SHORT COMMUNICATION

Efficacy of DEET and non-DEET-based insect repellents against bites of Simulium damnosum vectors of onchocerciasis


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Abstract. Coping strategies including smoke screens are used against nuisance bites of Simulium damnosum Theobald (Diptera:Simuliidae) in onchocerciasis endemic communities. To find more effective alternatives, the efficacy of commercially available N,N-diethyl-3-methylbenzamide (DEET) products with active concentrations of 9.5, 13, 25, 50 and 98.1–100% and ‘NO MAS,’ (active component: para-menthane-3,8-diol and lemon grass oil) were tested at Bui-Agblekame, Ghana. A Latin square study design was implemented using eight groups of two vector collectors each, who used repellents (treatment), mineral oil or nothing each day until the end of the study. Flies were caught and their numbers each hour recorded using the standard methods for onchocerciasis transmission studies. T-tests were used to compare the mean duration of protection and a one-way analysis of variance controlling for catcher and repellent effect was performed. Tukey’s test was used to compare protection by repellents and mineral oil. The highest percentage protection was 80.8% by NO MAS and the least 42.5% by the 13% DEET product. The period of absolute protection was 5 h by NO MAS and 1 h by 50% DEET product. No significant increase in protection was offered beyond 25% active DEET products and no significance was observed in terms of catcher × repellent effect (F = 1.731, d.f. = 48, P = 0.209).

Key words. Simulium damnosum, coping strategies, DEET, insect repellents, Onchocerciasis.

Introduction

Onchocerciasis or river blindness is a filarial disease caused by infection with Onchocerca volvulus and is transmitted to humans by the blackfly Simulium damnosum s.l. The disease in the chronic stage manifests as blindness, severe dermatitis and skin depigmentation.

Blackflies are day-biters, the period when most humans are active and productive. Nuisance biting can be perceived as a greater issue over the short term than the blindness which is slow to develop and tends to manifest later in life. In endemic areas of Ghana, biting rates from Simulium damnosum can be very high. In a recent study, individuals were estimated to experience as many as 5611 bites/person a month at certain sites in the Black Volta Basin (M. Osei-Atweneboana, 2011, unpublished data). In 2009, the bites of blackflies nearly led to the suspension of the building at the Bui Dam being constructed on the Black Volta river, with the workers and residents threatening to vacate the area as a result of the intolerable annoyance (http://www.modernghana.com/news/240480/1/black-flies-invade-bui-again.html).

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While the use of the microfilaricide ivermectin can limit onchocerciasis morbidity, there is currently no effective solution for preventing fly bites. Theoretically, protection from blackfly bites could be best achieved by either avoiding infested areas, wearing protective clothing or using a personal insect repellent. Although avoiding infested areas could explain the observed desertion of fertile river valleys during the pre-Onchocerciasis Control Programme era, this is not a practical option. Wearing protective clothing, although feasible, would be unlikely to succeed because of the high temperatures and humidity experienced in northern Ghana. The local inhabitants in the study area are mostly either farmers or fishermen and many household chores are conducted along the banks of rivers. Afflicted communities employ traditional coping measures such as creating an immersive smoky environment, smearing their bodies with plant products (e.g. chewed palm kernel nuts), chemical products such as engine oil and diesel, or mud to prevent bites. In endemic areas along the Black Volta river women literally ‘smoke’ their babies in attempts to prevent them from being bitten. In creating these smoke screens, fresh branches and leaves of plants are used, causing smoke inhalation with its attendant future health complications.

The use of fresh plant parts to generate the smoky environment may also contribute to environmental degradation.

As part of a major project employing an eco-health approach to onchocerciasis control in Ghana, community members and other stakeholders in 19 communities in the Black Volta River basin were surveyed to determine which interventions against onchocerciasis were most preferred. Interventions against incessant biting by *S. damnosum* s.l. vectors were regarded by respondents to be of utmost priority (I. Osei-Akoto, 2011, unpublished data). In discussions with communities, inhabitants affirmed that the smearing of the body with chewed palm nuts worked well but was not sufficiently long lasting.

