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Original article

Prospective study on the incidence of infection by *Borrelia burgdorferi* sensu lato after a tick bite in a highly endemic area of Switzerland

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ABSTRACT

The periurban forest of Neuchâtel (Switzerland) is a high-risk area for Lyme Borreliosis, due to a high density of infected *Ixodes ricinus* ticks. In this study, we evaluated the risk of subclinical (seroconversion) and clinical infection after a tick bite in Neuchâtel inhabitants from 2003 to 2005. Inhabitants have been invited, through media, to visit a physician after a tick bite. A questionnaire was filled out and two blood samples were taken at 8-week interval. EIA screening tests for IgM and IgG (IMX system, Abbott) were applied for paired sera. In case of a change in antibody titres between both samples, a homemade Western-blot using *Borrelia afzelii*, *B. burgdorferi* sensu stricto and *B. garinii* as antigens was performed. Participants were included into two groups. Group one included asymptomatic participants ($n = 255$). Among them, nine (3.5%) seroconverted with seroconversion rates varying between 6.8% in 2003, 2.1% in 2004 and 2.3% in 2005. Participants who developed clinical symptoms of LB were included into group two ($n = 14$). *Erythema migrans* (EM) was reported in 5.2% of participants (5.2%), varying between 7.5% in 2003, 5% in 2004 and 3.4% in 2005. Ticks obtained from 186 participants were examined for *B. burgdorferi* infection by PCR/Reverse Line Blotting, and by Real Time PCR and tick attachment duration was estimated. Among *I. ricinus* ticks collected from participants, 32.8% were infected by *B. burgdorferi* sensu lato. *B. afzelii* predominated among these ticks. Globally, 65.9% of nymphs remained attached for more than 24 h whereas only 38.3% of female ticks remained attached for more than 24 h. We observed that 6.6% and 2.4% of participants bitten by infected and uninfected ticks, respectively, developed EM.

Introduction

The tick *Ixodes ricinus* is the main European vector of spirochetes belonging to the complex *Borrelia burgdorferi* sensu lato (sl), the agent of Lyme Borreliosis (LB). In Europe, 8 different genospecies belonging to this complex have been reported: *B. burgdorferi* sensu stricto (ss), *B. afzelii*, *B. garinii*, *B. valaisiana*, *B. lusitaniae*, *B. bissettii*, *B. bavariensis* and *B. spielmanii* (Rauter and Hartung, 2005; Richter et al., 2006; Margos et al., 2009). Among them three genospecies, *B. burgdorferi* ss, *B. garinii* and *B. afzelii*, are the most frequently reported in humans. LB is a multisystemic disease and clinical manifestations of European LB range from the characteristic localized cutaneous form, erythema migrans (EM), occurring at the site of inoculation to more severe, systemic manifestations like neuroborreliosis, carditis, arthritis or acrodermatitis chronica atrophicans (Steere, 2001).

The risk of developing LB in an area depends on several factors, such as the density of tick population, their infection rate by *B. burgdorferi* sl and the frequency of human contacts with tick biotopes (forests, gardens, etc.). Indeed, outdoor activity for leisure or work is considered to be a major risk factor for LB in Europe. In Switzerland, studies in high-risk populations, such as orienteers, showed a high seroconversion rate and a low incidence of clinical manifestations (Fahrer et al., 1991). A several month follow-up of these Swiss orienteers showed that contacts with *B. burgdorferi* sl were frequent, but very few reported clinical manifestations (Fahrer et al., 1998; Zhioua et al., 1998). Moreover, the seroprevalence is not negligible in the general population, as shown by a seroprevalence rate of 9% in a population of blood donors (Nadal et al., 1989). Another important factor of risk is the duration of *I. ricinus* tick attachment. The risk of transmission increases with the duration of tick attachment but transmission of spirochetes may already occur during the first 24 h of *I. ricinus* attachment (Kahl et al., 1998; Crippa et al., 2002).

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Switzerland appears as a high-risk area in Europe, with an estimated annual incidence of 12,000 cases in 2008 and 8900 in 2009 (FOPH (Swiss Federal Office of Public Health), 2010). An epidemiological study conducted in Western Switzerland by Nahimana et al. (2000) showed that the highest incidence of LB (95 cases/100,000 inhabitants) occurred in the Neuchâtel region. These observations in addition to the fact that tick density in this area can be as high as 300 ticks/100 m^2 with an infection rate reaching 50% (Jouda et al., 2004a) drove us to carry out a prospective study to evaluate the risk of subclinical (seroconversion) and clinical infections after a tick bite among residents of this region. Very few studies evaluated the risk of developing LB after an infected versus uninfected tick bite in Europe (Fryland et al., 2011), therefore we also addressed the question of the risk of acquiring clinical LB and asymptomatic infection linked to the infection status of the tick.

