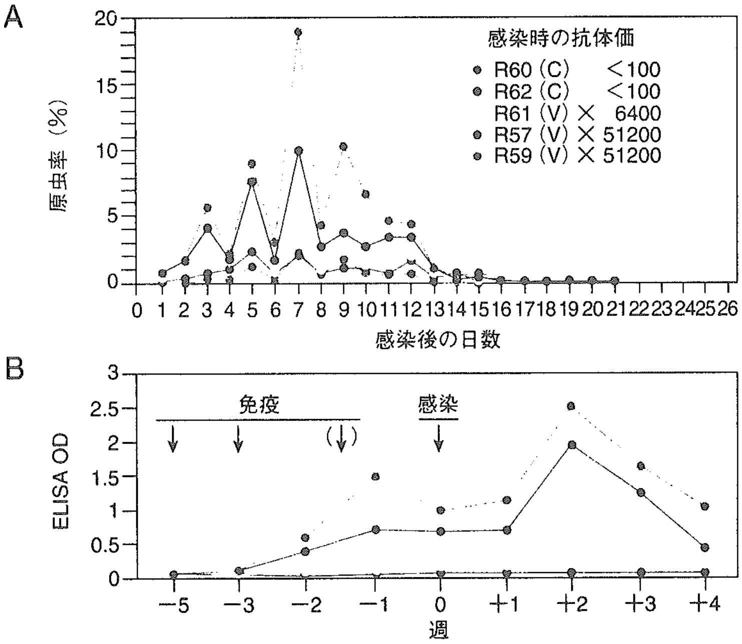

図8. SE36治験製剤によるリスザルを用いたワクチン試験

A：マラリア原虫感染後の原虫率の推移. マラリア原虫感染赤血球を静脈注射によって感染5頭のリスザルに感染させた. ワクチン群（V）で高い抗体価を示した2頭（R57, R59）では原虫率（全赤血球に対する感染赤血球の割合）が対照群（C）に比べて低く抑制されている. ヒト熱帯熱マラリア原虫の感染に対してリスザルは自然治癒する.

B：免疫前から感染経過の抗体価の推移. −5週に初回免疫を行い, −3週に追加免疫を施した. R61のリスザルは抗体価の上昇が低かったため, さらに1回の追加免疫を施した. ＋2週目においてSE36ワクチンによって誘導された抗体応答が原虫の感染によって増強（ブースト）されている. 対照群（C）においてはマラリア感染終了後においても抗SE36抗体価の上昇は見られない.

て増強（ブースト）されていることである（図8B）. これは感染したマラリア原虫によって提示されるSERAタンパク質によって増強されたものであり, 流行地域においてSE36ワクチンを接種した人では感染を受けることによってワクチン効果が増強されることを示唆するものである.

Ⅳ. 臨床試験を目指すSE36マラリアワクチン

これまで世界で開発が進められてきたマラリアワクチン抗原タンパク質とSE36タンパク質との違いは, SERAが数少ないマラリア原虫の“アキレス腱”の1つであり, また, 遺伝子操作によって生産したワクチン抗原, SE36タンパク質そのものに対する抗体が流行地において直接発症予防に働いていることである. したがって, SE36タンパク質のワクチン効果は大いに期待されるところであるが, 一方, ワクチン効果はワクチン接種対象者のマラリア経験によって大きく影響を受ける可能性がある. マラリア感染を繰り返す高度マラリア流行地域の住民においてすら, 抗SE36抗体保有者は50%にも満たないことから, 流行地域ではSE36ワクチンがかなりなマラリア発症抑止効果を与えるであろうと期待できる. 一方, 日本人のようにマラリア感染経験がない場合に, マラリア発症をどのレベルまで抑止できるかについての確実な予測はできない. 例えばSE36ワクチン免疫によって日本人の血中原虫率を1%以内に抑えることができたとしても, 発熱などの初期症状をどの程度軽減できるかについては不明である. しかしながら, マラリア感染経験がない人の場合でもSE36ワクチンによって血中原虫率をある程度抑制すれば, マラリア重症化による死亡を予防するものと期待している.

平成16年度に予定している第Ⅰ相臨床試験を目指して, 試作ワクチンの量産体制が（財）阪大微生物病研究会において確立された. 第Ⅰ相臨床試験において安全性と免疫原性が確認されれば, ウガンダ, および東南アジア地域において感作された人を対照とした第Ⅰ相臨床試験を行い, 続いて第Ⅱ相臨床試験において効果試験を行う予定である. 本プロジェクトにはWHO-TDR（世界保健機関熱帯病研究特別プロジェクト）も重大な感心を寄せており, 臨床開発を共同で推進する予定である.

文献

1) Li J, et al: Mol Biochem Parasitol (2002) 120: 177-186
2) Li J, et al: Parasitol Int (2002) 51: 343-352
3) Sugiyama T, et al: Vaccine (1996) 14: 1069-1076
4) Pang XL, et al: Infect Immun (1999) 67: 1821-1827
5) Pang XL, et al: Vaccine (1998) 16: 1299-1305
6) 堀井俊宏：蛋白質核酸酵素 (2001) 46: 2154-2162
7) Okech BA, et al: Am J Trop Med Hyg (2001) 65: 912-917
8) Aoki S, et al: J Biol Chem (2002) 277: 47533-47540
9) Morimatsu K, et al: Mol Biochem Parasitol (1997) 86: 249-254

SHORT COMMUNICATION

Diving Ability of *Anopheles gambiae* (Diptera: Culicidae) Larvae

NOBUKO TUNO,[1] KAORI MIKI,[2] NOBORU MINAKAWA,[3] ANDREW GITHEKO,[4] GUIYUN YAN,[3] AND MASAHIRO TAKAGI[1]

J. Med. Entomol. 41(4): 810–812 (2004)

ABSTRACT *Anopheles gambiae* Giles larvae usually live near the surface of shallow and temporary aquatic habitats. How deep the larvae can dive and how long they can submerge may be related to feeding efficiency and predator avoidance. This study examined diving behavior of *An. gambiae* larvae in the laboratory. We recorded diving depths and larval mortality of second and fourth instars in clean water and muddy water by using deep water (32-cm) and shallow water (20-cm) columns. In deep water columns with clean water, we found that 2% of second instars and 6% of fourth instars died from diving, whereas 3% of second instars and 11% of fourth instars died in muddy water. The fourth instars dived deeper in muddy water than in clean water. The mortality rates of the fourth instars subjected to diving stimulations were significantly higher than those in the shallow water columns. Therefore, larval diving behavior may offer the benefits of predator avoidance and food acquisition but also incur energetic costs and increased mortality.

KEY WORDS *Anopheles gambiae*, larval ecology, diving behavior, mosquito

MOST HABITATS OF *Anopheles gambiae* Giles and *Anopheles arabiensis* Patton are small, temporary, sunlit, turbid pools of water, whereas *Anopheles funestus* Giles is often found in permanent water bodies (Gillies and De Meillon 1968, Minakawa et al. 1999, Gimnig et al. 2001). *An. gambiae* larvae generally live near the surface of aquatic habitats, but they dive and remain at the bottom for some time if some mechanical disturbance takes place. While at the bottom, they often adopt a C-shaped posture, resembling dead insect carcasses. They also can move along the bottom, turning over particle after particle (N.T., unpublished data). These observations suggest that the diving ability of anopheline larvae (i.e., depths and duration of diving) may be related to predator avoidance and feeding.

Romoser and Lucas (1999) studied mosquito pupal diving behavior. They showed that diving behavior varied dramatically among mosquito genera: *Culex pipiens* L. and *Anopheles stephensi* Liston make short-duration, shallow dives, whereas *Aedes aegypti* (L.), *Aedes albopictus* (Skuse), and *Aedes triseriatus* (Say) make longer duration dives, typically to a depth at which they become neutrally or negatively buoyant. They suggested that pupal diving behavior helps avoid predation and the mechanical shock of a direct hit by a raindrop and prevents them from being washed away from their container habitats by overflowing water. In this article, we report a study on the diving ability of *An. gambiae* larvae and the effects of water condition and larval age on larval diving ability.

Materials and Methods

This study was conducted using *An. gambiae* larvae from a laboratory colony maintained in the Vector Biology Control and Research Center, Kenya Medical Research Institute, Kisumu, western Kenya. This strain was derived from wild mosquitoes collected in the vicinity of the Institute. All experiments were conducted in 500-ml graduated cylinders (34 cm height, 5 cm in inner diameter at room temperature [26 ± 2°C).

The diving ability of the second and fourth instars was examined under two water conditions to determine the effect of turbidity on larval diving behavior under two different water depths. Two water conditions were dechlorinated tap water (hereafter referred to as "clean water"), and mixture of local black cotton soil with dechorinated water (hereafter referred to as "muddy water"). The muddy water had ≈2–3-mm thick sediment at the bottom of the muddy water column and the turbidity was 170–400 Formazin turbidity units. In the deep water columns, individual larva was discharged into the 500-ml graduated cylinder with 32-cm-deep water from a plastic cup by using a pipette with 0.5 ml of water. In most cases, the discharging acts provoke a dive. When they did not dive, we discharged another 0.5 ml of water with a pipette at the surface and repeated until they started to dive (three stimulations were sufficient). Caution was taken not to touch the larvae and to maintain

---

[1] Department of Vector Ecology and Environment, Institute of Tropical Medicine, Nagasaki, University, Nagasaki 852-8523, Japan.

[2] Suemasa 34, Tatsuno, Tatsuno City, Hyogo 679-4125, Japan.

[3] Department of Biological Science, State University of New York, Buffalo, NY 14260.

[4] Kenya Medical Research Institute, P.O. Box 1578, Kisumu, Kenya.

0022-2585/04/0810–0812$04.00/0 © 2004 Entomological Society of America

Table 1. Mortality of the second and fourth instars of *An. gambiae* s.s. larvae in clean and muddy water column

Water depth (cm)	Age of larvae	Clean water			Muddy water		
		No. larvae tested	No. larvae reached to the bottom	No. larvae died	No. larvae tested	No. larvae reached to the bottom	No. larvae died
32	Second instar	100	10	2	100	9	3
	Fourth instar	100	16	6	100	21	11
20	Second instar	100	7	0	100	20	0
	Fourth instar	100	34	0	100	49	0

similar stimulation intensity for all larvae. The depth each larva reached and whether it returned to the surface were recorded. Those individuals that did not return to the surface immediately also were observed for survivorship within 6 h. In total, we measured the diving depths and mortalities for 100 second and 100 fourth instars in each of the two water conditions (clean and muddy water); thus, a total 400 larvae were used. In the shallow water columns, second and fourth instars were individually placed in the same 500-ml graduated cylinders that were filled with 20-cm-deep clean or muddy water. Their survivorship was examined within 6 h. A total of 400 mosquito larvae were used in the shallow water column group.

