HHS Public Access

Author manuscript

J Med Entomol. Author manuscript; available in PMC 2020 January 08.

Published in final edited form as:

J Med Entomol. 2019 January 08; 56(1): 199–214. doi:10.1093/jme/tjy138.

Impact of Wearing and Washing/Drying of Permethrin-Treated Clothing on Their Contact Irritancy and Toxicity for Nymphal *Ixodes scapularis* (Acari: Ixodidae) Ticks

Neeta P. Connally[1,4], **Dominic A. Rose**[2], **Nicole E. Breuner**[2], **Robert Prose**[2], **Amy C. Fleshman**[2], **Karen Thompson**[1], **Lisa Wolfe**[3], **Corey D. Broeckling**[3], and **Lars Eisen**[2]

[1]Department of Biological & Environmental Sciences, Western Connecticut State University, Danbury, CT 06810,

[2]Division of Vector Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, CO 80521,

[3]Proteomics and Metabolomics Facility, Colorado State University, Fort Collins, CO 80523

Abstract

Permethrin-treated clothing is available as consumer products to prevent bites by tick and insect pests. We used bioassays to examine the impact of wearing and washing/drying of permethrin-treated shirts, pants, and socks, and wearing of treated shoes, on their contact irritancy and toxicity for nymphal *Ixodes scapularis* Say (Acari: Ixodidae) ticks, the primary vectors in the eastern United States of the causative agents of Lyme disease, human anaplasmosis, and human babesiosis. Pristine permethrin-treated clothing displayed strong contact irritancy and toxicity toward *I. scapularis* nymphs, with 0–30% of ticks across clothing types and tick sources displaying normal movement 1 h after forced contact for 30–120 s with treated textile. Following 16 d of wear and 16 rounds of machine washing and drying, we recorded reduced concentrations (by 50–90%) of permethrin, compared with pristine treated clothing, from shirts, pants, and socks. This loss of permethrin was associated with reduced contact irritancy and toxicity for ticks after forced contact with worn and washed/dried treated clothing: 31–67% of ticks displayed normal movement 1 h after contact. Nevertheless, the worn and washed/dried treated clothing was still superior to nontreated textile, for which 90–100% of ticks displayed normal movement. Treated shoes, which were worn but not washed, remained as toxic to the ticks as pristine treated shoes. We caution that these laboratory bioassay results should not be interpreted as being directly indicative of the outcome of using washed/worn permethrin-treated clothing in daily life. Although wear and washing/drying did reduce the irritancy and toxicity of permethrin-treated clothing for *I. scapularis* nymphs more than we had expected, the remaining effect might still reduce the risk of tick bites in a real-life scenario.

Keywords

Ixodes scapularis; permethrin; clothing; toxicity; irritancy

[4]Corresponding author, connallyn@wcsu.edu.

The nymphal life stage of the black-legged tick, *Ixodes scapularis* Say (Acari: Ixodidae), is the primary vector in the eastern United States of the causative agents of Lyme disease, human anaplasmosis, and human babesiosis (Piesman and Eisen 2008, Eisen et al. 2017a). Use of personal protective measures is the first line of defense against this tick (Hayes and Piesman 2003, Eisen and Eisen 2018). Consumer products to reduce the risk of tick bites include a wide range of noncontact spatial tick repellents (e.g., N,N-Diethyl meta toluamide [DEET]-based products), tick-irritant permethrin sprays for treatment of clothing, and clothing factory-impregnated with permethrin (Dolan and Panella 2011, Eisen and Dolan 2016). A seminal study by Miller et al. (2011), where human volunteers allowed themselves to receive tick bites, demonstrated that wearing permethrin-impregnated clothing (shoes, socks, shorts, and shirts) not only reduced the number of laboratory-reared *I. scapularis* nymphs that attached to human volunteers but also led to the majority of attached nymphs dying within hours of their attachment. We further demonstrated that permethrin-impregnated clothing, in the form of shirts, pants, and socks from Insect Shield, LLC (Greensboro, NC), has both contact irritant and toxicant effects on *I. scapularis* nymphs (Eisen et al. 2017b, Prose et al. 2018). However, published studies are lacking for how wearing and washing/drying of permethrin-impregnated clothing may impact their contact irritancy and toxicity for *I. scapularis* nymphs.

Previous studies have evaluated the impact of repeated washing, or wearing and washing, of permethrin-impregnated military uniforms on their protective effect against the lone star tick, *Amblyomma americanum* (L.) (Acari: Ixodidae), or the common tick (a.k.a., castor bean tick), *Ixodes ricinus* (L.) (Acari: Ixodidae) (Schreck et al. 1978, 1982; Evans et al. 1990; Faulde et al. 2003, 2006; Faulde and Uedelhoven 2006). An early field trial where subjects wore permethrin-impregnated military uniforms in tick-infested areas showed that the uniforms remained highly protective against *A. americanum* ticks—as determined by recovery of only dead or moribund nymphal or adult ticks from clothing or skin of subjects wearing treated uniforms—for up to 132 h of wear stress or three washes in warm water with detergent followed by drying at high heat (Schreck et al. 1982). A few live or attached but dead ticks were recovered from subjects wearing uniforms having been washed four times. The concentration of permethrin in these textiles, which were treated by being saturated with an aqueous permethrin suspension with the goal of achieving absorption of 0.12–0.20 mg permethrin per cm^2 of textile, fell by only 5% following 132 h of being worn but was reduced by ~50% after four washes (Schreck et al. 1982). Another study using a similar impregnation process for military uniforms reported >50% decline in the concentration of permethrin after two washes and ~75% decline after four to five washes (Evans et al. 1990).

Subsequent refined methodology for impregnation of military uniforms employed polymerization of permethrin onto the fiber surface (polymer-coating method) rather than absorption of permethrin onto the fiber surface via dipping in a permethrin solution (absorption method) (Faulde et al. 2016). Military uniforms impregnated with permethrin via the polymer-coating method have far superior retention of permethrin following washing, with only ~10% decline in the concentration of permethrin after 10 washes at 60°C with a light-duty detergent and demonstrated potential to remain above a 0.20 mg permethrin per cm^2 of textile after 100 washes (Faulde et al. 2003, Faulde and Uedelhoven

2006). Bioassay challenges where field-collected *I. ricinus* nymphs were held in continuous contact with military uniform textiles impregnated with permethrin via the polymer-coating method resulted in 100% knockdown (inability to move/migrate) by 7 min of exposure for unwashed treated textile, increasing to 15 min after 100 washes (Faulde et al. 2003, Faulde and Uedelhoven 2006). Additional trials with uniforms worn under field conditions for several months and washed 70–100 times resulted in 100% knockdown after 6.8–19.5 min of continuous exposure with increasing knockdown time related to decreasing concentration of permethrin in the test textile samples (Faulde and Uedelhoven 2006, Faulde et al. 2006).

Similar bioassay studies on the impact of wear and washing/drying on the protective efficacy of factory-impregnated permethrin-treated consumer product clothing against ticks are missing from the published literature. However, some recent studies have evaluated the impact of repeated washes on the permethrin content of consumer product permethrin-treated clothing, including clothing treated by Insect Shield (DeRaedt Banks et al. 2015, Faulde et al. 2016, Richards et al. 2017). Machine washing resulted in successive loss of permethrin from treated clothing in two studies, with averages remaining above 0.20 mg permethrin per cm^2 of textile after 20 washes in one study (Faulde et al. 2016), but falling below 0.20 mg permethrin per cm^2 of textile after 15 washes in the other study (DeRaedt Banks et al. 2015). Handwashing in cold water also resulted in loss of permethrin from treated textiles (Richards et al. 2017). However, it is not clear how such losses of permethrin from the textiles impact the irritancy and toxicity for ticks coming into contact with the textile.

The primary purpose of our study was to examine the impact of wearing and washing/drying of permethrin-treated consumer product clothing on its contact irritancy and toxicity against *I. scapularis* nymphs. Additionally, we compared the concentration of permethrin present in pristine treated clothing versus clothing that were washed/dried or worn and washed/dried.

Materials and Methods

Source of Permethrin-Treated Clothing and Nymphal *I. scapularis* Ticks

Permethrin-treated clothing included in the study were either purchased as already impregnated retail products from Insect Shield (T-shirts and socks) or purchased untreated and then sent to Insect Shield for impregnation (pants and shoes) (Fig. 1). Hereafter, T-shirts are referred to simply as shirts. The Insect Shield shirts were 100% cotton, whereas the Insect Shield socks were a blend of cotton (49%), polyester (49%), rubber (3%), and spandex (1%). The pants (Carhartt, Inc., Dearborn, MI) were 100% cotton, and the ‘tongue’ portion of the high top shoes (Nike, Inc., Beaverton, OR) used in the bioassays was 100% cotton canvas. For shirts, pants, and socks, tick challenge bioassays were conducted with pieces of textile that were: 1) treated but neither worn nor washed/dried (designated Treatment 1, T1, representing pristine treated clothing); 2) treated and washed/dried but not worn (Treatment 2, T2); and 3) treated, worn, and washed/dried (Treatment 3, T3, representing normal use). The shoe tongue textiles challenged in the bioassays were either treated but neither worn nor washed/dried (T1) or treated and worn but not washed (designated T3 as normal use because shoes are worn but rarely washed). The nontreated control (designated C) was always a 100% cotton shirt textile.

Laboratory-reared *I. scapularis* ticks used in bioassays were second-generation nymphs (CT14) originating from females collected in Fairfield County, CT, and maintained at the Centers for Disease Control and Prevention, Division of Vector-Borne Diseases, Fort Collins, CO. Prior to being used, the nymphs were held within desiccators (90–95% RH) in a growth chamber maintained at 21–22°C with a 16:8 (L:D) h cycle. Laboratory-reared nymphs used in the bioassays were, with a few exceptions, 3.2- to 5.2-mo postmolt. Field-collected nymphal ticks (CT-field) of unknown age used in the bioassays originated from three collection locations within Fairfield County, CT, in May–June 2016. Field-collected nymphs were collected by dragging a 1 m by 0.5 m flannel tick flag through the leaf litter for 30-s intervals and then inspecting the flag after each flag sample. Field-collected nymphs were held in the laboratory at room temperature in desiccators (85–95% RH) for ≥2 wk before being used.

