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Tick infestation risk and *Borrelia burgdorferi* s.l. infection-induced increase in host-finding efficacy of female *Ixodes ricinus* under natural conditions

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Abstract An investigation of the risk of human tick infestation, together with the prevalence of *Borrelia burgdorferi* s.l. infection, was conducted in a sylvatic habitat in western Germany to provide data needed for future risk-benefit evaluations of acaricides used for clothing impregnation. Additionally, data were collected on behavioural changes in *Borrelia burgdorferi* s.l.-infected adult female *I. ricinus* ticks and the possible impact of such changes on host-finding efficacy. The risk of *I. ricinus*-infestation was determined by collecting from the protective clothing of volunteers and by dragging in known tick-infested sites in the Kühkopf Mountain area, Koblenz, Germany, from June through October 2006. The overall tick infestation rate per person per hour was 7.4 ± 5.5, with the following sex- and stage-specific differences: males 0.32 ± 0.37, females 1.1 ± 1.2, nymphs 3.6 ± 4.4, larvae 2.4 ± 3.5. Concurrent dragging revealed an average 19.4 ± 16.2 times higher infestation rate as well as a markedly lower infection rate with borreliae in adult *I. ricinus* ticks when compared to ticks collected from exposed human volunteers. Although the difference in infection rates was statistically significant ($P < 0.023$) only in adult female ticks, our data indicate that *B. burgdorferi* s.l. infection may increase host-finding efficacy in adult *I. ricinus*. The overall exposure risk was 1.0 *B. burgdorferi* s.l.-infected ticks per person per hour of exposure, or 0.25 ticks per 100 m walking distance in the study area.

Keywords *Ixodes ricinus* · Host-seeking activity · *Borrelia burgdorferi* s.l. · Human exposure risk

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Introduction

Among the increasing number of vector-borne diseases that are currently emerging or resurging worldwide, only a few are vaccine-preventable (e.g., tick-borne encephalitis, yellow fever, Japanese encephalitis, and plague). For this reason, personal protective measures against hematophagous vectors constitute the first line of defense against arthropod-transmitted diseases in endemic areas. In Europe, tick-borne diseases pose the greatest risk, with over 10,000 reported clinical cases of tick-borne encephalitis, and estimates of hundreds of thousands of clinical cases of Lyme disease occurring annually (Ginsberg and Faulde, in press). A major advance in protecting at-risk personnel, such as foresters, hikers and soldiers, against ticks and tick-borne diseases has been the development of topical repellents and residual acaricides that can be impregnated into clothing (Schreck et al. 1982; Rey 1998; Faulde and Uedelhoven 2006).

Since skin or fabric repellents are likely to be used by thousands of inexperienced people exposed to vector ticks, a safety factor should be calculated by analyzing the risk-benefit ratio of the biocides employed (Young and Evans 1998; WHO 2001a, b). In order to evaluate a substance's toxicology and the efficacy of its formulation for human use, it is necessary to quantify (1) the tick infestation rate on humans during field exposure in known tick habitats, (2) tick population densities, and (3) local prevalence of associated disease agents. Although toxicological evaluations of many skin repellents are available (Chavasse and Yap 1997; WHO 2001a, b), and studies of permethrin-based fabric repellent formulations are well under way (Snodgrass 1992; Rossbach et al. 2005), no quantitative study of the risk of human tick infestation, and associated disease agents, currently exists for Central Europe.

In Europe, the castor bean or sheep tick, *Ixodes ricinus*, is the most common tick species and the principal vector of *B. burgdorferi* s.s., *B. afzelii*, *B. garinii*, *B. spielmani*, *B. valaisiana*, *B. lusitaniae*, tick-borne encephalitis virus (Central European encephalitis), Uukuniemi virus, Erve virus, Eyach virus, *Anaplasma phagocytophilum*, and *Rickettsia helvetica* (Gern and Humair 2002; Süss and Schrader 2004; Süss et al. 2004). *Ixodes ricinus* is exophilic, passively questing from vegetation for its hosts, which include a wide range of reptiles, birds and mammals. Due to the medical importance, great abundance, and broad distribution of both vector and disease agent, *I. ricinus*-associated *Borrelia burgdorferi* s.l. infection constitutes an excellent model for investigating the impact of tick-borne diseases in Central Europe.

