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Research

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Protective Effectiveness of Long-Lasting Permethrin Impregnated Clothing Against Tick Bites in an Endemic Lyme Disease Setting: A Randomized Control Trial Among Outdoor Workers

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Abstract

Tick-borne diseases are a growing threat to public health in the United States, especially among outdoor workers who experience high occupational exposure to ticks. Long-lasting permethrin-impregnated clothing has demonstrated high initial protection against bites from blacklegged ticks, *Ixodes scapularis* Say (Acari: Ixodidae), in laboratory settings, and sustained protection against bites from the lone star tick, *Amblyomma americanum* (L.) (Acari: Ixodidae), in field tests. However, long-lasting permethrin impregnation of clothing has not been field tested among outdoor workers who are frequently exposed to blacklegged ticks. We conducted a 2-yr randomized, placebo-controlled, double-blinded trial among 82 outdoor workers in Rhode Island and southern Massachusetts. Participants in the treatment arm wore factory-impregnated permethrin clothing, and the control group wore sham-treated clothing. Outdoor working hours, tick encounters, and bites were recorded weekly to assess protective effectiveness of long-lasting permethrin-impregnated garments. Factory-impregnated clothing significantly reduced tick bites by 65% in the first study year and by 50% in the second year for a 2-yr protective effect of 58%. No significant difference in other tick bite prevention method utilization occurred between treatment and control groups, and no treatment-related adverse outcomes were reported. Factory permethrin impregnation of clothing is safe and effective for the prevention of tick bites among outdoor workers whose primary exposure is to blacklegged ticks in the northeastern United States.

Key words: tick-borne disease, permethrin, blacklegged tick, prevention, outdoor worker health

The rising incidence of vector-borne diseases in the United States, especially those transmitted by ticks, is a growing public health concern. In 2017, nearly 60,000 cases of tick-borne disease were reported to the Centers for Disease Control and Prevention (CDC), an increase of over 10,000 cases from 2016 and setting a record high for the United States (CDC 2019). The most frequently reported tick-borne disease in the United States is Lyme disease, with more than 30,000 incident cases reported each year, followed by anaplasmosis and ehrlichiosis, spotted fever rickettsiosis, and babesiosis (CDC 2019). The primary vector for three high incidence tick-borne diseases (Lyme disease, anaplasmosis, and babesiosis) is the blacklegged tick *Ixodes scapularis* (Say) in the Northeast and Mid-West or the western blacklegged tick *Ixodes pacificus* (Cooley and Kohls) (Acari: Ixodidae) along the Pacific coast of the United States (CDC 2019). Without an available vaccine for most tick-borne diseases in humans, prevention hinges on tick avoidance and use of repellents and insecticides to prevent bites (Vázquez et al. 2008, NIOSH 2017).

Outdoor workers, particularly those working in forested or brushy areas, experience high occupational exposure to tick bites and are at an increased risk of acquiring a tick-borne illness (Adamek et al. 2006, Adjemian et al. 2012, Wallace et al. 2016). To reduce the potential for infection, the National Institute for Occupational Safety and Health recommends that outdoor workers wear light colored protective clothing such as a long-sleeved shirt and long pants tucked into socks or boots, use repellents containing at least 20% N,N-diethyl-meta-toluamide (DEET), apply permethrin to clothing, and check skin and clothing for ticks daily (NIOSH 2017). While these measures can be effective, they are dependent on individual adherence and require daily application or maintenance (Adamek et al. 2006, Vázquez et al. 2008, Adjemian et al. 2012).

Permethrin self-application of clothing was first shown to be efficacious against a wide array of blood-feeding arthropods in 1978 (Schreck et al. 1978). Subsequent studies confirmed that permethrin impregnated cloth attained a near 100% mortality rate for tick species responsible for most tick-borne diseases in the United States, including *Amblyomma americanum* (L.) (Acari: Ixodidae) (Schreck et al. 1982, Mount and Snoddy 1983, Evans et al. 1990, Prose et al. 2018), *Dermacentor variabilis* (Say) (Acari: Ixodidae) (Mount and Snoddy 1983, Evans et al. 1990, Prose et al. 2018), *I. scapularis* (Schreck et al. 1986, Evans et al. 1990, Miller et al. 2011, Prose et al. 2018), and *I. pacificus* (Lane 1989). However, clothing treated in this manner lost protective effectiveness after a few launderings (Schreck et al. 1986). Additionally, adherence to self-application guidelines has been previously reported to be low, even among workers in high tick exposure areas (Adamek et al. 2006, Vázquez et al. 2008).