Materials and methods

Study design

Physicians (outpatient clinics, general practitioners and dermatologists) in the area received written information about the study and were invited to notify to us all persons who presented for a tick bite. In parallel, residents were informed about the study through local newspapers and radio and were invited to visit their physician or the outpatient clinic of the community hospital after a tick bite.

Each patient consulting for a tick bite was included in the study after informed consent and received information on the aims and the protocol of the study. A questionnaire was filled out by the physicians to determine history of a tick bite and clinical LB, a previous serological status for LB, date and place of the tick bite. A first blood sample was drawn (5 ml of whole blood) and the site of the tick bite was carefully examined. Ticks were removed and placed in a vial. Any local cutaneous reaction was recorded. EM was notified according to the definition of Stanek et al. (1996): "Expanding red and bluish-red patch often with central clearing. Advancing edge typically distinct, often intensely coloured, not markedly elevated". Careful patient information included a description of EM and patients were actively encouraged to visit their physician should EM-like lesions develop before the following scheduled visit. Eight weeks after the first visit, clinical examination was repeated, a second blood sample was taken and a second questionnaire was filled. Blood samples and ticks removed from participants were sent to the Institute of Biology, University of Neuchâtel, for examination. Serological tests were performed only in patients for whom 2 blood samples were available. The Ethical Committee of the Canton of Neuchâtel approved the study.

Inclusion criteria

Only participants with two blood samples were included and were divided into 2 groups. Group one included participants responding to the following criteria: no clinical manifestations, no antibiotic treatment, a maximal delay of 9 days between the tick bite and the first blood sampling, a maximal delay of 12 weeks between the first blood sampling and the second, no tick bite between the two blood samplings. Participants who developed a clinical manifestation were included into group two without taking into account the criteria for group 1, for example some patients included into group two received antibiotic treatment. The main criterion to be included into group two was development of clinical manifestations.

Table 1
Score for the IgG Western blot.

10 points	5 points	1 point
90 (93)	72	Others
31(OspA)	65 (66)	
21/OspC (21)	58 (58)	
	56	
	43 (45)	
	39 (39)	
	34 (OspB)	
	30 (30)	
	29 (28)	
	18 (18)	

Serum analyses

Blood samples were centrifuged at 0.8rcf for 10 min. Serum was collected in a sterile cryotube and frozen at −20 °C until analysis. Screening tests for both IgG and IgM, using a commercially available EIA, a Microparticule Enzyme Immunoassay (MEIA) (IMX system, Abbott) was applied at the same time for each paired sera. Antigens included in the IMX system were whole lysate of *B. burgdorferi* ss (ATCC35211) and flagellin recombinant of *B. burgdorferi* ss (ATCC35210) for IgG. For IgM, antigens were the same as for IgG with in addition OspC recombinant of *B. burgdorferi* ss (ATCC35210). Tests were performed and interpreted according to instructions of the manufacturer (indices).

For detection of significant antibody index changes, a control population of 21 persons who did not remember a tick bite during the previous year was included in the study. Two blood samples were drawn at 8-week interval during winter. According to the distribution of index differences between the two sera, the upper limit value of distribution confidence interval was calculated to have 95% of these differences below this value. Hence, an increase between the two indices corresponding to each serum in the study population equal or higher than 0.236 for IgM and 0.077 for IgG was considered as a significant change ($p < 0.05$). A seroconversion was defined as the changeover from a negative to a positive index, from equivocal to positive or from negative to equivocal but with an index increase corresponding to 0.236 for IgM and 0.077 for IgG.

In case of significant MEIA index changes as defined above, a homemade Western-blot (WB) using *B. afzelii* (strain NE17), *B. burgdorferi* ss (strain B31) and *B. garinii* (strain NE83) as antigens was performed. The *Borrelia* strain B31 was isolated from *I. scapularis*, NE83 from *I. ricinus* and the strain NE17 was isolated from a tick fed on a wild host. *Borreliae* were cultured in modified BSK-II medium (Sinsky and Piesman, 1989). The sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblot assays were slightly modified from Péter et al. (1997). In short, the suspension of washed spirochetes was electrophoresed on a 12.5% polyacrylamide gel. The proteins were transferred to nitrocellulose and immunoreactions were performed with human serum diluted 1:200. Fixed antibodies were revealed by a secondary antibody (anti-human IgG or anti-human IgM) conjugated to alkaline phosphatase (MaProline GmbH, Starrkirch-Will, Switzerland), followed by the addition of substrate BCIP/NBT (5-bromo-4-chloro-3-indoyl *p*-toluidine phosphate/*p*-nitro blue tetrazolium chloride).