Fisher's exact test was used to test the difference in larval mortality between the deep water and shallow water columns and between second and fourth instars under clean and muddy water conditions. The effects of water conditions and larval age on diving depth were analyzed using the two-way analysis of variance (ANOVA) and two-tailed *t*-tests.

Results

We observed both active and passive dives. An active dive involved twisting the body in a zig-zag manner, whereas a passive dive resembled a free-fall, in which the body was held straight and the larva sank to the bottom. Usually, the larvae actively swam downward (active diving) to break the adhesion with water surface and then changed to passive mode; however, passive and active modes were both observed at every depth. Some larvae attempted to ascend to the surface, but they were unable to sustain their activities and began to sink and eventually drowned. In the clean water columns (32-cm depth), among the 100 second instars tested, 10 (10%) reached to the bottom of the water column and two (2%) died (Table 1). The proportion of the second instars that reached the bottom and mortality rates in the clean water columns were similar to those in the muddy water columns (9% reached the bottom, 3% mortality rate). For the fourth instars, 16% reached to the bottom and 6% died in the clean water columns, similar to those in the muddy water columns (21% reached the bottom and 11% died in the muddy water columns, Table 1; Fisher's exact test, $P = 0.31$). In the shallow water group, higher proportion of mosquito larvae dived to the bottom of the 20-cm water column for both clean water and muddy water, but no mortality was observed (Table 1). Diving stress increased larval mortality in fourth instars in the 32-cm depth water columns, compared with the 20-cm depth water columns (Fisher's exact test: clean water, $P = 0.03$; muddy water, $P < 0.001$).

Table 2 shows the average diving depths after the drowned individuals were excluded from the analyses. ANOVA revealed that water turbidity and larval age significantly affected the diving depth (larval age: $F = 7.990$; df = 1, 374; $P < 0.01$ and turbidity: $F = 9.465$; df = 1, 374; $P < 0.01$). The fourth instars dived significantly deeper (mean ± SD, 16.90 ± 7.80) in the muddy water columns than in the clean water columns (12.83 ± 8.25) ($P = 0.001$; Table 2). However, the diving depth of the second instars was similar between muddy and clean water columns ($P = 0.404$). The water turbidity affected the diving depth of second and fourth instars differently. In clean water column, the diving depth of second and forth instars was similar ($P = 0.530$), whereas in muddy water the forth instars dived deeper than the second instars ($P < 0.001$).

Discussion

This study has demonstrated that the fourth instars of *An. gambiae* dived significantly deeper in muddy water columns than in clean water columns. The mortality rates of fourth instars in 32-cm depth water columns were significantly higher than those in shal-

Table 2. Diving depth of survivors of second and fourth instars of *An. gambiae* in clean and muddy water column

Age		Water turbidity		Comparison	*t*-Test		
		Clean	Muddy		*t*	df	*P*
Second instar	Mean	12.10	13.03	Second (muddy vs. clean)	− 0.837	193	0.404
	SD	7.78	7.77				
	N	98	97	Fourth (muddy vs. clean)	− 3.427	181	0.001
Fourth instar	Mean	12.83	16.90	Clean (second vs. fourth)	− 0.629	190	0.530
	SD	8.25	7.80				
	N	94	89	Muddy (second vs. fourth)	− 3.870	184	0.001

P, Bonferroni adjusted probability; *t*, pooled-variance *t*-test statistics.

lower depth (20-cm) water columns, regardless of water turbidity. The vertical movement of larvae and pupae is affected by buoyancy. The relative buoyancy density of anopheline larvae is >1.0, but <1 for pupae because pupae contain space for wing and proboscis development and considerable air is trapped inside the pupae (Romoser 1975, Clements 1999). Thus, mosquito larvae can sink passively to the bottom of the water column after detaching themselves from the water surface, whereas pupae consume considerable energy to dive (Lucas and Romoser 2001). In our study, we observed that *An. gambiae* larvae moved actively to detach from the water surface and then often passively sank. Unlike pupa, mosquito larvae do not have specific organs to stock air. As a consequence, a larva diving deeper will consume more energy to swim back to the water surface. Mosquito larvae would drowned if they did have the energy or ability to swim back to the air–water interface.

Gimnig et al. (2001) reported that water bodies inhabited by both of *An. gambiae* and *An. arabiensis* were shallower (mean depth 9.7 cm) than those that did not contain *An. gambiae* complex larvae (mean depth 22.9 cm). Water depth of larval habitats generally correlates with other biotic or abiotic factors such as habitat persistence, temperature, algae content, and predator presence (Minakawa et al. 1999). In this study, we demonstrated that water depth directly affected the mortality rate of *An. gambiae* when larvae were forced to submerge. It is not known whether female anophelines can detect habitat depth and thereby avoid laying eggs in deep habitats, because water depth may be correlated with many variables that directly or indirectly affect female oviposition behavior. Fourth instars dived deeper in muddy water than in clean water, whereas the second instars did not show such a difference. Perhaps *An. gambiae* larvae are adapted to reach to the muddy bottom where they could avoid predation or acquire food. Such adaptation might be more apparent for older larvae but not for younger ones. Therefore, larval diving behavior may not only offer the benefits of predator avoidance and food acquirement but also incur energy expenditure and increasing mortality.

Acknowledgments

We thank S. Juma and T. Otieno for technical assistance. We are grateful to Edward D. Walker and two anonymous reviewers for constructive criticism on the manuscript. This work is supported by National Institutes of Health grant R01 (AI) 50243 and KAKENHI15770012.

References Cited

Clements, A. N. 1999. The biology of mosquitoes, vol. 2 sensory reception and behaviour. Cambridge University Press, Cambridge, United Kingdom.

Gillies, M. T., and B. De Meillon. 1968. The Anophelinae of Africa south of the Sahara (Ethiopian zoogeographical region). Publ. South Afr. Instit. Med. Res. No. 54.

Gimnig, J. E., M. Ombok, L. Kamau, and W. A. Hawley. 2001. Characteristics of larval anopheline (Diptera: Culicidae) habitats in western Kenya. J. Med. Entomol. 38: 282–288.

Minakawa, N., C. M. Mutero, J. I. Githure, J. C. Beier, and G. Yan. 1999. Spatial distribution and habitat characterization of anopheline mosquito larvae in western Kenya. Am. J. Trop. Hyg. 61: 1010–1016.

Romoser, W. S. 1975. Buoyancy and ventilation in *Aedes aegypti* (L.) pupae (Diptera: Culicidae). J. Med. Entomol. 12: 547–550.

Romoser, W. S., and E. A. Lucas. 1999. Buoyancy and diving behavior in mosquito pupae. J. Am. Mosquito Contr. Assoc. 15: 194–199.

Lucas, E. A., and Romoser, W. S. 2001. The energetic costs of diving in *Aedes aegypti* and *Aedes albopictus* pupae. J. Am. Mosquito Contr. Assoc. 17: 56–60.

Received 12 August 2003; accepted 11 May 2004.

CHANGES IN MALARIA VECTOR DENSITIES OVER A TWENTY-THREE YEAR PERIOD IN MAE HONG SON PROVINCE, NORTHERN THAILAND

Wannapa Suwonkerd[1], Yoshio Tsuda[2], Hans J Overgaard[3], Srisucha Chawprom[1], Nobuko Tuno[4], Somsak Prajakwong[1] and Masahiro Takagi[4]

[1]Office of Disease Prevention and Control No. 10, Department of Diseases Control, Ministry of Public Health, Chiang Mai, Thailand; [2]Department of Medical Entomology, National Institute of Infectious Diseases, Tokyo, Japan; [3]Department of Entomology, Swedish University of Agricultural Science, Uppsala, Sweden; [4]Department of Vector Ecology, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan

Abstract. Mae Hong Son Province in northwestern Thailand has a long history of malaria. During the last two decades the province has had one of the highest malaria incidences of all provinces in Thailand. Data were analyzed to determine whether the vector populations were stable or increasing during the last two decades and to determine the seasonal prevalence of the main vectors, and whether or not they were related to the malaria transmission peak, in the wet season. We compiled and analyzed accumulated entomological records from 1977 to 1999. The aim was to investigate long-term changes in mean densities of malaria vectors between two periods (1977-1989 and 1990-1999), and the differences in vector densities between two seasons (wet and dry). A total of 141,144 adult anophelines of 29 species were collected on indoor and outdoor human baits and animal baits during the study period. Of the main malaria vectors, the densities of *Anopheles minimus* s.l. and *Anopheles maculatus* complex increased significantly. *Anopheles dirus* s.l., however, was stable between the two periods. These vector populations were associated with consistently high malaria incidence in the province during the last two decades. *An. minimus* s.l. density was not significantly different between seasons. However, in the second period, both *An. dirus* s.l. and *An. maculatus* complex showed a tendency for higher wet season densities. This can explain the high malaria incidence in the rainy season in Mae Hong Son. Environmental and climatic factors seem to have been favorable for supporting a consistently high vector population in the province, and consequently a high malaria transmission rate during the period of study.

INTRODUCTION

Long-term studies on mosquito abundance are not common, partly because regular mosquito control and surveillance activities in many countries are quite recent innovations. If standard sampling protocols have not been used, the data may be difficult to interpret and analyze. In Thailand, however, such long-term mosquito data do exist. Entomological surveillance was first described and established as a regular part of malaria control programs in the early 1950s. It became more organized in the 1970s and has continued to the present time. This entomological surveillance followed standard collection protocols, reporting the number of adult mosquitos collected on indoor and outdoor human baits, as well as on animal bait when cows or buffalos were available (Bhatia and Notananda, 1953). All collected anopheline mosquitos were counted and morphologically identified to species.

In several studies, the effects of climatic, environmental and socioeconomic factors have been used to explain long-term changes in numbers of malaria cases (Bouma *et al,* 1996; Van

---

Correspondence: Wannapa Suwonkerd, Vector Borne Disease Section, Office of Disease Prevention and Control No. 10, Department of Disease Control, Ministry of Public Health, 18 Boonruangrit Road, Chiang Mai 50200, Thailand.
Tel: +66-53-221529, Fax: +66-53-212389
E-mail: malar@chmai.loxinfo.co.th

der Hoek *et al*, 1997; Hu *et al*, 1998; Mouchet *et al*, 1998). However, none of these studies used detailed data on vector populations as a causal factor, although the probability of malaria transmission largely depends on the vectorial capacity of the mosquito population (Onori and Grab, 1980). Information from long-term entomological studies is valuable for analyzing the effect of temporal changes in vector densities on malaria incidence. Our intention was to describe changes in anopheline mosquito densities over 23 years in Mae Hong Son, a malaria hyper-endemic province in northwest Thailand.

