IN VIVO TEST METHOD for REPELLENT EFFECTIVENESS on THREE COMMON TICKS
(BLACKLEGGED TICK, AMERICAN DOG TICK, LONE STAR TICK)

A placebo controlled trial comparing 30% DEET And TICKS-N-ALL TICK REPELLENT

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ABSTRACT

The present paper describes a test procedure with human volunteers used for a comparative investigation of tick repellents. Two commercially available repellents, Ticks-N-All Tick Repellent (TNATR) and a 30% DEET repellent, the gold standard in insect repellents, were tested against the ticks Blacklegged Tick (Ixodes Scapularis), American Dog Tick (Dermacentor Variabilis), Lone Star Tick (Amblyomma Americanum), and several test criteria described. Ticks were placed on a cardboard plate situated on an arm treated with repellent. While the arm was kept vertically, it was observed whether or not a tick entered treated skin and walked a distance of at least 5 cm.

INTRODUCTION

Ticks are vectors transmitting pathogenic microorganisms to vertebrate hosts worldwide (Sonenshine, 1993; Estrada-Pena and Jongejan, 1999).[1] In many parts of Asia, America, and Europe, tick-borne diseases rank among the most frequent arthropod-borne diseases of humans (Korenberg and Kovalevskii, 1999; Shapiro and Gerber, 2000).[2] To avoid infection by such tick-borne pathogens, vector tick-bites have to be prevented. A practicable way to prevent tick bites is the use of repellents for personal protection. The simplest test used for ticks involves a tick-walking area with untreated and repellent-treated parts, (e.g., Dremova and Smirnova, 1970),[3] where repellency is indicated by the percentage of ticks entering the treated zone compared to a control. However, no in vitro test system developed so far can precisely predict the efficacy a repellent will show on the host. Here it becomes part of a complex pattern of volatile and contact host stimuli, and additionally exhibits interactions with skin chemistry. Therefore, a critical test using the vertebrate species to be protected still produces the most reliable results. With human volunteers, field tests as well as laboratory investigations have been performed. In field tests, the repellent may be applied to clothes (Lane, 1989; Evans et al., 1990)[4] or to skin (Schreck et al., 1995; Solberg et al., 1995).[5] The latter method puts higher demands on the repellent, because it has to counteract not only short-distance attractants such as warmth or skin volatiles but also contact attractants of the skin.

The procedure briefly involves application of a repellent on a vertically positioned arm, leaving an area above and below the arm untreated. Ticks are placed on the plate in the center of treated area and are monitored for entering/not entering the treated zone.

Once on a host, the natural behavior of ticks (particularly such species without feeding for prolonged periods) is to search for a suitable feeding site, thereby showing a tendency to walk upwards. The repellent in the mentioned assay has to counteract this tendency. Since the tick‘s motivation to walk up may not be as high as the motivation to enter a host at all, a modified procedure was developed with the aim to increase the demands on the tick repellent to be evaluated. The present paper focuses on test criteria critical for the evaluation of repellent action and discusses their respective meaningfulness.

MATERIALS and METHODS

Ticks

Dermacentor variabilis, Amblyomma americanum, Ixodes Scapularis were used at an age of 1 year post ecdysis. The ticks were kept at 22°C in a dark chamber for two days prior to experiment.

General Test Procedure

Tests were performed outdoors with temperatures ranging from 28 – 30°C. The volunteer washed their forearm with non-perfume soap, dried it, and then applied the repellent on the arm according to product label. Both products were sprayed one time using the same make of fine mist sprayer and dispersed in a 16 cm band around the arm. Equal amount of repellent applied was confirmed by weighing the repellent container before and after application. The skin area to be treated was measured by determining the length of the arm at its respective front and back as well as its circumference. A 2 cm circular cardboard plate was applied in the center of the treated skin area, using Vaseline. During a test, the arm was held vertically. Fifteen minutes after repellent application, one tick was placed on the plate. The tick was observed for 5 minutes and was evaluated whether: ticks entered the treated skin area or not; ticks fell down from plate or skin; ticks walked a distance of at least 5 cm and the direction (either up, down, or horizontally) the ticks walked when crossing the treated area. Every five minutes, a new tick was placed on the plate until a total of 10 ticks were observed per 60 minutes. This procedure was continued with a new tick every five minutes with a total of 12 ticks per hour for 6 hours or until a decreased efficacy of 25 percent. The skin was washed with non-perfume soap at the end of a trial.

Control Tests

Prior to product test, a control run with 12 ticks was performed under identical conditions, without repellent applied. This control served to demonstrate sufficient activity of the ticks on the test day (at least 11 of the 12 ticks should cross 5 cm of skin within 5 min.) and to make sure that there was no residual repellent activity in cases where tests had been performed on that arm previously.