We applied the following criteria for a positive WB with human IgG (based on the criteria of the Laboratory of Parasitic Diagnosis, Institute of Zoology, University of Neuchâtel, 1999; criteria adapted from Dressler et al., 1993): points were attributed for each antigenic band revealed on the strip (for example, 10 points were allotted to the 90 kDa protein band (antigen) (Table 1). The sum of the points for each strip was calculated and interpretation was as follows according to the score: >46 points: definite contact with *B. burgdorferi* sl; 35–45 points: equivocal contact with *B. burgdorferi* sl; <34 points: no contact with *B. burgdorferi* sl.

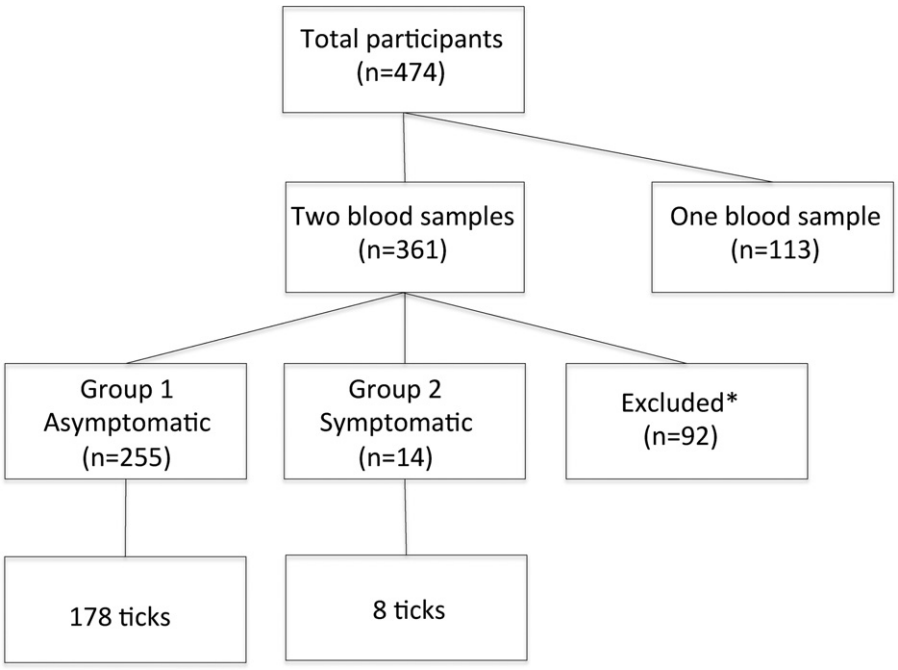

Fig. 1. Flow chart of the persons who participated in the study. * See criteria in the text.

Criteria for a positive WB with human IgM (based on the criteria of the Laboratory of Parasitic Diagnosis, Institute of Zoology, University of Neuchâtel, 1999; criteria adapted from Engström et al., 1995) were as follows: the intensity of the antigenic bands revealed on the strip was estimated. If there was only a slight trace on the antigenic band, 0.5 point was allotted, if the intensity of the antigenic band was low, 1 point, if the intensity of the band was strong, 2 points and if the intensity of the band was very strong, 3 points. A serum was regarded as positive if p21 (OspC), p41 (fla), p39 bands were present on the strip, even slightly, or if the intensity of the p21 (OspC) was strong or very strong, or if the total score of the strip was equal or higher than 3 with the presence of 2 bands. Moreover, the positivity was considered as strong if the total score of the strip was equal or higher than 4.5 points. The positivity was low if the total score of the strip lied between 1.5 and 2.5. If the intensity of a band was unclear, result of the strip was considered as "ambiguous". Criteria for positive IgM and IgG WB as well as sensitivity and specificity were the same as those used by our laboratory in a European multicentre study on immunoblotting organized by EUCALB (Robertson et al., 2000b).

In case of MEIA results showing significant index changes between the two blood samples as described above, the criteria for confirmation of WB for IgG and IGM were the appearance of new bands on the second serum compared with the first and the increase in intensity of bands (in particular OspC, OspA, 93 kDa for IgG and OspC, p39, p41 for IgM).

