

Tick bites in a Lyme borreliosis highly endemic area in Switzerland

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Abstract

The duration of tick feeding is an important indicator to evaluate the risk of *Borrelia burgdorferi* sensu lato transmission, which increases considerably with the blood meal duration. This blood meal duration may be estimated from scutal index, the ratio between body length (idiosoma) and scutum width. For the estimation of blood meal duration in *Ixodes ricinus*, nymphal and adult female ticks were detached at predetermined intervals (24, 48, 72, and 96 h) from laboratory mice and rabbits and their scutal index calculated. From this, non-linear regression equations were developed to determine the duration of attachment for nymphal and adult female *I. ricinus* ticks. As part of an epidemiological study addressing the risk of subclinical (seroconversion) and clinical infections after a tick bite in the Neuchâtel area (Switzerland) over 3 years (2003–2005), duration of tick attachment and anatomical site of bites collected on participants as well as seasonal distribution of tick bites were studied. Tick attachment duration was estimated in all ticks collected during this study ($n = 261$). Nymphs were attached for a mean (\pm standard error, SE) of 31.6 h (± 2.6) and females for a mean (\pm SE) of 29.6 h (± 3.2). Most nymphs were removed after 24 h of blood meal whereas most females were removed before 24 h. Legs were the major anatomical sites of bites for women (40.7%), men (44.4%), and almost all age classes. Only children < 10 years old were bitten more frequently on the head (41.2%) and on the neck (38.5%) than participants > 10 years. The majority of tick bites were recorded from May to July during the 3 years. Attachment sites can influence the discovery of ticks, hence the duration of the tick bite. A detailed body examination after each outing in forest and an early withdrawal of an attached tick is an effective way to prevent Lyme borreliosis.

Keywords: *Ixodes ricinus*; Scutal index; Duration of tick attachment

Introduction

Lyme borreliosis (LB) is the most prevalent human disease transmitted by ticks in Europe. LB is caused by

Borrelia burgdorferi sensu lato (sl) genospecies and can generate various clinical manifestations, varying from a skin rash (erythema migrans) to severe arthritic, dermatologic, neurological, and cardiac manifestations (Stanek et al., 2002). In Europe, 7 different genospecies belonging to *B. burgdorferi* sl have been reported: *B. burgdorferi* sensu stricto (ss), *B. afzelii*, *B. garinii*, *B. valaisiana*, *B. lusitaniae*, *B. bissettii* and *B. spielmanii*

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(Rauter and Hartung, 2005; Richter et al., 2006). Among them 3 genospecies, *B. burgdorferi* ss, *B. garinii*, and *B. afzelii*, are the most frequently reported as pathogenic for humans.

The principal vector of *B. burgdorferi* sl in Switzerland is *Ixodes ricinus* (*Ixodidae* family), which colonizes mixed deciduous forests and woodlands up to an altitude of 1500 m (Aeschlimann et al., 1987). The infection rate of *I. ricinus* ticks with *B. burgdorferi* sl varies between 9% and 40% for nymphs and between 22% and 47% for adults (Jouda et al., 2004a). These rates vary according to areas in Switzerland.

The risk of becoming infected with *B. burgdorferi* sl in an area depends on several factors, such as the density of tick populations, their infection rate, and the frequency of human contacts with tick biotopes. Another important factor of risk is the duration of *I. ricinus* tick attachment (here also referred to as blood meal duration), the risk of transmission increasing with the duration of tick attachment (Kahl et al., 1998; Crippa et al., 2002). Therefore, knowledge of the duration of tick attachment is an important tool to evaluate the risk of *B. burgdorferi* sl transmission. *B. burgdorferi* sl is not transmitted immediately after an infected tick bite. In Europe, the risk of transmission is already present during the first 24 h of tick feeding (Kahl et al., 1998; Crippa et al., 2002). Duration of feeding time (tick attachment to the host) can be estimated from the scutal index (SI), the ratio between body length (idiosoma) and scutum width, as reported by Falco et al. (1996) for *I. scapularis*. Since no SI was available to evaluate duration of attachment for *I. ricinus*, we determined an SI for nymphal and adult female *I. ricinus* ticks.

