

Long-lasting Permethrin Impregnated Uniforms

A Randomized-Controlled Trial for Tick Bite Prevention

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Background: Because of frequent exposure to tick habitats, outdoor workers are at high risk for tick-borne diseases. Adherence to National Institute for Occupational Safety and Health—recommended tick bite prevention methods is poor. A factory-based method for permethrin impregnation of clothing that provides long-lasting insecticidal and repellent activity is commercially available, and studies are needed to assess the long-term effectiveness of this clothing under field conditions.

Purpose: To evaluate the protective effectiveness of long-lasting permethrin impregnated uniforms among a cohort of North Carolina outdoor workers.

Design, setting, and participants: A double-blind RCT was conducted between March 2011 and September 2012. Subjects included outdoor workers from North Carolina State Divisions of Forestry, Parks and Recreation, and Wildlife who worked in eastern or central North Carolina. A total of 159 volunteer subjects were randomized, and 127 and 101 subjects completed the first and second years of follow-up, respectively.

Intervention: Uniforms of participants in the treatment group were factory-impregnated with long-lasting permethrin whereas control group uniforms received a sham treatment. Participants continued to engage in their usual tick bite prevention activities.

Main outcome measures: Incidence of work-related tick bites reported on weekly tick bite logs.

Results: Study subjects reported 1,045 work-related tick bites over 5,251 person-weeks of follow-up. The mean number of reported tick bites in the year prior to enrollment was similar for both the treatment and control groups, but markedly different during the study period. In our analysis conducted in 2013, the effectiveness of long-lasting permethrin impregnated uniforms for the prevention of work-related tick bites was 0.82 (95% CI=0.66, 0.91) and 0.34 (95% CI=-0.67, 0.74) for the first and second years of follow-up.

Conclusions: These results indicate that long-lasting permethrin impregnated uniforms are highly effective for at least 1 year in deterring tick bites in the context of typical tick bite prevention measures employed by outdoor workers.

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Introduction

In the U.S., more than 34,000 cases of tick-borne illnesses including Lyme disease, spotted fever group rickettsioses, ehrlichiosis, and anaplasmosis were reported in 2010.¹ The true incidence is likely to be higher because of under-reporting.² The incidence of tick-borne diseases is rising and new tick-borne pathogens are emerging.

Tick-borne diseases are an occupational risk for outdoor workers, particularly among forestry workers.³ A recent serosurvey of National Park Service employees showed that 22% of employees were seropositive for previous exposure to spotted fever group rickettsiae, 3% for *Ehrlichia chaffeensis*, and 8% for *Anaplasma phagocytophilum*.⁴

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Tick bite prevention methods recommended by the National Institute for Occupational Safety and Health for outdoor workers include wearing light-colored protective clothing (long sleeves, long pants, and a hat); tucking pants into socks or boots; regular application of insect repellent (at least 20% *N,N*-diethyl-*meta*-toluamide [DEET]) to exposed skin and clothing; spraying work clothing with permethrin; and thoroughly checking one's body for ticks daily.⁵ The most commonly used form of permethrin for clothing treatment is a self-applied permethrin aerosol spray. Under controlled conditions, self-application of permethrin to clothing can provide nearly 100% protection against questing ticks including *Amblyomma americanum*,^{6–9} *Dermacentor variabilis*,^{6,7} *Ixodes scapularis*,^{6,9,10} and *Ixodes pacificus*.¹¹ This high rate of protection, however, is not sustained over long periods of wear or multiple washings.^{8,11} Furthermore, adherence to these recommendations, even among those who work in highly endemic areas for tick-borne disease, appears to be poor.^{4,12–14} Thus, more effective and user-friendly tick bite prevention methods are needed.

A factory-based method for long-lasting permethrin impregnation (LLPI) of textiles using a proprietary formulation of permethrin has been shown to retain tick-repellent activity over 70 washes in laboratory studies.^{15,16} Clothing treated with this method is commercially available at many outdoor retailers and is a key component of the Department of Defense Insect Repellent System.^{17,18} An open-label pilot study was conducted to assess the effectiveness of LLPI clothing for the prevention of tick bites among 16 outdoor workers from the North Carolina Division of Water Quality.¹⁹ Subjects wearing LLPI clothing had 93% fewer tick bites compared to those using standard tick bite prevention measures. To evaluate this intervention in a more rigorous manner, a double-blind RCT of LLPI uniforms was conducted among outdoor workers from North Carolina's Divisions of Parks and Recreation, Forestry, and Wildlife.

