INDIAN JOURNAL OF MALARIOLOGY

Volume 31 **Number 1**

March 1994

MALARIA RESEARCH CENTRE
Indian Council of Medical Research
22-Sham Nath Marg
Delhi-110 054

INDIAN J. MALARIOL.

Quarterly

Year of Revival: 1981

SUBSCRIPTION RATE

Annual	India	Rs. 75.00*
	Other countries (including airmail postage)	$ 20.00

*25% discount would be admissible to individual subscribers on annual basis.

Subscription may be sent by a bank draft or postal order only, payable at Delhi to the Director, Malaria Research Centre, 22-Sham Nath Marg, Delhi-110 054.

The 'Indian Journal of Malariology' is indexed by 'BIOSIS', 'Drugs and Pharmaceuticals Current Indian Titles', 'Index Medicus', 'Indian Science Abstracts', 'Review of Applied Entomology', 'Protozoological Abstracts', 'Quarterly Bibliography of Major Tropical Diseases' and it is selectively abstracted by 'Tropical Diseases Bulletin'. This Journal is also accessible on the CAB Computer Database and MEDLINE.

INDIAN JOURNAL OF MALARIOLOGY

INDIAN JOURNAL OF MALARIOLOGY

CONTENTS

Volume 31
Number 1
March 1994

Laboratory Studies of Pyrethroid-Netting Combinations to Kill Mosquitoes 1

B.R. Jana-Kara, T. Adak, C.F. Curtis and *V.P. Sharma*

The Host Immune Responses to *Plasmodium falciparum*: Part II — T-cell Regulation of Human Immune Responses to Pf155/RESA, a Well-Defined Blood-Stage Antigen of *Plasmodium falciparum*: A Review 12

Lalitha Kabilan

Phytotoxicological Evaluation of *Tagetes erectes* on Aquatic Stages of *Anopheles stephensi* 21

Manik Sharma and *R.C. Saxena*

Response of *P. falciparum* to Chloroquine in Car Nicobar Island 27

A. Giri and *M.K. Das*

Short Notes

Breeding Habitats of Anopheline Mosquitoes in Assam 31

V. Dev

Incrimination of *Anopheles vagus* Donitz, 1902 as an Epidemic Malaria Vector in Bangladesh 35

N.P. Maheswary, S. Majumdar, A.R. Chowdhury, M.S. Faruque and *R.M. Montanari*

In-vitro Chloroquine Resistance of *P. falciparum* in Vellore, Tamil Nadu

E.A. Venkatesan, E. Mathai, A. Sudarsanam, U. Sitaram, Molly Thomas, A. Ganesh, Dilip Mathai and *T. Jacob John*

Note: The editor assumes no responsibility for the statements and opinions expressed by the contributors.

Indian Journal of Malariology
Vol. 31, March 1994, pp. 1-11.

Laboratory Studies of Pyrethroid-Netting Combinations to Kill Mosquitoes

B.R. JANA-KARA, T. ADAK, C.F. CURTIS[a] and V.P. SHARMA

Bioassays of cotton or synthetic netting, impregnated with one of two formulations of deltamethrin or a formulation of lambda-cyhalothrin, showed that the order of merit of these insecticides varied significantly with the type of netting used. Washing reduced the insecticidal power of all combinations of insecticide and netting. Halving the time of exposure and doubling the dose tended to increase the mortality. Different *An. stephensi* strains varied significantly in susceptibility. Netting (5 to 8 mm mesh) impregnated with deltamethrin was effective in killing mosquitoes which penetrated the netting in search of an animal host.

Keywords: *An. stephensi*, Deltamethrin, Impregnated bednets, Lambda-cyhalothrin, Pyrethroid-Netting combinations

INTRODUCTION

For most of the numerous trials of pyrethroid-impregnated bednets, an arbitrary choice has been made of which pyrethroid compound and formulation and which type of cotton or synthetic fibre netting to use. However, to make the method optimally cost-effective, information is required on the relative insecticidal performance of different pyrethroid-netting combinations. This information will have to be assessed in the light of data on costs, availability, preferences of the population concerned and side effects, if any, of the different materials.

Malaria Research Centre, 22-Sham Nath Marg, Delhi-110 054, India.
[a]London School of Hygiene and Tropical Medicine, London, U.K.

It would be hardly feasible to test all possible insecticide-netting combinations in the field. However, bioassays, involving exposure of mosquitoes to impregnated netting for timed intervals and subsequently recording mortality, are a useful preliminary screening method. The exposure should be only for a few minutes[1] and not for one hour conventionally used with less potent insecticides because: (i) one hour is not a realistic representation of the behaviour of free-flying mosquitoes which are irritated by pyrethroids and do not rest on them for long[2]; (ii) one hour's exposure to any deposit of a pyrethroid at a concentration likely to be used on bednets would kill 100%; under these conditions of "overkill" it would not be possible to distinguish an inferior from a superior pyrethroid-netting combination.

Hossain *et al.*[3] tested permethrin on a variety of fabrics and showed that it was more effective on synthetic fibres than on cotton. They also showed that, unlike with older insecticides, there was not an equivalence of time and exposure – halving the time and doubling the dose increased the mortality. *Anopheles gambiae* was found to be more susceptible than *Culex quinquefasciatus*.

Lindsay *et al.*[4] tested a range of pyrethroids on polyester netting and attempted to quantify the effect of washing the netting. The alpha-cyano pyrethroid lambda-cyhalothrin gave 100% mortality of *An. gambiae* after 3 min exposure to 25 mg/m^2 even after four washes. Deltamethrin is another alpha-cyano compound of high insecticidal power, and an emulsifiable concentrate was used to apply the same dose as for lambda-cyhalothrin (unlike all the other pyrethroids where a dose of at least 100 mg/m^2 was considered necessary). Deltamethrin deposits caused 100% mortality when unwashed, but mortality declined after washing.

Most projects have used emulsifiable concentrates for net impregnation but in Guangdong Province, China, wettable powder was used[5].

Some people in hot humid climates find that conventional bednets cause an intolerable restriction in ventilation. Therefore, Kurihara *et al.*[6] and Hossain and Curtis[7] studied impregnated broad-mesh netting which may be more comfortable for the user but still effective in preventing mosquito biting.

The possible evolution of pyrethroid resistance by *Anopheles* vector populations is of great concern. Laboratory selection has produced resistance in several strains of the urban malaria vector *An. stephensi*[8-10]. Enhanced natural tolerance of unselected strains may be indicative of a capacity to respond to selection after the introduction of widespread use of impregnated nets. This paper describes bioassays of two formulations of deltamethrin and one of lambda-cyhalothrin. The effects of varying time, dose, formulation,

washing and mosquito strain are illustrated and submitted to analysis of variance. The effectiveness of deltamethrin-impregnated broad-mesh netting is shown.

MATERIALS AND METHODS

Mosquito strains

Anopheles stephensi: (i) Delhi, collected in Okhla area in 1987 and colonised at the Malaria Research Centre by conventional methods until the tests were carried out in 1989; (ii) Assam, collected in Sonapur in 1988 and colonised as for the Delhi strain; and (iii) Madras, colonised in 1987 as for the Delhi strain.

Culex quinquefasciatus: Collected in Delhi in 1987 and colonised as for the *An. stephensi* strains.

Netting

Cotton: mesh 1.5 mm, thread diameter 0.1 mm.

Nylon: mesh 2 mm, thread diameter 0.2 mm.

Polyethylene: mesh 1.8 mm (irregular and loosely woven), thread diameter 0.1 mm.

Green, wide mesh nylon: mesh 5x3 mm, thread diameter 0.5 mm.

White fishing net: mesh 8 mm, thread diameter 0.8 mm.

Insecticides

Deltamethrin: (K-othrine from Roussel Uclaf) 2.5% flowable formulation and 2.5% wettable powder (WP).

Lambda-cyhalothrin: (Icon from ICI) 5% emulsifiable concentrate (EC).

Aqueous mixtures of each of the concentrates was made up at concentrations designed to give the desired dosages on the netting samples, taking into account the amount of liquid retained by the fabric concerned after dipping and hand wringing.

