Medical and Veterinary Entomology (2008) **22**, 144–151

Twelve-hour duration testing of cream formulations of three repellents against *Amblyomma americanum*

J. F. CARROLL[1], J. P. BENANTE[2], J. A. KLUN[3], C. E. WHITE[4], M. DEBBOUN[5], J. M. POUND[6] and W. DHERANETRA[2]

[1]Animal Parasitic Diseases Laboratory, United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Beltsville Agricultural Research Center, Beltsville, Maryland, U.S.A., [2]Division of Entomology, Walter Reed Army Institute of Research, Silver Spring, Maryland, U.S.A., [3]Invasive Insect Behavior and Biocontrol Laboratory, USDA, ARS, Beltsville Agricultural Research Center, Beltsville, Maryland, U.S.A., [4]Division of Medical A/V, Library and Statistical Services, Walter Reed Army Institute of Research, Silver Spring, Maryland, U.S.A., [5]Medical Zoology Branch, Department of Preventive Health Services, Academy of Health Sciences, US Army Medical Department Center and School, Fort Sam Houston, Texas, U.S.A. and [6]Knipling-Bushland US Livestock Insects Research Laboratory, USDA, ARS, Kerrville, Texas, U.S.A.

Abstract. The repellent efficacies of the U.S. military repellent 33% N,N-diethy l-3-methylbenzamide (deet), 10% and 20% (1S, 2′S) 2-methylpiperidinyl-3-cyclohexene-1-carboxamide (SS220) and 10% and 20% 1-methyl-propyl-2-(hydroxyethyl)-1-piperidinecarboxylate (Bayrepel) cream formulations on human volunteers against the lone star tick *Amblyomma americanum* (L.) were evaluated in a simulated forest floor environment over a 12-h testing period. At 2-h intervals, volunteers, with repellent applied in a 5-cm-wide band around each ankle, stood for 5 min in plastic tubs containing leaf litter and 100 host-seeking *A. americanum* nymphs. Ticks were allowed to remain on a volunteer's feet and ankles for an additional 5 min after the volunteer exited the tub. All repellent formulations provided high levels of protection for the entire 12 h. No ticks crossed 5-cm-wide bands of 20% SS220 and Bayrepel during any challenge, and thus 100% protection was afforded throughout the test. These formulations showed a long-lasting efficacy hitherto unknown in tick repellents intended for use on human skin.

Key words. Lone star tick, (1S, 2′S) 2-methylpiperidinyl-3-cyclohexene-1-carboxamide, 1-methyl-propyl-2-(hydroxyethyl)-1-piperidinecarboxylate, N,N-diethyl-3-methylbenzamide, Picaridin.

Introduction

Ixodid ticks are vectors of a variety of bacterial, viral and protozoan pathogens capable of causing debilitating and even fatal illnesses in humans (Sonenshine, 1993; Parola & Raoult, 2001). The lone star tick *Amblyomma americanum* rapidly responds to host-generated stimuli by moving toward the source (Waladde & Rice, 1982). In recent years, *A. americanum* has increased its range in the U.S.A. and has been incriminated in the transmission of the human pathogens *Ehrlichia chaffeensis* Anderson, Dawson, Jones & Wilson and *Ehrlichia ewingi* Anderson, Greene, Jones & Dawson (Childs & Paddock, 2003). Although increasingly effective area-wide tick control technologies are becoming available (Pound *et al.*, 2000), they are still in relatively limited use. Arthropod repellents, therefore, provide critical personal protection for people entering tick-infested habitats (Centers for Disease Control, 2002).

Permethrin has proven to be an effective repellent against ticks, but its use is limited to clothing treatments (Schreck *et al.*, 1982, 1986; Lane & Anderson, 1984; Evans *et al.*, 1990). For five decades, deet (N,N-diethyl-3-methylbenzamide) has been widely used in repellent products for use on human skin to protect against biting flies and ticks.

Using fingertip bioassays, Schreck *et al.* (1995) found that by 2.7 h post-application, 0.3 mg deet/cm^2 skin repelled $< 90\%$ of *A. americanum* nymphs. Jensenius *et al.* (2005) evaluated four

Correspondence: Dr J. F. Carroll, Animal Parasitic Diseases Laboratory, USDA, ARS, BARC-East, Building 1040, Beltsville, Maryland 20705, U.S.A. Tel.: + 301 504 9017; Fax: + 301 504 5306; E-mail: john.carroll@ars.barc.usda.gov

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE **2008**	2. REPORT TYPE	3. DATES COVERED **00-00-2008 to 00-00-2008**

4. TITLE AND SUBTITLE
Twelve-hour duration testing of cream formulations of three repellents against Amblyomma americanum

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
U.S. Department of Agriculture,Animal Parasitic Diseases Laboratory, Agricultural Research Service (ARS),Beltsville Agricultural Research Center,Beltsville,MD,20705

