SHORT COMMUNICATION

Different Activities and Footwear Influence Exposure to Host-Seeking Nymphs of *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae)

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J. Med. Entomol. 38(4): 596–600 (2001)

ABSTRACT The relative potential for a person accidentally acquiring host-seeking nymphs of the blacklegged tick, *Ixodes scapularis* Say, and lone star tick, *Amblyomma americanum* (L.), while wearing either of two types of footwear, walking, crawling on hands and knees, and sitting on large fallen logs in deciduous woods, was evaluated. Although flag samples indicated substantial populations of *I. scapularis* nymphs and low to moderate numbers of *A. americanum* at the study sites, relatively few *I. scapularis* and fewer still *A. americanum* nymphs were acquired during 30-s and 5-min walks. Significantly fewer *I. scapularis* were picked up when boots were worn with ankles taped (an anti-tick precaution) than when sneakers were worn with socks exposed during 5-min walks, but when thus attired, there was no significant difference between the number of nymphs acquired during 30-s walks. Nymphs of *I. scapularis* did not appear to accumulate incrementally on footwear or clothing during walks when boots were worn and ankles taped. Crawling for 30 s (≈3 m distance) yielded significantly more *I. scapularis* nymphs than walking for 30 s. During crawling, *I. scapularis* nymphs were picked up on 58% of the 30-s samples. Most ticks picked up during crawls were on pant legs. When a flannel flag cloth (0.5 by 0.5 m) was appressed to the upper surface of logs suitable to be sat upon by tired hikers, *I. scapularis* nymphs were found on 87% of the logs and in 36% of the samples. These data indicate that the potential for contact with host-seeking nymphs of *I. scapularis* occurring at these densities is greatly elevated by engaging in activities that involve contact with fallen logs and close contact of hands and knees with leaf litter.

KEY WORDS blacklegged tick, lone star tick, Lyme disease, risk

BLACKLEGGED TICKS, *Ixodes scapularis* Say, are the principal vectors of *Borrelia burdorferi* Johnson, Schmid, Hyde, Steigerwalt & Brenner, the causative agent of Lyme disease in the eastern United States (Burgdorfer et al. 1982, Johnson et al. 1984). *Ixodes scapularis* also transmits human granulocytic ehrlichiosis (Walker and Dumler 1996). Bites by infected *I. scapularis* nymphs account for most human cases of Lyme disease (Lane et al. 1991). Lone star ticks, *Amblyomma americanum* (L.) are sympatric with *I. scapularis* in many areas of the coastal plain of the mid-Atlantic states. Lone star ticks readily bite humans and are vectors of human monocytic ehrlichiosis (Walker and Dumler 1996).

The risk of Lyme disease transmission posed by *I. scapularis* nymphs depends in part on the infection rate in the tick population, density of the tick population, and how long a tick is attached (Piesman 1993, Piesman et al. 1987). Nymphs of *I. scapularis* are most abundant in wooded habitats (Falco and Fish 1989, Fish 1993) where they are associated with the leaf litter layer during their host-seeking phase. Lone star ticks share wooded habitats with *I. scapularis*, where both species can attach to persons venturing into tick-infested woods. Adult *I. scapularis* wait for hosts up to ≈1 m above the ground on vegetation, are readily picked up on the clothing (often the pant legs) of a person walking (Ginsberg and Ewing 1989), and can pose a risk to hikers (Oliver and Howard 1998). How *I. scapularis* nymphs, which are much smaller and quest for hosts near ground level, are accidently acquired by persons engaged in various activities in woodlands is less obvious. Having spent many hours standing and walking in places moderately to heavily populated with host-seeking *I. scapularis* nymphs, J.F.C. observed very few *I. scapularis* nymphs on his clothing, footwear, or skin when he left the woods. In contrast, if his activities included putting his hands in leaf litter or kneeling on it, he would soon detect *I. scapularis* nymphs on himself. The purpose of this study was to quantify the relative potential for accidentally acquiring *I. scapularis* and *A. americanum* nymphs when a person engages in any of three basic types of activity: walking, crawling on hands and knees, and sitting on logs. Persons who enter wooded

Standard precautions to prevent tick bite were followed by J.F.C.

habitats for work or recreation perform ≥1 of the aforementioned activities. As examples, hikers walk, and when tired, sit; and persons clearing brush on a wooded suburban home site are likely to repeatedly put their hands in leaf litter.