Bioassays

Bioassays of unwashed nets: Mosquitoes of the Delhi strains of *An. stephensi* and *Cx. quinquefasciatus* were tested with cotton, nylon and polyethylene netting impregnated with six doses ranging from 5 to 25 mg/m^2 with aqueous mixtures made from flowable or WP formulations of deltamethrin or EC of lambda-cyhalothrin. *An. stephensi* was exposed for 30 s, 1 min and 3 min. With *Cx. quinquefasciatus*, exposures of 5 and 10 min were also tested. Each combination of formulation, netting, dose and time was replicated three times.

Batches of 20-25 blood-fed, 3 to 5-day-old female mosquitoes were used for bioassays. Exposures were carried out in WHO susceptibility test kits lined with impregnated or unimpregnated control netting pieces stiffened by tap-

ing them to filter paper. Exposures were at 25°C. Knockdown was observed 1 h after the end of exposure and mortality was scored after 24 h. For some tests nets were washed with laundry soap under cold running water with hand rubbing for 2 min. Each test was replicated three times with concurrent controls. Where control mortality exceeded 20% the day's data were rejected and the test was repeated.

The broad-mesh, fishing net was tested in a double cage with the netting placed as a barrier between the two cages. The mosquitoes were introduced into cage A and a rabbit was placed as a bait in cage B. After 1 h the numbers of fed and unfed mosquitoes were recorded and all were removed to a holding cage. Mortality was recorded 24 h later.

RESULTS AND DISCUSSION

The results in terms of per cent knockdown at 1 h and per cent mortality at 24 h showed the same general pattern and in this paper only the 24 h mortality data are presented. The mortality data were initially subjected to log dose/probit mortality analysis, but it was found that there was a highly significant heterogeneity about the fitted regression lines, so that the confidence limits of LD50 values could not be reliably determined. Instead, the per cent mortalities were arcsine transformed, which improved their fit to normal distributions. The transformed data for each mosquito genus were submitted to factorial analysis of variance by dose, time of exposure, insecticide and type of netting with the results shown in Table 1 for *An. stephensi* and in Table 2 for *Cx. quinquefasciatus*. All the main effects were highly significant. However, the duration of exposure did not have a strong influence on mortality, e.g. for *An. stephensi* increasing exposure from 30 s to 3 min increased the overall mean mortality only from 37.3 to 58.0%. Table 3 shows pairs of instances where exposure time was halved and dose was doubled. In almost all cases this increased mortality, i.e. equivalence of dose and time do not apply in contrast to older, less potent insecticides[11]. This conclusion agrees with that of Hossain and Curtis[7] for permethrin. The fact that short exposures to pyrethroids are disproportionately effective is favourable to their efficiency on bednets which mosquitoes contact only fleetingly.

Tables 1 and 2 show highly significant netting insecticide interactions and Fig. 1(a) and (b) illustrate the fact that the order of merit of the insecticide formulations varies with the type of netting, e.g. deltamethrin WP is better than flowable on cotton but worse on synthetic fibres. In some parts of India, cotton nets are more widely available than synthetic ones and for a field trial of deltamethrin-impregnated nets in Assam deltamethrin WP on cotton was successfully used[12].

Effect of washing

The bioassays were repeated with *An. stephensi* on new netting samples

Table 1. Analysis of variance of % mortality of *An. stephensi* exposed to a range of doses, exposure times, netting materials and insecticide formulations

Source of variation	DF	SS	MS	F value
Main effects				
Dose	5	1.1861	0.2372	10.36*+
Time	2	1.2042	0.6021	26.29*+
Netting	2	1.4122	0.7060	30.83*+
Insecticide	2	1.2996	0.6498	28.37*+
Interactions				
Dose x Time	10	0.1863	0.0186	n.s.
Dose x Netting	10	0.3379	0.0338	n.s.
Time x Netting	4	0.0491	0.0123	n.s.
Time x Insecticide	4	0.2610	0.0653	2.85 n.s.
Dose x Insecticide	10	0.4493	0.0449	1.96 n.s.
Netting x Insecticide	4	2.0220	0.5055	22.07*+
Dose x Time x Netting	20	0.3200	0.0160	n.s.
Dose x Time x Insecticide	20	0.5947	0.0297	n.s.
Dose x Netting x Insecticide	20	0.4979	0.0249	n.s.
Time x Netting x Insecticide	8	0.1793	0.0224	n.s.
Residual	40	0.8553	0.0214	
Residual incorporating non-significant interactions	132	3.0205	0.0229	
Total	293	13.8754		

DF – Degrees of freedom; SS – Sum of squares; MS – Mean square; *$p<0.001$; +$p < 0.01$; n.s. – Nonsignificant; L.S.D. for netting/insecticide combination = 0.095; F based on residual incorporating nonsignificant interactions.

which were initially unwashed, as before, and, subsequently, washed. Analysis of variance (not shown) confirmed the above conclusions and also showed a highly significant effect of washing, which is illustrated in Fig. 2. It is notable that the ranking of the different combinations of netting and insecticide remains almost the same after washing as before. It might have been expected that WP would be more readily rubbed off netting during washing than flowable formulation, but this appears not to be the case. Nevertheless, bioassays of nets impregnated with WP and in domestic use showed a steep decline in insecticidal power over a nine-month period[12].

Comparison of different *An. stephensi* strains

Table 4 shows analysis of variance of data from deltamethrin flowable tested on the three different types of netting against three different Indian strains of *An. stephensi*. These strains differed highly significantly, with the Assam and Madras strains being much more tolerant than the Delhi strain (Fig. 3). So far, the introduction of impregnated nets in Assam has been in rural areas where *An. minimus* is the malaria vec-

Table 2. Analysis of variance of % mortality of *Cx. quinquefasciatus* exposed to a range of doses, exposure times, netting materials and insecticide formulations

Source of variation	DF	SS	MS	F value
Main effects				
Dose	5	2.6636	0.5327	204.9*+
Time	4	1.9610	0.4902	188.5*+
Netting	2	0.3347	0.1673	64.35*+
Insecticide	2	0.9669	0.4834	185.9*+
Interactions				
Dose x Time	20	0.0484	0.0024	n.s.
Dose x Netting	10	0.0164	0.0016	n.s.
Time x Netting	8	0.1029	0.0129	4.96*+
Dose x Insecticide	10	0.0805	0.0080	3.07*+
Time x Insecticide	8	0.3823	0.0478	18.38*+
Netting x Insecticide	4	1.2455	0.3113	119.7*+
Dose x Time x Netting	40	0.1407	0.0035	n.s.
Dose x Time x Insecticide	40	0.0798	0.0019	n.s.
Dose x Netting x Insecticide	20	0.1052	0.0053	2.04+
Time x Netting x Insecticide	16	0.3614	0.0226	8.69*+
Residual	80	0.2000	0.0025	
Residual incorporating non-significant interactions	190	0.4853	0.0026	
Total	459	9.1746		

DF — Degrees of freedom; SS — Sum of squares; MS — Mean square; *$p<0.001$; + $p<0.01$; n.s. — Nonsignificant; L.S.D. for netting/insecticide combination = 0.025; F based on residual incorporating nonsignificant interactions.

