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FIELD EVALUATION OF THREE PLANT-BASED INSECT REPELLENTS AGAINST MALARIA VECTORS IN VACA DIEZ PROVINCE, THE BOLIVIAN AMAZON

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ABSTRACT. The efficacy of repellents against *Anopheles darlingi*, the main malaria vector in Bolivia, was evaluated. This mosquito has a peak in biting activity early in the evening. Three natural repellents (1 eucalyptus based, 1 neem based, and 1 containing several repellent essential oils) were tested in comparison with 15% deet in human landing catches in Bolivia. The eucalyptus-based repellent containing 30% *p*-menthane-diol applied at a dose similar to those used in practice gave 96.89% protection for 4 h. Deet gave 84.81% protection. The other 2 products did not provide significant protection from mosquito bites.

KEY WORDS Repellent, *Anopheles darlingi*, neem, eucalyptus, Bolivia, mosquito

INTRODUCTION

Malaria is reemerging throughout the Amazon region of South America. Incidence has increased dramatically in endemic countries since the 1980s. In Peru, Brazil, and Bolivia the absolute number of slide-confirmed cases and the proportion of *Plasmodium falciparum* cases have risen unabated despite control programs. In Beni Province in northern Bolivia, 1,214 cases of malaria were confirmed in 1981, and 25,097 were confirmed in 1999 (Ministerio de Salud Bolivia 2000).

The increase in malaria prevalence is due to the development of forested areas, resulting in increased human–vector contact and the entry of nonimmune workers to endemic areas. Anopheline mosquitoes are present at low density in primary forest, but deforestation results in an increase in the number of species and abundance (Tadei et al. 1998). Of the human population of Riberalta, Vaca Diez, 73.3% live in rural and periurban areas, in close contact with the forest. Men of working age carry the greatest burden of disease. This indicates sylvatic transmission, where men work in agriculture or forestry.

The primary malaria vector in the Amazon, *Anopheles darlingi* (Root), is a highly efficient vector and is difficult to control. Tadei et al. (1998) observed that *An. darlingi* in the Brazilian Amazon rarely contact insecticide-treated surfaces in poorly constructed houses. No indoor-resting mosquitoes were observed in the study area (W. Gironda, personal communication). The breeding sites of *An. darlingi* are scattered and transient. Therefore, indoor residual spraying and larviciding are less suitable as a means of control for this species. This species is both exophagic and endophagic in this region (Gironda 1999), and is highly anthropophilic (Deane et al. 1949). This species bites throughout the night, with a peak in activity at dusk and dawn (Tadei et al. 1998). Tadei et al. (1988) found that 1% of *An. darlingi* biting between 1800 and 2200 h in the Brazilian Amazon had malaria salivary gland infections. Because of this early evening peak, personal protection may be beneficial to supplement widespread bed-net use in this region.

Natural insect repellents were tested along with deet (*N,N*-diethyl-*m*-toluamide), the gold standard synthetic repellent, because preliminary focus groups in Vaca Diez (Ruiz 2000) highlighted a greater cultural acceptability for plant-based products. Three natural repellents were tested: lemon eucalyptus (*Eucalyptus maculata citriodon*), which was shown by Trigg (1996) to provide protection against *Anopheles gambiae* (Giles) and *Anopheles funestus* (Giles); neem (*Azadirachta indica*) oil, which was effective against mosquitoes and sand flies in India (Sharma and Dhiman 1993, Sharma et al. 1993) and Venezuela (Caraballo 2000); and Treo® (Bioverama 1997), which contains a blend of essential oils in a cosmetic moisturizer. Field studies conducted in Bolivia found Treo to be effective against *Anopheles* (Slater and Avila, unpublished). Treo also is widely available. Deet (15%) was compared with the natural products because it provides 3 h of protection (Consumer Reports 2000), the minimum protection required if the repellent is used before people retire to their bed-nets.

MATERIALS AND METHODS

Study area: Vaca Diez is situated in northeastern Bolivia, bordering Brazil. The province has 2 urban centers, Riberalta (population 49,000) and Guayaramerin (population 28,000). Both of these towns are rapidly expanding because of immigration of workers.

The study took place in Warnes, a rural site located 30 km from Riberalta. The site was selected because of its proximity to *An. darlingi* breeding sites and a high incidence of malaria. The site was in a secondary forest clearing, with a small stream less than 30 m from the collecting site. Much of the area surrounding the clearing was planted with crops such as rice and maize. An additional breeding site was a river within 2 km of the site. Mosquitoes were collected over 25 nights in February and March 2001 at the end of the rainy season because mosquito densities are highest at this time (Klein and Lima 1990, Gironda 1999).