The malaria history in Mae Hong Son Province shows that transmission occurs throughout the year with a major peak during the early part of the wet season (May to August) and a smaller one in the dry season (November to January), and this general pattern did not change between the last two decades (OVBD2, 2000). This transmission coincides with intense agricultural activity after the first monsoon, when farmers stay in farm huts which rice cultivating and are exposed to vectors (Somboon *et al*, 1998). Furthermore, transmission is closely related to forest locations where there is an abundance of malaria vector breeding sites (Ketrangsee *et al*, 1991), various occupational factors encourage population movement or the influx of refugees (Singhanetra-Renard, 1986; Butraporn *et al*, 1995; Stern, 1998), socioeconomic factors affecting malaria (Fungladda *et al*, 1987), and a high degree of drug resistance (Wernsdorfer *et al*, 1994).

Mosquito collection data from northern Thailand have accumulated at the Office of Vector-Borne Disease Control No.2, Chiang Mai, without any formal analysis. We constructed a database of records from 1977 to 1999, with the objective of retrospectively studying temporal and seasonal variations in anopheline densities in Mae Hong Son Province. We investigated whether stable or increasing densities of the main malaria vectors during the study period were associated with the consistent high malaria incidence in the province. Malaria transmission in northern Thailand usually takes place during the wet season (Ismail *et al*, 1974). Therefore, we hypothesized that malaria vector density would be higher in this season than in the dry season.

MATERIALS AND METHODS

Study area

Mae Hong Son Province is located on the Thai-Myanmar border in northwestern Thailand (Fig 1). The province is geographically homogenous with 90% of the area covered with mountains and about 70% with mainly mixed deciduous and dry dipterocarp forests. The rapid socioeconomic changes in Thailand of recent decades has mainly taken place in larger population centers and focal areas in the central valley of Mae Hong Son Province. Many villages are remotely situated, with no public transport. Approximately 40% of the province has poor accessibility, and during the rainy season accessibility is reduced even more. In a large part of the province, there is limited land for paddy fields and the average size of farms is small. The province is administratively divided into 7 districts (amphoe), 45 cantons (tambon), and 395 villages. The population consists of Thai nationals and many hill tribes of various ethnic groups. Many refugee camps are situated along the border, housing a large number of displaced people from conflict areas within Myanmar. The population in 1977 was 123,816 and in 1999 it had increased by 58% to 195,209 (Office of Vector Borne Disease Control No.2, 1978-2000).

Available records and data analysis

Accumulated mosquito collection data at the Office of Vector Borne Disease Control No. 2 in Chiang Mai (OVBD2), from 1977 to 1999, were compiled and analyzed. Data from before 1977 were few and incomplete and were therefore not used. Each record included the name of the village where mosquitos had been collected, the number of adult anopheline species collected by indoor human bait, outdoor human bait and animal bait, number of collection nights, number of collectors, village population, and number of recorded malaria cases. In human bait collections, two persons collected mosquitos indoors and outdoors, respectively. Half-night collections were undertaken, *ie* from sunset to midnight. Simultaneous animal bait collections were conducted using one cow or buffalo, if available. Each record was standardized by calculating the density of mosquitos, given as the number of mosquitos per bait per half-night.

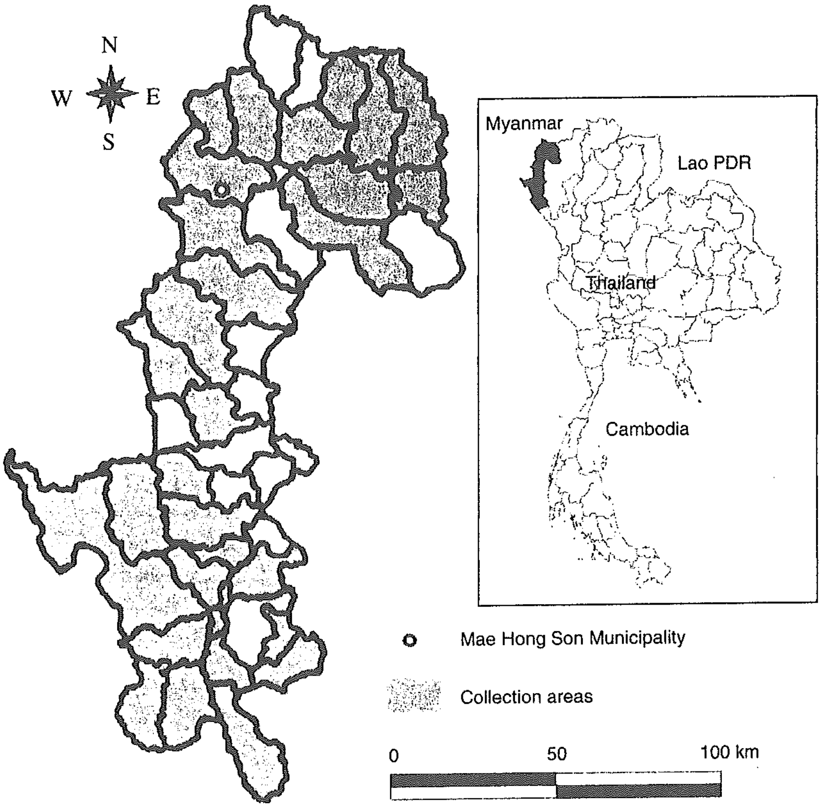

Fig 1–Location of Mae Hong Son Province and cantons (in gray) where entomological data were collected from 1977-1999.

Mosquito collections were undertaken in 97 villages (about 25% of all villages in the province) in 32 cantons (71% of all cantons) (Fig 1). We separated all records into two time periods, the first from 1977 to 1989 and the second from 1990 to 1999, and into two seasons, a wet season (May-September) and a dry season (October-April). Because of relatively few data from the early 1980s, we added available data from the end of the 1970s to the first period, so that replications were as much as possible comparable for each period. It was difficult to find long-term continuous records from single villages during the whole period. For this reason, in our analysis, villages were pooled into the next larger administrative unit, the canton. Thus, a total of 321 records of indoor human bait collections, 501 records of outdoor human bait collections, and 271 records of animal bait collections were available.

Mosquitos were morphologically identified to species using the keys of Peyton and Scanlon (1966); Sawadwongporn (1972); Rattanarithikul and Harrison (1973); Harrison and Scanlon (1975); Peyton and Harrison (1979); Harrison (1980); Rattanarithikul and Green (1986); Rattanarithikul and Panthusiri (1994). Recently, *An. minimus* and *An. dirus* were genetically found to be species complexes of at least three and five sibling species, respectively (Baimai, 1988; Baimai *et al*, 1988; Sucharit *et al*, 1988; Green *et al*, 1990; 1992; Sharpe *et al*, 1999, Walton *et al*, 1999). However, in this study these two species were morphologically identified as *An.minimus* s.l. and *An. dirus* s.l., respectively. Before 1989, all species belonging to the *An. maculatus* complex were collectively identified as *An. maculatus*. Since 1990, individual members of the complex have been morphologically identified as *An.*

Table 1
List of anopheline species collected in Mae Hong Son from 1977 to 1999, their mean densities (no. of mosquitos/bait/half-night) and ± SE.

Species	Mean densities ± SE		
	Human bait		Animal bait
	Indoor	Outdoor	
An. dirus s.l.	0.08 ± 0.02	0.15 ± 0.05	0.32 ± 0.01
An. minimus s.l.	2.11 ± 0.29	4.1 ± 0.27	6.9 ± 0.75
An. maculatus complex	0.55 ± 0.09	3.2 ± 0.18	12.3 ± 1.27
An. aconitus	0.19 ± 0.06	1.2 ± 0.18	8.4 ± 2.58
An. philippinensis	0.1 ± 0.05	0.5 ± 0.15	0.8 ± 0.33
An. barbirostris	0.1 ± 0.03	0.3 ± 0.06	9.3 ± 2.87
An. annularis	0.5 ± 0.14	2.6 ± 0.67	11.8 ± 2.32
An. barbumbrosus	<0.01	<0.01	<0.01
An. campestris	<0.01	0.01 ± 0.005	<0.01
An. culicifacies	0.01 ± 0.02	0.01 ± 0.003	1. 3 ± 0.81
An. hyrcanus group	0.21 ± 0.05	1.0 ± 0.15	9.6 ± 1.66
An. jamesii	<0.01	0.01 ± 0.003	<0.01
An. jeyporiensis	<0.01	0.06 ± 0.003	<0.1 ± 0.08
An. karwari	<0.01	<0.01	<0.01
An. kochi	0.1 ± 0.03	0.2 ± 0.03	9.8 ± 1.4
An. nivipes	<0.1	0.2 ± 0.05	2.2 ± 0.6
An. pseudojamesi	<0.01	<0.01	0.02 ± 0.02
An. splendidus	0.1 ± 0.03	0.3 ± 0.06	1.0 ± 0.47
An. tessellatus	<0.1 ± 0.004	0.1 ± 0.05	0.4 ± 0.07
An. vagus	<0.01	0.1 ± 0.01	15.4 ± 3.8
An. varuna	<0.01	0.1 ± 0.02	<0.01
An. umbrosus	<0.1±	<0.1±	<0.1±
Species of *An. maculatus* complex			
An. dravidicus	<0.01	0.1 ± 0.02	0.3 ± 0.06
An. maculatus s.s.	0.1 ± 0.02	1.2 ± 0.19	6.75 ± 1.13
An. notanandai	<0.01	<0.01	<0.01
An. pseudowillmori	0.05 ± 0.2	0.14 ± 0.04	0.2 ± 0.06
An. sawadwongporni	0.2 ± 0.04	0.7 ± 0.14	2.5 ± 0.47
An. willmori	0.12 ± 0.02	0.4 ± 0.05	2.01 ± 0.31
Species of *An. hyrcanus* group			
An. nigerrimus	<0.01	<0.01	<0.01
An. peditaeniatus	<0.01	<0.02 ± 0.01	0.61 ±0.19
An. sinensis	0	<0.01	<0.16 ± 0.12

maculatus s.s., *An. dravidicus*, *An. notanandai*, *An. pseudowillmori*, *An. sawadwongporni*, and *An. willmori* (Rattanarithikul and Green, 1986).

The three major malaria vectors, *An. dirus* s.l. (Scanlon and Sandhinand, 1965; Peyton and Harrison, 1979), *An. minimus* s.l. (Ayurakitkosol and Griffith, 1963), and the *An. maculatus* complex (Reid, 1968; Upatham *et al*, 1988; Rattanarithikul and Green, 1986) were subjected to statistical analysis. To determine differences in mean mosquito density, two-way analysis of variance was conducted. The variation of adult density found in indoor, outdoor, and animal bait collections was analyzed separately for each period (1st period: 1977-1989 or 2nd period: 1990-1999) and season (dry or wet). All statistical analyses were performed using SYSTAT statistical software (Wilkinson, 1996).