Borrelia detection in ticks and estimation of tick attachment duration

Ticks were examined for *B. burgdorferi* DNA using polymerase chain reaction (PCR), Reverse Line Blot (RLB) and Real Time-PCR. *Borrelia* DNA was extracted using 0.7 M ammonium hydroxide and denatured for 15 min at 100 °C (Guy and Stanek, 1991). The ammonia was evaporated by heating the samples at 100 °C for a further 15 min. An aliquot of 20 μl of DNA extract was added per PCR reaction. Primers B5S-Bor and 23S-Bor were used to amplify the variable spacer region between two repeated copies of the *23S* and *5S* ribosomal genes (Alekseev et al., 2001). DNA amplification was performed using a Whatman (Göttingen, Germany) Biometra TGradient Thermocycler 96. Isolates of *B. burgdorferi* ss (B31), *B. garinii* (NE11), *B. afzelii* (NE632), *B. lusitaniae* (PotiB3) and *B. valaisiana* (VS116) were used as positive controls. Negative controls for each tick batch consisted in NH_4OH without ticks for extraction and nanopure water for PCR. For RLB, following PCR amplification, samples were hybridized to 7 oligonucleotide probes (75 pmol) blotted in lines on an activated Biodyne C membrane (Pall Europe Ltd., Portsmouth, UK) using a Miniblotter 45 (Immunetics, Cambridge, MA). *B. burgdorferi* sl (SL), *B. burgdorferi* ss (SS), *B. afzelii* (AF) and *B. garinii* (GA) probes were described by Schouls et al. (1999) and Alekseev et al. (2001). *B. valaisiana* (VSNE), *B. garinii* (GANE) and *B. lusitaniae* (LusiNE) probes were described by Poupon et al. (2006). Hybridized products were detected by conjugation with Streptavidine-peroxydase (Dakopatts, Danemark) followed by chemiluminescent detection using the Amersham ECL detection system and visualized by exposure of the blot to X-ray film (Hyperfilm ECL; Amersham Biosciences, Otelfingen, Switzerland).

Real Time PCR was also used to analyse ticks removed from participants. This test is based on the quantitative amplification of a fragment of the chromosomal gene coding for the flagellin protein by 2 primers, FlaF1A and FlaR1, and by a probe, FlaProbe1 (Herrmann and Gern, 2010, modified from Schwaiger et al., 2001).

Duration of feeding time (tick attachment to the host) of ticks removed from participants was estimated from the scutal index (SI), the ratio between body length (idiosoma) and scutum width as reported by Huegli et al. (2009).

Statistical analysis

The Chi-square test was used to compare proportion of sera with serological movements according to year and proportion of cases with EM number according to year. Statistics were calculated with S-Plus© 7.0 for Windows.

Results

During the study period (February 2003–November 2005), 474 residents consulted for a tick bite. Among them 113 (23.8%) could

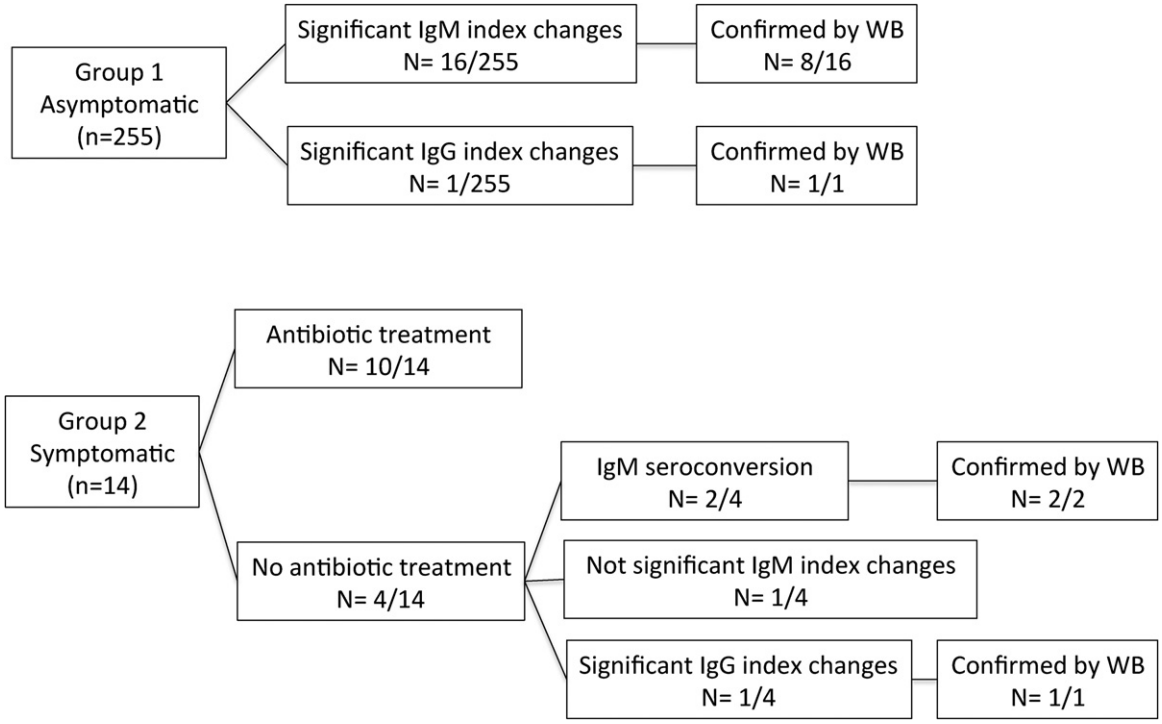

Fig. 2. Flow chart of the serological results of participants in the study.

not be included in the study because they gave only one blood sample (Fig. 1). Of the 361 participants who gave two blood samples, 255 (71%) fulfilled all the criteria for inclusion in study group one (asymptomatic participants) and 14 for inclusion in study group two (EM patients) (Fig. 1). Ninety-two residents gave two blood samples but were excluded because the interval between first and second blood samples was not respected. Hence, all blood samples obtained from group one ($n = 255$) and blood samples from patients who developed EM and did not receive treatment ($n = 4$) were tested for the presence of antibodies against *B. burgdorferi* sl (Fig. 2). The average age of the study population was 43 years (range <1–94 years). Ticks were obtained from 186 of 269 (69%) participants (Fig. 1).

