Title:

Human seroprevalence of antibodies to tick-borne microbes in southern Norway

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Abstract

The tick *Ixodes ricinus* is widespread along the coastline of southern Norway, but data on human exposure to tick-borne microbes are scarce. We aimed to assess the seroprevalence of IgG antibodies to various tick-borne microbes in the general adult population living in a Norwegian municipality where ticks are abundant. Søgne is a coastline municipality in the southernmost part of Norway, and has a high density of ticks. All individuals aged 18-69 years with residential address in Søgne municipality (n = 7424) were invited to give a blood sample and answer a questionnaire. Blood samples from 3568 individuals were available for analysis. All samples were analyzed for IgG antibodies to *Borrelia burgdorferi* sensu lato (*Bb*sl), and around 1500 samples for IgG antibodies to other tick-borne microbes. Serum IgG antibodies to *Bb*sl was present in 22.0% (785/3568) of the tested samples, tick-borne encephalitis virus (TBEV) in 3.1% (45/1453), *Anaplasma phagocytophilum* in 11.0% (159/1452), *Babesia microti* in 2.1% (33/1537), *Bartonella henselae/B. quintana* in 0.1% (2/1451) and *Rickettsia helvetica/R. conorii* in 4.2% (60/1445). Serum IgG antibodies to *A. phagocytophilum* and *R. helvetica/R. conorii* were significantly more prevalent (p = 0.010 and p = 0.016, respectively) among individuals with serum IgG antibodies to *Bb*sl than among individuals without. In conclusion, our study showed a high exposure to *Bb*sl in the general adult population living in a coastline municipality in the southernmost part of Norway. The population is also exposed to *A. phagocytophilum*, *R. helvetica/R. conorii*, *B. microti* and TBEV, but very rarely *B. henselae/B. quintana*.

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Introduction

There is an increasing attention to tick-borne infections and their impact on human health. In Norway, the tick *Ixodes ricinus* is widespread along most parts of the southern coastline (Jore et al., 2011). Lyme borreliosis caused by *Borrelia burgdorferi* sensu lato (*Bb*sl) complex species, and tick-borne encephalitis caused by the tick borne encephalitis-virus (TBEV), are well-known infections. Erythema migrans, the early local skin infection in Lyme borreliosis, is not notifiable to the Norwegian Surveillance System for Communicable Diseases (MSIS), but the incidence was estimated to 148/100 000/year in Norway in 2005-2009 (Eliassen et al., 2017). Disseminated *Borrelia* infections are notifiable, and the reported incidence was 5.0-8.2/100 000/year in Norway in 2011-2015 (Ocampo et al., 2016). TBEV infections are also notifiable, and the reported incidence was 0.2-0.4/100000/year in Norway in 2011-2015 (Ocampo et al., 2016).

The last years, several other microbes that may cause human disease, have been detected in ticks collected in Norway. These microbes are *Anaplasma phagocytophilum*, *Babesia* species, *Borrelia miyamotoi*, *Neoehrlichia mikurensis* and *Rickettsia helvetica* (Kjelland et al., 2018; Oines et al., 2012; Quarsten et al., 2015). Furthermore, *Bartonella* species have been detected in ticks collected in Denmark (Stensvold et al., 2015). A few cases of human disease caused by these so-called novel tick-borne microbes are reported in Norway (Frivik et al., 2017; Morch et al., 2015; Stuen and Bergstrom, 2008), but no incidence data exists, and the impact of such infections are unknown.

Population-based seroprevalence studies can provide important information about epidemiology and diagnostics of tick-borne infections. Firstly, as antibodies generally persist in blood for years after symptomatic or asymptomatic infections, the seroprevalence reflects cumulative exposure to the various microbes in humans. Secondly, knowledge about the background seroprevalence is crucial to correctly interpret an antibody test result in terms of diagnostic predictive value.

Routine diagnostics of Lyme borreliosis and other tick-borne infections is mainly based on detection of serum antibodies. However, antibody diagnostics is hampered by several factors: In early stages of the disease (up to 6-8 wk) antibodies can be absent, the test accuracy is not absolute, and antibodies can be present for years after cured infection (Dessau et al., 2018; Kalish et al., 2001; Leeflang et al., 2016). It is therefore important that the serological test results are interpreted in light of the clinical setting and background seroprevalence in which sampling takes place (Dessau et al., 2018).