In the situation described above, applying repellent to the skin may be the only feasible option to protect people from blackfly bites. Previous studies have reported protective effects of cocoa oil (Pitroipa et al., 2002) and coconut oil and palm oil (Sylla et al., 2003) although the exact degrees of protection were not determined. Other studies conducted in Nigeria have demonstrated significant repellent effects of roots and leaf extracts of plants achieving 78 and 78.1% protection with roots of *Occimum gratissimum* and leaves of *Pistia hyptis*, respectively (Sam-Wobo et al., 2011). Another Nigerian study this time using 20% (v/v) volatile oil of *O. gratissimum* and liquid paraffin applied topically could achieve as high as 90.2% protection against blackfly bites (Usip et al., 2006). Interestingly a repellent called ‘Simmo’, which is made from edible plant products, as either base-oil or a cream, has been tested in Ghana and the base-oil was found to offer 100% protection against *S. damnosum* s.l. for over 10 h (Opoku et al., 1986). However, the practicability of using these plant-derived products on a large scale to protect inhabitants in onchocerciasis-endemic areas is doubtful, which makes the case for testing commercially available repellents, which are known to provide reliable and prolonged protection from mosquito bites, including *N,N*-diethyl-3-methylbenzamide (DEET). DEET products are marketed in various formulations as lotions, and as impregnated wrist bands of different concentrations. Although it is known that DEET repellent products can provide predictable and prolonged protection from mosquito bites, its effect on blackflies to our knowledge, has not been previously evaluated in onchocerciasis endemic areas in Ghana. Rather Renz & Eyong (1983) tested DEET-impregnated garments and found a 90% reduction in the fly biting density over a period of 5–7 days (40–56 h of exposure). While DEET is one of the most widely used personal repellents, many sophisticated consumers in developed countries however are reluctant to apply DEET to their skin because of safety concerns and odour, and therefore deliberately seek out other repellent products.

In view of this, we included in the study a new water-based repellent called ‘NO MAS’ (NM) whose plant-derived active ingredients are PPD (para-menthane-3, 8-diol) and lemongrass oil. PPD is well known as an insect repellent and has been found in several studies to be comparable to DEET (Carroll & Loye, 2006).

This study reports on results obtained using commercially available DEET products and ‘NO MAS’ to protect against the bites of *Simulium damnosum* vectors of onchocerciasis.

Materials and methods

Study site

The site selected for the study was Bui Agblekame (Lat. 08.12087'N; Long. 02.03118'W) which is located on the Black Volta River downstream of the Bui Dam construction site. This site was selected because of the high daily biting rates recorded throughout the year (M. Osei-Atweneboana, 2011, unpublished data). The onchocerciasis vector species found there are predominantly *S. damnosum* s.s. and *S. sirbanum* (Boakye et al., 1998). Although we did not know of the maximum distance of host attraction of a single human to blackflies, the collectors were situated on the river bank at least 50 m apart to minimize the ‘relativity effect’, wherein flies must choose between two hosts simultaneously.

Selection of DEET products

We purchased and tested six DEET-based repellent lotions (ranging from 9.5 to 98.1–100% DEET). NO MAS was supplied by Mr Sam Darling (Del Cielo Project, Canada). Table 1 lists the trade names of the tested products.

Testing method

Using a Latin Square design (Bailey, 1996), every day for 8 days, eight groups each comprised of two vector collectors performed blackfly landing catches (Walsh et al., 1978) while seated at fixed positions along the bank of the river. Each group was rotated sequentially to a new position each day until the study was completed. On any given day, six groups used the repellents (treatment) while one used mineral oil and another.
nothing (controls). The vector collectors had both lower legs treated with either one of the repellents or mineral oil or nothing from the foot to the knee. Approximately 500 μL of the lotions i.e. NO MAS, ODOMOS™ (9.5% DEET) and Jungle Formula (50% DEET) and mineral oil were applied evenly on each leg, while in the case of the rest of the products the legs were sprayed to achieve complete coverage. Repellents and mineral oil were applied by field supervisors to remove operator bias and also to ensure uniformity in smearing of the repellents. Repellents and mineral oil were applied at 06.45 hours each morning, followed by catches from 07.00 to 18.00 hours each day with the collectors alternating each hour as the human bait. The number of flies caught each hour was recorded on a standard form based on that used by the Onchocerciasis Control Programme (OCP/Fiche 1) in the field.

All containers were covered with opaque masking tape and relabelled by code. Additionally, the collectors were made to wear long-sleeved shirts to ensure that the flies only had access to their lower legs. The numbers of days that each repellent was tested were not the same for all because of insufficient supply of both 9.5 and 98.1–100% DEET products.

