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Postprint available at: Linköping University Electronic Press http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-66879 Low risk of developing Borrelia burgdorferi infection in the South East of

Sweden after a bite by a Borrelia burgdorferi-infected tick

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Abstract

Objectives: The risk of developing Lyme borreliosis (LB) from Borrelia burgdorferi sensu

lato (Bb)-infected ticks in Sweden is largely unknown. In the current study, we investigated

the prevalence of Bb in ticks which have bitten humans and the risk of developing LB from

Bb-infected ticks.

Methods: Health questionnaires, blood samples and ticks were collected from 394 tick-bitten

study subjects in the County of Östergötland, Sweden, at time of the tick bite. Questionnaires

and blood samples were also collected three months later. Ticks were screened for Bb DNA

with PCR while sera were analysed for antibodies against Bb with two ELISA assays.

Seroconversion, i.e. at least twofold increase of anti-Bb antibodies after three months, was

confirmed by a line immunoblot.

Results: Seventy-five of 397 ticks collected from the study subjects were detected Bb-

positive. Sixty-four of the tick-bitten subjects had been bitten by Bb-infected ticks. Four of

them showed seroconversion and were thereby considered to have an active Bb infection.

None of them visited health care due to symptoms, but one reported symptoms.

Conclusion: Our data suggest that the risk of developing LB after bite by a Bb-infected tick is

low, and asymptomatic Bb infections seem to be more frequent than symptomatic.

Keywords: Borrelia burgdorferi sensu lato, Lyme borreliosis, tick-bite, anti-Borrelia

antibodies

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Introduction

The spirochete *Borrelia burgdorferi* sensu lato (Bb) is the causative agent of the tick-borne disease Lyme borreliosis (LB) ¹ of which at least four species; *Borrelia (B.) burgdorferi* sensu stricto, *B. garinii*, *B. afzelii* ² and the newly described *B. spielmanii* ³ are known to be pathogenic to humans. The disease is nationally notifiable in the USA, Japan and in several European countries, e.g. Estonia, Latvia, Lithuania, Croatia, Czech Republic, Italy, Portugal, Slovenia and Scotland ⁴. The highest frequencies of LB in Europe are reported from Austria, Germany, Slovenia and Sweden, whereas the highest frequencies in the USA are reported from the Northeastern and upper Midwest states ^{1,5}. The distribution of LB is heterogeneous with hyperendemic areas where the incidence is over 100 annual clinical LB infections per 100 000 inhabitants, such as the Åland Islands with 2 000/100 000/y ⁶ and the Swedish county of Blekinge with an incidence of 464/100 000/y ⁷. The incidence rate of LB in Sweden has not been fully investigated. Two previous studies have investigated the annual incidence of LB in the southeastern county of Blekinge (464/100 000 inhabitants) ⁷ and in seven southern counties (69/100 000 inhabitants) ⁸.

The clinical manifestations of the disease can be divided into three stages; early localised infection occurring in the skin and manifested by development of erythema migrans or lymphocytoma; early disseminated infection usually manifested by neurological (neuroborreliosis) or muscoskeletal symptoms (Lyme arthritis) and late disseminated infection in untreated patients who have developed acrodermatitis chronica atrophicans, chronic neuroborreliosis or Lyme arthritis. In addition, the clinical outcome of the infection differs between individuals. Individuals may have an infection which is principally asymptomatic ⁸⁻¹⁰, or a subacute infection defined as symptoms lasting less than six months, or symptoms

lasting more than six months despite well-recognised antibiotic treatment, i.e. LB with persistent symptoms post-treatment ¹¹⁻¹⁵.

