INTRODUCTION

Ticks are important vectors of numerous human infectious diseases, such as Lyme borreliosis and rickettsial diseases (1). In Japan, pathogens that cause diseases, such as anaplasmosis, ehrlichiosis, babesiosis, Lyme disease, tick-borne encephalitis, and Japanese spotted fever have been isolated from ticks (2–11). Moreover, severe fever with thrombocytopenia syndrome, 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 to use repellents. N,N-diethyl-m-toluamide (DEET) has a broad activity spectrum that includes acarians through skin or clothing treatment, none of the DEET repellents in Japan included directions for ling acarians through skin or clothing treatment, none of the DEET repellents in Japan included directions for repellency against ticks is an effective personal protection measure.

SUMMARY: The objective of this study was to clarify the efficacy of a currently available N,N-diethyl-m-toluamide (DEET) repellent against tick species in Japan. We performed 2 different field trials: “human trap,” and “flag-dragging.” In total, 482 ticks were collected from white flannel cloths in the field studies. The collected tick species were *Ixodes persulcatus* and *I. ovatus*, which accounted for 5.3% and 94.7% of the ticks 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 times for *I. persulcatus* and *I. ovatus* female adults from DEET-treated flannel cloths were determined. The median escape times for *I. persulcatus* and *I. ovatus* on DEET-treated flannel cloths were 48 s (95% confidence interval [CI]: 30–96) and 10 s (95% CI: 5–24), respectively. In contrast, many ticks remained on the untreated flannel cloths 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 an effective personal protection measure.

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 a formulation for liquefied petroleum gas-mediated spray; Ikeda Mohando Co., Ltd. Toyama, Japan) was applied to flannel cloths. 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 Pendant® Temperature/Light Data Logger 8 K (Onset Computer Corporation, Bourne, MA, USA). The field was mainly composed of bamboo grass that was approximately 1 m in height. Pieces of white flannel cloth (100% cotton, 72 × 72 cm) were spread on the ground and then one side of the cloth was evenly treated with the DEET formulation by spraying approximately 20 g of spray content per cloth at
15 cm above the ground. Each of the treated pieces of flannel was prepared just before 2 serial trials, which used the same cloth. The weight of the spray can before and after each spraying were recorded to calculate the DEET dosage per flannel cloth. The experiment was conducted by 2 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 they stood still on an asphalt road for 10 min. Attached ticks on each piece of flannel were then collected with tweezers. Then, 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 walking DEET-treated flannel cloth was reused twice. Thirteen walking repetitions were performed.

**Flag-dragging test:** The flag-dragging test was performed on a mountain trail along the Tottabetsu River, Obihi 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 × 72 cm) was treated with DEET using same method applied in the human trap test, with the exception that the DEET treatment was applied to both sides of the cloth. A flag was built up by fixing one side of a flannel cloth to a wooden stick (85 cm of the cloth. A flag was built up by fixing one side of a flannel cloth (72 × 72 cm) was treated with DEET using same method applied in the flag-dragging test, respectively.

**Laboratory test:** The laboratory tests used female adults of *I. persulcatus* and *I. ovatus* that had been collected during the human trap and flag-dragging trials in the field tests. Both sides of a white flannel cloth (72 × 72 cm) were treated with DEET by the same method described in the flag-dragging test and then cut into 10 × 10 cm pieces for laboratory testing using the central area of the original flannel. Two parallel sides of the flannel were attached to 2 arm broom handles by clothespins in order to form a 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 a broom handle and the flannel cloth mounted by ticks was left 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 compared to remaining ticks (lasting contacts) on untreated flannel pieces at the end of the trial period. It was assumed that the time of first contact with the DEET-treated and untreated flannel pieces was the same.

