Michael K. Faulde[1]*, Klaus G. Mross[2]

[1] Department of Medical Entomology / Zoology, Central Institute of the Bundeswehr, Medical Service, Koblenz, Germany
[2] Bundeswehr Regional Medical Command Ii. Occupational Medicine, Diez, Germany

Fabric impregnation using acaricides: effective and safe method for the prevention of tick-infestation and tick-borne diseases

Keywords
Fabric impregnation
Permethrin
Ixodes ricinus ticks
Tick prevention
Interruption of transmission modes

*Corresponding Author:

PD Dr. Michael K. Faulde

Department of Medical Entomology / Zoology
Central Institute of the Bundeswehr
Medical Service
P. O. Box 7340
56065 Koblenz

E-Mail: MichaelFaulde@bundeswehr.org

Summary

Background: Hard ticks are responsible for more cases of human disease than any other arthropod vector in the Northern Hemisphere. In Central Europe, over 60 % of all yet reported endemic arthropod- and rodent-borne diseases are transmitted by hard ticks, especially the castor bean tick, *Ixodes (I.) ricinus*. Among tick-borne diseases (TBDs), Lyme borreliosis occurs most frequently, with up to 100.000 clinical cases estimated annually in Germany, and hundreds of thousands of cases for Europe as a whole. In Central Europe, studies quantifying the risk of human *I. ricinus* infestation and associated *Borrelia burgdorferi* sensu lato infection have not yet been conducted under natural conditions, but these are necessary in order to calculate risk-benefit-ratios for protective measures. Of the European TBDs pathogenic to humans, only tick-borne encephalitis, caused by a flavivirus, is vaccine preventable. Consequently, individual protection from tick infestation and tick bite constitutes the first line of defence against TBDs. In addition to using skin and fabric repellents, individuals can protect themselves against tick attachment by tucking trousers into boots or socks and tucking shirts into trousers. Light-coloured clothing aids in detecting of dark-coloured ticks, which can be collected or removed, e.g., with forceps or commercial tape. The risk of acquiring TBDs rises when hard ticks attach to human clothing and begin to search for a suitable place to bite. It should be borne in mind that, because of their small size and cryptic colouration, attached larvae and nymphs can easily be overlooked on clothes. The development of permethrin-impregnated clothing constitutes a major advance in overcoming TBD risk. However, the protection afforded by such materials against *I. ricinus* tick infestation and associated *B. burgdorferi* s.l. infection has not yet been objectively analyzed especially under field conditions.

Method: In order to improve effectiveness, minimize loss of active ingredient during laundering, and reduce biocide exposure for inexperienced users unfamiliar with sprays or dips, a new factory-based, high-residue and toxicologically safe polymer-coating method has been developed and field-tested, utilizing specific polymerization of permethrin onto clothing prior to the tailoring process.

Results: Our investigation was conducted within a defined sylvatic habitat at a popular recreation area in western Germany and revealed an average *I. ricinus* infestation rate of 1,8 ± 1,3 ticks and a quantitative exposure rate of 0,25 *B. burgdorferi* s.l.-infected ticks per person per 100 m walking distance. The *B. burgdorferi* s.l. prevalence rate in *I. ricinus* ticks was significantly higher (p < 0,023) in adult females collected from human volunteers (56 % positive), compared with females collected by dragging (25 % positive). These data indicate that the actual human risk of infection by *B. burgdorferi* s.l. cannot be determined from prevalence rates obtained by the widely used dragging method. Factory-based permethrin-impregnated clothing provided a high mean protection rate of 95,5 % against questing *I. ricinus* ticks.

Conclusion: These data clearly demonstrate that such clothing is an important barrier to hard ticks and associated disease agents.

Introduction

The importance of hard ticks in the spread of disease has increased enormously in recent years in Germany too, both in qualitative and quantitative terms. Based on

present-day knowledge of the situation in Germany, hard ticks are responsible for transmission of 18 (62 %) of the 29 endemic arthropod-and rodentborne human pathogens [1,2,3]. In addition, they are responsible for the infections caused by the tickborne meningoencephalitis virus, uukuniemi virus, erve virus, eyach virus, tribec virus as well as the lipovnik virus [2]. As regards bacteria and parasites, they transmit the five *Borrelia burgdorferi* sensu lato genospezies *B. burgdorferi sensu stricto, B. afzelii, B. garinii, B. spielmanii,* und *B.valaisiana* (potentially pathogenic to humans), *Coxiella bumetii, Anaplasma phagocytophilum, Rickettsia (R.) slovaca, R. helvetica, Francisella tularensis* [3] as well as the parasites *Babesia (B.) divergens* [4] and *B. microti* [5]. The castor bean tick, *Ixodes (I.) ricinus,* is the most common and competent tick vector in Germany, and as such is by far the most commonly found tick on humans [6].