Several previous studies have assessed seroprevalence of IgG antibodies to *Bb*sl among blood donors from different regions in Norway. The seroprevalence among blood donors was 18.2% (45/247) in the south (Vest-Agder county) (Mygland et al., 2006), 9.6% (117/1213) in the west (Sogn og Fjordane county) (Hjetland et al., 2014), 9.2% (48/519) in the southeast (Vestfold county) and 0.5% (5/1048) in the north (Nordland, Troms and Finnmark counties) (Hvidsten et al., 2017). Similar test kit (Enzygnost Lyme link VIsE/IgG) was applied in all regions, except in Vest-Agder county (where Premier Human Lyme and Enzygnost Borreliosis were applied before and after 1997, respectively). In another Norwegian study of sera from a population aged two to > 50 years (in which 74.6% (2280/3057) were < 20 years), the overall seroprevalence of antibodies to *Bb*sl applying two different tests was 2.7% (84/3057) and

3.4% (104/3057), ranging from 0-7.6% and 1.0-9.6% in different counties (Vestrheim et al., 2016).

Norwegian seroprevalence data of antibodies to other tick-borne microbes than *Bb*sl are scarce. A few small studies from different parts of the country have assessed seroprevalence of IgG antibodies to TBEV, reporting rates at 0% (0/1213) in the west (Sogn og Fjordane county), 0.7% (3/461) in the southeast (Østfold county) and 2.4% (3/126) in the south (Tromøy, Aust-Agder county) (Hjetland et al., 2015; Larsen et al., 2014; Skarpaas et al., 2002). The seroprevalence of IgG antibodies to *A. phagocytophilum* has only been assessed in one Norwegian study, and was found to be 16.3% (49/301) among blood donors from western Norway (Sogn og Fjordane county) (Hjetland et al., 2015). A study from southern Sweden found the seroprevalence of IgG antibodies to *Babesia microti/B. divergens* to be 2.5% (5/197) among healthy individuals and 16.3% (14/86) among individuals seropositive to *Bb*sl antibodies (Svensson et al., 2019). For *R. helvetica*, two Swedish studies reported 3.8% (9/236) and 9.2% (19/206) IgG seropositivity among patients with symptoms of infectious disease after a tick-bite, and 0.6% (1/161) and 1.3% (1/80) among healthy control groups (Elfving et al., 2008; Lindblom et al., 2013). In another Swedish study among blood donors, the seroprevalence of IgG antibodies to two *Bartonella henselae* strains was 1.2% (6/498) and 2.0% (10/298), and for *Bartinella quintana* 0.2% (1/498) (McGill et al., 2005). No studies have so far assessed the human seroprevalence of antibodies to *Babesia*, *Bartonella* and *Rickettsia* species in Norway.

Most previous studies on seroprevalence of antibodies to tick-borne microbes have been restricted to blood donors or other selected population groups, and for several of the "novel" tick-borne infections, data on seroprevalence is lacking. In the present study, we therefore

aimed to assess the seroprevalence of IgG antibodies to various tick-borne microbes in an unselected Norwegian adult population living in an area where ticks are abundant.

Material and methods

Recruitment area

Søgne is a coastal municipality in Vest-Agder county, in the southernmost part of Norway. The municipality has a high density of ticks (Jore et al., 2011). *Borrelia burgdorferi* sensu lato was found in 22.3% of *Ixodes ricinus* ticks collected in Søgne (Kjelland et al., 2010), and the municipality has a high incidence of Lyme neuroborreliosis (19/100,000 annually in 1994-1999) (Ljostad et al., 2003). In January 2016, there was 11,260 inhabitants in Søgne, of whom 7,424 were aged from 18 to 69 years (population data from the National Registry). According to the MSIS-registry, 0-6 cases of disseminated Lyme borreliosis have been reported annually in Søgne the last 10 years (http://msis.no), buy only one case of TBEV-infection from 1994 to 2017 (https://www.fhi.no/en/el/insects-and-pests/ticks-and-tick-borne-diseases/skogflattencefalitt-tbe/).

Study participants and recruitment strategies

All individuals aged 18-69 years, with residential address in Søgne municipality, were invited to participate in the study. Participation entailed to give a blood sample and to answer a questionnaire. We used two different recruitment strategies; From June 2015 to January 2016, eligible individuals who attended the general practitioner's center were informed about the study and invited to participate. Then, from January to June 2016, we sent a letter with study information to all eligible individuals not already enrolled in the study and announced that they would be contacted by phone within a few weeks. Invitation requests were then made by phone, and time for blood sampling agreed. Written informed consent was obtained prior to

blood sampling and distribution of login key to a web-based questionnaire. Those who did not respond to the questionnaire within 2-6 wk after blood sampling were contacted once more by letter or phone for a reminder. Recruitment and blood sampling were conducted by Søgne medical center, which is the only general practitioner center in the municipality.

