Tick Bite Protection With Permethrin-Treated Summer-Weight Clothing

NATHAN J. MILLER,[1] ERIN E. RAINONE,[1] MEGAN C. DYER,[1] M. LILIANA GONZÁLEZ,[1,2] AND THOMAS N. MATHER[1,3]

J. Med. Entomol. 48(2): 327–333 (2011); DOI: 10.1603/ME10158

ABSTRACT The number of tick bites received by individuals wearing either permethrin-treated or untreated summer clothing (T-shirt, shorts, socks, and sneakers) was compared during a controlled indoor study. Pathogen-free nymphal *Ixodes scapularis* Say were placed on the left shoe, right leg, and left arm of 15 (5/treatment group/d) human volunteers wearing untreated outfits or outfits treated with permethrin either commercially or using a do-at-home treatment kit. The number and location of ticks attached to subjects' skin were recorded 2.5 h postinfestation. Subjects wearing outfits treated with permethrin received 3.36 times fewer tick bites than subjects wearing untreated outfits. No statistically significant differences in number of tick bites were detected between commercial permethrin treatment (19.33%) and the do-at-home permethrin application method (24.67%). The success of permethrin-treated clothing in reducing tick bites varied depending on the specific article of clothing. Subjects wearing permethrin-treated sneakers and socks were 73.6 times less likely to have a tick bite than subjects wearing untreated footware. Subjects wearing permethrin-treated shorts and T-shirts were 4.74 and 2.17 times, respectively, less likely to receive a tick bite in areas related to those specific garments than subjects wearing untreated shorts and T-shirts. Ticks attached to subjects were classified as alive or dead before removal. On subjects wearing untreated outfits, 97.6% of attached nymphs were alive, whereas significantly fewer (22.6%) attached nymphs were alive on subjects wearing repellent-treated outfits. Results of this study demonstrate the potential of permethrin-treated summer clothing for significantly reducing tick bites and tick-borne pathogen transmission.

KEY WORDS *Ixodes scapularis*, tick bite protection, permethrin, tick repellent clothing

Arthropod-borne infections significantly impact human populations, from worldwide epidemics of mosquito-transmitted malaria, yellow fever, dengue fever, and filariasis to more regional disease foci of Lyme borreliosis vectored by ixodid ticks. Significant resources, including funding, research, and human efforts, are dedicated to eradicating, or at least reducing, the impact of these infections. Extensive global and local programs instituted by agencies such as the World Health Organization and Centers for Disease Control and Prevention have broad-reaching integrated pest management approaches focusing on education, vector habitat reduction, indoor residual spraying, as well as significant public health regulatory and legislative frameworks (WHO 2004). In addition to these vector control strategies, people use a variety of techniques to protect themselves from disease-causing vectors; and these methods vary significantly depending on geographic location and specific arthropod pest. Among these personal protection strategies, insect repellents represent one of the first lines of defense against attacks by blood-feeding arthropods and the diseases they can transmit. The most desirable repellents are ones that are less toxic to humans, effective against a broad spectrum of arthropods, long lasting, and cost effective.

The most common repellents applied to skin contain the active ingredient DEET. DEET, along with active ingredients such as Picaridin and other novel compounds, have proven successful against mosquitoes. However, the effectiveness and duration of these compounds to repel ticks are variable, depending on formulation and method of testing (Jensenius et al. 2005, Carroll et al. 2008, Salafsky et al. 2000, Solberg et al. 1995). An alternative personal-use repellent strategy, available for decades, is to apply repellent or toxicant products to clothing or other fabrics rather than directly on the skin (Lane and Anderson 1984). This method has many positive features, including that chemicals are not applied to skin, potentially reducing chemical exposure as well as increased duration of efficacy lasting through multiple launderings. Synthetic pyrethroids (permethrin, in particular) are most commonly applied to fabrics such as bed nets and clothing because of their margin of safety, effective-

[1] Center for Vector-Borne Disease, University of Rhode Island, 9 East Alumni Avenue, Suite 7, Kingston, RI 02881.
[2] Department of Computer Science and Statistics, University of Rhode Island, Tyler Hall, Kingston, RI 02881.
[3] Corresponding author: Center for Vector-Borne Disease, University of Rhode Island, 9 East Alumni Avenue, Suite 7, Kingston, RI 02881 (e-mail: tmather@uri.edu).

ness to repel and kill a wide array of arthropods, and their durability once applied (Schreck et al. 1986, Evans et al. 1990, Brown and Hebert 1997, Faulde and Uedelhoven 2006, Faulde et al. 2006, Appel et al. 2008).