Although *I. ricinus* has been studied for more than 50 years, knowledge of this species' biology remains incomplete, partly because of geographical and genetic heterogeneity, but also because of the diversity of habitats in which it may be found (Gern and Humair 2002). Generally, *I. ricinus* is active only when the humidity exceeds 80%. By contrast, *Borrelia*-infected adults of *I. persulcatus*, the principal vector of *B. burgdorferi* s.l. in Eastern Europe and Asia, appear to be significantly more tolerant of lower relative humidity and higher air and soil temperatures than non-*Borrelia*-infected adults (Alekseev and Dubinina 2000). Additionally, exoskeleton anomalies in *Borrelia*-infected female *I. persulcatus* resulted in a 1.3-fold increase in locomotor activity (Alekseev et al. 2000). Whether *B. burgdorferi* s.l.-induced changes in the behaviour of *I. persulcatus* and *I. ricinus* result in greater questing and host seeking activity, leading to increased infestation efficacy, remains unknown.

This study was designed to quantitatively analyze human exposure to *I. ricinus* ticks, measuring tick density by dragging and determining the prevalence of *Borrelia burgdorferi* s.l. in ticks removed from humans and drags under field conditions in Germany.

Materials and methods

Tick field exposure and sampling

From early June until the end of October 2006, one female and five male volunteers were exposed to ticks at infested sites in the Kühkopf Mountain recreational area (50°18′30–50″ N; 7°33′00″ E; 310 m above sea level), near Koblenz, Germany. This area is characterized by deciduous forests within a region considered non-endemic for tick-bone encephalitis virus. The mesotrophic sylvatic Luzulo-Fagion-biotope (Rothmaler 1984) is dominated by beech trees (*Fagus silvatica*) with scattered oaks (*Quercus* spp.), plus lower (10–60 cm) vegetation consisting chiefly of hairy wood-rush (*Luzula pilosa*), wood melick (*Melica uniflora*) and dog's mercury (*Mercurialis perennis*), as well as raspberry (*Rubus idaeus*) and patches of leaf litter without vegetation (Fig. 1). Volunteers wore full-body protective professional garments (Kleenguard IPP, Kimberley-Clark GmbH, Mülheim-Kärlich, Germany) and leather military boots. "Buddy checks" were conducted every 5 min in order to prevent tick infestation and feeding. Continuous occupational health surveillance according to G 42 (national regulation on occupational exposure to infectious agents) was enforced (Lieboldt 2007). Field studies took place on non-rainy days with maximum daytime temperatures exceeding 19°C and relative humidity (r.h.) greater than 30%, as measured by a digital thermohygrometer (H 270, Orglmeister, Walluf, Germany). Monthly mean temperatures during the study period were: June 17.1°C, July 21.5°C, August 14.8°C, September 17.4°C, and October 12.4°C. The volunteers' legs were completely covered with untreated white bed linen, affixed with rubber bands; this material was also employed for dragging. During exposure, ticks that had attached to the linen were removed every 5 min, counted, identified, and transferred to 80% ethanol. Tick sampling with protective garments did not exceed 1.5 h (excluding time spent in detecting and collecting attached ticks) per person per day. One full hour of exposure corresponded to a 400 m walk within a tick-infested site. Simultaneously, another volunteer sampled ticks by dragging a blanket (Ferquel et al. 2006) at the same site. A 1 m^2 white cotton blanket was dragged over low vegetation and leaf litter, and ticks attaching to the blanket were removed, identified and

Fig. 1 Tick dragging in the mesotrophic sylvatic Luzulo-Fagion-biotope of the Kühkopf recreational area, Koblenz, Germany, during 2006

counted every 100 m. The "relative Tick Infestation Ratio" (rTIR)—the number of ticks attached to the blanket per 100 m^2 versus the number collected from an exposed volunteer per 100 m—is defined as follows:

$$\text{rTIR} = \frac{\text{Number of ticks attaching to drag per 100 m}^2}{\text{Number of ticks infesting an exposed human per 100 m}}$$

All ticks collected from humans (except one adult female, which escaped during collection) or by dragging were further investigated for *Borrelia burgdorferi* s.l. infection.