Factory impregnation with permethrin can lead to persistent protective activity (Faulde et al. 2003, Vaughn and Meshnick 2011, Vaughn et al. 2014). Long-lasting permethrin-impregnated (LLPI) garments are reported to retain insecticidal activity through 70 washes (Insect Shield 2019, Faulde et al. 2006); however, significant declines in permethrin content following as few as 5 wash cycles have been reported (Gopalakrishnan et al. 2014, Connally et al. 2019, Richards et al. 2018). Wash and wear associated reduction in permethrin contact toxicity has been observed for mosquitos, but has not been thoroughly tested for ticks. Despite this potential limitation, factory-impregnation of clothing with permethrin remains the longest lasting insecticide-based method for preventing insect bites with high protective effectiveness. Because of these properties, the US Department of Defense has used LLPI treatment for all uniforms distributed to members of the armed forces since 2013 (Proctor et al. 2019). Factory-treated LLPI clothing is also available for purchase and use by the general public from private companies such as Insect Shield LLC (Greensboro, NC).

LLPI clothing has repeatedly been shown to be safe to wear (Proctor et al. 2019, Sullivan et al. 2019). A recent study of LLPI uniforms issued to forestry workers in North Carolina found an average dose of <4 µg/kg of body weight of permethrin was absorbed following 3 mo of field wear exposure (Sullivan et al. 2019). Urine permethrin levels were well below the EPA limit of 250 µg/kg/d (EPA 2017) as well as the more conservative European limits of 50 µg/kg/d (Aylward et al. 2018). Thus, the daily wearing of LLPI clothing is well within the safe dose range established by protective regulatory agencies.

LLPI uniforms were shown to be highly protective against tick bites among outdoor workers in a recent double-blinded randomized controlled trial (RCT) among North Carolina outdoor workers (Vaughn et al. 2014). Subjects wearing LLPI uniforms had over 80% fewer tick bites in the first year of the trial compared to subjects wearing untreated uniforms; however, in the second year, the protective effectiveness fell to only 36%. The sharp decline in protection in the second year of the trial was unexpected as most treated garments had been washed fewer times than the reported 70-wash threshold. Additionally, almost all of the tick bites in this study were from lone star ticks, *A. americanum*, the dominant human-biting tick in North Carolina. A field test of the protective effectiveness of LLPI clothing against bites from *I. scapularis* in a region of high Lyme disease endemicity has not previously been conducted. To evaluate the effect of LLPI clothing against blacklegged tick bites among outdoor workers with high levels of exposure, we conducted a double-blind RCT of LLPI clothing among a cohort of outdoor workers in Rhode Island and southern Massachusetts spanning two tick seasons.

Materials and Methods

Study Design

A double-blind placebo-controlled randomized control study was employed to evaluate the effectiveness of permethrin treated uniforms in reducing tick bites among outdoor workers in Rhode Island and southern Massachusetts (Supp Fig. 1 [online only]). Participants were enrolled in two cohorts; each cohort was followed for two consecutive tick seasons, defined as March through December of 2016–2017 (first cohort), and 2017–2018 (second cohort). The study design and procedures were approved by the IRB of the University of North Carolina at Chapel Hill (IRB no.15–1770). All participants provided written informed consent prior to randomization.

Participants

Participants were selected from eligible employees of the National Park Service in Rhode Island, the Rhode Island Division of Environmental Management, Massachusetts Department of Conservation and Recreation, University of Rhode Island, US Department of Agriculture—Animal and Plant Health Inspection Service, and other local outdoor workers who were self-employed or employed by a private company in the study region. Inclusion criteria included age ≥18 yr, average ≥10 h/week of outdoor work during peak tick season, and written informed consent. Exclusion criteria included pregnancy or planned pregnancy during the study period, inability to speak English, and known allergy or sensitivity to insecticides. All participants were offered free LLPI treatment of clothing following completion of the study, free blood testing for tick-borne diseases, and free socks. Participants were also offered small monetary gift cards for completion of the following key study procedures: enrollment procedures, mailing clothing for treatment, end of year 1 survey, end of year 2 survey, and contribution of 1 clothing item for permethrin quantification (not described in present study).

Procedures

Prior to starting the study, all study identification numbers were block randomized to the treatment or control group at a 1:1 ratio. Randomization was conducted by the study statistician using a random number generator in block sizes of six. Randomized treatment assignments were shared only once with the clothing treatment manager and were not revealed again until after the final data analysis. None of the study personnel, investigators, or participants had access to randomization assignments, preserving the double-blind study design.

