Bite-Count Evaluation of the Repellency of *N,N*-Diethyl-3-methylbenzamide to Larval *Eutrombicula alfreddugesi* (Acari: Trombiculidae)

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ABSTRACT Larvae of the pest chigger mite *Eutrombicula alfreddugesi* Oudemans, 1910, exhibited a low repellent threshold for *N,N*-diethyl-3-methylbenzamide (deet) in a field setting. Use of a deet-based repellent significantly reduced chigger mite infestation. Efficacy of aerosol formulations was significantly diminished with 9% (AI) deet. *E. alfreddugesi* utilized both direct and indirect paths to infest the human body. Direct infestation led to the highest body region bite counts. Distribution of *E. alfreddugesi* bites over 15 regions of the human body fell into three zones of decreasing parasitism: the ankle/calf region (accessed through direct infestation); the groin, chest, back, armpits, and feet (accessed through indirect infestation); and the thighs, arms, buttocks, head, and neck, which were infrequently attacked or were unavailable to *E. alfreddugesi*.

KEY WORDS Arachnida, trombiculidae, *Eutrombicula alfreddugesi*, repellent

THE LARVAE OF *Eutrombicula alfreddugesi* Oudemans, 1910, are common acarine parasites of humans and most other mammals. These chiggers have been collected across much of eastern and central North America (Wharton & Fuller 1952). *E. alfreddugesi* is endemic to the southern tier of states, and populations have been reported as far north as Minot, ND; however, chigger populations decrease in abundance above latitude 40°N (Wharton & Fuller 1952, Loomis & Wrenn 1984). The parasitic relationship with the host is obligate but of short duration, producing a characteristic parasitope marked by dermal circumscribed inflammatory edema and intense pruritus. A mosquito parasitope is usually slightly punctate and short-lived, but a chigger parasitope is usually smooth and may remain visible for 14–21 d (Jenkins 1948).

Available acaricides have shown little promise for seasonal area control. Keller & Gouck (1957) demonstrated 10- to 14-wk control of *E. alfreddugesi* using toxaphene and dieldrin. In tests with 18 other acaricides, they observed small reductions in larval chigger population densities followed by rapid recovery of populations to before-treatment densities. Roberts & Zimmerman (1980) reported 83–90% average control of *E. alfreddugesi* over a 4-wk period in field tests using two pyrethroids. Although differences in efficacy were demonstrated among formulations and concentrations, the control trend remained the same: excellent initial control was followed by a rapid chigger population recovery. Reed et al. (1977) reported similar rapid recovery rates of larval *E. alfreddugesi* populations after a controlled burn of chigger-infested areas. They observed that the adults and eggs of *E. alfreddugesi* were found at minimal soil depths of 2–4 cm, forming a population reservoir that was protected by the soil and quickly replaced those exposed larvae that were destroyed by fire. Thus the endemic and protected nature of *E. alfreddugesi* populations precludes widespread chemical control and suggests the use of personal repellents to reduce human infestation by these pests.

Previous field studies have tested the repellent effects of barrier chemicals applied to human skin or impregnated into clothing. Nagayo et al. (1917) employed protective clothing in combination with phenol, sulfur ointment, or pyrethrum applied as a personal repellent in areas of endemic scrub typhus in northern Japan. Ewing (1925) developed naphtha- and sulfur-impregnated clothing for use against larval chigger infestation. Other workers have had varying degrees of success by focusing on chemically impregnated clothing as a control method for *E. alfreddugesi* (Madden et al. 1944; Bushland 1946a,b, 1948; Snyder & Morton 1946a,b; Snyder & Cross 1948).