Wear and Washing/Drying of Clothing

As noted above, the T3 shirts, pants, and socks were both worn and washed/dried, whereas the shoes were worn but not washed/dried. The T3 category included clothing worn by four different individual female human subjects (age range, 22–30) wearing similar clothing for outdoor fieldwork activities on 2 d/wk over an 8-wk period (total of 16 d) in late spring and early summer coinciding with the peak activity period for *I. scapularis* nymphs in Connecticut (Stafford 2007). Subjects conducted similar field activities while together in the same locations on the days wearing the treated clothing. Field sites included multiple locations in western Connecticut (Fairfield County), in public lands consisting primarily of deciduous or mixed deciduous and coniferous tree species with minimal to moderate understory vegetation. The shirts, pants, and socks were washed/dried twice weekly (16 total occasions) in a household washing machine using the medium heat setting (range 24.2–25.1°C, pH 6.4–6.5, chlorine 0–0.5 ppm, alkalinity 0–70 ppm) and All Free and Clear (Sun Products, New Providence, NJ). After being washed, the clothing was dried in a household dryer using the medium heat setting (approximately 57°C, per the manufacturer). The T2 shirt, pants, and socks were not worn but washed/dried 16 times using the same methods described above. The number of ticks detected on the clothing or body of human subjects was recorded daily. The component of this study involving humans wearing permethrin-treated clothing in the field was approved by the Institutional Review Board at Western Connecticut State University and by the Human Investigations Committee at the Connecticut Department of Public Health.

Contact Irritancy and Toxicity Bioassays With *I. scapularis* Nymphs

The contact irritancy and toxicity bioassays used to evaluate the impact of test textiles on *I. scapularis* nymphs were conducted using methods described in detail previously (Eisen et al. 2017b). Briefly, the contact irritancy assay employed a playing card (64 mm wide by 89 cm tall) covered by a solid piece of test textile (Fig. 1) and positioned at a 45° angle. Groups of 4–10 ticks were introduced in the center of the textile-covered card and the number of ticks still remaining on the textile was recorded at 1-min intervals over a 5-min period. Strong contact irritancy manifests as nymphal ticks becoming visibly agitated (‘hot-foot’ effect) and actively dislodging from treated textile by flipping over and tumbling downward along the textile-covered card until they fall off the bottom of the card. For trials with laboratory-

reared nymphs, each test textile was challenged with 5 groups of 10 ticks, for a total of 50 ticks (grand total of 1,390 ticks). Following the completion of the assays, ticks were held at room temperature in desiccators (90–95% RH) until scored for vigor (see section called 'Classification Scheme for Tick Vigor After Exposure to Test Textiles') at 1 and 24 h after exposure to the test textile. Assays with <80% of matched control ticks, originating from the same colony storage vials and tested on the same date, displaying normal activity at 24 h after exposure were excluded from data analysis relating specifically to tick vigor at 24 h after exposure to treated textile. For trials with field-collected nymphs, each test textile was challenged with groups of 4–5 nymphs, testing up to 40 ticks per textile (grand total of 338 ticks). Due to a shortage of field-collected nymphs, fewer ticks were tested upon sock and shoe textiles than for the shirt and pants textiles.

The toxicity assay exposed ticks to continuous contact with a test textile, cut into a 100-mm-diameter circle (Fig. 1) and placed within a horizontal petri dish, for a predetermined period of time. In this study, ticks were held in continuous contact with test textiles (prevented from moving off of the test textile) for 30, 60, or 120 s. These contact durations were found previously to severely impact the vigor of *I. scapularis* nymphs (Eisen et al. 2017b). For trials with laboratory-reared nymphs, each combination of test textile and exposure time was challenged with three groups of 10 ticks, for a total of 30 ticks (grand total of 2,320 ticks). Due to shortage of laboratory-reared ticks of uniform source and age, only T1 and T3 test textiles were challenged in the toxicity assay. Following the completion of the assays, ticks were held at room temperature in desiccators (90–95% RH) until they were scored for vigor at 1 and 24 h after exposure to the test textile. Assays with <80% of matched control ticks, originating from the same colony storage vials and tested on the same date, displaying normal activity at 24 h after exposure were excluded from data analysis relating specifically to tick vigor at 24 h after exposure to treated textile. Preliminary data analysis (not shown) revealed no consistent trends for a given type of clothing toward decreased proportions of ticks displaying normal behavior or willingness to ascend onto a finger with increasing exposure times within the 30–120 s exposure time range. The presented bioassay data therefore are combined across the 30, 60, and 120 s exposure times. For trials with field-collected nymphs, each test textile was challenged over a 60-s period with groups of 3–5 ticks, testing up to 21 ticks per textile (grand total of 120 ticks). Due to a shortage of available field-collected nymphs, toxicity assays were conducted only for shirt and pants textiles.

Classification Scheme for Tick Vigor After Exposure to Test Textiles

Our classification scheme for tick vigor at different time points after exposure to permethrin-treated textiles was described in detail previously (Eisen et al. 2017b) and is summarized here. Tick vigor following introduction onto a nontreated surface and stimulation of activity via gentle physical prodding and human breath was scored across four categories of capacity for movement: 1) tick completely motionless; 2) tick capable of some movement of the legs but unable to right itself or walk; 3) tick capable of righting itself but not able to move in a coordinated way or readily orient toward a stimulus; and 4) tick displaying normal movement and response to a stimulus. Ticks that were scored as displaying normal movement (4) were further assessed to determine if they would ascend onto a finger when

presented the opportunity. Our previous study (Eisen et al. 2017b) showed that *I. scapularis* nymphs exposed to permethrin-treated textiles may display normal movement but nevertheless be unwilling to ascend a human finger.

Quantification of Permethrin in Treated Textiles

Previous studies have expressed the concentration of permethrin present in a textile as mg permethrin per cm^2 or m^2 of textile (Schreck et al. 1978, 1982; Evans et al. 1990; Faulde et al. 2003, 2006, 2016; Faulde and Uedelhoven 2006; DeRaedt Banks et al. 2015; Richards et al. 2017). However, this is problematic when comparing textiles of different thickness or weave, or clothing made from different types of materials. We therefore prefer to express the concentration permethrin present in a textile as mg permethrin per g of textile. Pristine Insect Shield clothing are marketed as containing 0.52% permethrin (w/w), which equates to 5.2 mg of permethrin per g of textile.

Quantification of permethrin in treated test textiles used in the tick bioassays was done at the Colorado State University Proteomics and Metabolomics Facility (Fort Collins, CO). Three 1-cm^2 samples were cut from each treated test textile and their mass (g) was recorded. Three samples from a nontreated 100% cotton textile were included as a negative control. Aiming to recover ≥95% of the permethrin in the textile samples, a series of four consecutive extractions using 1 ml of solvent containing 100% methanol labeled with an internal standard, transpermethrin-(phenoxy-d5) (Sigma-Aldrich, St. Louis, MO) at 0.05 mg/ml, was performed. The samples were sonicated at room temperature for 15 min after each round of extraction. The average rate of recovery of permethrin from treated samples was estimated at 98.6%. Samples (4 ml total volume) were then stored at −80°C until further analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) performed using an Acquity UPLC system (Waters, Milford, MA) coupled with a Xevo TQ-S Tandem Quadrupole Mass Spectrometer (Waters). Chromatographic separation was achieved using a UPLC T3 C18 stationary phase (1 × 100 mm, 1.7 μM) column (Waters).

Mobile phases were 100% methanol (A) and water with 0.1% formic acid (B). The analytical gradient was as follows: time = 0 min, 80% B; time = 0.5 min, 80% B; time = 1 min, 99.9% B; time = 5 min, 99.9% B. The column was flushed to avoid carryover and re-equilibrated with a subsequent injection of 100% hexane with the following gradient: time = 0 min, 99.9% B; time = 2 min, 99.9% B; time = 2.5 min, 80% B; time = 5 min, 80% B. The flow rate was 220 μl/min and the injection volume was 1 μl for each sample and 5 μl for the hexane blank. Samples were held at 4°C in the autosampler and the column was maintained at 60°C. The mass spectrometry was operated in selected reaction monitoring and positive ionization modes, with the capillary voltage set to 3.2 kV. Interchannel delay was set to 3 ms. Transition ions for permethrin and trans-permethrin-d5 are 408 > 183, 355 and 413 > 188, 360, respectively. Collision energy was set at 10 volts and cone voltage was set to 30 volts for both target analyte and deuterated internal standard. The source temperature was 150°C and the desolvation temperature was 350°C. Desolvation gas flow was 1,000 liter/h, cone gas flow was 150 liter/h, and collision gas flow was 0.2 ml/min. The nebulizer pressure (nitrogen) was set to 7 Bar and argon was used as the collision gas. A calibration curve was generated for permethrin, ranging from 0 to 312 μg/ml, using the authentic permethrin

standard in 100% methanol solution. The internal standard concentration in the methanol curve was held constant at 0.05 mg/ml.

Resulting raw data files were imported into the Skyline open source software package (MacLean et al. 2010) and analytes were visually inspected for retention times and peak area integration. Peak areas were integrated between 1.5 and 2 min for all samples. Permethrin detected in treated test samples was normalized to the peak area of the deuterated internal standard present in each sample. Normalized peak areas were then quantified using the linear regression equation generated for permethrin from the calibration curve. Limits of detection (LOD) and limits of quantification (LOQ) represent the lower limits of detection and quantification for each compound in the matrix of this study. Two regression curves were plotted for this data set in order to accurately represent lower concentrations (i.e., less than 5 μg/ml). The permethrin LOD (0.05 μg/ml) and LOQ (0.16 μg/ml) were calculated as 3 times or 10 times the standard deviation (0.00022) of the blank divided by the slope (0.0135) of the low-range calibration curve (Broccardo et al. 2013). Finally, based on the mass (g) of each test sample, the uniform extraction volume (4 ml), and the permethrin concentrations in the extraction for the test samples (μg/ml), we calculated the concentration of permethrin in the test samples expressed as mg permethrin per g of textile. Outliers in the data for a given type of clothing (shirt, pants, sock, or shoe) and treatment were defined, using Tukey fences, as: values below $\text{Quartile}_1 - 1.5(\text{Quartile}_3 - \text{Quartile}_1)$ or above $\text{Quartile}_3 + 1.5(\text{Quartile}_3 - \text{Quartile}_1)$.

Bioassay Data Analysis

Pairwise statistical comparisons among treatments for a given type of clothing (shirt, pants, sock, or shoe) for the probability of ticks to remain on a test textile, display normal movement, or display normal movement and willingness to ascend onto a finger were based on likelihood ratio test, conducted in JMP Pro 13 (Cary, NC). Results were considered significant when $P < 0.05$, with Bonferroni correction to account for multiple pairwise comparisons for a given treatment and outcome measure.