Following enrollment, study participants were assigned a study identification number (previously randomized for treatment or control), and they were asked to label their uniforms or clothing with their study ID and submit them for 'treatment' in two shipments to the Insect Shield facility in Greensboro, NC. At the Insect Shield facility, the clothing treatment manager was provided access to randomization assignments for each study ID to ensure that the clothing was permethrin-impregnated or sham-treated correctly. In the event that new uniforms or clothing were obtained after study initiation, participants were asked to submit these garments to Insect Shield for treatment or sham-treatment prior to use. At the Insect Shield facility, garments were either treated with permethrin according to their proprietary LLPI process for clothing (active treatment arm) or laundered in a commercial washer/dryer (control arm) and returned to participants. Permethrin treatment of clothing has no odor (Schreck et al. 1978, Insect Shield 2019) and does not affect the look or feel of cloth (Insect Shield); treated clothing is indistinguishable from untreated clothing by human senses. Throughout the study, participants were asked to wash their clothes as they normally would and to maintain their routine tick bite prevention measures (e.g., protective clothing, tick checks, use of insect repellents on clothing and/or skin).

Occupational tick exposure was assessed through weekly tick logs administered to participants from March through December for two consecutive years starting the year of enrollment. Weekly logs were administrated through an online survey platform (SurveyMonkey), and reminders to complete the survey were sent to all participants weekly by one of the study coordinators. Study surveys were designed to assess work-related tick exposure and tick bites. To address this objective, weekly tick logs recorded data on the number of hours the participant worked that week, work time spent outdoors, the number of ticks encountered during work, the number of ticks found attached/biting during work, and any insect repellent use on clothing and skin. In addition to weekly tick logs, each participant was asked to complete three questionnaires: one at baseline to ascertain occupational exposure, demographic data, and any history of tick-borne disease; the second at the conclusion of the first study year to reevaluate work-related exposure profiles, ascertain the frequency of using specific articles of clothing (e.g., socks, shirt, pants, hat), tick bite prevention measures used during work (wore long pants, hat, long-sleeved shirt, pants tucked into boots/socks, tick check during day, tick check at the end of the day, and insect repellent application to skin and/or clothing), and the development of any tick-borne illness since starting the study; and a final questionnaire at the conclusion of the study (end of year 2) that was similar to the questionnaire at the end of the first year. To minimize loss to follow-up, the study coordinator provided weekly reminders for participants to mail clothing items for initial treatment for 1 mo following enrollment and a single reminder for retreatment of new clothing items between seasonal changes (i.e., summer to fall). Participants were administratively removed from the study after 3–4 wk of failed contact attempts.

All participants were instructed to seek immediate medical care following development of symptoms of a tick-borne illness such as fever, rash, or other flu-like symptoms 3 d to 3 wk following a tick bite. A study physician was available to participants at all times throughout the course of the study for consultation in such an event or referral to the participant's personal physician for testing and treatment. The study covered the cost of all testing for tick-borne pathogens during the study. For any adverse effects a participant suspected to be related to their clothing, the study physician was unblinded to identify the treatment assignment of the participants. In cases where adverse events were determined to be related to LLPI treatment of clothing, the study covered the cost of all clothing replacements and the participant was administratively censored from the study.

Statistical Analysis

Statistical comparisons between subjects in the control and treatment groups were conducted using Fisher exact test for binary variables and Pearson's χ^2 test for nominal variables with more than two categories. Kruskal–Wallis rank-sum test was used to compare nonnormally distributed continuous variables including age, duration of employment, and previous number of reported tick bites.

The primary outcome for this study was tick bites, defined as a tick attached with its mouthparts inserted into the skin. The secondary outcome was tick encounters, defined as a tick crawling on the skin or clothing of the subject. Incidence rates of tick bites and encounters were calculated from self-reported numbers per 100 outdoor working hours. Poisson regression was used to estimate the incidence rate difference and incidence rate ratio between treatment and control groups. Protective effectiveness was derived from calculating one minus the incidence rate ratio. The model included variables for treatment, year of follow-up, and the interaction between treatment and year of follow-up to explore the possible difference of treatment effect in the second year of the trial due to continued wear/washing. No other covariates were included in the model since no baseline variables had a >10% change in the estimates due to possible confounding. However, the model included outdoor work hours as an offset variable to account for the proportionality of tick bites and encounters to the length of exposure. A Wald-type 95% confidence interval for the incidence rate difference was reported, as well as for measures of protective effectiveness. P-values <0.05 were considered statistically significant. All analyses were performed using SAS, version 9.4 (SAS Institute Inc., Cary, NC). The analyses followed the intent-to-treat principle.