Ticks are also highly relevant in terms of the annual incidence of infectious diseases transmitted by them. In the northern hemisphere hard tick or Lyme borreliosis is the most common vector-associated infectious disease. The annual incidence in Europe is thought to be several 100,000 cases [7]. Germany is estimated to have between 20,000 and 60,000 [8] cases, and even up to 100,000 cases on occasion [9]. There can be enormous variations in the incidence from one area to another. For example, in the Oder-Spree region in the federal state of Brandenburg an average incidence of 89.3 cases per 100,000 inhabitants was registered for 2003. Here the local fluctuations were between 16 cases per 100,000 in the district of Erkner and 311 cases per 100.000 in the district of Brieskow-Finkenheerd [10]. Since 2002 confirmed cases of Lyme borreliosis in the six eastern federal states of Germany have been reported to the Robert Koch Institute in Berlin. The increase noted in the number of cases reported there between 2002 and 2006 permit the conclusion that at a European level, Central Europe has the highest level [11].

The second most common infectious disease spread by hard ticks in Germany is tickborne meningoencephalitis, which is a notifiable disease pursuant to the German Protection against Infection Act (IfSG), accounting for between 200 and 450 cases each year. At present there are no reliable data available on the incidence rate for the other tickborne diseases mentioned. The castor bean tick, *I. ricinus,* is by far the most common, and in geographic terms, most widespread and at the same time most important vector (Figure 1) [1,2,3]. It is possible that because of the trend towards greater spread, associated with environmental and climate changes, hard tick species that hitherto had been of no relevance to humans and the diseases spread by them will in future pose a greater threat. For example, in addition to geographic spread, an increased presence of the ornate dog tick, *Dermacentor reticulatus* has been observed in the state of Brandenburg since July 2006 on humans [12].

Both the frequency of the tick population as well as their pathogenic prevalence show considerable variation between tick habitats and the respective geographic endemic regions. It is thus very difficult to obtain valid data for endangerment analyses in terms of tick infestation rate per person per exposure time, or per exposure distance, in the tick habitat so as for example be able to draw conclusions on the infection pressure on the basis of the number of infected ticks per person per exposure time. Such investigations are also of paramount importance for risk-benefit evaluation of preventive measures.

Of the diseases transmitted by hard ticks endemic to Germany, only tickborne meningoencephalitis, which is widespread in Bavaria and Baden-Württemberg, is vaccine preventable [13]. All other diseases, such as Lyme borreliosis, which is endemic and common throughout Germany, can be effectively prevented in regional tick endemic areas only by avoiding a potentially infectious bite. To date, this could be done only by mechanical methods such as removal of ticks from light-coloured clothing, ensuring that the exposed skin areas were kept to a minimum or the use of various commercially available skin repellents, e.g. products based primarily on the synthetic substances N,N-diethyl-3-methylbenzamide (DEET), (N,N-butyl-N-acetyl)-aminopropionic acid ethyl ester (IR3535) and 1-(1-methyl-propoxyl)-2-(2-hydroxyethyl)-piperdine (KBR 3023, Bayrepel) [14,15]. These are applied topically to the skin so that ticks will as far as possible quantitatively avoid that skin area and a potentially infectious bite is prevented. Since ticks adhere first of all to the clothing and wander around for hours until they find direct access to the skin, the efficacy of the combined use of the prevention measures mentioned is considerably limited. If they are not noticed as they wander around on the clothing, or are overlooked when searching for them – something that can easily happen in the case of the very small (< 0.5 mm), almost colourless tick larvae (Figure 1) - they will attach themselves to body sites that have not been treated with a repellent. To prevent this, and the considerable infection risk posed by it, clothing was impregnated in different ways with the synthetic, acaricide pyrethroid permethrin as a protection against tick infestation [16,17,18]. However, so far no studies have been carried out on the efficacy of this method against *I. ricinus,* the main vector in Central Europe.

Using a recently developed and innovative application method, permethrin was initially applied to textile fabrics at the factory in a concentration of 1250 mg/m² [18]. In addition to a high level of wash resistance and residual activity, a particularly good efficacy was noted in the laboratory against the main tick vector, *I. ricinus* [19]. The latest toxicological tests carried out within the framework of a biomonitoring study attest to the user safety of the impregnation method thanks to a polymer coating, where in an unwashed state in a worst-case scenario a maximum of 20 % of the "tolerable daily quantity" of permethrin was absorbed mainly transdermally [20].