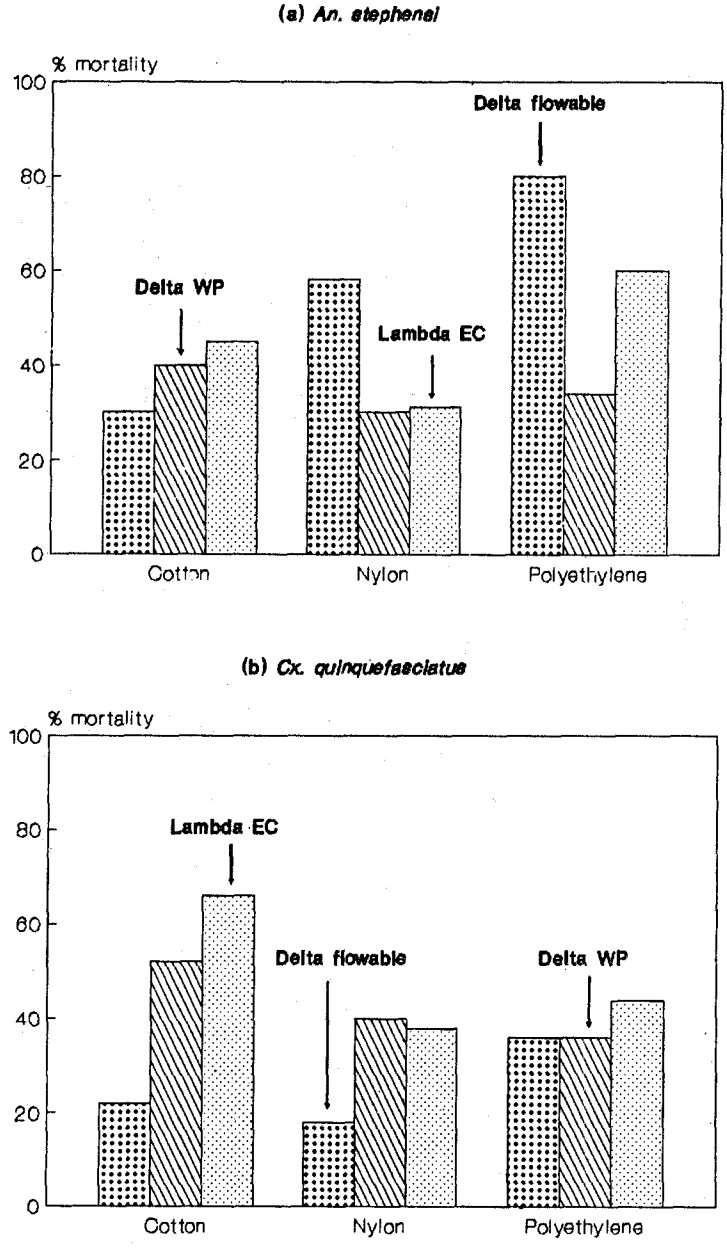


Fig. 1: Mean mortalities of (a) *An. stephensi* (30 s to 3 min exposures) and (b) *Cx. quinquefasciatus* (30 s to 10 min exposures) on different combinations of netting and insecticide formulation. The means are obtained by back-transforming the means of the arcsine values used in the analysis of variance

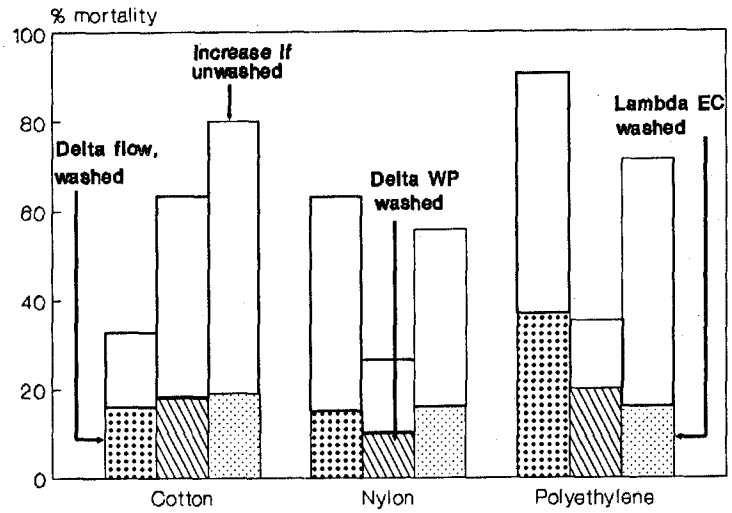


Fig. 2: Mean mortalities of *An. stephensi* on different combinations of insecticide and netting with or without washing; the white section of each column indicates the increase in mortality with unwashed, as compared with washed, nets of the same insecticide/netting category

Table 3. Time–dose–response relationship of *An. stephensi* and *Cx. quinquefasciatus* exposed to insecticide-impregnated netting materials

Exposure time	Dose (mg/m^2)	% mortality of *An. stephensi*	% mortality of *Cx. quinquefasciatus*
1 min	5	38.56	18.14
30 s	10	38.56 (0)	15.88 (-)
1 min	7.5	42.48	22.15
30 s	15	45.46 (+)	30.95 (+)
1 min	12.5	31.88	32.82
30 s	25	46.46 (+)	36.62 (+)
10 min	5	-	29.12
5 min	10	-	30.95 (+)
10 min	7.5	-	34.71
5 min	15	-	44.47 (+)
10 min	12.5	-	49.46
5 min	25	-	53.45 (+)

(+) indicates greater effect of the shorter exposure time with the higher dosage; (-) indicates lesser effect of the shorter time exposure with the higher dosage; (0) indicates no change on doubling the dose and halving the exposure time.

Table 4. Analysis of variance of % mortality of *An. stephensi* strains exposed to flowable deltamethrin-impregnated netting at 25 mg/m^2

Source of variation	DF	SS	MS	F value
Main effects				
Time	2	0.0442	0.0221	4.25 n.s.
Netting	2	0.7439	0.3719	71.52*+
Strain	2	0.6904	0.3452	66.38*+
Interactions				
Time x Netting	4	0.0354	0.0089	1.71 n.s.
Time x Strain	4	0.0463	0.0116	2.23 n.s.
Netting x Strain	4	0.4022	0.1005	19.23*+
Residual	8	0.0420	0.0052	
Total	26	2.0044		

DF — Degrees of freedom; SS — Sum of squares; MS — Mean square; *$p<0.001$; +$p<0.01$; n.s. — Nonsignificant.

tor and the urban vector *An. stephensi* has not been recorded[12].

Broad-mesh netting

Table 5 shows the results of cage tests of two types of broad-mesh netting impregnated with deltamethrin. The green 5 mm netting was more effective than the white fishing net in preventing mosquito penetration in search of an animal bait. However, with both types of impregnated net, very few mosquitoes fed or survived.

CONCLUSIONS

(1) The bioassays showed that the order of merit of different insecticide formulations may vary with the type of netting. This fact should be taken into account, especially where the choice of materials for field trials or operations is not constrained by local availability.

(2) Washing of impregnated nets reduces their insecticidal power and, as far as possible, communities should coordinate their washing of bednets at a time just before re-impregnation is due.

(3) Strains of *An. stephensi* vary in susceptibility to killing by impregnated nets. It seems possible that greater tolerance may be indicative of a higher potential to evolve resistance to a level which would seriously impede the effectiveness of impregnated bednets.

(4) Deltamethrin-impregnated broad-mesh netting is effective in pre-

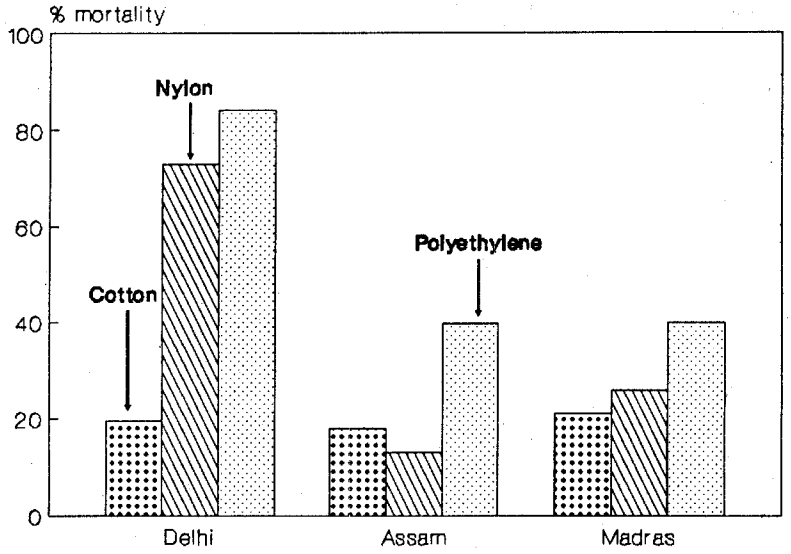


Fig. 3: Mortality of three different Indian strains of *An. stephensi* on flowable deltamethrin applied to three types of netting

Table 5. Results of *Anopheles stephensi* released in a cage containing a rabbit behind a wide mesh netting impregnated with deltamethrin flowable formulation, at 25 mg/m^2

Thread dia. and colour	Treated/ Untreated	% mosquitoes caught in cage B(*)	% excluded from cage B(*)	% fed	% reduction in feeding (*)	% survival	% mortality
0.5 mm green	Treated	18.9	67.6	0.8	98.3	1.6	98.2
	Untreated	58.3	–	47	–	88	–
0.8 mm white	Treated	39.7	17.5	1.6	96.5	3.5	96.4
	Untreated	48.1	–	46.2	–	97.5	–

(*) corrected for control : $100 - \left(\frac{\text{\% success in treated} \times 100}{\text{\% success in control}}\right)$

Note: Results derived from pooling the data of three replicates with a total of 500 to 630 mosquitoes.

venting feeding and in killing mosquitoes even though the mesh size is much too large to act as a physical barrier. Presumably the mosquitoes pause on the netting fibres before flying through and thus pick up a lethal dose.