Results

Characteristics of the Study Participants

In total, 132 subjects were enrolled and randomized in this study; 65 were randomly assigned for LLPI treatment of their clothing and 67 to the control, sham-treated, group. Subjects were excluded if they did not send in clothing (n = 44), or failed to complete any weekly tick logs (n = 6). In total, 82 participants completed at least 1 yr of the study and were included in analyses (Fig. 1). Baseline demographic characteristics, tick exposure histories—including self-reported tick-borne diseases—and tick bite prevention measures routinely used by subjects were not significantly different between the control and treatment groups (Table 1). Overall, most subjects were male (76%), had a bachelor's or graduate degree (68%), were employed by a governmental organization (60%), and wore uniform-type clothing during outdoor work hours (70%). Median duration of employment at the current job was 5 yr (IQR: 12.8 yr), and the median age was 40 yr with a range of 24–70 yr.

At enrollment, greater than one-third of participants (n = 29) reported a previous tick-borne disease diagnosis, of which 76% (22/29) were Lyme disease. Routinely used tick bite prevention measures were not significantly different between the control and treatment groups; over 90% of subjects in both groups reported wearing long pants, a long-sleeved shirt, and checking their body for ticks during the work day, and 100% of subjects reported checking themselves for ticks at the end of their work day. Significant differences were not observed for the application of DEET (P = 0.209) and permethrin to clothing (P = 0.428) between treatment and control groups. The frequency of using any insect repellent on skin did not differ between the two groups (P = 0.641). The median number of tick bites reported for the previous year was also similar between treatment and control groups (Table 1).

Thirty-one participants were lost to follow-up between the first and second study years, 16 subjects were in the treatment group and 15 in the control group. Individuals who dropped out of the study after the first year tended to be younger and have lower educational attainment than retained subjects. Subjects lost to follow-up were not significantly different from those who remained in the study in terms of median tick bites received, prior diagnosis with one or more tick-borne illnesses, median years of employment, or for any tick bite prevention measures (Supp Table 1 [online only]). Since participants who were lost did not differ significantly from those who remained with regard to treatment status and outcome frequency (median

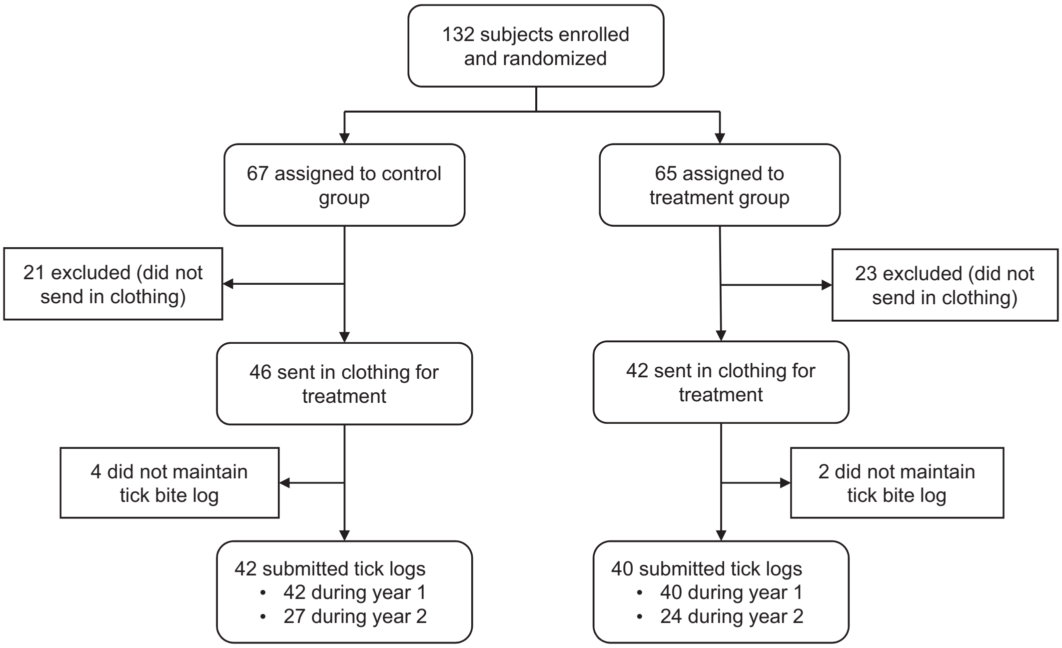

Fig. 1. Study participant enrollment randomization and follow-up.