Serological tests

Enzyme-linked immunosorbent assay (ELISA) tests were applied for detection of serum IgG antibodies to *Bb*sl (Enzygnost Lyme link VIsE/IgG, Siemens Healthcare Diagnostics Products GmbH, Erlangen, Germany) and TBEV (Serion ELISA classic TBE virus IgG, Institut Virion\Serion GmbH, Würzburg, Germany, positive and equivocal results were also confirmed with Enzygnost anti-TBE virus IgG, Siemens Healthcare Diagnostics Products GmbH). The manufacturer report a specificity at 98-100% for the Enzygnost Lyme link VIsE/IgG-test, 90-98% for the Serion ELISA classic TBE virus IgG-test, and 99.5% for the Enzygnost anti-TBE virus IgG-test. Confirmation by immunoblot was not performed. Classification of sera as negative, equivocal and positive was according to kit instructions. Indirect immunofluorescent assay (IFA) tests were used for detection of serum IgG antibodies to *A.phagocytophilum* (*Anaplasma phagocytophilum* IFA IgG, Focus Diagnostics, Cypress, California, USA), *B. microti* (*Babesia microti* IFA IgG, Focus Diagnostics), *B. henselae*/ *B. quintana* (*Bartonella* IFA IgG, Focus Diagnostics), and *R. helvetica*/*R. conorii* (*Rickettsia* Screen IFA IgG Antibody Kit, Fuller Laboratories, Fullerton, California, USA). Because of substantial cross reactivity for IgG antibodies to *B. henselae/B. quintana* and *R. helvetica/ R. conorii*, the results are reported summarized for the *Bartonella* species and the *Rickettsia* species respectively. All analyses and interpretation of results were performed according to the manufacturer's instructions. A sample dilution of 1:64 was applied for evaluation of IFA-tests. Further titration of positive sera was not performed. All IFA-slides were evaluated

separately by the same two investigators to ensure equal interpretation. Sera with an IF-brightness of > 2+ were classified as positive, a score of 1+ to 1.5+ as equivocal. All 3568 samples were analyzed for *Bb*sl IgG antibodies. Around 1500 samples were analyzed for IgG antibodies to other tick-borne infections, these samples were collected consecutively from the start of the study.

Questionnaire

Participants were encouraged to answer the questionnaire online, but a paper version was also available on request. The questionnaire included questions about demographics, exposure to tick-bites, previous tick-borne infections, other diagnoses, regular medication and vaccination against tick-borne encephalitis and yellow fever. The web-based questionnaire was designed so that each question had to be answered in order to proceed.

Statistics

Chi-square/Fisher's exact test was applied to compare proportions between groups, and independent samples t-test to compare mean-values between groups. A p-value <0.05 was considered statistically significant. All statistical analyzes were performed using IBM SPSS Statistics, version 25 (IBM Corporation, Armonk, New York, USA).

Ethics

The study was approved by the Regional Committee for Medical and Health Research Ethics (approval number 2013/2082 and 2014/449), and the Research Unit at Sørlandet Hospital. Written informed consent was obtained from all participants. Participants could at any time withdraw their consent. Seven randomly selected participants received a gift card valued NOK 500 for taking part in the study, otherwise there was no economic benefit of participation.

Results

Study participants and recruitment rates

Out of 7424 invited individuals, 3571 responded to the invitation. Serum from 3568 (48.1%) individuals were available for analysis, and they were included in the study. Out of the 3568 included individuals, 1221 (34.2 %) were recruited when visiting the general practitioner's center, 1606 (45.0 %) by invitation letter/phone, and 741 (20.8 %) by other/unknown way of recruitment. Recruitment rates according to gender and age groups are described in Table 1. Mean age in the study was 48.2 years versus 41.9 years in the whole Søgne population aged 18-69 years ($p < 0.001$). The proportion of females was 53.1% in the study versus 48.8% in the whole Søgne population aged 18-69 years ($p < 0.001$). Recruitment rates were lowest among younger age groups and males. Out of the 3568 participants, 2968 (83.2%) returned the questionnaire.

Self-reported exposure to tick-bites and tick-borne infections

Reported exposure to tick-bites and tick-borne infections earlier in life are described in Table 2. More males than females reported at least three tick-bites earlier in life (66.7% vs 61.5%, $p = 0.003$). There were no significant gender differences for reporting at least one tick-bite, nor for erythema migrans earlier in life. Five percent (147/2950) reported health complaints attributed (by the participant and/or their general practitioner) to tick-borne infection.

Vaccinations

Vaccination against TBEV was reported by 4.3% (127/2947) of respondents. For yellow fever, 11.7% (344/2944) reported being vaccinated, 37.8% (1113/2944) not being vaccinated, and 50.5% (1487/2944) did not know.

Prevalence of serum IgG antibodies to tick-borne microbes

The prevalence of serum IgG antibodies to different tick-borne microbes are listed in Table 3.