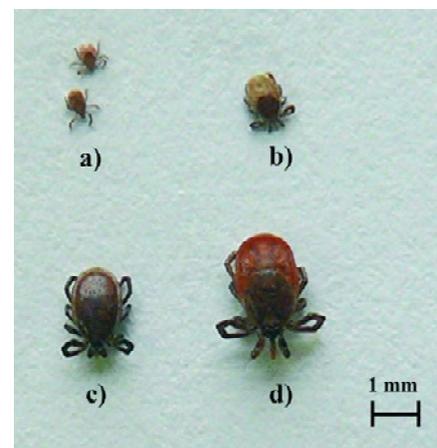*Figure 1: Gender dimorphism and size comparison of the different stages of the castor bean tick, Ixodes ricinus: a) 6-legged larva; b) nymph; c) adult male; d) adult female.*

The aim of the study was to investigate and quantify under field conditions, first, the infestation rate of *I. ricinus* ticks and, as such, the infection pressure associated with tickborne diseases using Lyme borreliosis by way of example and, second, to investigate the protection factor afforded by factory-based, permethrin-impregnation of clothing using a polymer coating method as a novel protection measure against tick infestation – and hence against tick bite.

Materials and Methods

Permethrin Impregnation of Textiles
Factory-based, permethrin-impregnated clothing was manufactured by UTEXBEL S.A. (Ronse, Belgium). Permethrin was applied at a final concentration of 1300 mg permethrin/m^2 (cis:trans=25:75) using a polymer technique [18,20]. The standard trouser fabric used was made of a mixture of 80 % cotton and 20 % polyester fibres with a specific weight of 300 g/m^2. Non-impregnated trouser fabric was used as a negative control.

Tick Trapping and Exposure
Tick trapping was carried out between June and October 2006 in known tick habitats in the leisure area of Kühkopf near Koblenz (7°33'00-10" E; 50°18'30-50" N; 310 meters above sea level). This region is outside the regions known to be endemic for tickborne meningoencephalitis. The tick habitat is characterised by what is known as a "mesotrophic sylvatic Luzulo-Fagion biotop" [21], comprising beech forests with occasional oak trees as well as lower level vegetation, primarily with hairy woodrush (Luzula pilosa) and raspberries (Rubus idaeus).

During tick trapping one female and five male volunteers wore a yellow suit offering complete protection against tick bite (KleenguardIPP, Kimberley-Clark GmbH, Mülheim-Kärlich, Germany) as well as leather boots. The trousers were tucked into the boots and sealed with a rubber band. Occupational medicine investigations into activities posing a risk of infection (G 42) were conducted in parallel [22]. Impregnated and non-impregnated fabric was affixed to the protective suit with rubber bands on the legs (upper and lower leg) (Figure 2a). At 5-minute intervals ticks found on the legs were collected,

documented and immediately transferred to 80 % ethanol. The total maximum time spent trapping ticks in a habitat did not exceed 1.5 hours. The speed of progress was deliberately kept slow and was 400 m per hour (without "collection breaks"). Parallel to tick trapping on the legs of exposed volunteers, ticks were collected in the habitat by means of "dragging" (Figure 2b). To that effect, a 1 m^2 sized, white cotton cloth that had not been impregnated with biocide and was secured to a stick was dragged across the vegetation. Ticks adhering to this were collected every 5 min, documented and transferred to 80 % ethanol. At the same time, ticks adhering to the non-impregnated fabrics on both legs were collected in a similar manner. The relative tick infestation rate (rT), as a comparative value between Dragging tick infestation pressure versus Human tick infestation pressure was defined as:

$$rZ = \frac{\text{Number of ticks from dragging per 100 } m^2}{\text{Number of ticks per person per 100 m}}$$

Tick trapping was conducted only on dry days at temperatures above 19 °C and relative ambient humidity of over 30 %. The temperature and relative humidity were measured using a thermohygrometer on site (H 270, Orglmeister, Walluf, Germany). The monthly average temperatures during the study period was: 17.1 °C in June, 21.5 °C in July, 14.8 °C in August, 17.4 °C in September, and 12.4 °C in October.

Borrelia burgdorferi s.l.-Detection in Ticks
Each tick was mechanically homogenised (MagNA Lyser, Roche Diagnostics Co., Mannheim, Germany). Following that, DNA was isolated using a High Pure PCR Template Preparation Kit (Roche Diagnostics Co., Mannheim, Germany) and investigated for *B. burgdorferi* s.l.