Methods

Study Design

A double-blind randomized intervention was conducted to determine whether wearing LLPI uniforms results in fewer tick bites among outdoor workers. The study included follow-up over two tick seasons (March–September 2011 and 2012). The IRB of the University of North Carolina at Chapel Hill approved the study protocol (IRB no. 10-1027). All subjects provided written informed consent.

Participants

Eligible participants included employees of the North Carolina Forest Service, North Carolina Division of Parks and Recreation,

North Carolina Wildlife Resources Commission, and North Carolina County and Local Parks and Recreation who worked in eastern or central North Carolina, were aged ≥ 18 years, spent an average of ≥ 10 hours/week working outdoors during tick season, and were required to wear a uniform while on the job. Exclusion criteria were pregnancy, being a non-English speaker, or having a known allergy to insecticides. Informational meetings were conducted at state and local parks, forestry, and wildlife offices throughout the study area between October 2010 and April 2011, and eligible employees were invited to enroll. Subjects were assigned a study identification (ID) number upon enrollment and given a small flashlight and six pairs of boot socks as a token of appreciation for their participation. After the first year of follow-up, subjects were given six additional pairs of socks as an incentive to complete the final year of follow-up.

Randomization and Masking

Prior to the start of enrollment, each ID number was assigned to either the treatment or control group through block randomization using block sizes of six, which were generated randomly by computer, with 1:1 allocation for the treatment or control interventions. The randomization list was generated by the study statistician, and none of the study personnel or investigators had access to this list until after data collection was completed.

Intervention

All subjects were asked to send all of their spring and summer work uniforms, including shirts, T-shirts, pants, shorts, hats, and socks, directly to the treatment facility. Once the uniforms were received, all items were labeled with the subject's ID number and either treated with permethrin according to the factory-based proprietary LLPI process for clothing (treatment) or simply washed and dried in a commercial washer/dryer (control). In addition, according to their treatment group, subjects were either given six pairs of treated or untreated boot socks at the start of each tick season. Subjects were instructed to send any new uniforms purchased during the study period to the treatment facility before wearing them. All subjects were instructed to launder their clothing as they normally would and to continue with their usual tick bite prevention measures (including use of repellents).

Data Collection

After providing informed consent, participants completed a baseline questionnaire, which collected demographic and occupational information, history of tick-bites and tick exposure in the past year, history of tick-borne disease, and use of tick bite prevention measures. The follow-up periods consisted of two consecutive tick seasons (2011 and 2012) starting the week of March 15, 2011, or the week the subject started wearing their study uniforms and continuing through the last week of September. During the follow-up periods, all subjects were asked to keep a diary of all tick bites (attached ticks), which were recorded on weekly tick bite logs. For each entry in the log, subjects recorded the date of the tick bite(s), number of tick bites, location of the tick bites on the body, county where the tick bite(s) were most likely to have been acquired, whether they were on the job when the tick bite(s) were acquired, whether they had been using insect repellent at the time of the bite(s) and the type of repellent, and whether the tick(s) were collected.

Subjects also recorded the number of work and non-work hours spent outdoors each week on their weekly tick bite logs. Subjects were provided a tick removal kit, including forceps and collection vials, and were encouraged to submit any attached ticks for identification. At the end of each year, all subjects were asked to complete a follow-up questionnaire. After data collection was completed, subjects were asked to guess whether they were in the treatment or control group, and provide the reason for their guess.

Adverse Events and Tick-Borne Illnesses

An adverse event was defined as any report of adverse effects that a participant believed to be related to their uniforms. In the case of an adverse event report, the study physician was unblinded to determine whether the subject was in the treatment group. If an adverse reaction occurred related to a subject's treated uniforms, the study would pay to replace all of the participants' uniforms. All subjects were also instructed to report any illnesses with symptoms of fever, rash, headache, muscle aches, or extreme tiredness within 3 weeks of a tick bite. In the case of illness, the subject consulted with the study physician or their own physician and sera were collected for testing against tick-borne pathogens.