Table 2

Comparison of mean densities (number of mosquitos/bait/half-night) of three main malaria vectors in Mae Hong Son, between two periods (1977-1989 and 1990-1999) and between two seasons (dry and wet); pairs of means in boxes and in boxes with highlight are significantly different ($p<0.05$).

Anopheles species	Seasons	Human bait indoor		Human bait outdoor		Animal bait	
		1st period	2nd period	1st period	2nd period	1st period	2nd period
An.minimus s.l.	Dry	0.825	2.002	4.044	4.502	3.143	7.097
	Wet	0.845	2.766	2.680	4.417	4.337	8.484
An. dirus s.l.	Dry	0.017	0.018	0.019	0.059	0.004	0.050
	Wet	0.005	0.181	0.231	0.231	0.000	0.033
An. macualtus	Dry	0.004	0.218	3.435	1.621	14.494	8.743
complex	Wet	0.010	1.120	2.171	4.859	11.223	14.289

1st period = 1977-1989, 2nd period = 1990-1999; dry season = October-April, rainy season = May-September.

RESULTS

During the study period, a total of 141,144 anophelines of 29 species were recorded in Mae Hong Son Province, of which 64,469 were collected on outdoor human bait, 63,416 on animal bait, and 13,259 on indoor human bait. The mosquito indices are expressed as the mean number of mosquitos per bait per half night. Table 1 gives a list of all anopheline species, and the mean densities (±SE) by the three different collection methods. Of all anophelines collected, *An. minimus* s.l. was predominantly biting on humans with a mean density of 2.1 and 4.1 from indoor and outdoor collection, respectively (Table 1). On animal bait, the most abundant species was *An. vagus* (15.4), followed by *An. maculatus* complex (12.3), and *An. annularis* (11.8) (Table 1).

Malaria vectors

***An. minimus* s.l.** The results of the ANOVA on the whole data set for this species showed that there were significant differences between periods (1977-1989 and 1990-1999) ($F_{1, 1093}=13.40$, $p<0.001$) and there were no significant interactions between any of the factors ($p>0.05$). Also, for each season, there were significant differences between periods (dry: $F_{1, 525}=4.69$, $p<0.05$; and wet: $F_{1, 568}=9.11$, $p<0.01$), but when separated by collection method, only an increase in outdoor human bait in the wet season was significant (Table 2). No significant differences were found between seasons in the whole data set ($F_{1, 1093}=0.27$, $p=0.60$), nor in each period ($p>0.2$).

***An. dirus* s.l.** In two-way analysis of all the data, there were no significant differences in density between any of the factors analyzed (period, season). However, when the periods were analyzed for this species separately, in the second period, a higher indoor density during the wet season was found (Table 2).

***An. maculatus* complex.** For this species complex, there was no difference in density between the two periods ($F_{1, 1093}=2.04$, $p>0.05$). However, there was a significant interaction between periods and seasons in the whole data set ($F_{1,1093}=5.05$, $p<0.05$), which is reflected by a significant difference between periods in the dry season ($F_{1,525}=9.61$, $p<0.01$), but not in the wet season ($F_{1, 568}=2.70$, $p>0.05$). Furthermore, we found contrasting results when analyzing differences between periods for each collection method and each season. For the outdoor human bait collections, there were significant decreases in the dry season and significant increases in the wet season (Table 2). The human indoor collections increased significantly between periods in both seasons. There were no differences between seasons in the first period, but in the second period there were significantly higher densities in the wet season for all collection methods.

DISCUSSION

We found that the densities of the three main malaria vectors either increased or were stable in Mae Hong Son Province during the study period. The density of *An. dirus* s.l., a more efficient vector than *An. minimus* s.l. (Gould *et al,* 1966; Ismail *et al,* 1974; 1975), was stable throughout the period. However, the density of *An. minimus* s.l. and *An. maculatus* complex had significantly increased from the first period (1977-1989) to the second period (1990-1999). Specifically, the density increase in the latter two species was mainly observed in the wet season. These facts support our hypothesis that large or increasing vector populations could be a contributing factor for the consistent high malaria incidence in the province.

We also found significantly higher densities of *An. dirus* s.l. and *An. maculatus* complex in the wet season than in the dry season, especially in the second period. High wet season densities of *An. dirus* s.l. and *An. maculatus* complex have also been reported in other studies (Ismail *et al,* 1974; 1975; Rosenberg, 1982; Upatham *et al,* 1988; Rosenberg *et al.* 1990; Suwonkerd *et al,* 1995; Takagi *et al,* 1995). It is believed that *An. dirus* s.l. usually retreats to dense humid forest areas during the dry season and returns to the forest fringe and populated areas during the rainy season (Rosenberg *et al,* 1990), thus being largely responsible for wet season transmission (Ismail *et al,* 1974). *An. maculatus* complex has generally been thought to be an important vector in southern Thailand (Upatham *et al,* 1988), and of little or no importance elsewhere in the country (Harbach *et al,* 1987). However, *An. maculatus* s.s. and *An. sawadwongporni*, which are members of this complex, were incriminated in Mae Hong Son Province (Somboon *et al,* 1994). *An. pseudowillmori*, also a member of this complex, was incriminated in the neighboring province of Tak (Green *et al,* 1991). It appears, therefore, the species of the *An. maculatus* complex may have played a larger role in malaria transmission in northern Thailand than previously assumed. The density of *An. minimus* s.l. was not significantly different between seasons. Previous studies found that *An. minimus* s.l. was prevalent throughout the year, including a major part of the dry, cool season, as it remained at high density from November to February, and for a shorter duration in the early part of the rainy season (Ismail *et al,* 1974; 1975; 1978). Therefore, it is seems that *An. minimus* s.l. is responsible for transmitting malaria throughout the year, thus reinforcing wet season transmission by *An. dirus* s.l. and *An. maculatus* complex. These results partly support our hypothesis that the seasonal prevalence of the main malaria vectors was higher in the wet season, thus being responsible for the higher transmission rates in this season.

Rainfall has been suggested as an important climatic factor affecting both mosquito population and malaria transmission (Bouma *et al,* 1996; Hu *et al,* 1998; Mouchet *et al,* 1998). In Mae Hong Son, total rainfall varied between 1,025 and 1,650 mm/year during the years 1970-1998 (Meteorological Department, 1970-1999). About 70%-90% of the rainfall in Mae Hong Son Province was recorded in the wet season. However, no significant difference in wet season rainfall was observed between the two study periods ($t=1.70$, $p=0.104$) (Fig 2). Therefore, it seems that variation in rainfall did not have much effect on malaria vector density in the study area during the study period.

Deforestation has been associated with either increases or decreases of vector transmitted diseases (Walsh *et al,* 1993). In Africa, where 90% of worldwide malaria morbidity and mortality occurs, deforestation is considered a factor in the increase in malaria, due to the development of anopheline larval sites exposed to the sun (Mouchet *et al,* 1988). By contrast, in Thailand, the ecological conditions in deforested areas become unfavorable for breeding of the main malaria vectors, suggesting a reduced malaria risk (Ismail *et al,* 1978; Rosenberg *et al,* 1990). This corresponds with a study in Yunnan, in southern China, where dense forests were associated with a high abundance of *An. minimus* s.l., and a high incidence of malaria (Hu *et al,* 1998). In Mae Hong Son Province, forest cover decreased from approximately 87% in 1976 to about 70% in 1998 (Royal Forest Department, 1999). However, since mosquito collections were mainly undertaken in more or less remotely located transmission areas, where, in general, little land use change has taken

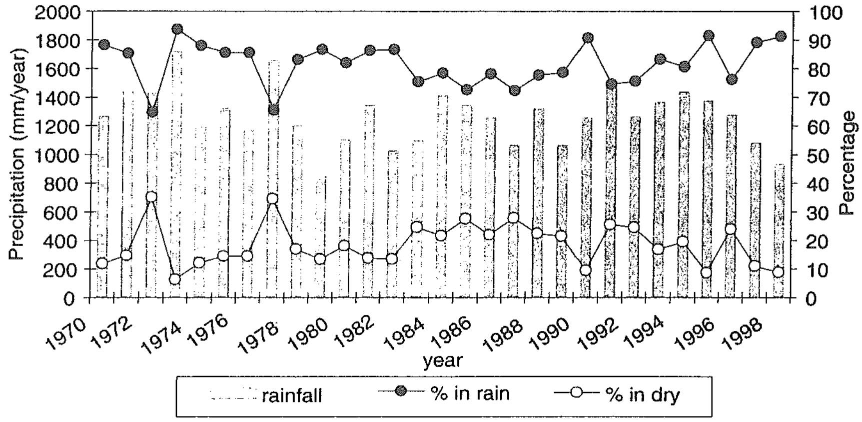

Fig 2–Yearly changes in precipitation and the proportion of rainfall recorded in wet and dry seasons from 1977-1998 in Mae Hong Son Province, northern Thailand.

place, it is reasonable to assume that there was a higher forest cover and a lower rate of deforestation in these locations. In a non-transmission area in the neighboring province of Chiang Mai, malaria vector density, especially that of *An. minimus* s.l., decreased between 1977 and 1999, and it was concluded that this might have been a result of increased landscape diversity and forest fragmentation (Suwonkerd *et al,* 2002). However, in transmission areas in Chiang Mai Province, forest cover was extensive, landscape diversity low, and malaria vector densities increased during the same period. In a comparative study of six areas in northern Thailand, of which two were situated in Mae Hong Son Province, Overgaard *et al* (in press), found that anopheline diversity and density were generally higher in forested areas with low landscape diversity than in agricultural areas with a high landscape diversity. Thus, it seems that the mosquito situation in Mae Hong Son over the last two decades resembles that of transmission areas in Chiang Mai Province.

However, apart from the density of malaria vectors, and climatic and environmental factors, there are other possible explanations for the observed high malaria rates in Mae Hong Son Province. In particular, much movement of refugees and others across the border to Myanmar, as well a high degree of multi-drug parasite resistance, may play an important role in malaria transmission in the province.

To conclude, the increases in malaria vector densities in Mae Hong Son Province suggest that entomological factors probably play a large role in malaria transmission in the province. Furthermore, environmental and climatic factors seem to have been favorable for a consistently high vector population and relatively stable malaria transmission rate. Understanding how different factors affect malaria transmission and mosquito populations will eventually lead to better planning of malaria control.