The following formula was used for calculation:

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

where \(C\) and \(T\) indicate the total numbers of ticks that attached to the untreated (control) and DEET-treated flannel cloths, respectively, in 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 the escape time. The factors considered were DEET treatment and species. Median escape time and confidence interval (CI) in the laboratory test were also estimated using a Kaplan-Meier survival analysis using the survival package in R (The R Foundation; https://www.r-project.org/). A log-rank test was used to analyze the survival curves on DEET-treated flannel cloths and untreated flannel cloths 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 the mean temperature 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 flannels 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 assumed 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 either *I. persulcatus* or *I. ovatus*, 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 collected from the treated flannel cloths in the human trap test was 26, significantly lower than the number collected from untreated flannel cloth \((q^2 = 99.3, df = 1, P < 0.05)\). The repellency of the DEET-treated flannel cloths was 84%.

Only one adult male *I. persulcatus* was collected from treated flannel cloths in the flag-dragging test. In contrast, at 292, the total number of ticks collected from untreated flannel cloths was significantly higher \((q^2 = 290.0, df = 1, P < 0.05)\). The repellency of DEET-treated flannel cloths according to 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/cm². From the results of the regression analysis, the adjusted hazard ratio of the escape time for *I. persulca-
Table 1. Number of collected ticks and percent repellency on human trap flag-dragging tests

<table>
<thead>
<tr>
<th>Species</th>
<th>Human trap</th>
<th>Flag-dragging</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DEET</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>DEET</td>
<td>Control</td>
</tr>
<tr>
<td>I. persulcatus</td>
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<td></td>
</tr>
<tr>
<td>Adult female</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Adult male</td>
<td>1</td>
<td>2</td>
</tr>
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<td>Nymph</td>
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<td>I. ovatus</td>
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<td></td>
</tr>
<tr>
<td>Adult female</td>
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<td>77</td>
</tr>
<tr>
<td>Adult male</td>
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</tr>
<tr>
<td>Nymph</td>
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<td>1</td>
</tr>
<tr>
<td>Combined</td>
<td>26</td>
<td>163</td>
</tr>
</tbody>
</table>

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.

DISCUSSION

This study assessed the bug repellent efficacy of the currently available medical drugs in Japan, which includes 12% DEET at its highest mixing rate. Instructions about how the DEET product and several other repellents could be used to protect users from ticks were first added in late 2013. The repellence of the Mushi-Pale "PS 12" 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, have been reported in several studies (15–18). At 84%, the total repellency shown in the present human trap test was almost equivalent to the 85% repellency against Amblyomma americanum when 25% DEET repellent in absolute ethanol was applied to skin at 0.5 mg/cm² (15). When a pressurized spray that contained 20% DEET was applied to clothing, the repellency to A. americanum, Dermacentor variabilis and I. dammini was 85%, 94%, and 86%, respectively (16,17). A pressurized spray that included 30% DEET also provided 92% repellency to I. dammini (17). In addition, Schreck et al. (18) showed that DEET repellency to A. americanum on military fatigue uniforms that had been dipped in a DEET acetone solution at 2.15 mg/cm² was 81%. These results indicate that increasing the DEET concentration...
in repellents does not dramatically improve repellency against ticks. The equivalent efficacy shown in the human trap test by the DEET spray in 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).

This study employed two different testing methods in the field: human trap and flag-dragging. Although a difference between the 2 tests was found with 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 the DEET-treated flannels in the flag-dragging test was high in comparison to 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. More of the DEET component could have been removed in the human trap test due to the intensity of the contact with the vegetation. The importance of the need for reaplication of repellent should therefore be considered during practical use in order to achieve greater efficacy. Another possible reason why the DEET repellency in the human trap test was not as efficacious as expected might be due to an aspect of its formulation that makes it suitable for clothing impregnants for protection from the lone star tick. This study demonstrated the efficacy of 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 when 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 indicated that although the repellents appear to prevent tick bites, the effect is incomplete (15–18). In other words, users should not rely only on DEET repellents. It is important to combine personal protection methods against 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).

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Conflict of interest None to declare.

REFERENCES