N,N-diethyl-3-methylbenzamide (deet) is marketed in numerous formulations to repel larval chigger mites. Buescher et al. (1984) reported deet and dimethyl phthalate to be significantly better than 10 other commercial repellents in a comprehensive laboratory assay against the lar-

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vae of *Leptotrombidium fletcheri* (Wormersly & Heaslip). Kulkarni (1977) tested deet against two species of larval trombiculid mites, *L. deliense* (Walch) and *L. akamushi* (Brumpt), and reported 100% repellence. Both studies were conducted with laboratory colonies and abiotic test surfaces.

There have been no thorough field trials to test the repellency of deet applied to skin to larval chigger mites. This study was conducted to determine the repellency of deet formulations to *E. alfreddugesi* using bite counts on human volunteers. Theoretical infestation routes were derived from the distribution of chigger bites on the body.

Materials and Methods

Experimental Protocol. Tests were conducted in June and July of 1987–1988 at Camp Cornhusker, Richardson County, NE, in cooperation with the Boy Scouts of America.

Chigger mite populations were sampled using a modified version of the black-plate method (Williams 1946) in 17 locations within the study site. Approximately 200 mites were prepared for scanning electron microscopy and identified as *E. alfreddugesi* at the University of Nebraska.

In 1987 there were four 6-d experimental periods. During the first three periods, bite counts were taken on human volunteers (males 9–13 yr of age) to establish the intensity and dispersal of chigger parasitopes. During the fourth period, bite counts were made on volunteers supplied with one of five repellent formulations. In 1988, repellents were tested in three 6-d experimental periods. Volunteers camped in semipermanent tents and slept on cots for the duration of each experimental period.

Each experimental period was initiated (day 1) by visually inspecting volunteers for chigger bites upon their arrival at camp (before-test bites). On the last afternoon (day 6: ≈122–125 h total exposure), volunteers were again examined for chigger bites. Bite counts in the groin and buttock regions were self-reported by the subjects using hand-held mirrors. In 1987, bites were tabulated for 15 designated body regions, whereas in 1988, only bites on the legs and ankles were tabulated. A total of 623 volunteers participated in the study over a 2-yr period.

The products tested were provided by Miles Laboratories (Chicago, IL); the dilution reagent (*N-N*-Propanol) was purchased from commercial sources. The deet present in products contained 95% of the *meta* isomer and 5% other isomers. All concentrations were volume/volume dilutions.

In 1987, five formulations of Cutter Insect Repellent were tested. Formulations and percentage deet were as follows: pump spray (18%), aerosol spray (22%), stick (33%), original cream (35%), maximum strength liquid (100%). In 1988, three formulations were tested in dilution series as follows: aerosol spray (9 and 22% deet), maximum strength liquid (1, 15, 25, 50, 75, and 100% deet), pump spray (1, 2.5, 4.5, 7, 9, 13.5, and 18% deet).

Aerosol sprays were prepared by Miles Laboratories. Pump spray and liquid dilution series were prepared using full-strength product and commercial-grade *N-N*-propanol as a dilutent. Each repellent container was etched with an identification number and weighed before testing. After field testing, weighing quantified the amount of repellent used by each volunteer. Repellents were issued on day 1 of each experimental period during before-bite screening. Volunteer names and container numbers were recorded to ensure volunteer-container fidelity. Participants were instructed to apply the repellent (according to labeled directions) to their legs, from hips to toes, each morning before breakfast and after swimming or showering.

On day 6, repellent containers were collected and chigger bites on each volunteer counted. Bites on thighs, calves, ankles, and feet were combined into a legs-only bite count.

All tests, except those with aerosol products, were conducted using a double-blind protocol. The exact composition of a product tested by an individual was known neither to the researcher nor the volunteer until tests were completed. Aerosol products were precluded from a double-blind protocol because the 9% deet aerosol carried a green test label, while the 22% deet aerosol carried an orange commercial label.

Aerosol spray and liquid dilution series studies were completed over a single testing week; the pump spray dilution series study was completed over two testing weeks. In the 1987 tests, volunteers from the first three periods served as untreated checks. In 1988, we were unable to obtain volunteers for an untreated check. The comparative nature of the 1988 studies made the use of an untreated check preferential, but not imperative.