ACKNOWLEDGEMENTS

We wish to thank the staff of the Malaria Research Centre laboratories at 2-Nanak Enclave, especially Bhopal, Uttam and Satya, for providing an endless supply of mosquitoes. The work was carried out during the tenure of an Indian Council of Medical Research postgraduate scholarship to B.R. J-K.

REFERENCES

1. WHO (1989). The use of impregnated bednets and other materials for vector-borne disease control. A report of the WHO/VBC informal consultation, Geneva WHO/VBC/89.981.
2. Miller, J.E. and G. Gibson (1994). Behavioural response of host-seeking mosquitoes to insecticide-impregnated bed netting: a new approach to insecticide bioassays. *J. Med. Entomol.*, **31** (in press).
3. Hossain, M.I., C.F. Curtis and J.P. Heekin (1989). Assays and bioassays of permethrin-impregnated fabrics. *Bull. Entomol. Res.*, **79:** 299-308.
4. Lindsay, S.W., M.I. Hossain, S. Bennett and C.F. Curtis (1991). Preliminary studies on the insecticidal activity and wash-fastness of twelve pyrethroids impregnated into bednetting assayed against mosquitoes. *Pesticide Sci.*, **32:** 397-411.
5. Li Zuzi, M. Zhang, Wu Yuguang, B. Zhong, G. Lin and H. Hui (1989). Trial of delatamethrin-impregnated bednets for the control of malaria transmitted by *An. sinensis* and *An. anthropophagus*. *Amer. J. Trop. Med. Hyg.*, **40:** 356-359.
6. Kurihara, T., K. Fujita and T. Suzuki (1985). Insecticide treatment of wide-mesh curtains for vector control and the effect upon behavioural responses of adult mosquitoes. *Japanese J. Sanit. Zool.*, **36:** 25-31.
7. Hossain, M.I. and C.F. Curtis (1989). Behavioural and killing effects of permethrin-impregnated bednets – a laboratory evaluation. *Med. Veter. Entomol.*, **3:** 367-376.
8. Omer, S.M., G.P. Georghiou and S.N. Irving (1980). DDT/pyrethroid resistance inter-relationships in *Anopheles stephensi*. *Mosq. News*, **40:** 200-209.
9. Curtis, C.F., N. Hill and S.H. Kasim (1993). Are there effective resistance management strategies for vectors of human disease? *Biol. J. Linn. Soc.*, **48:** 3-18.
10. Chakravorthy, B.C. and M. Kalyanasundaraman (1992). Selection of permethrin resistance in the malaria vector *Anopheles stephensi*. *Indian J. Malariol.*, **29:** 161-165.
11. Busvine, J.R. (1958). Experiments concerned with the development of the World Health Organization test for resistance in adult mosquitoes. *Indian J. Malariol.*, **12:** 279-286.
12. Jana-Kara, B.R., Wajiullah, B.R. Shahi, C.F. Curtis and V.P. Sharma (1994). Deltamethrin-impregnated bednets against *Anopheles minimus* transmitted malaria in Assam, India. *J. Trop. Med. Hyg.* (in press).

Indian Journal of Malariology
Vol. 31, March 1994, pp. 12-20.

The Host Immune Responses to *Plasmodium falciparum*: Part II — T-cell Regulation of Human Immune Responses to Pf155/RESA, A Well-Defined Blood-Stage Antigen of *Plasmodium falciparum*: A Review

LALITHA KABILAN

This review gives a brief summary of the T-cell epitopes in Pf155/RESA and how these T-cell epitopes on activation *in vitro* regulate Pf155/RESA-induced immune responses. It also discusses the genetic control of immune responses against Pf155/RESA.

Keywords: Immune response, Pf155/RESA, *Plasmodium falciparum*, T-cell

INTRODUCTION

Malaria control has had a high priority for the past 50 years in malaria-endemic areas and successful results were obtained for a period of time. However, malaria reappeared in most places for several reasons. At present, a vaccine against malaria is considered as a powerful tool in malaria control[1].

Acquired immunity by natural infection is a prerequisite for survival in endemic areas. The involvement of various effect or mechanisms in induction of protective immunity against malaria is complex and not yet clearly known. Although antimalarial immunity is both antibody- and cell-mediated[2], most antibody responses are T-cell-dependent. Since priming of T-cell system is essential for the development and

Malaria Research Centre (ICMR), 2, Nanak Enclave, Delhi-110 009, India.

maintenance of this immunity[3], the efficacy of an antigen as a malaria vaccine depends to a great extent on the T-cell recognition sites and the nature of the responses induced by these determinants[4]. Failure of vaccination trials in humans with circumsporozoite protein of *Plasmodium falciparum*[5] emphasizes the importance of T-cell-reactive sites in vaccine candidate antigens. An understanding of epitope-specific T-cell responses to an immunogen in populations naturally exposed to malaria is essential to select suitable epitopes from the immunogen to be included in a subunit vaccine.

The *P. falciparum* antigen Pf155/RESA (ring-infected erythrocyte surface antigen), which is deposited in the erythrocyte membrane at parasite invasion[6], is generally considered to be a subunit vaccine against blood stage[7]. The molecular structure of this antigen showed two extensive blocks of tandemly repeated amino acid sequences[8]. The 3' repeat region is shown to be conserved in different *Pf* isolates[9] and consists of an eight amino acid (EENVEHDA x 5) sequence followed by more than 30 times tandem repeat of four amino acid (EENV in one letter code) sequence. The 5' repeat block consists of DDEHVEEPTVA repeated twice and with minor variations another five times.

Studies have revealed the presence B-cell epitopes[7,10] in the immunodominant repeat domains. This review gives a brief summary of the T-cell epitopes in Pf155/RESA and how these T-cell epitopes on activation *in vitro* regulate Pf155/RESA-induced immune responses. It also discusses the genetic control of immune responses against Pf155/RESA.

T-Cell epitope mapping in Pf155/RESA

In order to define immunodominant determinants in Pf155/RESA recognized by T-cells which are suitable for inclusion in a subunit malaria vaccine, Pf155/RESA was mapped for T-cell-reactive sites. Crude *Pf* schizont preparation, semi-purified, enriched Pf155/RESA and synthetic peptides (15 to 30 amino acids long) representing different sequences from repeat and non-repeat regions of 3' and 5' terminal regions were used as stimulants to activate *in vitro* of T-cells from *Pf* primed donors. Peptides used for proliferation assay were selected on the basis of high alpha amphipathic score which is predicted to have T-cell sites[11]. *In vitro* T-cell proliferative responses were measured by thymidine incorporation and supernatants from T-cell cultures were tested for IFN-gamma release by ELISA[12,13]. Antibody reactivities in the plasmas collected from primed donors to the corresponding peptides used for T-cell activation were estimated by ELISA[7] (enzyme-linked immunosorbent assay). Donors with no previous history of exposure to malaria were used as controls.