Materials and Methods

The study was conducted during May and June at the Patuxent Wildlife Research Center, Prince George's County, MD. Five sites in primarily deciduous woodland were selected because they were known from previous surveillance to be populated with at least moderate numbers of *I. scapularis* nymphs. Dominant species of trees included red maple, *Acer rubrum,* white oak, *Quercus alba,* American beech, *Fagus americanum,* sweet gum, *Liquidambar styraciflua,* and some large spruce, *Picea* sp., associated with the ruins of an old building. The understory vegetation was sparse: characterized by a few species, such as greenbrier, *Smilax* sp., Japanese barberry, *Berberis thunbergii,* and New York fern, *Dryopteris noveboracensis.* Leaf litter ranged in depth from 2 to 10 cm. Each site was ≈0.5 ha, the dimensions determined by continuity of plant communities, a powerline cut, and a stream.

To obtain baseline population data, each site was sampled for host-seeking ticks by flagging 10 times with a 0.5 by 0.5-m flannel cloth for 30 s, while walking slowly and traveling ≈10 m/30 s. The flag samples were taken along two diagonal transects.

White coveralls were worn during all field activities. Pant legs of the coveralls were tucked into white cotton socks and masking tape (7.6 cm wide, Anchor Continental, Columbia, SC) was wrapped around the juncture. Two kinds of shoes commonly worn during outdoor activities, hiking boots and sneakers, were used to ascertain if the type of footwear influences the success of nymphs in catching hold of and remaining on shoes and lower legs. When boots were worn, masking tape was wrapped around each leg from ≈6 cm below the boot tops to ≈10 cm above the boot tops (sealing the pant leg-boot top juncture), so that no portion of the socks was exposed. Sneakers were worn with a 7- to 8-cm wide band of masking tape, affixed where pant legs and socks overlapped, to represent a less tick-preventive attire. An ≈10-cm vertical section of each sock was exposed. The lowest portion of exposed pant legs was ≈30 cm above ground level whether boots or sneakers were worn. The boots (Sierra Lite II, HI-Tech Sports USA, Modesto, CA) had rubber soles with suede and cloth uppers. The rubber sole extended ≈2 cm up the toe of each boot. The sneakers (711, New Balance Athletic Shoes, Boston, MA) also had rubber soles and cloth and smooth leather uppers. The hiking boots and sneakers worn were representative styles of these highly diversified classes of footwear. Immediately before walking, masking tape was appressed to the footwear to remove any ticks acquired while approaching the starting point of the walk.

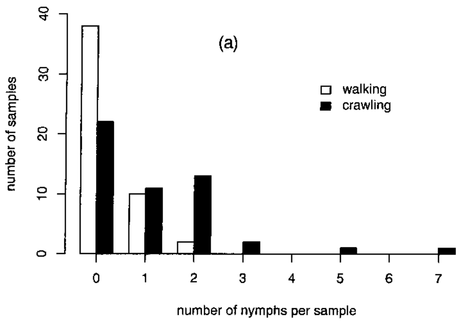
At each of the five sites, J.F.C. walked at a moderate hiking pace for 30 s (covering ≈30 m) 10 times. As soon as he stopped walking, he carefully examined his pant legs, socks, masking tape, and footwear for ticks. A piece of masking tape was then appressed to all surfaces (as far as possible) of the footwear except the bottoms of the soles, and the tape was checked for ticks. All ticks were removed and released except for some voucher specimens. The locations of the ticks were recorded as to whether they were on footwear, socks, pant legs, hands, or elsewhere. The 30-s walks were conducted along three transects across each site and ≥20 m from the nearest completed walk route. Five-minute walks were conducted in a similar manner. For these, J.F.C. walked the length of each site, returning in the opposite direction several meters from the path just traversed. This pattern was continued until 5 min had elapsed. A second 5-min walk was conducted at each site and did not cover any of the area traversed during the first walk. At the end of each 5-min sampling period, clothing, tape, and footwear were checked for ticks.