Tick analysis for B. burgdorferi infection

Among the 186 *I. ricinus* ticks obtained from both groups of participants, 3 (1.6%) were larva, 119 (64%) were nymphs, 62 (33.3%) were females and 2 (1.1%) were males. Sixty-one ticks (32.8%) were infected by *B. burgdorferi* sl using RLB and/or Real Time PCR. Four *Borrelia* species were detected in ticks: *B. afzelii* was the most frequent ($n = 25$), followed by *B. valaisiana* ($n = 3$), *B. garinii* ($n = 2$) and *B. burgdorferi ss* ($n = 1$). Mixed infections were observed in 2 ticks (*B. afzelii* and *B. garinii*, *B. burgdorferi ss* and *B. afzelii*).

Calculation of attachment duration of ticks was possible in 129 ticks (47 females and 82 nymphs). Nymphs were attached for a mean (±standard error) of 33.7 h (±2.6) and females for a mean of 27.3 h (±4.1). Among nymphs, 65.9% remained attached for more than 24 h whereas only 38.3% of female ticks remained attached for more than 24 h. The attachment duration of ticks varied between 1 h and 5 days. Infection rates were not significantly different between ticks attached for <24 h and those attached for >24 h (Chi test $p = 0.37$).

Serology results

Among the participants included into group one, 16/255 (6.3%) developed significant IgM index changes by MEIA (Fig. 2). Serum samples from these 16 participants were further analysed by WB. WB results of eight (50%) of them confirmed MEIA results. The second serum sample of 5/8 showed an increase in the intensity and/or the appearance of the p41 and OspC bands (Fig. 3) and a total score of ≥3 pts. Another participant displayed a reaction with p60 (on strain NE83), p41 (on the 3 strains) and OspC increased intensity in the 2nd serum (on strain NE83) (Fig. 4). Finally paired serum samples from another subject showed an increase in the intensity of the p41 and OspC (on strains B31, NE83 and NE17) and of p39 (on strains NE17) (Fig. 4) and the scores of the paired sera were 5.5 pts and 7 pts, respectively, on strain NE17.

One additional participant (0.4%), who developed an IgG seroconversion detected by MEIA, was tested by WB (Fig. 2). The increase in intensity and the appearance of several bands in the second serum sample (Fig. 5) confirmed the seroconversion. When the B31 strain was used, the protein bands corresponding to p90, p41, p39, increased in intensity. The bands corresponding to p72, p60, p54, p43 appeared. When strain NE83 was used, p90, p56, p43, p39, p30 and OspC increased in intensity. When strain NE17 was used, p90, p60, p56, p39, OspA, p30 and OspC increased in intensity in the second serum (Fig. 5). The scores of the paired sera were higher than 46 pts on strains NE17 and NE83.

Hence, among participants belonging to group one, nine had serum samples displaying significant index changes by MEIA between both serum samples that were confirmed by WB (Fig. 2). This gives an IgM and IgG seroconversion rate of 3.5% (9/255) varying between 6.8% (5/74) in 2003, 2.1% (2/95) in 2004 and 2.3% (2/86) in 2005. The difference between years is not significant (Chi square test, $p = 0.094$).