Data Analysis. The distribution of chigger bites among 15 untreated body regions was established using Protected Least Squares Means Pair-wise Comparisons (LSMEANS) (SAS Institute 1982). The repellent formulation, dilution series, and aerosol studies used Completely Random designs. Only the repellent formulation study included untreated volunteers. Data were analyzed using analysis of variance (ANOVA) and Protected LSMEANS as required (SAS Institute 1982). For the 1987 repellent formulation study, whole-body bite counts and legs-only bite counts were analyzed separately. All comparisons were made at $\alpha = 0.05$ level of significance. Protected LSMEANS in an unbalanced design are analogous to Fisher's least significant difference test. The two methods are equivalent within a balanced design (SAS Institute 1982).

Table 1. Mean *E. alfreddugesi* bite count and statistical grouping for 15 regions of the human body

Region	Mean bites
Right calf[a]	5.75a
Left calf[a]	5.69a
Chest	4.96b
Left foot	4.92b
Right foot	4.72b
Groin	4.58b
Back	4.48b
Left armpit	2.72c
Right armpit	2.68c
Buttocks	2.21c
Left arm	1.79c
Right arm	1.78c
Head/neck	1.60c
Right thigh	1.55c
Left thigh	1.49c

Data collected from 335 human males 9–13 yr of age in Richardson County, NE, June 1987. Data presented are means for 122–125 h exposure.

Means followed by the same letter are not significantly different under a Protected LSMEANS comparison ($P > 0.05$); those followed by different letters are significantly different ($P < 0.05$).

[a] The calf region extends downward from the midpoint of the kneecap, including the ankle but not the foot.

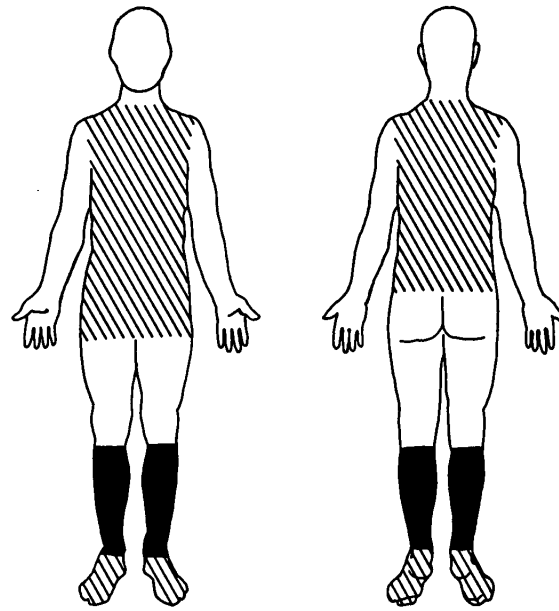

Fig. 1. The distribution of larval *E. alfreddugesi* parasitopes over the human body. ■, intense parasitism; ▧, moderate parasitism; □, negligible parasitism.

Calculations were completed using SAS release 5.16 (SAS Institute 1982). All data are reported as the mean per individual over 6 d.

Results

Bite Distribution—1987 18-d Study. Body-region bite counts for the untreated group, with Protected LSMEANS groupings, are given in Table 1. There were significant differences in the number of bites per body region (ANOVA: $F = 27.36$; df = 14, 4676; $P < 0.05$). Three areas of diminishing parasitic effect were distinguished: intense, moderate, and negligible areas of parasitism (LSMEANS: $P < 0.05$). Effects on the calves were intense; those on the chest, feet, groin, and back moderate; and those on the armpits, buttocks, arms, head and neck, and thighs negligible. Symmetrical body regions (i.e., right calf and left calf) were consistently grouped together. Fig. 1 depicts relative bite distribution over the human body.

