Advance Publication by J-STAGE

Japanese Journal of Infectious Diseases

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Kohei Ogawa, Osamu Komagata, Toshihiko Hayashi, Kentaro Itokawa, Shigeru Morikawa, Kyoko Sawabe, and Takashi Tomita

Received: January 29, 2015. Accepted: May 26, 2015
Published online: June 12, 2015
DOI: 10.7883/yoken.JJID.2015.038
Field and laboratory evaluations of the efficacy of DEET repellent against *Ixodes* ticks

Kohei Ogawa¹, Osamu Komagata¹, Toshihiko Hayashi¹, Kentaro Itokawa¹, Shigeru Morikawa², Kyoko Sawabe¹, and Takashi Tomita¹*

¹Department of Medical Entomology and ²Department of Veterinary Science, National Institute of Infectious Diseases, Tokyo 162-8640, Japan

*Corresponding author: Department of Medical Entomology, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan.
Tel: +81-03-4582-2743, Fax: +81-03-5285-1147, E-mail: tomita@nih.go.jp

Keywords: DEET, ticks, repellent, human trap, flag-dragging
小川浩平 1, 駒形修 1, 林利彦 1, 糸川健太郎 1, 森川茂 2, 沢辺京子 1, 富田隆史 1

1 〒162-8640 東京都新宿区戸山 1-23-1 国立感染症研究所昆虫医科学部

2 〒162-8640 東京都新宿区戸山 1-23-1 国立感染症研究所獣医科学部
The objective of this study was to clarify the efficacy of a currently-available DEET repellent against tick species in Japan. We performed two different field trials: “human trap,” and “flag-dragging.” In total, 482 ticks were collected from white flannel cloths in field studies. The species of collected ticks consisted of *Ixodes persulcatus* and *I. ovatus* which accounted for 5.3 and 94.7% in the human trap test and 31.4 and 68.6% in the flag-dragging test, respectively. The repellency levels of DEET-treated flannel cloths in the human trap and flag-dragging tests were 84.0 and 99.7%, respectively. The escape time of *I. persulcatus* and *I. ovatus* female adults from DEET-treated flannel cloth was measured. Median escape times for *I. persulcatus* and *I. ovatus* on DEET-treated flannel cloth were 48 s (95% CI: 30–96) and 10 s (95% CI: 5–24), respectively. In contrast, many ticks remained on untreated flannel for 10 min after mounting. These results indicate that DEET repellents appear to prevent tick bites and that the use of DEET repellents against ticks is effective as a personal protective measure.
INTRODUCTION

Ticks are important vectors of numerous human infectious diseases such as lyme borreliosis and rickettsial diseases (1). In Japan, pathogens such as anaplasmosis, ehrlichiosis, babesiosis, lyme disease, tick-borne encephalitis and Japanese spotted fever are isolated or found from ticks (2–11). Moreover, severe fever with thrombocytopenia syndrome (SFTS), which is caused by a novel bunyavirus that was first isolated in China, has recently been found to be prevalent in Japan (12). Given that effective vaccines are currently unavailable except for tick-borne encephalitis, the avoidance of tick bites is the most important preventive strategy against these tick-borne diseases.

A personal measure that can be taken to protect from tick bites is the use of repellents. DEET (N, N-diethyl-m-toluamide) has a broad activity spectrum that includes mosquitoes, biting flies, chiggers and ticks (13). It has been the most widely used arthropod repellent for over five decades. In the U.S., DEET-based products with concentrations ranging from 5-100% are commercially available for application to skin or clothing. It is estimated by the U.S. Environmental Protection Agency (EPA) that DEET is used by over one-third of the U.S. population (14).