Table 1. Demographic characteristics, history of tick bites and tick-borne disease, and tick bite prevent measures used among participants, by treatment status

	Control	Treatment	*P*-value
n	42	40	
Male (%)	35 (83.3)	27 (67.5)	0.158
Age (median [IQR])	40.00 [33.3, 53.0]	40.5 [32.8, 57.0]	0.686[a]
Education (%)			0.493
High school or less	2 (4.8)	3 (7.5)	
Some college	13 (31.0)	8 (20.0)	
Bachelor's or graduate degree	27 (64.3)	29 (72.5)	
Employer and Garment type[b] (%)			0.283
Government-uniform	26 (61.9)	18 (45.0)	
Government-no uniform	2 (4.8)	3 (7.5)	
Public-uniform	0 (0.0)	2 (5.0)	
Private-uniform	4 (9.5)	7 (17.5)	
Private-no uniform	7 (16.7)	4 (10.0)	
Self-employed-no uniform	3 (7.1)	6 (15.0)	
Duration of employment (median [IQR])	5.2 [2.0, 9.4]	4.9 [1.3, 15.1]	0.917[a]
Number of bites in previous year (median [IQR])	2.0 [0, 4]	1 [0, 3]	0.434[a]
Prior tick-borne disease diagnosis (%)			
Lyme	12 (27.9)	10 (25.0)	0.908
Babesiosis	2 (4.6)	1 (2.5)	1.000
Anaplasmosis	0 (0.0)	2 (5.0)	1.000
RMSF	1 (6.7)	0 (0.0)	0.453
Ehrlichiosis	0 (0.0)	1 (2.5)	0.980
Tick bite prevention measures (ever vs. never) (%)			
Long pants	41 (97.6)	40 (100.0)	1.000
Hat	33 (78.6)	35 (87.5)	0.435
Long-sleeved shirt	40 (95.2)	38 (95.0)	1.000
Tuck pants into boots/socks	20 (47.6)	25 (62.5)	0.258
Tick check during the day	42 (100.0)	37 (94.9)	0.442
Tick check at the end of day	42 (100.0)	40 (100.0)	NA
Insect repellent applied to skin	33 (78.6)	34 (85.0)	0.641
Insect repellent applied to clothes	35 (83.3)	35 (87.5)	0.825
Self-applied permethrin to clothing (%)	23 (62.2)	17 (50.0)	0.428
Use of DEET on clothing (%)	27 (73.0)	19 (55.9)	0.209

RMSF: Rocky Mountain Spotted Fever.

[a]Kruskal–Wallis rank-sum test used for nonparametric variables.

[b]Garment type classified as uniform-like or nonuniform. Uniform-like garments contained higher polyester content (>50%) relative to cotton.

tick bites received), participant loss was not considered a significant threat to the validity of the study results.

Protective Effectiveness Against Tick Bites: Primary Outcome

Throughout the first year, 40 subjects in the treatment group and 42 subjects in the control group completed weekly tick logs. All subjects logged the number of hours worked outdoors, the number of tick encounters, and the number of tick bites received each week. In the first study year, there were 24 tick bites reported in the treatment group (incidence rate, 0.13 per 100 outdoor work hours) and 78 bites reported in the control group (incidence rate, 0.37 per 100 outdoor work hours), for a protective effectiveness of 65% (95% CI: 45–78%). In the second year, 16 subjects in the treatment group and 15 in the control group were lost to follow-up. For the 24 subjects who remained in the treatment group, 36 bites were reported (incidence rate, 0.25 per 100 outdoor work hours), and among the 27 subjects remaining in the control group, 88 tick bites were reported (incidence rate, 0.50 per 100 outdoor work hours). The protective effectiveness of LLPI treatment in the second year was 50% (95% CI: 27–66%).

Overall, the 2-yr protective effectiveness of LLPI clothing against tick bites in the treatment group compared to the control group was 58% (95% CI: 43–69%). An absolute decrease in protective effectiveness against tick bites of 15 percentage-points was observed from the first to second year of the study (Table 2).

Protective Effectiveness Against Tick Encounters: Secondary Outcome

During the first study year, a total of 2,205 tick encounters were recorded over 39,901 outdoor working hours. Among the treated group, the incidence rate of tick encounters was 4.24 per 100 outdoor work hours compared with 6.67 encounters per 100 outdoor work hours among controls. The year 1 protective effectiveness of the treatment against tick encounters was 36% (95% CI: 31–42%). In the second study year, the total number of tick encounters and total outdoor work hours dropped to 1,770 encounters across 31,729 work hours. The incidence rate among the treatment group was 3.81 encounters per 100 outdoor work hours and among the control group, 7.04 encounters per 100 outdoor work hours for a protective effectiveness of 46% (95% CI: 40–51%) in the second year. The combined 2-yr protective effectiveness of LLPI clothing against all tick encounters was 41% (95% CI: 37–44%) with a 10% absolute increase in protective effectiveness observed against tick encounters from year 1 to year 2 of the study among the treated (Table 3).