One commonly perceived limitation to clothing-only repellents, especially for tick bite protection, is the belief that treatment will only be effective if users wear long pants, long sleeve shirts, and boots, such as military-issued battle dress uniforms (our unpublished observations). Most people choose not to dress in such a manner during spring and summer periods when tick activity generally is greatest (Nicholson and Mather 1996, Piesman et al. 1987b), seemingly precluding efficient tick bite protection using clothing-only repellents. To date, there have been no studies that assess the potential efficacy of using permethrin-based clothing-only repellents for preventing tick bites when individuals are wearing casual summer clothing, including sneakers, socks, shorts, and T-shirt. Accordingly, we conducted a small clinical trial to evaluate nymphal *Ixodes scapularis* biting success on people wearing either commercially treated or self-applied permethrin-repellent summer clothing and compared that with people wearing clothing with no repellent treatment. In particular, study participants were infested with 30 pathogen-free nymphal ticks and were examined for the number of ticks biting 2 h later.

Materials and Methods

Pathogen-Free Ticks. Pathogen-free nymphal *I. scapularis* used in this study were laboratory-reared, F_1 generation. Rhode Island field-collected adult *I. scapularis* were blood fed on New Zealand white rabbits, and gravid females were held individually in three dram vials (>90% RH; 23°C; 14:10 light:dark cycle) until oviposition. Resulting larvae from a single egg mass were blood fed on pathogen-free Syrian golden hamsters, and replete larvae were stored in three dram vials (>90% RH; 23°C; 14:10 light:dark cycle) until molting to the nymphal stage. Nymphal ticks were 5–6 mo postmolt when used for this study. All procedures involving animals, including euthanasia, were performed under the guidelines of the University of Rhode Island's Institutional Animal Care and Use Committee.

As a result of previously documented potential transovarial pathogen transmission of *Borrelia miyamotoi,* a subsample of the tick cohort used in this study was screened for infection by polymerase chain reaction (PCR) using methods previously described (Scoles et al. 2001). Briefly, total DNA was extracted from ticks using Isoquick nucleic acid extraction kit (Orca Research, Bothell, WA). Fifty nymphal ticks were crushed in separate pools of five in 1.5-ml microcentrifuge tubes containing 100 μl of sample buffer provided in the extraction kit. Remaining procedures were performed following manufacturer's instructions. Rehydrated, total DNA was stored at 4°C until assaying. Target DNA amplification was performed by adding 2.5 μl of DNA sample to a 25 μl reaction mixture containing 12.5 μl of 2× PCR Master Mix (Promega, Madison, WI) consisting of *Taq* polymerase, dNTPs, and $MgCl_2$ buffer. Primers FLA181 F (5′-CCA GCA TCA TTA GCT GGA A-3′) and FLA400R (5′-CAC CTT GAA CTG GAG CGG CT-3′) were added to each sample. Amplification was performed in a MJ Research (Waltham, MA) thermocycler under the following conditions: initial 5-min 94°C denaturizing step, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 s, and elongation at 72°C for 30 s. The amplification cycles were followed by a final elongation at 72°C for 5 min. PCR samples, including positive (*B. miyamotoi* DNA) and negative (water) controls, were held at 4°C, and then 5 μl of each sample was visualized on an ethidium bromide-stained 2% agarose gel.

Human Subjects. Fifteen volunteers were recruited from the University of Rhode Island community through an e-mail blast to faculty, staff, and students. From eligible respondents to the solicitation, eight females and seven males, ranging in age from 18 to 50, were selected randomly for inclusion. All subjects were fully informed of their involvement in this research project. Study procedures, including recruitment, were performed according to the University of Rhode Island's Institutional Review Board guidelines (HU0607-150; approved 1 May 2007). Subjects were compensated for their participation at the completion of the study.