Borrelia burgdorferi s.l. detection in ticks

Single ticks were mechanically homogenized using a MagNA Lyser device (Roche Diagnostics Co., Mannheim, Germany). DNA was isolated using a High Pure PCR Template Preparation Kit (Roche Diagnostics Co., Mannheim, Germany) and subsequently tested for *B. burgdorferi* s.l.

Specific *B. burgdorferi* s.l. PCR was carried out using a LightCycler 2.0 (Roche Diagnostics Co., Mannheim, Germany), as described by Rauter et al. (2002), targeting the three *Borrelia* genospecies: *B. burgdorferi* s.s., *B. garinii*, and *B. afzelii*. *Borrelia burgdorferi* s.l.-specific fluorogenic primer and probe sequences, targeting the *Osp A* gene, as well as positive controls of *B. burgdorferi* s.s., were purchased from TibMolBiol Co. (Berlin, Germany); DNA from *B. garinii*, and *B. afzelii* was kindly provided by the Landesgesundheitsamt Baden-Württemberg (Stuttgart, Germany). Positive controls and a negative (*Borrelia*-DNA-free) λ-DNA control (LightCycler Fast Start DNA Master HybProbe, Roche Diagnostics Co.), simultaneously acting as a run-specific internal amplification control, were employed during each run.

All *B. burgdorferi* s.l. positive, borderline, and unclear results were independently confirmed by a second LC-PCR run using the commercially available RealArt Borrelia LC PCR Kit (Artus GmbH, Hamburg, Germany) for combined detection of *B. burgdorferi* s.s., *B. garinii*, and *B. afzelii*.

Data analysis

Values were reported as mean ± SD. Differences in mean *B. burgdorferi* s.l. infection rates by tick sex and stage were analyzed using Pearson's χ^2 test, including error degrees of freedom (df), and two-sided asymptotic significances against the residual error at the 5% level (statistical significance) (SPSS 8.0 program, SPSS Software GmbH, Munich, Germany).

Results

During 18 h of human exposure in known tick-infested habitats, 132 ticks were collected; of these, 6 (4.5%) were males, 16 (12.1%) females, 64 (48.5%) nymphs, and 46 (34.9%) larvae. Figure 2 shows the tick infestation rates obtained by human field exposure. The overall tick infestation rate per person per hour was 7.4 ± 5.5 (range 0–23), with the following sex- and stage-specific differences: males 0.32 ± 0.37 (range 0–2), females 1.1 ± 1.22 (range 0–4), nymphs 3.6 ± 4.4 (range 0–17), larvae 2.45 ± 3.5 (range 0–12).

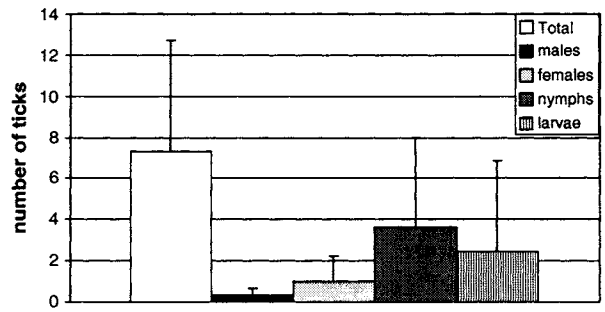

Fig. 2 Mean sex- and stage-specific tick attachment rates per person per hour of exposure, constituting the crude risk of tick infestation, measured in the Kühkopf recreational area, Koblenz, Germany, during 2006

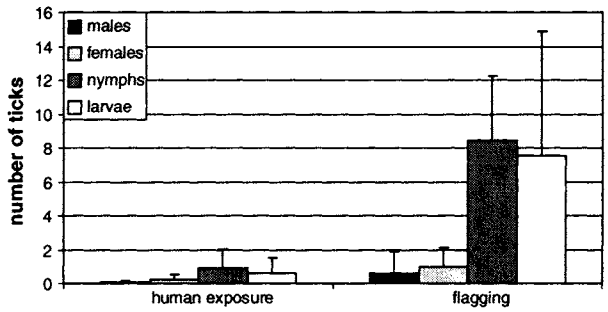

Fig. 3 Mean sex- and stage-specific tick infestation rates per person per 100 m walking distance versus dragging results, measured in the Kühkopf recreational area, Koblenz, Germany, during 2006