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The repellent efficacies of the U.S. military repellent 33% N,N-diethy l-3-methylbenzamide (deet), 10% and 20% (1S, 2 ′ S) 2-methylpiperidinyl-3-cyclohexene- 1-carboxamide (SS220) and 10% and 20% 1-methyl-propyl-2-(hydroxyethyl)-1-piperidinecarboxylate (Bayrepel) cream formulations on human volunteers against the lone star tick Amblyomma americanum (L.) were evaluated in a simulated forest floor environment over a 12-h testing period. At 2-h intervals, volunteers, with repellent applied in a 5-cm-wide band around each ankle, stood for 5 min in plastic tubs containing leaf litter and 100 host-seeking A. americanum nymphs. Ticks were allowed to remain on a volunteer ? s feet and ankles for an additional 5 min after the volunteer exited the tub. All repellent formulations provided high levels of protection for the entire 12 h. No ticks crossed 5-cm-wide bands of 20% SS220 and Bayrepel during any challenge, and thus 100% protection was afforded throughout the test. These formulations showed a longlasting efficacy hitherto unknown in tick repellents intended for use on human skin.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**8**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

commercially available lotions containing deet against *Amblyomma hebraeum* Koch, a species that occurs in sub-Saharan Africa and the West Indies. They found that, in fingertip bioassays, lotions consisting of 19.5%, 31.6% and 80% deet repelled ≥77% *A. hebraeum* nymphs at 2 h post-treatment and < 70% at 4 h post-treatment. A 2% deet formulation containing 1% citronella oil had only 59% repellency against *A. hebraeum* at 1 h post-treatment. Bayrepel (1-methyl-propyl-2-[hydroxyethyl]-1-piperidinecarboxylate) (also known as Picaridin and KBR 3023) has shown effectiveness in repelling mosquitoes and ticks (Klun *et al.*, 2003; Pretorius *et al.*, 2003).

To be of significance as a personal protective measure, a repellent must act against the target organisms over an extended period. However, little is known of the extended efficacy (≥8 h) of commonly used and promising repellents against ticks. Pretorius *et al.* (2003) compared 20% lotions of deet and KBR 3023 (Bayrepel) in fingertip bioassays and found that both repelled > 85% of *A. hebraeum* nymphs at 1 h after treatment. Although repellency fell to 71% and 54% efficacy, respectively, by 4 h post-treatment, deet and Bayrepel were clearly highly repellent to the ticks tested for ≥1 h. (1S, 2′S) 2-methylpiperidinyl-3-cyclohexene-1-carboxamide (SS220) repelled ≥94% *A. americanum* and *Ixodes scapularis* Say nymphs in fingertip bioassays 10–20 min after application (Carroll *et al.*, 2005).

The Walter Reed Army Institute of Research (WRAIR) Arthropod Repellent Program is directed toward finding an improved arthropod repellent formulation that: (a) provides protection equal to or better than that of the current military insect repellent, a sustained-release, polymer formulation, known as the extended duration topical insect and arthropod repellent (EDTIAR), containing 33% deet; (b) prevents bites for ≥12 h under a variety of environmental conditions; (c) is safe to use, and (d) is acceptable to the user and pleasant to apply on human skin (Debboun *et al.*, 1999).

The ultimate test of a repellent's efficacy is how well it performs under field conditions. In a field test using human volunteers, Solberg *et al.* (1995) found that, at 0.5 mg/cm^2 skin, deet repelled 85% of *A. americanum* nymphs and adults during 30 min exposure immediately after treatment, but only 55% during a similar exposure 6 h post-treatment. By contrast, the racemic 2-methylpiperidinyl-3-cyclohexene-1-carboxamide (AI3-37220) repelled > 90% of *A. americanum* 6 h after treatment.

Field trials involving multiple concentrations of multiple repellents can be difficult and often uninformative. Adequate replicated exposure of each of several treatments can be compromised by time limitations, volunteer scheduling, weather, variable tick densities, and distributions (Schulze *et al.*, 2002). Rain and wet vegetation inhibit tick acquisition and consequent trial postponements can be a concern if volunteers must travel to a distant location. Walking with bare lower legs through such vegetation to contact ticks also exposes volunteers to scratches from thorns and skin rashes from plants, such as poison ivy (*Toxicodendron radicans*).

To address the need for combined efficacy and longevity in safe and acceptable repellents, we used an alternative to a classic (outdoor) field trial. We tested cream formulations of deet, SS220 and Bayrepel against *A. americanum* under simulated field-contact conditions over a 12-h period. Bayrepel and SS220 were chosen on the basis of the high level of repellent activity they have shown against ticks and mosquitoes (multi-pest protection is an important quality in a repellent) in a variety of studies (Debboun *et al.*, 2007).

Materials and methods

Ticks

The lone star tick nymphs used in the trials were from a colony maintained at the United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Knipling-Bushland Livestock Insects Research Laboratory, Kerrville, TX. They were kept at 24 °C, ≈97% RH and LD 16 : 8 h until testing. All tests coincided with the photophase in which ticks were kept. Ticks were tested at 8–10 weeks after their last molt.