Treatment Groups and Treated Clothing. Each subject was provided summer-weight clothing, which included cotton shorts, T-shirt, and socks, as well as low-top canvas sneakers. All outfits were light colored and purchased to fit each subject. Shorts, T-shirts, and socks either remained untreated or were treated with permethrin in one of the following two ways. 1) Do-at-home treatment kit (soaking): permethrin treatment was performed by the research team 2 d before initiating the study using Sawyer Military Style Clothing Treatment kit (Sawyer Products, Safety Harbor, FL) following manufacturer's instructions. 2) Commercially treated: clothing was treated with permethrin by Insect Shield, LLC (Greensboro, NC), using their proprietary impregnation method. All sneakers (provided by Converse, North Reading, MA) worn by subjects wearing either do-at-home or commercially treated clothing were sprayed similarly by the research team using a 0.5% permethrin aerosol spray (Sawyer Products) and allowed to air dry 1 d before the study. Sneakers worn by subjects in the control group remained untreated. Each treated and untreated outfit, including sneakers, were kept in individual, sealed plastic bags to avoid cross contamination. All subjects were exposed to a tick challenge each day of the 2-d trial. On the first day of the trial, each treatment group (untreated, do-at-home, or commercial treatment) contained five randomly assigned subjects. On the second day, subjects were logically assigned to a different treatment group so that no subject received the same treatment 2 d in a row. New sets of treated and untreated clothing were provided to each subject on day 2.

Table 1. Percentage of nymphal *Ixodes scapularis* ticks attaching to a specific body region (related to where ticks were applied) on subjects wearing permethrin-treated or untreated clothing

Treatment	No. subjects	No. ticks applied	% attached ticks		
			Shoes (n = 100)	Shorts (n = 100)	T-shirt (n = 100)
At-home treatment	10	300	1	17	56
Commercial treatment	10	300	0	24	34
Untreated	10	300	27	55	64

Ten ticks were applied to each of three separate locations (shoes, leg, and arm) on each subject.

Tick Challenge. Thirty ticks were placed on each subject during each day of the trial. On day 1, 10 ticks were placed on each of three body locations, as follows: 1) over the laces of the left shoe; 2) on the right leg above the right knee; and 3) on the left arm just above the elbow. This arrangement allowed us to distinguish the likely tick origination site when examining subjects for any attached ticks. Subjects sat in a chair placed on top of a 1-m^2 white flannel sheet. Ten ticks were counted out onto a 4-cm-diameter cotton cosmetic pad that was then loosely applied to each respective infestation location by a piece of tape. This tick application procedure was repeated until each subject had one cotton pad attached to each of the three body locations. After 15 min, cotton pads were removed; if any ticks remained on the cotton pad, they were placed on the individual at the respective location. Ten minutes after cotton pads were removed, subjects were asked to perform a series of activities, including the following: standing, sitting, walking in place, bending, and stretching for ≈5 min. These same activities were repeated at 20-min intervals for the next 2 h.

Subjects and the white flannel sheets under their chairs were closely monitored by a team of observers throughout the tick challenge. Ticks that fell off each subject while sitting or during the activity regimen were collected, held in an individual vial, and classified as unattached. At the end of the activity regimen, each subject was examined by a member of the research team and the exact location on the body of any attached ticks was recorded. Attached ticks were carefully removed using fine-pointed tweezers and stored in a separate labeled vial. The tick challenge was performed similarly on the same subjects (with subjects in their new treatment group) on the next day. One exception was made based on anecdotal evidence from day 1 that most of the ticks attached to subjects wearing treated clothes appeared dead. Thus, each attached tick was categorized as alive or dead before removing ticks from subjects during the second day of the trial. This decision was made by trained observers looking for tick leg movement with the aid of a magnifying glass.

Statistics. Data were analyzed using SAS, version 9.2. All analyzed variables were categorical, and the dependent variables were always dichotomous (e.g., ticks attached, nonattached; alive, dead). Under a logistic regression framework, we used the likelihood ratio χ^2 statistic to test associations of categorical variables with the dichotomous dependent variable. Additionally, odds ratios and confidence intervals for odds ratios were found using PROG LOGISTIC in SAS, and confidence intervals for differences in proportions were obtained by using PROC FREQ. Odds ratio confidence intervals were nonsignificant if they contained the number 1, whereas differences in proportions were nonsignificant if zero was contained in their confidence interval.