Determination of Test Criteria

Duration of efficacy. The time point after repellent application when 75 percent of ticks had crossed at least 5 cm of treated skin, arbitrarily chosen as the end point of efficacy. The end of test was determined when efficacy decreased to 25 percent or 6 hours maximum test period was reached.

Quantitative repellency. Quantitative repellency was determined based on: the number of ticks entering the treated skin, and on the number of ticks crossing at least 5 cm of treated skin. Repellency (R) was computed by the formula: R = 100 - NR/N * 100 ; whereby NR is the number of ticks entering or crossing treated skin, and N the respective tick numbers of the control trial.

Tick walking direction. The walking direction of ticks crossing 5 cm of skin was determined at the point when traversing the marked circle as either upwards, downwards, or horizontally. For statistical evaluation, the number of ticks walking upwards vs. not upwards (horizontally or downwards) was compared with the respective control by a G-test (see below).

Test Volunteers

There were four volunteers consisting of two women and two men. Volunteers were advised not to use any perfume or consume any caffeinated drinks on days of experiments.

Products Tested

We tested Ticks-N-All Tick Repellent (TNATR) and a commercially available product containing 30% DEET. Both products were tested by the same four people and each volunteer used a new package. The sequence of products was determined by random for each Volunteer.

Data Analysis

Differences in the duration of repellency between products as well as between time periods were investigated using one-way ANOVA followed by the conservative Scheffé-Test. A p-value <0.05 was regarded significant. The repellency of a product was tested by the G-test (Sokal and Rohlf, 1981)[6] which was written in an Excel spreadsheet.

RESULTS

Duration of Efficacy

Table 1 shows the durations of efficacy of the products down to a degraded amount of 25% efficacy. Which is the considered unsatisfactory for preventing insect bites that can lead to disease.

Quantitative Repellency

When the repellency was tested quantitatively, products showed repellent action (G-test, Figure 1). Figure 1 shows the repellency of the products in relation to their respective controls within the specified time period after application.

Table 1 Adjusted mean duration of efficacy of commercial tick repellents tested with nymphs of *I. Scapularis*

	Duration of efficacy			
Product	Time Interval	Ticks Tested	Ticks/Walked	% of efficacy
TNATR	30	24	0	100.0
	60	48	0	100.0
	90	48	0	100.0
	120	48	0	100.0
	150	48	0	100.0
	180	48	1	95.8
	210	48	4	83.3
	240	48	6	74.9
	270	48	10	58.3
	300	48	13	45.8
	330	48	16	33.3
	360	48	19	20.8
DEET	30	24	0	100.0
	60	48	1	95.8
	90	48	3	87.4
	120	48	9	62.5
	150	48	13	45.8
	180	48	18	24.9
	210	48	19	20.8

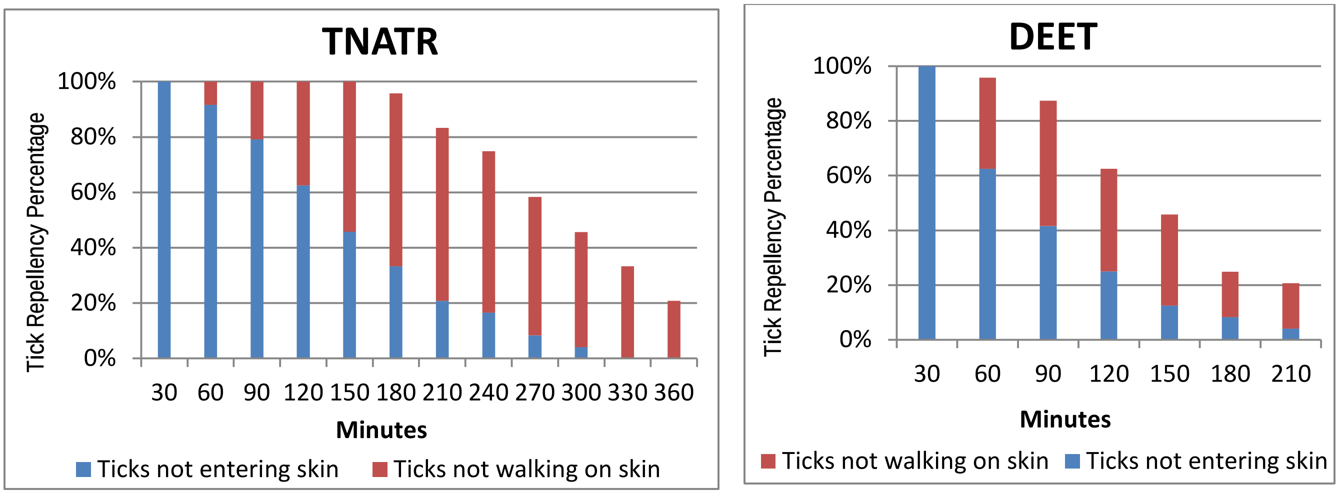

Figure 1. Repellency of the investigated products, expressed as the combined percentage of ticks not entering treated skin and the percentage of ticks, once on the skin, not traversing a distance of at least 5 cm compared to the respective control.

