DEET AND PERMETHRIN AS PROTECTANTS AGAINST MALARIA-INFECTED AND UNINFECTED *ANOPHELES STEPHENSI* MOSQUITOES[1]

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ABSTRACT. Deet and permethrin were evaluated as protectants against *Plasmodium falciparum*-infected, *P. berghei*-infected and uninfected *Anopheles stephensi* mosquitoes. Deet 50% effective dose (ED_{50}) values were 3.2 $\mu g/cm^2$ for *P. falciparum*-infected and 1.9 $\mu g/cm^2$ for uninfected mosquitoes; permethrin values were 0.5 $\mu g/cm^2$ and 0.6 $\mu g/cm^2$, respectively. Deet ED_{50} values were 2.3 $\mu g/cm^2$ for *P. berghei*-infected and 1.3 $\mu g/cm^2$ for uninfected mosquitoes; the permethrin values were both 0.5 $\mu g/cm^2$. There were no significant differences in the protective efficacy of deet or permethrin between malaria-infected and uninfected *An. stephensi* mosquitoes.

Anopheles infected with malaria parasites can exhibit a variety of pathological features (Maier et al. 1987). Some investigators have reported reduced longevity and increased mortality; however, others have seen no significant changes (Chege and Beier 1990, Klein et al. 1982, Maier et al. 1987). Differences in behavior have been reported between sporozoite-infected and uninfected mosquitoes. These include increased intradermal probing time (Rossignol et al. 1984) and increased biting rates (Rossignol et al. 1986) for infected as compared with uninfected mosquitoes.

It is important to know whether malaria-infected and uninfected mosquitoes differ in their response to repellents and insecticides. Such information would contribute to our understanding the role these chemicals may play as personal protectants in reducing the risk of malaria. We therefore conducted studies to determine the comparative efficacy of *N,N*-diethyl-3-methylbenzamide (deet) and permethrin against uninfected, *Plasmodium falciparum*-infected and *P. berghei*-infected *Anopheles stephensi* Liston (India strain) mosquitoes.

Anopheles stephensi were infected with cultured *P. falciparum* NF54 (Burkot et al. 1984). When gametocytes were present in the infected cultures, mosquitoes from the same brood were allowed to feed to repletion on both *P. falciparum*-infected and uninfected (control) red blood cell cultures using a membrane feeder (Burkot et al. 1984). *Anopheles stephensi* were infected with *P. berghei* NK65 by feeding on anesthetized DUB-ICR white mice with circulating gametocytes (Nussenzweig et al. 1969). Control mosquitoes were fed on uninfected mice of the same age.

Infected and control mosquitoes were transferred to separate 4-liter mesh-topped paper cartons (200 females/carton) and given access to 10% sucrose solution and a slice of apple. The *P. falciparum*-infected and control mosquitoes were maintained at 27°C and 75% RH; *P. berghei*-infected and control mosquitoes were held at 20°C and 75% RH.

Infection rates were determined 20 days after the bloodmeal by examining salivary glands of 10 mosquitoes from each container for the presence of sporozoites. Only groups of mosquitoes with infection rates of 80–100% were used. After tests to assess mosquito response were completed, the insects were frozen and held at −70°C until the level of infection in individual mosquitoes was estimated using enzyme-linked immunosorbent assays (ELISAs). The amount of circumsporozoite (CS) protein in each *P. falciparum*-infected mosquito was determined as described by Wirtz et al. (1987). The same method was used in a *P. berghei* ELISA based on capture and peroxidase-conjugated monoclonal antibodies 5G5.3 (10 $\mu g/ml$) (Wirtz et al. 1987) and 3.28.1 (2 $\mu g/ml$) (Egan et al. 1987), respectively. The amount of CS protein in each mosquito was estimated from the linear part of the positive control curve and expressed as sporozoite equivalents. The detection limits of the *P. falciparum* and *P. berghei* ELISAs were ca. 20 and 125 sporozoite equivalents, respectively. The amount of CS protein was not directly equivalent to the number of sporozoites in the salivary glands because CS protein is also present on parasites in the hemocoel and in developing oocysts.

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The white-rabbit test procedure utilized to determine the effective doses (EDs) of the repellents was similar to the "free choice" method described by Buescher et al. (1982) and modified by Robert et al. (1991). Each cage (4 × 5 × 18 cm) had 5 circular 29-mm diam openings that aligned with 5 areas on a rabbit's shaved belly, treated at random with ethanol (solvent control) and 4 serial dilutions of the test chemical in absolute ethanol. Two cages each of malaria-infected and uninfected mosquitoes were tested on each treated area. A coin toss determined whether a test started with infected or uninfected mosquitoes. Five or 6 cages of malaria-infected and an equal number of cages containing uninfected mosquitoes were used for each test series. Each cage contained 20 female mosquitoes.