Specific *B. burgdorferi* s.l. PCR was carried out using a Lightcycler 2.0 (Roche Diagnostics Co., Mannheim, Germany) and the primer and probe systems presented by Rauter et al. [23] to detect the specific *OspA* gene of the *Borrelia* genospecies *B. burgdorferi* s.s., *B. garinii* and *B. afzelii*. Primer and probe synthesis was carried out by TibMolBiol Co. (Berlin, Germany). Positive *Borrelia* controls as well as *Borre-*

lia DNA-free, internal λ-DNA-amplification control (LightCycler Fast Start DNA Master HybProbe, Roche Diagnostics Co.) was included for each test. All *Borrelia*-positive and unclear results were confirmed using a subsequent second LC-PCR (RealArt Borrelia LC PCR Kit, Artus GmbH, Hamburg, Germany).

Statistical Calculations
All values were given as means ± standard deviation. Differences in the mean *B. burgdorferi* s.l. infection rates among tick genders and the different tick stages were investigated using Pearson's χ2 test in respect of a residual error probability to a 5 % level (statistical significance level) (SPSS 8.0 program, SPSS Software GmbH, Munich, Germany).

Results

Following a total exposure of 36 h in the tick habitat, 132 hard ticks were collected on the non-treated fabric, all belonging to the species *I. ricinus*. Of these 6 (4.5 %) were adult males 16 (12.1 %) adult females, 64 (48.5 %) nymphs and 46 (34.9 %) larvae. The total average tick infestation rate per person and hour was 7.4 ± 5.5 showing the following gender and stage-specific distribution: 0.32 ± 0.37 adult males; 1.1 ± 1.22 adult females; 3.6 ± 4.4 nymphs and 2.45 ± 3.5 larvae. Calculated on the basis of 100 m migration distance in the tick habitat, this translated into a total average infestation rate per person of 1.82 ± 1.3 ticks with the following gender and stage-specific distribution: adult males: 0.08 ± 0.09; adult females: 0.25 ± 0.3 ; nymphs 0.9 ± 1.1; larvae: 0.61 ± 0.87 (Figure 3).

In the control group only six ticks (2 adult females, 4 nymphs, no adult male or larvae) were found during the same period of time and under similar conditions on the permethrin-impregnated clothing. These were characterised by faster movement, apparently due to the excitatory or repellent effect produced by the acaricide. The total protection effect conferred against tick infestation with *I. ricinus* was 95.5 %. Making a distinction between gender and stages, the following protective effects were calculated as percentages: adult males: 100 %; adult females: 88.8 %; nymphs: 94.1 %; larvae: 100 % (Figure 4).

Figure 2: Tick trapping on a) impregnated and non-impregnated uniform material as well as b) by means of dragging in the tick habitat, in the Kühkopf leisure area, near Koblenz, 2006.

Dragging of ticks which was carried out at the same time yielded an average of 17.6 ± 9.2 ticks per 100 m², with the following gender and stage-specific distribution: adult males: 0.6 ± 1.3; adult females: 1.0 ± 1.1; nymphs: 8.45 ± 3.8; larvae: 7.6 ± 7.3 (Figure 3). Compared with the human infestation rate, an average of 19.4 ± 16.2 times higher infestation rates were found from dragging over the ground / vegetation. The mean rT was 19.4 with the following gender and stage-specific distribution: adult males: 15.2; adult females: 8.3; nymphs: 18.9; larvae: 25.0. Hence human "infestation efficiency" was markedly higher for adult female ticks found in the habitat.

From the ticks collected from the non-impregnated fabric worn by exposed volunteers, *B. burgdorferi* s.l. infection was detected in 33.3 % (2/6) adult males,

56.25 % (9/16) adult females, 9.4 % (6/64) nymphs and 0 % (0/46) larvae (Figure 5). The mean *B. burgdorferi* s.l.-prevalence was 12.9 %. With a mean positivity rate of 10.2 % for the ticks collected by dragging, *B. burgdorderi* s.l. was detected in 17.8 % (5/28) adult males, 25 % (11/44) of adult females, 8.9 % (10/112) of nymphs and 2.9 % (3/102) of larvae (Figure 5). Accordingly, *B. burgdorferi* s.l. prevalence rates for ticks collected from humans versus ticks collected through dragging was statistically significantly higher in female adults (x^2 = 5.2; df = 1; P < 0.023), but not in adult males (x^2 = 0.7; df = 1; P < 0.395), nymphs (x^2 = 0.14; df = 1; P < 0.71) or larvae (x^2 = 1.4; df = 1; P < 0.24). The mean exposure risk was (0.99) *B. burgdorferi* s.l.-infected *I. ricinus* tick per person per hour or 0.25 per person per

100 m. All six of the ticks collected from the permethrin-impregnated clothing were *B. burgdorferi* s.l. negative.