Statistical Analysis

The analyses, conducted in 2013, followed the intent-to-treat principle. Baseline characteristics of the treatment and control groups were compared using the Pearson chi-square test for dichotomous variables, Mantel–Haenszel chi-square test for ordinal variables, and Student's *t* test for continuous variables. *P*-values <0.05 were considered statistically significant. Protective effectiveness (1 – the incidence rate ratio)²⁰ and 95% CIs for comparing reported tick bites between the treatment and control groups were calculated using a generalized estimating equations

(GEE) approach for Poisson regression. The use of GEE methods accounted for the within-subject correlation due to repeated measures using a working correlation matrix. The GEE model used to calculate the protective effectiveness of the LLPI clothing used a Poisson distribution with a log link, and included terms for treatment, year of follow-up, and the interaction of treatment and year of follow-up, with an offset variable for log outdoor work hours. No other covariates were included in the model based on our evaluation of possible confounding by baseline variables using a 10% change in estimate criteria. The incidence of tick bites was calculated as the total number of work-related tick bites per 100 hours spent working outdoors. Estimates were stratified by year of follow-up to examine whether the treatment effect waned with continued wear/washing. Incidence rate differences and CIs were calculated by inverse weighting of tick bites by the average outdoor work hours in the corresponding treatment group, so that the variance of the rate difference could be estimated using normal approximation. Secondary outcomes, including chigger (larval-stage mites in the family *Trombiculidae*) bites and mosquito bites, were compared using the Pearson chi-square test for dichotomous variables. The success of blinding was assessed using Bang's blinding index, with values between –0.2 and 0.2 used as the threshold for successful blinding.²¹ All analyses were performed using SAS, version 9.2 (SAS Institute Inc., Cary NC), or Stata, version 12 (StataCorp LP, College Station TX).

Results

Study Population

During 2 years of recruitment, 159 subjects were enrolled and randomized. Twenty-six of 159 enrolled subjects (16%) never sent in their uniforms and were excluded (Figure 1). At baseline, there were no significant differences

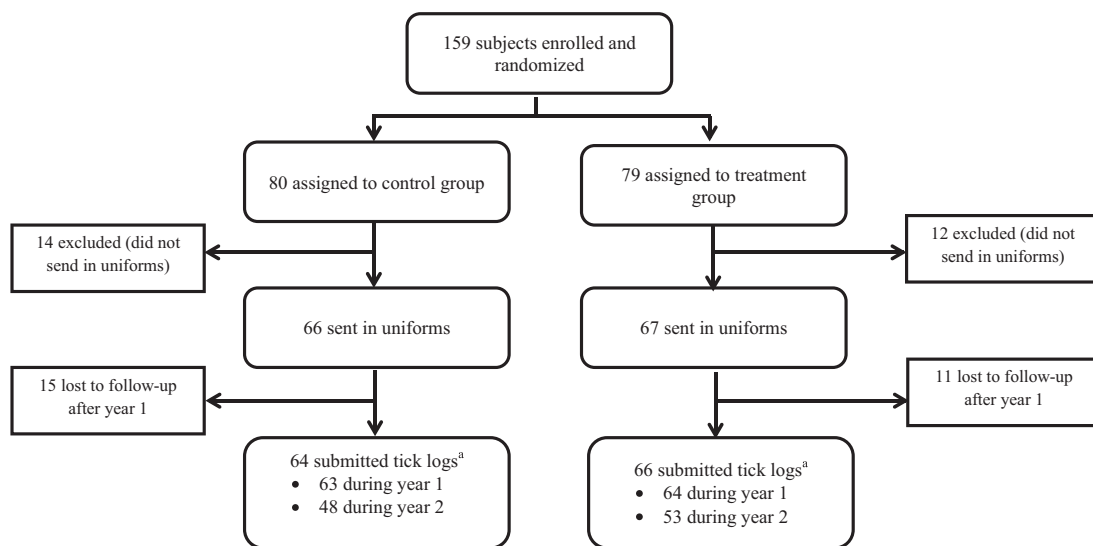


Figure 1. Study participant randomization and follow-up

^aSix subjects enrolled after the first year were only followed for Year 2. Two subjects did not submit any tick logs during Year 1 but began submitting logs in Year 2. Three subjects did not submit any tick logs.

in demographic and other characteristics between the treatment and control groups (Table 1). The majority of subjects were white men with a college degree who had been working in their current position for an average of 8 years. In the year prior to enrollment, the mean number of tick bites reported on the baseline questionnaire was 19.3 in the treatment group and 19.6 in the control group. Twenty-six subjects were lost to follow-up after the first year, including 15 in the control group and 11 in the treatment group (Figure 1). Loss to follow-up was primarily due to subjects who moved or were transferred to jobs outside the study area ($n=12$). Subjects who were lost to follow-up did not differ from those who remained in the study according to treatment status, demographic characteristics, and outcome (mean tick bites per week); thus, we felt that the missing-completely-at-random assumption was reasonable and missing data due to loss to follow-up were treated as non-informative and ignorable (Supplemental Table 1).