All samples were analyzed for serum IgG antibodies to *Bb*sl, and the prevalence according to gender and age groups are listed in Table 4. The prevalence of serum IgG antibodies to *Bb*sl was significantly higher among males than females (27.7% vs 17.0%, $p < 0.001$), and tended for both genders to increase with increasing age. Adjusted for gender and age (based on the gender and age stratified seroprevalences listed in Table 4), the estimated overall seroprevalence of IgG antibodes to *Bb*sl in the Søgne population aged 18-69 years was 19.5%. The prevalence of serum IgG antibodies to *Bb*sl in relation to exposure to tick-bites and tick-borne infections earlier in life as reported by the participants were as follows: No tick-bite, 13.4% (59/439); one tick-bite, 16.9% (62/366); two tick-bites, 15.8% (41/259); three or more tick-bites, 27.2% (513/1883); tick-bite in the last year, 33.1% (326/984); and *Bb*sl-infection earlier in life, 32.1% (244/761). Among participants recruited when attending the general practitioner's center the seroprevalence of IgG antibodies to *Bb*sl was 21.9% (267/1221), whereas among participants recruited by invitation it was 22.9% (367/1606), and among participants with other/unknown way of recruitment it was 20.4% (151/741) ($p = 0.401$). The seroprevalence of IgG antibodies to *Bb*sl was 22.1% (340/1537) among samples tested for at least one other tick-borne pathogen, and 21.9% (445/2031) among samples tested only for *Bb*sl ($p = 0.880$). Among individuals not vaccinated against TBEV and/or yellow fever, the seroprevalence of IgG antibodies to TBEV was only 1.4% (6/419). We found no gender differences for the seroprevalence of IgG antibodies to TBEV (among individuals not vaccinated against TBEV and/or yellow fever), *A. phagocytophilum*, *B. microti*, *B. henselae/B. quintana* and *R. helvetica/R. conorii*. The seroprevalence of IgG antibodies to *A. phagocytophilum* was highest in the age group 60-69 years (16.9%) and lowest in the age group 40-49 years (6.7%). There were no differences in seroprevalence of IgG antibodies to TBEV (among individuals not vaccinated against TBEV and/or yellow fever), *B. microti*, *B. henselae/B. quintana* and *R. helvetica/R. conorii* according to age groups.

Serum IgG antibodies to *A. phagocytophilum* and *R. helvetica/R. conorii* was more prevalent among individuals with serum IgG antibodies to *Bb*sl than among individuals without (p = 0.010 and p = 0.016, respectively). Serum IgG antibodies to TBEV, *B. microti* and *B. henslae/B. quintana* did not show significant association with the presence of serum IgG antibodies to *Bb*sl. Among individuals not vaccinated against TBEV and/or yellow fever, the seroprevalence of IgG antibodies to TBEV was 2.0% (2/101) among individuals with serum IgG antibodies to *Bb*sl and 1.3% (4/318) among individuals without (p = 0.634).

Out of 1359 samples tested for all six microbes, 5.5% (75/1359) were seropositive to two microbes, 0.4% (6/1359) seropositive to three microbes, and none seropositive to more than three microbes.

Discussion

This is the first large-scale population based study of human exposure to tick-borne microbes in Norway. The study participants reported a high exposure to tick-bites; 63.9% reported at least three tick-bites earlier in life, and 33.4% reported at least one tick-bite the last year. We also found a high seroprevalence of IgG antibodies to *Bb*sl (22.0%) among the study participants. This was within the range reported in a previous study among blood donors (18.2%) from the same county (Mygland et al., 2006), but higher than previously found in other Norwegian regions (Hjetland et al., 2014; Hvidsten et al., 2017; Vestrheim et al., 2016). We did not confirm the results of the ELISA-test by immunoblot because the improvement of specificity by two-tier testing is limited (Dessau et al., 2018). However, a slight overestimation of the seroprevanence due to lack of confirmation of the positive samples by immunoblot, cannot be excluded.

The seroprevalence of IgG antibodies to *Bb*sl tended to increase with age (11.9% in the age group 18-29 years vs 33.5% in the age group 60-69 years), and was higher among males than females (27.7% vs 17.0%, respectively). An increasing seroprevalence with increasing age was also found in several previous studies from Norway and other European countries (Carlsson et al., 1998; Hjetland et al., 2014; van Beek et al., 2018; Vestrheim et al., 2016; Wilking et al., 2015), and is attributed to a cumulative exposure to tick-bites and prolonged persistence of serum IgG antibodies after infection. Previous studies from Norway (Hjetland et al., 2014; Vestrheim et al., 2016) and other European countries (Carlsson et al., 1998; van Beek et al., 2018; Wilking et al., 2015) also report higher seroprevalences of antibodies to *Bb*sl among males than females. This may be due to differences in exposure to tick-bites, or immunological factors. More males than females reported exposure to at least three tick-bites earlier in life (66.7% vs 61.5%, respectively), but there were no significant gender differences regarding reporting at least one tick-bite or tick-borne infection earlier in life. In our study, the gender difference in seroprevalence of IgG antibodies to *Bb*sl therefore seems to be greater than the gender difference in reported exposure to tick-bites and tick-borne infections can explain. More unnoticed tick-bites among males than females, a tendency that males notice and remove ticks later than females (Wilhelmsson et al., 2013), or differences in immunological factors, are possible causes for this discrepancy.