Repellents

Deet (33%) was formulated as standard military use formulation (EDTIAR) by 3M (St Paul, MN, U.S.A.). Bayrepel (10% and 20%) and SS220 (10% and 20%) were formulated by Avon Products, Inc. (Suffern, NY, U.S.A.). The repellent creams were individually formulated for each arthropod repellent product (personal communication, A. Pechko, Avon Products, Inc.). Because there was no single cream common to all the repellent formulations, a blank cream control was not used.

Volunteers

Human experimentation guidelines and protocols defined by the National Institute of Health and the WRAIR Human Use Review Board, specifically Human Use Protocol No. 970 (Field Evaluation of Tick Repellents and Repellent Formulations Using Human Volunteers) were adhered to in all aspects of this study. The 10 volunteers (four women, six men) who participated in the trials were all > 21 years old. One female volunteer was used for 1 day only as a replacement for a scheduled volunteer who was unable to participate that day. On the day of each trial, all scheduled volunteers wore shorts. Footwear was removed at the start of each challenge.

Design

Each formulation was tested on five or six volunteers. Four volunteers were used on each of 9 days. On each day three volunteers received a formulation and the fourth served as a control (Table 1). The assignment of treatments was withheld from volunteers. Because the control was bare skin, volunteers were aware when they had not received a repellent. The applications were staggered at about 20 min intervals. Challenges were timed at 2, 4, 6, 8, 10 and 12 h after application. Between

Table 1. Schedule of volunteers and the repellent treatments they received.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
Volunteer									
1		Control		20% S					
2	20% B	20% S			33% D		Control		10% B
3	20% B	10% S			33% D		10% B	20% S	Control
4			33% D		Control	10% B		10% S	
5				10% S		10% B	20% B	Control	20% S
6	20% B	20% S	10% S	Control					
7	Control			20% B		33% D			
8			10% S			Control			10% B
9			Control				20% S	33% D	
10					33% D				

B, Bayrepel; D, deet; S, SS220.

challenges, volunteers were allowed to engage in normal activities but avoided contact between the bands of repellent cream and other surfaces, including other areas of their own legs.

Bioassay

The circumferences of the ankles at 5 cm and 10 cm above the lateral projection of the ankle bone were measured on both legs of each volunteer. Based on the average of these measurements for each leg, the surface area of the intervening 5-cm-wide band around the leg was calculated. Using a fine-tipped marker, two horizontal lines were drawn 5 cm apart around each of a volunteer's legs, with the lower of the lines about 5 cm above the ankle bone, to mark the boundaries of the treatment band. Quantities of repellent cream providing a coverage of 1.92 mg cream/cm^2 of skin on the leg were weighed on plastic weigh boats about 5 min before application. The repellent cream was removed from the weigh boat on the gloved index finger of one person (throughout the trial) and evenly applied to the skin between the lines marked on a volunteer's leg. The glove and weigh boat were weighed before receiving the cream, with the cream and after the application to ascertain the quantity of cream applied.

Most ticks are acquired from leaf litter and low vegetation (≤ 1 m above ground level). Dry leaf litter, consisting predominantly of leaves from oaks (*Quercus rubra*, *Quercus falcata, Quercus alba*), sweet gum (*Liquidambar styraciflua*), black gum (*Nyssa sylvatica*), red maple (*Acer rubrum*), sassafras (*Sassafras albidum*) and Virginia pine (*Pinus virginiana*), was collected from woodlands in Prince George's and Montgomery counties, Maryland. Leaf collection sites were ostensibly favourable for the occurrence of *A. americanum*; however, leaves were only taken from portions of the sites where no ticks were captured by pre-collection flagging with a flannel cloth. Leaves were stored in closed plastic bags until used. Leaf litter was spread to a depth of about 7 cm in a plastic tub (0.92 × 0.54 × 0.13 m) (Gracious Living Industries, Woodbridge, ON, Canada). A continuous band of masking tape (7.5 cm wide) was affixed around the rim of each tub so that about 5 cm of sticky surface projected above the rim. One hundred *A. americanum* nymphs were transferred using artist's paint brushes from holding vials to the surface of the leaf litter in the central portion of the tub (≥ 10 cm from the walls of the tub). Within 3 min of the ticks being distributed in the tub, a barefoot volunteer stepped into the tub and shuffled into the leaf litter to maximize contact of the upper surface of the feet and

Table 2. Average percentage protection among all volunteers challenged at 2-h intervals. Volunteers acted as their own controls.

	Hours post-application					
Treatment	2 h	4 h	6 h	8 h	10 h	12 h
Bare skin*	35.17	−3.04	−23.31	0.05	−5.93	19.45
33% deet	100	97.39	97.39	100	100	97.65
10% Bayrepel	97.90	98.94	99.23	98.23	98.53	98.44
20% Bayrepel	100	100	100	100	100	100
10% SS220	100	100	100	99.23	98.17	98.97
20% SS220	100	100	100	100	100	100

*The bare skin (no repellent cream) row compares a volunteer's count of ticks for individual untreated skin challenges with the average of all bare skin counts for that person. Individual results are expected to range between 100% and − 100%.

