of 1.5 from this area contrasts with 5.6–7.2 from the other locations.

*Age and gender*

The number of tick bites increased with age, and there was no gender difference in overall analyses (Table 3). In the younger age groups, however, males reported more bites than females (Fig. 2). In subjects older than 50 years old, this was reversed, with females reporting more tick bites then males. When testing for effect modification by gender on the relation between age group and tick bites, we found a statistically significant interaction both for total ($p = 0.008$) and recent tick bites ($p = 0.001$).

*Other risk factors*

Adjusted analyses further showed that both recent and total tick bites were more common among participants with the highest educational level, increased outdoor activity, and among hunters and owners of domestic animals (Table 3). Daily smokers reported fewer total tick bites.

*Consequences of tick bites*

Simple medical outcomes of tick bites are listed in Table 4. As most responders reported many bites, these percentages refer to the life-time risk of ever having experienced the symptoms and do not reflect the risk after each single bite. The most common occurrence was a rash around the bite, reported by 22.7% of the bitten respondents. Joint pain or swollen joints was reported by 2.0%, followed by headache (1.0%), fever (0.5%), and palsy in the face or elsewhere (0.5%). A total of 12.7% of the bitten persons reported to have seen a doctor because of tick bite or a consequence thereof, while 7.7% had received antibiotics.

Avoidance of certain areas because of concern for tick bites was reported by 15.7% of 1194 respondents in this study, 22.2% of women and 10.3% of men, respectively ($p < 0.001$, data not shown). There was no significant age difference.

**Table 2**
Self-perceived occurrence of ticks and reported tick bites among 1213 participants in the Tick-borne Infection Study in Sogn and Fjordane, 2010, according to geographical area (blood bank).

| | Total[a] | Blood bank | | | | $p^b$ |
|---|---|---|---|---|---|---|
| | | Førde *n* (%) | Florø *n* (%) | Lærdal *n* (%) | Nordfjordeid *n* (%) | |
| **Self-perceived occurrence of ticks in living area** | | | | | | <0.001 |
| *n* | 1193 (100.0) | 604 (50.6) | 350 (29.3) | 72 (6.0) | 167 (14.0) | |
| None (%) | 10.1 | 12.9 | 7.4 | 6.9 | 7.2 | |
| Little (%) | 21.5 | 22.7 | 19.1 | 37.5 | 15.6 | |
| Some (%) | 51.0 | 49.0 | 49.7 | 45.8 | 62.9 | |
| Many (%) | 17.4 | 15.4 | 23.7 | 9.7 | 14.4 | |
| $p^c$ | | 0.003 | 0.001 | 0.009 | 0.012 | |
| **Total tick bites ever experienced** | | | | | | <0.001 |
| *n* | 1194 (100.0) | 608 (50.9) | 349 (29.2) | 72 (6.0) | 165 (13.8) | |
| None (%) | 34.3 | 32.7 | 29.8 | 73.6 | 32.1 | |
| 1 (%) | 18.0 | 18.9 | 20.3 | 12.5 | 12.1 | |
| 2–5 (%) | 23.8 | 25.2 | 24.6 | 9.7 | 23.0 | |
| 6–20 (%) | 14.7 | 14.5 | 15.2 | 1.4 | 20.6 | |
| >20 (%) | 9.2 | 8.7 | 10.0 | 2.8 | 12.1 | |
| $p^c$ | | 0.594 | 0.282 | <0.001 | 0.037 | |
| Estimated mean no. of tick bites[4] | 5.7 | 5.6 | 6.0 | 1.5 | 7.2 | |
| **Recent tick bites (past 12 months)** | | | | | | 0.006 |
| *n* | 1194 (100.0) | 608 (50.9) | 349 (29.2) | 72 (6.0) | 165 (13.8) | |
| None (%) | 70.0 | 70.4 | 64.2 | 90.3 | 72.1 | |
| 1 (%) | 14.8 | 15.3 | 18.1 | 2.8 | 11.5 | |
| 2–5 (%) | 11.6 | 11.2 | 12.3 | 5.6 | 13.9 | |
| 6–20 (%) | 2.8 | 2.3 | 4.6 | 1.4 | 1.2 | |
| >20 (%) | 0.8 | 0.8 | 0.9 | 0.0 | 1.2 | |
| $p^c$ | | 0.859 | 0.017 | 0.002 | 0.309 | |
| Estimated mean no. of tick bites[d] | 1.2 | 1.1 | 1.5 | 0.4 | 1.1 | |

[a] Numbers do not add to 1213 due to missing data.
[b] *p* value for association between blood bank and self-reported tick occurrence/tick bites by using chi-square test.
[c] *p* value for difference between actual blood bank and the total of the others by Fisher's exact test.
[d] Number of tick bites were estimated by using midpoint averages as described in the Materials and methods section.

## Discussion

The main findings of this study are that about two thirds of healthy blood donors in Sogn and Fjordane had experienced tick bites, and about 30% during the preceding 12 months. There were significant differences according to geography, age, gender, education, outdoor time, animal contact, and smoking. Among bitten subjects, 22.7% had experienced a rash surrounding the bite.

Tick bite is a sine qua non for acquisition of tick-borne diseases. Whether or not an infectious agent is actually transmitted from tick to human depends on several additional factors, such as presence of transmissible agents in the tick and duration of tick feeding (Sood et al., 1997).

Data on tick bites and potential risk factors were collected from blood donors using a questionnaire. Blood donors are not completely representative of the general population. They are healthy, and children and persons over 70 years are not represented. As seen in Table 1, there was, however, a fair distribution regarding gender and age groups. The area surrounding the innermost part of the Sognefjord in our study was somewhat underrepresented, as were some municipalities distant from the blood banks. According to Norwegian blood bank regulations, persons that have been bitten by ticks should not donate blood for 4 weeks after the bite, and persons with suspected or verified Lyme borreliosis should not donate blood until 6 months after adequate treatment has been given (Comelli et al., 2011). Donors with recent tick bites and/or Lyme borreliosis may therefore be underrepresented. Not all tick bites are recognized by the bitten person, and thus will not be reported in a questionnaire survey like this (i.e., information bias). Responses to several of the questions are based on the respondents' subjective estimations, and the reliability of data must therefore be interpreted with caution.

Comparing our results with studies from other countries, we found that a lower percentage of the population has experienced tick bites than in Åland (Wahlberg, 1990), 66% as contrasted to 85% (Table 2). In a survey in Alsace, France, 16.5% of the general population had experienced one or more tick bites during the preceding year (Mitschler et al., 2004), which is lower than reported in our study (30%). Also Leiby and co-workers' findings from Connecticut and Wisconsin indicated a lower frequency of tick bites in blood donors than we found (Leiby et al., 2002). Explanations for these differences may include variations in tick abundance, general awareness of ticks, and outdoor and recreational habits in the respective populations.

Two Dutch and one Belgian study showed an uneven geographical distribution of tick bites in the different areas within the respective countries (de Mik et al., 1997; den Boon et al., 2004; Vanthomme et al., 2012). The difference in sampling methods (general practice versus blood donors) precludes any direct comparison with our findings other than stating that local geographical differences are an important risk factor for tick bites.

In accordance with current estimates of tick distribution as well as the variation of notified cases of systemic borreliosis within the county, we found a significantly lower number of tick bites in the blood donors from the easternmost blood bank of Lærdal. Although entirely based on their subjective estimate, the respondents from this blood bank also reported a correspondingly low occurrence of ticks in their living area.

As expected, we found a significant age-related trend in reported total tick bites. The somewhat lower reported number in the oldest group of 60–69 years may be related to sampling variation, a cohort effect in the oldest group reflecting a change in outdoor habits in the general population, or an increased density of ticks during the past decades. The same age trend was also present for recent tick bites.

Bennett and co-workers found in south-eastern Sweden that women aged 40 years or older had a 48% higher risk than men in the same age group for contracting tick bites, and a 42% higher risk

**Table 3**
Reported tick bites ever and past 12 months in 1213 blood donors in the Tick-borne Infection Study in Sogn and Fjordane, 2010, in relation to demographics and risk factors.

| Characteristics | *n* (%)[a] | Tick bites ever | | | Tick bites in the past 12 months | | |
|---|---|---|---|---|---|---|---|
| | | Odds ratio[b] (95% CI) | Adjusted odds ratio[c] (95% CI) | Estimated mean number of tick bites[d] | Odds ratio[b] (95% CI) | Adjusted odds ratio[c] (95% CI) | Estimated mean number of tick bites[d] |
| **Gender** | | | | | | | |
| Female | 535 (44.8) | 1 | 1 | 5.3 | 1 | 1 | 1.0 |
| Male | 659 (55.2) | 1.1 (0.9–1.3) | 1.1 (0.9–1.3) | 6.0 | 1.0 (0.8–1.3) | 1.1 (0.8–1.3) | 1.3 |
| *p* difference | | 0.500 | 0.634 | | 0.994 | 0.933 | |
| **Age** | | | | | | | |
| 19–29 | 79 (6.7) | 1 | 1 | 2.4 | 1 | 1 | 0.3 |
| 30–39 | 233 (19.9) | 1.7 (1.0–2.7) | 1.6 (1.0–2.6) | 4.7 | 3.1 (1.5–6.3) | 2.9 (1.4–6.1) | 1.2 |
| 40–49 | 411 (35.1) | 2.0 (1.3–3.1) | 1.9 (1.2–2.9) | 5.3 | 2.8 (1.4–5.7) | 2.8 (1.4–5.7) | 1.1 |
| 50–59 | 340 (29.0) | 3.1 (2.0–4.9) | 3.0 (1.9–4.8) | 7.6 | 4.3 (2.1–8.6) | 4.4 (2.2–9.0) | 1.5 |
| 60–69 | 108 (9.2) | 2.4 (1.4–4.2) | 2.4 (1.4–4.1) | 6.3 | 2.2 (1.0–4.8) | 2.3 (1.0–5.1) | 1.0 |
| *p* trend | | <0.001 | <0.001 | | 0.014 | 0.006 | |
| **Education** | | | | | | | |
| Primary school ≤9 years | 83 (7.0) | 1 | 1 | 3.8 | 1 | 1 | 0.7 |
| Secondary school | 593 (49.9) | 1.5 (1.0–2.3) | 1.9 (1.2–2.9) | 6.1 | 1.7 (1.0–3.0) | 1.7 (1.0–3.1) | 1.3 |
| University/college 1–4 years. | 338 (28.4) | 1.4 (0.9–2.1) | 1.8 (1.2–2.9) | 5.1 | 1.9 (1.0–3.4) | 2.1 (1.1–3.8) | 1.1 |
| University/college >4 years | 175 (14.7) | 1.9 (1.2–3.1) | 2.6 (1.6–4.2) | 6.5 | 2.6 (1.4–4.8) | 2.8 (1.5–5.3) | 1.2 |
| *p* trend | | 0.056 | 0.004 | | 0.003 | 0.001 | |
| **Household yearly gross income (EUR)** | | | | | | | |
| <50,000 | 154 (13.2) | 1 | 1 | 4.3 | 1 | 1 | 0.8 |
| 50–99,000 | 640 (54.7) | 1.4 (1.0–2.0) | 1.4 (1.0–1.9) | 5.9 | 1.6 (1.0–2.4) | 1.5 (1.0–2.3) | 1.1 |
| 100–150,000 | 352 (30.1) | 1.5 (1.1–2.1) | 1.4 (1.0–2.0) | 6.0 | 1.6 (1.0–2.5) | 1.4 (0.9–2.3) | 1.3 |
| >150,000 | 25 (2.1) | 0.8 (0.4–1.8) | 0.6 (0.3–1.4) | 3.3 | 1.5 (0.6–3.7) | 1.0 (0.4–2.6) | 1.2 |
| *p* trend | | 0.176 | 0.593 | | 0.097 | 0.500 | |
| **Daily smoking** | | | | | | | |
| No | 928 (82.0) | 1 | 1 | 5.9 | 1 | 1 | 1.3 |
| Yes | 204 (18.0) | 0.7 (0.6–1.0) | 0.7 (0.5–0.9) | 4.7 | 0.7 (0.5–1.0) | 0.7 (0.5–0.9) | 0.7 |
| *p* difference | | 0.032 | 0.007 | | 0.089 | 0.024 | |
| **Outdoor hours per week during summertime** | | | | | | | |
| ≤5 | 275 (23.1) | 1 | 1 | 4.1 | 1 | 1 | 0.5 |
| 6–10 | 409 (34.3) | 1.2 (0.9–1.6) | 1.2 (0.9–1.7) | 4.8 | 1.5 (1.1–2.2) | 1.6 (1.1–2.3) | 0.9 |
| >10 | 508 (42.6) | 1.7 (1.3–2.2) | 1.8 (1.3–2.3) | 7.2 | 2.0 (1.5–2.9) | 2.2 (1.6–3.1) | 1.7 |
| *p* trend | | <0.001 | <0.001 | | <0.001 | <0.001 | |
| **Hunting past 12 months** | | | | | | | |
| No | 956 (80.3) | 1 | 1 | 5.0 | 1 | 1 | 0.8 |
| Yes | 235 (19.7) | 1.8 (1.4–2.3) | 2.3 (1.7–3.1) | 8.5 | 2.6 (2.0–3.5) | 3.7 (2.7–5.2) | 2.5 |
| *p* difference | | <0.001 | <0.001 | | <0.001 | <0.001 | |
| **Ever active orienteer** | | | | | | | |
| No | 1124 (94.5) | 1 | 1 | 5.5 | 1 | 1 | 1.1 |
| Yes | 65 (5.5) | 1.5 (1.0–2.4) | 1.5 (0.9–2.3) | 8.3 | 1.8 (1.1–3.0) | 1.8 (1.1–3.0) | 2.3 |
| *p* difference | | 0.054 | 0.110 | | 0.015 | 0.025 | |
| **Cat or dog owner** | | | | | | | |
| No | 637 (53.7) | 1 | 1 | 5.4 | 1 | 1 | 1.1 |
| Yes | 549 (46.3) | 1.2 (1.0–1.5) | 1.2 (1.0–1.5) | 6.1 | 1.4 (1.1–1.8) | 1.4 (1.1–1.8) | 1.2 |
| *p* difference | | 0.076 | 0.086 | | 0.007 | 0.011 | |
| **Domestic animals** | | | | | | | |
| No | 1024 (86.6) | 1 | 1 | 5.4 | 1 | 1 | 1.1 |
| Yes | 159 (13.4) | 1.5 (1.1–2.0) | 1.6 (1.2–2.2) | 7.7 | 1.7 (1.3–2.4) | 1.9 (1.3–2.7) | 1.6 |
| *p* difference | | 0.010 | 0.002 | | 0.001 | <0.001 | |