An epidemiological study was conducted from 2003 to 2005 on the incidence of infection with *B. burgdorferi* sl after a tick bite in the Neuchâtel region (Switzerland) (Huegli et al., unpublished), an area with a high density of *B. burgdorferi* sl-infected *I. ricinus* ticks (Jouda et al., 2004b; Morán Cadenas et al., 2007). Here, we present data on anatomical site of bites, tick bite duration, and seasonal distribution of tick bites for all ticks collected during this study.

Materials and methods

Determination of the attachment duration

Collection of ticks

Questing nymphal and female *I. ricinus* ticks were sampled by dragging a white flannel flag over the low vegetation in mixed deciduous forests at a periurban site of Neuchâtel (St-Blaise and Hauterive), Switzerland. A first sampling was realised in autumn 2002 and a

second one in spring 2003. Ticks were placed in humidified containers, until they were used to infest laboratory mice and rabbits. Twenty unfed nymphs and 20 unfed female ticks of each seasonal sampling were placed in 70% ethanol to provide unengorged tick controls.

Laboratory mice and rabbits infestation

Twenty-five nymphs were fed on each of 4 Swiss mice. Ticks were placed inside capsules glued to the body of each host. This capsulation permitted easy retrieval of ticks and minimized loss due to host grooming. Ten female ticks were placed on each ear of two New Zealand white rabbits and contained by a cloth bag attached over the ear. After 1 h of infestation, tick attachment was checked and all unattached ticks were removed. Nymphs and female ticks were removed from their host 24, 48, 72, and 96 h after attachment. All ticks were stored in 70% ethanol until they were measured. This experiment was replicated in autumn 2002 and in spring 2003.

Tick measurement and SI determination

Ticks were measured using a stereo microscope (Olympus, Switzerland) and Analysis software (Soft Imaging System GmbH, Münster, Germany). We measured the body length from the base of the basis capitulum to the anterior tip of the abdomen (idiosoma) and the maximum width of the scutum, according to Falco et al. (1996). SI was expressed as the ratio between body length (idiosoma) and scutum width and was calculated for nymphal and female *I. ricinus* ticks. SI provides a measurement of increased body size, because body length increases while scutal width remains constant during tick feeding.

Epidemiologic study

As part of an epidemiological study conducted from 2003 to 2005 in a high risk area (Neuchâtel region, Switzerland) (Huegli et al., unpublished), physicians (general practitioners, dermatologists, etc.) were informed about the study and were invited to notify us all persons who presented for a tick bite. In parallel, inhabitants 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 experiencing a tick bite. A questionnaire was filled up by the participant to obtain information about age, gender, the anatomic site where a tick attached and the date of the bite (if known). Removed ticks were measured and duration of feeding time (tick attachment to the host) was estimated from SI, as mentioned above.

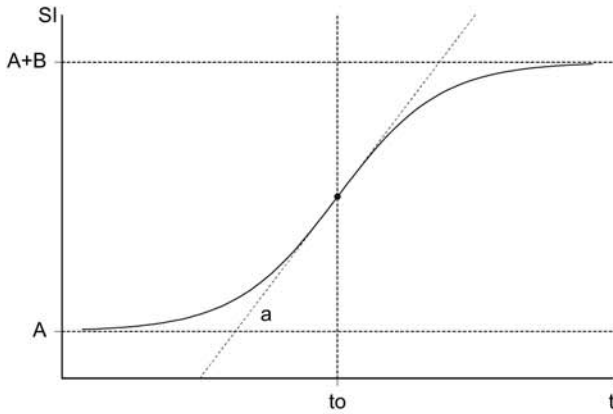


Fig. 1. The non-linear regression model predicting the duration of tick attachment based on scutal index (SI): $SI = A + B / (1 + \exp[-C(t-t_0)])$. $tg \alpha = (B \cdot C) / 4$.

Statistical analysis

The effect of time on tick stage size was evaluated by analysis of variance on SI. Non-linear regression equations (Fig. 1) were developed for SI with S-Plus[®] 7.0 for Windows. For nymphs and females, SI is a sigmoid function of feeding time. The relationships between attachment time and victim age, between attachment time and attachment site, between anatomical site of bites and victim gender were evaluated with Fisher's exact test. The χ^2 test was used for attachment times > 24 h, comparisons between nymphs and adults, and for anatomical site comparisons between children < 10 years old and participants > 10 years. All statistics were calculated with S-Plus[®] 7.0 for Windows.