Discussion

On comparing the insecticidal and acaricidal efficacy of permethrin-impregnated fabrics against silver fishes (*Lepisma saccharina*), yellow fever mosquitoes (*Aedes aegypti*) and the castor bean tick (*Ixodes ricinus* nymphs), a particularly high level of activity was identified in laboratory tests against hard ticks [18]. Theoretically, this permits the conclusion that permethrin-treated fabrics can provide good protection against tickborne diseases, especially against Lyme borreliosis and tickborne meningoencephalitis [19,24]. On using an application concentration of 1300 mg permethrin / m² , it was possible to achieve an excellent protective effect against the hard tick *Amblyomma americanum* in North America [16] and in France against the hard tick genus *Dermacentor* [18], which is commonly encountered there. Our own results obtained on using a novel polymerisation method of application confirmed a protective effect of over 95 % against infestation with *I. ricinus*, which is by far the most common and most important tick vector in Central Europe. To date it is unknown, and has not been studied so far, to what extent this protection rate is directly related to the number of prevented tickborne diseases or whether, for example due to irreversible toxicological damage to the ticks following exposure to the acaricide, this protection rate should be set at a higher level. In that respect it must be pointed out that the very small (< 0.5 mm), relatively common, 6-legged tick larvae, which are of an inconspicuous colour and are difficult to notice even when attached to the human body may be *B. burgdorferi* s.l. positive due to transovarial transmission (vertical transmission from infected mother tick to the egg). While the role of tick larvae in transmitting pathogens has been widely dismissed as being negligible, this transmission channel could at least explain why there was no sign of tick bite in more than 50 % of Lyme borreliosis patients [11].

Of particular interest in terms of epidemiology and infection biology is the fact that adult female *I. ricinus* ticks infected
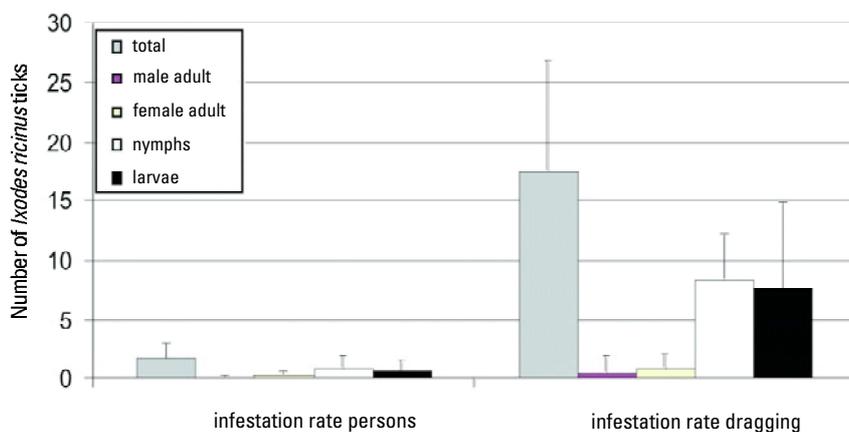

Figure 3: Total infestation rate per person per 100m vs dragging per 100m with the castor bean tick, Ixodes ricinus, in the Kühkopf leisure area, near Koblenz, 2006

with *B. burgdorferi* s.l. are found statistically significantly more often on humans than through simultaneously conducted dragging. This could mean, on the one hand, that the regional *B. burgdorferi* s.l. prevalence rates identified in ticks through dragging cannot be directly extrapolated to the infection pressure on humans, but rather the actual infection pressure on exposure in the tick habitat is higher. On the other hand, this finding would suggest that *B. burgdorferi* s.l. infection in *I. ricinus* ticks initiates one or several changes in behaviours in the reproductive active adult females such that the probability of finding a host or host finding efficiency – and, accordingly, the associated transmission of *Borrelia* to a new host is markedly in-

creased. In principle, based on the knowledge gleaned hitherto, *I. ricinus* is active only when the relative humidity in the microhabitat is more than 80 % [6]. However, Alekseev and Dubinina [25] have noted that *I. persulcatus*, which is closely related to *I. ricinus* and principle vector of Lyme borreliosis in Eastern Europe and Asia, was statistically significantly more resistant to low ambient humidity following infection with *B. burgdorferi* s.l. that its non-infected counterparts. Furthermore, *Borrelia* infection in this tick species can give rise to anomalies in the exoskeleton and, in turn, to a 1.3-faster movement. To what extent similar changes are induced by *B. burgdorferi* s.l. in *I. ricinus*, and what implications this

might have for host finding efficiency, has not been studied to date.