[a] Numbers do not add to 1213 due to missing data. Those who responded answered both questions (tick bites ever and past 12 months).
[b] Odds ratio was estimated using proportional odds ratio models (logistic regression model with more than two categorical outcomes).
[c] Adjusted for gender, age group, and blood bank.
[d] Number of tick bites were estimated by using midpoint averages as described in the method.

**Table 4**
Symptoms, doctor visits and antibiotic treatment ever following a tick bite among 875 bitten subjects in the Tick-borne Infection Study in Sogn and Fjordane, 2010.

| **Symptoms ($n$ = 785)** | $n$ (%) |
|---|---|
| A rash around the bite | 178 (22.7) |
| Palsy in face or elsewhere | 4 (0.5) |
| Fever | 4 (0.5) |
| Headache | 8 (1.0) |
| Joint pain or swollen joints | 16 (2.0) |
| **Seen a doctor because of tick bite ($n$ = 778)** | 99 (12.7) |
| **Received antibiotics because of tick bite or consequence thereof ($n$ = 777)** | 60 (7.7) |

than younger women (Bennet et al., 2007). A similar tendency is also apparent in our study for subjects 50 years or older (Fig. 2). A plausible hypothesis for the increased risk in elderly women could be the traditional, but now declining custom of picking wild berries (e.g. blueberries, cloudberries, lingonberries) for the household, showing a similar distribution in age and gender (Vaage, 2004). Unfortunately, questions on this were not included in the questionnaire. Other hypotheses could be related to other behavioural or biological aspects of postmenopausal women, as discussed by Bennet et al. (2007). The reported drop in risk of tick bites in elderly men as opposed to women is not easy to explain and might be due to behavioural differences in exposure to ticks or to recollection errors. Cohort effects might be important.

We found that tick bites were more common among participants with the highest educational level. This is in accordance with observed life style differences in other studies (Vaage, 2004). We found no relation between risk of tick bites and household yearly gross income.

After adjustment for gender, age, and blood bank, daily smoking seemed to protect against tick bites according to our material ($p = 0.007$), but after statistical correction also for outdoor hours per week, this association was no longer significant ($p = 0.017$).

As expected, the number of outdoor hours per week during summertime showed a significant relationship to the risk of tick bites (Table 3). The estimated mean number of recent tick bites increased from 0.5 in those spending <5 h to 1.7 in subjects spending more than 10 h outdoors per week. Similarly, Stjernberg and Berglund found a 4% risk of being tick-bitten per 10 hours spent outdoors in an endemic area in Sweden (Stjernberg and Berglund, 2002).

Tick bites were more common among participants having domestic animals (sheep, cows, horses, etc.). This group of respondents are probably mostly full or part-time farmers, expected to spend much time in tick habitats.

Hunting, especially for deer, is a common leisure activity in Sogn and Fjordane. More than 11,000 deer were hunted in the county in 2009. We found an increased risk of tick bites in the group of hunters, even after adjustment for "hours spent outdoors per week in summertime" ($p < 0.001$ both for total and recent bites). However, this question does not discriminate between tick-infested and non-infested outdoor areas, and there is reason to believe that the increased risk in hunters reflects their activity in tick habitats.

Among our respondents, 65 (5.5%) reported to have been active orienteers. A Swiss study published in 1991 found that 78% of 995 orienteers had experienced at least one tick bite (Fahrer et al., 1991). In our material, that was the case for 92.3% (data not shown). A Swedish study found that 53% of Swedish orienteers were bitten by ticks in one year (Gustafson et al., 1993), and we found a similar rate of 53.8% (data not shown). We found that orienteers reported more tick bites than non-orienteers, but this association was not statistically significant at alpha level 0.01, possibly due to small numbers (Table 3).

Several reports have investigated the risk for developing disease after a single tick bite, and most of them find the risk to be low (Fryland et al., 2011; Jacobs et al., 2008; Maiwald et al., 1998; Nadelman et al., 2001; Nahimana et al., 2004; Shapiro et al., 1992; Stjernberg and Berglund, 2002).

Wahlberg (1990) found that out of 441 bitten subjects, 14 reported ever having had erythema chronicum migrans, and 73 had had other rashes around the tick bite, all together 19.7%. In our material, we found a similar frequency of "rash" of 22.7%. We did not try to discern between erythema migrans and other rashes, as we believe that to be an error-prone task in a retrospective questionnaire survey.

Outdoor activities are in general regarded as positive for public health and quality of life, and it would be a concern if the population chose to avoid certain recreational outdoor areas in fear of tick bites. In our study, 15.7% reported such avoidance. Whether this is a sensible approach is difficult to ascertain. The marked gender difference, with avoiding behaviour in twice as many women than men, is not easy to explain. It may reflect a general gender difference in risk avoidance.

In conclusion, a majority of blood donors in the western part of Norway had experienced tick bites. Age, gender, outdoor activities, animal contact, and geography affected the risk. Symptoms of disease were seldom reported.

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## References

Andreassen, A., Jore, S., Cuber, P., Dudman, S., Tengs, T., Isaksen, K., Hygen, H., Viljugrein, H., Anestad, G., Ottesen, P., Vainio, K., 2012. Prevalence of tick borne encephalitis virus in tick nymphs in relation to climatic factors on the southern coast of Norway. Parasites Vectors 5, 177.

Armstrong, B.G., Sloan, M., 1989. Ordinal models for epidemiologic data. Am. J. Epidemiol. 129, 191–204.

Bakken, J.S., Krueth, J., Tilden, R.L., Dumler, J.S., Kristiansen, B.E., 1996. Serological evidence of human granulocytic ehrlichiosis in Norway. Eur. J. Clin. Microbiol. Infect. Dis. 15, 829–832.

Bennet, L., Stjernberg, L., Berglund, J., 2007. Effect of gender on clinical and epidemiologic features of Lyme borreliosis. Vector Borne Zoonotic Dis. 7, 34–41.

Comelli, H.L., Andreassen, Å., Dao, P., Dudman, S., Noraas, S., Ottesen, P., Sandbu, S., Skarpaas, T., Soleng, A., Sundal, J., Vainio, K., Aaberge, I., Vold, L., 2011. Annual Report. Ticks and Tick-borne Diseases 2010. The Norwegian Institute of Public Health (in Norwegian).

de Mik, E.L., van Pelt, W., Docters-van Leeuwen, B.D., van der Veen, A., Schellekens, J.F., Borgdorff, M.W., 1997. The geographical distribution of tick bites and erythema migrans in general practice in The Netherlands. Int. J. Epidemiol. 26, 451–457.

den Boon, S., Schellekens, J.F., Schouls, L.M., Suijkerbuijk, A.W., Docters van Leeuwen, B., van Pelt, W., 2004. Doubling of the number of cases of tick bites and Lyme borreliosis seen by general practitioners in the Netherlands. Ned. Tijdschr. Geneeskd. 148, 665–670 (in Dutch).

Fahrer, H., van der Linden, S.M., Sauvain, M.J., Gern, L., Zhioua, E., Aeschlimann, A., 1991. The prevalence and incidence of clinical and asymptomatic Lyme borreliosis in a population at risk. J. Infect. Dis. 163, 305–310.

Fryland, L., Wilhelmsson, P., Lindgren, P.E., Nyman, D., Ekerfelt, C., Forsberg, P., 2011. Low risk of developing *Borrelia burgdorferi* infection in the south-east of Sweden after being bitten by a *Borrelia burgdorferi*-infected tick. Int. J. Infect. Dis. 15, e174–e181.

Gustafson, R., Forsgren, M., Gardulf, A., Granström, M., Svenungsson, B., 1993. Antibody prevalence and clinical manifestations of Lyme borreliosis and tick-borne encephalitis in Swedish orienteers. Scand. J. Infect. Dis. 25, 605–611.

Jacobs, J.J., Noordhoek, G.T., Brouwers, J.M., Wielinga, P.R., Jacobs, J.P., Brandenburg, A.H., 2008. Small risk of developing Lyme borreliosis following a tick bite on Ameland: research in a general practice. Ned. Tijdschr. Geneeskd. 152, 2022–2026 (in Dutch).

Jore, S., Viljugrein, H., Hofshagen, M., Brun-Hansen, H., Kristoffersen, A., Nygard, K., Brun, E., Ottesen, P., Saevik, B., Ytrehus, B., 2011. Multi-source analysis reveals

latitudinal and altitudinal shifts in range of *Ixodes ricinus* at its northern distribution limit. Parasites Vectors 4, 84.

Kjelland, V., Stuen, S., Skarpaas, T., Slettan, A., 2010. Prevalence and genotypes of *Borrelia burgdorferi* sensu lato infection in *Ixodes ricinus* ticks in southern Norway. Scand. J. Infect. Dis. 42, 579–585.

Kristiansen, B.E., Jenkins, A., Tveten, Y., Karsten, B., Line, O., Bjöersdorff, A., 2001. Human granulocytic ehrlichiosis in Norway. Tidsskr. Nor. Laegeforen. 121, 805–806 (in Norwegian).

Leiby, D.A., Chung, A.P., Cable, R.G., Trouern-Trend, J., McCullough, J., Homer, M.J., Reynolds, L.D., Houghton, R.L., Lodes, M.J., Persing, D.H., 2002. Relationship between tick bites and the seroprevalence of *Babesia microti* and *Anaplasma phagocytophila* (previously *Ehrlichia* sp.) in blood donors. Transfusion 42, 1585–1591.

Maiwald, M., Oehme, R., March, O., Petney, T.N., Kimmig, P., Naser, K., Zappe, H.A., Hassler, D., von Knebel Doeberitz, M., 1998. Transmission risk of *Borrelia burgdorferi* sensu lato from *Ixodes ricinus* ticks to humans in southwest Germany. Epidemiol. Infect. 121, 103–108.

Mehl, R., 1983. The distribution and host relations of Norwegian ticks (*Acari Ixodides*). Fauna Norv. Ser. B 30, 46–51.

Mitschler, A., Grange, F., Lipsker, D., Jaulhac, B., Piemont, Y., Belanger, P., Pagnon, X., Mayer, O., Guillaume, J.C., 2004. Knowledge and prevention of tick-bite borreliosis: survey of the population in Alsace, an endemic area. Ann. Dermatol. Venereol. 131, 547–553 (in French).

Nadelman, R.B., Nowakowski, J., Fish, D., Falco, R.C., Freeman, K., McKenna, D., Welch, P., Marcus, R., Aguero-Rosenfeld, M.E., Dennis, D.T., Wormser, G.P., 2001. Prophylaxis with single-dose doxycycline for the prevention of Lyme disease after an *Ixodes scapularis* tick bite. N. Engl. J. Med. 345, 79–84.

Nahimana, I., Gern, L., Blanc, D.S., Praz, G., Francioli, P., Péter, O., 2004. Risk of *Borrelia burgdorferi* infection in western Switzerland following a tick bite. Eur. J. Clin. Microbiol. Infect. Dis. 23, 603–608.

Scott, S.C., Goldberg, M.S., Mayo, N.E., 1997. Statistical assessment of ordinal outcomes in comparative studies. J. Clin. Epidemiol. 50, 45–55.