Results

Scutal index and determination of the attachment duration

Mean SI (\pm standard error, SE) for attachment intervals of experimentally fed nymphs and females are given in Table 1. Analysis of variance on the normally distributed data indicates a significant effect of attachment time on SI ($p < 0.05$) for nymphs and females. Analysis of variance on SI of ticks removed at 0 and 24 h showed no significant effect of time on tick stage size ($p > 0.05$). However, when comparing tick sizes at 24 vs. 48 h, 48 vs. 72 h, and 72 vs. 96 h, there are significant differences for each pair of time groups ($p < 0.05$) both for nymphs and adults.

Non-linear regression equations were developed from SI to determine the duration of attachment for nymphal and adult female *I. ricinus* ticks. For nymphs and females, this result is a positive relation: $SI = A + B /$

Table 1. Mean scutal indices (SI) for unengorged *I. ricinus* and those fed on mice (nymphs) and rabbits (females) for each attachment interval

Hours attached	Nymphs			Females		
	No.	SI	\pm SE	No.	SI	\pm SE
0	46	1.99	0.013	51	1.85	0.010
24	46	2.05	0.014	23	1.91	0.011
48	46	2.92	0.034	21	2.30	0.029
72	33	4.36	0.033	24	2.96	0.040
96	21	4.53	0.032	18	3.83	0.052

SE: standard error.

Table 2. Parameter estimates for the four-parameter logistic model

	Nymphs			Females		
	Value	\pm SE	<i>t</i> -Value	Value	\pm SE	<i>t</i> -Value
A	1.959	0.053	36.8	1.758	0.101	17.35
B	2.62	0.26	10.1	3.18	0.725	4.39
C	0.106	0.026	4.11	0.045	0.01	3.87
t_0	51.65	2.18	23.67	74.978	9.24	8.11

SE: standard error.

$[1 + \exp(-C[t-t_0])]$, where SI is a four-parameter logistic function of feeding time *t* (Fig. 1). Parameters *A*, *B*, *C*, and t_0 are reported in Table 2.

For nymphs and females, the non-linear regression equation resulted in a positive relation: $SI = 1.96 + 2.62 / [1 + \exp(-0.106[t-51.6])]$ and $SI = 1.76 + 3.18 / [1 + \exp(-0.0456[t-75.0])]$, respectively.

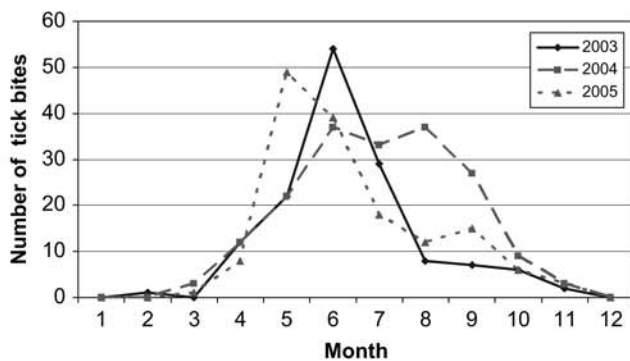
Epidemiological study

During the study period (February 2003–November 2005), 474 inhabitants (237 women, 236 men, 1 unknown) of the Neuchâtel area consulted a physician for a tick bite to participate in the study. The average age of the study population was 43 years (range < 1–94 years), and all age classes were represented: Age class of 50–59 years (19.6%) was the most frequent, followed by the age class of 30–39 years (15.8%) (Table 3). About 8% of the participants were < 10 years old. The age class of ≥ 80 years (2.1%) was the least represented (Table 3).