Results

Subjects wearing treated summer-weight outfits (sneakers, socks, shorts, T-shirt) were 3.36 times (odds ratio = 3.36 with a 95% confidence interval (CI) [2.499, 4.526]) less likely to have nymphal *I. scapularis* attach to their body than subjects wearing untreated clothing. Both clothing impregnation methods showed significant protective benefits when compared with the untreated group (likelihood ratio = 64.8117, df = 1, $P < 0.0001$). Subjects wearing commercially treated clothing experienced fewer tick attachments (58 of 300, 19.33%) than did the do-at-home treatment group (74 of 300, 24.67%), but this difference was not significant (CI for difference [−0.1195, 0.0128], Fisher exact test $P = 0.1391$), and tick attachment results for subjects wearing both types of treated outfits were combined.

When considering the tick bite protection effectiveness of each article of permethrin-treated clothing (shoes/socks, shorts, T-shirts), subjects wearing permethrin-treated shoes and socks experienced significantly fewer tick attachments (1 of 200, 0.5%) than subjects wearing untreated shoes and socks (27 of 100, 27%) (estimated difference between population proportions = 26.5%, 95% CI [17.74%, 35.26%], Fisher exact test $P < 0.0001$) (Table 1). The odds of nymphal attachment, below the waist on the leg where ticks were applied to shoes, were 74 times less (odds ratio = 73.60, 95% CI [2.4, 551.45]) for the permethrin-treated group than the untreated group (see Fig. 1). Similarly, nymphal tick attachment to subjects wearing permethrin-treated shorts (41 of 200, 20.5%) and T-shirts (90 of 200, 45%) was significantly less when compared with subjects wearing untreated shorts (55 of 100, 55%) and T-shirts (64 of 100, 64%), respectively. The estimated difference of nymphal tick attachment between permethrin-treated and untreated shorts was 34.5% (95% CI]23.26%, 45.74%], Fisher exact test $P <$ 0.0001), whereas a difference of 19% (95% CI [7.34%, 30.66%], $P = 0.0022$) was observed between subjects wearing permethrin-treated and untreated T-shirts.