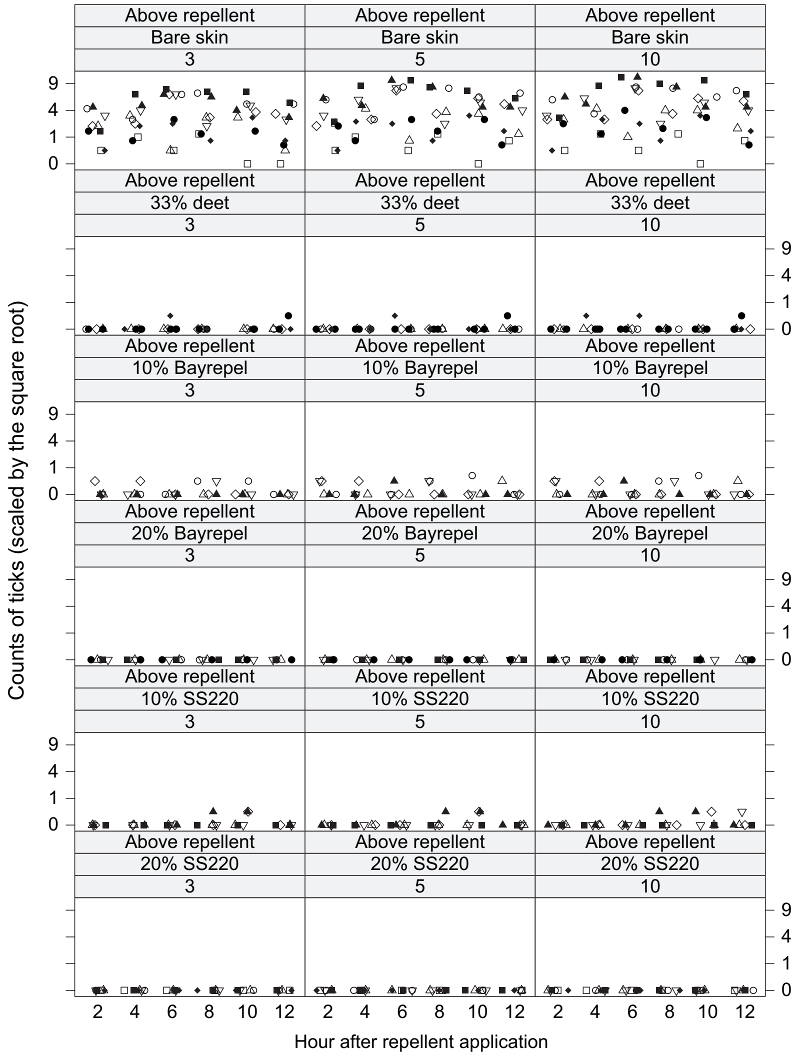

Fig. 1. Number of ticks above the treatment bands per volunteer at 3, 5 and 10 min after the start of each exposure. (Ticks that crossed the repellent treatment bands on volunteers' legs were considered not repelled.). Because ticks that ascended above the treatment bands were immediately removed for safety reasons, 10-min counts represent the sum of all ticks that crossed during the specific challenge period. Each symbol denotes the count on an individual volunteer. Note that no ticks crossed 20% Bayrepel or 20% SS220.

lower ankles with the ticks. Stepping downward on the leaf litter was avoided in order not to trap ticks beneath the feet. At 3 min after a volunteer entered the tub, the numbers of ticks on the volunteer's legs were recorded according to location: below the treatment band; on the treatment band, and above the treatment band. As soon as a tick climbed completely across a treatment band, it was removed from the volunteer's leg with a piece of masking tape, thus eliminating a risk to the volunteers. Ticks that crossed through a treatment band were considered not repelled. With many ticks crawling on the volunteer's legs and feet, two persons (counters/removers) were needed to obtain a simultaneous 360-degree view of the volunteer's legs. The counters/removers approached to within 1.5 m of the volunteer only to count or remove ticks. At 5 min after entering the tub, the volunteer stepped out of the tub onto a mat of plastic-backed absorbent paper (plastic side up) and ticks were counted again according to location. As soon as ticks dropped or crawled from the volunteer onto the mat, they were removed on tape. The volunteer stood on the mat for an additional 5 min, at which point a final count was made and all ticks removed. After each challenge, the leaves and ticks were dumped into plastic garbage bags, which were sealed and later autoclaved. During the exposures, the ambient temperature was 21–24 °C and RH 18–48%.

Statistical methods

Graphics, simple summary statistics, and generalized linear mixed models were used to analyse these data. Simple summary statistics included point estimates for average percentage protection across volunteers. For this calculation, each volunteer acted as his/her own control. Percentage protection per volunteer was calculated as 1 – (total number of ticks that had crawled completely across a particular repellent band during a particular count) / (average number of ticks found on the bare skin control) × 100.