These results correspond to a mean tick infestation rate of 1.85 ± 1.3 ticks per person per 100 m walking distance, of which 0.08 ± 0.09 were males, 0.25 ± 0.03 were females, 0.91 ± 1.1 were nymphs, and 0.61 ± 0.87 were larvae (Fig. 3). During 2006, *I. ricinus* questing activity was bimodal, peaking in June and September.

Dragging, conducted concurrently with human exposure, yielded a mean of 17.6 ± 9.2 ticks per 100 m^2 and the following distribution by tick sex and stage: males 0.6 ± 1.3, females 1.0 ± 1.1, nymphs 8.45 ± 3.8, larvae 7.6 ± 7.3 (Fig. 3). Dragging resulted in a 19.4 ± 16.2-times ($n = 10$, range 2.7–52) higher tick infestation rate when compared to the numbers collected from humans. The mean rTIR was 19.4, with the following sex- and stage-specific differences: males 15.2, females 8.3, nymphs 18.9, and larvae 25.0.

Among the ticks collected from exposed volunteers (Table 1), *B. burgdorferi* s.l. infection was detected in 33.3% (2/6) of males, 56.25% (9/16) of females, 9.4% (6/64) of nymphs, and 0% (0/46) of larvae, with a mean positivity rate of 12.9%. Out of the 17 *Borrelia*-positive tick samples, 7 (41.2%) were *B. burgdorferi* s.s., 5 (29.4%) were *B. garinii*, and 4 were *B. afzelii* (23.5%), including one (5.9%) *B. burgdorferi* s.s./*B. afzelii* double infection (Table 1). These results amount to an overall exposure risk of 1.0 *B. burgdorferi* s.l.-infected tick per person per hour of exposure, or 0.25 per 100 m walking distance in the tick-infested sites studied. Ticks collected by dragging showed the following *B. burgdorferi* s.l. positivity rates: males 17.8% (5/28), females 25% (11/44), nymphs 8.9% (10/112), and larvae 2.9% (3/102), with a mean infection rate of 10.2%. *Borrelia* genospecies differentiation showed the following pattern: 15 (51.7%) *B. burgdorferi* s.s., 8 (27.6%) *B. garinii*, and 6 (20.7%) *B. afzelii*. Differences in *B. burgdorferi* s.l. infection in ticks sampled from humans versus dragging were statistically significant in females ($\chi^2 = 5.2$, df = 1, $P < 0.023$), but not in males ($\chi^2 = 0.7$, df = 1, $P < 0.395$), nymphs ($\chi^2 = 0.14$, df = 1, $P < 0.71$), and larvae ($\chi^2 = 1.4$, df = 1, $P < 0.24$) (Fig. 4).

Table 1 Overview on tick pattern collected by exposed volunteers, associated *B. burgdorferi*-positive *Ixodes ricinus* ticks, and climatic conditions

Volunteer no.	Total tick exposure (h):	No. of ticks collected during exposure (M/F/N/L):	Corresponding *Borrelia*-positive ticks (M/F/N/L):	Temperature (°C)/relative humidity (%) during exposure
1	4	12 (0/0/2/10)	0/0/0/0	23/51
		4 (0/0/2/2)	0/0/0/0	23/54
		4 (0/0/0/4)	0/0/0/0	25/49
		6 (0/0/0/6)	0/0/0/0	23/53
2	4	14 (0/2/0/12)	0/1 Ba/0/0	23/51
		4 (2/0/0/2)	1 Bgss/0/0/0	23/54
		4 (2/0/2/0)	1 Bgss/0/0/0	25/49
		0 (0/0/0/0)	0/0/0/0	23/53
3	3	1 (0/1/0/0)	0/0/0/0	25/40
		6 (0/0/6/0)	0/0/1 Ba/0	24/45
		8 (2/4/2/0)	0/1 Bgss + 2 Bg/1 Bg/0	23/50
4	3	6 (0/0/6/0)	0/0/0/0	20/50
		7 (0/2/5/0)	0/1 Bgss + 1 Ba/1 Bgss + 1 Ba/0	34/32
		12 (0/0/8/4)	0/0/1 Bg/0	34/36
5	3	8 (0/2/4/2)	0/1 Bgss + 1 Bg/1 Bgss/0	28/30
		3 (0/1/2/0)	0/0/0/0	28/36
		10 (0/2/8/0)	0/0/0/0	23/50
6	1	23 (0/2/17/4)	0/1 Bgss-Bg double infection/0/0	23/50