To simulate outdoor activities, such as clearing brush or gardening, that involve placing one's hands and legs in leaf litter, J.F.C. crawled slowly on hands and knees for 30 s, 10 times (≈3 m) at each site. After each crawl, he checked his hands, legs, tape, socks, and footwear for ticks. Sneakers were the only footwear worn during crawl samples. Bands of masking tape doubled over on itself, sticky side out, were worn around his wrists to prevent ticks from ascending his arms unseen.

The flagging and the four different types of walks and the crawl were done in random order at each site. All activities were carried out under similar meteorological conditions (sunny to partly cloudy, and 26–34°C). In most cases, the entire set of activities was not completed at any given site on the same day. No activity was done in the same part of a site <20 min after the previous activity was concluded.

To assess the potential for acquiring ticks by persons sitting on large fallen logs, as weary hikers might do, three logs at each of the five sites were sampled for ticks. A 0.5 by 0.5-m flannel flag cloth was appressed for ≈5 s to the upper surface of large logs (≥1 m circumference) at five areas on each log where the absence of branch spikes allowed full extension of the cloth. Ticks on the cloth were counted and some kept to verify identification.

For statistical analysis, we assumed that our samples were Poisson in nature, but perhaps over or under dispersed. We used a generalized linear model with a Poisson link (McCullagh and Nelder 1983), with quasi-likelihood estimation to allow for over- or under-dispersed Poisson data. We used the *R* statistical package (Free Software Foundation, Boston, MA, http://www.gnu.org/) to perform model estimation and statistical tests. The program generated approximate F values, their associated P values, and contrast estimates (and their standard errors). Counts for the 5-min walks were divided by 10 to put them on the



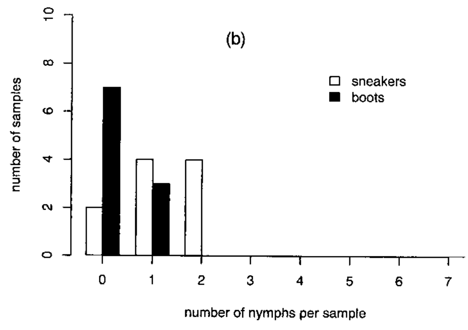


Fig. 1. Numbers of *I. scapularis* nymphs acquired during (a) 30 s of walking or crawling while wearing sneakers and (b) 5 min (300 s) of walking while wearing tick preventive (boots and tape) or nonpreventive (sneakers and minimal tape) footwear.

same time scale as the other treatments. Contrasts were made to the 30-s walk with boots.

Results

Flag sampling showed a significant difference in the abundance of the two species of ticks ($P < 0.001$). Host-seeking nymphs of *I. scapularis* (total count = 239) were found significantly more frequently than *A. americanum* nymphs (total count = 34). Some sites differed significantly ($P < 0.05$) from others in tick abundance. However, there were no significant treatments by location interactions. Thus, treatment differences were consistent across sites. Overall, relatively few ticks were picked up and retained during walks (Fig. 1). The distributions of counts were highly right skewed with many zero counts, as is typical of a Poisson distribution characterized by a relatively small parameter. For both species of ticks, the only 30-s treatment that differed from the 30-s walk with boots was crawling. The latter activity yielded significantly more ticks for *I. scapularis* ($P < 0.001$) and for *A. americanum* ($P < 0.05$). Nymphs of *I. scapularis* were picked up and retained on 58% of the 30-s crawls compared with 22 and 24% of 30-s walks wearing boots and sneakers, respectively. We analyzed the 5-min walk data separately and found that walking with boots yielded significantly fewer nymphs than did walking with sneakers for *I. scapularis* ($P < 0.05$) but not for *A. americanum* ($P > 0.05$). A total of only three *I. scapularis* nymphs was acquired in the 5-min walks with boots.