A repellent either had the effect that ticks did not enter treated skin, or, if ticks indeed had entered the skin, they were unable to cross it properly.

From Figure 2 it is apparent, that most of the ticks that did not cross treated skin either fell down from the plate or from the skin.

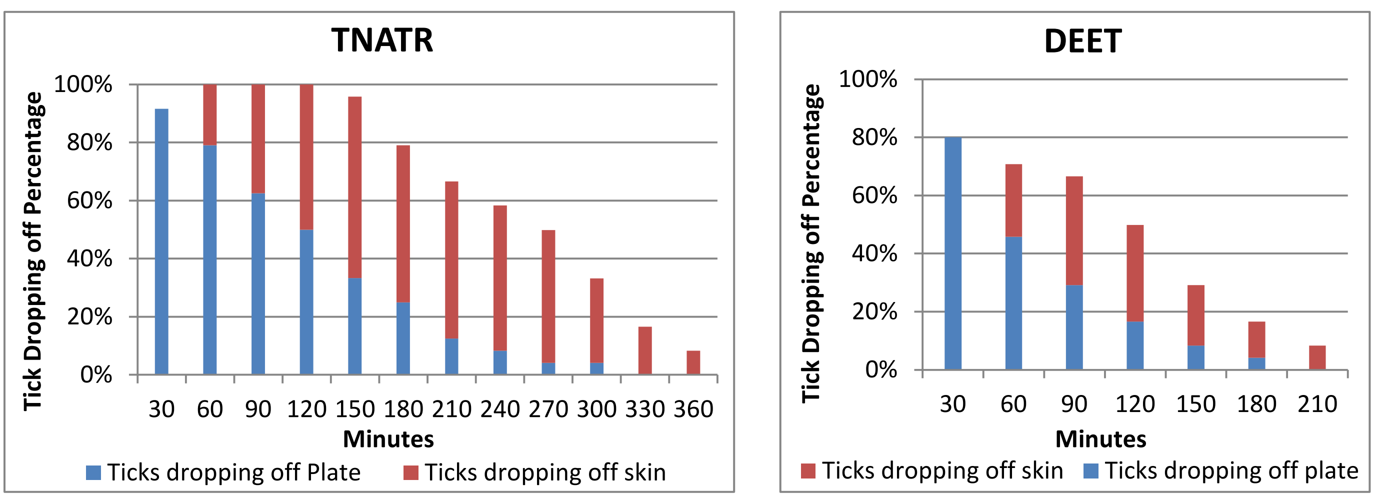

Figure 2. Percentages of ticks either dropping off the plate or off the skin in the course of the repellent test.

Table 2. Results of the G-tests evaluating the proportion of insects traversing repellent treated skin at defined time set after application vs. control (untreated skin).

Product	Time Interval	G-Value	Significance
TNATR	30	0	62.4
	60	0.95	62.4
	90	0.874	62.4
	120	0.625	62.4
	150	0.458	62.4
	180	0.259	65.1
	210	0.248	74.9
	240	N/A	N/A
	270	N/A	N/A
	300	N/A	N/A
	330	N/A	N/A
	360	N/A	N/A
DEET	30	0	62.4
	60	0.958	65.13
	90	0.874	71.39
	120	0.625	79.8
	150	0.458	85.2
	180	0.256	1.0
	210	0.248	1.0

Tick Walking Direction

Control ticks showed a strong tendency to walk upwards. Taken together (Table 2), 88.5% of the control ticks (n=96) walked up, 11.45% walked to either side, and 4.1% walked down. In contrast, ticks walking on treated skin preferred to walk downwards on the host but as efficacy started to degrade the percentages switched and corresponded with the control tendency to walk upward. This was apparent in both products.

Table 3: Shows number of ticks (n) traversing 5 cm of skin and the respective percentage walking upwards, downwards or horizontally, before repellent application (Control) and at time intervals of every 30 minutes after application of repellent.