There was considerable variation of the responses to the peptides among the primed donors. None of the peptides elicited a positive response in all the donors. A majority of the donors responded to Pf155/RESA, whereas only 40% of the donors elicited a positive response to peptides suggesting that the protein may contain T-cell epitopes not covered by the peptide[12]. In general, donors who responded to oligopeptide also responded to native Pf155/RESA. A few exceptions responded to only peptides and not to the native protein. Whether these responses are due to cross-reactions to other *Pf* antigens needs to be investigated. However, cross-reactions at cellular level between peptides have been reported[14]. Whether non-responsiveness to the malarial antigen seen among some primed donors in these experiments is due to genetic control, or to the reflection of the immune status of individuals, is yet to be proved.

More donors responded to epitopes in the repeat region than to the non-repeating sequences[10]. Two peptides, $(EENVEHDAEENVEENV)_2$ and (YDEENVEEHDEEYDE), from the 3' repeat region were the best stimulators. Proliferative experiments with peptides containing either EENVEHDA or EENV or a mixture of these two revealed that the mixture had additive effects suggesting the presence of two or more distinct or cross-reacting epitopes in the EENVEHDAEENVEENV sequence. None of unprimed donors responded to any of the peptides[10,12].

IFN-gamma responses

Cytokines considered to be of interest in malaria are the interferons. Antigen-induced IFN-gamma release may be a reflection of cell-mediated antibody-independent immunity. IFN-gamma has been shown to be involved in the protective immune responses to malaria[15] and therefore measurement of IFN-gamma secretion *in vitro* may be a useful and sensitive indicator of cellular immunity in *P. falciparum* malaria. Both the intact Pf155/RESA and synthetic peptides induced T-cells from primed donors to release IFN-gamma, whereas no IFN-gamma was produced by T-cells from control donors with no previous malaria exposure[10,12,13]. Such a response may indicate the existence of specific cellular mechanisms involved in protective immunity to malaria. However, only 30 to 40% of the donors elicited a positive response in these assays (ELISA).

To estimate the number of antigen-specific responding T-cells, ELISPOT (enzyme-linked immunospot assay)[13], an assay in which the actual number of responding cells with specific function can be enumerated, has been used. Oligopeptides from the 3' repeat region induced T-cells from primed donors to secrete IFN-gamma which was detected by ELISPOT. The secreted cytokine was

determined by ELISA. There was a significant correlation between these two assays. However, ELISPOT enables estimation of malaria-specific memory T-cells even when these are present at numbers too low for determination of IFN-gamma secretion in the culture supernatant by ELISA[13].

T-cell regulation of humoral response to Pf155/RESA *in vitro* production of anti-malarial antibody in response to Pf155/RESA is dependent upon T-helper cells. To establish the presence of T-helper epitopes in Pf155/RESA which on activation can help B-cells, parallel cultures of autologous T-cells, B-cells and T/B-cell mixtures (2:1), from *Pf* and *P. vivax* primed donors were activated with crude *Pf* antigen, RBC (red blood cell) control antigen, Pf155/RESA-enriched antigen, and the culture supernatants were harvested and assayed for *in vitro* production of immunoglobulins (Ig) and specific antimalarial antibody in ELISA and in immunofluorescence respectively[16]. Antimalarial activity to total parasite was determined on air-dried unfixed parasite monolayers, whereas anti-Pf155/RESA antibodies were determined by EMIF (erythrocyte membrane immunofluorescence) on 1% GDA (glutaraldehyde)-fixed air-dried monolayers of ring-infected erythrocytes[6].

Very little immunoglobulin and no specific antibody secretion were detected in the supernatants from T-cell cultures, or from B-cell cultures in the absence of T-cells reflecting the T-cell dependency. Anti-*Pf* antibodies were found only in the cultures containing T/B-cell mixtures stimulated with *Pf* antigen[16]. Using T/B-cell cooperation system, synthetic peptides from Pf155/RESA were reported to induce production of parasite-specific antibodies *in vitro*[17].

The induced specific antibodies were primarily of IgG isotype suggesting that the responses elicited here reflect the secondary responses of primed cells *in vivo*. This conclusion was also supported by the finding that secretion of anti-Pf155/RESA was found in Pf155/RESA seropositive donors with elevated antibody titres to Pf155/RESA. The importance of priming *in vivo* for the outcome of experiments *in vitro* is being reported elsewhere in another antigen system[18].

In murine model, T-helper (TH) cells were shown to belong to two subsets. Upon activation, T-helper cells, designated as TH1, produce IL-2 and IFN-gamma and TH2 produce IL-4 and IL-5[19]. To investigate the possible role of TH1- and TH2-like cells in *Pf* primed donors, T-cells were activated with Pf155/RESA-derived peptides and activation was measured by proliferation IFN-gamma release, transcription or translation of IL-4 mRNA. In *Pf* exposed donors, IL-4 secretion or IL-4 mRNA expression by Pf155/RESA peptides-induced T-cells was shown to correlate with anti-peptide antibody

level in the corresponding sera of T-cell donors, indicating that T-cell epitopes in Pf155/RESA can induce TH2-like T-cell subsets[20] which can produce IL-4 and thus help B-cells secrete antibodies.

Correlation analysis of T-cell responses

In individual donor, there was no association between proliferation and IFN-gamma secretion. Both these responses were negatively correlated with the concentration of serum antibodies to the corresponding peptides used for T-cell activation[10,12]. This reinforces the importance of including several parameters of T-cell activation in order to estimate responses induced by different epitopes. Lack of correlation between humoral response and proliferation may suggest that proliferation assays do not measure the activation of T-cells that help B-cells[16]. A significant association between RESA peptide-induced expression of IL-4 and the presence of antibodies to the same peptide suggest a causal relationship between the activation of IL-4 producing T-cell subset and production of anti-Pf155/RESA antibodies in donors who attain clinical immunity following natural infection[20].

Lack of disassociation between Pf155/RESA-induced proliferation and IL-4 and IFN-gamma secretion indicates the presence of multipotential (THO) cells capable of producing different lymphokines or heterogeneous malaria-specific T-cells from both TH1 and TH2. Although human T-helper cells appear to be heterogeneous with regard to surface marker and lymphokine production, the question of TH1- and TH2-like subsets in humans remains controversial.

Immune response to a conjugate of MSA-1 (merozoite surface antigen-1) and Pf155/RESA

Sequences from the 3' repeat region of Pf155/RESA (EENVEHDAEENVEENV) were conjugated with the 36 amino acid long conserved sequences from the N' terminal region of MSA-1 and tested for *in vitro* proliferation and IFN-gamma release. The hybrid peptide was found to induce more frequent responses than the peptide containing the MSA-1 sequence alone suggesting that Pf155/RESA can increase the immunogenicity if conjugated with other immunogens[21] derived from other stages of the parasite as reported elsewhere[22].

Influence of *Pf* transmission seasons over Pf155/RESA-induced immune responses

Investigations of immune responses (T- and B-cell responses) to Pf155/RESA in different geographical regions of the world which differ in malaria transmission were carried out. In The Gambia, where the transmission is seasonal, it has been reported that Pf155/RESA peptide-specific T-cell responses per-

sisted, whereas the antibody responses showed a tendency to decrease during the nontransmission season. In Liberia, where the transmission is perennial and intense, no differences were seen either in T-cell or in antibody (B) responses, suggesting that different requirements are needed for the boosting of T- and B-cells[10]. It is claimed that persistent exposure to malaria is required to maintain antibody responses[10]. However in Madagascar, a decrease in proliferative responses during nontransmission season has been reported[17]. In this area malaria has reappeared after twenty years of disappearance, where the adult population is not considered immune[17].

Are immune responses to Pf155/RESA genetically controlled?