The risk of developing LB from a Bb-infected tick is not well-studied in Sweden, although several studies in other countries have addressed this issue ¹⁶⁻²⁰ and LB is the most commonly reported arthropod-borne disease in both the USA and Europe ⁴. Several studies have shown antibodies against Bb in individuals who have no experience of symptoms possibly associated with LB ^{10, 21}. This may indicate that the immune system eradicates the spirochete fast and efficiently. Thus, asymptomatic infections, where symptoms associated with LB are absent, could be frequent after Bb infection. However, this, has to our knowledge, not been thoroughly investigated. Nadelman et al. 18 have previously shown that 3.2% of the healthy newly tick-bitten control subjects in an antibiotic prophylaxis study performed in an American hyperendemic area, developed erythema migrans during the study period of six weeks. Surprisingly, none of the study's control subjects seemed to have an asymptomatic Bb infection. However, B. burgdorferi sensu stricto is the only human pathogenic strain of Bb in the USA. Therefore, the disease outcome of healthy newly tickbitten individuals bitten by infected ticks in Europe, where B. afzelii and B. garinii are prevalent, needs to be further investigated in order to study the risk of developing LB and the occurrence of asymptomatic infections in Europe. Therefore, we wanted to follow tick-bitten individuals for a period of three months after the tick bite, in order to study the rate of infection and the clinical outcome. Expanding the knowledge concerning the risk of developing LB is also important for the understanding of the different disease outcomes.

The aim of this study was to evaluate the risk of developing LB after a bite by a Bb-infected tick. We also wanted to investigate if asymptomatic Bb infection occurs by studying if there are individuals whom, after bitten by infected ticks, develop anti-Bb antibodies but show no symptoms possibly associated with LB. Finally, we wanted to study if the number of

spirochetes in the infected ticks and the time elapsed before the tick removal is correlated to the development of LB.

Material and methods

Study design

The study was initiated in the early summer of 2007 by advertisement in local television and newspapers. Between June 2007 and January 2008, the population belonging to nine Primary health care (PHC) centres in the County of Östergötland in South East Sweden and the Department of Infectious Diseases at the University Hospital in Linköping (UHL) were asked to participate in the study if they had been bitten by a tick. The study subjects were asked to bring their tick/s to their PHC centre, where they signed informed consent, answered a standardized health questionnaire with relevant questions, and blood samples were collected. The participants were further asked to return to the PHC centre for a second visit three months later for new blood sampling and a new health questionnaire. Two sets of blood samples, the ticks and questionnaires were sent to the UHL for processing and storage. The study subjects could discontinue their participation at any time, and samples would be discarded at request from the individual. The study was approved by the Ethical Committee of the Faculty of Health Sciences at Linköping University (Dnr.M132-06).

Questionnaires

The first questionnaire included questions about the time of the tick bite, estimated time of tick-exposure and previous treatments for Bb infection. The second questionnaire included questions about new tick bites during the past three months, the general health condition during the period, symptoms possibly associated with LB, and if they had attended their PHC centre because of the symptoms. The listed symptoms possibly associated with LB were

headache, fatigue, fever, neck pain, loss of appetite, nausea, weight loss, vertigo, cognitive difficulties, radiating pain, myalgia/arthralgia and numbness. In addition, medical records were obtained from study subjects who had attended health care for symptoms possibly associated with LB, including erythema migrans (EM).

Inclusion and exclusion criteria

Individuals of 18 years of age or older were recruited to the study if they recently had been bitten by a tick and brought it to the first visit at the PHC centre, and signed a consent to participate. Individuals who were on antibiotic treatment during the time of inclusion, who suffered from similar symptoms as those associated with LB, or received treatments for diseases that would require medications that could influence the immune response, were not included in the study.

Study subjects

Three hundred and ninety-seven ticks were collected from 394 tick-bitten study subjects during June 2007-January 2008 (Fig.1). Fifty-three of the 394 study subjects were excluded from further analysis due to not attending to the follow-up visit in spite of having received a reminder (n=46), suffering from similar symptoms as those associated with LB at the time of inclusion (n=2), being on antibiotic treatment while included in the study (n=2), blood samples were not analysed by both ELISA assays (n=2) and one subject whose blood samples were damaged during the transport, ending up with 341 subjects completing the study. One of the authors (PF), with long time clinical experience of LB, scrutinized medical records from study subjects, who during the study period attended health care for symptoms possibly associated with LB, in order to determine current Bb infection or symptoms resulting from or associated with other diseases.