			Walking Direction		
Product	Time Interval	N	Up	Side	Down
TNATR	Control	48	89.5	10.4	4.1
	30	0	0.0	0.0	0.0
	60	0	0.0	0.0	0.0
	90	0	0.0	0.0	0.0
	120	0	0.0	0.0	0.0
	150	0	0.0	0.0	0.0
	180	1	0.0	0.0	100.0
	210	4	25.0	25.0	50.0
	240	6	16.6	33.3	50.0
	270	10	30.0	20.0	50.0
	300	13	38.4	23.0	38.4
	330	16	43.7	18.7	37.5
	360	19	42.1	26.3	31.5
DEET	Control	48	87.5	12.5	4.1
	30	0	0.0	0.0	0.0
	60	1	0.0	100.0	0.0
	90	3	0.0	33.3	66.6
	120	9	55.5	22.2	22.2
	150	13	61.5	23.0	15.3
	180	18	77.7	11.1	11.1
	210	22	81.8	13.6	4.5

To test the claim of kills by contact, this procedure involves application of a repellent, both the DEET product and TNATR on the overall inside surface of their own clear plastic container. Twelve ticks are placed in each treated container and twelve in an untreated container. After 5 minutes of exposure, the twelve ticks are removed from their treated containers to untreated containers to represent a more life-like scenario. Removed insects are maintained, monitored and observed for one complete life cycle. The number of ticks to die during the observed life cycle is described in the table below.

Product	**Mortality %**			
	<5 mins	<10 mins	<15 mins	<20 mins
TNATR	50%	83.33%	91.66%	100%
DEET	8.33%	25%	41.67%	66.67%
Control	0%	0%	0%	0%

DISCUSSION

A good repellent assay should challenge a product in a situation as close as possible to conditions of intended use. Thus it should test the critical properties a repellent must exhibit. In order to prevent infection by tick-borne pathogens, it should theoretically suffice if a repellent just induces a tick not to bite. If a chemical could render human skin unacceptable as a blood source, there would be no need to repel the tick to move away from the repellent source. This, however, would be insufficient under practice conditions. Using such a chemical, the complete body, including the head would have to be treated, because otherwise, a tick could walk across the body until it finds an untreated area and bite there. Such whole body treatments are unlikely to be accepted by the consumer. As a consequence, a repellent must have the property to prevent ticks from accessing untreated skin.

This could be achieved either by preventing the tick from clinging to the body at all, or by inducing the tick to drop off once it landed on treated skin. The described protocol performs a rigorous test for this situation and proved able to discriminate the repellent efficacy of two products developed for use on human skin. The ratio of the described assay is as follows: Ticks placed on the cardboard plate are on the one hand exposed to certain host stimuli such as body warmth and volatile skin kairomones, but on the other are still not yet on the host itself. In this situation, a tick has to make a choice between staying off the host and walking onto treated skin. If, alternatively, the tick had been placed not on the plate but on untreated skin surrounded by treated skin, the choice would be less rigorous for the tick, namely untreated versus treated skin. Once on the skin, it was further observed whether a tick could walk vertically a distance of 5 cm or not. A tick unable to walk properly would drop off the vertical area. As shown, the great majority of ticks not crossing the area dropped off (compare figures 1 and 2), whereas only single specimens either walked back to the plate or remained stationary. That is, besides the criteria of entering/not entering skin and crossing/not crossing a certain distance, the dropping off of ticks should be a suitable criterion for repellent tests.

Another criterion, albeit for a more subtle repellent effect, may be the direction the tick walks on treated skin. The data show that ticks placed on a human body tend to walk upwards. This tendency was reversed by the test products, suggesting that ticks not forced to drop off still might have the motivation to leave the host. If this interpretation is right, then a substantial proportion of ticks walking down might be indicative of a somewhat weaker repellent effect. This is in accordance with the observation that the proportion of ticks walking down decreased with increasing time passed after application of repellent, i.e., with decreasing concentration of the repellent (Table 2). A further criterion that might be indicative for a certain repellent effect is the time period recorded between placing the tick on the copper plate and the time point when the tick entered the skin.

Even very hungry ticks, however, do not bite immediately when placed on the skin, and no such tick bites were observed in the course of the experiments. In summary, the described test procedure investigates critical properties of a tick repellent and produces results that allow judging the protective power of a test product in terms of quantitative repellency and duration of efficacy.

CONCLUSION

In the present test, TNATR outperformed the efficacy of the DEET product by a ratio of 3 to 1. TNATR was able to prevent ticks from entering treated skin for 180 minutes compared to 30 minutes with the DEET product. The duration of efficacy of TNATR lasted 58% longer than DEET. TNATR had 6 hours of acceptable repellency as to 3.5 hours of DEET.

References

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