Protective Effectiveness Against Tick Bites

The mean number of person-weeks of follow-up was similar for the treatment and control groups (41.1 person-weeks and 42.2 person-weeks, respectively). The number of work-related tick bites (bites reported as having been acquired on the job) reported by group and incidence of tick bites per person-week stratified by year of follow-up are shown in Table 2. In total, 1,045 work-related tick bites were reported over 5,251 weeks of follow-up. The incidence of tick bites in Year 1 was 0.24 bites per 100 outdoor work hours in the treatment group and 1.37 bites per 100 outdoor work hours in the control group, with an incidence rate difference of -1.13 (95% CI= $-1.78, -0.50$). In Year 2, the incidences were 0.69 and 1.05 tick bites per 100 outdoor work hours in the treatment and control groups, respectively, with an incidence rate difference of -0.36 (95% CI= $-1.12, 0.40$). The incidence of tick bites was significantly lower among subjects in the treatment group during the first year of follow-up, with a protective effectiveness of 0.82 (95% CI= $0.66, 0.91$, $p<0.001$) against tick bites for subjects wearing LLPI uniforms compared to those using their usual tick bite prevention measures. During the second year of follow-up, the protective effectiveness was 0.34 (95% CI= $-0.67, 0.74$, $p=0.38$). The overall protective effectiveness for both years of follow-up was 0.65 (95% CI= $0.29, 0.82$, $p=0.004$). Two outliers were observed in which a subject reported ≥ 50 tick bites in a single week. These were likely to be larval ticks and it is uncertain whether they represented true bites (attachment). When these outliers were excluded, the protective

effectiveness for Year 1 was 0.78 (95% CI= $0.60, 0.88$) and that for Year 2 was 0.52 (95% CI= $0.01, 0.77$) (Supplemental Table 2).

Secondary Outcomes

In support of the tick bite log data, 867 ticks were collected and submitted by subjects over both years, more from subjects in the control group (581 ticks) than from subjects in the treatment group (286 ticks). More than 90% of the submitted ticks were lone star ticks (*A. americanum*). Nearly all subjects reported having chigger and mosquito bites in the year prior to enrollment, with no significant differences between those assigned to the treatment or control groups. During both years of follow-up, the risk of having any chigger bites was significantly reduced among subjects in the treatment group (Year 1, risk ratio [RR]= 0.66 , $p<0.001$; and Year 2, RR= 0.71 , $p=0.002$) (Table 3). Almost all subjects continued to report having been bitten by mosquitoes during the follow-up, although the proportion who reported having frequent mosquito bites was lower among those in the treatment group (Year 1, RR= 0.66 , $p=0.08$; Year 2, RR= 0.56 , $p=0.08$).

Masking

To assess the degree of unmasking, all subjects were asked to guess their treatment status at the end of the study. Of the 97 subjects who responded, 41/51 (80.4%) of subjects in the treatment group and 27/46 (58.7%) in the control group guessed correctly (Supplemental Table 3). Most subjects related their guess to the frequency of tick and chigger bites they experienced and the behavior of ticks on their uniforms. Bang's blinding index, which can be interpreted as the proportion of unmasking in each group, was 0.74 for the treatment group and 0.28 for the control group, indicating unmasking was high for both groups, although less so for the control group.

Adverse Events and Tick-Borne Illnesses

There were no adverse events reported related to the subjects' uniforms. Five subjects reported illnesses suspected to be tick-related, two were confirmed (one case of ehrlichiosis and one case of spotted fever rickettsiosis), both among subjects in the control group. The other reported illnesses were a local reaction to a tick bite, viral mononucleosis, and a mild viral illness.