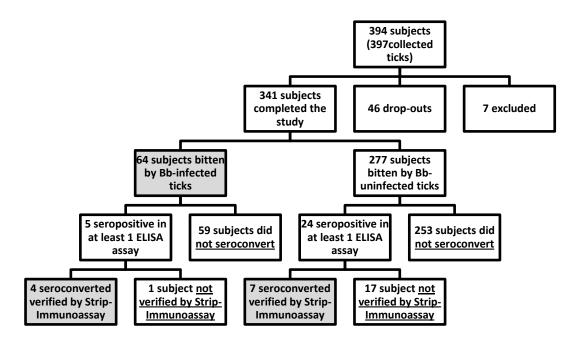


Fig. 1 Flow chart over the tick-bitten study subjects who participated in the study and their antibody responses

Analyses of Borrelia antibodies and seroconversion

The blood samples were centrifuged at 1 250g for 10 minutes at room temperature upon the arrival to the UHL, and serum aliquots were frozen and stored at -70°C until further analysis. Serum samples from both the time of tick bite and the second visit to the PHC centre were analysed for both anti-flagellum and anti-C6 antibodies. Enzyme-linked immunosorbent assay (ELISA) IDEIATM Borrelia IgG (Oxoid, Cambs, UK) was used for the analysis of IgG antibodies directed against the Bb flagellum. C6 Lyme ELISATM kit (Immunetics Inc., Cambridge, MA, USA) was used for analysis of IgM and IgG antibodies directed against the synthetic C6 peptide derived from the Bb protein VIsE (variable major protein-like sequence, expressed). A Bio-Rad CODA apparatus (Bio-Rad Laboratories, Hercules, CA, USA) was used for the C6-analysis. Both the ELISA assays were performed according to manufacturer's instructions with the exception of the IDEIATM Borrelia IgG, which was analysed on single

samples instead of duplicates, as is the standard procedure at the Department of Clinical Microbiology at the UHL. Seroconversion, i.e. the development of new antibodies, indicating a current infection, was defined as either seronegative to seropositive or a minimum of twofold increase in arbitrary units (anti-flagellum) or Lyme index (anti-C6 antibodies). All serum samples from study subjects who showed seroconversion in any or both ELISAs were further analysed with the line immunoblot recomLine Borrelia IgG (Mikrogen, Neuried, Germany) in order to confirm conversion. This detects antibodies directed against p100 (B. afzelii), VIsE (fusion protein from different genospecies), p58 (B. garinii), p41 (B. burgdorferi sensu stricto), mp39 (B. afzelii), OspA (B. afzelii), OspC (B. burgdorferi sensu stricto, B. afzelii, B. garinii, B. spielmanii) and p18 (B. burgdorferi sensu stricto, B. afzelii, B. garinii 1, B. garinii 2, B. spielmanii). The results and the occurrence of seroconversion from the recomLine Borrelia IgG were interpreted according to manufacturer's instructions. The serological assessment of a current Bb infection, further defined as subacute or asymptomatic infection, was done by one of the authors (DN), who have long time clinical experience of LB at the hyperendemic Åland Islands. Subjects, who showed seroconversion in at least one of the ELISA assays verified by seroconversion also in the line immunoblot assay, were considered as having a probable current Bb infection.

Handling of ticks

On arrival to the UHL, the ticks were washed in 70% ethanol, followed by washing with phosphate buffered saline (PBS, Medicago, Uppsala, Sweden). The ticks were then dried for a few seconds on a paper handkerchief and longitudinally bisected. The bisected ticks were stored in -20°C before subjected to DNA-extraction, followed by real-time PCR for detection and quantification of Borrelia spp.

PCR assays for detection, quantification, and species determination of *Borrelia* spp. in ticks detached from humans

Detection and quantification of Bb were performed using a real-time PCR assay based on the Light Upon eXtensionTM (LUX) technique (Invitrogen Corporation), as previously described by Wilhelmsson P, et.al. ²². The Bb was detected and quantified using the group-specific primers B16S_FL and B16S_R, targeting all *Borrelia*-species so far detected in Europe ²², covering a 131 base pair long, genus-specific region of the 16S rRNA gene. The primer pair was validated using DNA extracted from strains of 11 different *Borrelia* species as well as DNA from unrelated bacterial species, human DNA, and DNA from ticks. The PCR products obtained was nucleotide sequenced in order to verify the expected specificity. The results from the validation showed that the primer pair possessed the expected specificity. Furthermore, the LUX primer pair was compared to an already published TaqMan real-time PCR assay ²³, showing that the LUX real-time PCR assay detected further three positive samples (out of totally 399 DNA extracts from ticks analysed) than the TaqMan real-time PCR assay. The detection limit for the LUX real-time PCR assay was less than 10 gene copies, which is equivalent to the number of copies that exists in one Borrelia cell ²³. In addition, compared to the TaqMan assay, the LUX assay had a higher mean efficiency. The Bb positive samples were further species determined by two nested, conventional PCR