While the human infestation rates with *I. ricinus* cannot be directly compared per distance per time unit with the tick population density collected through dragging per surface area per time unit, conclusions on the magnitude involved can be inferred from these data. With a population density of 17.6 ± 9.2 ticks per $100 \ m^2$, an average quantitative exposure of 0.25 *Borrelia*-infected ticks per person per 100 m was observed per migration distance in the infestation region. In Central Europe tick densities of over 100 per $100 \ m^2$ have been reported following dragging. The highest densities were reported for the Munster and Guebwiller region in Elsass, France, with 105 and 114 ticks per $100 \ m^2$ [26]. Accordingly, the extrapolated quantitative exposure risk for the same *Borrelia* prevalence and population density would be more than 6-fold higher. The average *Borrelia* prevalence in Elsass was 19.3 % for the *I. ricinus* ticks studied, but was markedly higher than our values so that in this tick focus an overall 10- to 12-fold higher exposure (corresponding to 2.5 to 3 *Borrelia*-infected ticks per person per 100 m migration distance) can be mathematically inferred.

Bearing in mind that, of the infectious diseases spread by endemic ticks, only meningoencephalitis can be prevented by vaccine, personal protective measures against tick infestation and tick bite are of paramount importance. At present, repellents applied to the exposed skin areas are the most commonly used method to protect humans against ticks.

Noteworthy here is the behaviour evinced by *Ixodes*, which while on the lookout for a suitable attachment site on the body can often wander around for hours on the clothing and, in general, can avoid the skin areas treated with a repellent. It is therefore very important to inspect the body for ticks when clothing has not been treated with an acaricide. A novel, highly effective approach to tick prevention is the impregnation of clothing with permethrin, which is seen to be an effective acaricide. Permethrin impregnation of clothing is also possible using spray or dip applications of commercially available products as well as, recently, factory-based impregnation using a polymer coating method. While the
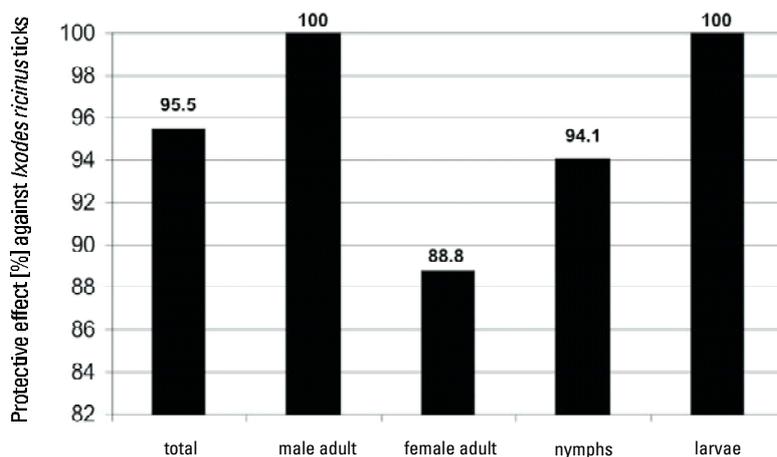

Figure 4: Protective effect, as percentage, of factory-based permethrin-impregnated clothing against infestation with various stages and male/female genders of the castor bean tick, Ixodes ricinus, in the Kühkopf leisure area, near Koblenz, 2006.
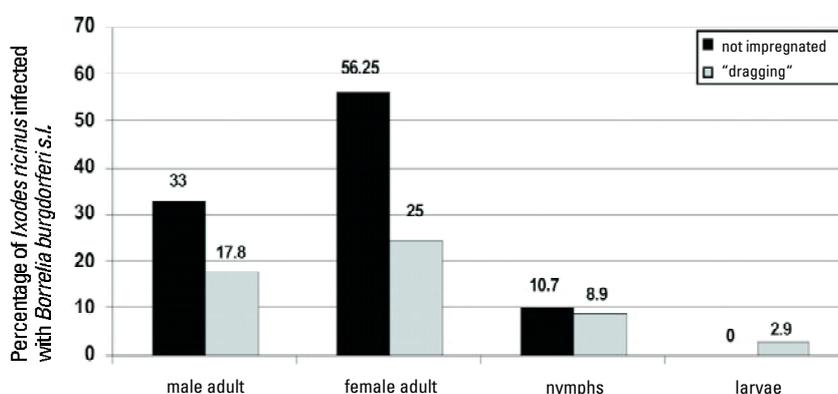