Discussion

Prevention of tick bites is critical among outdoor workers and others with extensive exposure to ticks. This study demonstrated that in the first year of wear, LLPI uniforms significantly reduced tick bites by $>80\%$ among

Table 1. Demographic characteristics, history of tick bites, tick-borne illness, and preventive measures usage by treatment group, *n* (%) unless otherwise noted

	Treatment group (<i>n</i> =67)	Control group (<i>n</i> =66)
Gender		
Men	52/66 (78.8)	55/66 (83.3)
Women	14/66 (21.2)	11/66 (16.7)
Age (years; M [SD])	39.1 (9.2)	38.8 (9.3)
Race		
White	56/58 (96.6)	58/59 (98.3)
Black	2/58 (3.4)	0/59 (0.0)
Other	0/58 (0.0)	1/59 (1.7)
Education		
High school or less	5/65 (7.7)	10/66 (15.2)
Some college	20/65 (30.8)	14/66 (21.2)
Bachelor or graduate degree	40/65 (61.5)	42/66 (63.6)
Work division		
NC Forest Service	21/66 (31.8)	27/66 (40.9)
NC Division of Parks and Recreation	31/66 (47.0)	25/66 (37.9)
NC Wildlife Resources Commission	9/66 (13.6)	10/66 (15.2)
NC Local or County Parks and Recreation	5/66 (7.6)	4/66 (6.1)
Years in current position (M [SD])	7.9 (6.7)	8.2 (6.8)
Number of tick bites in previous year (M [SD])	19.3 (32.0)	19.6 (39.3)
Previous diagnosis with a tick-borne illness^a		
Lyme disease	4/67 (6.0)	4/66 (6.1)
Rocky Mountain spotted fever	6/67 (9.0)	7/66 (10.6)
Ehrlichiosis	2/67 (3.0)	3/66 (4.6)
Anaplasmosis	0/67 (0.0)	0/66 (0.0)
Babesiosis	0/67 (0.0)	0/66 (0.0)
Use of tick bite prevention measures^b		
Long pants	62/66 (93.9)	62/65 (95.4)
Long sleeves	48/57 (84.2)	52/59 (88.1)
Hat	43/63 (68.2)	38/64 (59.4)
Tucked or taped pant legs	5/60 (8.3)	7/59 (11.9)
Repellent on skin	22/64 (34.4)	22/61 (36.1)
Repellent on clothing	38/65 (58.5)	35/65 (53.8)
Tick checks after working outdoors	58/66 (87.9)	60/65 (92.3)
Use of self-applied permethrin on clothing	30/67 (44.8)	34/66 (51.5)

(continued on next page)

outdoor workers even when usual tick bite prevention measures were employed by both groups. The effectiveness of the LLPI uniforms declined in Year 2. Based on laboratory knock-down studies of mosquito and tick species after exposure to treated fabric, the repellency of the LLPI clothing used in this study is registered by the U.S. Environmental Protection Agency to last through 70 launderings.¹⁶ The estimated numbers of launderings of the subjects' uniforms in this study, based on questionnaire data, were generally below 70 washes after the first year (Supplemental Table 4). Therefore, we believe that environmental conditions in the field (not present in laboratory studies) may also play a role in loss of effectiveness. Subjects spent many outdoor hours in their uniforms; this continued exposure to various environmental conditions (sunlight, rain, and heat) and heavy wear of uniforms may have contributed to the observed loss of effectiveness. Future studies should focus on the effects of environmental factors on permethrin loss and knockdown capacity of LLPI clothing.

Adherence to the assigned treatment (wearing only "study" uniforms) was likely to be highest in the first year of follow-up. The workers in this study typically purchase a number of new uniforms every year and we asked that any new uniforms purchased during the study be sent for "treatment" before

Table 1. Demographic characteristics, history of tick bites, tick-borne illness, and preventive measures usage by treatment group, *n* (%) unless otherwise noted (*continued*)

	Treatment group (<i>n</i> =67)	Control group (<i>n</i> =66)
Number of uniforms submitted (M [SD])		
Shirts	15.4 (10.6)	13.9 (9.9)
Pants	9.1 (5.1)	9.2 (6.5)
Socks	8.4 (6.0)	8.5 (6.7)
Hats	1.7 (2.0)	1.3 (1.5)

^aBased on self-report^bReported using these measures at least 50% of the time while working outdoors

being worn. Approximately half of the subjects in each group submitted additional uniforms after the first year; therefore, it is likely that some subjects wore “non-study” uniforms during the second year, which could bias the treatment effect toward the null. Permethrin-treated socks have been shown to be particularly effective in preventing tick bites,²² as questing ticks will often encounter socks and footwear first after finding a human host. Subjects were only provided six pairs of treated (or sham-treated) socks at the start of each year, and although most subjects submitted additional socks of their own at the start of the study, only 12 subjects submitted additional socks in the second year. Because socks tend to wear out more quickly than other pieces of clothing, it is unlikely that all subjects wore “study” socks exclusively. Failure to wear permethrin-treated socks

self-applied repellents more frequently, which could have led to an underestimation of the protective effectiveness of the LLPI uniforms ([Supplemental Table 5](#)).