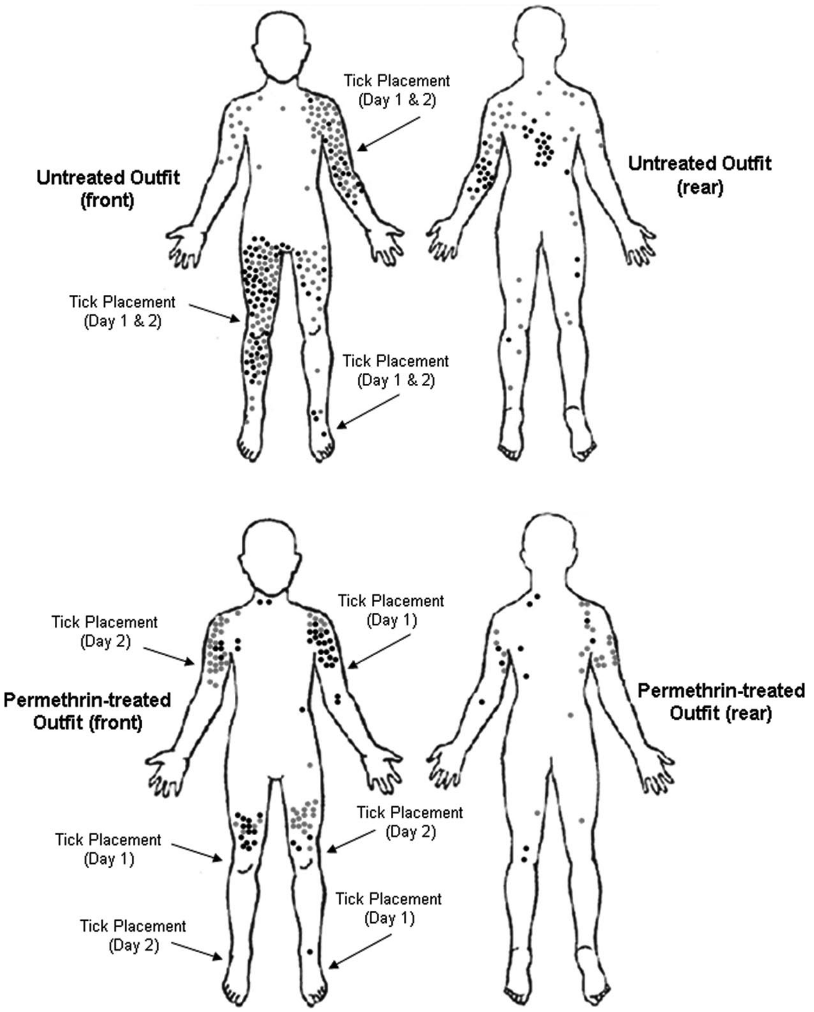

Fig. 1. Number and location of attached nymphal *I. scapularis* ticks on subjects wearing untreated or permethrin-treated summer-weight clothing. Dots represent attached tick location, as follows: day 1, black dots; day 2, gray dots.

The odds of nymphal tick attachment, below the waist on the same leg to which ticks were applied, were ≈5 times less (odds ratio = 4.74, 95% CI [2.81, 7.99]) for subjects wearing permethrin-treated shorts, and the odds of ticks attaching to the arm and upper body in relation to where ticks were applied to the arm were ≈2 times less (odds ratio = 2.17, 95% CI [1.3, 3.56]) for subjects wearing permethrin-treated T-shirts. The majority (90 of 132, 68%) of ticks attached to subjects wearing treated outfits were found on the arms and upper body, whereas 56% (82 of 146) of ticks attached to subjects wearing untreated outfits were found below the waist.

After the 2.5-h test period on the second day of the trial, an attempt was made to distinguish between live and dead attached nymphs as they were removed from subjects (the analysis of this part of the experiment only considers five subjects/treatment [total of 15 subjects] with 30 nymphs applied to each subject). Of the 450 ticks applied to subjects on day 2 of the study, a total of 159 (35%) was found attached to subjects at the end of the test period. From five subjects wearing untreated clothes, 97.6% (82 of 84) of attached nymphs were classified as alive at the time of removal. Only 22.7% (17 of 75) of attached nymphs were classified as alive on the 10 subjects wearing treated outfits (estimated difference of population proportion of 74.95%, 95% CI [64.93, 84.97]). Ticks attached to subjects wearing permethrin-treated clothing are more likely to be found dead than ticks attached to subjects wearing untreated clothing (Fisher exact test $P < 0.0001$).

Comparing tick attachment on all patients, irrespective of treatment, between days 1 and 2 of the trial, we found that significantly more ticks ($n = 159$) attached on day 2 than on day 1 ($n = 119$) (95% CI [−14.90%, −2.88%]). Although there was no statistical difference in the number of ticks attached to patients wearing treated clothing on day 1 ($n = 57$) compared with day 2 ($n = 75$) (95% CI [−12.61%, 0.6%]), there was a significant difference in the number of ticks attached to patients wearing untreated clothing, with fewer ticks attached on day 1 ($n = 62$) than on day 2 ($n = 84$) (95% CI [−25.86%, −3.48%]).

Discussion

In a clinical setting, we demonstrated that wearing permethrin-treated summer clothing and footwear has potential to be an effective strategy for reducing tick bites and transmission of tick-borne infections. Overall, individuals wearing permethrin-treated T-shirt, shorts, socks, and low-top sneakers were nearly 3.5 times less likely to be bitten by nymphal *I. scapularis* than individuals wearing untreated clothing. When we evaluated the treatment effect based on specific articles of clothing, subjects wearing permethrin-treated sneakers were 74 times less likely to have a tick bite than subjects wearing untreated shoes. This observation alone potentially has great public health importance because nymphal *I. scapularis* are principal vectors for the agents causing Lyme disease, human babesiosis, and human anaplasmosis (Spielman et al. 1985, Telford et al. 1996), and they typically quest close to the ground in leaf litter (Ginsberg and Ewing 1989). Because of this, the initial site of nymphal *I. scapularis* encounter for humans is likely to be on lower extremities, especially footwear.