Participant Adherence to Study Clothing

Overall, the most frequently worn items of study clothing were socks, which were reported as being worn most or all of the time while at work by 84% of participants in the first year and 86% in the second year. Adherence was also high for study pants, which were worn most or all of the time while at work by 83% of participants in year 1 and 77% in year 2, as well as the study shirt which was reported as worn most or all of the time at work by 81% of participants in year 1 and 71% of participants in year 2. For both study years, zero participants reported never wearing their study pants and shirt and only one participant reported never wearing the study socks. Hats had the lowest reported frequency of being worn (33% reported wearing a study hat most or all of the time in year 1 and 14% in year 2); however, hats were not a required uniform item for all participants and were only treated for participants who mailed them in. The poor adherence to wearing a study hat is likely due to the small number of participants who ever wore a hat and sent one in for treatment. These results are summarized in Supp Table 2 [online only].

Adverse Events and Tick-Borne Diseases

No adverse events related to treatment of participants' clothing or uniforms were reported throughout the study. Three participants were treated with doxycycline, prescribed by their personal physician, for an incident tick-borne disease. All three participants were in the control group.

Discussion

In this randomized controlled trial of 82 outdoor workers in Rhode Island and southern Massachusetts, LLPI clothing exhibited a 58% protective effectiveness against tick bites over 2 yr compared with untreated clothing. In the first year of the study, LLPI treated clothing significantly reduced tick bites by 65%, even when participants in both groups utilized other preventative tick bite measures. From the first to second year of the study, the protective effectiveness of LLPI clothing against tick bites decreased by 15 percentage-points to a 50% reduction in tick bites in the second year.

While the decrease in protective effectiveness against tick bites over time was expected and has been shown previously (Vaughn et al. 2014), the magnitude of reduction was smaller than was observed in a previous study among North Carolina outdoor workers (Vaughn et al. 2014). However, the 65% protective effectiveness

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

	N	Total tick bites	Total outdoor work hours	Tick bites per 100 outdoor work hours	Incidence rate difference (95% CI)	Protective effectiveness (95% CI)	*P*-value
Year 1							
Treatment	40	24	18,769	0.13	−0.24 (−0.34, −0.15)	0.65 (0.45, 0.78)	<0.001
Control	42	78	21,132	0.37			
Year 2							
Treatment	24	36	14,352	0.25	−0.25 (−0.55, 0.04)	0.50 (0.27, 0.66)	<0.001
Control	27	88	17,377	0.50			
Years 1 and 2							
Treatment	40	60	33,121	0.18	−0.25 (−0.42, −0.07)	0.58 (0.43, 0.69)	<0.001
Control	42	166	38,550	0.43			

CI: confidence interval.

Table 3. Incidence of work-related tick encounters by treatment group and year of follow-up

	N	Total tick encounters	Total outdoor work hours	Tick encounter incidence per 100 outdoor work hours	Incidence rate difference (95% CI)	Protective effectiveness (95% CI)	*P*-value
Year 1							
Treatment	40	796	18,769	4.24	−2.42 (−2.88, −1.97)	0.36 (0.31, 0.42)	<0.001
Control	42	1,409[a]	21,132	6.67			
Year 2							
Treatment	24	547[b]	14,352	3.81	−3.22 (−4.34, −2.11)	0.46 (0.40, 0.51)	<0.001
Control	27	1,223	17,377	7.04			
Years 1 and 2							
Treatment	40	1,343	33,121	4.05	−2.78 (−3.52, −2.03)	0.41 (0.37, 0.44)	<0.001
Control	42	2,631	38,509	6.83			

CI: confidence interval.

[a]One subject reported 100 tick encounters in 1 wk.

[b]One subject reported 200 tick encounters in 1 wk.

against tick bites achieved in the first year of this study is less than the 80% protective effectiveness that was observed during the first year of LLPI uniform wear by Vaughn and colleagues.

There are a few possible explanations for the differences in effectiveness between the results reported here and the results of the NC study. First, it is possible that blacklegged ticks, the primary human biting tick in our study areas of Rhode Island and southern Massachusetts, were less affected by the permethrin-treated clothing than the lone star tick most commonly observed in the NC study. However, evidence from a recent laboratory-based contact irritancy study demonstrated that LLPI clothing had a stronger and faster knockdown effect on *I. scapularis* nymphs and adults than their *A. americanum* counterparts (Prose et al. 2018). Second, there was a greater variety of garment types and fabrics used in the current study than in the NC study. In the present study, 57 participants (70%) wore high polyester content, uniform-like outfits for work, as opposed to the exclusive use of uniforms (65% polyester/35% cotton) in the NC study. It has been previously shown that initial permethrin content and retention rates depend on fabric material (Richards et al. 2018), thus it is possible that some of the fabric types used in this study initially absorbed less permethrin and/or lost permethrin content at a faster rate. Finally, the hotter climate and lower latitude in NC could have led to faster release of permethrin from fabric either by a direct effect (temperature, UV exposure) or indirect effect (perspiration), possibly explaining the larger decline in protective effectiveness between the first and second study years in NC compared to the present study.