assays, using primers targeting the genetically diverse regions within the 5S-23S intergenic spacer (IGS) and 16S-23S IGS. The outer primer pair used to amplify the region of 5S-23S IGS was earlier described by Postic et al. ²⁴ and the inner primer pair was reported by Wilhelmsson P, et al ²². The primer set used to amplify the region of 16S-23S IGS was described by Bunikis et al. ²⁵. Macrogen Inc. (Seoul, Korea) performed nucleotide sequencing of the PCR products obtained from these two PCR assays based on BigDye chemistry.

Statistical methods

Fisher's exact test was used to compare the percentage distribution of data between the two groups of study subjects bitten by Bb-infected and Bb-uninfected ticks. The age was assumed to be normally distributed, and therefore Student's t-test was used to compare the age distribution within the two groups of tick-bitten study subjects. Spearman rank correlation test was used to calculate correlation between the serology of the two ELISA assays. All statistical calculations were done with the SPSS 15.0 for windows. P-values < 0.05 were considered significant.

Results

A flow-chart which summaries the results of the study is shown in Figure 1.

Prevalence of Bb in the collected ticks

Seventy-five (19%) of all the collected ticks were infected with Bb, whereas the remaining ones did not contain any detectable levels of the spirochetes ²². Sixty-four (19%) of the 341 tick-bitten study subjects who completed the study had been bitten by a Bb-infected tick, while 277 (81%) study subjects had been bitten by a tick that did not contain any detectable Bb spirochetes (Fig 1).

Few individuals seroconverted

Of the initial 341 blood samples, 136 (40 %) had detectable levels of anti-C6 antibodies and 39 (11%) samples had anti-flagellum antibodies, as expected in tick-exposed persons in an endemic region. Of the 136 individuals who were seropositive for Bb antibodies at the time of enrolment, 56 individuals reported a past history of symptomatic LB. C6 and flagellum antibodies were correlated in the serology (rho=0.585, p<0.01), i.e. positive detection of anti-Bb antibodies in the first blood sample. The test for antibodies to native flagellum protein

showed significantly less sensitivity, 29% (95% CI 22-37%), than the C6 antibody test (p<0.0001). Five of the 64 subjects (7.8%) who were bitten by a Bb-infected tick showed seroconversion for anti-flagellum, anti-C6 or both antibodies as detected by ELISA during the three months they participated in the study (subjects 1-5 in Table 1). In addition, 24 subjects among the 277 bitten by Bb-uninfected ticks (8.7%), also showed seroconversion (subjects 6-29 in Table 1). The samples from subjects displaying seroconversion in any of the ELISA assays were further analysed with a line immunoblot assay in order to confirm the finding. The seroconversion among the five subjects bitten by Bb-infected ticks was verified by the development of new antibodies also in the line blot in four of the subjects. These four were consequently considered as having a probable current Bb infection (subjects 1-4 in Table 1). Two of the subjects who seroconverted had previously had LB, although not within the previous year before participation in the study. Out of the 24 subjects bitten by Bb-uninfected ticks, and where seroconversion was determined by at least one of the two ELISA assays, conversion was verified by the line blot assay in seven study subjects (subjects 6-12 in Table 1).