The apparent protection provided by treated shoes (odds ratio = 73.60) was considerably higher than the protection provided by treated shorts (odds ratio = 4.74) and T-shirts (odds ratio = 2.17). Given the strong protective effect observed for treated footwear, we were surprised not to find a higher overall combined level of tick bite protection for subjects wearing treated outfits (sneakers, socks, shorts, T-shirt) compared with untreated outfits. The outcome of a subpopulation of data is not always reflected in the outcome of the whole population when the subpopulations are not directly comparable (Julious and Mullee 1994). That may be true for this experiment; nymphal *I. scapularis* were applied directly to the shoes and received an immediate dose of permethrin, whereas ticks applied to the leg and arm were temporarily shielded by a cotton pad. This 15 min of coverage may have provided some ticks the opportunity to attach before permethrin exposure. This effect can be seen in Fig. 1, in which many of the ticks that did successfully attach to subjects wearing treated clothing were found at or near the tick application site as compared with the distribution pattern of ticks attached to subjects wearing untreated outfits, particularly the ticks that were placed on the shoes. Early, initial trials exposing nymphal *I. scapularis* to permethrin-treated clothing demonstrated rapid tick morbidity leading to mortality, even when exposures were for 10–20 s (our unpublished data). Based on this, finding 132 of 600 ticks attached to subjects wearing permethrin-treated clothing was unexpected and, as discussed above, our method for applying ticks to the subjects may have contributed to this greater than expected level of tick attachment.

This experiment provides evidence supporting the potential of permethrin-treated summer-weight clothing to reduce risk of tick-borne pathogen transmission. Although not checked on the first day, during the second day of the experiment, we observed that the majority of ticks attached to subjects wearing permethrin-treated clothing were dead (58 of 75, 77.3%) 2.5 h after their initial attachment, whereas nearly all ticks (82 of 84, 97.6%) found attached to subjects wearing untreated clothing were alive. Although ticks used in this trial were pathogen free, in another study, *Borrelia burgdorferi*-infected nymphal *I. scapularis* attached for up to 24 h before topical permethrin treatment failed to transmit infection to hamsters (Mather 1994). Transmission of *B. burgdorferi* as well as other tick-transmitted pathogens, such as *Babesia microti*, requires that ticks be attached to hosts for longer than 24 h (Piesman et al. 1987a). Certain tick-borne viruses may be transmitted more rapidly (Ebel and Kramer 2004), and studies would be needed to determine whether the relatively rapid killing of ticks potentially able to attach to a person wearing tick-repellent clothing would also protect them from infection.

The efficacy of permethrin-treated fabrics to repel and kill ticks, mosquitoes, and other biting insects is well documented; however, insect repellents of any kind are not well embraced by the general public because of perceived low effectiveness and safety concerns (Herrington 2004). Permethrin compounds are used extensively for pest control in agricultural, commercial, and residential settings; the Environmental Protection Agency (EPA) estimates that >2 million pounds of active ingredient are used annually (unpublished market data from United States Food and Drug Administration and technical registrants). This widespread use has driven aggressive investigations into permethrin's safety profile. The reregistration eligibility decision for permethrin released in April 2006 (revised December 2007) by the EPA reviewed current and historical studies and reported on the general safety of permethrin as well as specifically addressing the safety of permethrin-treated fabrics (http://www.epa.gov/oppsrrd1/REDs/permethrin_red.pdf). Mean exposure scenarios concerning permethrin-treated fabrics were found to be 24 times below the EPA's level of concern. The main risk that individuals experience wearing either permethrin-treated clothing or other repellents is dermal exposure. Direct application studies of various liquid and cream permethrin formulations indicate that dermal absorption is low. In a clinical study examining the effect of applying a 5% (a.i. permethrin) cream, the typical treatment for scabies mites, little or no dermal reactions occurred (Schultz et al. 1990). In several studies investigating permethrin absorption after dermal application, typically <2% of total active ingredient applied was found in the body (Tomalik-Scharte et al. 2005, Snodgrass 1992). Additionally, permethrin metabolites (*m*-phenoxy-benzyl alcohol and *m*-phenoxybenzoic acid) may be found in low levels, but possess low toxicity levels similar to permethrin and are completely cleared from the body within days of exposure (Anadón et al. 1991). When permethrin is used as a clothing-only repellent, the compound is tightly bound to the fabric as demonstrated by wash off data (Faulde et al. 2006). Thus, exposure to active ingredient would be considerably less than the total amount approved for clothing applications, and this amount is significantly less than levels used in preclinical toxicity studies. Even assuming that all of the permethrin applied to an outfit constituted an exposure level, individuals would be exposed to active ingredient at a rate 38 times below the no observed adverse effect level for dermal exposure to permethrin. In our study, we observed no apparent dermal carryover effect from day 1 to day 2; more ticks successfully attached to patients wearing untreated clothes on day 2 than day 1. All of the patients wearing untreated clothing on day 2 had worn either commercially treated or treat-at-home clothes on day 1 of the study.