Repellents and application: The study consisted of single-blind man-landing catches based on a 5 × 5 Latin square design. Five experienced insect collectors sat in 5 different positions over 25 nights and tested 5 treatments. The Bolivian Ministry of Health and Social Welfare granted full ethics approval, and prophylaxis was offered. The treatments consisted of 3 natural repellents, deet, and a negative control. The 1st natural repellent was lemon eucalyptus in isopropanol containing 30% *p*-menthane-diol, citronellal, geraniol, and D-pineol. The 2nd natural repellent was Riddance Bug Repellent (NeemCo Ltd.; Irvine, UK). This product has never been field tested, although preliminary laboratory studies confirmed a good level of protection against *An. albimanus.* The product is a liquid composed of 2% neem oil in alcohol. The 3rd natural repellent was Treo (Bioveramar 1997), which is registered as a repellent in the USA, where it has been marketed for a number of years. The formulation is comprised of 0.05% citronella, 0.06% geraniol, 0.08% rhodinol extra, 0.06% terpineol, and less than 0.5% *p*-menthane-diol in a moisturizing cream. The deet formulation consisted of 15% deet in ethanol. The negative control was 10% baby oil in ethanol.

Repellents were applied at 1630 h. Applications of 3 ml of product were made by hand to each leg as evenly as possible onto the area from the knee to the ankle. Shorts and shoes were worn to standardize the exposed area. The products were measured with a micropipette and applied while wearing a glove. At the field site, the testers sat on low stools under trees, at the forest edge, 10 m apart. Collections were performed between 1830 h and 1930 h and between 1930 and 2030 h, 2–3 and 3–4 h after treatment. Landing mosquitoes were aspirated into plastic cups containing moist filter paper and cotton wool soaked in 10% glucose solution. Cups were replaced each hour to record hourly rates of biting. The mosquitoes were killed with ethyl acetate and identified with a field microscope the morning after collection. Each individual received a different treatment each night, and sat in a different position every 5th night. The volunteers washed their legs with soap after testing, and again the following morning. Washing and the use of soap or deodorant after midday were prohibited.

Data were normalized by using natural log +1 then analyzed with a general linear model (GLM) in the Minitab Statistical Software package (Minitab Inc., State College, PA) and Stata 6 (Stata Corp., College Station, TX). The effects of treatment, individual, and position were measured. Possible additive effects from interactions between individual and treatment, position and individual, and treatment and position were also analyzed.

RESULTS

A total of 8,855 mosquitoes were caught in 47 h over 25 nights. Three collections were not included because of poor weather conditions. The catch included 81.29% *An. darlingi.* The remainder of the catch contained nonvector *Aedes, Mansonia, Coquilettidia, Culex,* and *Anopheles mediopunctatus* (Theobald). A few (0.58%) *Anopheles allopha* (Peryassu), a secondary malaria vector (Faran and Linthicum 1981), were collected.

The GLM analysis on data for all mosquitoes captured showed significant effects for treatment ($F = 60.67$, $P \geq 0.001$), position ($F = 3.92$, $P = 0.007$), and individual ($F = 6.05$, $P \geq 0.001$). The data for *An. darlingi* alone were tested and showed significance for treatment ($F = 53.68$, $P \geq 0.001$), position ($F = 5.01$, $P = 0.002$), and individual ($F = 4.58$, $P = 0.003$). No significant interaction was found between factors for total mosquitoes caught. However, a significant interaction was found between individual and position when the data for *An. darlingi* alone were tested ($F = 2.59$, $P = 0.004$), although the treatments and positions were rotated to reduce such bias. The interaction between treatment and person also was significant ($F = 2.12$, $P = 0.016$). The results showed that the protection afforded by the repellents does not vary among individuals over a period when tested against all mosquitoes, although the attractiveness of individuals to mosquitoes does differ. Variations of up to 30 times in the amount of short-chained aliphatic acids produced in the sweat of volunteers of differing attractiveness to *An. gambiae* have been recorded (Knols et al. 1997). The significant interaction between individuals and repellent for *An. darlingi* may reflect the highly anthropophilic nature of this species. A significant difference was found in the number of mosquitoes caught in different positions (Table 1). This likely was related to the location of houses in the clearing. Positions 1, 2, and 3 captured a nightly average of 100, 103, and 84 mosquitoes, respectively, whereas positions 4 and 5 captured an average of 40 and 34 mosquitoes, respectively. The collection positions varied from 10 to 50 m away from each house.