Data were analyzed by the probit plane procedure (Finney 1971, Buescher et al. 1982). Each analysis yielded an ED_{50}, ED_{95}, 95% confidence intervals (calculated by Fieller's Theorem) and slope value. Significant differences were determined by comparing the 95% confidence intervals between EDs.

The *P. falciparum*- and *P. berghei*-infected mosquitoes had infection rates of 91.7 and 91.9%, respectively (Table 1). The mosquitoes were highly infected, although the mean number of sporozoite equivalents in the *P. berghei*-infected mosquitoes (95,431) was significantly higher compared with the *P. falciparum*-infected mosquitoes (3,013) (Table 1). Burkot et al. (1987) reported a geometric mean of 4,000 *P. falciparum* sporozoite equivalents for wild-caught *An. punctulatus* Dönitz complex members in Papua New Guinea. Wild-caught *An. gambiae* Giles in Kenya contained a geometric mean of 962 *P. falciparum* gland sporozoites, determined by microscopic examination of salivary glands (Beier et al. 1991).

The ED_{50} and ED_{95} values for deet were similar to those previously reported for this strain of *An. stephensi* (Robert et al. 1991). However, the EDs reported for permethrin were lower than those reported by Robert et al. (1991). One possible explanation for the differences in EDs of permethrin between previous research and the present study is the difference in the age of the mosquitoes used. Robert et al. (1991) used 5- to 10-day-old nulliparous females, whereas 20- to 24-day-old blood-fed females were used in the present study. Vladimirova (1970) reported that the sensitivity of *Aedes aegypti* (Linn.) to

Table 1. Malaria infection levels in *Anopheles stephensi* used in efficacy tests.

Sporozoite* equivalent	*Plasmodium falciparum*-infected			*Plasmodium berghei*-infected		
	Number of mosquitoes	% total	Mean sporozoite** equivalent	Number of mosquitoes	% total	Mean sporozoite equivalent
Negative ***	28	8.3	—	26	8.1	—
20–124	27	8.0	59	—	—	—
125–999	79	23.5	461	6	1.9	800
1,000–9,999	186	55.4	3,655	64	20.1	4,100
10,000–100,000	16	4.8	13,144	178	55.8	37,000
>100,000	0	0.0	—	45	14.1	257,000
Total	336	100.0	3,013	319	100.0	95,431

* Sporozoite equivalent is a measure of the amount of circumsporozoite protein present in each mosquito, expressed as an equivalent number of salivary gland sporozoites.

** Mean number of sporozoite equivalents.

*** The detection limits of the *P. falciparum* and *P. berghei* ELISAs were 20 and 125 sporozoite equivalents, respectively.

Table 2. Effective doses of deet and permethrin in $\mu g/cm^2$ to protect against bites of malaria-infected and uninfected *Anopheles stephensi* mosquitoes.*

Treatment group	Deet		Permethrin	
	ED_{50} (95% C.I.)**	ED_{95} (95% C.I.)	ED_{50} (95% C.I.)	ED_{95} (95% C.I.)
P. falciparum-infected	3.2 (0.3–7.5)	11.1 (5.6–16.0)	0.5 (0.3–0.7)	2.5 (1.5–8.4)
Uninfected	1.9 (0.6–3.0)	8.3 (5.8–14.6)	0.6 (0.3–1.5)	4.6 (1.7–86.3)
P. berghei-infected	2.3 (1.1–3.4)	10.1 (7.2–18.3)	0.5 (0.3–0.7)	5.6 (2.3–23.4)
Uninfected	1.3 (1.5–2.5)	8.6 (6.4–19.6)	0.5 (0.1–1.2)	3.2 (1.3–***)

* 480 *P. berghei*-infected, 220 *P. falciparum*-infected and equal numbers of uninfected mosquitoes were tested.

** 95% confidence interval.

*** Not determined.

repellents increased with age with the highest sensitivity reported at 3 wk.

There was no significant difference in the comparative effectiveness of deet or permethrin against *P. falciparum*-infected, *P. berghei*-infected or uninfected *An. stephensi* mosquitoes (Table 2). The authors are unaware of any other information on the effectiveness of repellents against pathogen-infected mosquitoes compared with controls. However, recent studies have reported no significant differences in the response of pathogen-infected and uninfected mosquitoes to some insecticides. Ganushkina (1987) reported no significant differences in sensitivity of *P. berghei*-infected and uninfected *An. stephensi* and *P. gallinaceum*-infected and uninfected *Ae. aegypti* mosquitoes exposed to *Bacillus sphaericus* and *B. thuringiensis* (H-14). Also, Rawlins et al. (1988) demonstrated that dengue-infected and control *Ae. albopictus* (Skuse) mosquitoes did not significantly differ in their response to malathion.