Figure 5: Percentage of various stages and male/female genders of the castor bean tick, Ixodes ricinus infected with Borrelia burgdorferi s.l. on non-impregnated human clothing as well as on the dragging cloth in the Kühkopf leisure area, near Koblenz, 2006.

spray and dip method can pose a high risk of contamination to users, in particular if not correctly applied, this problem does not occur for factory-treated clothing [19,20]. Unlike the polymerisation technique, completely homogeneous perm e-thrin coating is not possible with the spray or dip method, there is a marked high risk of cross-contamination when laundered in a machine with other clothing and, furthermore, depending on the product re-impregnation is needed after 6 to 50 washes [27]. Thanks to the "depot effect" mediated by incorporation of the active substance into the polymer coating, adequate bioactivity against vectors can be guaranteed in at laboratory level even after 100 washes as well as under military combat conditions. Following biomonitoring tests among users of unwashed clothing initially impregnated with 1300 mg/m^2 permethrin, metabolites at a concentration of 5–6 mg permethrin/kg body weight and day was quantified in urine [20]. Based on an oral permethrin-absorption rate of 50 % this would correspond to a total recalculated intake quantity of around 20 % permethrin of the statutorily stipulated tolerable daily intake (TDI) of 50 mg permethrin/kg body weight and day [20]. From the subsequent toxicological evaluation is concluded that when used properly, clothing impregnated by means of the polymerisation technique is not expected to give rise to any clinically relevant side effects in humans [20].

Factory-based permethrin impregnation using a polymer coating method represents a novel and toxicologically safe protection method against ticks and other biting vector arthropods since it helps to effectively prevent vectorborne diseases. This can be used in conjunction with skin repellents on an individual basis, especially by occupationally exposed persons such as forestry workers or soldiers, but also by persons exposed to ticks during leisure periods as well as visitors to tropical countries.

Conflict of Interest

The authors declare that there is no conflict of interest as understood by the International Committee of Medical Journal Editors.

References

1. Faulde M, Hoffmann G: Vorkommen und Verhütung vektorassoziierter Erkrankungen des Menschen in Deutschland unter Berücksichtigung zoonotischer Aspekte. Bundesgesundheitsbl - Gesundheitsforsch - Gesundheitsschutz 2001; 44: 116–136.

2. Süss J, Schrader C: Durch Zecken übertragene humanpathogene und bisher als apathogen geltende Mikroorganismen in Europa. Teil I: Zecken und Viren. Bundesgesundheitsbl - Gesundheitsforsch - Gesundheitsschutz 2004; 47: 392–404.

3. Süss J, Fingerle V, Hunfeld K-P, Schrader C, Wilske B: Durch Zecken übertragene humanpathogene und bisher als apathogen geltende Mikroorganismen in Europa. Teil II: Bakterien, Parasiten und Mischinfektionen. Bundesgesundheitsbl - Gesundheitsforsch - Gesundheitsschutz 2004; 47: 470–486.

4. Häselbarth K, Tenter AM, Brade V, Krieger G, Hunfeld KP: First case of human babesiosis in Germany – Clinical presentation and molecular characterization of the pathogen. Int J Med Microbiol 2007; 297: 197–204.

5. Hildebrandt A, Hunfeld KP, Baier M, Krumbholz A, Sachse S, Lorenzen T, Kiehntopf M, Fricke HJ, Straube E: First confirmed autochthonous case of human *Babesia microti* infection in Europe. Eur J Clin Microbiol Infect Dis 2007; 26: 595–601.

6. Gern L, Humair P-F: Ecology of *Borrelia burgdorferi sensu lato* in Europe. In: Gray, J.S., Kahl, O., Lane, R.S., Stanek, G. (Eds). Lyme borreliosis – biology, epidemiology and control. CABI international; 2002: 149–174.

7. Ginsberg HS, Faulde MK: Ticks. In: Urban Pests and Health. WHO, European Centre for Environment and Health, Bonn Office, Bonn, Germany, (im Druck).