Treo provided an average of 18.76% protection, Riddance provided 56.75% protection, 15% deet provided 84.81% protection, and lemon eucalyptus provided 96.89% protection for 4 h. The data for total mosquitoes were analyzed because nuisance biting is a significant motivation in repellent utilization for individuals.

DISCUSSION

The estimated economic loss in Bolivia due to malaria was U.S. $18,768,000 in 1997 (Ministerio de Salud Bolivia 2000). The use of repellents is relevant because of the difficulty in reducing numbers of *An. darlingi* and the biting activity of this

Table 1. Effect of treatment on mosquitoes (means of total mosquitoes captured 3 and 4 h after application of repellent).[1]

Treatment	Arithmetic mean	−95% CI	Williams'[2] mean	+95% CI
Negative control	150.64 a	59.80	93.53	145.98
Treo	122.38 a	65.83	92.69	130.41
2% neem oil	65.90 a	44.40	67.83	103.33
15% deet	22.87 b	7.73	12.74	20.62
Lemon eucalyptus	4.68 c	1.32	2.91	4.53

[1] Differing letters denote significant differences; $P > 0.001$.
[2] Williams 1937.

species. Working men comprised 63.1% of the reported cases of malaria in 1998 (Instituto Nacional de Laboratorios de Salud 1999). This group is also at greatest risk of contracting mucocutaneous leishmaniasis, caused by *Leishmania* (*Viannia*) *braziliensis braziliensis*. The prevalence in Beni was 0.6% in 1993 and is increasing (David et al. 1993). The local vectors of this disease, *Lutzomyia* (*Psychodopogus*) *llansmartinsii* (Fraiha and Ward) and *L.* (*P.*) *yucumensis* (Le Pont), are not susceptible to control by insecticides because they are exclusively sylvatic. The best method of disease control is prevention of human–vector contact by use of repellents, long clothing, and fine-mesh bed-nets, although the latter may be unpopular because they restrict airflow. Repellents also may prevent transmission of yellow fever in the sylvatic cycle transmitted by day-biting *Haemagogus* and *Sabathes*.

The WHO (1995) recommends that "all travellers should be told that protection from biting mosquitoes is their first line of defence against malaria." This also applies to people living in endemic areas, particularly those exposed to mosquito bites early in the evening. Of the workforce in Vaca Diez, 3.1% is employed gathering Brazil nuts (Lenglet 2001). The majority of field workers use bednets when they are away from home, whether sleeping indoors or outdoors, to protect themselves from mosquitoes (Quintana and Jove 1999). Although bed-net use is 99% in rural areas (Lenglet 2001), Ruiz (2000) found that the rural and periurban adult human population go to bed between 2200 and 2300 h. Forest workers go to bed between 2100 and 2200 h because they are irritated by insects. *Anopheles darlingi* has a peak in activity in Vaca Diez between 1800 and 2100 h, so people are exposed to infectious bites for more than 3 h before retiring to the protection of their bed-nets.

Ruiz (2000) interviewed 111 men and women from rural, periurban, urban, and indigenous communities. Of the people interviewed, 74% preferred a repellent cream as a form of personal protection. Only 14% of respondents favored permethrin soap, because of its smell and application method. In rural communities, water is not always available. Insecticidal soap for clothing was not considered useful, because people do not wear long clothing.

This study has shown that lemon eucalyptus high in *p*-menthane-diol and 15% deet are effective repellents against the South American malaria vector *An. darlingi* and nuisance mosquitoes for 4 h. These repellents provide a high degree of protection against mosquitoes in this region—96.88% and 84.81%, respectively. The protection period also is sufficient for the evening exposure of residents to mosquitoes before they go to bed. If men working in forests use deet, it may be useful to utilize a stronger formulation to prolong the protection period and to counteract the effect of increased sweating, because this is likely to reduce the longevity of repellents.

Men and women in periurban and rural tend to wash at around 1800 to 1900 h (Ruiz 2000). Health education targeted at these populations may encourage the application of repellents after washing each evening. Men in rural areas also expressed interest in insect repellents because of the biting nuisance they experience when working in the field. Lenglet (2001) found that only 1.9% of the population is aware of repellents as a method of malaria prevention; therefore, health education will be an essential component of the implementation of the marketing and provision of repellents. However, the implementation of skin repellents to complement bed-net use is likely to prove a useful tool in combating the social and economic burden of vectorborne disease in South America.

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