Results

Quantification of Permethrin in Treated Textiles

The average concentrations of permethrin per g of textile cut from the pieces of pristine (T1), washed/dried (T2), or worn and washed/ dried (T3) treated clothing that were used in the bioassays are shown in Table 1. Nontreated control samples were below the limit of quantification for permethrin. Although the sample sizes were judged too small for statistical analysis, it is evident from the data that permethrin was lost from shirts, pants, and socks due to washing/drying of treated clothing. After 16 rounds of washing/drying (T2), approximately 90% of the permethrin appeared to have been lost, compared with pristine treated textile (T1), from shirts, with smaller percentage losses due to washing/drying for pants (~60%) and socks (~50%). Additional loss of permethrin that could be attributed to 16 d of wear, by comparing worn and washed/dried clothing (T3) with washed/dried but unworn clothing (T2), was not observed for either socks or shoes, whereas it was minimal (<2%) for shirts and limited (<15%) for pants.

Samples from pristine treated shirts, pants, and socks (T1) were found to contain, on average, 8–13 mg permethrin per g of textile (Table 1). After 16 washes and 16 d of wear, the average concentration of permethrin per g of textile remained above 5.2 mg for the socks but had fallen below 2.2 mg for the pants and below 1.2 mg for the shirts. Moreover, the concentration of permethrin per g of textile tended to be more variable (higher coefficient of variation) among worn and washed/dried samples (T3) compared with pristine samples (T1) from the same type of treated clothing (Table 1). High- or low-end outliers in the data for concentration of permethrin per g of worn and washed/dried textile were recorded for pants and shoe samples, which were purchased untreated and then sent in for treatment, but not for shirts and socks, which were purchased already impregnated.

Contact Irritancy Assay With Laboratory-Reared *I. scapularis* Nymphs (CT14)

Irritancy to ticks—The proportions of ticks still remaining on test textiles in the vertical contact irritancy assay at 1-min intervals over the 5-min observation period are illustrated in Fig. 2, and the results at the end of the 5-min observation period are summarized in Table 1. One notable finding for pristine treated textiles is that the ticks dislodged at a slower rate from socks than from shirts, pants, or shoes. Approximately half of the ticks had dislodged from shirts, pants, or shoes by 1 min after introduction, whereas this did not occur until after 5 min for socks despite the concentration of permethrin in pristine treated socks being comparable to that in shirts and pants (Fig. 2; Table 1).

Compared with the baselines for nontreated textile (C), the proportions of ticks remaining after 5 min on pristine permethrin-treated clothing (T1) were significantly reduced for all types of clothing (Table 1). The reduction in percentage points of ticks remaining on test textiles after 5 min ranged from 74–78% for the shoe and pants to 66% for the shirt and 34% for the sock. Following wear and washing/drying to mimic normal use (T3), the irritant effect was reduced from that of pristine clothing (T1), including significant reductions for the shirt, pants, and sock (Table 1). The reduction in percentage points of ticks remaining on test textiles after 5 min ranged from 58% for the shirt to 31–34% for the pants and sock. Worn and washed/dried clothing (T3) was still significantly more of an irritant than nontreated textile (C) in the case of the pants and the shoe (percentage point reductions of 43 and 72%, respectively) but not for the shirt or sock (Table 1). Washing/drying alone was found to impact the irritancy of the permethrin-treated clothing: significant increases in the proportion of ticks remaining on permethrin-treated clothing that specifically can be attributed to washing/drying (comparing T1 pristine and T2 washed/dried clothing) were recorded both for the shirt and pants (Table 1). Finally, permethrin-treated clothing that was worn and washed/dried (T3) performed similarly to clothing that was washed/dried but not worn (T2) for pants and socks, whereas the irritant effect was significantly reduced for the worn and washed/dried shirt (Table 1).

Tick vigor after exposure—Results for assessments of tick vigor at 1 and 24 h after exposure for variable time periods (<1–5 min) in the vertical contact irritancy assay are shown in Tables 2 and 3. Following exposure to nontreated textile (C), ≥97% of ticks displayed normal movement 1 h later and ≥90% still displayed normal movement after 24 h. This is in stark contrast to exposure to pristine treated clothing (T1), which resulted in

significantly lower proportions of ticks displaying normal movement after 1 h (0–4% across clothing types) with strong effects remaining after 24 h (0–16%). Notably, worn and washed/dried treated clothing (T3) was significantly less toxic to the ticks compared with pristine treated clothing (T1) for several textiles, including the shirt and sock at both 1 and 24 h, and the pants at 1 h (Tables 2 and 3). The increase in percentage points of ticks displaying normal activity after exposure to worn and washed/dried clothing (T3) ranged from 79% for the shirt at 1 h to 34–54% for the pants and sock at 1 h and the shirt and sock at 24 h. No similar increase was seen for the shoe, which was worn but not washed (Tables 2 and 3). Finally, although loss of toxicity resulted from the wear and washing/drying, it is important to note that worn and washed/dried clothing (T3) in the form of shirt, pants, and sock still were significantly more toxic to the ticks compared with nontreated textile (C) (Tables 2 and 3). The decrease in percentage points of ticks displaying normal activity after exposure to worn and washed/dried clothing (T3) ranged from 46–58% for the pants and sock to 19% for the shirt at 1 h; and from 50–71% across clothing types at 24 h.

The general patterns were similar across textile treatments for ticks with normal movement that also were willing to seek out and ascend a human finger (Tables 2 and 3). However, one notable finding for the 1 h time point was that many ticks displaying normal movement after exposure to a treated textile were unwilling to ascend onto a finger, including 46–56% of the ticks displaying normal movement after exposure to worn and washed/dried (T3) shirt and sock, and 80% for pants (Table 2). By 24 h, similar failure of ticks displaying normal movement to ascend onto a finger was observed only for 18–24% of ticks exposed to worn and washed/dried (T3) shirt and sock and 38% for pants (Table 3). Another interesting observation when comparing tick vigor at 1 and 24 h after exposure was that the percentage of ticks displaying normal movement decreased distinctly from 1 to 24 h, due to slowly developing toxic effects, after exposure to the worn and washed/dried (T3) shirt (from 79 to 39%) or pants (from 42 to 19%) but not for the sock (from 54 to 50%).

Contact Irritancy Assay With Field-Collected *I. scapularis* Nymphs (CT-Field)

Irritancy to ticks—The field-collected nymphs were less active in the vertical assay compared with the laboratory-reared nymphs: the proportion of field-collected nymphs remaining on nontreated control textile after 5 min (97% of 99 nymphs) was significantly higher than for laboratory-reared nymphs (78% of 240 nymphs) (likelihood ratio test; $P < 0.0001$). This may have contributed to the overall trend of the field-collected ticks being more likely than the laboratory-reared ticks to remain on treated clothing over the 5-min observation period (Table 1). Compared with the baselines for nontreated textile (C), the proportions of field-collected ticks remaining after 5 min on pristine permethrin-treated clothing (T1) were significantly reduced for all types of clothing, with percentage point reductions ranging from 46–47% for the shirt, pants, and shoe to 33% for the sock (Table 1). The data for worn and washed/dried treated clothing (T3) are suggestive of intermediate contact irritancy between nontreated textile (C) and pristine permethrin-treated clothing (T1).

Tick vigor after exposure—Results for assessments of tick vigor at 1 and 24 h after exposure in the contact irritancy assay are shown in Tables 4 and 5. The proportion of ticks

displaying normal movement 1 or 24 h after exposure to nontreated textile (C) was significantly higher (78–100%) across all clothing types and time points than for pristine permethrin-treated clothing (T1) (0–8%) (Tables 4 and 5). Similar results for worn and washed/dried treated clothing (T3) were intermediate, ranging from 6 to 60% of ticks displaying normal movement after exposure to shirt, pants, or sock. The worn and washed/dried treated clothing (T3) was significantly more toxic than nontreated textile (C) across all clothing types and time points (range of 40–82 percentage points reduction for ticks displaying normal movement) but significantly less toxic than pristine permethrin-treated clothing (T1) in the case of shirt at 1 and 24 h and sock at 1 h (33–60 percentage points increase).

Toxicity Assay With Laboratory-Reared *I. scapularis* Nymphs (CT14)

Results for assessments of tick vigor at 1 and 24 h after forced exposures (30–120 s) to textiles in the horizontal toxicity assay are shown in Tables 6 and 7. Following exposure to nontreated textile (C), all 580 examined ticks displayed normal movement 1 h later and 93–99% still displayed normal movement after 24 h. At 24 h, 77–84% of the ticks exposed to nontreated textile (C) displayed normal movement and ascended onto a human finger. In striking contrast, none of 360 ticks held in contact with pristine permethrin-treated shirt, pants, sock, or shoe textile (T1) displayed normal movement at 1 h (Table 6). By 24 h, only 1 of these 360 ticks (0.3%) displayed normal movement, and this tick was not willing to ascend a finger (Table 7). Across shirt, pants, and sock textile, worn and washed/dried treated clothing (T3) were significantly less toxic than pristine treated clothing (T1) but still significantly more toxic than nontreated textile (C), regardless of whether the outcome measure was ticks displaying normal movement (at 1 or 24 h) or displaying normal movement and ascending onto a human finger (24 h) (Tables 6 and 7). The increase in percentage points of ticks displaying normal activity after exposure to worn and washed/dried treated clothing (T3), compared with pristine treated clothing (T1), ranged from 59% for the shirt at 1 h to 29–42% for the shirt at 24 h and the pants and sock at 1 or 24 h. On the other hand, the decrease in percentage points of ticks displaying normal activity after exposure to worn and washed/dried treated clothing (T3), compared with nontreated textile (C), ranged from 58–69% for the shirt at 24 h and the pants and sock at 1 or 24 h, and to 41% for the shirt at 1 h. The toxicity of the T3 shoe textile, which was worn but not washed, was similar to the pristine (T1) shoe textile.

Two notable observations from the vertical assay also held true in the horizontal assay. At the 1 h time point most ticks displaying normal movement were unwilling to ascend onto a finger (Table 6). Moreover, when comparing tick vigor at 1 and 24 h after exposure, the percentage of ticks displaying normal movement decreased distinctly from 1 to 24 h, due to slowly developing toxic effects, after exposure to the washed and worn shirt (from 59 to 29%).

Toxicity Assay With Field-Collected *I. scapularis* Nymphs (CT-Field)

Results for assessments of tick vigor at 1 and 24 h after forced exposures (60 s) to shirt and pants textiles in the horizontal toxicity assay are shown in Tables 8 and 9. Exposure to pristine treated clothing (T1) or worn and washed/dried treated clothing (T3) resulted in

significant reductions in the proportion of ticks displaying normal movement compared with nontreated textile (C), both for shirt and pants at 1 and 24 h.