Among the 474 participants, 495 tick bites were recorded. No significant difference was observed when the anatomical site of bites was compared with victim gender (Fischer's exact test, $p = 0.113$). Legs were the major anatomical sites of bites for women (40.7%, 100/246), men (44.4%, 110/248), and all age classes (data not shown), followed by trunk [29.7% (73/246) and 31.0%

Table 3. Attachment duration of *I. ricinus* with respect to victim age, Neuchâtel area, 2003–2005

Age class (years)	Nymphs			Females		
	No.	No. >24 h	% >24 h	No.	No. >24 h	% >24 h
0–9	14	7	50	6	5	83
10–19	7	6	86	6	1	17
20–29	22	14	64	8	2	25
30–39	28	12	43	19	6	32
40–49	30	22	73	12	3	25
50–59	31	25	81	19	8	42
60–69	18	14	78	15	10	67
≥70	13	8	62	6	3	50

**Fig. 2.** Seasonal distribution of tick bites in 2003, 2004, and 2005 in the Neuchâtel area.

(77/248), respectively] and arms [16.7% (41/246), 13.7% (34/248) respectively]. Children <10 years were bitten more frequently on the head (41%, 7/17) and on the neck (38.5%, 10/26) than participants >10 years [2.2% (10/454) and 3.5% (16/454), respectively] (χ^2 test, $p < 0.0001$).

The majority of tick bites were recorded from May to July during the 3 years. Moreover, many bites took place in autumn, particularly in 2004 and 2005 (Fig. 2).

Duration of tick attachment

A total of 352 ticks were removed from 341 persons. Among them, 67.9% (239/352) were nymphs and 29.8% (105/352) were female ticks. Calculation of attachment duration of ticks using the SI developed for *I. ricinus* was possible in 261 ticks (90 females and 171 nymphs). Nymphs were attached for a mean (\pm SE) of 31.6 h (\pm 2.6). Among these nymphs, 36.3% (62/171) were attached for <24 h and 63.7% (109/171) removed after >24 h (Table 4). When attachment times were analysed in accordance with victim age, no differences were found (Fisher's exact test, $p > 0.05$), although the age class 10–19 years accounted for the highest percentage of nymphs (86%) attached for >24 h, followed by the age class 50–59 years (81%) (Table 3). When the sites of

Table 4. Attachment durations of *I. ricinus* nymphs ($n = 171$) and females ($n = 90$) removed from participants of the epidemiological study in the Neuchâtel area, 2003–2005

Hours of attachment	Nymphs		Females	
	No.	%	No.	%
0–24	62	36.3	52	58
>24–48	66	38.4	18	20
>48–72	36	21.1	9	10
>72–96	4	2.3	7	8
>96	3	1.8	4	4

Table 5. Attachment duration of *I. ricinus* with respect to attachment site, Neuchâtel area, 2003–2005

Body site	Nymphs			Females		
	No.	No. >24 h	% >24 h	No.	No. >24 h	% >24 h
Arms	25	14	56	6	2	33
Neck	8	8	100	6	4	67
Legs	79	45	57	35	9	26
Head	5	3	60	5	3	60
Trunk	46	33	72	34	17	50
Unknown	8	7	88	5	3	60

attachment were examined, no differences were observed with respect to duration of attachment (Fisher's exact test, $p > 0.05$), although neck (100%) and trunk (71.7%) had relatively high percentages of nymphs attached for >24 h (Table 5).

For female ticks, a mean attachment duration (\pm SE) of 29.6 h (\pm 3.2) was observed, 58% (52/90) of females removed within 24 h, and 42% (38/90) after >24 h (Table 4). When attachment times were analysed, no differences were found (Fisher's exact test, $p > 0.05$) regarding to victim age, although the age class 0–9 years accounted for the highest percentage of females attached

for >24 h (83%, Table 3). When the sites of attachment were examined, no differences were found with respect to duration of attachment (Fisher's exact test, $p > 0.05$), although the neck (67%) had a relatively high percentage of females attached for >24 h (Table 5). More nymphs than adults were detected after 24 h of attachment (χ^2 test, $p = 0.001$).

Discussion

The epidemiology of LB is governed by 3 principal factors: density of tick populations, their prevalence of infection with *B. burgdorferi* sl, and frequentation of the forest environment. In their leisure time or professionally, a great number of people visit forests, especially during the tick activity periods (spring and autumn) (Morán Cadenas et al., 2007). During those, the number of bites is important, whereas it decreases during the periods when the ticks are inactive (winter). Another important factor influencing the exposure to ticks is the personal behaviour, which itself is influenced by seasonal climatic conditions. Thus, during June 2003, which was very warm (<http://www.meteoschweiz.admin.ch/web/en/climate.html>), a greater number of inhabitants went to the forest to search refreshment, and as a result the number of tick bites was higher, although the free-living tick population density was decreasing (Morán Cadenas et al., 2007).