Use of permethrin-treated clothing for tick bite protection and disease prevention is not well known or widely used even in areas where tick-borne diseases are highly endemic (Malouin et al. 2003, Gould et al. 2008). Studies such as this one, demonstrating the potential for significant protection against known vector species, should help support people's decision to more regularly use this potentially effective strategy.

Acknowledgments

We thank Insect Shield, LLC, and Sawyer Products for providing repellent clothing and repellent for use in the study, and Converse for donating sneakers. We also thank Nancy Doyle-Moss RN, Karen Frost, Lyndsay Knoblock-Fast, Kristen Motel, and Wendy Coy for their assistance with the study, and the 15 volunteers for their participation. This work was supported in part by USDA-CSREES SRG 2003-34520-13949 and gifts from Insect Shield and the Beech Tree Trust. This is Contribution 5154 of the Rhode Island Agricultural Experiment Station.

References Cited

Anadón, A., M. R. Martinez-Larrañaga, and M. J. Díaz. 1991. Toxicokinetics of permethrin in the rat. Toxicol. Appl. Pharmacol. 110: 1–8.

Appel, K. E., U. Gundert-Remy, H. Fischer, M. Faulde, K. G. Mross, S. Letzel, and B. Rossbach. 2008. Risk assessment of Bundeswehr (German Federal Armed Forces) permethrin-impregnated battle dress uniforms (BDU). Int. J. Hyg. Environ. Health 211: 88–104.

Brown, M., and A. A. Hebert. 1997. Insect repellents: an overview. J. Am. Acad. Dermatol. 36: 243–249.

Carroll, J. F., J. P. Benante, J. A. Klun, C. E. White, M. Debboun, J. M. Pound, and W. Dheranetra. 2008. Twelve-hour duration testing of cream formulations of three repellents against *Amblyomma americanum.* Med. Vet. Entomol. 22: 144–151.

Ebel, G. D., and L. D. Kramer. 2008. Short report: duration of tick attachment required for transmission of Powassan virus by deer ticks. Am. J. Trop. Med. Hyg. 71: 268–271.

Evans, S. R., G. W. Korch, and M. A. Lawson. 1990. Comparative field evaluation of permethrin and DEET treated military uniforms for personal protection against ticks (Acari). J. Med. Entomol. 27: 829–834.

Faulde, M., and W. M. Uedelhoven. 2006. A new clothing impregnation method for personal protection against ticks and biting insects. Int. J. Med. Microbiol. 296 (Suppl. 40): 225–229.

Faulde, M. K., W. M. Uedelhoven, M. Malerius, and R. G. Robbins. 2006. 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. 171: 472–477.

Ginsberg, H. S., and C. P. Ewing. 1989. Comparison of flagging, walking, trapping, and collecting from hosts as sampling methods for northern deer ticks, *Ixodes dammini*, and lone-star ticks, *Amblyomma americanum* (Acari: Ixodidae). Exp. Appl. Acarol. 7: 313–322.

Gould, L. H., R. S. Nelson, K. S. Griffith, E. B. Hayes, J. Piesman, P. S. Mead, and M. L. Cartter. 2008. Knowledge, attitudes, and behaviors regarding Lyme disease prevention among Connecticut residents, 1999–2004. Vector Borne Zoonotic Dis. 8: 769–776.

Herrington, J. E. 2004. Risk perceptions regarding ticks and Lyme disease: a national survey. Am. J. Prev. Med. 26: 135–140.

Jensenius, M., A. M. Pretorius, F. Clarke, and B. Myrvang. 2005. Repellent efficacy of four commercial DEET lotions against *Amblyomma hebraeum* (Acari: Ixodidae), the principal vector of *Rickettsia africae* in southern Africa. Trans. R. Soc. Trop. Med. Hyg. 99: 708–711.