Generalized linear mixed models were fit under R (R Development Core Team, 2005) using the optional lme4 (Bates & Sarkar, 2006) and Matrix (Bates & Maechler, 2006) packages. Fixed effect components for models were selected when they were

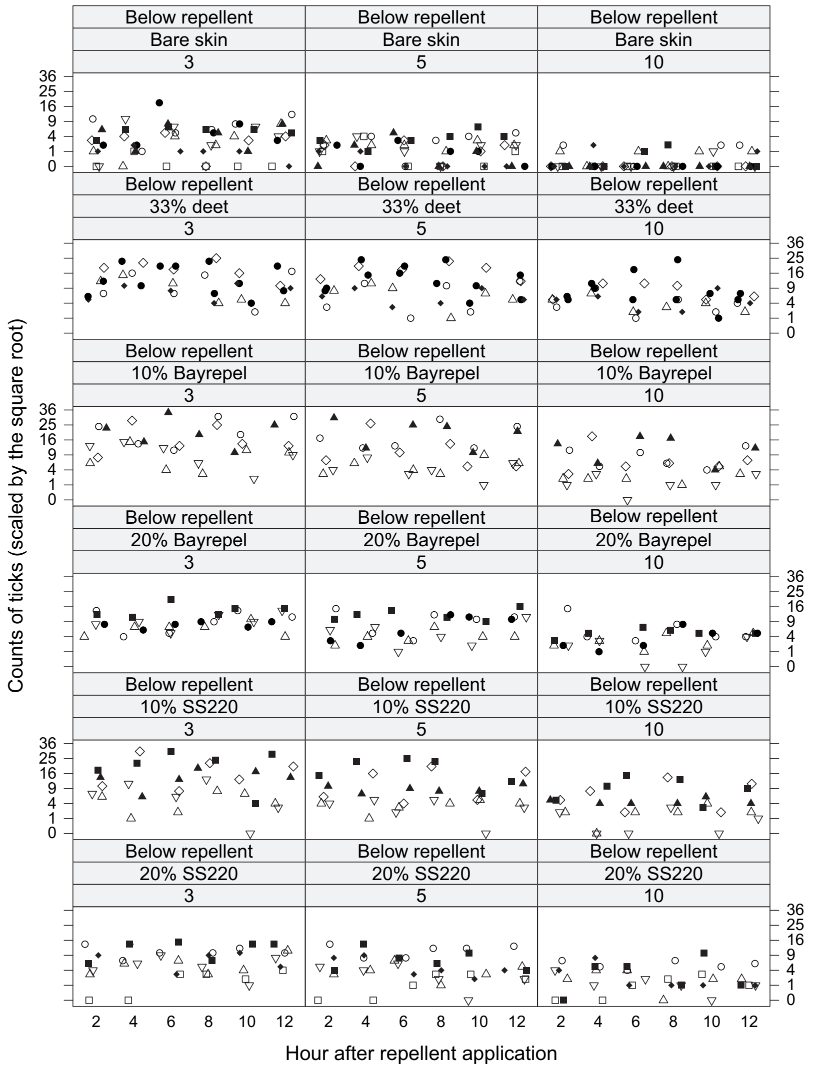

Fig. 2. Tick counts on legs and feet below the treatment bands of individual volunteers. In the case of controls, ticks crawled up the volunteers' legs above the level of the treatment band. With the repellents, ticks were often observed dropping from and crawling off the volunteers' legs and feet, accounting for the lower counts at 5 and 10 min. That ticks tended to leave repellent-treated volunteers rather than remain on their untreated skin is important to personal protection. Each symbol denotes the count on an individual volunteer.

found to be statistically significant in comparison with a mechanistically selected base model. The fixed effect for the base model was hour after application. The random effects for the base model were day of study and volunteer. Distributions were modelled as either Poisson or quasi-Poisson.

Results

A tick was considered repelled if it failed to ascend completely across the 5-cm-wide treatment area encircling each ankle of the volunteers. On legs that did not receive a repellent treatment, ticks generally climbed rapidly up the ankle and through the marked 5-cm band that corresponded with the treatment bands (Fig. 1). All treatments repelled greater (statistically significant) numbers of ticks than the control ($P = 2.2 \times 10^{15}$, quasi-Poisson distribution). All repellent treatments were effective through 12 h post-application, with an average protection by challenge period (2, 4, 6, 8, 10, 12 h post-application) for all treatments of ≥97.39% (Table 2). In no case was the percentage protection < 85% for an individual volunteer during a particular counting period. Both 20% SS220 and 20% Bayrepel creams provided 100% protection for 12 h, with no tick crossing the treatment bands on any volunteer for any count for any challenge (Table 1). At 10%, SS220 and Bayrepel creams were only slightly less effective, repelling 92–97% of ticks. The 33% deet formulation (EDTIAR) repelled > 85% of the ticks throughout the 12-h testing period for each volunteer tested (Fig. 2). All the alternative repellents were estimated to repel more ticks than deet, although not at a statistically significant level. The 20% Bayrepel showed the largest difference with deet ($P \approx 0.057$), followed by 10% Bayrepel ($P \approx 0.477$), 20% SS220 ($P \approx 0.376$), and 10% SS220 ($P \approx 0.374$). The high levels of effectiveness of all treatments make practical comparisons difficult.