Bioassay Outcomes for Laboratory-Reared *I. scapularis* Nymphs (CT14) in Relation to Concentration of Permethrin in the Test Textile

The relationships, across all individual examined treated test textiles, between the concentration of permethrin per g of test textile and contact irritancy or tick vigor in the vertical and horizontal assays are illustrated in Figs. 3–5. These relationships should be interpreted with caution as the underlying data include multiple types of clothing (shirts, pants, socks, and shoes) and treatments (pristine, washed/dried, and worn and washed/dried). Nevertheless, some clear patterns emerge. First, data points for socks consistently are outliers as the socks appear to underperform in terms of irritancy and impact on tick vigor in relation to the concentration of permethrin they contain (Figs. 3–5). Disregarding the socks, the observed distributions across shirt, pants, and shoe textiles tend to be L-shaped with consistently high irritancy and low tick vigor after exposure to test samples with >5 mg permethrin per g of textile but variable irritancy and tick vigor when the permethrin concentrations fell below 5 mg per g of textile (Figs. 3–5).

Discussion

Pristine permethrin-treated clothing displayed strong irritancy and toxicity toward *I. scapularis* nymphs. This is consistent with the results from previous studies for *I. scapularis* exposed to permethrin-treated consumer product clothing (Miller et al. 2011, Eisen et al. 2017b, Prose et al. 2018) as well as for *I. scapularis* or closely related ticks, *Ixodes pacificus* Cooley and Kohls or *I. ricinus*, exposed to treated uniforms or coveralls (Schreck et al. 1986; Lane 1989; Evans et al. 1990; Faulde et al. 2003, 2006, 2008, 2014; Faulde and Uedelhoven 2006; Jordan et al. 2012). Washing/drying of treated clothing resulted in loss of permethrin from the textiles and decreased irritancy and toxicity for *I. scapularis* nymphs, but no additional substantial impact over that attributable to washing alone was evident from wearing the clothing. Loss of permethrin resulting primarily from washes rather than wear was reported previously for treated uniforms (Schreck et al. 1982). We only evaluated clothing after it had been worn (total of 16 d) and washed/dried (16 occasions total) over an 8-wk period (the approximate period of time for peak seasonal activity of *I. scapularis* nymphs in the Northeast), and therefore lack data for loss of permethrin over successive occasions of washing/drying. Other studies do provide such data for machine-washed permethrin-treated consumer product clothing: although the results vary across studies, they all indicate substantial loss of permethrin over the first 15 washes (DeRaedt Banks et al. 2015, Faulde et al. 2016, Richards et al. 2017).

Following wear and washing/drying, approximately 90% of the permethrin was lost from the shirt samples and 50–60% was lost from pants and sock samples. However, we caution that these reductions relate to the concentration of permethrin in the treated textile rather than to permethrin present on the surface of the fibers. The ‘dose’ of permethrin a tick receives while in contact with a treated textile likely results from a combination of factors, including how much permethrin is present on the surface of the fibers (and thus accessible to the tick),

the weave of the textile (impacting which surfaces of the tick make contact with the fibers, and their ability to cling onto textile fibers in a vertically oriented assay; Prose et al. 2018), and the behavior of the tick (simply walking or also flipping over to escape the irritant textile). Consequently, concentration of permethrin present in treated textile samples was not necessarily predictive of bioassay results. For example, laboratory-reared nymphs were more likely to remain in contact for 5 min with vertically oriented worn and washed/dried treated socks (82%) compared with shirts (64%) despite a fivefold higher concentration of permethrin in the sock test samples (Table 1). Moreover, the probability of ticks displaying normal movement and being willing to ascend a finger 1 and 24 h after timed exposure was similar for worn and washed/dried treated socks (15 and 27%, respectively) and shirts (22 and 21%). Although they are labor-intensive, bioassays thus remain the gold standard for assessing the potential impact of a permethrin-treated textile on tick behavior and vigor.

Despite the permethrin-treated clothing losing both contact irritancy and toxicity against *I. scapularis* nymphs following washing/drying (16 occasions) and wear (16 d), it is important to note that they still provided some protection compared to nontreated clothing. The data presented in Table 6 for vigor of ticks 1 h after timed exposure to test textiles from shirts, pants, or socks are of particular interest in this respect. The proportion of ticks displaying normal movement (and thus being potentially able to bite) 1 h after exposure was 100% for nontreated textile and 0% across textile types for pristine treated textile, whereas it was intermediate (31–59%) across treated textile types for worn and washed/dried treated clothing. Moreover, the proportions of ticks displaying normal movement and being willing to ascend a finger (and thus likely to be able to bite) 1 h after exposure to different types of worn and washed/dried treated clothing were even lower (7–22%). Similar patterns occurred for ticks examined for vigor 24 h after exposure (Table 7), when the percentage points for likelihood of displaying normal movement or displaying normal movement and being willing to ascend a finger were 50–68% lower across textile types for worn and washed/dried treated clothing compared to nontreated textile. We conclude that our wear and wash/drying scheme did result in reduced contact irritancy and toxicity of permethrin-treated clothing for *I. scapularis* in the bioassays but that they still far outperformed nontreated textiles. This agrees with previous studies on treated military uniforms where repeated washing resulted in reduced concentration of permethrin and increased time needed to incapacitate *I. ricinus* nymphs held in continuous contact with the textile (Faulde et al. 2003, Faulde and Uedelhoven 2006).

Plotting the concentration of permethrin in the textile samples against linked bioassay outcomes across all examined textile types revealed some intriguing trends (Figs. 3–5). Most importantly, we recorded consistently high irritancy and low tick vigor after exposure to test samples with >5 mg permethrin per g of textile but variable irritancy and tick vigor when the permethrin concentrations fell below 5 mg per g of textile. We also noted that the treated sock samples consistently stood out from shirt, pants, and shoe samples in that the sock samples had a less pronounced impact on the ticks in relation to their concentration of permethrin. We speculate that this may be related to the looser weave of this particular sock allowing ticks to move across the fibers with less of their body surface in contact with the treated textile. Alternatively, less of the permethrin may be located on the surface of the fibers in the socks compared to the other textile types. It is also possible that the treatment of

the synthetic blend of fibers in the sock is somehow different compared to the other textile types that were 100% cotton.

The study had several notable limitations. Because we did not examine a successive series of numbers of wash/dry occasions and days of use for the treated clothing, we have no information for the rate of loss in contact irritancy and toxicity between 1 wash/drying occasion and 1 d of wear and 16 wash/drying occasions and 16 d of wear. This would be interesting to explore in future studies. Moreover, we focused primarily on clothing made from cotton (except for the socks that were a cotton/polyester blend) and it is possible that the results would have been different for treated clothing made from synthetic materials or cotton/synthetic blends. Based on the divergent results for sock textile in the vertical contact irritancy assay, future studies would benefit from control textiles used in this bioassay being identical (but nontreated) to the treated test textiles. We also cannot rule out that a different regimen of washing or drying temperatures, or washing followed by air-drying rather than machine-drying, would have reduced the loss of tick contact irritancy and toxicity for the treated clothing. Drying clothes at high heat is generally recommended for killing ticks that go unnoticed on clothing worn outdoors (Carroll 2003, Nelson et al. 2016), but if such drying negatively impacts the killing efficacy of permethrin-treated clothing, this may be counterproductive in this special case. With regards to permethrin concentration, it also should be noted that tested samples from washed and worn clothing did not come from the same individual garments as the samples tested in a pristine treated state. Although unlikely, we therefore cannot rule out the possibility of some minor differences in permethrin concentration between the pristine treated clothing tested and the pristine clothing tested only later after being washed and worn. Limitations related to the permethrin quantification method are discussed above and general limitations of the bioas-says and tick vigor assessment, including the use of laboratory-reared versus field-collected ticks, were discussed previously (Eisen et al. 2017b, Prose et al. 2018).

Finally, we caution that laboratory bioassay results should not be interpreted as being directly indicative of the outcome of actually using permethrin-treated clothing in daily life. Even if wear and washing/drying did reduce the irritancy and toxicity of permethrin-treated clothing for *I. scapularis* nymphs more than we had expected, the remaining effect might still reduce the risk of tick bites in a real-life scenario. A prospective intervention study with human subjects wearing permethrin-treated and nontreated clothing during their daily activities in habitats where ticks may be encountered is needed to determine the potential for consumer product permethrin-treated clothing to reduce tick bites and tick-borne illness.

Acknowledgments

We thank Marc Dolan and Charlie Hoxmeier of the Centers for Disease Control and Prevention (CDC) and research interns at Western Connecticut State University for field and technical support; Brad Biggerstaff and Kalanthe Horiuchi of the CDC for statistical assistance; and Rebecca Eisen of the CDC for helpful discussions. Work conducted at Western Connecticut State University was supported by CDC through TickNET and the Emerging Infections Program cooperative agreement (5U50CK000195–05).

References Cited

Broccardo CJ, Schauer KL, Kohrt WM, Schwartz RS, Murphy JP, and Prenni JE. 2013 Multiplexed analysis of steroid hormones in human serum using novel microflow tile technology and LC-MS/MS. J. Chromatogr. B 934: 16–21.

Carroll JF 2003 A cautionary note: survival of nymphs of two species of ticks (Acari: Ixodidae) among clothes laundered in an automatic washer. J. Med. Entomol 40: 732–736. [PubMed: 14596290]

DeRaedt Banks S, Orsborne J, Gezan SA, Kaur H, Wilder-Smith A, Lindsey SW, and Logan JG. 2015 Permethrin-treated clothing as protection against the dengue vector, Aedes aegypti: extent and duration of protection. PLoS Negl. Trop. Dis 9: e0004109. [PubMed: 26440967]

Dolan MC, and Panella NA. 2011 A review of arthropod repellents, pp. 1–19. In Paluch GE and Coats JR (eds.), Recent developments in invertebrate repellents. American Chemical Society, Washington, DC.

Eisen L, and Dolan MC. 2016 Evidence for personal protective measures to reduce human contact with blacklegged ticks and for environmentally based control methods to suppress host-seeking blacklegged ticks and reduce infection with Lyme disease spirochetes in tick vectors and rodent reservoirs. J. Med. Entomol 53: 1063–1092.