Repellent Studies—1987 Formulation Study. Whole-body and legs-only bite counts for treated and untreated groups are given in Table 2. There was a significant difference in whole-body bite count (ANOVA: $F = 5.65$; df = 5, 428; $P < 0.05$) and legs-only bite count (ANOVA: $F = 6.64$; df = 5, 428; $P < 0.05$) among five formulation groups and the untreated check. LSMEANS groupings (Table 2) distinguished significant differences between the five formulation groups and the untreated check, but did not distinguish significant differences among the five formulation groups. Whole-body and legs-only bite-count surveys expressed the same result: although there was no significant difference in bite counts among the five treatment groups using a personal repellent, all repellent groups showed significantly reduced bite counts in comparison with the untreated check.

Weight of active ingredient applied among six treatments, with LSMEANS groupings, are given in Table 2. Significantly different weights of active ingredient were applied among the five formulations tested and the untreated check (ANOVA: $F = 163.61$; df = 5, 428; $P < 0.05$). Four groups of decreasing application rate were distinguished (LSMEANS: $P = 0.05$). The group testing aerosol applied the largest amount of deet, followed by the groups testing pump spray and maximum strength, and the groups testing stick and cream. The untreated check applied no repellent products.

Repellent Studies—1988 Dilution Series Study. In the 1988 aerosol repellent dilution tests, a significantly higher legs-only bite count was observed in the 9% deet group (6.41 bites, $n = 17$) than in the 22% deet group (3.24 bites, $n = 21$) (ANOVA: $F = 6.04$; df = 1, 36; $P < 0.05$). The following gross repellent and active ingredient weights were observed: 9% deet, gross repellent 114.66 g, AI 10.31 g; 22% deet, gross repellent 69.52 g, AI 15.29 g. Although the 9% deet group applied significantly more repellent formulation (ANOVA: $F = 13.21$; df = 1, 36; $P < 0.05$), the 22% deet group applied significantly more active ingredient (ANOVA: $F = 6.13$; df = 1, 36; $P < 0.05$).

Table 2. Mean whole-body and legs-only *E. alfreddugesi* bite count and sample size for five deet-based repellent formulations

Formulation	% AI	n[a]	Mean bite count		$\bar{x}$ wt AI[c]
			Whole-body	Legs-only[b]	
Aerosol spray	22	17	15.82a	10.00c	15.47e
Pump spray	18	19	12.37a	8.20c	9.49f
Stick	33	19	17.21a	10.20c	4.87g
Cream	35	20	16.50a	9.15c	3.94g
Maximum strength	100	21	22.86a	10.10c	11.03f
Untreated control	0	338	51.27b	24.20b	0.00h

Formulations tested on human males 9–13 yr of age in Richardson County, NE, July 1987. Data presented are means for 122–125 h exposure.

Means in the same column followed by the same letter are not significantly different under a Protected LSMEANS comparison ($P > 0.05$); those followed by different letters are significantly different.

[a] Number of human volunteers in treatment group.

[b] The leg region extends downward from the articulation of the hip and femur and includes the foot.

[c] Mean weight (g) of deet applied per individual.

Legs-only bite count, gross repellent and active ingredient weight applied, and sample size for six dilutions in the 1988 liquid repellent tests are given in Table 3. There was no significant difference in bite count among the six groups (ANOVA: $F = 0.76$; df = 5, 33; $P > 0.05$). Although there was no significant difference in the weight of repellent formulation applied among the six groups (ANOVA: $F = 0.32$; df = 1, 33; $P > 0.05$), there was a significant difference in the weight of active ingredient applied (ANOVA: $F = 7.42$; df = 5, 33; $P < 0.05$). The amount of active ingredient applied decreased with increase in dilution factor.