Shapiro, E.D., Gerber, M.A., Holabird, N.B., Berg, A.T., Feder Jr., H.M., Bell, G.L., Rys, P.N., Persing, D.H., 1992. A controlled trial of antimicrobial prophylaxis for Lyme disease after deer-tick bites. N. Engl. J. Med. 327, 1769–1773.

Skarpaas, T., Ljøstad, U., Sundøy, A., 2004. First human cases of tickborne encephalitis, Norway. Emerg. Infect. Dis. 10, 2241–2243.

Sood, S.K., Salzman, M.B., Johnson, B.J., Happ, C.M., Feig, K., Carmody, L., Rubin, L.G., Hilton, E., Piesman, J., 1997. Duration of tick attachment as a predictor of the risk of Lyme disease in an area in which Lyme disease is endemic. J. Infect. Dis. 175, 996–999.

Stjernberg, L., Berglund, J., 2002. Risk of acquiring tick bites in south-eastern Sweden. Scand. J. Infect. Dis. 34, 840–844.

Tambs-Lyche, H., 1943. *Ixodes ricinus* og piroplasmosen i Norge (Meddelelse fra Bergens Museums zoologiske avdeling). Norsk Veterinærtidsskrift 55, 337–542 (in Norwegian).

Vaage, O.F., 2004. Training, exercise and outdoor activities, Reports 2004/13 Statistics Norway, (in Norwegian).

Vanthomme, K., Bossuyt, N., Boffin, N., Van Casteren, V., 2012. Incidence and management of presumption of Lyme borreliosis in Belgium: recent data from the sentinel network of general practitioners. Eur. J. Clin. Microbiol. Infect. Dis. 31, 2385–2390.

Wahlberg, P., 1990. Incidence of tick-bite in man in Aland Islands: reference to the spread of Lyme borreliosis. Scand. J. Infect. Dis. 22, 59–62.

# II

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# 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 burgorferi*; 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. burdgdorferi* sensu lato in blood

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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 Immunetics C6 Lyme ELISA kit (Immunetics, 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 Euro-Blot 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 statistical 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 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).

The associations between exposure variables, i.e. self-reported tick bites and other risk factors, and the outcomes, i.e. the prevalence rates for antibodies, were analysed by binary logistic regression. The odds ratios with 95% confidence intervals were estimated with and without adjustment for the participants' age, gender, and blood bank location as appropriate.

## 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 (%[1]) | Blot IgG, n (%[2]) | | | Blot IgM, n (%[2]) | | |
|---|---|---|---|---|---|---|---|
| | | – | +/– | + | – | +/– | + |
| IgG+ | 117 (9.6) | 30 (25.6) | 13 (11.1) | 74 (63.2) | 71 (60.7) | 12 (10.3) | 34 (29.1) |
| IgM+ | 99 (8.2) | 41 (41.4) | 24 (24.2) | 34 (34.3) | 27 (27.3) | 12 (12.1) | 60 (60.6) |
| C6+ | 102 (8.4) | 22 (21.6) | 10 (9.8) | 70 (68.6) | 66 (64.7) | 8 (7.8) | 28 (27.5) |
| IgG and/or IgM and/or C6+ | 198 (16.3) | 86 (43.4) | 34 (17.2) | 78 (39.4) | 109 (55.1) | 20 (10.1) | 69 (34.8) |
| IgG+, IgM+, C6+ | 31 (2.6) | 0 (0.0) | 2 (6.5) | 29 (93.5) | 6 (19.4) | 4 (12.9) | 21 (67.7) |
| IgG+, IgM+, C6– | 13 (1.1) | 4 (30.8) | 6 (46.2) | 3 (23.1) | 5 (38.5) | 1 (7.7) | 7 (53.8) |
| IgG+, IgM–, C6+ | 44 (3.6) | 3 (6.8) | 2 (4.5) | 39 (88.6) | 38 (86.4) | 3 (6.8) | 3 (6.8) |
| IgG+, IgM–, C6– | 29 (2.4) | 23 (79.3) | 3 (10.3) | 3 (10.3) | 22 (75.9) | 4 (13.8) | 3 (10.3) |
| IgG–, IgM+, C6+ | 1 (0.1) | 0 (0.0) | 1 (100.0) | 0 (0.0) | 0 (0.0) | 1 (100.0) | 0 (0.0) |
| IgG–, IgM+, C6– | 54 (4.5) | 37 (68.5) | 15 (27.8) | 2 (3.7) | 16 (29.6) | 7 (13.0) | 31 (57.4) |
| IgG–, IgM–, C6+ | 26 (2.1) | 19 (73.1) | 5 (19.2) | 2 (7.7) | 22 (84.6) | 1 (3.8) | 3 (11.5) |
| 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.
[1]Percentages of the total population of 1213.
[2]Percentages within this group.

**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 and Fjordane, Norway, 2010 (n = 1213)

| | n[1] (%) | IgG | | | IgM | | |
|---|---|---|---|---|---|---|---|
| | | Positive IgG (%) | Odds ratio[2] (95% CI) | Adjusted odds ratio[3] (95% CI) | Positive IgM (%) | Odds ratio[2] (95% CI) | Adjusted odds ratio[3] (95% CI) |
| Tick bites ever | | | | | | | |
| None | 409 (34.3) | 4.9 | 1 | 1 | 5.4 | 1 | 1 |
| One | 215 (18.0) | 9.3 | 2.0 (1.0–3.8) | 1.9 (1.0–3.7) | 7.0 | 1.3 (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 (1.2–4.0) | 8.5 | 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 (1.2–4.6) | 11.9 | 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 (1.7–6.6) | 12.7 | 2.6 (1.3–5.2) | 2.3 (1.1–4.8) |
| p trend | | | <0.001 | <0.001 | | 0.001 | 0.004 |
| Tick bites last 12 months | | | | | | | |
| None | 836 (70.0) | 7.7 | 1 | 1 | 6.2 | 1 | 1 |
| One | 177 (14.8) | 14.1 | 2.0 (1.2–3.3) | 1.9 (1.1–3.3) | 13.6 | 2.4 (1.4–4.0) | 2.3 (1.4–3.9) |
| 2–5 | 138 (11.6) | 10.9 | 1.5 (0.8–2.7) | 1.6 (0.9–2.9) | 10.1 | 1.7 (0.9–3.2) | 1.8 (0.9–3.3) |
| 6–20 | 33 (2.8) | 21.2 | 3.2 (1.4–7.8) | 3.0 (1.2–7.6) | 12.1 | 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 (1.4–22.9) | 20.0 | 3.8 (0.8–18.2) | 3.6 (0.7–17.6) |
| p trend | | | <0.001 | 0.001 | | 0.003 | 0.005 |

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

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.

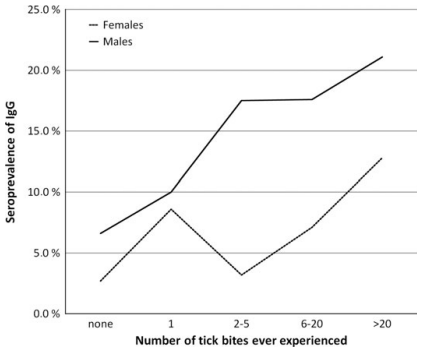


**Fig. 1.** Relationship of number of tick bites experienced with seroprevalence of IgG against *Borrelia burgdorferi* s.l. in the Tick-borne Infections Study in Sogn og Fjordane, Norway, 2010 (n = 1194).

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, and conversely, all C6 stronger than 465% of the cut off were positive in IgG. Similarly, we found that IgG reactions stronger than 252% of the cut off (34 U/mL) were positive in IgG 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 IgG 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 IgG 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 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] |
|---|---|---|---|---|---|---|
| | | Førde | Florø | Lærdal | Nordfjordeid | |
| n (%) | 1213 (100.0) | 614 (50.6) | 355 (29.3) | 73 (6.0) | 171 (14.1) | |
| IgG (%) | 9.6 | 10.7 | 7.6 | 2.7 | 12.9 | 0.028 |
| p[2] | | 0.111 | 0.073 | 0.021 | 0.084 | |
| IgM (%) | 8.2 | 8.8 | 8.2 | 4.1 | 7.6 | 0.617 |
| 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] | | 1.000 | 0.367 | 1.000 | 0.188 | |
| IgG only (%) | 6.0 | 6.5 | 4.8 | 2.7 | 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 | 0.015 |
| p[2] | | 0.004 | 0.053 | 0.081 | 0.768 | |

[1]p-value for association between blood bank and antibody prevalence by using chi-squared test.
[2]p-value for difference between actual blood bank and the total of the others by Fisher's exact test.

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 Immunetics 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).

Borrelia 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 Førde 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

**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[1] (%) | IgG | | | IgM | | |
|---|---|---|---|---|---|---|---|
| | | Positive IgG (%) | Odds ratio[2] (95% CI) | Adjusted odds ratio[3] (95% CI) | Positive IgM (%) | Odds ratio[2] (95% CI) | Adjusted odds ratio[3] (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 | | 0.768 | 0.845 |
| 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 | | 0.016 | 0.017 |
| 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 |

Table 4 *(continued)*

| Characteristic | n[1] (%) | IgG | | | IgM | | |
|---|---|---|---|---|---|---|---|
| | | Positive IgG (%) | Odds ratio[2] (95% CI) | Adjusted odds ratio[3] (95% CI) | Positive IgM (%) | Odds ratio[2] (95% CI) | Adjusted odds ratio[3] (95% CI) |
| Cat or dog owner | | | | | | | |
| No | 639 (53.5) | 11.6 | 1 | 1 | 7.8 | 1 | 1 |
| Yes | 555 (46.5) | 6.8 | 0.6 (0.4–0.8) | 0.6 (0.4–0.9) | 8.1 | 1.0 (0.7–1.6) | 1.1 (0.7–1.7) |
| p difference | | | 0.006 | 0.027 | | 0.857 | 0.659 |
| Domestic animals | | | | | | | |
| No | 1030 (86.6) | 9.6 | 1 | 1 | 8.3 | 1 | 1 |
| Yes | 159 (13.4) | 7.5 | 0.8 (0.4–1.4) | 0.8 (0.4–1.6) | 6.9 | 0.8 (0.4–1.6) | 0.9 (0.4–1.6) |
| p difference | | | 0.406 | 0.646 | | 0.566 | 0.610 |

[1]Numbers do not add to 1213 due to missing data.
[2]Odds ratio was estimated using binary logistic regression.
[3]Adjusted for gender, age group and blood bank, as appropriate.

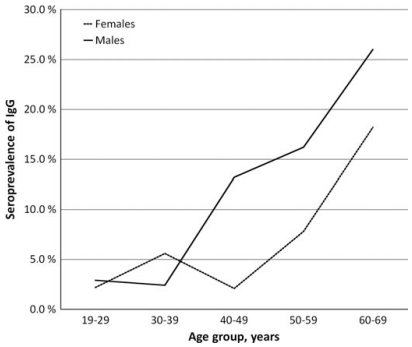


**Fig. 2.** Prevalence of *Borrelia* IgG (Enzygnost) according to gender and age group in the Tick-borne Infection Study in Sogn and Fjordane, Norway, 2010 (n = 1183).

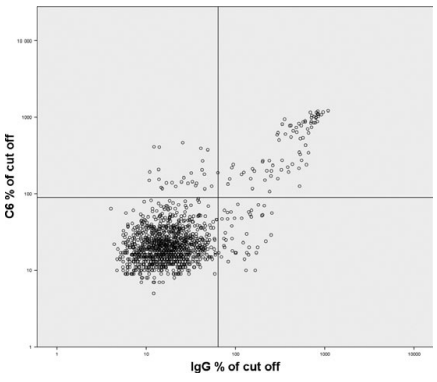


**Fig. 3.** Relationship between quantitative results of *Borrelia* antibodies in Enzygnost IgG and C6 ELISA in the Tick-borne Infections Study in Sogn og Fjordane, Norway, 2010 (n = 1213). The *X*-axis represents Enzygnost IgG reactivity, while the *Y*-axis represents C6 reactivity, both in percentage of cut off. The vertical and horizontal lines mark the boundary between negative and grey-zone results for the two tests, at 64% and 90% of the cut-off value, respectively. Note the logarithmic scale of both axes.