Eisen RJ, and Eisen L. 2018 The blacklegged tick, Ixodes scapularis: an increasing health concern. Trends Parasitol. 34: 295–309. doi:10.1016/j.pt.2017.12.006. [PubMed: 29336985]

Eisen RJ, Kugeler KJ, Eisen L, Beard CB, and Paddock CD. 2017a Tick-borne zoonoses in the United States: persistent and emerging threats to human health. ILAR J. 58: 319–335. doi:10.1093/ilar/ilx005. [PubMed: 28369515]

Eisen L, Rose D, Prose R, Breuner NE, Dolan MC, Thompson K, and Connally N. 2017b Bioassays to evaluate non-contact spatial repellency, contact irritancy, and acute toxicity of permethrin-treated clothing against nymphal Ixodes scapularis ticks. Ticks Tick. Borne Dis 8: 837–849. [PubMed: 28754599]

Evans SR, Korch GW, Jr., and Lawson MA. 1990 Comparative field evaluation of permethrin and deet-treated military uniforms for personal protection against ticks (Acari). J. Med. Entomol 27: 829–834. [PubMed: 2231620]

Faulde M, and Uedelhoven W. 2006 A new clothing impregnation method for personal protection against ticks and biting insects. Int. J. Med. Microbiol 296 (Suppl 40): 225–229. [PubMed: 16524779]

Faulde MK, Uedelhoven WM, and Robbins RG. 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. [PubMed: 14765673]

Faulde MK, Uedelhoven WM, Malerius M, and Robbins RG. 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. [PubMed: 16808123]

Faulde M, Scharninghausen J, and Tisch M. 2008 Preventive effect of permethrin-impregnated clothing to Ixodes ricinus ticks and associated Borrelia burgdorferi s.l. in Germany. Int. J. Med. Microbiol 298 (S1): 321–324.

Faulde MK, Rutenfranz M, Keth A, Hepke J, Rogge M, and Görner A. 2014 Pilot study assessing the effectiveness of factory-treated, long-lasting permethrin-impregnated clothing for the prevention of tick bites during occupational tick exposure in highly infested military training areas, Germany. Parasitol. Res 114: 671–678. [PubMed: 25416331]

Faulde MK, Pages F, and Uedelhoven W. 2016 Bioactivity and laundering resistance of five commercially available, factory-treated permethrinimpregnated fabrics for the prevention of mosquito-borne diseases: the need for a standardized testing and licensing procedure. Parasitol. Res 115: 1573–1582. [PubMed: 26738734]

Hayes EB, and Piesman J. 2003 How can we prevent Lyme disease? N. Engl. J. Med 348: 2424–2430. [PubMed: 12802029]

Jordan RA, Schulze TL, and Dolan MC. 2012 Efficacy of plant-derived and synthetic compounds on clothing as repellents against Ixodes scapularis and Amblyomma americanum (Acari: Ixodidae). J. Med. Entomol 49: 101–106. [PubMed: 22308777]

Lane RS 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. [PubMed: 2743839]

MacLean B, Tomazela DM, Shulman N, Chambers M, Finney GL, Frewen B, Kern R, Tabb DL, Liebler DC, and MacCoss MJ. 2010 Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. Bioinformatics 26: 966–968. [PubMed: 20147306]

Miller NJ, Rainone EE, Dyer MC, González ML, and Mather TN. 2011 Tick bite protection with permethrin-treated summer-weight clothing. J. Med. Entomol 48: 327–333. [PubMed: 21485369]

Nelson CA, Hayes CM, Markowitz MA, Flynn JJ, Graham AC, Delorey MJ, Mead PS, and Dolan MC. 2016 The heat is on: killing blacklegged ticks in residential washers and dryers to prevent tickborne diseases. Ticks Tick. Borne Dis 7: 958–963. [PubMed: 27156138]

Piesman J, and Eisen L. 2008 Prevention of tick-borne diseases. Annu. Rev. Entomol 53: 323–343. [PubMed: 17877457]

Prose R, Breuner NE, Johnson TL, Eisen RJ, and Eisen L. 2018 Contact irritancy and toxicity of permethrin-treated clothing for Ixodes scapularis, Amblyomma americanum and Dermacentor variabilis ticks (Acari: Ixodidae). J. Med. Entomol 55: 55. doi:10.1093/jme/tjy062.

Richards SL, Balanay JAG, Harris JW, Banks VM, and Meshnick S. 2017 Residual effectiveness of permethrin-treated clothing for prevention of mosquito bites under simulated conditions. J. Environ. Health 79: 8–15.

Schreck CE, Posey K, and Smith D. 1978 Durability of permethrin as a potential clothing treatment to protect against blood-feeding arthropods. J. Econ. Entomol 71: 397–400. [PubMed: 690316]

Schreck CE, Mount GA, and Carlson DA. 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. [PubMed: 6123602]

Schreck CE, Snoddy EL, and Spielman A. 1986 Pressurized sprays of permethrin or deet on military clothing for personal protection against Ixodes dammini (Acari: Ixodidae). J. Med. Entomol 23: 396–399. [PubMed: 3735345]

Stafford KC, III. 2007 Tick management handbook An integrated guide for homeowners, pest control operators, and public health officials for the prevention of tick-associated disease. The Connecticut Agricultural Experiment Station, New Haven, CT, Bulletin No. 1010.

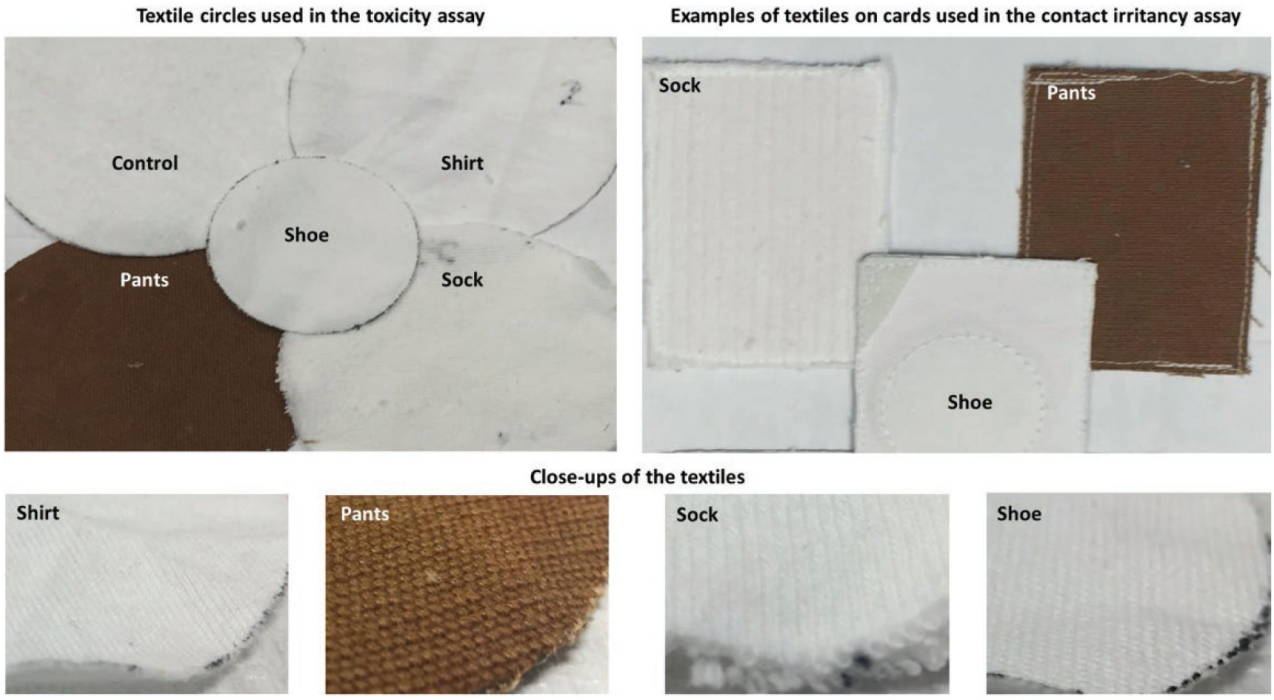

Fig. 1.
Types of permethrin-treated textiles included in the study.

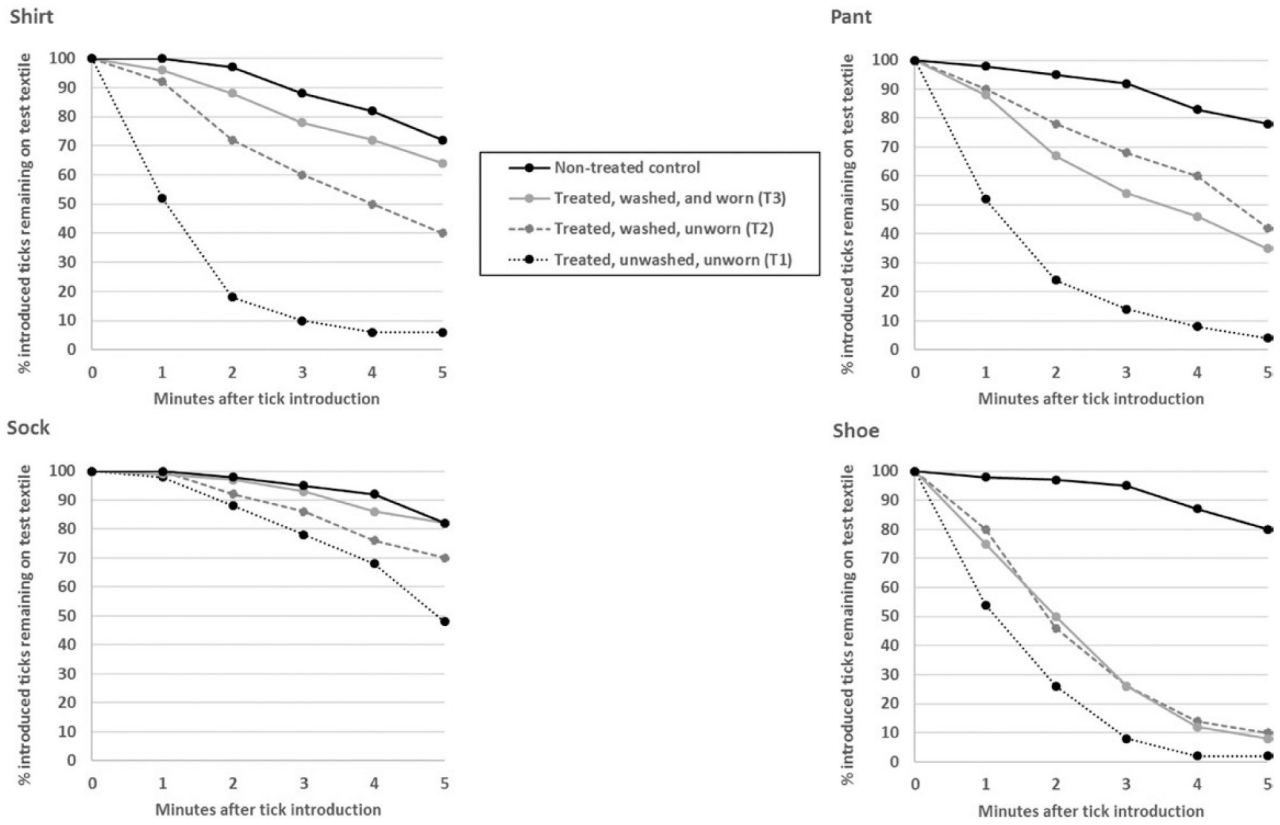

Fig. 2.