Some ticks repeatedly circled a leg at the lower boundary of a repellent band, whereas others entered the treatment and turned back or fell from the leg. Ticks were observed in the treatment bands of all formulations for at least one 3-, 5- and 10-min count except for 10-min counts for 20% Bayrepel. During the 12-h period, at least one tick crossed through the

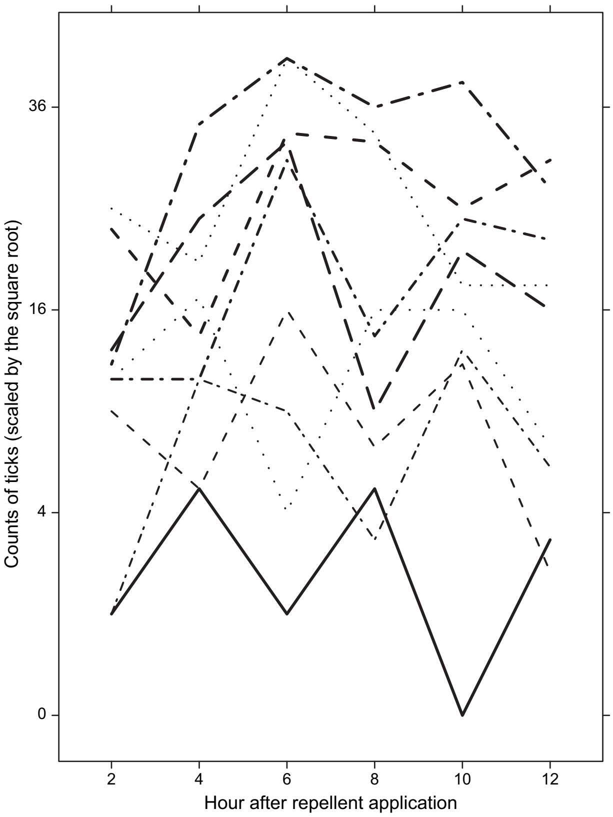

Fig. 3. Tick counts for individual volunteers during the six challenges when that person was a control. Individual volunteers acquired characteristically high, low or intermediate numbers of ticks. This is shown in the 10-min counts of ticks on a volunteer's legs and feet on the day that volunteer served as untreated control. Ticks were removed for safety reasons as soon as they crossed the marked band and were included in the 10-min count.

treatment band on five volunteers treated with 10% Bayrepel, three with 10% SS220, and two with 33% deet (Fig. 1). With 10% Bayrepel, at least one tick crossed through a treatment band of at least one volunteer during each of six challenge periods. By contrast, all the crossings of 10% SS220 occurred during the 8-, 10- and 12-h challenges. Ticks often fell or crawled off the feet, ankles and lower legs of volunteers treated with repellent creams. Counts of ticks below the treatment band tended to decrease between the 3- and 10-min counts. When a volunteer had received a repellent, many ticks crawled onto and then left the volunteer, whereas when a volunteer acted as a control, the ticks climbed through the marked band and were removed. Consistent individual variation was evident among the volunteers in the numbers of ticks counted on them, whether they had received a repellent or were acting as control (Fig. 3). Counts for each volunteer can be tracked by treatment over 12 h in Figs 1, 2.

Discussion

Foremost among our findings was the persistently high level of repellency evidenced by all the cream formulations throughout the 12-h trials. Long-lasting effectiveness of insect repellents applied to human skin has been reported against mosquitoes (Frances *et al.*, 2002). However, this is the first demonstration of such prolonged efficacy of insect-repellent products used on human skin against ticks. A long-lasting repellent allows users to remain in tick habitats for extended periods of time and requires fewer reapplications to maintain protection. In the present study, 33.3% deet cream provided ≥85% repellency against *A. americanum* for each challenge period throughout the 12 h and the Bayrepel and SS220 formulations provided equal or greater levels of repellency for the same duration. Using technical repellent in ethanol solutions, Schreck *et al.* (1995) found that the effectiveness of deet (0.3 mg repellent/cm^2 skin) against *A. americanum* nymphs fell to < 90% by 2.7 h post-application. We surmise that formulation chemistry was primarily responsible for the prolonged effectiveness of deet and the other repellent compounds observed in this study. Except for 10% SS220, no trend was discerned indicating that the efficacy of any of the repellent formulations diminished over time during the 12-h trials.

As in a field trial, the total number of ticks contacted and acquired by a volunteer during a challenge was not known. Three snapshot observations (counts) were made, at 3 and 5 min, with the volunteer in the tub, and at 10 min, with the volunteer out of the tub. The counts at 10 min tended to be lower than those at 3 and 5 min. As a volunteer stood in the tub, some ticks left the volunteer at the same time as others were moving onto the volunteer, and in the case of controls ticks crawled beyond the marked band and were removed. A tendency for *A. americanum* to drop off vertical surfaces when they encounter deet or racemic 220 was reported by Carroll *et al.* (2004) and is of significance in that it is preferable that repelled ticks leave a potential host completely rather than relocating on untreated skin or remaining sequestered on untreated clothing.