Self-reported symptoms, need for medical attention and Lyme borreliosis diagnosis

Self-experienced symptoms possibly associated with LB were reported by 91 tick-bitten subjects (Fig. 2 and Table 2). Although both study subjects bitten by infected and uninfected ticks reported symptoms that may have been due to a current Bb-infection (Table 2), the frequency of subjects reporting symptoms were higher in the group bitten by Bb-infected ticks (27/64) compared to subjects bitten by uninfected ticks (64/277), 42% (95% CI 30-54%) and 23% (95% CI 18-28%), respectively, p=0.003 (Fig. 2). There were no differences between subjects bitten by a Bb positive tick and subjects bitten by a negative tick when comparing the frequency of reported experience of several symptoms. Among the four subjects bitten by Bb-infected ticks and considered as carrying a probable, current Bb-infection, one reported

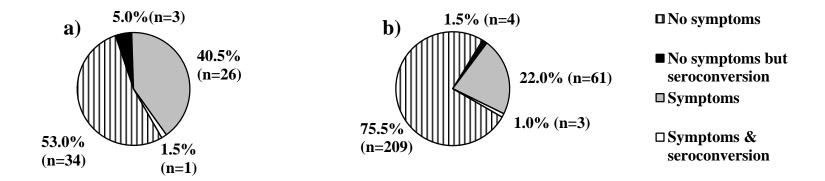


Fig. 2 Pie chart showing frequency of self-reported symptoms in study subjects bitten by Bb positive (n=64) or negative (n=277) ticks. Ninetyone of the 341 subjects in the study reported experience of symptoms possibly associated with Lyme borreliosis during the three months time of the study. Twenty-seven of these subjects had been bitten by Bb-infected ticks. Few of the subjects who had seroconverted experienced any symptoms possibly associated with LB. Bb, *Borrelia burgdorferi* sensu lato. a) subjects bitten by Bb positive ticks; b) subjects bitten by Bb negative ticks.

symptoms possibly associated with LB, but did not attended PHC during the study time due to the reported symptoms (subject 1 in Table 1). In contrast, two of the study subjects who seroconverted after a bite by an uninfected tick, searched care for their symptoms (subjects 6 and 16 in Table 1). One of the latter cases was diagnosed with an EM (subject 6 in Table 1).

Number of spirochetes in the ticks and time of tick exposure

Ticks from six of the 64 individuals bitten by Bb positive ticks were engorged (three adults and three nymphs) at the time of collection (data not shown). Two study subjects who had been bitten by two of the engorged adult ticks had seroconverted at the 3-month follow-up, but not developed symptoms possibly associated with LB. The other two study subjects who had seroconverted were bitten by adult ticks that were not engorged. Furthermore, among the four individuals considered as carrying a probable current Bb-infection, three had been bitten by ticks containing high numbers of Bb (subjects 1, 2 and 4 in Fig. 3), whereas the tick collected from the fourth subject contained low numbers of Bb spirochetes (individual 3 in Fig. 3). Three of these ticks contained B. afzelii while the fourth subject's tick contained B. garinii, as reported elsewhere ²². The only one of the four individuals who reported symptoms possibly associated with LB had been bitten by a tick containing high numbers of Bb spirochetes (individual 1 in Fig. 3). Estimated exposure for the tick (the time from the bite until the tick was removed) varied from less than one minute to 34 hours, as reported by the subjects bitten by a Bb-positive tick (data not shown). Among the four subjects considered as having a probable current Bb infection, the individual bitten by the tick with the lowest numbers of spirochetes reported the shortest time of exposure (5 minutes) (individual 3 in Fig. 3). The individual who reported symptoms estimated the time of exposure to almost nine hours (individual 1 in Fig. 3). The other two subjects reported exposure for less than one

minute and six hours (the individual was bitten by two ticks, of which one was negative for detection of Bb) and less than 16 hours, respectively (individuals 2 and 4 in Fig. 3).

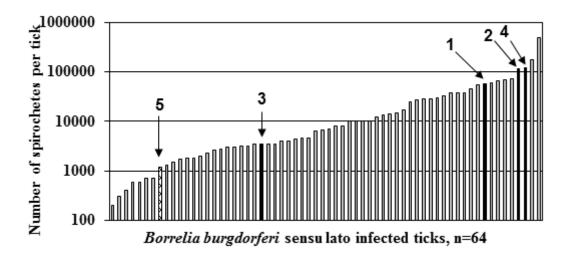


Fig. 3 Number of spirochetes in the ticks collected from tick-bitten study subjects

The bars represent the number of Bb spirochetes in the collected Bb-infected ticks. Black bars represent the four of the 64 study subjects who had seroconverted, i.e. had at least a twofold increase of anti-Bb antibodies as measured by ELISA and confirmed by the line immunoblot, during the study period. One of the subjects experienced symptoms possibly associated with LB (no 1). The fifth bar marked with an arrow represents the study subject with uncertain seroconversion. Bb, *Borrelia burgdorferi* sensu lato; 1, seroconversion and symptoms (fatigue and myalgia/arthralgia); 2, seroconversion; 3, seroconversion; 4, seroconversion; 5, uncertain seroconversion.