In 2013, when the present study was conducted, despite the availability of a few chigger control products for repelling acarins by skin or clothing treatment, none of the DEET repellents in Japan included directions for tick control. Furthermore, evaluations of the tick repellents registered from 2013 to 2014 have not been published. The purpose of this study was to clarify the efficacy of currently-available DEET repellent in Japan.
MATERIALS AND METHODS

Repellent

In this study, a commercially available DEET repellent (Mushi-Pale® PS: which includes 12% DEET as an active ingredient per formulate concentrate; in formulation for liquefied petroleum gas-mediated spray; Ikeda Mohando Co., Ltd. Toyama, Japan) was used for the processing of flannel sheets. The directions for use of this product were as follows: (I) Shake 5-10 times before use and spray from a distance of approximately 15 cm. (II) Do not spray continuously for more than 3 s at the same site. (III) For chiggers, reapply the spray every 4-6 h. One of the characteristics of this product is improved stability due to the attachment of DEET to a silicon dioxide matrix.

Human trap test

The human trap test was performed in Karikachi Toge, Shintoku Town, Hokkaido Prefecture (43°14’N, 142°77’E), between 11:00 a.m. and 3:00 p.m. on June 24, 2013. The temperature at 1 m above ground was repeatedly recorded during the field test using an HOBO Pendent® Temperature/Light Data Logger 8K (Onset Computer Corporation, Bourne, Mass). The field was mainly composed of bamboo grass of approximately 1 m in height. A piece of white flannel cloth (100% cotton, 72 x 72 cm) was spread on the ground and then only one side of the cloth was evenly treated with the DEET formulation by releasing approximately 20 g of spray can content per cloth toward the cloth at 15 cm above the ground. Each of the treated pieces of flannel was prepared just before two serial trials, which reused the same cloth. The weights of the spray can before and after each spraying were recorded to calculate
the DEET dosage per flannel cloth. The experiment was conducted by two testers. The left leg of each tester was fitted with the DEET-treated flannel cloth and the right leg with an untreated flannel cloth. The testers walked bow-legged in a field for 5 min in order to avoid the transfer of attached ticks between left and right legs and then stood still on an asphalt road for 10 min. Attached ticks on each piece of the flannel were then collected with tweezers. Afterwards, the species and developmental stages of the sampled ticks were identified in a laboratory under a stereomicroscope. Data, including the number of ticks, their species and their developmental stage were recorded for each trial. Each piece of DEET-treated flannel cloth was reused up to twice. Thirteen replications were performed.

**Flag-dragging Test**

The flag-dragging test was performed on a mountain trail along Tottabetsu river, Obihiro City, Hokkaido Prefecture (42°72’N, 142°90’E), between 9:30 a.m. and 3:30 p.m. on June 25, 2013. Temperatures during the field test were recorded as above. Both sides of the trail had weed growth of approximately 30 cm in height. A piece of white flannel cloth (72 x 72 cm) was treated with DEET by the same method as applied to the human trap test, with the exception that DEET treatment was applied to both sides. A flag was built up by holding a flannel cloth at one side with a wooden stick (85 cm x φ2.0 cm). Flags of DEET-treated or untreated flannel cloth were dragged by two testers over vegetation within 1 m of the trail edge while walking for 5 min at approximately 26 m/min and were held suspended in the air for 10 min. The ticks attached to each flannel were then collected by tweezers. Data pertaining to the collected ticks were then recorded in the same manner as described in the human trap
test. A DEET-treated flannel cloth was reused up to two times. Twenty-two replications were performed with DEET-treated and untreated (control) flannel cloths.

**Laboratory Test**

Laboratory tests used female adults of *Ixodes persulcatus* and *I. ovatus* that were collected during human trap and flag-dragging trials in field tests. Both sides of a white flannel cloth (72 x 72 cm) were treated with DEET by the same method as the flag-dragging test and then cut into 10 x 10 cm pieces for laboratory testing using the central area of the original flannel. Each of two parallel sides of the flannel was hooked with clothespins by two arm boom stands in order to form U-shape flexure for the cloth piece. Ten ticks, which had been paralyzed on ice, were transferred onto the center of the cloth flexure. One of the cloth sides was released from pinching and the flannel cloth mounted by ticks was vertically suspended. The time at which ticks dropped from the flannel was recorded, on an individual basis, for 10 min. Twenty-six female adult-stage *I. persulcatus* and 27 female adult-stage *I. ovatus* were used for each of the DEET-treated and -untreated pieces of flannel. Laboratory tests were performed at 25°C and 60% relative humidity.