Association between MHC (Major Histocompatibility Complex) class II types and immune responses to Pf155/RESA

Non-responsiveness to an immunogen seen in primed individuals has been attributed to genetic (MHC) restriction. An association between immune response to CSP and different haplotypes has been established in murine model[5]. Attempts are being made to look for association between various haplotypes and immune responses to different malaria immunogens in humans[23]. In a sero-typing study of 10 Gambian donors, an association between DRw13 and a disassociation between DQ3 was shown with lympho-proliferative responses to the TH2R and TH3R epitopes of the *Pf* CSP protein[24]. Analysis of immune responses showed that only 70% of clinically immune West African population had antibodies to Pf155/RESA. Whether the variations in immune responses to Pf155/RESA seen among different *Pf* primed donors reflect genetic restrictions imposed on immune responses by MHC II alleles was investigated. T-cell responses from primed Gambian were divided into high, medium and low, and their haplotypes were determined. No restriction to any particular allele was seen with either high, or low or medium responders. In fact, frequently they carried the same haplotype. Because of high polymorphism of the HLA molecule and the haplotype variation among different population races and ethnic groups, a large number of donors need to be studied to detect any association between a particular immune response and HLA class II types[23].

Studies in twins

Studies of antimalaria immune responses in monozygotic and dizygotic twins revealed that T-cell responses and antibody responses to Pf155/RESA were concordant among monozygotic pairs as compared to the dizygotic pairs. Thus, it appears that immune responses induced by Pf155/RESA in

Pf primed donors are genetically controlled. However, investigation for an association between immune response and class II alleles did not reveal any association between class II haplotypes and immune response from monozygotic twins. Thus, the influence of MHC class II allele diversity on cellular and humoral responses against immunodominant epitopes Pf155/RESA is weak and hard to demonstrate in outbred human populations[25].

CONCLUSIONS

The design of a vaccine will not only require identification of immunodominant antigens but also investigations on the role of the various epitopes on activation, that constitute the candidate antigens. Pf155/RESA, one of the major vaccine candidates against asexual blood-stage parasite of *Plasmodium falciparum*, consists of antibody binding sites (B-cell epitopes). The immunodominant conserved amino acid repeat region of this molecule contains multiple epitopes which can induce *in vitro* T-cells from *Pf* primed donors to proliferate, produce IFN-gamma, and synthesize IL-4. Lack of association seen between different T-cell activities underlines the importance of including several functional parameters of T-cell activation, when estimating the proportion of individuals responding to a given epitope. Although malaria-specific T-cells are activated in a classically restricted manner, it was difficult to demonstrate genetic restriction of immune response against Pf155/RESA in outbred human population. It is apparent that the immune responses are genetically regulated, possibly by genes outside the MHC region. However, further studies are urgently required to understand the involvement of the other non-MHC linked genetic factors in the regulation of Pf155/RESA-induced immune responses.

REFERENCES

1. Perlmann, P. (1987). Malaria vaccines — Are they approaching reality? *Nature*, **328**: 205-207.
2. Troye-Blomberg, M. and P. Perlmann (1988). T-cell functions in *Plasmodium falciparum* and other malarias. *Prog. Allergy*, **41**: 253-287.
3. Cavacini, L.A., L.A. Parke and W.O. Weidanz (1990). Resolution of acute malarial infections by T-cell-dependent non-antibody-mediated mechanisms of immunity. *Infect. Immun.*, **58**: 2946-2950.
4. Good, F.M. and L.H. Miller (1989). Involvement of T-cells in malaria immunity-implications for vaccine development. *Vaccine*, **7**: 3-9.
5. Good, M.F., I.A. Quakyi, A. Saul, J.A. Berzofsky, R. Carter and L.H. Miller (1987). Genetic control of the immune response in mice to a *Plasmodium falciparum* sporozoite vaccine; widespread non-responsiveness to single malaria T-epitope in highly repetitive vaccine. *J. Exp. Med.*, **164**: 655-660.
6. Perlmann, H., K. Berzins, M. Wahlgren, J. Carlsson, A. Björkman, M.E. Patarroyo

and P. Perlmann (1984). Antibodies in malarial sera to parasite antigens in the membrane of erythrocytes infected with early asexual stages of *Plasmodium falciparum*. *J. Exp. Med.*, **159:** 1686-1704.

7. Perlmann, H., K. Berzins, B. Wåhlin, M. Troye-Blomberg, M. Hagstdt, I. Andersson. B. Högh, E. Petersen and A. Björkman (1989). Dissection of the human antibody response to the malaria antigen Pf155/ RESA into epitope-specific compartments. *Immunol. Rev.*, **112:** 115-131.

8. Favaloro, J.M., R.L. Coppel, L.M. Corcoran, S.J. Foote, G.V. Brown, R.F. Anders and D.J. Kemp (1986). Structure of the RESA gene of *Plasmodium falciparum*. *Nucl. Acids Res.*, **14:** 8265-8277.

9. Perlmann, H., K. Berzins. B. Wåhlin, R. Udomsangpetch, W. Ruanghrachuporn, M. Wahlgren and P. Perlmann (1987). Absence of antigenic diversity in Pf155/ RESA, a major parasite antigen in membranes of erythrocytes infected with *Plasmodium falciparum*. *J. Clin. Microbiol.*, **25:** 2347-2354.

10. Troye-Blomberg, M., E.M. Riley, H. Perlmann, G. Andersson, Å. Larsson, R.W. Snow, S.J. Allen, R.A. Houghten, 0. Olerup, B.M. Greenwood and P. Perlmann (1989). T- and B-cell responses of *Plasmodium falciparum* malaria-immune individuals to synthetic peptides corresponding to sequences in different regions of the *P. falciparum* antigen Pf155/RESA. *J. Immunol.*, **143:** 3043-3048.

11. Margalit, H., J.L. Spouge, J.L. Cornette, K.B. Cease, C. DeLisi and J.A. Berzofsky (1987). Prediction of immunodominant helper T-cell antigenic sites from the primary sequences. *J. Immunol.*, **138:** 2213-2229.

12. Kabilan. L., M. Troye-Blomberg, H. Perlmann, G. Andersson, B. Högh, E. Petersen. A. Björkman and P. Perlmann (1988). T-cell epitopes in Pf155/RESA, a major candidate for a *Plasmodium falciparum* malaria vaccine. *Proc. Natl. Acad. Sci. USA*, **85:** 5659-5663.

13. Kabilan, L., M. Troye-Blomberg, G. Andersson, E.M. Riley, H.P. Ekre, H.C. Whittle and P. Perlmann (1990). Number of cells from *Plasmodium falciparum* immune donors that produce gamma-interferon *in vitro* in response to Pf155/ RESA, a malaria vaccine candidate antigen. *Infect. Immun.*, **58**(9): 2989-2994.

14. Rzepczyk, C. M., R. Ramasamy, D.A. Mutch, P. C.-L. Ho, D. Battistutta, K.L. Anderson, D. Parkinson, T.J. Doren and M. Honeyman (1989). Analysis of human T-cell response to two *Plasmodium falciparum* merozoite surface antigens. *Eur. J. Immunol.*, **19:** 1797-1802.

15. Schofield, L., J. Villaquiran, A. Ferreira, H. Schellekens, R. Nussenzweig and V. Nussensweig (1987). Gamma-interferon, CD8+ T-cells and antibodies required for immunity to malaria sporozoites. *Nature*, **330:** 664-666.

16. Kabilan, L., M. Troye-Blomberg, M.E. Patarroyo, A. Björkman and P. Perlmann (1987). Regulation of the immune response in *Plasmodium falciparum* malaria : Pt IV-T-cell dependent production of immunoglobulin and anti-*P. falciparum* antibodies *in vitro*. *Clin. Exp. Immunol.*, **68:** 288-297.

17. Astagneau, P., J.P. Lepers, C. Chougnot, C. Gaudobout, M.D.A. Rason, B. Larouze and P. Deloron (1991). Assessment of the protective value of antibodies to the *Plasmodium falciparum* ring infected erythrocyte surface antigen (RESA) : An epidemiologic study in Madagascar. *American J. Epidemiol.*, **133:** 177-183.

18. Volkman, D.J., S.P. Allyn and A.S. Fauci (1982). Antigen-induced *in vitro* antibody production in humans: tetanus toxoid-specific antibody synthesis. *J. Immunol.*, **129:** 107-118.