Additional tick bites during the study period

Thirty-three of the 64 (52%) study subjects bitten by a Bb-positive tick reported, according to the questionnaires, that they had been bitten by at least one more tick during the study period, whereas 139 of the 277 (50%) subjects bitten by a Bb-negative tick reported additional tick bites.

Sex and age distribution in the tick-bitten groups

More women than men participated in this study (63% women), but no differences in sex distribution could be seen between the group of study subjects bitten by Bb-infected ticks and

those bitten by uninfected ticks (64% and 63% women, respectively) (data not shown). The study subjects had a mean age of 62 years (range 19-91) with women having a mean age of 61 years (range 19-84) and men of 65 years (range 36-91) (data not shown). Those study subjects who showed seroconversion were older than those who did not produce new antibodies (p=0.029) (data not shown).

Discussion

The main finding of this study was that only four out of 64 individuals (6%) bitten by a confirmed Bb-infected tick showed definite seroconversion for Bb-antibodies three months after the tick bite. Out of these four subjects, considered to have a probable, current Bb infection, only one reported experience of symptoms possibly associated with LB, but did not attend PHC because of the symptoms. Taken together, these results indicate that the risk of obtaining a Bb infection, even after a bite from a Bb-infected tick, is small and furthermore, that the risk for development of clinical LB is even smaller, and that asymptomatic Bb infection does occur. Asymptomatic infection have previously been described in studies screening for anti-Bb antibodies in blood donors ¹⁰ and in a vaccine trial ²¹, but not in a prospective study of tick-bitten individuals like this.

The number of Bb spirochetes in the infected ticks varied largely ²². Few of the subjects bitten by Bb-infected ticks had serological evidence of Bb infection and no one developed clinical LB, making this study too small to draw firm conclusions about spirochetal load in the tick and the risk of infection. However, the majority of the study subjects reported that they had removed the tick within in 12 hours. Experimental data have shown that the spirochete is confined to the lumen of the tick gut 24 hours after host attachment ²⁶ and that the migration towards the salivary glands starts approximately 36 hours after the beginning of the tick feeding ²⁷. However, one should keep in mind that the time of tick exposure was

estimated by the study subjects, and may therefore not be entirely accurate. Interestingly, two of the study subjects bitten by Bb positive ticks and who seroconverted had been bitten by ticks that were not engorged suggesting that transmission may have occurred early. However, previous studies have shown that transmission during tick feeding may commence earlier in Ixodes ricinus than in Ixodes scapularis ^{28, 29}. This may explain the seroconversion in the subjects bitten by ticks that were not engorged.

The frequency of Bb-infected ticks (19%) ²² among ticks that have bitten humans in this study is in accordance with a previous Dutch study ¹⁶, where 20% of the ticks collected from patients contained Bb DNA. Another study from Switzerland ¹⁹, found a lower frequency, 10% of the ticks collected from persons who attended physicians concerning tick bites. However, both the previous studies were designed to enrol tick-bitten individuals who contacted general practitioners, which may result in a risk of attaining a biased study group due to differences in anxiousness of the person and concern about the risk of developing a disease. We therefore chose to recruit study subjects by advertisement of a scientific study, and thereby getting a more mixed study group with a decreased risk of bias due to anxiousness. Furthermore, the subjects were prospectively followed for three months.