The seroprevalence of IgG antibodies to *A.phagocytophilum* (11.0%) was lower than previously found among blood donors from western Norway (16.3%) (Hjetland et al., 2015), but higher than we would expect from a 0-4% prevalence of *A. phagocytophilum* in ticks from our region (Kjelland et al., 2018; Quarsten et al., 2015) and a presumed low transmission rate (Henningsson et al., 2015). The cut-off titer recommended by the IFA-test manufacturer (i.e. 1:64) may also be low, but we did not perform further titration of positive sera as the optimal cut-off value is debated (Hjetland et al., 2015). Furthermore, serological reactivity to *A.*

phagocytophilum has been reported in 22% (4/18) of patients infected with *Neoehrlichia mikurensis* (Wass et al., 2018), indicating that cross-reactivity may be present. *N. mikurensis* has been detected in 10-19% of ticks collected along the southern coast of Norway (Kjelland et al., 2018), in 11% of ticks in northern Norway (Larsson et al., 2018), and in the blood of 10% of patients (from Aust-Agder and Vest-Agder counties) with erythema migrans or flu-like symptoms after a tick-bite (Quarsten et al., 2017), but today no commercial serology test is available. The seroprevalence of IgG antibodies to *R. helvetica/R. conorii* (4.2%) was higher than reported for *R. helvetica* among blood donors in Sweden (0.6-1.3%) (Elfving et al., 2008; Lindblom et al., 2013), but much lower than among recently tick-bitten individuals in Sweden and Åland Islands (44.0%) (Lindblom et al., 2016). Cross-reactivity to other *Rickettsia* species within the spotted-fever group may also be present as the tests have low specificity within the *Rickettsia* spotted-fever group according to the manufacturer's instructions. Seropositivity to *A.phagocytophilum* and *R. helvetica/R. conorii* was both significantly more prevalent among participants seropositive to *Bb*sl than among participants seronegative to *Bb*sl, indicating tick-borne transmission of these infections. There was a low seroprevalence of IgG antibodies to TBEV, 3.1% among all tested participants and only 1.4% among participants reporting no vaccination against TBEV and/or yellow fever. Vaccination against Japanese encephalitis was not charted in our questionnaire, but according to the Norwegian prescription registry, the number of vaccinations is very low in the area (http://reseptregisteret.no). The seroprevalence of IgG antibodies to *B. microti* was also low (2.1%), and in the same range as reported for *B. microti*/B. *divergens* among healthy individuals in Sweden (2.5%) (Svensson et al., 2019). However, the overall seroprevalence of antibodies to *Babesia* species may be underestimated in our study since the IFA-test applied is designed for *B. microti*, and cross-reactivity for other *Babesia* species is probably low (Lempereur et al., 2015; Sayama et al., 2018). *B. microti* has not been identified in Norwegian

ticks (Oines et al., 2012), but was the most prevalent *Babesia* species in ticks in both a Danish and a Swedish study (Karlsson and Andersson, 2016; Stensvold et al., 2015). The *Babesia* species found in the Norwegian study of ticks were *B. venatorum, B. divergens* and *B. capreoli* (Oines et al., 2012). Tick-borne transmission of *Babesia* infection may exist in the study area, although there was no significant difference in seroprevalence of IgG antibodies to *B. microti* between the *Bb*sl seropositive and seronegative group. Only two participants (0.1%) were seropositive to *B. henselae/B. quintana*. In a previous study of microbes in ticks from the region, *Bartonella* species was not found (Quarsten et al., 2015), and it was also not found in a recent study from Sweden and Åland Islands (Cronhjort et al., 2019). Whether other Bartonella species may induce antibodies that cross-react with *B. henselae*/*B. quintana* is not known. Seropositivity to more than one other tick-borne pathogen than *Bb*sl was rare.

The strength of our study is the large number of participants from a general adult population in a geographically well-defined area. A weakness of our study is that we have not charted travel history, and some travel-related exposure to tick-borne microbes may be present. Another potential weakness is possible selection biases due to our recruitment method. However, there was no significant differences in seroprevalence of IgG antibodies to *Bb*sl between those recruited when attending the general practitioner's center, those recruited by invitation, and those with other/unknown way of recruitment (participants were asked about way of recruitment in the questionnaire, and classified as "unknown way of recruitment" if the question was not answered). The recruitment rate was lower among the younger age groups, and lower among males than females. Of the seroprevalences to the different tick-borne microbes, only the seroprevalence of antibodies to *Bb*sl showed a clear gender and age related association, and this was adjusted for when estimating the overall seroprevalence of IgG antibodies to *Bb*sl among the Søgne population aged 18-69 years (estimated to 19.5% vs 22.0% among all the study participants). The seroprevalence of IgG antibodies to *Bb*sl did not

differ significantly between the samples tested for at least one other pathogen than *Bb*sl and the samples tested only for *Bb*sl. We therefore consider the selection of samples tested for other microbes than *Bb*sl as representative with respect to exposure to tick-borne infections.