The proclivity of *A. americanum* to quickly move toward host-associated cues makes them good experimental subjects for behavioural bioassays with repellents. Host-seeking *A. americanum* are strongly attracted to CO_2 (Wilson *et al.*, 1972). They possess eyes, which, although simple (Phillis & Cromroy, 1977), may have the capacity to detect movements of hosts silhouetted against contrasting backgrounds. There is some evidence that higher concentrations of active ingredient are needed to repel *A. americanum* than *I. scapularis* (Carroll *et al.*, 2004, 2005, 2007), although this may not be true in all cases (Schreck *et al.*, 1995). *Ixodes scapularis* is too slow-moving for its use in a bioassay such as that described here to be practical. Even in fingertip bioassays, it is necessary to screen *I. scapularis* nymphs to determine which individuals are active climbers (Schreck *et al.*, 1995; Carroll *et al.*, 2005).

Under the simplified conditions of this study, all volunteers were exposed repeatedly to the same number of ticks, yet there was a tendency for individual volunteers to have consistently relatively high, intermediate or low counts. Individual variation in the attractiveness of humans to mosquitoes has been documented by several studies and was attributed to the mediation of host-seeking behaviour by host-produced chemical cues (Costantini *et al.,* 2001; Bernier *et al.*, 2002). Although *A. americanum* is an active 'hunter-type' species (Waladde & Rice, 1982) that will move toward a host, in nature tick–host contact is often a consequence of a host brushing against a stationary questing tick. The impact of individual variation among hosts, particularly humans, on the attraction and retention of ticks is poorly understood. Our observations suggest the need for an ample pool of volunteers or screening volunteers when conducting tick repellent trials.

The simulated field test showed that a high level of longterm (12 h) protection against an aggressive tick species, that is difficult to repel, is possible with cream formulations of 33% deet, 10% and 20% Bayrepel, and 10% and 20% SS220 applied at a recommended rate. Discrimination among these formulations as to which is the most effective against ticks may require tests lasting longer than 12 h. However, our results clearly encourage expectation of repellent products with improved protection times against tick bites.

Acknowledgements

We are especially grateful to the volunteers, without whose cooperation the repellent trials would not have been possible. We also thank T. E. Rowland, Walter Reed Army Institute of Research, K. W. Young and A. M. Abrams, United States Department of Agriculture, Agricultural Research Service, Animal Parasitic Diseases Laboratory, for their valuable assistance in conducting the trials. The opinions or assertions contained herein are the private views of the authors, and are not to be construed as official, or as reflecting true views of the US Department of the Army or the Department of Defense.

References

Bates, D.M. & Maechler, M. (2006) *Matrix: a Matrix Package for R. R Package Version 0.995-8*, R Foundation for Statistical Computing.

Bates, D.M. & Sarkar, D. (2006) *lme4: Linear Mixed-Effects Models using S4 Classes. R Package Version 0.995-2*, R Foundation for Statistical Computing.

Bernier, U.R., Kline, D.L., Schreck, C.E., Yost, R.A. & Barnard, D.R. (2002) Chemical analysis of human skin emanations: comparison of volatiles from humans that differ in attraction of *Aedes aegypti* (Diptera: Culicidae). *Journal of the American Mosquito Control Association*, **18**, 186–195.

Carroll, J.F., Solberg, V.B., Klun, J.A., Kramer, M. & Debboun, M. (2004) Comparative activity of deet and AI3-37220 repellents against the ticks *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) in laboratory bioassays. *Journal of Medical Entomology*, **41**, 249–254.

Carroll, J.F., Klun, J.A. & Debboun, M. (2005) Repellency of two species of ticks (Acari: Ixodidae) by deet and SS220 applied to skin involves olfactory sensing. *Medical and Veterinary Entomology*, **19**, 101–106.

Carroll, J.F., Cantrell, C.L., Klun, J.A. & Kramer, M. (2007) Repellency of two terpenoid compounds isolated from *Callicarpa americana* (Lamiaceae) against *Ixodes scapularis* and *Amblyomma americanum* ticks. *Experimental and Applied Acarology*, **41**, 215–224.

Centers for Disease Control (2002) *Lyme Disease*, p. 12. Department of Health and Human Services, Centers for Disease Control and Prevention, Fort Collins, CO.

Childs, J.E. & Paddock, C.D. (2003) The ascendancy of *Amblyomma americanum* as a vector of pathogens affecting humans in the United States. *Annual Review of Entomology*, **48**, 307–337.

Costantini, C., Birkett, M.A., Gibson, G. *et al*. (2001) Electroantennogram and behavioural responses of the malaria vector *Anopheles gambiae* to human-specific sweat components. *Medical and Veterinary Entomology*, **15**, 259–266.