Percentages of laboratory-reared *I. scapularis* nymphs (CT14) remaining on test textiles over a 5-min period in the vertical contact irritancy assay. Note the exception that T3 shoes were worn but not washed.

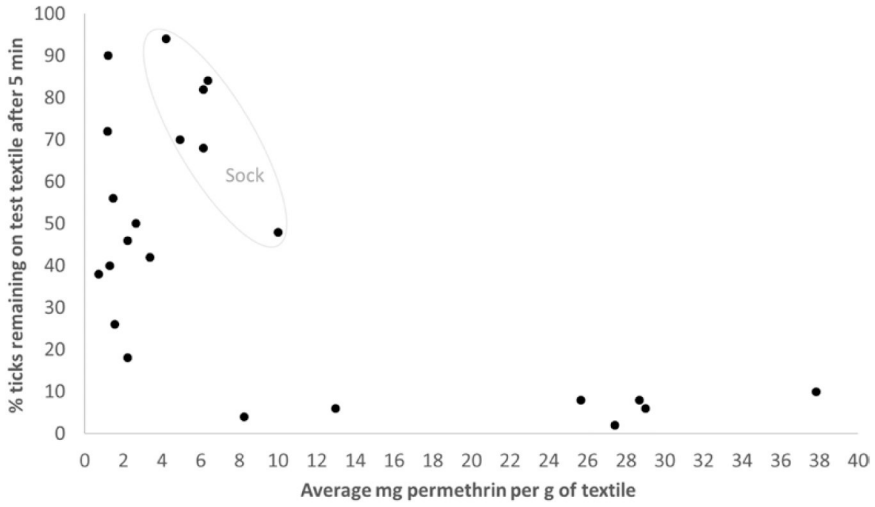

Fig. 3.

Ability of laboratory-reared *I. scapularis* nymphs (CT14) to remain in contact with treated textile in the vertical contact irritancy assay in relation to the permethrin concentration of the textile. Data for sock samples cluster as indicated within the elliptical shape overlaid on the graph.

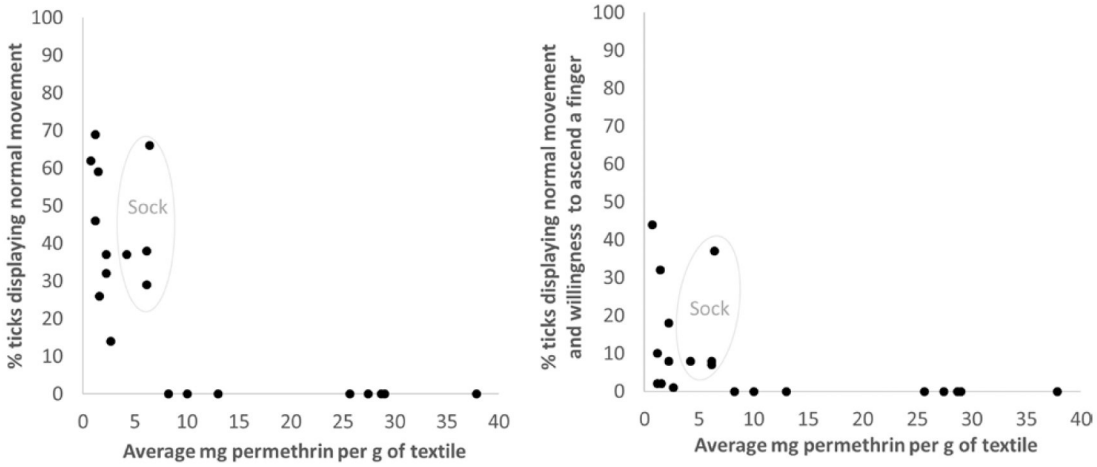

Fig. 4.

Vigor of laboratory-reared *I. scapularis* nymphs (CT14) 1 h after timed exposures (30–120 s) in the horizontal contact irritancy assay in relation to the permethrin concentration of the textile. Data for sock samples cluster as indicated within the elliptical shape overlaid on the graph.

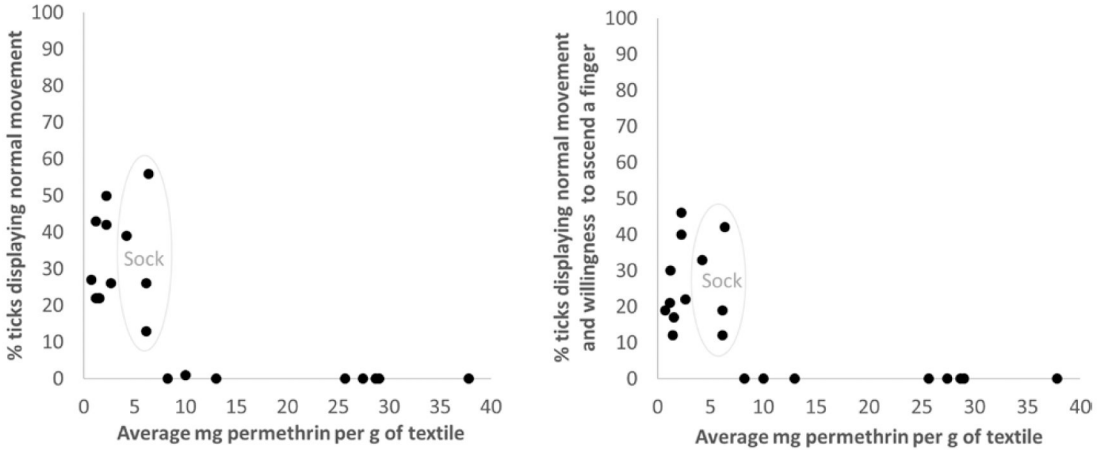

Fig. 5.

Vigor of laboratory-reared *I. scapularis* nymphs (CT14) 24 h after timed exposures (30–120 s) in the horizontal contact irritancy assay in relation to the permethrin concentration of the textile. Data for sock samples cluster as indicated within the elliptical shape overlaid on the graph.

Table 1.

Results of a contact irritancy assay challenging laboratory-reared (CT14) or field-collected (CT-field) *I. scapularis* nymphs introduced onto vertically oriented nontreated or permethrintreated clothing to remain in contact with the test textile over a 5-min period

		Laboratory-reared (CT14) ticks				Field-collected (CT-field) ticks			
		Outcome for ticks introduced onto test textile and observed over a 5-min period				Outcome for ticks introduced onto test textile and observed over a 5-min period			
			Still remaining on test textile after 5 min				Still remaining on test textile after 5 min		
Test textile and treatment	**Average (SD) permethrin concentration (mg permethrin per g textile) for three textile sample pieces**	**No. introduced onto test textile**	**No.**	**%**	**Statistically significant difference for % ticks remaining compared with other test textiles of the same type of clothing[a]**	**No. introduced onto test textile**	**No.**	**%**	**Statistically significant difference for % ticks remaining compared with other test textiles of the same type of clothing[a]**
Shirt									
Treated (Tl)	12.98 (2.25)	50	3	6	<T2, <T3, <C	40	19	48	<T3, <C
Treated and washed/dried (T2)	1.31 (0.08)	50	20	40	>T1, <T3, <C	10	7	70	None
Treated, worn, and washed/dried (T3)	1.14 (0.33)	200	128	64	>T1, >T2	40	32	80	>T1
Nontreated control (C)[b]	ND[C]	60	43	72	>T1, >T2	40	38	95	>T1
Pants									
Treated (Tl)	8.24 (0.94)	50	2	4	<T2, <T3, <C	30	16	53	<C
Treated and washed/dried (T2)	3.36 (0.82)	50	21	42	>T1, <C	10	8	80	None
Treated, worn, and washed/dried (T3)	2.19 (0.62)	200	70	35	>T1, <C	30	25	83	<C
Nontreated control (C)[b]	ND	60	47	78	>T1, >T2, >T3	30	30	100	>T1, >T3
Sock									
Treated (Tl)	10.01 (1.46)	50	24	48	<T3, <C	15	10	67	<C
Treated and washed/dried (T2)	4.93 (1.10)	50	35	70	None	10	8	80	None
Treated, worn, and washed/dried (T3)	5.72 (2.44)	200	164	82	>T1	20	15	75	None
Nontreated control (C)[b]	ND	60	49	82	>T1	15	15	100	>T1
Shoe									
Treated (Tl)	27.43 (3.63)	50	1	2	<C	15	7	47	<C
Treated and worn (T3)	30.28 (13.05)	200	16	8	<C	19	11	58	None
Nontreated control (C)[b]	ND	60	48	80	>T1, >T3	14	13	93	>T1

[a] Based on pairwise likelihood ratio test for different treatments within a given type of clothing (shirt, pants, sock, or shoe); statistical significance level adjusted (Bonferroni correction) to account for multiple pairwise comparisons

across textile treatments: from $P < 0.05$ to $P < 0.0083$ for six pairwise comparisons within a given type of clothing, or to $P < 0.0167$ for three pairwise comparisons within a given type of clothing.

[b] The nontreated control textile was always a 100% cotton shirt.

[c] ND, not determined.

J Med Entomol. Author manuscript; available in PMC 2020 January 08.

Table 2.