When testing a population of mosquitoes for sensitivity to repellents or insecticides there is a variation in response. In the present study, there were no statistical differences between malaria-infected and uninfected *An. stephensi*. This suggests that deet and permethrin may be used in the field with equal success against both malaria-infected and uninfected *An. stephensi* mosquitoes.

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REFERENCES CITED

Beier, J. C., F. K. Onyango, M. Ramadhan, J. K. Koros, C. M. Asiago, R. A. Wirtz, D. K. Koech and C. R. Roberts. 1991. Quantitation of malaria sporozoites in the salivary glands of wild Afrotropical *Anopheles*. Med. Vet. Entomol. 5:63–70.

Buescher, M. D., L. C. Rutledge, R. A. Wirtz, K. B. Glackin and M. A. Moussa. 1982. Laboratory tests of repellents against *Lutzomyia longipalpis* (Diptera: Psychodidae). J. Med. Entomol. 19:176–180.

Burkot, T. R., P. M. Graves, J. A. Cattani, R. A. Wirtz and F. D. Gibson. 1987. The efficiency of sporozoite transmission in the human malarias, *Plasmodium falciparum* and *P. vivax*. Bull. W.H.O. 65:375–380.

Burkot, T. R., J. L. Williams and I. Schneider. 1984. Infectivity to mosquitoes of *Plasmodium falciparum* clones grown *in vitro* from the same isolate. Trans. R. Soc. Trop. Med. Hyg. 78:335–338.

Chege, G. M. M. and J. C. Beier. 1990. Effects of *Plasmodium falciparum* on the survival of naturally infected Afrotropical *Anopheles* (Diptera: Culicidae). J. Med. Entomol. 27:454–458.

Egan, J. E., J. L. Weber, W. R. Ballou, M. R. Hollingdale, W. R. Majarian, D. M. Gordon, W. L. Maloy, S. L. Hoffman, R. A. Wirtz, I. Schneider, G. R. Woollett, J. F. Young and W. T. Hockmeyer. 1987. Efficacy of murine malaria sporozoite vaccines: implications for human vaccine development. Science 236:453–456.

Finney, D. J. 1971. Probit analysis. Cambridge Univ. Press, Cambridge.

Ganushkina, L. A. 1987. Evaluation of the effect of larvicides based on *Bacillus thuringiensis* var. *israelensis* H-14 and *Bacillus sphaericus* on the susceptibility of adult mosquito to *Plasmodium*. (In Russian). Med. Parazitol. Parazit. Boleznei. 1:10–13.

Klein, T. A., B. A. Harrison, R. G. Andre, R. E. Whitmire and I. Inlao. 1982. Detrimental effects of *Plasmodium cynomolgi* infections of the longevity of *Anopheles dirus*. Mosq. News 42:266–271.

Maier, W. A., H. Becker-Feldman and H. M. Seitz. 1987. Pathology of malaria-infected mosquitoes. Parasitol. Today 3:216–218.

Nussenzweig, R., J. Vanderberg and H. Most. 1969. Protective immunity produced by the injection of X-irradiated sporozoites of *Plasmodium berghei*. IV. Dose response, specificity and humoral immunity. Military Med. 134:1176–1182.

Rawlins, S. C., D. A. Eliason, C. G. Moore and E. G. Campos. 1988. Effects of dengue-1 infection in *Aedes albopictus* on its susceptibility to malathion. J. Am. Mosq. Control Assoc. 4:372–373.

Robert, L. L., J. A. Hallam, D. C. Seeley, L. W. Roberts and R. A. Wirtz. 1991. Comparative sensitivity of four *Anopheles* (Diptera: Culicidae) to five repellents. J. Med. Entomol. 28:417–420.

Rossignol, P. A., J. M. C. Riberio and A. Spielman. 1984. Increased intradermal probing time in sporozoite-infected mosquitoes. Am. J. Trop. Med. Hyg. 33:17–20.

Rossignol, P. A., J. M. C. Riberio and A. Spielman. 1986. Increased biting rate and reduced fertility in sporozoite-infected mosquitoes. Am. J. Trop. Med. Hyg. 35:277–279.

Vladimirova, V. V. 1970. Sensitivity of mosquitoes to repellents in relation to their physiological state. (In Russian). Med. Parazitol. Parazit. Bolezn. 39:49–53.

Wirtz, R. A., F. Zavala, Y. Charoenvit, G. H. Campbell, T. R. Burkot, I. Schneider, K. M. Esser, R. L. Beaudoin and R. G. Andre. 1987. Comparative testing of *Plasmodium falciparum* sporozoite monoclonal antibodies for ELISA development. Bull. W.H.O. 65:39–45.