In conclusion, our study indicates a high exposure to tick-bites and tick-borne microbes in the general adult population living in a coastline municipality in the southernmost part of Norway. The seroprevalence of IgG antibodies to *Bb*sl was high. Further, our study indicates that the population also is exposed to *A.phagocytophilum*, *R. helvetica/R. conorii*, *B. microti* and TBEV, but very rarely to *B. henselae/B. quintana*. In case of symptoms of infectious disease after a tick-bite, or fever without known cause, other tick-borne infections than *Bb*sl and TBEV should therefore be considered. However, serological testing should be interpreted carefully since a positive test for serum IgG antibodies cannot separate current from past infection.

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Declarations of interest:

None.

References:

Carlsson, S.A., Granlund, H., Nyman, D., Wahlberg, P., 1998. IgG seroprevalence of Lyme borreliosis in the population of the Aland Islands in Finland. Scand. J. Infect. Dis. 30, 501-503.
Cronhjort, S., Wilhelmsson, P., Karlsson, L., Thelaus, J., Sjodin, A., Forsberg, P., Lindgren, P.E., 2019. The Tick-Borne Diseases STING study: Real-time PCR analysis of three emerging tick-borne pathogens in ticks that have bitten humans in different regions of Sweden and the Aland islands, Finland. Infect. Ecol. Epidemio.l 9, 1683935.
Dessau, R.B., van Dam, A.P., Fingerle, V., Gray, J., Hovius, J.W., Hunfeld, K.P., Jaulhac, B., Kahl, O., Kristoferitsch, W., Lindgren, P.E., Markowicz, M., Mavin, S., Ornstein, K., Rupprecht, T., Stanek, G., Strle, F., 2018. To test or not to test? Laboratory support for the diagnosis of Lyme borreliosis: a position paper of ESGBOR, the ESCMID study group for Lyme borreliosis. Clin. Microbiol. Infect. 24, 118-124.
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.
Frivik, J.O., Noraas, S., Grankvist, A., Wenneras, C., Quarsten, H., 2017. A man in his sixties from Southern Norway with intermittent fever. Tidsskr. Nor. Laegeforen. 137.
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.
Hjetland, R., Henningsson, A.J., Vainio, K., Dudman, S.G., Grude, N., Ulvestad, E., 2015. Seroprevalence of antibodies to tick-borne encephalitis virus and *Anaplasma phagocytophilum* in healthy adults from western Norway. Infect. Dis. (Lond) 47, 52-56.
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.
Hvidsten, D., Mortensen, L., Straume, B., Arsenovic, M.G., Pedersen, A.B., Lyngas, G., Gray, J.S., Grude, N., 2017. Blood donor *Borrelia burgdorferi* sensu lato seroprevalence and history of tick bites at a northern limit of the vector distribution. Apmis. 125, 717-724.
Jore, S., Viljugrein, H., Hofshagen, M., Brun-Hansen, H., Kristoffersen, A.B., Nygard, K., Brun, E., Ottesen, P., Saevik, B.K., 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.