Active/new production of antibodies was also found in seven individuals bitten by ticks that did not contain detectable numbers of Bb DNA, and one of them was clinically diagnosed with an EM. The new production of anti-Bb specific antibodies is most probably explained by the occurrence of other tick bites close upon the relevant tick bite for the study or of tick bites within the three months of the study period. Two of the seven individuals reported that they had been bitten by at least one more tick during the study period. Another explanation might be that these ticks contained Bb spirochetes not detected by the PCR assay. Additional tick bites were also reported by the four subjects who seroconverted after a bite from Bb-infected ticks, and we can thus not be sure that Bb was transmitted by the tick we analysed. However,

since we used stringent criteria for current Bb-infection, i.e. an infection during the study period, the conclusions from this study regarding the risk of developing LB after a bite by a Bb-infected tick and the occurrence of asymptomatic Bb-infection still holds true, even if Bb was transmitted by another tick. In fact, if Bb was transmitted by another tick, the risk of developing LB after a single tick bite is even smaller, since this means that the tick included in this study did not transfer the spirochetes despite being infected.

Bb infection is traditionally diagnosed mainly by appearance of clinical manifestations and by serology ³⁰. However, the serology-based diagnostics is complicated, since there is no "golden standard" method and serology is negative for several weeks in the early stage of infection. There are many commercial kits available detecting IgM and/or IgG antibodies against different native, synthetic or recombinant Bb antigens ³⁰. The background seropositivity level of the population used as control group is also of importance in relation to the specificity of an antibody test. Second-stage tests with immunoblot are not widely used in Sweden. In order to ensure sensitivity as well as specificity we instead used two different commercial ELISA assays, which detect antibodies against different Bb antigens, followed by immunoblot analyses of samples from subjects showing seroconversion in either of the ELISA-tests. Both the anti-flagellum and the anti-C6 assays used in this study have previously been shown to perform at least as good as other commercial assays for detecting anti-Bb antibodies ³¹⁻³⁴. Furthermore, both Philipp et al. 2001 and Tjernberg et al. 2007, have shown that the detection of anti-C6 antibodies correlates well with ongoing LB both in experimental animals ³⁵ and in humans ^{35, 36}. However, the level of C6 antibodies after treatment can neither be used as an indicator of treatment success, nor of active disease ³⁷. The anti-flagellum assay is routinely used for clinical diagnosis of LB at the UHL, and the anti-C6 is used clinically at the Åland Central Hospital. In order to ensure specificity, sera from all subjects showing seroconversion in one or both of the ELISA assays were further

analyzed by the line immunoblot assay, detecting antibodies against 15 different Bb antigens from different Bb-species. However, since we were interested in the seroconversion and not the seroprevalence in the study group we did not use the immunoblot assay for those subjects who were seropositive but did not increase their antibody titres over the study. Only subjects in which seroconversion was confirmed by the line immunoblot were considered to have a probable current Bb-infection, which most likely ensures the specificity of the study, i.e. that the subjects we defined having a Bb infection actually were infected. The frequency of repeated tick bites and the background seroprevalence was surprisingly high, considering that the county of Östergötland previously have not been considered as an endemic area. We have previously reported a Bb infection prevalence of 5.8% among healthy blood donors in a study conducted in an inland town district in the county of Östergötland ¹⁰. One possible explanation for the increased seroprevalence in the current study might be that this was not limited only to the previous inland district but to several other parts of the county with different geographical locations, in order to better cover areas with increased risk of tick exposure. In addition, the anti-flagellum ELISA test for detection of Bb antibodies used in the first study is routinely used by the health care in the County of Östergötland. The anti-C6 ELISA, however, has to our knowledge, not been used either by the health care or for seroprevalence studies in the county of Östergötland. Therefore, the surprisingly high seroprevalence found with especially the anti-C6 assay may be due to differences in sensitivity between the two different tests.

The symptoms associated with LB are mainly non-specific and frequent in the general population. In the current study, although the frequency of subjects experiencing symptoms was increased among subjects bitten by Bb positive ticks, the total number of reported symptoms was similar in both groups. This support the findings in a previous study by Cerar

et al.³⁸, who reported significant symptomatology in normal non-Borrelia-infected clinical subjects in a Slovenian study.

The current data indicate an age difference between individuals concerning seroconversion, with higher age in the group of study subjects who seroconverted after a bite by a Bb-infected tick. The findings may be explained by a weakening of the innate immune response with age ^{39, 40}, resulting in an infection that is not cleared before the adaptive response is activated. Another explanation may be the age-related increase of certain subclasses of immunoglobulins due to cumulative exposure to antigens over time ^{41, 42}.