Legs-only bite count, gross repellent and active ingredient weight applied, and sample size for seven dilutions in the 1988 pump spray repellent tests are given in Table 4. There was no significant difference in bite count among the seven groups (ANOVA: $F = 1.83$; df = 6, 161; $P > 0.05$). Although there was no significant difference in the weight of repellent formulation applied among the seven groups (ANOVA: $F = 0.37$; df = 6, 161; $P > 0.05$), there was a significant difference in the weight of active ingredient applied (ANOVA: $F = 31.92$; df = 6, 161; $P < 0.05$). The amount of active ingredient applied decreased with increase in dilution factor.

Table 3. Mean weights of gross liquid repellent and active ingredient (deet) applied and mean legs-only *E. alfreddugesi* bite count for six dilution formulations

Deet (%)	n[b]	Mean wt[a]		Mean bite count
		Repellent	AI	Legs-only[c]
1	8	12.20	0.12	4.63
15	5	14.14	2.12	12.50 (8.80)[d]
25	7	16.49	4.12	3.71
50	7	16.28	8.14	9.29 (4.33)[e]
75	6	13.10	9.83	4.50
100	7	12.60	12.60	5.86

Formulations tested on human males 9–13 yr of age in Richardson County, NE, June–July 1988. Data presented are means for 122–125 h exposure.

[a] Mean weight (g) applied per individual.

[b] Number of human volunteers in treatment group.

[c] The leg region extends downward from the articulation of the hip and femur and includes the foot.

[d] One individual had a total of 31 bites. The corrected (outlier dropped) mean legs-only bite count is presented parenthetically.

[e] One individual had a total of 39 bites. The corrected (outlier dropped) mean legs-only bite count is presented parenthetically.

Discussion

Field observations of chigger mite host-seeking behavior indicate that *E. alfreddugesi* displays "Ueno's phenomenon" (Sasa 1961). Larvae remain low in the substrate and move to the highest point on the substrate to form rapidly moving clusters of mites in response to an approaching vertebrate. This behavior is apparently elicited by vertebrate respiratory carbon dioxide (Sasa 1961). If all the chigger mites parasitizing a human infested the body from ground

Table 4. Mean weights of gross pump spray repellent and active ingredient (deet) applied and mean legs-only *E. alfreddugesi* bite count for seven dilution formulations

Deet (%)	n[b]	Mean wt[a]		Mean bite count
		Repellent	AI	Legs-only[c]
1.0	23	75.08	0.75	14.71 (12.74)[d]
2.5	26	69.55	1.74	7.00
4.5	25	69.04	3.10	5.36
7.0	23	73.38	5.14	6.74
9.0	22	70.22	6.32	6.95
13.5	25	65.00	8.78	6.32
18.0	24	79.78	14.36	6.33

Formulations tested on human males 9–13 yr of age in Richardson County, NE, June–July 1988. Data presented are means for 122–125 h exposure.

[a] Mean weight (g) applied per individual.

[b] Number of human volunteers in treatment group.

[c] The leg region extends downward from the articulation of the hip and femur and includes the foot.

[d] One individual had a total of 60 bites. The corrected (outlier dropped) mean legs-only bite count is presented parenthetically.

level as a result of Ueno's phenomenon, a decreasing gradient of parasitism (feet to chest) would be expected. However, the distribution of chigger bites over the human body indicates zones of parasite intensity. This suggests that the movement of *E. alfreddugesi* is arrested on contact with a fleshy substrate and that both direct and indirect infestation routes are utilized.

The calves and ankles were parasitized more intensely than any other region of the body. Study participants generally wore socks with shoes or low boots in the field. The calves or ankles were the first exposed tissue available to mites moving upward in search of an attachment site. Field observations and the data presented here support the hypothesis that chiggers utilize a direct infestation route, as suggested by Ueno's phenomenon, and tend to infest the calf and ankle region heavily as a result of arrested movement on contact with host flesh (Clopton 1989).