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

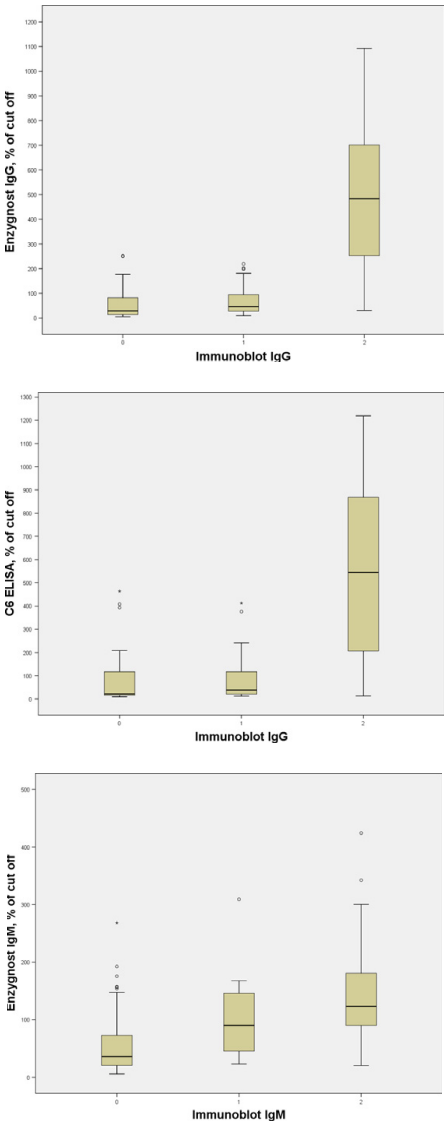


**Fig. 4.** Box plots of the relationship between strength of reaction in ELISA and result of immunoblot in 198 sera reactive in one or more ELISAs. The circles denote outliers and the stars are extreme values. 0 = negative, 1 = indeterminate, 2 = positive.

statistically significant difference between the genders across all age groups was found, although men reported some more tick bites (4). Also, women

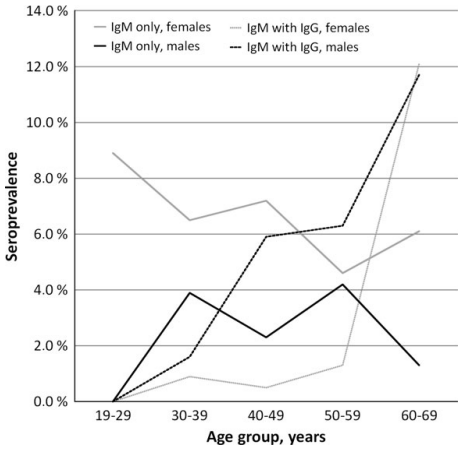


**Fig. 5.** Prevalence of *Borrelia* IgM alone and accompanying IgG (Enzygnost) according to gender and age group in the Tick-borne Infection Study in Sogn and Fjordane, Norway, 2010 (n = 1183).

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 Dehnert 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. Immunoblots 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, immunoblots 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 IgM 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-adv', 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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## REFERENCES

1. Jore S, Viljugrein H, Hofshagen M, Brun-Hansen H, Kristoffersen A, Nygard K, et al. Multi-source analysis reveals latitudinal and altitudinal shifts in range of Ixodes ricinus at its northern distribution limit. Parasit Vectors 2011;4:84.
2. Mehl R. The distribution and host relations of Norwegian ticks (Acari, Ixodides). Fauna norvegica Series B 1983;30:46–51.
3. Tambs-Lyche H. Ixodes ricinus og piroplasmosen i Norge (Meddelelse fra Bergens Museums zoologiske avdeling). Norsk veterinærtidsskrift 1943;55:337–42.
4. Hjetland R, Eliassen KE, Lindbaek M, Nilsen RM, Grude N, Ulvestad E. Tick bites in healthy adults from western Norway: occurrence, risk factors, and outcomes. Ticks Tick Borne Dis 2013;4:304–10.
5. Wormser GP, Schriefer M, Aguero-Rosenfeld ME, Levin A, Steere AC, Nadelman RB, et al. Single-tier testing with the C6 peptide ELISA kit compared with two-tier testing for Lyme disease. Diagn Microbiol Infect Dis 2013;75:9–15.
6. Mhalu FS, Matre R. Serological evidence of Lyme borreliosis in Africa: results from studies in Dar es Salaam, Tanzania. East Afr Med J 1996;73:583–5.
7. Tjernberg I, Kräger G, Eliasson I. C6 peptide ELISA test in the serodiagnosis of Lyme borreliosis in Sweden. Eur J Clin Microbiol Infect Dis 2007;26:37–42.
8. Dessau R, Eliasson I, Skarpaas T, Nyman D. Testing for Lyme borreliosis in the Nordic countries - variations in strategies and rate of seropositivity. Report from a survey of laboratory methods used in Scandinavia. 2011. Available at http://www.dskm.dk/pdf/rapporter/Serological%20testing%20for%20Lyme.pdf (accessed 03 June 2013).
9. Mygland A, Skarpaas T, Ljostad U. Chronic polyneuropathy and Lyme disease. Eur J Neurol 2006;13:1213–5.
10. Centers for Disease Control and Prevention. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. JAMA 1995;274:937.
11. Brouqui P, Bacellar F, Baranton G, Birtles RJ, Bjoersdorff A, Blanco JR, et al. Guidelines for the diagnosis of tick-borne bacterial diseases in Europe. Clin Microbiol Infect 2004;10:1108–32.
12. Ang C, Notermans D, Hommes M, Simoons-Smit A, Herremans T. Large differences between test strategies for the detection of anti-Borrelia antibodies are revealed by comparing eight ELISAs and five immunoblots. Eur J Clin Microbiol Infect Dis 2011;30:1027–32.
13. Dessau RB, Bangsborg JM, Jensen TP, Hansen K, Lebech AM. Andersen CO [Laboratory diagnosis of infection caused by Borrelia burgdorferi]. Ugeskr Laeger 2006;168:2805–7.
14. Grude N, Tveten Y, Stutzer A, Hoddevik G, Aaberge IS. Strategimøte 3. November 2011: Laboratoriediagnostikk ved borreliose. Oslo: Norwegian Institute of Public Health, 2012.

15. Harbo S. Diagnostikk og behandling av Lyme borreliose. Rapport til Helsedirektoratet fra arbeidsgruppen. November 2009. Oslo: Helsedirektoratet, 2009.
16. Steere AC, McHugh G, Damle N, Sikand VK. Prospective study of serologic tests for lyme disease. Clin Infect Dis 2008;47:188–95.
17. Branda JA, Linskey K, Kim YA, Steere AC, Ferraro MJ. Two-tiered antibody testing for Lyme disease with use of 2 enzyme immunoassays, a whole-cell sonicate enzyme immunoassay followed by a VlsE C6 peptide enzyme immunoassay. Clin Infect Dis 2011;53:541–7.
18. Branda JA, Strle F, Strle K, Sikand N, Ferraro MJ, Steere AC. Performance of United States serologic assays in the diagnosis of Lyme borreliosis acquired in Europe. Clin Infect Dis 2013;57:333–40.
19. Schoen RT. Editorial commentary: better laboratory testing for Lyme disease: no more Western blot. Clin Infect Dis 2013;57:341–3.
20. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977;33:159–74.
21. Stanek G, Wormser GP, Gray J, Strle F. Lyme borreliosis. Lancet 2012;379:461–73.
22. Panelius J, Lahdenne P, Heikkila T, Peltomaa M, Oksi J, Seppala I. Recombinant OspC from Borrelia burgdorferi sensu stricto, B. afzelii and B. garinii in the serodiagnosis of Lyme borreliosis. J Med Microbiol 2002;51:731–9.
23. Ulvestad E, Kanestrom A, Sonsteby LJ, Jureen R, Omland T, Edvardsen B, et al. Diagnostic and biological significance of anti-p41 IgM antibodies against Borrelia burgdorferi. Scand J Immunol 2001;53:416–21.
24. Berglund J, Eitrem R, Ornstein K, Lindberg A, Ringer A, Elmrud H, et al. An epidemiologic study of Lyme disease in southern Sweden. N Engl J Med 1995;333:1319–27.
25. Strle F, Nadelman RB, Cimperman J, Nowakowski J, Picken RN, Schwartz I, et al. Comparison of culture-confirmed erythema migrans caused by Borrelia burgdorferi sensu stricto in New York State and by Borrelia afzelii in Slovenia. Ann Intern Med 1999;130:32–6.
26. Jarefors S, Bennet L, You E, Forsberg P, Ekerfelt C, Berglund J, et al. Lyme borreliosis reinfection: might it be explained by a gender difference in immune response? Immunology 2006;118:224–32.
27. Bennet L, Stjernberg L, Berglund J. Effect of gender on clinical and epidemiologic features of Lyme borreliosis. Vector Borne Zoonotic Dis 2007;7:34–41.
28. Strle F, Wormser GP, Mead P, Dhaduvai K, Longo MV, Adenikinju O, et al. Gender disparity between cutaneous and non-cutaneous manifestations of lyme borreliosis. PLoS ONE 2013;8:e64110.
29. Dehnert M, Fingerle V, Klier C, Talaska T, Schlaud M, Krause G, et al. Seropositivity of Lyme borreliosis and associated risk factors: a population-based study in Children and Adolescents in Germany (KiGGS). PLoS ONE 2012;7:e41321.
30. Hubalek Z. Epidemiology of lyme borreliosis. Curr Probl Dermatol 2009;37:31–50.
31. Weinstein A. Editorial commentary: laboratory testing for Lyme disease: time for a change? Clin Infect Dis 2008;47:196–7.
32. Marangoni A, Sparacino M, Cavrini F, Storni E, Mondardini V, Sambri V, et al. Comparative evaluation of three different ELISA methods for the diagnosis of early culture-confirmed Lyme disease in Italy. J Med Microbiol 2005;54:361–7.
33. Philipp MT, Marques AR, Fawcett PT, Dally LG, Martin DS. C6 test as an indicator of therapy outcome for patients with localized or disseminated Lyme borreliosis. J Clin Microbiol 2003;41:4955–60.
34. Philipp MT, Wormser GP, Marques AR, Bittker S, Martin DS, Nowakowski J, et al. A decline in C6 antibody titer occurs in successfully treated patients with culture-confirmed early localized or early disseminated Lyme borreliosis. Clin Diagn Lab Immunol 2005;12:1069–74.
35. Fleming RV, Marques AR, Klempner MS, Schmid CH, Dally LG, Martin DS, et al. Pre-treatment and post-treatment assessment of the C(6) test in patients with persistent symptoms and a history of Lyme borreliosis. Eur J Clin Microbiol Infect Dis 2004;23:615–18.
36. Peltomaa M, McHugh G, Steere AC. Persistence of the antibody response to the VlsE sixth invariant region (IR6) peptide of Borrelia burgdorferi after successful antibiotic treatment of Lyme disease. J Infect Dis 2003;187:1178–86.
37. Probst C, Ott A, Scheper T, Meyer W, Stocker W, Komorowski L. N-terminal disulfide-bridging of Borrelia outer surface protein C increases its diagnostic and vaccine potentials. Ticks Tick Borne Dis 2012;3:1–7.
38. Dessau RB, Bangsborg JM, Ejlertsen T, Skarphedinsson S, Schonheyder HC. Utilization of serology for the diagnosis of suspected Lyme borreliosis in Denmark: survey of patients seen in general practice. BMC Infect Dis 2010;10:317.

IV

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# Subjective health complaints are not associated with tick bites or antibodies to *Borrelia burgdorferi* sensu lato in blood donors in western Norway: a cross-sectional study

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## Abstract

**Background:** There is controversy about chronic health consequences of tick-borne infections, especially Lyme borreliosis. This study aims to assess whether general function, physical fitness and subjective health complaints are associated with tick bites or antibodies to *Borrelia burgdorferi* sensu lato in blood donors.

**Methods:** Sera from 1,213 blood donors at four different blood banks in Sogn and Fjordane county in western Norway were obtained during January to June 2010, and analysed for specific IgG and IgM antibodies. A questionnaire including questions on tick bites, subjective health complaints, general function and physical fitness was completed.

**Results:** Tick bites had been experienced by 65.7 % of the study population. 78 (6.4 %) were positive for IgG (9.7 % in men, 2.4 % in women), and 69 (5.7 %) for IgM (6.1 % in men, 5.1 % in women), verified by immunoblot. No association between number of experienced tick bites or seropositivity for Borrelia antibodies and subjective health complaints, reduced general function or reduced physical fitness was found.

**Conclusion:** The results do not support any association between tick bites or Borrelia antibodies and subjective health complaints in blood donors in an endemic area for Lyme borreliosis.

**Keywords:** Lyme disease, Lyme borreliosis, Blood donors, Subjective health complaints, Tick bites

## Background

There is much controversy about chronic health consequences of treated and untreated Lyme borreliosis (LB) [1–4]. Symptoms from many organ systems are ascribed to "chronic LB", including fatigue, stiff neck, migrating arthralgias, myalgia, chest pain, palpitations, abdominal pain, nausea, back pain, headaches, etc. [3]. In addition, other agents transmitted by ticks, e.g., *Rickettsia* spp., are increasingly claimed to cause health problems [5].

In follow-up studies of patients with Lyme neuroborreliosis (LNB), several symptoms have been found to persist in some of the patients compared to controls, including malaise, fatigue, memory problems, concentration difficulties and paresthesias [6, 7].