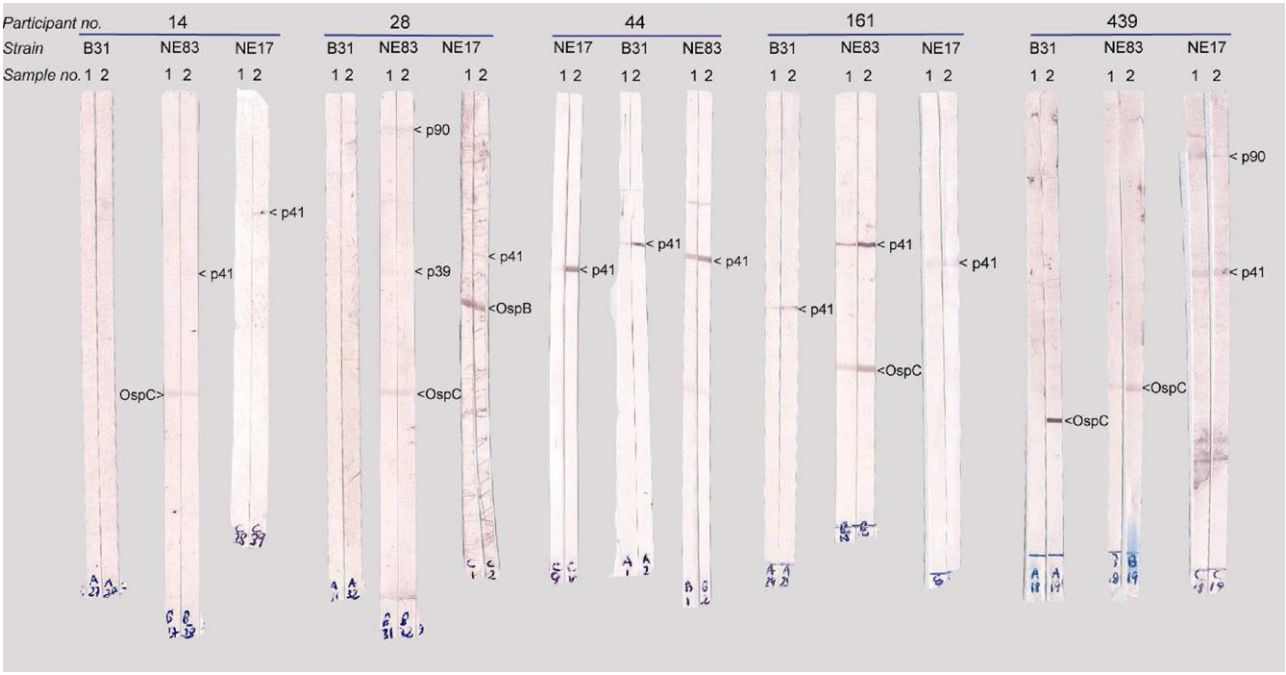

Fig. 3. Western blot of paired sera from participants with significant IgM index changes.

Clinical manifestations, treatment and tick infection

Among the participants of the study (groups one and two, $n = 269$), 14 (5.2%) developed EM lesions (group two). This rate varied among years: 7.5% (6/80) in 2003, 5% (5/100) in 2004 and 3.4% (3/89) in 2005.

Ten of 14 persons with EM received antibiotic treatment: one received tetracycline (10%), seven received doxycycline (70%), and the precise antibiotic treatment was not indicated for two persons (Table 2). Treatment duration varied from 7 to 21 days. Among the 4 persons with EM who were not treated with antibiotics, two

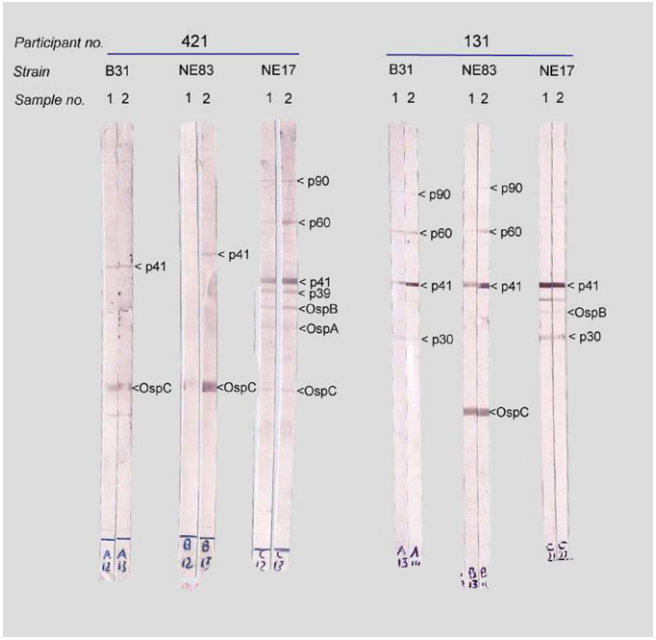

Fig. 4. Western blot of paired sera from participants with significant IgM index changes.