M = males; F = females; N = nymphs; L = larvae; Bbss = *Borrelia burgdorferi* sensu stricto; Ba = *Borrelia afzelii*; Bg = *Borrelia garinii*

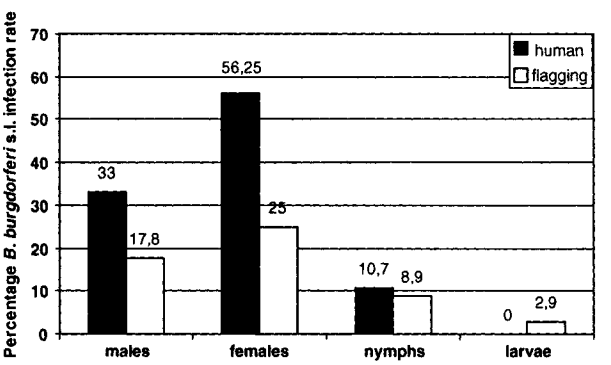

Fig. 4 Sex- and stage-specific differences in *B. burgdorferi* s.l. infection in *Ixodes ricinus* ticks collected from humans subsequent to field exposure versus collection by dragging, measured in the Kühkopf recreational area, Koblenz, Germany, during 2006

Discussion

For occupational health and safety reasons, human field exposure to *I. ricinus* ticks was conducted by walking very slowly in known tick-infested areas using full-body protection and leather military boots plus "buddy checks" designed to prevent tick bite and possible transmission of endemic tick-borne diseases. It is therefore impossible to ascertain whether

unprotected humans have a differing stimulatory effect on tick host seeking and infestation due to more rapid volitalization of attractants. Additionally, different human exposure behaviours and footwear may result in varying tick infestation ratios, as reported for both nymphs of the western black-legged tick, *Ixodes pacificus*, and the lone star tick, *Amblyomma americanum* (Carroll and Kramer 2001; Lane et al. 2004). Because of the relatively high mobility of *I. ricinus* ticks on our volunteers' legs, we did not attempt to determine this species' "attachment area" or "attachment height". Given the density of the local *I. ricinus* population, as measured by dragging, the risk of tick infestation, tick bite, and tick-borne disease transmission may exceed our estimates. Moreover, in light of micro-ecological differences, tick dragging is not directly comparable with human exposure. Nonetheless, the mean rTIR values for male *I. ricinus* indicate that male host-seeking efficacy is considerably lower than it is in females. Larvae showed the highest rTIR value, which corresponds to the lowest host-seeking efficacy with respect to humans, but their great abundance during the 2006 activity season resulted in the second highest infestation rate on humans, exceeded only by nymphs. It should be borne in mind that, because of their small size and cryptic colouration, attached larvae can easily be overlooked on the human body while remaining potential vectors of human pathogens acquired via transovarial transmission.

All larvae collected from humans tested negative for *B. burgdorferi* s.l. infection, whereas three of eight larvae collected by dragging tested positive for *B. burgdorferi* s.s. Interestingly, all three larvae were collected at a single location on the same day and time and were infected with the same *Borrelia* genotype, indicating that they were almost certainly the progeny of the same female. Our field data underscore the focal nature of transovarial transmission and support recent findings that the efficacy of transovarial transmission is usually low but, when it occurs, the prevalence of infection may be high, ranging from 43 to 100% (Bellet-Edimo 1997; Gern and Humair 2002).