Population based follow-up studies of patients treated for LB in general have given discrepant results regarding long-term consequences. Some American studies have reported that LB patients experience long-lasting symptoms [8, 9], while other investigations found that symptoms at follow-up did not exceed that of a control population [10–14].

European studies have also shown that patients having suffered from early LB report a number of symptoms; however, these did not exceed the corresponding symptoms in matched controls [15, 16].

In a recent Swedish study, patients bitten by *Borrelia*-infected ticks were compared to patients bitten by non-infected ticks [17]. The frequency of subjects reporting symptoms was higher in the group bitten by *Borrelia*-

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infected ticks compared to subjects bitten by uninfected ticks. There were, however, no differences between subjects bitten by a *Borrelia*-positive tick and subjects bitten by a *Borrelia*-negative tick when comparing the frequency of reported experience of several symptoms, such as fatigue, myalgia/arthralgia, headache, and neck pain.

It thus seems that long-lasting symptoms after LNB are frequent in adults; however, in properly controlled studies, lasting symptoms are rarely seen after early disease manifestations such as erythema migrans or after bites with *Borrelia*-infected ticks.

A majority of blood donors in Sogn and Fjordane county have experienced tick bites [18], and the seroprevalence of antibodies to *B. burgdorferi* s.l. is substantial [19]. The aim of this study was to assess the association, if any, of general function, physical fitness and subjective health complaints with reported tick bites and antibodies to *B. burgdorferi* sensu lato in this group of healthy adults.

## Methods

### Study population

During the period January 13th to June 15th 2010, blood donors at the four blood banks in Sogn and Fjordane were asked to participate in the Tick-borne Infection Study in Sogn and Fjordane. A total of 1,213 blood donors participated, a response rate of 76 %. The mean age was 45.8 years, ranging from 19 to 69. Men constituted 55.2 %. Further characteristics of the participants are presented elsewhere [18]. Informed consent was obtained from each participant, and the study was approved by the Regional Committee for Medical Research Ethics. This investigation included a questionnaire in addition to testing serum samples for antibodies to *B. burgdorferi* s.l., tick-borne encephalitis virus and *Anaplasma phagocytophilum*. The reported occurrence of tick bites and the results of the antibody tests in this study population have been published elsewhere [18–20].

### Questionnaire

The questionnaire included questions about demographics such as gender, age, marital status, education, household income and occupation. Questions on pet animals, farm animals, hours spent outdoors during summertime, hunting, orienteering, smoking, and symptoms and treatment after tick bites were also included.

The questionnaire included two questions on tick bites; tick bites ever experienced, and tick bites experienced during the last 12 months. The responses for both questions were given in the categories "none", "one", "2-5", "6-20" and "more than 20". The number of tick bites ever experienced was used in the present study.

Another section of the questionnaire included the Subjective Health Complaints (SHC) Inventory, a set of questions designed to measure common and prevalent health complaints in the general population [21–23]. The respondents reported to what extent they had been affected by 29 different health disturbances during the last month. The response options were "Not at all" (score 0), "A little" (score 1), "Some" (score 2), and "Serious" (score 3). In addition, the respondents were asked to record the duration of these symptoms (1–30 days).

The question related to general function was "How do you assess your ability to perform ordinary activity, your general function, is today?" The response options were "Good, as it usually is" (score 0), "Hardly reduced at all" (score 1), "Not much reduced" (score 2), "Moderately reduced" (score 3) and "Much reduced" (score 4), adapted from [24].

Physical fitness was assessed by the question "«During the past 2 weeks: What was the hardest physical activity you could do for at least 2 min?», with the response alternatives «Very heavy (ex. run, at fast pace)» (score 0), « Heavy (ex. jog, at slow pace)» (score 1), « Moderate (ex. walk, at a fast pace)» (score 2), «Light (ex. walk, at a medium pace)» (score 3), and «Very light (ex. walk, at a slow pace or not able to walk)» (score 4). This question was taken from the COOP/WONCA charts [25].

The questionnaires were answered anonymously, and most were completed during the time spent at the blood bank for routine donation.

### Laboratory methods

For testing of antibodies to *B. burgdorferi* s.l., all sera were tested in Enzygnost Lyme link VlsE/IgG and Enzygnost Borreliosis IgM (DADE Behring, Marburg, Germany) and in Immunetics C6 Lyme ELISA kit (Immunetics, 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) immunoblots. Further details are presented elsewhere [19]. The results of the immunoblots are used in the present study, and are here-after referred to as IgG and IgM.

### Risk factors

The three risk factors included in the present analyses were the number of self-reported tick bites ever experienced, positive IgG, and positive IgM for *B. burgdorferi* sensu lato. With the exeption of the questions on general function and physical fitness (Table 1), the numbers of tick bites were dichotomised into less than two versus two or more in the analyses.

**Table 1** Overall function and physical fitness in relation to tick bites and Borrelia antibodies

| Risk factor | | Reduced overall function | | Reduced physical fitness | |
|---|---|---|---|---|---|
| | | Odds ratio[2] (95 % CI) | Adjusted odds ratio[3] (95 % CI) | Odds ratio[2] (95 % CI) | Adjusted odds ratio[3] (95 % CI) |
| Total tick bites ever experienced | | | | | |
| n[1] | | 1167 | 1145 | 1179 | 1157 |
| None (%) | 34.1 | 1 | 1 | 1 | 1 |
| 1 (%) | 18.1 | 1.9 (1.0 - 3.5) | 1.7 (0.9 - 3.2) | 1.2 (0.9 - 1.6) | 1.1 (0.8 - 1.5) |
| 2-5 (%) | 23.7 | 0.7 (0.4 - 1.5) | 0.7 (0.4 - 1.6) | 0.8 (0.6 - 1.0) | 0.7 (0.5 - 0.9) |
| 6-20 (%) | 15 | 1. 9 (1.0 - 3.5) | 1.8 (0.9 - 3.5) | 0.6 (0.5 - 0.9) | 0.5 (0.4 - 0.7) |
| >20 (%) | 9.2 | 2.1 (1.0 - 4.4) | 2.0 (0.9 - 4.2) | 0.9 (0.6 - 1.3) | 0.7 (0.4 - 1.0) |
| p trend | | 0.086 | 0.117 | 0.012 | <0.001 |
| IgG | | | | | |
| n[1] | | 1179 | 1157 | 1191 | 1169 |
| negative (%) | 93.8 | 1 | 1 | 1 | 1 |
| positive (%) | 6.2 | 1.6 (0.8 - 3.5) | 1.7 (0.8 - 3.7) | 1.1 (0.7 - 1.7) | 1.1 (0.7 - 1.7) |
| p difference | | 0.216 | 0.204 | 0.725 | 0.688 |
| IgM | | | | | |
| n[1] | | 1179 | 1157 | 1191 | 1169 |
| negative (%) | 94.3 | 1 | 1 | 1 | 1 |
| positive (%) | 5.7 | 1.5 (0.7 - 3.5) | 1.5 (0.7 -3.4) | 1.1 (0.7 - 1.7) | 0.9 (0.6 - 1.5) |
| p difference | | 0.296 | 0.340 | 0.760 | 0.726 |

[1]Numbers do not add to 1,213 due to missing data
[2]Odds ratio was estimated using proportional odds ratio models (logistic regression model with more than two categorical outcomes)
[3]Adjusted for gender, age group and blood bank

### Outcome

The outcome variables in this study were the responses to questions on subjective health complaints (SHC), general function, and physical fitness.

For general function and physical fitness, the response was categorized in the categories 0–3, i.e., categories 3 and 4 were merged, because of few respondents in category 4.

For the SHC-questions, we calculated the total number of complaints reported by each participant. In addition, a total SHC score was computed as the sum of the severity scores of all 29 items in the questionnaire, the computation of this score was allowed if 18 or more of the questions were answered.

The proportion of subjects reporting any complaint within five subscales are reported as well; these include "musculoskeletal" complaints (headache, neck pain, shoulder pain, pain in arms, pain in upper back, low back pain, leg pain, and pain in the feet during exercise), "pseudoneurological" complaints (extra heartbeats, heat flushes, sleep problems, tiredness, dizziness, anxiety and sadness/depression), "gastrointestinal" complaints (heartburn, stomach discomfort, ulcer/non-ulcer dyspepsia, stomach pain, bloating, diarrhoea and constipation), "allergic" complaints (asthma, breathing difficulties, eczema, allergies and chest pain) and "flu" (cold/flu and cough). These subscales are based on factor analysis and the factors have shown good reliability in previous studies [21]. The computation of these proportions allowed for a certain number of missing values, which were ignored if constituting less than half of the questions within the subscale.

For the statistical evaluation of each individual SHC we chose to dichotomize all responses into "no complaint" versus any degree of complaint, as very few scored the two most serious responses 2 and 3.

### Statistical analysis

We used IBM SPSS Statistics version 21 (SPSS Inc., Chicago, Illinois) for statistical analyses except for Table 3, where Stata/SE version 13.1 for Windows (StataCorp LP, College Station, Texas) was used.

Immunoblot results for IgG and IgM were categorized as positive or negative, with grey-zone results included as negatives.

For the questions on general function and physical fitness, the association between the score and number of self-reported tick bites was analysed by using proportional odds models, i.e., logistic regression models with more than two ordinal categorical outcomes, with and without adjustment for gender, age group and location of blood bank [26].

For SHC, both individually for each complaint as well as for the five sub-scales, the associations between the risk factors and outcome variables were analysed by binary logistic regression, with and without adjustment for gender, age group and location of blood bank.

Differences in the total number of complaints and the total SHC score between groups with and without the risk factors were estimated by using linear regression model with robust variance estimation. All p values were two-sided and values below 0.05 were considered statistical significant.

## Results

Among participants, 65.7 % had experienced tick bites during their life time. One tick bite was reported by 18.0 %, 2–5 bites by 23.8 %, 6–20 by 14.7 %, and more than 20 bites by 9.2 %. The estimated mean number of tick bites was 5.7, as published elsewhere [18].

Borrelia IgG antibodies verified by immunoblot were present in 6.4 % (9.7 % in men and 2,4 % in women), whereas Borrelia IgM antibodies were demonstrated in 5.7 % (6.1 % in men and 5.1 % in women) [19].

Overall, 5.0 % reported to have received antibiotics because of tick bite or consequences thereof. Among IgG and/or IgM blot positives, antibiotics had been received by 11.9 %.

The results for the relationship between the risk factors tick bites ever experienced, *Borrelia* IgG and *Borrelia* IgM, and the outcomes general function and physical fitness are presented in Table 1. The proportion reporting reduced general function did not differ significantly between subjects with and without the risk factors. The number of tick bites was positively associated with good physical fitness (adjusted p for trend < 0.001).

The relationship between the proportion reporting any complaint within the five subscales of subjective health complaints and the risk factors are presented in Table 2, and results for each of the individual 29 complaints are presented in the Additional file 1.

There were no significant associations between the proportion reporting "musculoskeletal pain" or the individual related complaints with any of the risk factors.

For the group of "pseudoneurological" complaints, there were fewer complaints in the subjects with *Borrelia* IgG than in those without these antibodies (adjusted

**Table 2** Odds ratio for reporting any degree of subjective health complaint (SHC), grouped in five subscales

| Complaint/Risk factor (RF) | n[1] | Proportion with complaint (%) | | Odds ratio[2] (OR) for complaint when risk factor present | | | |
|---|---|---|---|---|---|---|---|
| | | RF absent | RF present | Unadjusted | p[3] | Adjusted[4] | p[3] |
| Musculoskeletal pain | | | | | | | |
| Tick bites >1 | 1139 | 66.6 | 68.1 | 1.1 (0.8 - 1.4) | 0.583 | 1.1 (0.8 - 1.4) | 0.578 |
| IgG + | 1151 | 67.1 | 69.0 | 1.1 (0.6 - 1.8) | 0.743 | 1.3 (0.7 - 2.2) | 0.374 |
| IgM + | 1151 | 66.9 | 72.6 | 1.3 (0.7 - 2.3) | 0.359 | 1.5 (0.8 - 2.7) | 0.172 |
| Pseudoneurology | | | | | | | |
| Tick bites >1 | 1136 | 40.8 | 39.2 | 0.9 (0.7 - 1.2) | 0.570 | 0.9 (0.7 - 1.1) | 0.264 |
| IgG + | 1148 | 41.1 | 23.9 | 0.5 (0.3 - 0.8) | 0.005 | 0.5 (0.3 - 0.8) | 0.010 |
| IgM + | 1148 | 40.2 | 37.1 | 0.9 (0.5 - 1.5) | 0.624 | 0.8 (0.5 - 1.5) | 0.522 |
| Gastrointestinal complaints | | | | | | | |
| Tick bites >1 | 1136 | 41.5 | 42.1 | 1.0 (0.8 - 1.3) | 0.829 | 1.0 (0.8 - 1.3) | 0.991 |
| IgG + | 1148 | 42.0 | 38.0 | 0.8 (0.5 - 1.4) | 0.515 | 0.9 (0.5 - 1.4) | 0.557 |
| IgM + | 1148 | 41.4 | 46.8 | 1.2 (0.7 - 2.1) | 0.408 | 1.1 (0.7 - 1.9) | 0.632 |
| Allergy | | | | | | | |
| Tick bites >1 | 1136 | 20.2 | 19.8 | 1.0 (0.7 - 1.3) | 0.870 | 0.9 (0.7 - 1.2) | 0.534 |
| IgG + | 1148 | 20.2 | 16.9 | 0.8 (0.4 - 1.5) | 0.497 | 0.8 (0.4 - 1.5) | 0.415 |
| IgM + | 1148 | 19.7 | 25.8 | 1.4 (0.8 - 2.6) | 0.245 | 1.4 (0.7 - 2.5) | 0.324 |
| Flu | | | | | | | |
| Tick bites >1 | 1161 | 29.3 | 31.4 | 1.1 (0.9 - 1.4) | 0.434 | 1.2 (0.9 - 1.5) | 0.264 |
| IgG + | 1174 | 29.7 | 36.5 | 1.4 (0.8 - 2.2) | 0.222 | 1.3 (0.8 - 2.2) | 0.273 |
| IgM + | 1174 | 29.5 | 42.2 | 1.7 (1.0 - 2.9) | 0.033 | 1.3 (0.8 - 2.2) | 0.273 |

[1]n does not reach 1213 because of missing data
[2]Odds ratio for subjects without the risk factor is 1
[3]Binary logistic regression
[4]Adjusted for gender, age group and blood bank location

$p = 0.010$). None of the individual complaints in this group of questions were significantly associated with any of the risk factors. However, for all questions there were fewer in the IgG positive group reporting symptoms than among IgG negatives (Additional file 1).