Children <10 years old accounted for only 8% of the participants. Previous studies reported that children were more often bitten than adults (Falco et al., 1996; Robertson et al., 2000). The difference is that in these two studies no blood sample was taken. In our case, the difficulty to take two blood samples from each participant as required in our epidemiological study (Huegli et al., unpublished) from young children probably dissuaded a great number of parents and physicians to include them in the study.

All age classes showed a majority of bites on legs. However, children <10 years old were bitten mainly in the neck and on the head. Similar observations were made by Robertson et al. (2000). Differences between adults and children in the distribution of tick bites on the body could reflect behavioural and physiological differences between these two groups of population.

The higher frequency of nymphal (67.7%) compared to females bites partially reflects the higher abundance of nymphs. In fact, the density of nymphs in the investigated area may be 2–30 times higher (depending on the year) than adult density (Jouda et al., 2004a, b; Morán Cadenas et al., 2007). Larval bites were not taken into account because their role in the transmission of *B. burgdorferi* sl does not seem important, transovarial transmission being rare in *I. ricinus* (Bellet-Edimo et al., 2005).

One of the most important factors of transmission risk of *B. burgdorferi* sl after a tick bite is the duration of attachment. Indeed, the risk of transmission increases with the duration of attachment (Kahl et al., 1998; Crippa et al., 2002). In this study, most nymphs were removed after >24 h of blood meal, whereas most females were removed within 24 h. The small size of the nymphs enables them to be attached longer before being detected and removed (Logar et al., 2002). The risk of infection is thus greater after a nymph bite than after a female bite, even if females have higher infection prevalences (Jouda et al., 2004a, b). Moreover, the risk of being bitten by a nymph is greater, considering their greater abundance compared to that of females (Jouda et al., 2004a, b; Morán Cadenas et al., 2007).

The duration of tick attachment was previously evaluated by determination of SI for *I. scapularis* (Falco et al., 1996). Here, we used non-linear equation to determine attachment time from SI for *I. ricinus*. For both nymphal and female *I. ricinus*, SI showed slow changes in body dimensions at 0–24 h and appeared to be too insensitive during the first hours of feeding. This was also reported by Falco et al. (1996) for *I. scapularis*. Recently, Gray et al. (2005) devised a new index, the coxal index (CI), which detects the changes in tick body dimensions that occur early in feeding. CI presented a greater sensitivity than the SI during the first 24 h but not later. Another advantage of the use of CI is that damaged ticks can be measured, whereas the anterior part of the tick should be complete to allow an accurate determination of SI.

In our study, if we considered the duration of attachment according to the age of the victim, no significant difference was observed. However, young children (0–9 years old) as well as the age class of the 30–39 years old represented the two age classes showing the highest percentage (50% and 43%, respectively) of nymphs detected and removed within 24 h of attachment. In another study in North America, it was observed that the duration of tick attachment increased significantly with the age of the victim (Falco et al., 1996). Young children represented the age class showing the lowest percentage of nymphs attached for >48 h, suggesting that they were inspected during the summer months. Paradoxically this same age class, as well as the age class of ≥ 60 years, showed the highest percentage of females removed after only 48 h of attachment. The same holds true for our study, although there was no significant difference: 83% and 62%, respectively, of females were removed after 24 h of attachment in these two age classes.

Attachment sites can influence the detection of ticks, hence the duration of the tick bite. Indeed, a tick attached on the head, the neck, or in the back is detectable with more difficulty, in particular for a nymph. Although there are no significant differences,

the highest proportion of nymphs attached for >24 h were found on the neck and the head.

Of female *I. ricinus* ticks attached to the head and to the neck, 60% and 67%, respectively, were detected after >24 h. But these data are not significantly different (Fisher's exact test, $p > 0.05$) and do not suggest that the site of attachment influences the probability of early or late finding. However, Falco et al. (1996) reported in their study that the duration of *I. scapularis* tick bites is influenced by the site of attachment.

Detailed body examinations after each outing in a forest and an early removal of an attached tick is an important aspect of prevention and is highly recommended, because it should significantly decrease the risk of transmission and the risk of developing LB as described in the epidemiological part of this study (Huegli et al., unpublished).

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