8. Wagner B: Borreliose und FSME: Gefahr durch Zeckenstiche! Der Hausarzt 1999; 8: 34.

9. Siegmund-Schultze N: Zweifelhafte Borreliose-Tests. Deutsches Ärzteblatt 2007; 104: 1891–1893.

10. Talaska T: Zur Lyme-Borreliose im Land Brandenburg. Epidemiologisches Bulletin 2005; 20/2005: 173–178.

11. Robert Koch-Institut, Lyme-Borreliose: Zur Situation in den östlichen Bundesländern. Analyse der Meldedaten aus den östlichen Bundesländern. Epidemiologisches Bulletin 2007; 38/2007: 351–355.

12. Talaska T, Faulde M, Dalitz B: Ein möglicher Vektor – Auwaldzecke – *Dermacentor reticulatus*. Epidemiologisches Bulletin 2007, Nr. 14/2007: 117–118.

13. Robert Koch-Institut, FSME: Risikogebiete in Deutschland. Epidemiologisches Bulletin 2007; Nr. 15/2007: 129–137.

14. Faulde M: Repellentien. In: Therapeutische Verfahren in der Dermatologie (Korting HC, Sterry W, Eds.) 2001: 727–741. Blackwell Wissenschafts-Verlag Berlin, Germany.

15. Faulde M: Insektizide, Akarizide und Repellentien. In: Krankheiten durch Arthropoden. (Aspöck H, Ed): (im Druck).

16. Schreck CE, Mount GA, Carlson DA: Pressurized sprays of permethrin on clothing for personal protection against the lone star tick (Acari: Ixodidae). J Econ Entomol 1982; 75: 1059–1061.

17. Ho-Pun-Cheung T, Lamarque D, Josse R, Perez-Eid C, Niel L, Martenot G, Auzanneau G, Rey JL: Effet protecteur de vêtements imprégnés de perméthrine vis-à-vis de D. reticulatus et D. marginatus dans un biotope ouvert du centre-ouest de la France. Bull Soc Pathol Exot 1999; 92: 337–340.

18. Faulde MK, Uedelhoven WM, Robbins RG: Contact toxicity and residual activity of different permethrin-based fabric impregnation methods for *Aedes aegypti* (Diptera: Culicidae), *Ixodes ricinus* (Acari: Ixodidae), and *Lepisma saccharina* (Thysanura: Lepismatidae). J Med Entomol 2003; 40: 935–941.

19. Faulde M, Uedelhoven W: A new clothing impregnation method for personal protection against ticks and biting insects. Int J Med Microbiol 2006; 296 Suppl. 1: 225–229.

20. Appel KE, Gundert-Remy U, Fischer H, Faulde M, Mross KG, Letzel S, Rossbach B: Risk Assessment of Bundeswehr (German Federal Armed Forces) Permethrin-impregnated battle dress uniforms (BDU). Int J Hyg Envir Heal 2008; 211: 88–104.

21. Rothmaler W: Exkursionsflora. Vol. 2. Volk und Wissen Volkseigener Verlag, Berlin, 1984: 44–54.

22. Lieboldt T: Anwendung der Biostoffverordnung bei Borrelienexposition: Auswirkungen auf den Dienstbetrieb der Bundeswehr. Wehrmed Wehrpharm 2007; 31: 66–69.

23. Rauter C, Oehme R, Diterich I, Engele M, Hartung T: Distribution of clinically relevant *Borrelia* genospecies in ticks assessed by a novel, single-run, Real-Time PCR. J Clin Microbiol 2002; 40: 36–43.

24. Mencke N: Acaricidal and repellent properties of permethrin, its role in reducing transmission of vector-borne pathogens. Parasitologia 2006; 48: 130–140

25. Alekseev AN, Dubinina HV: Abiotic parameters and diel and seasonal activity of *Borrelia*-infected and uninfected *Ixodes persulcatus* (Acarina: Ixodidae). J Med Entomol 2000; 37: 9–15.

26. Ferquel E, Garnier M, Marie J, Bernede-Bauduin C, Baranton G, Perez-Eid C, Postic D: Prevalence of *Borrelia burgdorferi* sensu lato and Anaplasmataceae member in *Ixodes ricinus* ticks in Alsace, a focus of Lyme borreliosis endemicity in France. Appl Environ Microbiol 2006; 72: 3074–3078.

27. Faulde MK, Uedelhoven WM, Malerius M, Robbins RG: Factory-based permethrin impregnation of uniforms: Residual activity against *Aedes aegypti* and *Ixodes ricinus* in battle dress uniforms worn under field conditions, and cross contamination during the laundering and storage process. Mil Med 2006; 171: 472–477.