In the subscale "gastrointestinal" complaints, some more subjects with more than one tick bite reported ulcer- and non-ulcer dyspepsia (adjusted $p = 0.079$), and there was a trend towards more IgM positives reporting constipation (Additional file 1; adjusted $p = 0.081$).

There were no significant associations between the number of subjects reporting complaints within the "allergy" subscale or the individual related complaints with any of the risk factors.

The "flu"-related questions all showed a higher percentage of IgM positives reporting symptoms, however, this associations were weakened when adjusted for gender, age group and blood bank location.

We found no significant associations between the total number of subjective health complaints or the total SHC score and any of the risk factors (Table 3) when adjusted for gender, age, and location of blood bank.

The additional questions on duration of the different symptoms were answered by only 60 % (38-73 %) of the subjects reporting any degree of the complaints. Therefore, these data were not considered satisfactory for statistical evaluation and are not shown.

## Discussion

The main finding in this study was the lack of association between the risk factors tick bites and Borrelia antibodies with the outcomes subjective health complaints, reduced

**Table 3** Number of subjective health complaints and total SHC score relative to tick bites and Borrelia antibodies

| | No. subjects[1] | Median (IQR)[2] | Mean (SEM)[3] | Adjusted mean (SEM)[3,4] |
|---|---|---|---|---|
| **Number of complaints** | | | | |
| Tick-bites | | | | |
| ≤1 | 624 | 3.0 (1.0-5.0) | 3.59 (0.14) | 3.62 (0.13) |
| >1 | 570 | 3.0 (1.0-5.0) | 3.64 (0.13) | 3.60 (0.14) |
| p difference | | | 0.785 | 0.903 |
| IgG | | | | |
| - | 1135 | 3.0 (1.0-5.0) | 3.62 (0.10) | 3.63 (0.10) |
| + | 78 | 2.0 (1.0-4.0) | 3.29 (0.50) | 3.42 (0.51) |
| p difference | | | 0.527 | 0.603 |
| IgM | | | | |
| - | 1144 | 3.0 (1.0-5.0) | 3.58 (0.10) | 3.60 (0.10) |
| + | 69 | 3.0 (1.0-5.0) | 3.94 (0.51) | 3.87 (0.51) |
| p difference | | | 0.480 | 0.600 |
| **Total SCH score[5]** | | | | |
| Tick-bites | | | | |
| ≤1 | 595 | 3.0 (1.0-6.0) | 4.71 (0.24) | 4.76 (0.25) |
| >1 | 541 | 3.1 (1.0-6.0) | 4.58 (0.20) | 4.51 (0.20) |
| p difference | | | 0.672 | 0.440 |
| IgG | | | | |
| - | 1077 | 3.0 (1.0, 6.0) | 4.62 (0.14) | 4.60 (0.14) |
| + | 71 | 3.0 (1.0, 5.0) | 5.22 (1.31) | 5.36 (1.26) |
| p difference | | | 0.644 | 0.550 |
| IgM | | | | |
| - | 1086 | 3.0 (1.0-6.0) | 4.57 (0.14) | 4.57 (0.14) |
| + | 62 | 3.1 (2.0-7.0) | 6.06 (1.41) | 5.94 (1.35) |
| p difference | | | 0.295 | 0.313 |

[1]The number of subjects does not reach 1213 because of missing data
[2]Interquartile range (IQR)
[3]Standard error of the mean (SEM) predicted by linear regression with robust variance estimation
[4]Adjusted for gender, age and blood bank location
[5]SCH: Subjective health complaints. Total SHC score: See text

general function and reduced physical fitness. On the contrary, we found a positive association between tick bites and good physical fitness, and between presence of *Borrelia* IgG and low occurrence of "pseudoneurological" complaints. For most complaints, however, we found no association.

The risk factor "number of tick bites" was included in this study to explore the possibility that seronegative LB or other tick-borne infectious agents not tested for, e.g. *Anaplasma phagocytophilum*, *Rickettsia* spp., etc., could give rise to chronic health problems of some magnitude.

IgG and IgM antibodies to *B. burgdorferi* s.l. verified by blot were chosen as risk factors in this study in order to avoid including false positive ELISA results. However, biologically false positive IgM blot results still are a problem, and American guidelines thus argue against using IgM blot in the second-tier testing when disease duration is longer than one month [27]. A proportion of the IgM blot positive specimens in this study are probably biologically false positive, as discussed elsewhere [19]. Also, many will lose their antibodies after a while, whether treated or not. However, antibodies against *B. burgdorferi* s.l., especially IgG, should be an indicator of former or present infection with this organism.

In several studies of symptoms after LB, the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) has been used [6, 8–10]. In addition, several questionnaires for assessing somatic symptoms in the general population exist [28]. The unique contribution of the current study was to include the SHC questionnaire, which was designed to measure common and prevalent health complaints in the general population, and has been used in several studies of the general population as well as different patients groups [29–32].

Compared to other surveys using the SHC Inventory in the general population, summarised by Eriksen et al. in 1999 [21], and for a working population more recently by Ihlebæk and co-workers [29], the proportion of subjects with any complaint were lower in our material for most complaints, reflecting the overall healthy status of the blood donors (Additional file 1).

Comparing our results to those of other studies is not straight forward, of two main reasons. Firstly, the selection of study population differs between studies, e.g., blood donors, subjects having been bitten by ticks, patients having suffered from LB, patients having suffered from LNB, etc. Secondly, the choice of questionnaire also varies. We are not aware of other studies using the SHC inventory in blood donors or in relation to LB. Nevertheless, in the following, a tentative comparison is attempted.

In a study of 1156 male military recruits in Germany, Treib and co-workers found that *Borrelia* IgG positive subjects reported significantly more fatigue, general malaise and limb pain, compared to seronegatives [33]. This is in contrast to our findings, where musculoskeletal pain in different locations as well as "tiredness", "reduced general function" and "reduced physical fitness" was reported as often by seronegatives as by seropositives for IgG.

Studies by Shadic and co-workers from 1994 and 1999 found that persons with a history of LB had more musculoskeletal impairment and a higher prevalence of verbal memory impairment when compared with those without a history of LB [8, 9]. As these reports are based on a different study population as well as a different questionnaire, they are not directly comparable with our present study. However, our results do not corroborate these findings, as musculoskeletal pain and reduced general function were not found associated with seropositivity or number of tick bites.

In agreement with our results, Seltzer and co-workers [10] reported that persons having suffered from LB generally did not report more symptoms than controls, including memory problems, numbness, fatigue, swollen joints, headaches, neck pain, or problems with sleeping and exercise. Also, supporting our findings, in two recent Slovenian studies of patients treated for erythema migrans, the researchers did not find any association between cases and controls in non-specific symptoms 6 months after treatment [15, 16]. In long-term follow-up of patients with culture-confirmed LB, Wormser and co-workers did not find evidence of severe fatigue or fibromyalgia attributable to LB [11, 12], and summary scores of physical and mental health were similar to those of the general population [14].

As part of the Swedish "STING-study", Fryland and co-workers [17] compared symptoms in patients bitten by ticks infected with *Borrelia burgdorferi* sensu lato with patients bitten with non-infected ticks. They found a higher frequency of patients reporting any symptom in the former group, but no differences between the groups when comparing the frequency of each of several symptoms. In the present study, we found that the count of subjective health complaints had no relation with any of the risk factors (Table 3).

In a prospective investigation of patients treated for LNB in Norway, Eikeland and co-workers found that LNB-treated patients scored lower on all the SF-36 subscores except on bodily pain, and they reported fatigue, memory problems and concentration difficulties more often than matched controls [6]. Notable was that in contrast to the results from Shadic et al., pain was not more common among LNB-treated patients than among controls, neither assessed by direct question nor by SF-36. These data are not comparable to our study, as very few, if any, of our study subjects have suffered from LNB [18].

According to alternative views on LB, there are many undiagnosed patients with nonspecific chronic symptoms attributable to the disease [3]. Among the listed symptoms, some are included in the SHC inventory. The prevalence of these was not significantly higher for any of the risk factors tick bites, IgG or IgM in our study. However, a number of symptoms are not explicitly included in the SHC inventory. If these symptoms were of any significance in our study population, it should be reflected in the score on "reduced general function" in subjects with the risk factors. This was, however, not the case.

The clear correlation between the number of tick bites and physical fitness is not surprising. Persons more exposed to ticks are presumably more involved in outdoors activities such as hiking and hunting, and are in a generally good physical condition.

The negative association of "pseudoneurological" complaints and Borrelia IgG antibodies, but not number of tick bites or IgM, is difficult to explain. Also here, a spurious relationship connected to confounding lifestyle factors can be suspected.

The strength of this study is the relatively large representation of healthy subjects from Sogn and Fjordane county, the good response rate, and the frequent occurrence of tick bites and seropositivity to *B. burgdorferi* s.l., as well as the scope of the questionnaire, covering a broad range of health complaints. Thus, major chronic health effects of tick bites and seropositivity to *B. burgdorferi* s.l. should be detected.

On the other hand, blood donors are not completely representative of the general population. They are healthy, and children and persons over 70 years are not represented. There was, however, a fair distribution regarding gender and age groups. According to Norwegian blood bank regulations, persons that have been bitten by ticks should not donate blood within four weeks of the bite, and persons with suspected or verified LB should not donate until six months after adequate treatment has been given [34]. Donors with recent tick bites and/or LB may therefore be underrepresented. Not all tick bites are recognized by the bitten person, and thus will not be reported in a questionnaire survey like this (i.e., information bias). This is supported by the seroprevalence rate for immunoblot verified IgG antibodies to *B. burgdorferi* s.l. of 2.4 % in subjects not reporting any tick bite [19].

It is to be expected that persons with significant chronic health problems do not volunteer as blood donors, or will be excluded. Persons of this category are therefore probably underrepresented in this material. However, given the commonness of these "soft" symptoms also in blood donors (Table 2), one would expect that mild degrees of such complaints should be represented.

## Conclusions

This study of blood donors in western Norway assessed the association between three risk factors – the number of tick bites ever experienced and the presence of Borrelia IgG and IgM antibodies – with the questionnaire-based outcomes general function, physical fitness and 29 different subjective health complaints.

A limitation of this study is that very few of the study subjects reported ever having had symptoms indicative of systemic LB.

There was no association between general function and the risk factors. However, we found a clear correlation between the number of tick bites ever experienced and good physical fitness. This most probably represents a spurious relationship as both risk factor and outcome are related to a healthy life style.

We did not find any association between more subjective health complaints and tick bites or Borrelia antibodies. Seeing the «pseudoneurological» group of complaints together, there were fewer complaints in subjects with Borrelia IgG antibodies, also probably a non-causal relationship.

The results do not support any association between tick bites or Borrelia antibodies and subjective health complaints in blood donors in an endemic area for Lyme borreliosis.

## Additional file

**Additional file 1: Individual subjective health complaints relative to tick bites and Borrelia antibodies.**

#### Competing interests

The authors declare that they have no competing interests.

#### Authors' contributions

RH conceived and designed the study and drafted the manuscript. CI, HR, NG and EU helped in the conception of the study. RMN helped in the statistical analyses. All authors read, revised and approved the final manuscript.