19. Langhorne, J., S. Gillard, B. Simon, S. Slade and K. Eichmann (1989). Frequencies of CD4+ T-cells reactive with *Plasmodium chabaudi chabaudi:* distinct response kinetics for cells with Th1 and Th2 characteristics during infection. *Infect. Immun.*, **1:** 416-424.

20. Troye-Blomberg, M., E.M. Riley, L. Kabilan, M. Holmberg, H. Perlmann, U. Andersson, C.H. Heusser and Perlmann (1990). Production of activated human T-cells of interleukin-4 but not IFN-gamma is associated with elevated levels of serum antibodies to activating malaria antigens. *Proc. Natl. Acad. Sci. USA*, **87:** 5484-5488.

21. Kabilan. L., V.P. Sharma, P. Kaur, S.K. Ghosh, R.S. Yadav and V.S. Chauhan (1994). Cellular and humoral immune responses to well-defined blood-stage antigens (major merozoite surface antigens) (MSA-1) of *Plasmodium falciparum* in adults from stable malaria zone of India. *Infect. Immun.*, **62:** 2.

22. Patarroyo, M.E., P. Romero, M.L. Torres, P. Clavijo, A. Moreno, A. Martinez, R. Rodriguez, F. Guzman and E. Cabezas (1987). Induction of protective immunity against experimental infection with malaria using synthetic peptides. *Nature*, **328:** 629-632.

23. Troye-Blomberg, M., O. Olerup, A. Larsson, K. Sjöberg, H. Perlmann, E.M. Riley, J.-P. Lepers and P. Perlmann (1991). Failure to detect MHC class II associations of the human immune response induced by repeated malaria infections to the *Plasmodium falciparum* antigen Pf155/RESA. *Int. Immunol.*, **3**(10): 1210-1219.

24. De Groot, A.S., A.H. Johnson, W.L. Maloy, I.A. Quakyi, E.M. Riley. A. Menon, S.M. Banks, J.A. Bezofsky and M.F. Good (1989). Human T-cell recognition of polymorphic epitopes from malaria circumsporozoite protein. *J. Immunol.*, **142:** 4000-4005.

25. Sjöberg, K. (1991). Ph.D. Thesis. Department of Immunology, Stockholm University, Stockholm, Sweden.

Indian Journal of Malariology
Vol. 31, March 1994, pp. 21-26.

Phytotoxicological Evaluation of *Tagetes erectes* on Aquatic Stages of *Anopheles stephensi*

MANIK SHARMA and R.C. SAXENA

Petroleum ether (5%) in ethyl acetate fraction of *Tagetes erectes* showed toxic activity against second and fourth instar larvae of *Anopheles stephensi* below 100 ppm concentrations. The LC50 values for second and fourth instar larvae were calculated to be 43 and 58 ppm respectively. The second instar larvae were more susceptible than the fourth instar larvae. The extract did not affect the larval developmental period. The growth index of treated mosquitoes was significant in comparison with that of the control and untreated sets ($p<0.001$). Treatment with the extract had a significant effect on the mortality and reduction in the adult emergence of the vector.

Keywords: Adult emergence, *Anopheles stephensi*, Growth regulation, *Tagetes erectes*

INTRODUCTION

Natural products of plant origin have been tried in the recent past for a variety of insect pests and vectors[1-4]. But insecticides of plant origin have been extensively used on agricultural pests and, to a very limited extent, against insect vectors of public health importance. Plant insecticides, therefore, deserve a careful and thorough screening as biocides for vector control[5]. We have studied the phytotoxicological activity of *Tegetes erectes* (Compositae) against a major malaria vector *An. stephensi*.

P.G. Department of Zoology, S.S.L. Jain College, Vidisha-464 001, India.

MATERIALS AND METHODS

The plant *Tagetes erectes* L., a scandent found in the gardens and in pots throughout the country, is known for its repellent property. Its flowers were collected in the full blooming season in the months of November to February from the Indira Complex residential area in Vidisha town of Madhya Pradesh. After proper identification by a botanist a voucher specimen was deposited in the laboratory for record. The plant material was washed thoroughly with tap water and kept for drying in shade for more than a month. The air-dried material was powdered to about 40-60 mesh size and extracted with petroleum ether in a soxhlet apparatus until exhaustion. The crude extract was filtered and evaporated to dryness under reduced pressure at 40°C.

The crude extract was purified by column and thin-layer chromatographic techniques. A known quantity (500 mg) of crude extract was slurried with a small quantity of petroleum ether and was allowed to adsorb on a silica gel column packed in ethyl acetate and was chromatographed over different adsorbents. Six fractions (260 ml) were collected. All fractions were monitored over TLC until a single spot was obtained. These fractions were taken in glass vials and the solvent was allowed to dry, and the dried fractions were weighed and stored in a refrigerator for bioassay experiments.

The bioassay technique

To carry out bioassay experiments, laboratory-cultured strains of *An. stephensi* L. were maintained with a photoperiod of 14 h of light and 10 h of darkness at 27±2°C and 75-85% RH. The larvae were fed with a diet of finely ground brewer's yeast and dog biscuits (3:1). Adults were fed *ad libitum* on 10% sucrose solution as well as water-soaked raisins. Females were allowed to blood-feed from a restrained rabbit three days after emergence.

After preliminary experiments with different concentrations of crude and fractions, the purified fraction, coded as T_4, was found effective and selected for further bioassay experiments. Different concentrations ranging from 10 to 100 ppm were applied against second and fourth instar larvae of the mosquito, following the WHO methodology[6]. Observations were recorded at an interval of 24 h till the adults emerged. Deformities, if any, and larval, pupal and adult developmental periods were recorded. The growth index of the treated mosquitoes was calculated in accordance with Saxena and Sumithra's procedure[7]. Statistical evaluation of data was carried out by probit analysis[8] and the level of significance by Duncan's multiple range test[9].

RESULTS AND DISCUSSION

The purified fraction obtained from column chromatography and TLC us-

Table 1. Toxicity of purified fraction (T_4) of *Tagetes erectes* on second and fourth instar larvae of *An. stephensi*

Treated aquatic stages	Conc.	Larval mortality %	Regression equation ($Y = a+bx$)	Chi-square [χ^2 (n-2)]	LC50 (ppm)	Log LC50 ± SD	95% FL (ppm)
Second instar	20	32	$Y = 0.495 + 3.36x$	0.407	52	1.6329 ± 0.021	LL = 48
	40	48					UL = 63
	60	56					
	80	82					
	100	100					
	Control	4					
	Untreated	0					
Fourth instar	20	28	$Y = 0.544 + 2.473x$	0.067	78	1.8017 ± 0.030	LL = 60
	40	38					UL = 86
	60	46					
	80	68					
	100	100					
	Control	0					
	Untreated	0					

FL — Fiducial limit; LL — Lower limit; UL — Upper limit; $p < 0.05$.

Table 2. Effect of fraction T_4 of *Tagetes erectes* on growth and metamorphosis of *An. stephensi*

Conc. (ppm)	Larval mortality (%)	Av. larval period (days)	Pupal mortality (%)	Av. pupal period (days)	Adult emergence (%) (*a*)	Av. development period (days) (*b*)	Total mortality (%)	Growth index (*a*/*b*)
20	32	10.5	10	1.5	60	12.0	40	5.0
40	48	10.5	14	1.5	42	12.0	58	3.5
60	56	11.0	16	1.5	28	12.5	72	2.24*
80	82	11.0	20	1.5	8	12.5	92	0.64*
100	100	–	–	–	–	–	–	–
Control	4	15.0	0	2.0	96	17.0	4	5.64
Untreated	8	15.5	0	2.5	92	18.0	8	5.25

*Growth index was significantly different in comparison with that of the control and untreated groups ($p < 0.05$); 25 second instar larvae were treated at each concentration in an average of four consistent replicates.

ing 5% petroleum ether in ethyl acetate as solvent (coded as T_4) was found effective with 24 h LC50 of 52 and 83 ppm for second and fourth instar larvae respectively. The second instar larvae were more susceptible than the fourth instars. The statistical data regarding LC50, regression equation, chi-square, log LC50±SD and 95% FL (fiducial limits) are given in Table 1. The results of the treatment with the fractions on second and fourth instar larvae were found significantly different from those of the control and untreated sets ($p<0.05$). Several such reports pertaining to the toxic properties of plant products against mosquitoes and other insect pests are available[1,10-13]. The results with the fractions showed a high concentration, i.e. more than 40 ppm concentration was effective against the aquatic stages of *An. stephensi*. The findings of our study confirm the earlier reports of Saxena and Sumithra[7] who found that second instar larvae of *An. stephensi* were more susceptible than third and fourth instar larvae when the acetone extract of *Ipomea cornea fistulosa* was tested.