For most model estimates, the dispersion parameter was slightly greater than unity, and greater for *A. americanum* (2.1) than for *I. scapularis* (1.3). However, the difference between the two species was due entirely to one sample (crawling) with a count of 15 *A. americanum* nymphs. With that sample removed, the dispersion parameter for *A. americanum* was estimated to be only slightly greater than one (1.14). The interpretation for dispersion parameters near unity is that one is sampling from a population distributed randomly in space with higher dispersion parameters indicating more clustering. The dispersion parameter for the 5-min walks, analyzed apart from the other data, was estimated to be much less than one (0.09 for *I. scapularis* and 0.06 for *A. americanum*), implying that counts of ticks from 5-min walks are samples from a more regular (under-dispersed) distribution.

Most *I. scapularis* nymphs picked up during walking were found on the footwear in both the 30-s and 5-min sessions (Table 1). Nymphs of *A. americanum* were distributed on shoes, socks, and pant legs. After 30-s crawls, most *I. scapularis* nymphs were found on legs and hands, whereas *A. americanum* nymphs were predominantly on legs.

Nymphs of *I. scapularis* were found on 87% of the large logs suitable for hikers to use as seats. Of the 75 samples taken on logs, 36% captured ≥1 *I. scapularis* nymph, with a total capture of 48 nymphs. No *A. americanum* nymphs were captured on the log samples.

Discussion

Walking in woods infested with moderate numbers of host-seeking *I. scapularis* and low numbers of *A. americanum* incurs some risk of acquiring nymphs. The numbers of *I. scapularis* nymphs acquired were influenced by the type of footwear worn while walking. Current fashions in outdoor footwear fall into two

Table 1. Numbers and locations of *I. scapularis* and *A. americanum* (in parentheses) nymphs acquired during the designated activities

	Hands	Shoes	Socks/ Ankle Tape	Pantlegs
30-s walk[a] (boots)	—	15 (5)	0 (0)	0 (3)
30-s walk[a] (sneakers)	—	9 (3)	0 (4)	5 (2)
5-min walk[b] (boots)	—	3 (0)	0 (0)	0 (2)
5-min walk[b] (sneakers)	—	8 (2)	1 (4)	3 (0)
30-s crawl[a]	14 (4)	4 (5)	1 (6)	36 (16)

[a] 10, 30-s samples at each of five sites ($n = 50$).
[b] 2, 5-min samples at each of five sites ($n = 10$).

main categories, hiking boots and sneakers. In recent years, both types of footwear have undergone a figurative explosion in variation of the basic type. To attempt to evaluate even a small fraction of the different models of outdoor footwear for their capacity to pick up ticks would be a major undertaking. In this study we compared a tick preventive type of footwear (boots with tape extending past the juncture of sock and pant leg) with a relatively nonpreventive type of footwear (sneakers with exposed socks, and a narrow band of tape at the juncture of sock and pant leg). Boots with taped over socks acquired fewer ticks in 5-min walks than did the sneakers with *I. scapularis* but not *A. americanum* (the sparseness of the *A. americanum* data set is a possible reason for the difference). Also, when boots with tape were worn, *I. scapularis* were not found on pant legs as they were when sneakers were worn. Some *A. americanum* nymphs were found on pant legs when boots were worn in 30-s walks. Lone star tick nymphs are more active and faster than *I. scapularis* nymphs and may also use higher questing sites. Possibly, a few nymphs crawled to the footwear while J.F.C. was standing examining his attire for ticks.