ACKNOWLEDGEMENTS

The authors are grateful to Drs Barbara Ekbom, Michael Boots, Pradya Somboon, Ralph E Harbach, and Kriengsak Limkitikul for their valuable comments and suggestions on the manuscript. We thank the staff the of entomology team of the Vector Borne Diseases Section, Office of Disease Prevention and control No. 10 (former Malaria Center 2), Chiang Mai, Thailand, and to the numerous malaria personnel who conscientiously performed their work that made this study possible. We also thank the Department of Disease Control, Ministry of Public Health, Thai Government for use of the accumulated entomological data and to all related offices for providing additional valuable data for this study. This study was supported by the RONPAKU program of the Japanese Society for the Promotion of Science.

REFERENCES

Ayurakitkosol L, Griffith ME. Progress toward malaria eradication in Thailand. Bangkok, Thailand: Proceedings of the 9th Pacific Scientific Congress, 1963: 122-36.

Baimai V. Population cytogenetic of the malaria vector *Anopheles leucosphyrus* group. *Southeast Asian J Trop Med Public Health* 1988; 19: 667-80.

Baimai V, Kijchalao U, Sawadwongporn P, Green CA. Geographic distribution and biting behaviour of four species of the *Anopheles dirus* complex (Diptera: Culicidae) in Thailand. *Southeast Asian J Trop Med Public Health* 1988; 19: 151-61.

Bhatia ML, Notananda V. Malaria control demonstration project, entomological report, Thailand. Report of the Thai government WHO/UNICEF, SEA/MAL, unpublished report to WHO, New Delhi, India. 1953: 79.

Bouma MJ, Dye C, Van der Kaay HJ. Falciparum malaria and climate change in the northwest frontier province of Pakistan. *Am J Trop Med Hyg* 1996; 55: 131-7.

Butraporn P, Prasittisuk C, Krachaiklin S, Chareonjai P. Behaviours in self-prevention of malaria among mobile population in East Thailand. *Southeast Asian J Trop Med Public Health* 1995; 2: 213-8.

Fungladda W, Sornmani S, Klongkamnuankarn K. Sociodemographic and behavioral factors associated with malaria patients in Kanchanaburi, Thailand. *J Trop Med Hyg* 1987; 90: 233-7.

Gould JD, Scanlon JE, Ward RA. Anopheles vectors of malaria in Southeast Asia. US Military Academy, West Point, NY: Proceedings of the Army Science Conference. 1966: 1-362.

Green CA, Gass RF, Munstermann LE, Baimai V. Population-genetic evidence for two species in *Anopheles minimus* in Thailand. *Med Vet Entomol* 1990; 4: 25-34.

Green CA, Munstermann LE, Tan SG, Panyim S, Baimai V. Population genetic evidence for species A, B, C and D of the *Anopheles dirus* complex in Thailand and enzyme electromorphs for their identification. *Med Vet Entomol* 1992; 6: 29-36.

Green CA, Rattanarithikul R, Pongparit S, Sawadwongporn P. A newly-recognized vector of human malarial parasites in the Oriental region: *Anopheles (Cellia) pseudowillmori* (Theobald, 1910). *Trans R Soc Trop Med Hyg* 1991; 85: 35-6.

Harbach RE, Gingrich JB, Pang LW. Some entomological observations on malaria transmission in a remote village in northwestern Thailand. *J Am Mosq Control Assoc* 1987; 3: 296-301.

Harrison BA. Medical entomology studies XIII. The Myzomyia series of Anopheles (Cellia) in Thailand, with emphasis on intra-interspecific variation (Diptera: Culicidae). *Contrib Am Entomol Inst* 1980; 17: 1-195.

Harrison BA, Scanlon JE. Medical entomology studies II. The subgenus Anopheles in Thailand (Diptera:Culicidae). *Contrib Am Entomol Ins* 1975; 12: 1-307.

Hu H, Singhasivanon P, Salazar NP, *et al*. Factors influencing malaria endemicity in Yunnan Province, PR. China (Analysis of spatial pattern by GIS). *Southeast Asian J Trop Med Public Health* 1998; 29: 191-200.

Ismail IAH, Notananda V, Schepens J. Studies on malaria and response of *Anopheles balabacensis balabacensis* and *Anopheles minimus* to DDT residual spraying in Thailand. Part I. Pre-spraying observations. *Acta Trop* 1974; 31: 129-64.

Ismail IAH, Notananda V, Schepens J. Studies on malaria and response of *Anopheles balabacensis balabacensis* and *Anopheles minimus* to DDT residual spraying in Thailand. Part II. Post-spraying observations. *Acta Trop* 1975; 32: 206-31.

Ismail IAH, Phinichpongse S, Boonrasri P. Responses of *Anopheles minimus* to DDT residual spraying in a cleared forested foothill area in central Thailand. *Acta Trop* 1978; 35: 69-82.

Ketrangsee S, Suvannadabba S, Thimasarn K, Prasittisuk C, Rooney W. Malaria situation in Thailand with special reference to forest related malaria. Forest malaria in Southeast Asia. Sharma VP, Kondrashin AV, eds. New Delhi, India: Proceedings of an Informal Consultative Meeting WHO/MRC, 18-22 February, 1991: 193-220.

Meteorological Department, Climatology Division, Bangkok, Thailand. Rainfall yearly records, 1970-1999; 29 issues.

Mouchet J, Manguin S, Sircoulon J, *et al*. Evolution of malaria in Africa for the past 40 years: impact of climate and human factors. *J Am Mosq Control Assoc* 1998; 14: 121-30.

Office of Vector Borne Diseases Control No. 2 Department of Communicable Disease Control, Ministry of Public Health, Thai Government. Plan of Action, 1978-2000: 22 issues.

Office of Vector Borne Diseases Control No. 2. Ministry of Public Health, Thai Government, Chiang Mai. Annual report. 2000: 1-155.

Onori E, Grab B. Indicators for the forecasting of malaria epidemics. *Bull WHO* 1980; 58: 91-8.

Peyton EL, Harrison BA. *Anopheles (Cellia) dirus*, a new species of the Leucosphyrus Group from Thailand (Diptera: Culicidae). *Mosq Sys* 1979; 11: 40-52.

Peyton EL, Scanlon JE. Illustrated key to the female Anopheles mosquitos of Thailand. US Army Medical Component, Southeast Asia Treaty Organization. 1966: 1-79.

Rattanarithikul R, Green CA. Formal recognition of the species of the *Anopheles maculatus* group (Diptera: Culicidae) occurring in Thailand, including the descriptions of two new species and a preliminary key to females. *Mosq Sys* 1986; 18: 246-78.

Rattanarithikul R, Harrison BA. An illustrated key to the *Anopheles* larvae of Thailand. US Army Medical Component, South East Asia Treaty Organization, 1973: 1-125.

Rattanarithikul R, Panthusiri P. Illustrated keys to the medical important mosquitos of Thailand. *Southeast Asian J Trop Med Public Health* 1994; 25: 1-66.

Reid JA. Anopheline mosquitos of Malaya and Borneo. Studies of the Institute of Medical Research, Malaysia. 1968: 1-520.

Rosenberg R. Forest malaria in Bangladesh. III. Breeding habits of *Anopheles dirus. Am J Trop Med Hyg* 1982; 31: 192-201.

Rosenberg R, Andre RG, Somchit L. Highly efficient dry season transmission of malaria in Thailand. *Trans R Soc Trop Med Hyg* 1990; 84: 22-8.

Royal Forest Department, Ministry of Agriculture and Co operatives, Thai Government, Bangkok. Annual report. 1999: 150.

Sawadwongporn P. Pictorial key of Anopheles in Thailand. Malaria Center Region 2, Chiang Mai, Department of Communicable Diseases Control, Ministry of Public Health, Thai Government, Thailand. 1972: 1-55.

Scanlon JE, Sandhinand U. The distribution and biology of *Anopheles balabacensis* in Thailand (Diptera: Culicidae). *J Med Entomol* 1965; 2: 61-9.

Sharpe RG, Hims MM, Harbach RH, Butlin RK. PCR-based methods for identification of species of the *Anopheles minimus* group: allele-specific amplification and single-strand conformation polymorphism. *Med Vet Entomol* 1999; 13: 265-73.

Singhanetra-Renard A. Population movement, socio-economic behaviour and the transmission of malaria in northern Thailand. *Southeast Asian J Trop Med Public Health* 1986; 17: 396-405.

Somboon P, Aramrattana A, Lines JD, Webber R. Entomological and epidemiological investigations of malaria transmission in relation to population movements in forest areas of north-west Thailand. *Southeast Asian J Trop Med Public Health* 1998; 29: 3-9.

Somboon P, Suwonkerd W, Lines JD. Susceptibility of Thai zoophilic Anophelines and suspected malaria vectors to local strains of human malaria parasites. *Southeast Asian J Trop Med Public Health* 1994; 25m: 766-70.

Stern A. International population movements and public health in the Mekong region: an overview of some issues concerning mapping. *Southeast Asian J Trop Med Public Health* 1998; 29: 201-12.

Sucharit S, Komalamisra N, Leemingsawat S, Apiwathanasorn C, Thongrungkiat S. Population genetic studies on the *Anopheles minimus* complex in Thailand. *Southeast Asian J Trop Med Public Health* 1988; 19: 717-23.

Suwonkerd W, Takagi M, Amg-Ung B, Prajakwong S. Seasonal and spatial distribution of 3 malaria vectors at three mountainous villages in north Thailand. *Jpn J Trop Med Hyg* 1995; 23: 183-7.

Suwonkerd W, Overgaard HJ, Tsuda Y, Prajakwong S, Takagi M. Malaria vector densities in transmission and non-transmission areas during 23 years and land use in Chiang Mai province, Northern Thailand. *Basic Appl Ecol* 2002; 3: 197-207.

Takagi M, Suwonkerd W, Tsuda Y, *et al.* Seasonal density and malaria vector competence of *Anopheles minimus* and other anophelines at a shallow valley in north Thailand. *Jpn J Trop Med Hyg* 1995; 23: 177-82.

Upatham ES, Prasittisuk C, Ratanatham S, *et al.* Bionomics of *Anopheles maculatus* complex and their role in malaria transmission in Thailand. *Southeast Asian J Trop Med Public Health* 1988; 19: 259-69.

Van der Hoek W, Konradsen F, Perera D, Amerasinghe PH, Amersinghe FP. Correlation between rainfall and malaria in the dry zone of Sri Lanka. *Ann Trop Med Parasitol* 1997; 8: 945-9.

Walsh JF, Molyneux DH, Birley MH. Deforestation: effects on vector-borne disease. *Parasitology* 1993; 106: S55-S75.