Our study has several limitations. Primarily, the study protocol followed an intent-to-treat design, which assumed that participants exclusively wore their treated or untreated clothing for all outdoor work, but imperfect adherence to the study treatment plan could have biased measures of protective effectiveness toward the null. Secondly, the study experienced loss to follow-up between years 1 and 2 (38% of participants overall were lost). Characteristics of participants who were lost to follow-up did not differ significantly between study arms with regard to tick exposure history, prior diagnosis with a tick-borne disease, or any of the tick bite prevention measures assessed (Supp Table 1 [online only]); thus, we do not expect that loss of these subjects strongly influenced the results of the study. Third, self-application of permethrin to study clothing was reported by 40 participants at baseline. Of these, 23 were in the control group and 17 in the treatment group, which was not significantly different (Table 1). The duration and magnitude of effectiveness that self-application of permethrin to clothing offers against tick bites varies by treatment method, brand, and adherence to directions (Faulde et al. 2003, Faulde and Uedelhoven 2006). Because of this, a reliable measure of effect that self-applied permethrin treatment may have had on this study could not be estimated and was not corrected for in our analyses. Finally, unmasking of treatment status by participants was not assessed. However, permethrin has been documented as odorless (Schreck et al. 1978, Insect Shield 2019) and does not alter the appearance of clothing once applied (Insect Shield), thus treated and sham-treated clothing were indistinguishable by sight and smell.

This study is the first RCT to field test LLPI clothing among outdoor workers in an environment where *I. scapularis* is the dominant human biting tick. Prior studies have shown factory permethrin impregnation of uniforms to be highly effective at preventing tick bites from the lone star tick among outdoor workers (Vaughn and Meshnick 2011, Vaughn et al. 2014). However, the effectiveness of the same treatment method under field test conditions against blacklegged ticks—the vector of the three most frequently reported tick-borne diseases in the United States—had yet to be evaluated and was the primary impetus for this study. As such, we have shown that long-lasting permethrin impregnated clothing provides significant levels of protection from bites of blacklegged ticks among outdoor workers and retains moderate protective effectiveness through 2 yr of routine field work wear and laundering. In conclusion, our study indicates that that LLPI clothing is a low maintenance, long lasting, and effective method of achieving protection against tick bites for outdoor workers.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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References Cited

Adamek, B., A. Ksiaiek, A. Szczerba-Sachs, J. Kasperczyk, and A. Wiczkowski. 2006. Tick-borne diseases exposure of forestry workers and preventive methods usage. Przegl. Epidemiol. 60: 11–15.

Adjemian, J., I. B. Weber, J. McQuiston, K. S. Griffith, P. S. Mead, W. Nicholson, A. Roche, M. Schriefer, M. Fischer, O. Kosoy, et al. 2012. Zoonotic infections among employees from Great Smoky Mountains and Rocky Mountain National Parks, 2008-2009. Vector Borne Zoonotic Dis. 12: 922–931.

Aylward, L. L., K. Irwin, A. St-Amand, A. Nong, and S. M. Hays. 2018. Screening-level biomonitoring equivalents for tiered interpretation of urinary 3-phenoxybenzoic acid (3-PBA) in a risk assessment context. Regul. Toxicol. Pharmacol. 92: 29–38.

CDC. 2019. Tickborne Disease Surveillance Data Summary. https://www.cdc.gov/ticks/data-summary/index.html/and/Tickborne/Diseases/of/the/United/States./Available/from/https://www.cdc.gov/ticks/diseases/index.html

Connally, N. P., D. A. Rose, N. E. Breuner, R. Prose, A. C. Fleshman, K. Thompson, L. Wolfe, C. D. Broeckling, and L. Eisen. 2019. Impact of wearing and washing/drying of permethrin-treated clothing on their contact irritancy and toxicity for nymphal *Ixodes scapularis* (Acari: Ixodidae) ticks. J. Med. Entomol. 56: 199–214.

(EPA) Environmental Protection Agency. 2017. Repellent-treated clothing. https://www.epa.gov/insect-repellents/repellent-treated-clothing

Evans, S. R., G. W. Korch, Jr, 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. Uedelhoven. 2006. A new clothing impregnation method for personal protection against ticks and biting insects. Int. J. Med. Microbiol. 2: 225–229.