**Statistical Analysis**

Repellency was defined as the proportion of tick drop-offs on DEET-treated flannel pieces to remaining ticks (lasting contacts) on untreated flannel pieces at the end of trial time. It was assumed that the time of first contact with the DEET-treated and untreated flannel pieces was even.
The following formula was used for calculation:

\[ \text{Repellency (\%)} = \left( \frac{C - T}{C} \right) \times 100 \]

C and T indicate the total numbers of ticks that attached with untreated (control) and DEET-treated flannel cloths, respectively, in each of the human trap and flag-dragging tests. In each test, repellency was analyzed by the chi-square goodness-of-fit test in order to assess whether there was a significant difference in the ratio of observed tick numbers between the DEET-treated and untreated flannel cloths.

The Cox proportional hazards regression test was used to determine the factors affecting on the escape time. The factors considered were DEET treatment and species. Median escape time and confidence interval in the laboratory test were also estimated by Kaplan-Meier survival analysis using the survival package in R. A log-rank test was used to analyze the survival curves on DEET-treated flannel cloth and untreated flannel cloth for each species.

**RESULTS**

**Field Test**

Mean temperature (min/max) during the human trap test was 20.3°C (minimum: 17.4°C; maximum: 25.7°C), whereas that observed in the flag-dragging test was 19.7°C (minimum: 18.2°C; maximum: 20.8°C). The mean DEET doses per single side ± standard deviation on DEET-treated flannel in the human trap and flag-dragging tests were 0.291 ± 0.056 mg/cm² and 0.283 ± 0.027 mg/cm², respectively. It should be noted that these concentrations are expressed in theoretical values and assume that all of the released active ingredient was attached to the flannel cloths.
In total, 482 ticks were collected from the flannel cloths in the field tests (Table 1). The collected ticks were of the *I. persulcatus* and *I. ovatus* species, which accounted for 5.3 and 94.7% of the ticks collected in the human trap test and 31.4 and 68.6% of those in the flag-dragging test, respectively.

The total number of ticks that collected from treated flannel cloths in the human trap test was 26, significantly lower than the number collected from untreated flannel cloth ($\chi^2 = 99.3$, df = 1, $P < 0.05$). The repellency of DEET-treated flannel cloth was 84%. Only one adult male *I. persulcatus* was collected from treated flannel cloth in the flag-dragging test. In contrast, at 292, the total number of ticks collected from untreated flannel cloth was significantly higher ($\chi^2 = 290.0$, df = 1, $P < 0.05$). The repellency of DEET-treated flannel cloth on the flag–dragging test was 99.7%.

**Laboratory Test**

The DEET dose per single side on treated flannel cloths in the laboratory test was 0.268 mg/cm$^2$. From the results of the regression analysis, the adjusted hazard ratio of the escape time for *I. persulcatus* to that for *I. ovatus* was estimated to be 0.31 (95% CI: 0.19–0.50, $P<0.05$). In addition, the adjusted hazard ratio of the escape time for DEET-treated flannel cloth to that for untreated flannel cloth was estimated to be 10.40 (95% CI: 6.04–17.91, $P<0.05$).

Median escape times for *I. persulcatus* and *I. ovatus* on DEET-treated flannel cloth were 48 s (95% CI: 30–96) and 10 s (95% CI: 5–24), respectively (Fig. 1). Although most ticks on DEET-treated flannel cloth escaped within 10 min, one adult female *I. persulcatus* remained on a DEET-treated flannel cloth until counting. In
contrast, many ticks remained on the untreated flannel cloth for 10 min after mounting. The median escape time of *I. ovatus* from untreated flannel cloth was estimated to be 164 s (95% CI: 150–NA), while that for *I. persulcatus* could not be calculated since more than a half of the individuals did not escape within 10 min.