**Table 1.** List of repellent products with concentrations of active ingredients used for the study.

<table>
<thead>
<tr>
<th>Code</th>
<th>Product, Manufacturer</th>
<th>Concentration and active ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>ODOMOS™</td>
<td>9.5% DEET</td>
</tr>
<tr>
<td>B</td>
<td>MEDISOF®</td>
<td>13% DEET</td>
</tr>
<tr>
<td>C</td>
<td>OFF™ Deep Woods® SC  Johnson Co.</td>
<td>25% DEET</td>
</tr>
<tr>
<td>D</td>
<td>Jungle Formula® Chefaro UK Ltd</td>
<td>50% DEET</td>
</tr>
<tr>
<td>E</td>
<td>Ben’s® 100 Max Formula, TENDER Corporation, USA and REPEL® 100</td>
<td>98.1 and 100% DEET</td>
</tr>
<tr>
<td>F</td>
<td>NO MAS</td>
<td>16% PMD and 2% Lemongrass oil</td>
</tr>
<tr>
<td>G</td>
<td>Mineral oil, (Cat No. M5904, Sigma, USA)</td>
<td>Inert control</td>
</tr>
<tr>
<td>H</td>
<td>None treatment</td>
<td>Control</td>
</tr>
</tbody>
</table>

Nothing (controls). The vector collectors had both lower legs treated with either one of the repellents or mineral oil or nothing from the foot to the knee. Approximately 500 μL of the lotions i.e. NO MAS, ODOMOS™ (9.5% DEET) and Jungle Formula (50% DEET) and mineral oil were applied evenly on each leg, while in the case of the rest of the products the legs were sprayed to achieve complete coverage. Repellents and mineral oil were applied by field supervisors to remove operator bias and also to ensure uniformity in smearing of the repellents. Repellents and mineral oil were applied at 06.45 hours each morning, followed by catches from 07.00 to 18.00 hours each day with the collectors alternating each hour as the human bait. The number of flies caught each hour was recorded on a standard form based on that used by the Onchocerciasis Control Programme (OCP/Fiche 1) in the field.

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**Ethical considerations**

We recruited consenting volunteers from the community who, based on the location of their domicile, are already exposed to blackfly bites and potential onchocerciasis transmission. We chose volunteers who had prior experience catching blackflies, having been involved in previous onchocerciasis studies that we have conducted in the area. A form outlining the procedure was given to each person to ensure that they had full understanding of the potential risks of a study of this kind. The insect repellents all contained active ingredients that have been approved by various regulatory bodies and are commercially available. Full ethical approval was obtained from the Institutional Review Board of Noguchi Memorial Institute for Medical Research (FWA no.: 00001824; 046/11-12). In addition, collectors who had missed the previous round of treatment provided within the community were given ivermectin after the study.

**Data analysis**

The biting pressure expressed as the mean daily biting rate (number of flies/person/day) was estimated from the number of flies captured for each treatment, and the percentage protection of each repellent was estimated using the following equation: percentage protection = [(T – C)/C] × 100 (where T = flies landing on treatments and C = flies landing on controls). Microsoft Excel was used to perform this analysis.

Two-way t-test comparisons between each set of repellent was done using Minitab version 16 (Minitab Inc., College State, PA, USA). A univariate analysis of variance involving two factors (catchers and repellent) was also conducted, with total flies caught as the dependent variable, followed by Tukey’s tests (Sokal & Rohlf, 1981) to compare the total numbers of flies caught for all the tested repellents. A P-value of less than 0.05 was considered to be statistically significant. SPSS version 16.0 (SPSS Inc., Cary, NC, USA) was used for this analysis.

**Results**

The no treatment controls caught a total of 953 flies over 9 days for a mean daily biting pressure (DBP) of 105.9 flies. The catchers who used NO MAS caught a total of 224 flies over 11 days which translated into a mean daily biting pressure of 20.4 flies per person. The duration of complete protection (time to first bite) achieved with NO MAS was 5 h (range = 5–7 h). The estimated overall protection achieved with NO MAS was 80.8%. The 98.1–100% DEET group caught 289 flies over 7 days (DBP = 41.3), the minimum time to first bite was 3 h (range 3–7 h), and the estimated total protection was 61%. For the 50% DEET group a total of 383 flies were caught over 9 days (DBP = 42.6), the recorded minimum time to first bite was 1 h (range = 1–5 h) and the estimated total protection was 59.8%. The 25% DEET group caught 360 flies over 8 days (DBP = 45), the minimum time to first bite was 2 h (range = 2–6 h) and the estimated total protection was 57.5%. The 13% DEET group caught 487 flies over 8 days (DBP = 60.9), the minimum time to first bite was 2 h (range 2–5 h) and the estimated total protection was 42.5%. The 9.5% DEET group caught 446 flies over 9 days (DBP = 55.8), the minimum time to first bite was 2 h (range = 2–4 h) and the estimated total protection was 47.4%. A total of 289 flies were caught over 7 days by catchers who used mineral oil (DBP = 40) and the estimated total protection was 62.2%.