Debboun, M., Strickman, D., Klein, T.A. *et al*. (1999) Laboratory evaluation of AI3-37220, AI3-35765, CIC-4, and deet repellents against three species of mosquitoes. *Journal of the American Mosquito Control Association*, **15**, 342–347.

Debboun, M., Frances, S.P. & Strickman, D. (2007) *Insect Repellents: Principles, Methods, and Uses*, pp. 495. CRC Press, Boca Raton, FL.

Evans, S.R., Korch, G.W. Jr & Lawson, M.A. (1990) Comparative field evaluation of permethrin and deet-treated military uniforms for personal protection against ticks (Acari: Ixodidae). *Journal of Medical Entomology*, **27**, 829–834.

Frances, S.P., Van Dung, N., Beebe, N.W. & Debboun, M. (2002) Field evaluation of repellent formulations against daytime and night-time biting mosquitoes in a tropical rainforest in northern Australia. *Journal of Medical Entomology*, **39**, 541–544.

Jensenius, M., Pretorius, A.-M., Clarke, F. & Myrvang, B. (2005) Repellent efficacy of four commercial DEET lotions against *Amblyomma hebraeum* (Acari: Ixodidae), the principal vector of *Rickettsia africae* in southern Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **99**, 708–711.

Klun, J.A., Khrimian, A., Margaryan, A., Kramer, M. & Debboun, M. (2003) Synthesis and repellent efficacy of a new chiral piperidine analogue: comparison with deet and Bayrepel activity in human-volunteer laboratory repellent assays against *Aedes aegypti* and *Anopheles stephensi*. *Journal of Medical Entomology*, **40**, 293–299.

Lane, R.S. & Anderson, J.R. (1984) Efficacy of permethrin as a repellent and toxicant for personal protection against the Pacific coast tick and the pajaroello tick (Acari: Ixodidae and Argasidae). *Journal of Medical Entomology*, **21**, 692–702.

Parola, P. & Raoult, D. (2001) Ticks and tick-borne diseases in humans: an emerging infectious threat. *Clinical Infectious Diseases*, **32**, 897–928.

Phillis, W.A. & Cromroy, H.L. (1977) The microanatomy of the eye of *Amblyomma americanum* (Acari: Ixodidae) and resultant implications of its structure. *Journal of Medical Entomology*, **13**, 685–698.

Pound, J.M., Miller, J.A., George, J.E. & LeMeilleur, C.A. (2000) The '4-poster' passive topical treatment device to apply acaricide for controlling ticks (Acari: Ixodidae) feeding on white-tailed deer. *Journal of Medical Entomology*, **37**, 588–594.

Pretorius, A.-M., Jensenius, M., Clarke, F. & Ringertz, S.H. (2003) Repellent activity of DEET and KBR 3023 against *Amblyomma hebraeum* (Acari: Ixodidae). *Journal of Medical Entomology*, **40**, 245–248.

R Development Core Team. (2005) *R: A Language and Environment for Statistical Computing, Reference Index Version 2.2.1.* R Foundation for Statistical Computing, Vienna. http://www.R-project.org.

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

Schreck, C.E., Snoddy, E.L. & Spielman, A. (1986) Pressurized sprays of permethrin or deet on military clothing for personal protection against *Ixodes dammini* (Acari: Ixodidae). *Journal of Medical Entomology*, **23**, 396–399.

Schreck, C.E., Fish, D. & McGovern, T.P. (1995) Activity of repellents applied to skin for protection against *Amblyomma americanum* and *Ixodes scapularis* ticks (Acari: Ixodidae). *Journal of the American Mosquito Control Association*, **11**, 136–140.

Schulze, T.L., Jordan, R.A. & Hung, R.W. (2002) Effects of microscale habitat physignomy on the focal distribution of *lxodes scapularis* and

Amblyomma americanum (Acari: Ixodidae) nymphs. *Environmental Entomology*, **31**, 1085–1090.

Solberg, V.B., Klein, T.A., McPherson, K.R., Bradford, B.A., Burge, J.R. & Wirtz, R.A. (1995) Field evaluation of deet and a piperidine repellent (AI3-37220) against *Amblyomma americanum* (Acari: Ixodidae). *Journal of Medical Entomology*, **32**, 870–875.

Sonenshine, D.E. (1993) *Biology of Ticks*. Vol. 2, pp. 465. Oxford University Press, New York.

Waladde, S.M. & Rice, M.J. (1982) The sensory basis of tick feeding behaviour. *Physiology of Ticks* (ed. by F. D. Obenchain & R. Galun), pp. 71–118. Pergamon, New York.

Wilson, J.G., Kinzer, D.R., Sauer, J.R. & Hair, J.A. (1972) Chemo-attraction in the lone star tick (Acarina: Ixodidae). I. Response of different developmental stages to carbon dioxide administered via traps. *Journal of Medical Entomology*, **9**, 245–252.

Accepted 30 January 2008