Julious, S. A., and M. A. Mullee. 1994. Confounding and Simpson's paradox. BMJ 309: 1480–1481.

Lane, R. S., and J. R. Anderson. 1984. Efficacy of permethrin as a repellent and toxicant for personal protection against the Pacific Coast tick and the Pajaroello tick (Acari: Ixodidae and Argasidae). J. Med. Entomol. 21: 692–702.

Malouin, R., P. Winch, E. Leontsini, G. Glass, D. Simon, E. B. Hayes, and B. S. Schwartz. 2003. Longitudinal evaluation of an educational intervention for preventing tick bites in an area with endemic Lyme disease in Baltimore County, Maryland. Am. J. Epidemiol. 157: 1039–1051.

Mather, T. N., inventor; Harvard University, Assignee. 1994. Method for the prevention of ectoparasite-mediated pathogen transmission. U.S. patent 5,283,259.

Nicholson, M. C., and T. N. Mather. 1996. Methods for evaluating Lyme disease risks using geographic information systems and geospatial analysis. J. Med. Entomol. 33: 711–720.

Piesman, J., T. N. Mather, R. J. Sinsky, and A. Spielman. 1987a. Duration of tick attachment and *Borrelia burgdorferi* transmission. J. Clin. Microbiol. 25: 557–558.

Piesman, J., T. N. Mather, G. J. Dammin, S. R. Telford III, C. C. Lastavica, and A. Spielman. 1987b. Seasonal variation of transmission risk of Lyme disease and human babesiosis. Am. J. Epidemiol. 126: 1187–1189.

Salafsky, B., Y. X. He, J. Li, T. Shibuya, and K. Ramaswamy. 2000. Short report: study on the efficacy of a new long-acting formulation of *N*, *N*-diethyl-*m*-toluamide (DEET) for the prevention of tick attachment. Am. J. Trop. Med. Hyg. 62: 169–172.

Schreck, C. E., E. L. Snoddy, and A. Spielman. 1986. Pressurized sprays of permethrin or DEET on military clothing for personal protection against *Ixodes dammini.* J. Med. Entomol. 23: 396–399.

Schultz, M. W., M. Gomez, R. C. Hansen, J. Mills, A. Menter, H. Rodgers, F. N. Judson, G. Mertz, and H. H. Handsfield. 1990. Comparative study of 5% permethrin cream and 1% lindane lotion for the treatment of scabies. Arch. Dermatol. 126: 167–170.

Scoles, G. A., M. Papero, L. Beati, and D. Fish. 2001. A relapsing fever group spirochete transmitted by *Ixodes scapularis* ticks. Vector Borne Zoonotic Dis. 1: 21–34.

Snodgrass, H. L. 1992. Permethrin transfer from treated cloth to the skin surface: potential for exposure in humans. J. Toxicol. Environ. Health 35: 91–105.

Solberg, V. B., T. A. Klein, K. R. McPherson, B. A. Bradford, J. R. Burge, and R. A. Wirtz. 1995. Field evaluation of DEET and a piperidine repellent (AI3-37220) against *Amblyomma americanum* (Acari: Ixodidae). J. Med. Entomol. 32: 870–875.

Spielman, A., M. L. Wilson, J. F. Levine, and J. Piesman. 1985. Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease. Annu. Rev. Entomol. 30: 439–460.

Telford, S. R. III, J. E. Dawson, P. Katavolos, C. K. Warner, C. P. Kolbert, and D. H. Persing. 1996. Perpetuation of the agent of human granulocytic ehrlichiosis in a deer tick-rodent cycle. Proc. Natl. Acad. Sci. USA 93: 6209–6214.

Tomalik-Scharte, D., A. Lazar, J. Meins, B. Bastian, M. Ihrig, B. Wachall, A. Jetter, I. Tantcheva-Poór, G. Mahrle, and U. Fuhr. 2005. Dermal absorption of permethrin following topical administration. Eur. J. Clin. Pharmacol. 61: 399–404.

[WHO] World Health Organization. 2004. Global strategic framework for integrated vector management. World Health Organization, Geneva, Switzerland.

Received 14 June 2010; accepted 3 October 2010.