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## References

1. Feder Jr HM, Johnson BJ, O'Connell S, Shapiro ED, Steere AC, Wormser GP. A critical appraisal of "chronic Lyme disease". N Engl J Med. 2007;357(14):1422–30.
2. Cairns V, Godwin J. Post-Lyme borreliosis syndrome: a meta-analysis of reported symptoms. Int J Epidemiol. 2005;34(6):1340–5.
3. Cameron D, Gaito A, Harris N, Bach G, Bellovin S, Bock K, et al. Evidence-based guidelines for the management of Lyme disease. Expert Rev Anti Infect Ther. 2004;2(1 Suppl):S1–13.
4. Shapiro ED, Dattwyler R, Nadelman RB, Wormser GP. Response to meta-analysis of Lyme borreliosis symptoms. Int J Epidemiol. 2005;34(6):1437–9. author reply 1440–1433.
5. Berghoff W. Chronic Lyme disease and Co-infections: differential diagnosis. Open Neurol J. 2012;6:158–78.
6. Eikeland R, Mygland A, Herlofson K, Ljostad U. European neuroborreliosis: quality of life 30 months after treatment. Acta Neurol Scand. 2011;124(5):349–54.
7. Henningsson AJ, Malmvall BE, Ernerudh J, Matussek A, Forsberg P. Neuroborreliosis–an epidemiological, clinical and healthcare cost study from an endemic area in the south-east of Sweden. Clin Microbiol Infect. 2010;16(8):1245–51.
8. Shadick NA, Phillips CB, Logigian EL, Steere AC, Kaplan RF, Berardi VP, et al. The long-term clinical outcomes of Lyme disease. A population-based retrospective cohort study. Ann Intern Med. 1994;121(8):560–7.
9. Shadick NA, Phillips CB, Sangha O, Logigian EL, Kaplan RF, Wright EA, et al. Musculoskeletal and neurologic outcomes in patients with previously treated Lyme disease. Ann Intern Med. 1999;131(12):919–26.
10. Seltzer EG, Gerber MA, Cartter ML, Freudigman K, Shapiro ED. Long-term outcomes of persons with Lyme disease. JAMA. 2000;283(5):609–16.
11. Wormser GP, Weitzner E, McKenna D, Nadelman RB, Scavarda C, Farber S, et al. Long-term assessment of fibromyalgia in patients with culture-confirmed Lyme disease. Arthritis Rheumatol. 2014. doi:10.1002/art.38972.
12. Wormser GP, Weitzner E, McKenna D, Nadelman RB, Scavarda C, Nowakowski J. Long-term assessment of fatigue in patients with culture-confirmed Lyme disease. Am J Med. 2015;128(2):181–4.
13. Auwaerter PG. Life after Lyme disease. Clin Infect Dis. 2015. doi:10.1093/cid/civ279.
14. Wormser GP, Weitzner E, McKenna D, Nadelman RB, Scavarda C, Molla I, et al. Long-term assessment of health-related quality of life in patients with culture-confirmed early Lyme disease. Clin Infect Dis. 2015. doi:10.1093/cid/civ277.
15. Cerar D, Cerar T, Ruzic-Sabljic E, Wormser GP, Strle F. Subjective symptoms after treatment of early lyme disease. Am J Med. 2010;123(1):79–86.
16. Stupica D, Lusa L, Ruzic-Sabljic E, Cerar T, Strle F. Treatment of erythema migrans with doxycycline for 10 days versus 15 days. Clin Infect Dis. 2012;55(3):343–50.
17. Fryland L, Wilhelmsson P, Lindgren PE, Nyman D, Ekerfelt C, Forsberg P. Low risk of developing Borrelia burgdorferi infection in the south-east of Sweden after being bitten by a Borrelia burgdorferi-infected tick. Int J Infect Dis. 2011;15(3):e174–181.
18. Hjetland R, Eliassen KE, Lindbaek M, Nilsen RM, Grude N, Ulvestad E. Tick bites in healthy adults from western Norway: occurrence, risk factors, and outcomes. Ticks Tick Borne Dis. 2013;4(4):304–10.
19. Hjetland R, Nilsen RM, Grude N, Ulvestad E. Seroprevalence of antibodies to Borrelia burgdorferi sensu lato in healthy adults from western Norway: risk factors and methodological aspects. APMIS. 2014;122(11):1114–24.
20. Hjetland R, Henningsson AJ, Vainio K, Dudman SG, Grude N, Ulvestad E. Seroprevalence of antibodies to tick-borne encephalitis virus and Anaplasma phagocytophilum in healthy adults from western Norway. Infect Dis. 2015;47(1):52–6.
21. Eriksen HR, Ihlebaek C, Ursin H. A scoring system for subjective health complaints (SHC). Scand J Public Health. 1999;27(1):63–72.
22. Ihlebaek C, Eriksen HR, Ursin H. Prevalence of subjective health complaints (SHC) in Norway. Scand J Public Health. 2002;30(1):20–9.
23. Filipkowski KB, Smyth JM, Rutchick AM, Santuzzi AM, Adya M, Petrie KJ, et al. Do healthy people worry? Modern health worries, subjective health complaints, perceived health, and health care utilization. Int J Behav Med. 2010;17(3):182–8.
24. Reiso H, Nygard JF, Brage S, Gulbrandsen P, Tellnes G. Work ability assessed by patients and their GPs in new episodes of sickness certification. Fam Pract. 2000;17(2):139–44.
25. Weel Cv, König-Zahn C, Touw-Otten FWMM, Duijn NP, Meyboom-de Jong B. Measuring functional health status with the COOP/WONCA Charts. A manual. Northern Centre of Health Care Research: Groningen; 1995.
26. Scott SC, Goldberg MS, Mayo NE. Statistical assessment of ordinal outcomes in comparative studies. J Clin Epidemiol. 1997;50(1):45–55.
27. From the Centers for Disease Control and Prevention. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. JAMA 1995, 274(12):937.
28. Zijlema WL, Stolk RP, Löwe B, Rief W, White PD, Rosmalen JGM. How to assess common somatic symptoms in large-scale studies: a systematic review of questionnaires. J Psychosom Res. 2013;74(6):459–68.
29. Ihlebaek C, Brage S, Eriksen HR. Health complaints and sickness absence in Norway, 1996–2003. Occup Med. 2007;57(1):43–9.
30. Indregard AM, Ihlebaek CM, Eriksen HR. Modern health worries, subjective health complaints, health care utilization, and sick leave in the Norwegian working population. Int J Behav Med. 2013;20(3):371–7.
31. Morken MH, Lind RA, Valeur J, Wilhelmsen I, Berstad A. Subjective health complaints and quality of life in patients with irritable bowel syndrome following Giardia lamblia infection: a case control study. Scand J Gastroenterol. 2009;44(3):308–13.
32. Stubhaug B, Tveito TH, Eriksen HR, Ursin H. Neurasthenia, subjective health complaints and sensitization. Psychoneuroendocrinology. 2005;30(10):1003–9.
33. Treib J, Grauer MT, Haass A, Langenbach J, Holzer G, Woessner R. Chronic fatigue syndrome in patients with Lyme borreliosis. Eur Neurol. 2000;43(2):107–9.
34. Comelli HL, Andreassen Å, Dao P, Dudman S, Noraas S, Ottesen P, Sandbu S, Skarpaas T, Soleng A, Sundal J et al.. Årsrapport. Flått og flåttbårne sykdommer 2010. Nasjonalt folkehelseinstitutt; 2011.