Kalish, R.A., McHugh, G., Granquist, J., Shea, B., Ruthazer, R., Steere, A.C., 2001. Persistence of immunoglobulin M or immunoglobulin G antibody responses to *Borrelia burgdorferi* 10-20 years after active Lyme disease. Clin. Infect. Dis. 33, 780-785.
Karlsson, M.E., Andersson, M.O., 2016. *Babesia* species in questing *Ixodes ricinus*, Sweden. Ticks Tick Borne Dis. 7, 10-12.
Kjelland, V., Paulsen, K.M., Rollum, R., Jenkins, A., Stuen, S., Soleng, A., Edgar, K.S., Lindstedt, H.H., Vaino, K., Gibory, M., Andreassen, A.K., 2018. Tick-borne encephalitis virus, *Borrelia burgdorferi* sensu lato, *Borrelia miyamotoi*, *Anaplasma phagocytophilum* and *Candidatus Neoehrlichia mikurensis* in *Ixodes ricinus* ticks collected from recreational islands in southern Norway. Ticks Tick Borne Dis. 9, 1098-1102.
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.
Larsen, A.L., Kanestrom, A., Bjorland, M., Andreassen, A., 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.
Larsson, C., Hvidsten, D., Stuen, S., Henningsson, A.J., Wilhelmsson, P., 2018. "*Candidatus Neoehrlichia mikurensis*" in *Ixodes ricinus* ticks collected near the Arctic Circle in Norway. Parasit Vectors 11, 620.
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.
Lempereur, L., Shiels, B., Heyman, P., Moreau, E., Saegerman, C., Losson, B., Malandrin, L., 2015. A retrospective serological survey on human babesiosis in Belgium. Clin. Microbiol. Infect. 21, 96.e91-97.
Lindblom, A., Wallmenius, K., Nordberg, M., Forsberg, P., Eliasson, I., Pahlson, C., Nilsson, K., 2013. Seroreactivity for spotted fever rickettsiae and co-infections with other tick-borne agents among habitants in central and southern Sweden. Eur. J. Clin. Microbiol. Infect. Dis. 32, 317-323.
Lindblom, A., Wallmenius, K., Sjowall, J., Fryland, L., Wilhelmsson, P., Lindgren, P.E., Forsberg, P., Nilsson, K., 2016. Prevalence of *Rickettsia* spp. in Ticks and Serological and Clinical Outcomes in Tick-Bitten Individuals in Sweden and on the Aland Islands. PLoS One 11, e0166653.
Ljostad, U., Mygland, A., Skarpaas, T., 2003. Neuroborreliosis in Vest-Agder. Tidsskr. Nor. Laegeforen. 123, 610-613.
McGill, S., Wesslen, L., Hjelm, E., Holmberg, M., Auvinen, M.K., Berggren, K., Grandin-Jarl, B., Johnson, U., Wikstrom, S., Friman, G., 2005. *Bartonella* spp. seroprevalence in healthy Swedish blood donors. Scand. J. Infect. Dis. 37, 723-730.
Morch, K., Holmaas, G., Frolander, P.S., Kristoffersen, E.K., 2015. Severe human *Babesia divergens* infection in Norway. Int. J. Infect. Dis. 33, 37-38.
Mygland, A., Skarpaas, T., Ljostad, U., 2006. Chronic polyneuropathy and Lyme disease. Eur. J. Neurol. 13, 1213-1215.
Ocampo, J.M.F., Jore, S., Andreassen, Å.K., Wiklund, B.S., Dudmann, S., Ottesen, P., Sandbu, S., Soleng, A., Vainio, K., Aaberge, I., Nygård, K., Vold, L., 2016. Årsrapport flått og flåttbårne sykdommer i 2015. Norwegian institute of public health.
Oines, O., Radzijevskaja, J., Paulauskas, A., Rosef, O., 2012. Prevalence and diversity of *Babesia* spp. in questing *Ixodes ricinus* ticks from Norway. Parasit Vectors 5, 156.
Quarsten, H., Grankvist, A., Hoyvoll, L., Myre, I.B., Skarpaas, T., Kjelland, V., Wenneras, C., Noraas, S., 2017. *Candidatus Neoehrlichia mikurensis* and *Borrelia burgdorferi* sensu lato detected in the blood of Norwegian patients with erythema migrans. Ticks Tick Borne Dis. 8, 715-720.

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.
Sayama, Y., Zamoto-Niikura, A., Matsumoto, C., Saijo, M., Ishihara, C., Matsubayashi, K., Nagai, T., Satake, M., 2018. Analysis of antigen-antibody cross-reactivity among lineages and sublineages of *Babesia microti* parasites using human babesiosis specimens. Transfusion 58, 1234-1244.
Skarpaas, T., Sundoy, 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.
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.
Stuen, S., Bergstrom, K., 2008. Human anaplasmosis--a hidden disease in Norway?. Tidsskr. Nor. Laegeforen. 128, 2579-2581.
Svensson, J., Hunfeld, K.P., Persson, K.E.M., 2019. High seroprevalence of *Babesia* antibodies among *Borrelia burgdorferi*-infected humans in Sweden. Ticks Tick Borne Dis. 10, 186-190.
van Beek, J., Sajanti, E., Helve, O., Ollgren, J., Virtanen, M.J., Rissanen, H., Lyytikainen, O., Hytonen, J., Sane, J., 2018. Population-based *Borrelia burgdorferi* sensu lato seroprevalence and associated risk factors in Finland. Ticks Tick Borne Dis. 9, 275-280.
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.
Wass, L., Grankvist, A., Mattsson, M., Gustafsson, H., Krogfelt, K., Olsen, B., Nilsson, K., Martensson, A., Quarsten, H., Henningsson, A.J., Wenneras, C., 2018. Serological reactivity to *Anaplasma phagocytophilum* in neoehrlichiosis patients. Eur. J. Clin. Microbiol. Infect. Dis. 37, 1673-1678.
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.
Wilking, H., Fingerle, V., Klier, C., Thamm, M., Stark, K., 2015. Antibodies against *Borrelia burgdorferi* sensu lato among Adults, Germany, 2008-2011. Emerg. Infect. Dis. 21, 107-110.