Walton C, Handley M, Kuvangkadilok C, *et al.* Identification of five species of the *Anopheles dirus* complex from Thailand, using allele-specific polymerase chain reaction. *Med Vet Entomol* 1999; 13: 24-32.

Werndorfer WH, Chongsuphajaisiddhi T, Salazar NP. Special report: a symposium on containment of mefloquin-resistant falciparum malaria in Southeast Asia with special reference to border malaria. *Southeast Asian J Trop Med Public Health* 1994; 25: 11-8.

Wilkinson L. SYSTAT version 6.0 for Windows: Statistics. Chicago, IL: SPSS Inc, 1996.

Journal of the American Mosquito Control Association, 20(3):292–298, 2004

LABORATORY AND FIELD EVALUATION OF SPATIAL REPELLENCY WITH METOFLUTHRIN-IMPREGNATED PAPER STRIP AGAINST MOSQUITOES IN LOMBOK ISLAND, INDONESIA

HITOSHI KAWADA,[1] YOSHIHIDE MAEKAWA,[1] YOSHIO TSUDA[2] AND MASAHIRO TAKAGI[1]

ABSTRACT. Spatial repellency of a new multilayer paper strip impregnated with metofluthrin, a newly synthesized pyrethroid, was evaluated in the laboratory and in the field at Kerandangan, Lombok Island, Indonesia, with the use of cow- and human-baited double nets. Spatial repellency was observed in both cow- and human-baited collections. Metofluthrin treatment reduced mosquito collection by >80% during the 1st 4 weeks. However, repellency seemed to reduce with the loss of metofluthrin by evaporation within 6 wk after treatment.

KEY WORDS Metofluthrin, spatial repellency, *Anopheles balabacensis, Culex quinquefasciatus,* Lombok Island

INTRODUCTION

Since the indoor residual spraying of insecticides lost its reliability because of the development of physiological or behaviorological resistance in mosquitoes, one of the major innovations in the field of malaria vector control during the past 2 decades has been insecticide-impregnated mosquito nets. Recent development of pyrethroids and its formulation technologies have accelerated the development of long-lasting insecticidal net (WHO 2000, N'Guessan et al. 2001). It will be the most promising measure for controlling malaria mosquitoes at low cost and high sustainability, as opposed to residual spraying. However, the exophagic behavior of mosquitoes to shift their biting preference to earlier in the evening still presents a challenge. In such cases, active people outside and inside of houses could still be exposed to the danger of malaria transmission. Mosquito coils, mats, and other formulations that prevent mosquito bites are broadly and successfully used inside houses in wealthy communities. Use of these devices is, however, limited in the poor communities because of lack of convention, electricity, and money. Moreover, their uses seem limited outside of houses because they sometimes are unable to maintain sufficient aerial concentration of active ingredient in outdoor conditions.

Metofluthrin, 2,3,5,6-tetrafluoro-4-methoxymethylbenzyl (*EZ*)-(1*RS*,3*RS*;1*RS*,3*SR*)-2,2-dimethyl-3-(prop-1-enyl) cyclopropanecarboxylate (S-1264) is a newly synthesized pyrethroid with demonstrated knockdown and lethal activity against mosquitoes (Shono et al. 2004, Sugano et al. 2004). The vapor pressure of metofluthrin (1.87×10^{-3} Pa at 25°C) is ca. >2 times and >100 times larger than *d*-allethrin and permethrin, respectively. Metofluthrin vaporizes at normal temperature without heating, whereas the other conventional pyrethroids need heating for evaporation. High vapor pressure and insecticidal activity of metofluthrin could lead new mosquito controlling devices that need no external energy for vaporization with low cost and long time efficacy.

In this paper, we report the results of laboratory and field studies that evaluate the insecticidal activity of metofluthrin-impregnated paper strip against *Anopheles balabacensis* Baisas, which is a major malaria vector in Lombok Island, Indonesia. We also report on the possibility of the use of metofluthrin-impregnated paper strip in outdoor conditions against mosquitoes, including *Anopheles* spp. and *Culex quinquefasciatus* Say.

MATERIALS AND METHODS

Formulation of metofluthrin-impregnated paper strip: Metofluthrin multilayer paper strip devices were supplied by Sumitomo Chemical Co., Ltd. (Takarazuka, Hyogo, Japan). Metofluthrin (200 mg) diluted with acetone was uniformly applied to the paper strip device, which has a multilayer and foldable structure (Fig. 1), and acetone was allowed to vaporize under ambient conditions. The folded device was compact (9 × 7 cm, ~3 mm thick), and total surface area of the unfolded paper was ~2,000 cm^2.

Laboratory evaluation against An. balabacensis: Knockdown activity of metofluthrin-impregnated paper strips was evaluated in an office of NTB Dinas Kesehatan Propinsi (Lombok, Indonesia). Room dimensions were 2.7 m wide, 3.9 m deep, and 3.2 m high. Field-collected larvae of *An. balabacensis* were reared in the laboratory and emerged adults were used in the test. Five females (3–7 days old) were released into stainless steel cages (210 × 170 × 150 mm, 11 mesh). Four pairs of cages were hung in a diagonal line at 1 m and 2.4 m (in the corner of the room) from the center of the room at a height of 130 and 30 cm, respectively, from the floor. A multilayer paper strip was hung at the center of the ceiling such that the bot-

---

[1] Department of Vector Ecology and Environment, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Nagasaki 852-8523, Japan.

[2] Laboratory of Taxonomy and Ecology, Department of Medical Entomology, National Institute of Infectious Diseases, Shinjuku-ku, Tokyo 162-8640, Japan.

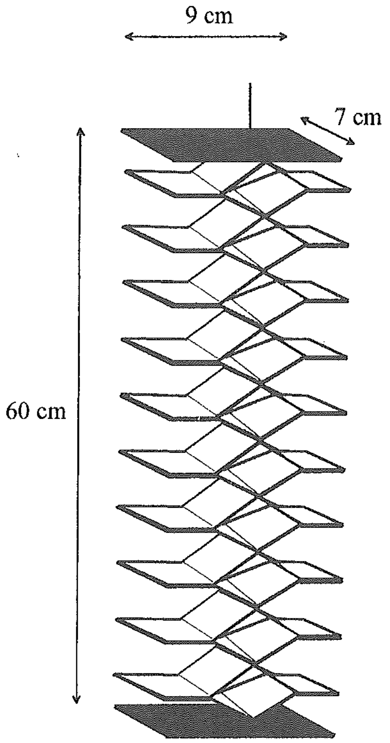

Fig. 1. Multilayer paper strip device impregnated with 200 mg of metofluthrin.

tom end of the strip was 160 cm from the floor. Knockdown of mosquitoes was observed for 1 h, and mortality was recorded at 24 h. There was no air-conditioner or ventilation system in the room, and temperature and relative humidity (RH) were recorded at 28–30°C and >70%, respectively.

Field collection of mosquitoes in Kerandangan: Weekly collection of mosquitoes was carried out at Kerandangan, a coastal area about 15 km northwest of Mataram, Lombok Island, Indonesia. A house was used for indoor human-baited collection (HBI). A bed net was hung in a room with 2 human volunteers inside, and collection of mosquitoes was made outside the bed net. Outdoor human-baited collection was carried out with the use of a double net (HBO). Collection of mosquitoes was made inside of the outer net (3 × 3 × 2 m) and outside of the inner net (1 × 1 × 2 m) in which 2 humans lay. Outdoor cow-baited collection also used a double net (CBO). Collection of mosquitoes was made inside of the outer net (6 × 6 × 2 m) and outside of the inner net (4 × 4 × 2 m) in which a cow lay. Half-night (1800–2400 h) collection of mosquitoes by aspirator was carried out at 1-h intervals. Collection time was 45 min for HBI and HBO (e.g., 1800–1845 h) and 15 min for CBO (e.g., 1845–1900 h). Field collection was carried out once a week from November 12, 2002, to February 11, 2003. Species and number of collected mosquitoes were recorded the day after collection.

Evaluation against mosquitoes in the field: Evaluation was carried out at the same place as mosquito collection in Kerandangan. Trial design is shown in Fig. 2. A room of similar size (volume 15–18 m^3, floor area 5.5–8.5 m^2) in 3 different houses was used for the indoor human-baited collection (the HBI-1 house was used for the 2nd, 3rd, and 4th tests and the HBI-2 house was used only for the 1st test; the HBI-3 house was used for the 1st, 2nd, 3rd, and 4th tests). The door and windows of each room were kept open during the test. Two sites were used for outdoor human-baited collection with a double net. Two sites were used for outdoor cow-baited collection with a double net. Test strips were set before the test started (1800 h). Strips were hung 10–30 cm below the ceiling of the house outside the bed nets for HBI (Fig. 3A) and in a space between the inside net and outside net of the double nets for HBO and CBO (Fig. 3B, 3C), respectively. The number of test samples hung were 1 for HBI, 2 for HBO, and 4 for CBO. The number of test samples in HBO and CBO was roughly decided according to the sizes of both nets. Half-night (1800–2400 h) collection of mosquitoes by aspirator was carried out at 1-h intervals in the same manner as in the weekly collection. In every test, 1 site was used for the treatment and the other site was left untreated. Each test was repeated 2 times on successive days at a different site. The 1st test (just after unfolding of strips) was carried out on December 22, 2002 (1800–2400 h), and December 24, 2002 (1800–2400 h); the 2nd test (after 2 wk) on January 7, 2003 (1800–2400 h), and January 8, 2003 (1800–2100 h; the test was stopped at 2100 h because of rain); the 3rd test (after 4 wk) on January 21, 2003 (1800–2400 h; replication could not be carried out because of rain); and the 4th test (after 6 wk) on February 4, 2003 (1800–2400 h), and February 6, 2003 (1800–2400 h). Test strips were preserved in unfolded condition in the room in Mataram city for the next test. Average temperature and relative humidity recorded ranged from 25 to 30°C and 70 to 90% RH, respectively. The rainy season started at the end of October 2002 and lasted throughout the test period. The number of collected mosquitoes and species identified was recorded every test day.