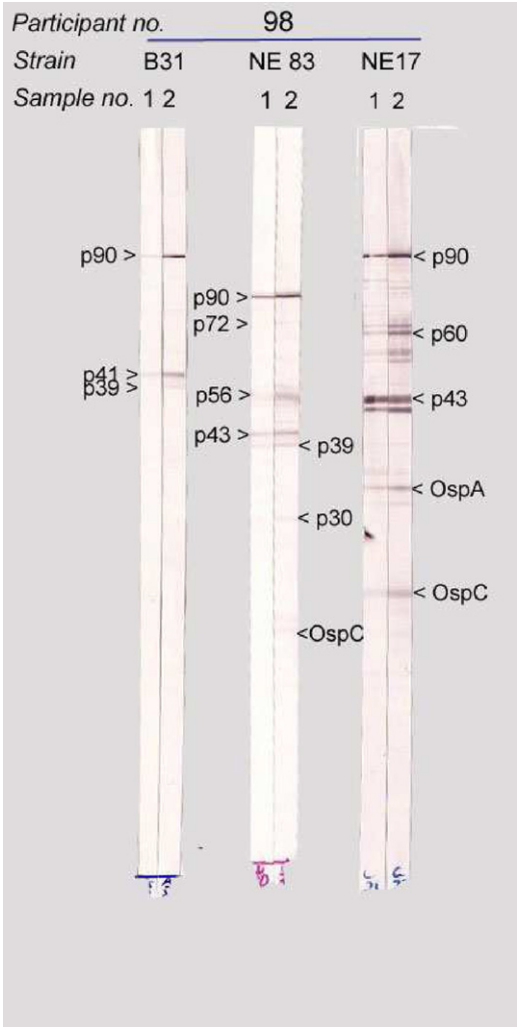

Fig. 5. Western blot of paired sera from the participant with IgG seroconversion.

Table 2
Characteristics of participants with EM bitten by an infected (1–4) or an uninfected tick (5–8).

	Western blot					
	1/2* IgM	1/2* IgG	Clinical manifestation	Antibiotics	Attachment duration (h)	*Borrelia* genospecies
1	−/−	−/−	EM	Yes	0	*B. garinii*
2	−/−	−/−	EM	Yes	32.4	*B. afzelii*
3	−/−	−/−	EM	Yes	38.3	*B. afzelii*
4	−/−	−/−	EM	Yes	49.9	*B. afzelii*
5	−/−	−/−	EM	Yes	>100	–
6	−/−	−/−	EM	Yes	27.8	–
7	+/+	−/−	EM	No	8.3	–
8	−/−	−/−	EM	No	NA	–

1/2*: 1st serum, 2: 2nd serum; NA: not available.

showed an IgM seroconversion by MEIA confirmed by WB (Table 2). A third person showed a rise in IgG in MEIA and WB. The fourth patient demonstrated two IgM positive sera in MEIA and confirmed by WB but without serological changes in MEIA and WB.

Among the 14 participants who developed EM, 8 provided a tick (Fig. 2). Four of these 8 ticks were infected by *Borrelia* (Table 2). Three of 4 infected ticks remained attached for longer than 24 h. Three ticks were infected with *B. afzelii* and one with *B. garinii*. Two of the 4 uninfected ticks remained attached for longer than 24 h (Table 2). One patient whose tick was not infected was IgM positive (first and second blood samples).

Risk of subclinical and clinical infection after a tick bite

Data on the serological or clinical evolution were available for 186 participants whose tick was examined for *B. burgdorferi* sl. Among them 61 (32.8) were bitten by infected ticks and 125 (67.2%) by uninfected ticks. In the group of persons bitten by an infected tick ($n = 61$), one (1.6%) showed a significant IgM index change confirmed by WB and 4 (6.6%) developed an EM. In contrast, among the 125 persons whose tick was uninfected, 3 (2.4%) developed a significant IgM index change confirmed by WB and 4 (3.2%, 4/125) developed an EM. Overall, the risk, after an infected tick bite, to develop asymptomatic infections or clinical symptoms of LB was 8.2% (5/61) whereas it was 5.6% (7/125) after an uninfected tick bite.

Discussion

One of the most important factors of *B. burgdorferi* sl transmission risk after a tick bite is the duration of tick attachment. Indeed, the risk of transmission increases with the duration of *I. ricinus* attachment (Crippa et al., 2002; Kahl et al., 1998). In this study, we observed that the majority of nymphs were removed after 24 h of blood meal (65.9%), in contrast to females that were detected earlier (61.7% removed before 24 h of blood meal) (Huegli et al., 2009). The small size of the nymphs enables them to remain attached longer before being detected and removed (Logar et al., 2002). The risk of transmission is thus greater after a bite of a nymphal tick than after the bite of a female tick, even if females show higher infection prevalence (Jouda et al., 2004a,b). Moreover, the risk to be bitten by a nymph is greater, because they are more abundant than females (Jouda et al., 2004a,b).

The infection prevalence by *B. burgdorferi* sl of the ticks collected on the participants is relatively close to that of questing ticks collected in the same area (Jouda et al., 2004a; Morán Cadenas et al., 2007). In other studies where ticks were examined after a blood meal, the infection prevalence of the ticks collected on the patients was usually much lower than that of the questing ticks (Robertson et al., 2000a; Nahimana et al., 2004). As mentioned below, we did not observe any inhibition effect due to blood feeding in our study. *B. afzelii* predominated in the ticks collected on the participants, as it also predominated in the questing ticks (unpublished data). However the prevalence of *B. afzelii* was higher in the ticks removed from the participants.