With respect to the overall *Borrelia* genospecies pattern detected in *I. ricinus*, *B. burgdorferi* s.s. prevailed in 47.8% of cases, *B. garinii* in 28.3%, and *B. afzelii* in 21.7%; there was also one (2.2%) *B. burgdorferi* s.s./*B. afzelii* double infection. Kipp et al. (2006), working in six different habitats in Thuringia, Germany, determined that the prevalence and genetic diversity of *B. burgdorferi* s.l. are highly variable and habitat-specific. Depending on area and habitat investigated, *B. burgdorferi* s.s. prevalence varied from 0 to 20%, *B. garinii* from 0 to 39%, and *B. afzelii* from 7 to 52%. Clearly, the prevalence of *B. burgdorferi* s.s. is unusually high within the mesotrophic sylvatic Luzulo-Fagion-biotopes along the Rhine Valley in Rhineland-Palatinate, while *B. garinii* and *B. afzelii* prevalence rates fit the expected range.

Nymphs are the stage of *I. ricinus* most often found on humans, followed by larvae, females, and males. The unexpectedly high peak in larval questing during 2006 may have been due to the harsh winter and spring of 2005/2006 (the mean temperature between January and March 2006 was 0.1°C, whereas the 10-year average for this area is 2.5°C), which may have delayed tick activity until late April/early May. Simultaneous dragging revealed that the mean rTIR was 19.4 times (range 2.7–52) higher on low vegetation and leaf litter, indicating that tick infestation risk increases dramatically in persons who come into contact with that microhabitat (Carroll and Kramer 2001; Lane et al. 2004), especially for prolonged periods (e.g., soldiers, hunters, foresters). Under such conditions, tick avoidance measures should include a combination of skin and fabric repellents (Faulde et al. 2006). In Central Europe, the highest tick densities that have been reported to date are 105 and 114 per 100 m^2 at foci in Alsace, France (Ferquel et al. 2006); by comparison, the tick density in the Kühkopf area, 17.6 ± 9.2 ticks per 100 m^2, is moderate. In highly

infested areas, a tick infestation rate exceeding 100 per person per exposure hour can be expected. But if we consider the corresponding *B. burgdorferi* s.l. positivity rates, which vary between 9.6% (nymphs) and 36.4% (adults), with a reported mean of 19.4%, then the number of attached *B. burgdorferi* s.l.-infected ticks may exceed 20 per person per hour of exposure within the Alsatian foci, almost 20 times the risk measured in the Kühkopf recreational area.

Intriguingly, adults of *I. ricinus* collected from humans showed a higher *B. burgdorferi* s.l. infection rate than ticks collected by the dragging method, although this difference was statistically significant only in females. Confirmation of this observation is needed because of the relatively small numbers of adult ticks in our study. Apparently, infection with *B. burgdorferi* s.l. may induce behavioural changes in adult ticks, especially adult females, possibly leading to an increase in host-finding efficacy as well as an underestimation of their impact in *B. burgdorferi* s.l. transmission.

As yet it is unclear how far the suppression of locomotor activity, which occurs in adult and immature *Borrelia burgdorferi* s.l.-infected *I. ricinus* as opposed to uninfected specimens (Alekseev et al. 2000), may positively or negatively influence host seeking efficacy. If *B. burgdorferi* s.l. infection can cause behavioural changes in adult *I. ricinus*, leading to a preference for higher air, soil and subsoil temperatures and lower relative humidity, as has been reported for the sister species *I. persulcatus* (Alekseev and Dubinina 2000), then host acquisition may be facilitated and, with it, transmission of disease agents. Whether pathogens other than *B. burgdorferi* s.l. also influence tick behaviour, especially as co-infections, remains unknown.

Our data on tick infestation and *B. burgdorferi* s.l. prevalence rates in ticks attaching to humans at infested sites in western Germany underscore the considerable risk of acquiring Lyme borreliosis in Central Europe. Since no licensed vaccine exists for Lyme borreliosis worldwide, personal protective measures remain the first line of defense against tick bite and tick-borne diseases (Young and Evans 1998; WHO 2001a, b; Mencke 2006). It is hoped that the results reported here will prove useful in future risk-benefit analyses of acaricides formulated for use on fabrics.

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