**DISCUSSION**

This study was conducted to assess the efficacy of a bug repellent of the currently available medical drugs in Japan, which includes 12% DEET at its highest mixing rate, and first added instructions to protect users from ticks in late 2013 along with several other repellents. The repellence of this DEET product was tested by human bait and flag-dragging methods using DEET-treated and untreated flannel cloths. Tick repellency data on DEET, derived from similar human trap field tests, has been reported in several studies (15-18). At 84%, the total repellency shown in the present human trap test was almost equivalent to 85% repellency against *Amblyomma americanum* when 25% DEET repellent in absolute ethanol was applied to skins at 0.5 mg/cm$^2$ (15). When pressurized spray including 20% DEET was applied to clothing, the repellency to *A. americanum*, *Dermacentor variabilis* and *I. dammini* was 85, 94 and 86%, respectively (16, 17). Pressurized spray including 30% DEET also provided 92% repellency to *I. dammini* (17). In addition, Schreck et al. (18) reported that the repellency of DEET to *A. americanum* on military fatigue uniforms which were dipped in a DEET acetone solution at 2.15 mg/cm$^2$ was 81%. These results indicate that the increase of DEET concentration in repellents does not dramatically improve repellency against ticks. The equivalent efficacy shown in the human trap test by the DEET spray
of the present study might be due to an aspect of its formulation that makes it suitable for clothing treatment (a silicon dioxide base was used in the formulation).

The present study utilized two different testing methods in the field: human trap and flag-dragging. Although a difference between the two tests was found in regard to the species composition of the collected ticks, this difference is probably due to the differences in the distribution of ticks at the study sites. Furthermore, it should be noted that the repellency of DEET-treated flannel in the flag-dragging test was high in comparison to that in the human trap test. Similar results were obtained from previous field tests in which the repellencies shown by human trap and flag-dragging tests were 79.6% and 94.6%, respectively (19). During the present human trap test, the pieces of DEET-treated flannel coating the testers’ legs were scratched intensely by bamboo. In contrast, the DEET-treated flannel flags used in the flag-dragging test were gently rubbed on the vegetation. The DEET component could have been more exhausted in the human trap test due to the intensity of the contact with the vegetation. The importance of the need for reapplication of repellent should therefore be considered in practical use in order to achieve greater efficacy. There is another possibility to which the imperfect DEET repellency in the human trap test might be attributed: that an attractant factor, such as CO$_2$ or body odor, was present in human testers. The importance of either repellent exhaustion or human attraction as repellency-limiting factors needs to be clarified in further studies.

Although the escape time showed significant differences in laboratory test results between the two tested species (I. persulcatus and I. ovatus), half of the tested individuals of each species were repelled from the DEET-treated cloth within 1 min,
indicating rather immediate expression of repellency of the DEET product to the two *Ixodes* species. This study demonstrated the efficacy of a DEET repellent against *Ixodes* ticks, however, there is a need to investigate its efficacy against other genera including *Amblyomma, Haemaphysalis, Dermacentor* and *Rhipicephalus*.

In the United States, a matched case-control study to assess the effectiveness of personal preventive measures in Connecticut, indicated that the use of tick repellents on skin or clothing for crossing tick habitats had 20% effectiveness in the prevention of Lyme disease (20). In addition to the present study, previous studies on the efficacy of DEET repellents against ticks indicate that although DEET repellents appear to prevent tick bites, the effect is incomplete. In other words, users should not place too much trust in DEET repellent. It is important to combine personal protection methods in the prevention of tick bites such as the use of both repellent and protective clothing, the avoidance of tick–infested areas, tick control, and educational activities about ticks (21).

**Acknowledgments** This work was supported in part by Grants-in-Aid from the Ministry of Health, Labour and Welfare (H24-Shinko-Ippan-007 and H25-Shinko-Shitei-009).

**Conflict of interest:** None to declare.
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Figure legends

Fig. 1. Kaplan-Meier plots indicating estimated escape times in female adult-stage *I. persulcatus* (A) and *I. ovatus* (B) on DEET-treated and untreated flannel cloths.
Table 1. Number of collected ticks and percent repellency on human trap and flag-dragging tests

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<th></th>
<th>Flag-dragging</th>
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<td>Control</td>
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Fig. 1