Among the 255 participants without clinical manifestations (group one), a seroconversion rate of 3.5% was observed and confirmed by WB, which varied between 2.1 and 6.8% according to years. Only 50% of MEIA IgM positive results were confirmed by WB showing the importance of the use of a second serological test. Variable rates of seroconversion have been reported in different populations: 4.6% was observed in a general population in Sweden (Gustafson et al., 1992), 1.5% in a population of Dutch military personnel (Vos et al., 1994) and in the order of 5–7% in a population of German forestry (Rath et al., 1996). However, few studies reported the seroconversion rate after a tick bite. Such a study conducted in Western Switzerland revealed a seroconversion rate of 4.5% (Nahimana et al., 2004). The comparison of these different rates however should be critically evaluated, due to different study designs. Asymptomatic seroconversions seem to be frequent in Switzerland, where seroprevalence is relatively high among residents living in endemic areas and in populations at risk (Gern et al., 1989; Fahrer et al., 1991, 1998; Nahimana et al., 2004). Seropositive asymptomatic persons do not seem to have a higher risk to develop late clinical manifestations as suggested by Fahrer et al. (1998). These authors did not observe a higher risk to develop clinical manifestations between seropositive asymptomatic persons, and seronegative ones in a population of Swiss orienteers over a period of seven years. The follow up period in our study is obviously too short to allow any conclusion about the evolution of subjects with asymptomatic seroconversion.

EM is the only clinical signs of LB diagnosed in this study. Physicians did not report early neuroborreliosis or borrelial lymphocytoma. The frequency of EM during the three study years was 5.2% (14/269), varying between 3.4% and 7.5% according to year. It is important to note that clinical incidence is probably underestimated. With a follow-up of 2 months, it is not possible to take census of late clinical manifestations and a surveillance of participants during several months would provide more information.

Among participants with EM who were not treated, 2/4 developed an IgM seroconversion. The third showed a positive IgG titre change, while the levels of IgM remained negative. This could represent a case of reinfection, as described by Eiffert et al. (1996): immune response being reactivated and showing a faster appearance of antibodies than during a first infection. This second infection could be caused by another *Borrelia* genospecies and immune response, which seems to be species-specific, does not protect against a new infection, if this one is caused by a different genospecies (Eiffert et al., 1996).

We observed that 1.6% of persons bitten by an infected tick seroconverted without development of clinical signs of LB and that 6.6% developed an EM. These results confirm that asymptomatic infections occur after an infected tick bite. A recent study in Sweden also reports asymptomatic infections after an infected tick bite but with a higher rate (6.3%) (Fryland et al., 2011). In contrast the risk to acquire clinical LB was higher (6.6% developed EM) in our study compared to the Swedish study (1.6%, 1/64 participant self reported symptoms considered as probable LB but was not diagnosed by a physician).

Interestingly, both studies observed seroconversion in persons bitten by uninfected ticks with very similar rates (2.5% in Sweden and 2.4% in the present study) although serological tests differed between studies (Fryland et al., 2011). In the present study 3.2% participants bitten by uninfected ticks developed an EM diagnosed by a physician suggesting the occurrence of tick-bites that remained unnoticed. This corroborates the fact that patients who acquire LB frequently do not remember a tick bite. In the Swedish study only 2 subjects bitten by uninfected ticks sought care for the symptoms they developed and only one (0.4%) was diagnosed with EM (Fryland et al., 2011). Because the risk of acquiring LB after an infected tick bite in our study area is higher than in Sweden, it is not surprising that the occurrence of LB after an uninfected tick-bite was also higher among the Swiss residents.

Although the risk to develop asymptomatic infection and clinical symptoms of LB is slightly higher among persons bitten by infected ticks (8.2%) than among those bitten by uninfected ticks (5.6%), recommendation to analyse the *Borrelia* infection of the detected tick and to give an antibiotic treatment based only on the *Borrelia* infection of the tick does not seem justified in this area. It is important to note that participants detected the tick rather early after attachment, which probably reduced the risk of transmission of *B. burgdorferi* sl by the tick (Kahl et al., 1998; Crippa et al., 2002). In addition, among the 14 ticks removed from participants who developed EM, only 50% were infected by *B. burgdorferi* sl. Most probably the analysed tick was not the one that caused EM, another one, which remained unnoticed, most likely transmitted the spirochetes to the participant, which also explains development of LB among persons bitten by uninfected ticks. Another explanation could be that the tests used were not sensitive enough or that the blood present in some ticks inhibited the PCR. However since the infection prevalence between semi-engorged and fully engorged ticks was not significantly different, this suggests that blood did not inhibit the PCR. All this together indicates that examination for *Borrelia* of ticks detected by patients is not recommended.

In conclusion, this seroepidemiological study enabled us to determine an asymptomatic seroconversion rate of 3.5% and a clinical incidence of LB of 5.2% after a tick bite in the Neuchâtel area. However, this study did not address the question of the incidence of late clinical manifestations, in fact we have no information on late clinical manifestation that may have occurred after the end of this study. Data on the incidence of late clinical manifestations would give us more acute information for the recommendation of a prophylactic treatment.

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