Although there is potential for bias due to exclusion of subjects who did not submit uniforms, the proportions excluded from those assigned to the treatment and control group were similar. Subjects who were excluded tended to be older and had significantly fewer tick bites in the year prior to enrollment ([Supplemental Table 1](#)). Excluded subjects also were less likely to wear long pants, use repellent on their skin, and use self-applied permethrin on their clothing. We speculate that these subjects may have chosen not to send in their uniforms because of low perceived risk of tick bites and reluctance to use repellents. Given the evidence that those who opted to participate had more frequent exposure to ticks, our

could also have contributed to the loss of measured effectiveness in Year 2.

The high degree of unmasking among subjects is a potential source of bias. However, there were no significant differences observed in the proportion of subjects in each group performing regular tick checks and using other tick bite prevention measures during follow-up. The control group tended to use

Table 2. Incidence of work-related tick bites by treatment group and year of follow-up

	<i>n</i>	Total tick bites	Total outdoor work hours	Tick bite incidence per 100 outdoor work hours	Incidence rate difference (95% CI)	Protective effectiveness (95% CI)	<i>p</i> -value
Year 1							
Treatment	64	84	34,628.0	0.24	−1.13 (−1.78, −0.50)	0.823 (0.655, 0.910)	< 0.001
Control	63	493 ^a	35,750.8	1.37			
Year 2							
Treatment	53	181 ^b	26,171.5	0.69	−0.36 (−1.12, 0.40)	0.341 (−0.670, 0.740)	0.379
Control	48	287	27,353.0	1.05			
Years 1 and 2							
Treatment	66	265 ^b	60,799.5	0.44	−0.79 (−1.34, −0.26)	0.646 (0.288, 0.824)	0.004
Control	64	780 ^a	63,103.8	1.23			

Note: Boldface indicates statistically significant *p*-values.^aOne subject reported 102 tick bites in 1 week.^bOne subject reported 50 tick bites in 1 week.

Table 3. Proportion of subjects reporting any chigger bites by treatment group and follow-up year

Year	Treatment group	Control group	Risk ratio (95% CI)	p-value
Baseline	65/66 (98.5)	65/66 (98.5)	1.00 (0.96, 1.04)	1.0
Year 1	32/57 (56.1)	50/59 (84.8)	0.66 (0.51, 0.85)	<0.001
Year 2	32/50 (64.0)	45/50 (90.0)	0.71 (0.57, 0.89)	0.002

Note: Boldface indicates statistically significant p-values.

estimates of protective effectiveness are most relevant for those at high risk of tick bites.

The vast majority of the ticks collected by subjects were lone star ticks (*A. americanum*), the most common human-biting tick in North Carolina. Clothing that has been freshly treated with permethrin provides high rates of protection against several species of ticks, including *I. scapularis*, *I. pacificus*, *Ixodes ricinus*, *D. variabilis*, and *A. americanum*.^{6–11,22–24} Thus, LLPI clothing is likely to be protective against different tick species, but additional long-term studies are needed in other locations.

This study is the first RCT to evaluate the effectiveness of LLPI clothing with an extended follow-up period. Efficacy of permethrin impregnated clothing against ticks has been demonstrated in the laboratory and in short-duration field trials.^{6–11,22–26} However, traditional self-applied spray and dipping methods lose effectiveness unless reapplied every three to five washes.^{8,11,27} In an open-label pilot study, subjects wearing LLPI clothing had 93% fewer ($p < 0.0001$) tick bites compared with subjects using standard tick bite prevention measures over one tick season.¹⁹ The current study was designed to provide a more accurate and precise estimate over a longer duration of follow-up. The results of this study demonstrate that among high-risk individuals, LLPI uniforms are highly effective for at least 1 year against tick bites compared to existing tick bite prevention measures. Based on these findings, we recommend that wearing LLPI uniforms or clothing should be included as a standard tick bite prevention measure in addition to other recommended prevention measures for outdoor workers with substantial exposure to ticks, with retreatment or replacement of garments after 1 year if they are worn on a regular basis.

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Appendix

Supplementary data

Supplementary data associated with this article can be found at <http://dx.doi.org/10.1016/j.amepre.2014.01.008>.