RESULTS

Laboratory test: evaluation of metofluthrin-impregnated paper strip against An. balabacensis*:* Knockdown activity of a metofluthrin-impregnated paper strip against female adult *An. balabacensis* is shown in Table 1. All insects were knocked down within 30 min after treatment of the strip, and mortality at 24 h was 100% irrespective of the height and position of cages. Knockdown seemed to be faster in the cages at a distance of 2.4 m (in the

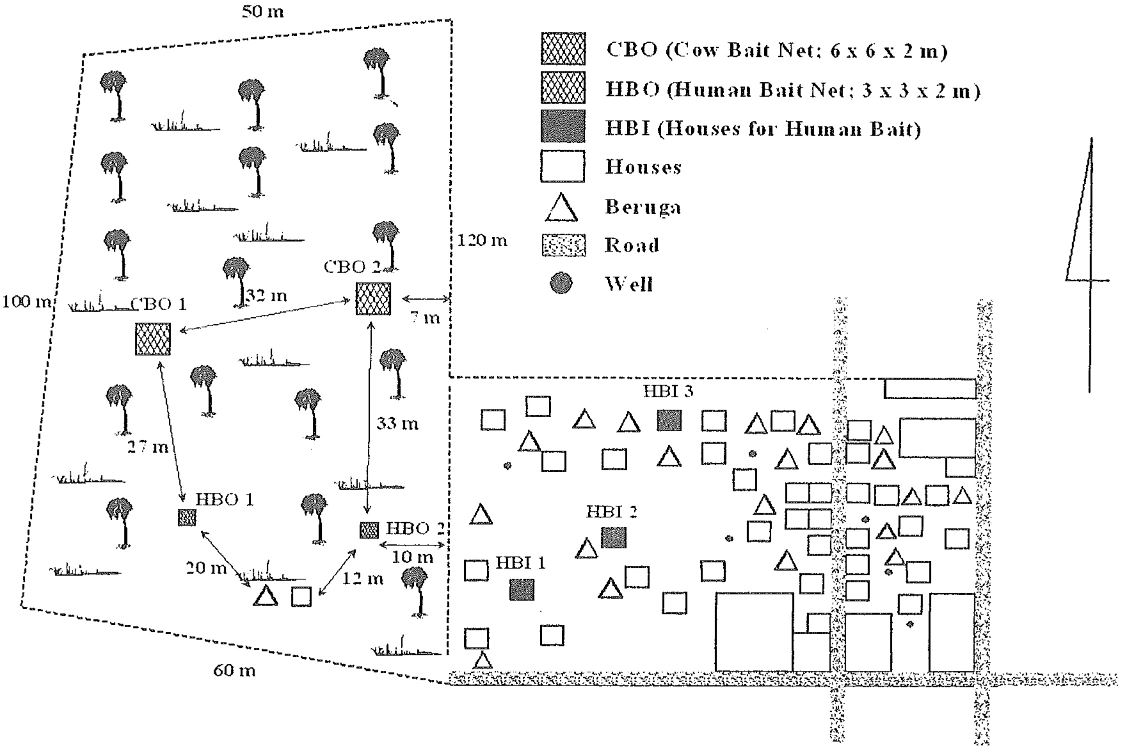

Fig. 2. Outline map of the test sites at Kerandangan. All houses are 1-story, made of wood and mud bricks, and with >2 rooms. A beruga is an arbor hut made of palm leaves; it has no walls or sometimes has simple screens in the back and on both sides.

corner of the room) than those in the cages 1 m from the strip.

Field test: population density and species composition of mosquitoes during the test period: Species composition at each untreated test site from November 12, 2002, to February 11, 2003, are shown in Table 2. Species compositions were almost the same in HBI and HBO; >80% of the mosquitoes collected by human bait were *Culex* spp., among which, *Cx. quinquefasciatus* was dominant (>90%), and the 3-species complex of the Pyretophorus series (i.e., *Anopheles vagus* Doenitz, *Anopheles indefinitus* (Ludlow), and *Anopheles subpictus* Grassi) ranked next after *Culex* spp. The proportions of *An. balabacensis* and *Anopheles sundaicus* (Rodenwaldt), which are thought to be the main malaria vectors, were low (<1%) in human-baited collection during the test period. The difference in mosquito density at the different collection sites was significant by the Friedman Test ($\chi^2 = 6.42$, 1 df, $P < 0.011$). Mosquito density was >6 times higher in HBO than in HBI, which indicates the exophagy of mosquitoes. Species composition in the cow-baited collection, on the other hand, was different from those in the human-baited collections; ~70% of the collection comprised the 3-species complex of Pyretophorus series, and the proportion of *Culex* spp. was lower than in the human-baited collections. The proportions of *An. balabacensis* and *An. sundaicus*, however, were low, as in the human-baited collections. Mosquito density was highest in CBO (461 per night), which was ~4 times and 20 times more than in HBO and HBI, respectively. *Anopheles* spp. and *Culex* spp. peaked in December 2002 and then gradually decreased by the end of February 2003.

Spatial repellency of metofluthrin-impregnated paper strips against mosquitoes: The number of *Anopheles* spp. and *Culex* spp. collected in metofluthrin-treated and untreated collection sites and overall changes in the total number of mosquitoes collected per hour in metofluthrin-treated and untreated sites by 3 different collections are shown in Table 3 and Fig. 4, respectively. A significant reduction in mosquito density in metofluthrin-treated sites was maintained in HBI (2-way ANOVA, F = 7.42, 1 df, $P = 0.034$), HBO (F = 7.30, 1 df, $P = 0.036$), and CBO (F = 10.12, 1 df, $P = 0.019$), respectively, although the density in the untreated sites of HBI and HBO were lower than those in CBO. Spatial repellency was prominent in the cow-baited collection, in which higher numbers of mosquitoes were collected than in human-baited collections. Percent reduction of mosquito density in the

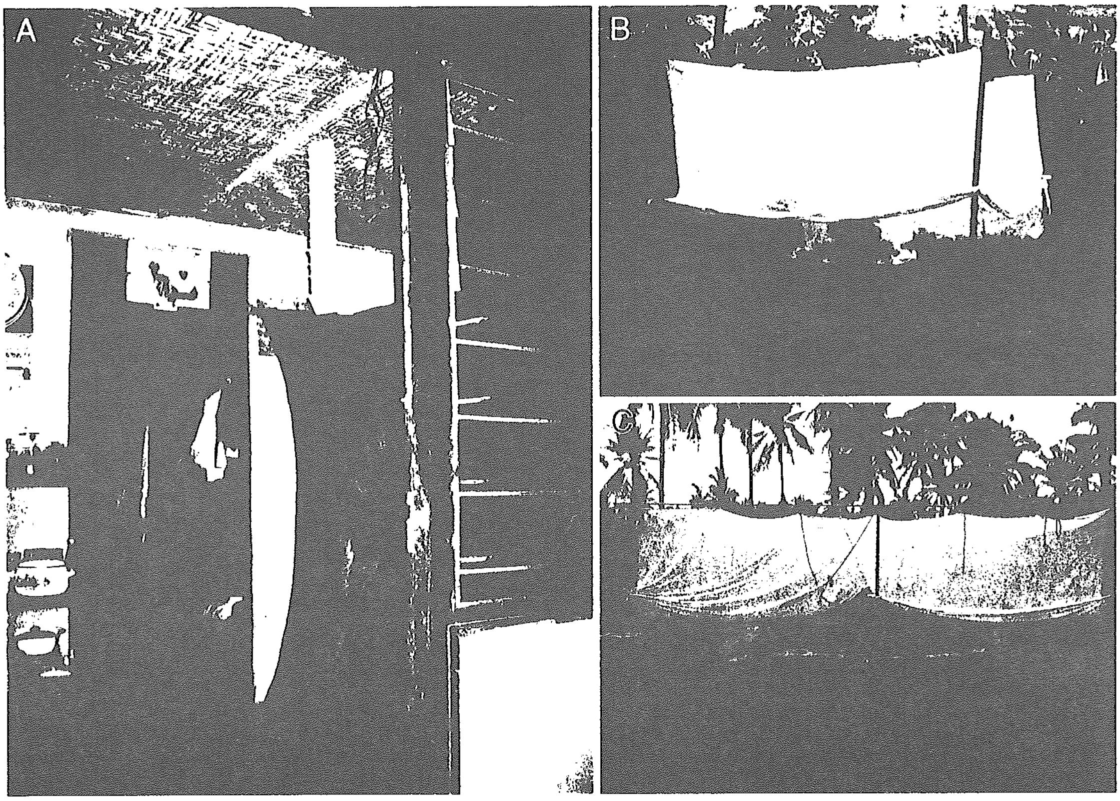

Fig. 3. Field test scene of each test site. (A) Indoor human-baited collection (HBI; a strip was hung 10–30 cm below the ceiling of the house outside the bed net). (B) Outdoor human-baited collection with a double net (HBO; 2 strips were hung in a space between the inside and outside nets). (C) Cow-baited collection with a double net (CBO; 4 strips were hung in a space between the inside and outside nets).

metofluthrin-treated CBO sites versus the untreated controls was 90.7% for *Anopheles* spp. and 92.6% for *Culex* spp. at the initial test. Percent reduction was low for *Anopheles* spp. after 2 wk (58.0%), but it recovered to 90.9% after 4 wk. For *Culex* spp., on the other hand, percent reduction was >90% until 4 wk after treatment. Percent reduction decreased after 6 wk for both groups of mosquitoes. The difference between percent control in metofluthrin-treated sites at 6 wk and at 0, 2, and 4 wk were significant by the chi-squared test on the basis of the number of mosquitoes collected in CBO. For

Table 1. Knockdown activity of metofluthrin-impregnated paper strip against caged female adults of *Anopheles balabacensis* at 0, 10, 30, and 60 min.

Distance from strip (m)	Height (m)	Replicate	No. knocked down				% mortality at 24 h
			0	10	30	60	
1	1.3	1	0	3	5	5	100
		2	0	1	5	5	
		%	0	40	100	100	
	0.3	1	0	1	5	5	100
		2	0	1	5	5	
		%	0	20	100	100	
2.4	1.3	1	0	5	5	5	100
		2	0	3	5	5	
		%	0	80	100	100	
	0.3	1	0	5	5	5	100
		2	0	4	5	5	
		%	0	90	100	100	

Table 2. Species composition of mosquitoes at each collection site from November 12, 2002, to February 11, 2003.

Mosquito species	Species composition (%)[1]		
	HBI	HBO	CBO
An. vagus + *An. indefinitus* + *An. subpictus*	8.9	8.2	69.8
An. tessellatus	0.3	0.1	0.6
An. annularis	0	0.8	0.5
An. barbirostris	0	0.4	0.8
An. maculatus	0	0.1	0.3
An. flavirostris	0.3	0	0.1
An. sundaicus	1.0	0.2	0.3
An. kochi	0	0	0.0[4]
Aedes spp.	1.3	1.4	0.9
Armigeres spp.	0.3	2.5	1.4
Culex spp.[2]	87.8	86.4	25.3
Total[3]	100 (20.2)	100 (123)	100 (461)

[1] HBI, indoor human-baited collection; HBO, outdoor human-baited collection; CBO, cow-baited collection.
[2] >90% was *Culex quinquefasciatus*.
[3] Number in parenthesis is average number of mosquitoes collected per night.
[4] Calculated value was <0.05%.