**Additional file Individual subjective health complaints relative to tick bites and Borrelia antibodies**

| Complaint/ risk factor (RF) | n[1] | Proportion with RF (%) | Proportion with complaint (%) | | Odds ratio (OR) for complaint when risk factor present (binary logistic regression) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | RF absent | RF present | Unadjusted | p | Adjusted[2] | p |
| Headache | | | | | | | | |
| Tick bites >1 | 1148 | 47.8 | 36.4 | 34.2 | 0.9 (0.7 - 1.2) | 0.447 | 1.0 (0.8 - 1.3) | 0.841 |
| IgG + | 1159 | 6.1 | 35.7 | 31.0 | 0.8 (0.5 - 1.4) | 0.425 | 1.2 (0.7 - 2.1) | 0.469 |
| IgM + | 1159 | 5.3 | 35.7 | 29.0 | 0.7 (0.4 - 1.3) | 0.285 | 0.9 (0.5 - 1.6) | 0.726 |
| Neck pain | | | | | | | | |
| Tick bites >1 | 1137 | 47.8 | 25.9 | 25.6 | 1.0 (0.8 - 1.3) | 0.900 | 1.0 (0.7 - 1.3) | 0.949 |
| IgG + | 1149 | 6.2 | 26.1 | 21.1 | 0.8 (0.4 - 1.4) | 0.358 | 0.8 (0.5 - 1.5) | 0.572 |
| IgM + | 1149 | 5.5 | 25.8 | 25.4 | 1.0 (0.5 - 1.8) | 0.946 | 1.0 (0.5 - 1.8) | 0.974 |
| Upper back pain | | | | | | | | |
| Tick bites >1 | 1121 | 47.5 | 10.4 | 13.1 | 1.3 (0.9 - 1.9) | 0.152 | 1.2 (0.8 - 1.8) | 0.325 |
| IgG + | 1133 | 6.2 | 11.8 | 11.4 | 1.0 (0.5 - 2.1) | 0.934 | 1.0 (0.4 - 2.1) | 0.953 |
| IgM + | 1133 | 5.6 | 11.7 | 12.7 | 1.1 (0.5 - 2.4) | 0.808 | 1.0 (0.5 - 2.3) | 0.934 |
| Lower back pain | | | | | | | | |
| Tick bites >1 | 1137 | 47.6 | 30.9 | 32.7 | 1.1 (0.8 - 1.4) | 0.505 | 1.0 (0.8 - 1.4) | 0.726 |
| IgG + | 1149 | 6.1 | 31.5 | 34.3 | 1.1 (0.7 - 1.9) | 0.629 | 1.1 (0.7 - 1.9) | 0.679 |
| IgM + | 1149 | 5.3 | 32.1 | 24.6 | 0.7 (0.4 - 1.3) | 0.224 | 0.7 (0.4 - 1.3) | 0.287 |
| Arm pain | | | | | | | | |
| Tick bites >1 | 1127 | 47.4 | 17.2 | 16.1 | 0.9 (0.7 - 1.3) | 0.622 | 0.8 (0.6 - 1.1) | 0.178 |
| IgG + | 1139 | 6.1 | 16.6 | 15.9 | 1.0 (0.5 - 1.8) | 0.881 | 0.8 (0.4 - 1.6) | 0.572 |
| IgM + | 1139 | 5.4 | 16.2 | 24.2 | 1.7 (0.9 - 3.0) | 0.101 | 1.5 (0.8 - 2.9) | 0.172 |
| Shoulder pain | | | | | | | | |
| Tick bites >1 | 1143 | 48.0 | 27.3 | 30.2 | 1.2 (0.9 - 1.5) | 0.269 | 1.0 (0.8 - 1.4) | 0.792 |
| IgG + | 1155 | 6.1 | 28.6 | 28.6 | 1.0 (0.6 - 1.7) | 1.000 | 1.0 (0.5 - 1.7) | 0.877 |
| IgM + | 1155 | 5.3 | 28.1 | 37.7 | 1.6 (0.9 - 2.6) | 0.107 | 1.5 (0.9 - 2.6) | 0.129 |
| Migraine | | | | | | | | |
| Tick bites >1 | 1126 | 47.5 | 6.3 | 8.0 | 1.3 (0.8 - 2.1) | 0.248 | 1.2 (0.7 - 1.9) | 0.447 |
| IgG + | 1138 | 6.2 | 7.0 | 7.0 | 1.0 (0.4 - 2.6) | 0.997 | 1.1 (0.4 - 2.9) | 0.864 |
| IgM + | 1138 | 5.4 | 7.0 | 8.2 | 1.2 (0.5 - 3.1) | 0.714 | 1.2 (0.5 - 3.3) | 0.651 |
| Pain in feet after strain | | | | | | | | |
| Tick bites >1 | 1138 | 47.8 | 9.9 | 11.2 | 1.1 (0.8 - 1.7) | 0.483 | 1.1 (0.7 - 1.6) | 0.754 |
| IgG + | 1150 | 6.3 | 10.3 | 13.9 | 1.4 (0.7 - 2.8) | 0.338 | 1.3 (0.6 - 2.8) | 0.433 |
| IgM + | 1150 | 5.4 | 10.3 | 14.5 | 1.5 (0.7 - 3.1) | 0.295 | 1.4 (0.7 - 3.0) | 0.376 |
| Palpitations | | | | | | | | |
| Tick bites >1 | 1136 | 47.6 | 7.6 | 8.7 | 1.2 (0.8 - 1.8) | 0.488 | 1.1 (0.7 - 1.8) | 0.566 |
| IgG + | 1148 | 6.1 | 8.4 | 2.9 | 0.3 (0.1 - 1.3) | 0.115 | 0.3 (0.1 - 1.4) | 0.140 |
| IgM + | 1148 | 5.4 | 7.8 | 12.9 | 1.7 (0.8 - 3.8) | 0.159 | 1.7 (0.8 - 3.7) | 0.200 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Heat flushes | | | | | | | | | | |
| Tick bites >1 | 1138 | 47.8 | | 10.1 | 10.5 | | 1.0 (0.7 - 1.5) | 0.834 | | 0.7 (0.4 - 1.0) | 0.075 |
| IgG + | 1150 | 6.1 | | 10.5 | 8.6 | | 0.8 (0.3 - 1.9) | 0.615 | | 1.2 (0.5 - 3.3) | 0.665 |
| IgM + | 1150 | 5.4 | | 10.3 | 10.3 | | 1.1 (0.5 - 2.5) | 0.802 | | 1.0 (0.4 - 2.6) | 0.973 |
| Sleep problems | | | | | | | | | | | |
| Tick bites >1 | 1137 | 47.8 | | 17.7 | 16.0 | | 0.9 (0.7 - 1.2) | 0.457 | | 0.8 (0.6 - 1.1) | 0.142 |
| IgG + | 1149 | 6.2 | | 17.3 | 11.3 | | 0.6 (0.3 - 1.3) | 0.191 | | 0.6 (0.3 - 1.3) | 0.208 |
| IgM + | 1149 | 5.4 | | 17.3 | 11.3 | | 0.6 (0.3 - 1.4) | 0.225 | | 0.6 (0.3 - 1.3) | 0.203 |
| Tiredness | | | | | | | | | | | |
| Tick bites >1 | 1140 | 47.8 | | 24.7 | 25.7 | | 1.1 (0.8 - 1.4) | 0.703 | | 1.0 (0.8 - 1.4) | 0.826 |
| IgG + | 1152 | 6.2 | | 25.8 | 15.5 | | 0.5 (0.3 - 1.0) | 0.056 | | 0.6 (0.3 - 1.1) | 0.103 |
| IgM + | 1152 | 5.3 | | 25.1 | 25.8 | | 1.0 (0.6 - 1.9) | 0.906 | | 1.0 (0.6 - 1.9) | 0.957 |
| Dizziness | | | | | | | | | | | |
| Tick bites >1 | 1135 | 47.8 | | 7.1 | 6.1 | | 0.9 (0.5 - 1.4) | 0.501 | | 0.8 (0.5 - 1.3) | 0.427 |
| IgG + | 1147 | 6.2 | | 6.8 | 5.6 | | 0.8 (0.3 - 2.3) | 0.708 | | 0.8 (0.3 - 2.4) | 0.702 |
| IgM + | 1147 | 5.4 | | 6.5 | 9.7 | | 1.5 (0.6 - 3.7) | 0.341 | | 1.6 (0.6 - 3.9) | 0.313 |
| Anxiety | | | | | | | | | | | |
| Tick bites >1 | 1138 | 47.6 | | 3.9 | 2.6 | | 0.7 (0.3 - 1.3) | 0.228 | | 0.6 (0.3 - 1.2) | 0.148 |
| IgG + | 1150 | 6.2 | | 3.3 | 2.8 | | 0.8 (0.2 - 3.6) | 0.813 | | 0.8 (0.2 - 3.5) | 0.759 |
| IgM + | 1150 | 5.5 | | 3.2 | 3.2 | | 1.5 (0.4 - 5.0) | 0.508 | | 1.4 (0.4 - 4.6) | 0.628 |
| Depressed | | | | | | | | | | | |
| Tick bites >1 | 1136 | 47.6 | | 6.2 | 6.3 | | 1.0 (0.6 - 1.6) | 0.963 | | 1.0 (0.6 - 1.7) | 0.881 |
| IgG + | 1148 | 6.2 | | 6.3 | 4.2 | | 0.7 (0.2 - 2.1) | 0.482 | | 0.7 (0.2 - 2.5) | 0.609 |
| IgM + | 1148 | 5.4 | | 6.0 | 9.7 | | 1.7 (0.7 - 4.1) | 0.245 | | 1.7 (0.7 - 4.1) | 0.252 |
| Heartburn | | | | | | | | | | | |
| Tick bites >1 | 1144 | 47.6 | | 19.0 | 17.8 | | 0.9 (0.7 - 1.2) | 0.611 | | 0.9 (0.6 - 1.2) | 0.369 |
| IgG + | 1156 | 6.2 | | 18.2 | 23.6 | | 1.4 (0.8 - 2.4) | 0.252 | | 1.2 (0.7 - 2.1) | 0.589 |
| IgM + | 1156 | 5.4 | | 18.1 | 25.4 | | 1.5 (0.9 - 2.8) | 0.151 | | 1.3 (0.7 - 2.5) | 0.343 |
| Stomach discomfort | | | | | | | | | | | |
| Tick bites >1 | 1134 | 47.4 | | 4.9 | 5.4 | | 1.1 (0.7 - 1.9) | 0.689 | | 1.0 (0.6 - 1.7) | 0.918 |
| IgG + | 1146 | 6.2 | | 5.3 | 4.2 | | 0.8 (0.2 - 2.6) | 0.694 | | 0.6 (0.2 - 2.1) | 0.423 |
| IgM + | 1146 | 5.4 | | 5.4 | 3.2 | | 0.6 (0.1 - 2.5) | 0.470 | | 0.5 (0.1 - 2.1) | 0.353 |
| Ulcer and non-ulcer dyspepsia | | | | | | | | | | | |
| Tick bites >1 | 1134 | 47.7 | | 0.5 | 1.7 | | 3.3 (0.9 - 12.4) | 0.073 | | 3.6 (0.9 - 14.8) | 0.079 |
| IgG + | 1146 | 6.2 | | 0.9 | 4.2 | | 4.7 (1.3 - 17.5) | 0.021 | | 3.0 (0.7 - 12.7) | 0.140 |
| IgM + | 1146 | 5.4 | | 7.8 | 6.6 | | 3.3 (0.7 - 15.0) | 0.131 | | 3.3 (0.6 - 16.9) | 0.159 |
| Stomach pain | | | | | | | | | | | |
| Tick bites >1 | 1132 | 47.6 | | 7.1 | 8.2 | | 1.2 (0.8 - 1.8) | 0.493 | | 1.2 (0.8 - 2.0) | 0.357 |
| IgG + | 1144 | 6.1 | | 7.5 | 10.0 | | 1.4 (0.6 - 3.1) | 0.456 | | 1.7 (0.7 - 3.9) | 0.248 |
| IgM + | 1144 | 5.3 | | 7.8 | 6.6 | | 0.8 (0.3 - 2.4) | 0.733 | | 0.9 (0.3 - 2.6) | 0.835 |
| Bloating | | | | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Tick bites >1 | 1142 | 47.6 | 24.9 | 23.7 | 0.9 (0.7 - 1.2) | 0.636 | 0.9 (0.7 - 1.2) | 0.375 |
| IgG + | 1154 | 6.2 | 24.5 | 22.2 | 0.9 (0.5 - 1.6) | 0.664 | 0.9 (0.5 - 1.6) | 0.688 |
| IgM + | 1154 | 5.5 | 24.4 | 23.8 | 1.0 (0.5 - 1.8) | 0.918 | 0.8 (0.4 - 1.5) | 0.456 |
| Diarrhoea | | | | | | | | |
| Tick bites >1 | 1136 | 47.7 | 10.3 | 10.5 | 1.0 (0.7 - 1.5) | 0.891 | 1.1 (0.7 - 1.6) | 0.688 |
| IgG + | 1148 | 6.2 | 10.3 | 12.7 | 1.3 (0.6 - 2.6) | 0.528 | 1.3 (0.6 - 2.8) | 0.450 |
| IgM + | 1148 | 5.4 | 10.7 | 6.5 | 0.6 (0.2 - 1.6) | 0.296 | 0.6 (0.2 - 1.6) | 0.302 |
| Constipation | | | | | | | | |
| Tick bites >1 | 1133 | 47.7 | 3.4 | 4.1 | 1.2 (0.7 - 2.2) | 0.541 | 1.2 (0.6 - 2.4) | 0.518 |
| IgG + | 1145 | 6.1 | 3.7 | 4.3 | 1.2 (0.3 - 3.8) | 0.810 | 1.4 (0.4 - 4.9) | 0.623 |
| IgM + | 1145 | 5.3 | 3.5 | 8.2 | 2.5 (0.9 - 6.5) | 0.069 | 2.5 (0.9 - 6.8) | 0.081 |
| Asthma | | | | | | | | |
| Tick bites >1 | 1133 | 47.7 | 3.5 | 2.0 | 0.6 (0.3 - 1.2) | 0.132 | 0.5 (0.2 - 1.1) | 0.089 |
| IgG + | 1145 | 6.1 | 2.9 | 2.9 | 1.0 (0.2 - 4.2) | 0.990 | 0.9 (0.2 - 3.9) | 0.879 |
| IgM + | 1145 | 5.4 | 2.7 | 6.5 | 2.5 (0.9 - 7.4) | 0.095 | 2.3 (0.8 - 7.0) | 0.130 |
| Breathing difficulties | | | | | | | | |
| Tick bites >1 | 1135 | 47.6 | 2.0 | 2.0 | 0.8 (0.3 - 2.0) | 0.662 | 0.7 (0.3 - 1.7) | 0.453 |
| IgG + | 1147 | 6.2 | 1.9 | 2.8 | 1.5 (0.4 - 6.7) | 0.571 | 1.0 (0.2 - 4.4) | 0.962 |
| IgM + | 1147 | 5.4 | 1.8 | 4.8 | 2.9 (0.8 - 9.9) | 0.099 | 1.9 (0.5 - 7.0) | 0.310 |
| Eczema | | | | | | | | |
| Tick bites >1 | 1137 | 47.6 | 8.7 | 10.2 | 1.2 (0.8 - 1.8) | 0.406 | 1.1 (0.7 - 1.6) | 0.693 |
| IgG + | 1149 | 6.1 | 9.7 | 4.3 | 0.4 (0.1 - 1.3) | 0.142 | 0.4 (0.1 - 1.2) | 0.104 |
| IgM + | 1149 | 5.4 | 9.2 | 12.9 | 1.5 (0.7 - 3.2) | 0.334 | 1.4 (0.6 - 3.1) | 0.394 |
| Allergies | | | | | | | | |
| Tick bites >1 | 1141 | 47.8 | 9.6 | 8.4 | 0.9 (0.6 - 1.3) | 0.509 | 0.9 (0.6 - 1.4) | 0.590 |
| IgG + | 1153 | 6.2 | 9.1 | 8.5 | 0.9 (0.4 - 2.2) | 0.843 | 1.1 (0.4 - 2.7) | 0.851 |
| IgM + | 1153 | 5.4 | 8.8 | 14.5 | 1.8 (0.8 - 3.7) | 0.133 | 1.8 (0.9 - 3.9) | 0.114 |
| Chest pain | | | | | | | | |
| Tick bites >1 | 1135 | 47.6 | 2.9 | 2.6 | 0.9 (0.4 - 1.9) | 0.785 | 0.7 (0.3 - 1.5) | 0.381 |
| IgG + | 1147 | 6.2 | 2.6 | 5.6 | 2.2 (0.8 - 6.6) | 0.143 | 1.5 (0.5 - 4.5) | 0.504 |
| IgM + | 1147 | 5.4 | 2.6 | 6.5 | 2.6 (0.9 - 7.7) | 0.083 | 2.0 (0.7 - 6.3) | 0.211 |
| Cold, flu | | | | | | | | |
| Tick bites >1 | 1152 | 47.9 | 25.8 | 26.6 | 1.0 (0.8 - 1.4) | 0.759 | 1.1 (0.8 - 1.4) | 0.504 |
| IgG + | 1165 | 6.3 | 25.6 | 32.9 | 1.4 (0.9 - 2.4) | 0.175 | 1.4 (0.8 - 2.4) | 0.181 |
| IgM + | 1165 | 5.4 | 25.6 | 34.9 | 1.6 (0.9 - 2.7) | 0.103 | 1.5 (0.9 - 2.6) | 0.153 |
| Cough | | | | | | | | |
| Tick bites >1 | 1140 | 47.8 | 11.9 | 11.7 | 1.0 (0.7 - 1.4) | 0.921 | 1.0 (0.7 - 1.4) | 0.929 |
| IgG + | 1151 | 6.3 | 11.9 | 9.7 | 0.8 (0.4 - 1.8) | 0.585 | 0.7 (0.3 - 1.7) | 0.493 |
| IgM + | 1151 | 5.6 | 11.3 | 18.8 | 1.8 (0.9 - 3.5) | 0.076 | 1.5 (0.8 - 3.0) | 0.239 |

[1] n does not reach 1213 because of missing data

[2] Adjusted for gender, age group and blood bank location