Vigor of laboratory-reared *I. scapularis* nymphs (CT14) 1 h after exposure for variable time periods (<1–5 min) to vertically oriented nontreated or permethrin-treated clothing

Test textile and treatment	No. examined	Ticks displaying normal movement[a]			Ticks displaying normal movement and willingness to ascend a human finger[b]		
		Normal movement		Statistically significant difference for % ticks with normal movement compared with other test textiles of the same type of clothing[C]	Normal movement and willing to ascend a human finger		Statistically significant difference for % ticks with normal movement and willingness to ascend finger compared with other test textiles of the same type of clothing[C]
		No.	%		No.	%	
Shirt							
Treated (T1)	50	0	0	<T2, <T3, <C	0	0	<T2, <T3
Treated and washed/dried (T2)	50	40	80	>T1, <C	22	44	>T1
Treated, worn, and washed/dried (T3)	200	157	79	>T1, <C	85	43	>T1
Nontreated control (C)[d]	60	59	98	>T1, >T2, >T3	ND[e]	ND	ND
Pants							
Treated (T1)	50	2	4	<T2, <T3, <C	0	0	None
Treated and washed/dried (T2)	50	13	26	>T1, <C	0	0	None
Treated, worn, and washed/dried (T3)	200	83	42	>T1, <C	16	8	None
Nontreated control (C)[d]	60	60	100	>T1, >T2, >T3	ND	ND	ND
Sock							
Treated (T1)	50	0	0	<T3, <C	0	0	<T3
Treated and washed/dried (T2)	50	3	6	<T3, <C	0	0	<T3
Treated, worn, and washed/dried (T3)	200	108	54	>T1, >T2, <C	48	24	>T1, >T2
Nontreated control (C)[d]	60	60	100	>T1, >T2, >T3	ND	ND	ND
Shoe							
Treated (T1)	50	0	0	<C	0	0	None
Treated and worn (T3)	200	0	0	<C	0	0	None
Nontreated control (C)[d]	60	58	97	>T1, >T3	ND	ND	ND

Average permethrin concentrations for given test textiles and treatments are the same as in Table 1.

[a]Excluding ticks that were not capable of any movement, capable only of some movement of the legs, or capable of righting themselves but not able to move in a coordinated way or orient toward a stimulus.

[b] Excluding ticks that were not capable of any movement, capable only of some movement of the legs, capable of righting themselves but not able to move in a coordinated way or orient toward a stimulus, or displaying normal movement but failing to ascend onto a human finger when provided the opportunity.

[c] Based on pairwise likelihood ratio test for different treatments within a given type of clothing (shirt, pants, sock, or shoe); statistical significance level adjusted (Bonferroni correction) to account for multiple pairwise comparisons across textile treatments: from $P < 0.05$ to $P < 0.0083$ for six pairwise comparisons within a given type of clothing, or to $P < 0.0167$ for three pairwise comparisons within a given type of clothing.

[d] The nontreated control textile was always a 100% cotton shirt.

[e] ND, not determined.

Table 3.

Vigor of laboratory-reared *I. scapularis* nymphs (CT14) 24 h after exposure for variable time periods (<1–5 min) to vertically oriented nontreated or permethrin-treated clothing

		Ticks displaying normal movement[a]			Ticks displaying normal movement and willingness to ascend a human finger[b]		
		Normal movement		Statistically significant difference for % ticks with normal movement compared with other test textiles of the same type of clothing[c]	Normal movement and willing to ascend a human finger		Statistically significant difference for % ticks with normal movement and willingness to ascend finger compared with other test textiles of the same type of clothing[C]
Test textile and treatment	No. examined	No.	%		No.	%	
Shirt							
Treated (T1)	50	0	0	<T2, <T3, <C	0	0	<T2, <T3, <C
Treated and washed/dried (T2)	50	14	28	>T1, <C	10	20	>T1, <C
Treated, worn, and washed/dried (T3)	200	78	39	>T1, <C	64	32	>T1, <C
Nontreated control (C)[d]	60	59	98	>T1, >T2, >T3	55	92	>T1, >T2, >T3
Pants							
Treated (T1)	50	3	6	<T2, <C	2	4	<T2, <C
Treated and washed/dried (T2)	50	19	38	>T1, >T3, <C	12	24	>T1, <C
Treated, worn, and washed/dried (T3)	200	37	19	<T2, <C	23	12	<C
Nontreated control (C)[d]	60	54	90	>T1, >T2, >T3	51	85	>T1, >T2, >T3
Sock							
Treated (T1)	50	8	16	<T2, <T3, <C	5	10	<T2, <T3, <C
Treated and washed/dried (T2)	50	24	48	>T1, <C	24	48	>T1, <C
Treated, worn, and washed/dried (T3)	200	100	50	>T1, <C	76	38	>T1, <C
Nontreated control (C)[d]	60	60	100	>T1, >T2, >T3	57	95	>T1, >T2, >T3
Shoe							
Treated (T1)	50	0	0	<C	0	0	<C
Treated and worn (T3)	150	4	3	<C	2	1	<C
Nontreated control (C)[d]	50	48	96	>T1, >T3	47	94	>T1, >T3

Average permethrin concentrations for given test textiles and treatments are the same as in Table 1.

[a]Excluding ticks that were not capable of any movement, capable only of some movement of the legs, or capable of righting themselves but not able to move in a coordinated way or orient toward a stimulus.

[b] Excluding ticks that were not capable of any movement, capable only of some movement of the legs, capable of righting themselves but not able to move in a coordinated way or orient toward a stimulus, or displaying normal movement but failing to ascend onto a human finger when provided the opportunity.

[c] Based on pairwise likelihood ratio test for different treatments within a given type of clothing (shirt, pants, sock, or shoe); statistical significance level adjusted (Bonferroni correction) to account for multiple pairwise comparisons across textile treatments: from $P < 0.05$ to $P < 0.0083$ for six pairwise comparisons within a given type of clothing, or to $P < 0.0167$ for three pairwise comparisons within a given type of clothing.

[d] The nontreated control textile was always a 100% cotton shirt.

J Med Entomol. Author manuscript; available in PMC 2020 January 08.

Table 4.

Vigor of field-collected *I. scapularis* nymphs (CT-field) 1 h after exposure for variable time periods (<1–5 min) to vertically oriented nontreated or permethrin-treated clothing

Test textile and treatment	No. examined	Ticks displaying normal movement[a]			Ticks displaying normal movement and willingness to ascend a human finger[b]		
		Normal movement		Statistically significant difference for % ticks with normal movement compared with other test textiles of the same type of clothing[c]	Normal movement and willing to ascend a human finger		Statistically significant difference for % ticks with normal movement and willingness to ascend finger compared with other test textiles of the same type of clothing[c]
		No.	%		No.	%	
Shirt							
Treated (T1)	35	0	0	<T3, <C	0	0	<T3, <C
Treated and washed/dried (T2)	5	1	20	<C	0	0	<C
Treated, worn, and washed/dried (T3)	30	18	60	>T1, <C	12	40	>T1, <C
Nontreated control (C)[d]	35	35	100	>T1, >T2, >T3	33	94	>T1, >T2, >T3
Pants							
Treated (T1)	25	2	8	<C	0	0	<C
Treated and washed/dried (T2)	5	0	0	<C	0	0	<C
Treated, worn, and washed/dried (T3)	30	6	20	<C	3	10	<C
Nontreated control (C)[d]	25	25	100	>T1, >T2, >T3	23	92	>T1, >T2, >T3
Sock							
Treated (T1)	10	0	0	<T3, <C	0	0	<T3, <C
Treated and washed/dried (T2)	5	0	0	<C	0	0	<C
Treated, worn, and washed/dried (T3)	16	8	50	>T1, <C	7	44	>T1, <C
Nontreated control (C)[d]	10	10	100	>T1, >T2, >T3	9	90	>T1, >T2, >T3
Shoe							
Treated (T1)	10	0	0	<C	0	0	<C
Treated and worn (T3)	14	1	7	<C	0	0	<C
Nontreated control (C)[d]	9	8	89	>T1, >T3	7	78	>T1, >T3

Average permethrin concentrations for given test textiles and treatments are the same as in Table 1.

[a]Excluding ticks that were not capable of any movement, capable only of some movement of the legs, or capable of righting themselves but not able to move in a coordinated way or orient toward a stimulus.

[b] Excluding ticks that were not capable of any movement, capable only of some movement of the legs, capable of righting themselves but not able to move in a coordinated way or orient toward a stimulus, or displaying normal movement but failing to ascend onto a human finger when provided the opportunity.

[c] Based on pairwise likelihood ratio test for different treatments within a given type of clothing (shirt, pants, sock, or shoe); statistical significance level adjusted (Bonferroni correction) to account for multiple pairwise comparisons across textile treatments: from $P < 0.05$ to $P < 0.0083$ for six pairwise comparisons within a given type of clothing, or to $P<0.0167$ for three pairwise comparisons within a given type of clothing.

[d] The nontreated control textile was always a 100% cotton shirt.

J Med Entomol. Author manuscript; available in PMC 2020 January 08.

Table 5.

Vigor of field-collected *I. scapularis* nymphs (CT-field) 24 h after exposure for variable time periods (<1–5 min) to vertically oriented nontreated or permethrin-treated clothing

Test textile and treatment	No. examined	Ticks displaying normal movement[a]			Ticks displaying normal movement and willingness to ascend a human finger[b]		
		Normal movement		Statistically significant difference for % ticks with normal movement compared with other test textiles of the same type of clothing[c]	Normal movement and willing to ascend a human finger		Statistically significant difference for % ticks with normal movement and willingness to ascend finger compared with other test textiles of the same type of clothing[c]
		No.	%		No.	%	
Shirt							
Treated (T1)	35	0	0	<T3, <C	0	0	<T3, <C
Treated and washed/dried (T2)	5	0	0	<C	0	0	<C
Treated, worn, and washed/dried (T3)	30	10	33	>T1, <C	9	30	>T1, <C
Nontreated control (C)[d]	35	34	100	>T1, >T2, >T3	33	94	>T1, >T2, >T3
Pants							
Treated (T1)	25	0	0	<C	0	0	<C
Treated and washed/dried (T2)	5	0	0	<C	0	0	<C
Treated, worn, and washed/dried (T3)	30	7	23	<C	3	10	<C
Nontreated control (C)[d]	25	23	92	>T1, >T2, >T3	20	80	>T1, >T2, >T3
Sock							
Treated (T1)	10	0	0	<C	0	0	<C
Treated and washed/dried (T2)	5	0	0	<C	0	0	<C
Treated, worn, and washed/dried (T3)	16	1	6	<C	1	6	<C
Nontreated control (C)[d]	10	8	80	>T1, >T2, >T3	8	80	>T1, >T2, >T3
Shoe							
Treated (T1)	10	0	0	<C	0	0	<C
Treated and worn (T3)	14	0	0	<C	0	0	<C
Nontreated control (C)[d]	9	7	78	>T1, >T3	6	67	>T1, >T3

Average permethrin concentrations for given test textiles and treatments are the same as in Table 1.

[a]Excluding ticks that were not capable of any movement, capable only of some movement of the legs, or capable of righting themselves but not able to move in a coordinated way or orient toward a stimulus.

[b] Excluding ticks that were not capable of any movement, capable only of some movement of the legs, capable of righting themselves but not able to move in a coordinated way or orient toward a stimulus, or displaying normal movement but failing to ascend onto a human finger when provided the opportunity.