In conclusion, this study shows that the risk of developing LB after a tick bite by a documented Bb-infected tick is small. Furthermore, asymptomatic Bb infection, documented by seroconversion in absence of symptoms possibly associated with LB, seems to be frequent among individuals who become infected.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1 Anti-*Borrelia burgdorferi* antibody serology and clinical manifestations in study subjects showing seroconversion during the study time, n=29

			Anti-Bb antibody seroconversion ^a					Self-	Current
Study Infected		Anti-flagellum,		Anti-C6,		Line blot, IgG			
subject	subject tick		IgG		IgG/M			reported	Bb-
no.								symptoms	infection
		sample	sample	sample	sample	sample	sample		
		Ι	II	Ι	II	Ι	II		
1	Yes	+	++	+	+	+	++	+ ^{d,e}	P
2	Yes	-	+	+	++	-	+	-	P
3	Yes	+	++	+	+	+	++	-	P
4	Yes	-	-	-	+	-	+	-	P
5	Yes	-	+	+	+	-	-	-	U
6	No	-	+	-	+	-	+	+ ^f	Yes ^{b,c}
7	No	-	+	-	+	+	++	+ ^d	P
8	No	+	++	+	+	+	++	$+^{\mathbf{d},\mathbf{g}}$	P
9	No	-	+	+	++	-	+	-	P
10	No	-	+	-	+	-	+	-	P
11	No	-	+	+	+	+	++	-	P
12	No	-	-	+	++	-	+	-	P

13	No	-	+	+	+	+	+	$+^{\mathbf{d},\mathbf{f}}$	U
14	No	-	-	-	+	-	-	$+^{\mathbf{d},\mathbf{g},\mathbf{h},\mathbf{i}}$	U
15	No	-	-	-	+	-	-	+ ^{e,g,h}	U
16	No	-	+	-	-	-	-	+ ^e	$\mathbf{U^b}$
17	No	+	++	+	+	+	+	-	U
18	No	+	++	+	+	+	+	-	U
19	No	+	++	+	+	+	+	-	U
20	No	+	++	+	+	+	+	-	U
21	No	+	++	+	+	+	+	-	U
22	No	-	+	+	+	+	+	-	U
23	No	-	+	+	+	+	+	-	U
24	No	-	+	+	+	+	+	-	U
25	No	-	+	+	+	+	+	-	U
26	No	-	+	+	+	-	-	-	U
27	No	-	-	+	++	-	-	-	U
28	No	-	-	-	+	-	-	-	U
29	No	-	-	-	+	-	-	-	U

Bb, Borrelia burgdorferi; P, probable; U, uncertain

Serology results presented as:

-, anti-Bb antibody-negative; +, anti-Bb antibody-positive; ++, at least twofold increase of anti-Bb antibody titre or, for Line blot assay, sera where anti-Bb antibodies against new antigens were detected.

^a at least a twofold increase of arbitrary units in the second blood sample or detection of antibodies not found in the first sample; ^bvisit to primary health care centre; ^cclinically diagnosed erythema migrans

^d Fatigue; ^e Myalgia/athralgia; ^f Vertigo; ^g Neck pain; ^h Headache; ⁱ Numbness

Positive findings are bolded

Table 2 Self-reported symptoms in 341 tick-bitten study subjects

Frequency of different symptoms reported by tick-bitten study subjects^a

_			
	All tick-	Subjects bitten	Subjects bitten
	bitten	by a Bb-	by a Bb-
	subjects	infected tick	uninfected tick
	(n=341)	(n=64)	(n=277)
No. of subjects	n=91	n=27	n=64
reporting			
symptoms			
Reported			
symptoms (%)			
Fatigue	18	17	19
Myalgia/arthralgia	17	16	17
Headache	14	15	14
Neck pain	11	13	10
Numbness	10	9	10
Vertigo	9	10	7
Concentration	5	6	5
difficulties			
Nausea	5	6	5

Radiating pain	4	3	5
Fever	3	0	4
Loss of appetite	2	4	1
Loss of weight	2	1	3

^a several individuals reported more than one symptom. Erythema migrans was not included in the list of symptoms in the questionnaire.