Figure 1 shows the hourly biting trends of the treatments and controls observed during the study period.

Two-way t-tests for each pair of repellents and controls, using total bites per day as the outcome, revealed that NO MAS provided significantly higher protection than all of the DEET products and mineral oil (P = 0.05). Among the DEET products the only significant difference found was between
Fig. 1. Graph showing the biting trends observed with the various treatments from 07.00 hours until the end of the period of catching.

Fig. 2. Plot of protection achieved by the various concentrations of DEET products which reveal that it does not improve significantly beyond 25% of the active ingredient.

The 98.1–100% and 13% DEET products ($T$-value = -8.66, d.f. = 10, $P = 0.000$). Indeed, the repellency effect of DEET products did not increase significantly after 25% concentration of the active ingredient (Fig. 2). Table 2 lists all of the $T$-values and the corresponding $P$-values for each pairwise comparison.

The tests of between subject effects found significance among catchers ($F = 4.04$, $P = 0.034$) and for repellents ($F = 21.652$, $P = 0.000$), but it was not significant for catcher $\times$ repellents effects ($F = 1.731$, $P = 0.209$).

Discussion

In endemic onchocerciasis areas, biting pressures may be extremely intense. As blackflies are day biters, durable repellent products that provide long-lasting effects are desirable. From this study, NO MAS appears to provide a sufficient duration and completeness of protection to make daily use practical and affordable. Not only did it provide complete protection for at least 5 h, its repellency was found to wane slowly compared with DEET products. It is therefore likely that twice-daily applications of NO MAS a day may provide full protection for users.

A recent study found NO MAS to provide 9–11 h protection against *Anopheles* vectors of malaria in Ghana (Dadzie et al., 2012), thus, the duration of protection against blackfly bites was lower. However, actual protection will vary based on conditions such as temperature, perspiration and water exposure, and repellency may be compromised. The inhabitants in these onchocerciasis endemic areas are mainly farmers and in these humid tropical environments, sweating could impair the persistence of repellency during the day when blackflies are active. Also, inhabitants of affected communities include fishermen and household chores such as laundry are performed by washing and wading in rivers, which may also influence the duration of repellency. The current study employed catchers who were stationary and therefore did not suffer these conditions. It would be necessary to evaluate NO MAS under real-life conditions to determine its operational efficacy against blackflies.

DEET is an effective active ingredient found in many repellent products with diverse formulations. It is generally assumed that the higher the concentrations of DEET, the greater the protection thus DEET products of up to 100% active ingredient are available on the market. For example, a study by Fradin & Day (2002) found products containing 23.8% DEET and 20% DEET to provide an average of 5 and 4 h of protection from mosquito bites respectively, whereas a product with 6.65% DEET and one with 4.75% DEET provided almost
2 and roughly 1 and a half hours of protection, respectively. Thus, the finding that the repellency effect of DEET products plateaus after 25% active ingredient was a surprise. Other previous studies have also observed a similar relationship in dosage and repellent efficacy in DEET with the duration of activity levelling off at concentrations near or greater than 50% for *Aedes aegypti* (Buescher et al., 1983), thus it is likely that the efficacy levels of DEET concentrations vary among different insect vectors.

A recent field study tested NO MAS against mosquito vectors of malaria and also investigated community and user acceptance after 37 710 user-days that involved 419 participants. Of these 96.1% of respondents said they would like to continue using the repellent. A user-acceptance rate of 96.7% was estimated. In the community, 81.8% said that NO MAS protected them from mosquito bites and more than 85% found NO MAS to have an appealing smell. Many (87.0%) users of NO MAS did not have any problems applying the repellent every day (Dadzie et al., 2012). We recommend a similar study be conducted in an onchocerciasis endemic area, as well as further investigations to estimate NO MAS repellency and its impact on the risk of onchocerciasis.

### Acknowledgements

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