[c] Based on pairwise likelihood ratio test for different treatments within a given type of clothing (shirt, pants, sock, or shoe); statistical significance level adjusted (Bonferroni correction) to account for multiple pairwise comparisons across textile treatments: from $P < 0.05$ to $P < 0.0083$ for six pairwise comparisons within a given type of clothing, or to $P < 0.0167$ for three pairwise comparisons within a given type of clothing.

[d] The nontreated control textile was always a 100% cotton shirt.

J Med Entomol. Author manuscript; available in PMC 2020 January 08.

Table 6.

Vigor of laboratory-reared *I. scapularis* nymphs (CT14) 1 h after continuous exposure for 30–120 s to horizontally oriented non-treated or permethrin-treated clothing

			Ticks displaying normal movement[a]			Ticks displaying normal movement and willingness to ascend a human finger[b]		
			Normal movement		Statistically significant difference for % ticks with normal movement compared with other test textiles of the same type of clothing[c]	Normal movement and willing to ascend a human finger		Statistically significant difference for % ticks with normal movement and willingness to ascend finger compared with other test textiles of the same type of clothing[c]
Test textile and treatment	Exposure time (s)	No. examine	No.	%		No.	%	
Shirt								
Treated (T1)	30–120	90	0	0	<T3, <C	0	0	<T3
Treated, worn, and washed/dried (T3)	30–120	360	212	59	>T1, <C	80	22	>T1
Nontreated control (C)[d]	120	150	150	100	>T1, >T3	ND[e]	ND	ND
Pants								
Treated (T1)	30–120	90	0	0	<T3, <C	0	0	<T3
Treated, worn, and washed/dried (T3)	30–120	360	112	31	>T1, <C	26	7	>T1
Nontreated control (C)[d]	120	150	150	100	>T1, >T3	ND	ND	ND
Sock								
Treated (T1)	30–120	90	0	0	<T3, <C	0	0	<T3
Treated, worn, and washed/dried (T3)	30–120	360	152	42	>T1, <C	53	15	>T1
Nontreated control (C)[d]	120	150	150	100	>T1, >T3	ND	ND	ND
Shoe								
Treated (T1)	30–120	90	0	0	<C	0	0	None
Treated and worn (T3)	30–120	300	0	0	<C	0	0	None
Nontreated control (C)[d]	120	130	130	100	>T1, >T3	ND	ND	ND

Average permethrin concentrations for given test textiles and treatments are the same as in Table 1.

[a]Excluding ticks that were not capable of any movement, capable only of some movement of the legs, or capable of righting themselves but not able to move in a coordinated way or orient toward a stimulus.

[b] Excluding ticks that were not capable of any movement, capable only of some movement of the legs, capable of righting themselves but not able to move in a coordinated way or orient toward a stimulus, or displaying normal movement but failing to ascend onto a human finger when provided the opportunity.

[c] Based on pairwise likelihood ratio test for different treatments within a given type of clothing (shirt, pants, sock, or shoe); statistical significance level adjusted (Bonferroni correction) to account for multiple pairwise comparisons across textile treatments: from $P < 0.05$ to $P < 0.0167$ for three pairwise comparisons within a given type of clothing.

[d] The nontreated control textile was always a 100% cotton shirt.

[e] ND, not determined.

J Med Entomol. Author manuscript; available in PMC 2020 January 08.

Table 7.

Vigor of laboratory-reared *I. scapularis* nymphs (CT14) 24 h after continuous exposure for 30–120 s to horizontally oriented non-treated or permethrin-treated clothing

			Ticks displaying normal movement[a]			Ticks displaying normal movement and willingness to ascend a human finger[b]		
			Normal movement			Normal movement and willing to ascend a human finger		
Test textile and treatment	Exposure time (s)	No. examined	No.	%	Statistically significant difference for % ticks with normal movement compared with other test textiles of the same type of clothing[c]	No.	%	Statistically significant difference for % ticks with normal movement and willingness to ascend finger compared with other test textiles of the same type of clothing[c]
Shirt								
Treated (T1)	30–120	90	0	0	<T3, <C	0	0	<T3, <C
Treated, worn, and washed/dried (T3)	30–120	360	103	29	>T1, <C	74	21	>T1, <C
Nontreated control (C)[d]	120	150	145	97	>T1, >T3	119	79	>T1, >T3
Pants								
Treated (T1)	30–120	90	0	0	<T3, <C	0	0	<T3, <C
Treated, worn, and washed/dried (T3)	30–120	330	126	38	>T1, <C	112	34	>T1, <C
Nontreated control (C)[d]	120	140	134	96	>T1, >T3	117	84	>T1, >T3
Sock								
Treated (T1)	30–120	90	1	1	<T3, <C	0	0	<T3, <C
Treated, worn, and washed/dried (T3)	30–120	360	120	33	>T1, <C	96	27	>T1, <C
Nontreated control (C)[d]	120	150	149	99	>T1, >T3	118	79	>T1, >T3
Shoe								
Treated (T1)	30–120	90	0	0	<C	0	0	<C
Treated and worn (T3)	30–120	270	0	0	<C	0	0	<C
Nontreated control (C)[d]	120	120	112	93	>T1, >T3	92	77	>T1, >T3

Average permethrin concentrations for given test textiles and treatments are the same as in Table 1.

[a]Excluding ticks that were not capable of any movement, capable only of some movement of the legs, or capable of righting themselves but not able to move in a coordinated way or orient toward a stimulus.

[b] Excluding ticks that were not capable of any movement, capable only of some movement of the legs, capable of righting themselves but not able to move in a coordinated way or orient toward a stimulus, or displaying normal movement but failing to ascend onto a human finger when provided the opportunity.

[c] Based on pairwise likelihood ratio test for different treatments within a given type of clothing (shirt, pants, sock, or shoe); statistical significance level adjusted (Bonferroni correction) to account for multiple pairwise comparisons across textile treatments: from $P < 0.05$ to $P < 0.0167$ for three pairwise comparisons within a given type of clothing.

[d] The nontreated control textile was always a 100% cotton shirt.

J Med Entomol. Author manuscript; available in PMC 2020 January 08.

Table 8.

Vigor of field-collected *I. scapularis* nymphs (CT-field) 1 h after continuous exposure for 60 s to horizontally oriented nontreated or permethrin-treated clothing

			Ticks displaying normal movement[a]			Ticks displaying normal movement and willingness to ascend a human finger[b]		
			Normal movement			Normal movement and willing to ascend a human finger		
Test textile and treatment	Exposure time (s)	No. examined	No.	%	Statistically significant difference for % ticks with normal movement compared with other test textiles of the same type of clothing[c]	No.	%	Statistically significant difference for % ticks with normal movement and willingness to ascend finger compared with other test textiles of the same type of clothing[c]
Shirt								
Treated (T1)	60	10	3	30	<C	1	10	<T3, <C
Treated and washed/dried (T2)	60	10	7	70	<C	6	60	None
Treated, worn, and washed/dried (T3)	60	21	14	67	<C	12	57	<C
Nontreated control (C)[d]	60	20	20	100	>T1, >T2, >T3	19	95	>T1, >T3
Pants								
Treated (T1)	60	10	1	10	<C	1	10	<C
Treated and washed/dried (T2)	60	10	5	50	<C	4	40	<C
Treated, worn, and washed/dried (T3)	60	20	9	45	<C	9	45	<C
Nontreated control (C)[d]	60	19	18	95	>T1, >T2, >T3	17	89	>T1, >T2, >T3

Average permethrin concentrations for given test textiles and treatments are the same as in Table 1.

[a]Excluding ticks that were not capable of any movement, capable only of some movement of the legs, or capable of righting themselves but not able to move in a coordinated way or orient toward a stimulus.

[b]Excluding ticks that were not capable of any movement, capable only of some movement of the legs, capable of righting themselves but not able to move in a coordinated way or orient toward a stimulus, or displaying normal movement but failing to ascend onto a human finger when provided the opportunity.

[c]Based on pairwise likelihood ratio test for different treatments within a given type of clothing (shirt, pants, sock, or shoe); statistical significance level adjusted (Bonferroni correction) to account for multiple pairwise comparisons across textile treatments: from $P < 0.05$ to $P < 0.0083$ for six pairwise comparisons within a given type of clothing.

[d]The nontreated control textile was always a 100% cotton shirt.

Table 9.

Vigor of field-collected *I. scapularis* nymphs (CT-field) 24 h after continuous exposure for 60 s to horizontally oriented nontreated or permethrin-treated clothing

			Ticks displaying normal movement[a]			Ticks displaying normal movement and willingness to ascend a human finger[b]		
			Normal movement			Normal movement and willing to ascend a human finger		
Test textile and treatment	Exposure time (s)	No. examine	No.	%	Statistically significant difference for % ticks with normal movement compared with other test textiles of the same type of clothing[c]	No.	%	Statistically significant difference for % ticks with normal movement and willingness to ascend finger compared with other test textiles of the same type of clothing[c]
Shirt								
Treated (T1)	60	10	3	30	<T3, <C	1	10	<C
Treated and washed/dried (T2)	60	10	0	0	<C	0	0	<C
Treated, worn, and washed/dried (T3)	60	21	0	0	>T1, <C	0	0	<C
Nontreated control *(C)*[d]	60	20	17	85	>T1, >T2, >T3	17	85	>T1, >T2, >T3
Pants								
Treated (T1)	60	10	2	20	<C	0	0	<C
Treated and washed/dried (T2)	60	10	1	10	<C	0	0	<C
Treated, worn, and washed/dried (T3)	60	20	0	0	<C	0	0	<C
Nontreated control (C)[d]	60	19	19	100	>T1, >T2, >T3	18	95	>T1, >T2, >T3

Average permethrin concentrations for given test textiles and treatments are the same as in Table 1.

[a]Excluding ticks that were not capable of any movement, capable only of some movement of the legs, or capable of righting themselves but not able to move in a coordinated way or orient toward a stimulus.

[b]Excluding ticks that were not capable of any movement, capable only of some movement of the legs, capable of righting themselves but not able to move in a coordinated way or orient toward a stimulus, or displaying normal movement but failing to ascend onto a human finger when provided the opportunity.

[c]Based on pairwise likelihood ratio test for different treatments within a given type of clothing (shirt, pants, sock, or shoe); statistical significance level adjusted (Bonferroni correction) to account for multiple pairwise comparisons across textile treatments: from $P < 0.05$ to $P < 0.0083$ for six pairwise comparisons within a given type of clothing.

[d]The nontreated control textile was always a 100% cotton shirt.