The torso (chest, back, and groin) and the feet are sites of intermediate levels of chigger parasitism. In this study, these areas were usually protected from direct infestation; however, the data presented here and additional observations by Clopton (1989) indicate that these areas are available to *E. alfreddugesi* via indirect infestation routes. Field observations confirm that towels, shirts, bedding, and other personal articles left in suitable mite habitat are infested by questing chigger mite larvae. Unless driven back to the substrate by increasing temperature or low humidity, or both, infesting mites will become quiescent and remain in these articles (Williams 1946, Sasa 1961, Clopton 1989). Chiggers are placed in direct contact with a person's body when these articles are retrieved and used. This is defined as an indirect transmission route and seems to be a major path of infestation for the moderate zones of parasitism.

The data place the armpits in the negligible zone of parasitism. This is probably an artifact of the sampling method, which divided the body into physical regions of unequal surface area. An assessment using bites per unit area per region probably would place the armpits in the moderate zone of parasitism.

E. alfreddugesi appears to have a low repellent threshold for deet. The data indicate that the use of a deet-based repellent, with little regard for formulation or concentration, can significantly reduce parasitism by chigger mites. None of the products tested provides 100% repellency. Whole-body and legs-only bite counts seem to provide comparable resolution in this type of repellent test. Legs-only bite counts are less labor-intensive and may prove to be the most efficient way to field-test chigger repellents.

Percentage repellence of all the formulations tested was less than the 100% benchmark established in the laboratory by Kulkarni (1977); however, the repellency observed in our field study should be a good indication of how the product can be expected to perform when used by the general public. Given the age of the study participants (9–13 yr), these evaluations could be considered "worst-case" scenarios for product performance in a "real-world" situation.

Generally, an increase in active ingredient did not significantly reduce bite counts associated with a repellent product. The 9% deet aerosol repellent testing group seems to be an exception to this trend. Bite counts from the 9% deet aerosol test group were well within the range of those of other products tested and much lower than bite counts from untreated individuals. The bite counts from the 9% deet aerosol test group, when compared with the 1987 untreated group (6.41 bites versus 24.20 bites), do not seem to indicate product failure. These data do indicate a loss of efficacy with lowered concentrations of deet in an aerosol product. Equivalent control in the liquid and pump spray dilution series test groups (to 1.0% [AI] deet) suggest insufficient product deposition in the 9% deet aerosol repellent test.

The high application pressure of a pump or aerosol spray jet is designed to maximize turbulent eddies and force droplet shearing, which reduces droplet size. Ideally, reduced droplet size provides a more uniform surface application of the product. In the 9% aerosol product, vortex losses to eddy diffusion, spray jet shearing, and product volatilization during application may have reduced the amount of repellent retained on the skin surface to subthreshold levels. At low deet concentrations, aerosol formulations may suffer greater vortex losses than pump spray formulations because of apparent differences in spray jet velocity.

The aerosol dilution test was the only test in which different repellent concentrations yielded a significant difference in the gross weight of repellent product used. Differences in label color excluded this test from a double-blind protocol, suggesting that excessive repellent use may have been induced as an artifact of product expectations rather than product performance.

Short-term area control is possible with available acaricides but involves expense, equipment, personnel, and potential environmental impact not associated with repellent use. Area control is not a feasible solution in all human exposure cases (e.g., primitive camping, wilderness recreation). Personal repellents (AI: deet) offer an inexpensive, convenient alternative to area control and can reduce human exposure in areas of high chigger mite population density.

Indirect infestation routes may be reduced or eliminated with sensible clothing use and storage. Clothing, towels, backpacks, and other personal items appear to serve as "inanimate mechanical vectors" of chigger mite larvae. These items should be stored away from the substrate to prevent chigger infestation. Anecdotal obser-

vations indicate that chigger mite larvae can remain active in clothing for several days but are eliminated by laundering. A commercial deet-based repellent used according to label directions, coupled with good judgment in clothing use and storage, should significantly reduce the incidence of chigger infestation for persons exposed to *E. alfreddugesi*.

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