The data suggest that *I. scapularis* nymphs do not steadily accumulate on a person continuously walking through tick-infested habitat, because the dispersion factor for 5-min walks was estimated to be much less than one. For example, when boots were worn, a total of 15 *I. scapularis* nymphs was acquired in 30-s walks, but for 5-min walks, a total of only three nymphs was acquired (total times were the same). We suggest two (not necessarily conflicting) hypotheses that may account for the small dispersion parameter found in 5-min walks.

According to the first hypothesis, *I. scapularis* nymphs may change their behavior a short time after being picked up. A nymph may adhere tightly (dislodgment unlikely), then later start moving about the host (dislodgment more likely). For this reason, a hiker might start losing nymphs after a minute or so of walking, so that accumulation of new nymphs is balanced by losing those acquired earlier.

A second hypothesis, can be conceptualized as follows. The final count of nymphs is a combination of two processes, an addition process (picking up nymphs) and a subtraction process (losing nymphs). The key point is that the subtraction process cannot start until a nymph has been picked up. For simplicity, assume that the pickup rate is constant and that the chance a nymph is lost is time independent (not dependent on when a nymph is picked up, unlike the first hypothesis). Then, the chance that a nymph will still be on the walker decreases as the time since it was picked up increases (the probability of still harboring a nymph acquired a relatively long time a ago is small). If the probability of nymph dislodgment is high (approximately that of picking up nymphs), the total number of nymphs on a hiker will quickly approach a steady state. For the 30-s walks, not enough time had elapsed for many nymphs to be lost, so only the addition process is important. For the 5-min walks, there is sufficient time to lose nymphs picked up earlier, so both processes were important. Thus, longer walks would result in a fairly constant number of nymphs (under-dispersed distribution of number of nymphs) on the hiker.

Regardless of the reason nymphs are lost, nymphs picked up on boots are likely to be later brushed off as a person continues to walk through the leaf litter. This may happen to ticks on drag cloths in drag sampling. The type of footwear seemed to make a difference in the number of *I. scapularis* nymphs retained in 5 min walks when four times more nymphs were acquired with sneakers (12) than boots (3).

The depth of the leaf litter may affect the number of nymphs that are picked up or brushed off footwear while a person walks. Deeper leaf litter may allow ticks to make contact higher on the shoe, sock or even pant leg and give them some advantage in catching hold and being retained. The leaf litter at the five sites used in this study was quite variable in depth (2–10 cm), within each given site and its effects were not addressed in this study.

As risky as kneeling or placing one's hands in leaf litter appears to be, sitting on large logs could have greater potential for picking up *I. scapularis* nymphs. The logs sampled often harbored several nymphs of *I. scapularis* (2.6 nymphs/m^2). Host-seeking nymphs of *I. scapularis* have been found to be somewhat concentrated near stone walls (Stafford and Magnarelli 1993). Like stone walls, hollow logs provide nesting sites for mice and other rodents. Many larval ticks feeding on these mice probably drop off in or near nest sites and would remain nearby when they later begin seeking hosts as nymphs (Matuschka et al. 1991).

Although footwear may influence the numbers of *I. scapularis* nymphs picked up and retained, what one wears on one's feet and ankles appears to be less important in acquiring *I. scapularis* nymphs than whether a person stands, kneels, or sits in the woods. Campers, brush clearers, and children playing all frequently engage in activities that involve putting their hands and knees in leaf litter. Nymphs of *I. scapularis* and *A. americanum* can occur in greater densities than those observed at the sites where this study was conducted. Further studies are warranted to determine whether the observations reported here hold true where *I. scapularis* and *A. americanum* nymphs are numerous, and to better determine which sorts of footwear accommodate or prevent picking up nymphs.

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Received for publication 8 February 2000; accepted 12 March 2001.