Faulde, M. K., W. M. Uedelhoven, and R. G. Robbins. 2003. 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. 40: 935–941.

Gopalakrishnan, R., A. K. Chaurasia, I. Baruah, and V. Veer. 2014. Evaluation of permethrin-impregnated military uniforms for contact toxicity against mosquitoes and persistence in repeated washings. Int. J. Environ. Sci. Technol. 11:1855–1860.

Insect Shield. 2019. Research: knockdown testing overview. www.insectshield.com/ourTechnology/Research.aspx

Lane, R. S. 1989. Treatment of clothing with a permethrin spray for personal protection against the western black-legged tick, *Ixodes pacificus* (Acari: Ixodidae). Exp. Appl. Acarol. 6: 343–352.

Miller, N. J., E. E. Rainone, M. C. Dyer, M. L. González, and T. N. Mather. 2011. Tick bite protection with permethrin-treated summer-weight clothing. J. Med. Entomol. 48: 327–333.

Mount, G. A., and E. L. Snoddy. 1983. Pressurized sprays of permethrin and deet on clothing for personal protection against the lone star tick and the American dog tick (Acari: Ixodidae). J. Econ. Entomol. 76: 529–531.

(NIOSH) National Institute for Occupational Safety and Health. 2017. Tick-borne Diseases. https://www.cdc.gov/niosh/topics/tick-borne/recommendation.html

Proctor, S. P., A. L. Maule, K. J. Heaton, B. S. Cadarette, K. I. Guerriere, C. C. Haven, K. M. Taylor, M. M. Scarpaci, M. Ospina, and A. M. Calafat. 2019. Permethrin exposure from wearing fabric-treated military uniforms in high heat conditions under varying wear-time scenarios. J. Expo. Sci. Environ. Epidemiol.: 1–12.

Prose, R., N. E. Breuner, T. L. Johnson, R. J. Eisen, and L. Eisen. 2018. Contact irritancy and toxicity of permethrin-treated clothing for *Ixodes scapularis*, *Amblyomma americanum*, and *Dermacentor variabilis* ticks (Acari: Ixodidae). J. Med. Entomol. 55: 1217–1224.

Richards, S. L., N. Agada, J. A. G. Balanay, and A. V. White. 2018. Permethrin treated clothing to protect outdoor workers: evaluation of different methods for mosquito exposure against populations with differing resistance status. Pathog. Glob. Health. 112: 13–21.

Schreck, C. E., K. Posey, and D. Smith. 1978. Durability of permethrin as a potential clothing treatment to protect against blood-feeding arthropods. J. Econ. Entomol. 71: 397–400.

Schreck, C. E., G. A. Mount, and D. A. Carlson. 1982. Wear and wash persistence of permethrin used as a clothing treatment for personal protection against the lone star tick (Acari: Ixodidae). J. Med. Entomol. 19: 143–146.

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* (Acari: Ixodidae). J. Med. Entomol. 23: 396–399.

Sullivan, K. M., A. Poffley, S. Funkhouser, J. Driver, J. Ross, M. Ospina, A. M. Calafat, C. B. Beard, A. White, J. A. Balanay, et al. 2019. Bioabsorption and effectiveness of long-lasting permethrin-treated uniforms over three months among North Carolina outdoor workers. Parasit. Vectors. 12: 52.

Vaughn, M. F., and S. R. Meshnick. 2011. Pilot study assessing the effectiveness of long-lasting permethrin-impregnated clothing for the prevention of tick bites. Vector Borne Zoonotic Dis. 11: 869–875.

Vaughn, M. F., S. W. Funkhouser, F. C. Lin, J. Fine, J. J. Juliano, C. S. Apperson, and S. R. Meshnick. 2014. Long-lasting permethrin impregnated uniforms: a randomized-controlled trial for tick bite prevention. Am. J. Prev. Med. 46: 473–480.

Vázquez, M., C. Muehlenbein, M. Cartter, E. B. Hayes, S. Ertel, and E. D. Shapiro. 2008. Effectiveness of personal protective measures to prevent Lyme disease. Emerg. Infect. Dis. 14: 210–216.

Wallace, J. W., W. L. Nicholson, J. L. Perniciaro, M. F. Vaughn, S. Funkhouser, J. J. Juliano, S. Lee, M. L. Kakumanu, L. Ponnusamy, C. S. Apperson, and S.R. Meshnick. 2016. Incident tick-borne infections in a cohort of North Carolina outdoor workers. Vector Borne Zoonotic Dis. 16: 302–8.