Web-references:

The Norwegian Surveillance System for Communicable Diseases (MSIS), Norwegian Institute of Public Health. http://msis.no. Accessed 20 January 2020.
Tick-borne encephalitis, Norwegian Institute of Public Health. https://www.fhi.no/en/el/insects-and-pests/ticks-and-tick-borne-diseases/skogflattencefalitt-tbe/. Accessed 20 January 2020.
The Norwegian prescription registry, Norwegian Institute of Public Health. http://reseptregisteret.no. Accessed 20 January 2020.

Table 1: Study participants and recruitment rates

	Study participants (n)	Recruitment rates (%)
All participants	3568	48.1
Gender		
Males	1674	44.0
Females	1894	52.3
Age (years)		
18-29	369	19.6
30-39	594	43.5
40-49	875	52.2
50-59	825	59.5
60-69	905	80.9

Table 2: Self-reported exposure to tick-bites and tick-borne infections earlier in life

	n	%*
No tick-bite	439	14.9
One tick-bite	366	12.4
Two tick-bites	259	8.8
Three or more tick-bites	1883	63.9
Tick-bite in the last year	984	33.4
Erythema migrans	723	24.6
Neuroborreliosis	29	1.0
Borrelia arthritis	14	0.5
Tick-borne encephalitis	3	0.1
Other tick-borne infection	87	2.9

*n = 2942-2968 responders to the questions

Table 3: Prevalence of serum IgG antibodies to different tick-borne microbes

	All samples n	IgG positive % (95% CI)	IgG equivocal % (95% CI)	IgG negative % (95% CI)
Borrelia burgdorferi sensu lato	3568	22.0 (20.6-23.4)	5.7 (4.9-6.4)	72.3 (70.9-73.8)
Tick-borne encephalitis virus	1453	3.1 (2.2-4.0)	0.6 (0.2-1.0)	96.3 (95.3-97.3)
Anaplasma phagocytophilum	1452	11.0 (9.3-12.6)	6.1 (4.9-7.4)	82.9 (81.0-84.9)
Babesia microti	1537	2.1 (1.4-2.9)	3.2 (2.3-4.1)	94.7 (93.5-95.8)
Bartonella henselae or/and B. *quintana*	1451	0.1 (-0.1-0.3)	1.7 (1.1-2.4)	98.2 (97.4-98.8)
Rickettsia helvetica or/and R. *conorii*	1445	4.2 (3.1-5.2)	5.1 (3.9-6.2)	90.8 (89.3-92.3)

Abbreviations: CI: Confidence Interval

Table 4: Prevalence of serum IgG antibodies to *Borrelia burgdorferi* sensu lato according to age and gender.

Age (years)	Males		Females		Males and females	
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
18-29	20	12.7 (7.5-17.8)	24	11.4 (7.1-15.7)	44	11.9 (8.6-15.2)
30-39	47	18.8 (14.0-23.6)	40	11.6 (8.2-15.0)	87	14.6 (11.8-17.5)
40-49	92	23.0 (18.9-27.1)	61	12.8 (9.8-15.9)	153	17.5 (15.0-20.0)
50-59	117	27.7 (23.5-32.0)	81	20.1 (16.2-24.0)	198	24.0 (21.1-26.9)
60-69	187	42.1 (37.5-46.7)	116	25.2 (21.2-29.1)	303	33.5 (30.4-36.6)
18-69	463	27.7 (25.5-29.8)	322	17.0 (15.3-18.7)	785	22.0 (20.6-23.4)

Abbreviations: CI: Confidence Interval

Table 5: Prevalence of serum IgG antibodies to other tick-borne microbes among individuals with and without antibodies to *Borrelia burgdorferi* sensu lato

	Proportion IgG positive % (n/N)				
	TBEV	*Anaplasma*	*Babesia*	*Bartonella*	*Rickettsia*
Borrelia IgG positive	2.8 (9/323)	14.9 (48/322)	3.2 (11/340)	0.0 (0/323)	6.5 (21/323)
Borrelia IgG negative or equivocal	3.2 (36/1130)	9.8 (111/1130)	1.8 (22/1197)	0.2 (2/1128)	3.5 (39/1122)
P-value	0.715	0.010	0.117	1.000	0.016

Abbreviations: *Borrelia*: *Borrelia burgdorferi* sensu lato, TBEV: Tick-borne encephalitis virus, *Anaplasma*: *Anaplasma phagocytophilum*, *Babesia*: *Babesia microti*, *Bartonella*: *Bartonella henselae* or/and *B. quintana*, *Rickettsia*: *Rickettsia helvetica* or/and R. *conorii*.