Anopheles spp., $\chi^2 = 106.3$ for week 0 versus week 6 (1 df, $P < 0.0001$), $\chi^2 = 17.4$ for week 2 versus week 6 (1 df, $P = 0.00003$), and $\chi^2 = 19.6$ for week 4 versus week 6 (1 df, $P = 0.00001$). For *Culex* spp., $\chi^2 = 32.3$ for week 0 versus week 6 (1 df, $P < 0.0001$), $\chi^2 = 38.6$ for week 2 versus week 6 (1 df, $P < 0.0001$), and $\chi^2 = 12.7$ for week 4 versus week 6 (1 df, $P = 0.00037$).

DISCUSSION

Metofluthrin and its impregnated multilayer paper strip showed promising spatial repellency against mosquitoes in the laboratory and in field conditions. Spatial repellency is thought to be caused by high knockdown activity and an intrinsic sublethal effect that also is present in other pyre-

Table 3. Number of mosquitoes collected in the metofluthrin-treated and untreated collection sites at Kerandangan.

Test site	Weeks after treatment	Replicate (day of collection)	No. collected[1]					
			Anopheles spp.			*Culex* spp.		
			Untreated	Treated	% control	Untreated	Treated	% control
Human bait indoor	0	1 (Dec. 22)	1	0	100	5	1	83.3
		2 (Dec. 24)	0	0		7	1	
	2	1 (Jan. 7)	1	0	100	8	0	100
		2 (Jan. 8)[2]	1	0		1	0	
	4	1 (Jan. 21)	0	0	—	3	0	100
		—[3]	—	—		—	—	
	6	1 (Feb. 4)	0	0	—	0	0	100
		2 (Feb. 6)	0	0		7	0	
Human bait outdoor	0	1	0	0	—	39	4	86.0
		2	0	1		11	3	
	2	1	0	0	100	10	0	100
		2[2]	1	0		2	0	
	4	1	2	0	100	22	0	100
		—[3]	—	—		—	—	
	6	1	2	1	33.3	20	14	24.1
		2	1	1		9	8	
Cow bait	0	1	194	22	90.7 a	83	6	92.6 a
		2	96	5		52	4	
	2	1	56	15	58.0 b	84	2	98.1 a
		2[2]	25	19		20	0	
	4	1	22	2	90.9 a	37	2	94.6 a
		—[3]	—	—		—	—	
	6	1	9	9	−53.6 c	48	20	48.3 b
		2	19	34		41	26	

[1] % control = 100 × (No. untreated − No. treated)/No. untreated. Values in the same column followed by the same letter are not significantly different (χ^2 test, $P > 0.05$).
[2] Collection was terminated at 2100 h because of rain.
[3] Collection was not carried out because of rain.

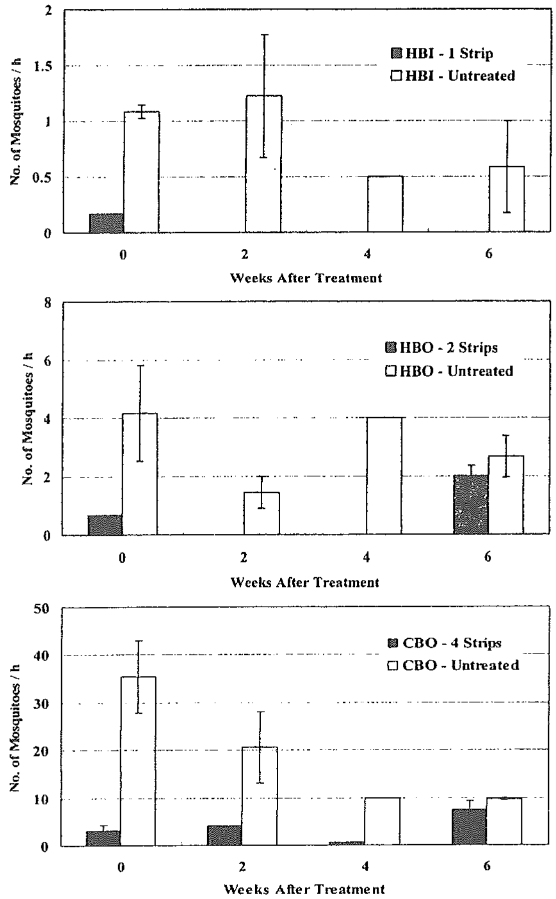

Fig. 4. Changes in total number of mosquitoes collected per hour at each test site. HBI, indoor human-baited collection; HBO, outdoor human-baited collection; CBO, cow-baited collection. Each line on the bar indicates the standard deviation.

throids. The laboratory test showed that mosquitoes were affected by airborne metofluthrin vapor and not by direct contact with the strip. The long-lasting effect of more than 4 wk in open field conditions is outstanding, even though the double net could have interfered with natural air circulation under our test conditions. Spatial repellency seemed to decrease with the loss of effective metofluthrin by evaporation within 6 wk of treatment. According to net area and number of strips treated in this study, the effective area affected by a metofluthrin-impregnated strip was estimated to be >5.5 m^2 for HBI, >4.5 m^2 for HBO, and >9 m^2 for CBO. A higher amount of active ingredient, improvement of slow-release devices, and additional protection for the active ingredient against degradation by external factors, such as oxidation and light irradiation, are required for the practical and successful use of metofluthrin in the field.

Our preliminary investigation on the ecology and biting habit of mosquitoes in Meninting County, Lombok Island, including the test area of this study, has shown that mosquitoes are exophagous and their biting seems to peak in the early night (Maekawa et al., unpublished data), and it is likely that malaria transmission occurs in the early night when most people are active outside. The people of Lombok Island, like other people in tropical areas, have arbor huts made of palm leaves, called "berugas," which have no walls or have only simple screens. People use berugas to nap, pray, and converse in the evening with neighbors. Control or prevention of mosquitoes in berugas, therefore, seems to be ideal for malaria control. Use of traditional and inexpensive practices, such as mosquito coils and mats, insecticide-impregnated or untreated bed nets, and curtains have mainly been focused on the prevention of mosquito bites inside houses (Yap et al. 1990, Aikins et al. 1994, Hewitt et al. 1996). There are very few reports of the efficacy of antimosquito products in the outdoors. Jensen et al. (2000) reported that only mosquito coils and *N,N*-diethyl-3-methylbenzamide (deet) products significantly reduced mosquito landing rates relative to untreated controls in the field among the several commercial antimosquito products, including mosquito attractant, mosquito coils, ultrasonic repeller, citronella candles, and mosquito plant. Pates et al. (2002) reported unique attempts with a kerosene oil lamp to vaporize transfluthrin, a new volatile pyrethroid. They modified the lamp by mixing transfluthrin with vegetable oil and heating it to 120°C in a tin can held just above the flame to avoid decomposition of the insecticide by heat. More than 90% protection was achieved with a higher concentration of transfluthrin (0.5%) in the vegetable oil, whereas 0.1% transfluthrin gave <75% reduction in typical houses of Dar es Salaam, Tanzania. Their device will offer a cost-effective alternative to mosquito coils and mats because kerosene lamps are cheap and used widely in tropical areas. Both kerosene lamps and mosquito coils could be applicable for outdoor use. The above 2 devices, however, need heat by burning of solvents or coil materials for evaporation and diffusion of active ingredients, which have the potential danger of fire, trouble in handling, and occasional side effects for human health by smoke.

The multilayer paper strip used in this study is thought to be an ultimate device that needs no external energy for heating and no other materials for evaporation and diffusion of active ingredients with low cost and high convenience. It is noteworthy that the metofluthrin-impregnated paper strips showed significant spatial repellency against mosquitoes in open field conditions for a month at 200 mg concentration. Further evaluation in more practical conditions, such as berugas, and further studies to enhance the duration of activity will promote development of the devices for the suppression of mosquito-borne diseases.

ACKNOWLEDGMENTS

We express our deep gratitude to Komang Tusta, Suharman Wardanang, Made Sutawa, Mettry Ishak, and Kozue Shimabukuro for their assistance in this study.

REFERENCES CITED

Aikins MK, Pickering H, Greenwood BM. 1994. Attitudes to malaria, traditional practices and bednets (mosquito nets) as vector control measures: a comparative study in five west African countries. *J Trop Med Hyg* 97:81–86.

Hewitt SE, Farhan M, Urhaman H, Muhammad N, Kamal M, Rowland MW. 1996. Self-protection from malaria vectors in Pakistan: an evaluation of popular existing methods and appropriate new techniques in Afghan refugee communities. *Ann Trop Med Parasitol* 90:337–44.

Jensen T, Lampman R, Slamecka MC, Novak RJ. 2000. Field efficacy of commercial antimosquito products in Illinois. *J Am Mosq Control Assoc* 16:148–52.

N'Gessan, R, Darriet F, Doannio JMC, Chandre F, Carnevale P. 2001. Olyset Net® efficacy against pyrethroid-resistant *Anopheles gambiae* and *Culex quinquefasciatus* after 3 years' field use in Côte d'Ivoire. *Med Vet Entomol* 15:97–104.

Pates HV, Line JD, Keto AJ, Miller JE. 2002. Personal protection against mosquitoes in Dar es Salaam, Tanzania, by using a kerosene oil lamp to vaporize transfluthrin. *Med Vet Entomol* 16:277–84.

Shono Y, Kubota S, Sugano M, Yap HH, Tsushima K. 2004. Field evaluation of papers strip and mosquito coil formulation impregnated metofluthrin for mosquito control in Malaysia. In: *The abstract book 70th annual meeting of American Mosquito Control Association 2004* February 21–26; Savannah, GA. Eatontown, NJ: American Mosquito Control Association. p 40, 57.

Sugano M, Shono Y, Ishiwatari T, Tsushima K. 2004. Metofluthrin, new synthetic pyrethroids, an innovative mosquito control agent. In: *The abstract book, 70th annual meeting of American Mosquito Control Association* 2004 February 21–26; Savannah, GA. Eatontown, NJ: American Mosquito Control Association. p 6, 57.

WHO [World Health Organization]. 2000. *Report of the second meeting of the Global Collaboration for Development of Pesticides for Public Health (GCDPP).* 2000 April 6–7; WHO/HQ, Geneva. Document WHO/CDS/WHOPES/GCDPP/2000.4. World Health Organization, Geneva.

Yap HH, Han HT, Yahaya AM, Baba R, Loh PY, Chong NL. 1990. Field efficacy of mosquito coil formulations containing *d*-allethrin and *d*-transallethrin against indoor mosquitos especially *Culex quinquefasciatus* Say. *Southeast Asian J Trop Med Public Health* 21:558–63.