Table 2 shows a significant decrease in the average larval, pupal and developmental periods ($p<0.05$) as compared to control and untreated sets. The growth index of the treated mosquitoes was also shorter than that of the control and untreated sets ($p< 0.001$). This resulted in 40-92% mortality (fall in population). This may be due to the involvement of certain growth-regulating hormones in the fraction. Several deformities, such as larval-pupal intermediate and half-ecdysed adults, were also observed during the course of our study which are in concurrence with our earlier report of mosquito larvicidal and growth disrupting activities of *Annona squamosa* extract against *An. stephensi* in which the extract caused a significant difference in average larval, pupal and adult developmental periods of the treated mosquitoes[3]. Other investigators have also reported homologous results[7,14]. Saxena *et al.*[14] have also described that the petroleum ether extract of *Ageratum conyzoides* and four other plants of family Compositae induced manifold defects and decrease in the growth index of the filaria vector *Culex quinquefasciatus*.

The findings of our study therefore confirm that 5% petroleum ether in ethyl acetate fraction (T_4) causes toxic and growth-regulating activities and that it could be sucessfully employed for reducing the vector density in the aquatic ecosystem, particularly in polluted water.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. Pritam Singh, Principal, Jain College, for facilities and to Dr. P. Jaitley for her help and encouragement.

REFERENCES

1. Sujatha, C.H., V. Vasuki, T. Mariappan, M. Kalyanasundaram and P.K. Das (1988). Evaluation of plant extracts for biological activity against mosquitoes. *Intl. Pest Contr.*, **30:** 122-124.
2. Sharma, R.N., V.S. Tare and S.G. Despande (1990). New chemicals, natural products and their permutations and combinations to combat insect pests. In *Impacts of environment on animals and aquaculture* edited by G.K. Manna and B.B. Jana: 97-100.
3. Saxena, R.C., V. Harshan, A. Saxena, P. Sukumaran, M.C. Sharma and M. Lakshamana Kumar (1993). Larvicidal and chemosterilant activity of *Annona squamosa* alkaloids against *Anopheles stephensi*. *J. Amer. Mosq. Contr. Assoc.*, **9**(1): 84-87.
4. Saxena, A. and R.C. Saxena (1992). Effect of *Ageratum conyzoides* extract on the developmental stages of malaria vector *Anopheles stephensi* (Diptera: Culicidae). *J. Environ. Biol.*, **13**(3): 207-209.
5. Sukumar, K., J. Perich Michael and R. Boobar Lewis (1991). Botanical derivatives in mosquito control: A review. *J. Amer. Mosq. Contr. Assoc.*, **7**(2): 210-237.
6. WHO (1971). Instruction for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO/VBC/755.83 (Mimeograph).
7. Saxena, S.C. and L. Sumithra (1985). Laboratory evaluation of leaf extract of a new plant to suppress the population of malaria vector *Anopheles stephensi* Liston. *Curr. Sci.*, **54:** 201-202.
8. Finney, D.J. (1971). *Probit analysis*, Third edn (Cambridge University Press, London).
9. Duncan, J. (1963). Post-treatment effects of sublethal doses of dieldrin on the mosquito *Aedes aegypti* L. *Ann. Appl. Biol.*, **52:** 1-6.
10. Saxena, S.C. and R.S. Yadav (1982). New mosquito larvicides from indigenous plants, *Delonix regia* and *Oligochaeta ramosa*. *Adv. Bios.*, **1**(1): 92-94.
11. Jondiko, I.J.O. (1986). A mosquito larvicide in *Spilanthes mauritiana*. *Phytochem.*, **25**(10): 2289-2290.
12. Pathak, A.K. and V.K. Dixit (1987). Insecticidal and insect repellent activity of essential oil of *Tridax procumbens* and *Cyathocline lyrata*. *Fitotherapia*, **59**(3): 211-214.
13. Thangam, T.S. and K. Kathiresan (1988). Toxic effect of seaweed extracts on mosquito larvae. *Indian J. Med. Res.*, **88:** 35-37.
14. Saxena, R.C., O.P. Dixit and S. Padma (1992). Laboratory assessment of indigenous plant extracts of anti-juvenile hormone activity in *Culex quinquefasciatus*. *Indian J. Med. Res.*, **95:** 204-207.

Indian Journal of Malariology
Vol. 31, March 1994, pp. 27-30.

Response of *P. falciparum* to Chloroquine in Car Nicobar Island

A. GIRI and M.K. DAS

A study was conducted to determine the status of susceptibility of *Plasmodium falciparum* to chloroquine in Car Nicobar Island during September 1991 to September 1992. Out of 66 patients selected for the study, 7 showed resistance according to the standard WHO extended field test. Among them five showed RI level of resistance and one each showed RII and RIII levels of resistance.

Keywords: Car Nicobar Island, Chloroquine resistance, Malaria, *Plasmodium falciparum*

INTRODUCTION

Resistance to chloroquine in *Plasmodium falciparum* has been reported from different parts of the world[1,2]. In India it has also been reported from many parts[3-8]. However, there has been no record of any chloroquine resistance from Car Nicobar Island though it contributes a high number of falciparum malaria cases every year. The study was, therefore, undertaken in MRC field station, Car Nicobar Island, to determine the status of chloroquine resistance. The results of the study are reported here.

MATERIALS AND METHODS

Car Nicobar Island is situated in the Bay of Bengal within the latitudes of 6°-10°N and longitudes of 92°-94°E, covering an area of 187 sq km. It has a tribal population of 19,252 (1991

Malaria Research Centre (Field Station), Malacca, Car Nicobar-744 301, India.

census) of Mongoloid race.

The study was carried out from September 1991 to September 1992 according to the 28-day *in-vivo* test method laid down by WHO[9].

Blood smears were collected from the patients suffering from fever by biased fever survey conducted in all the 19 villages of this island. The cases suffering from *P. falciparum* were selected for the study, taking into consideration their age, severity of illness, consumption of antimalarials, etc. Young children and old people were excluded. Those suffering from severe illness and chronic patients with trophozoites and gametocytes were also excluded from the test. Urine examination was carried out in the selected patients in order to determine the presence of chloroquine by Dill and Glazko method[10]. Those found positive were excluded from the test.

RESULTS

A total 10,435 blood smears were collected for selecting the patients. Among them, 527 were positive for malaria parasite, giving a slide positivity rate of 5.05. Among the positives, 279 had *P. falciparum* and 248 *Plasmodium vivax.*

Out of 279 *P. falciparum* cases, 73 were selected for the study. The WHO *in-vivo* test could be completed in 66 patients. The results of the tests are given in Table 1.

From the table it can be seen that 59 (89.3%) showed normal susceptibility to chloroquine. Five (7.5%) showed RI response and only 1(1.5%) each showed RII and RIII levels of resistance. Seven patients out of 73 originally selected had to be taken out of the test within 48 h of observation as their condition deteriorated and they had to be given I.V. quinine or alternate antimalarial drugs. According to the criteria of Rieckmann test[11], these cases can be categorised as poor response or RIII level of resistance.

DISCUSSION

At present, chloroquine resistance in *P. falciparum* is widely prevalent in different parts of India. This state of affairs occurred during the last three decades after the first reported appearance of this phenomenon in 1973 in Assam[3]. Das *et al.*[5] reported 10.2% RII level chloroquine resistance from Little Andaman Island and 10.5% RI level from Great Nicobar Island in 1981. However, we found 7.5% RI and 1.5% RII and RIII levels of resistance in Car Nicobar Island.

The problem of chloroquine resistance can be reasonably solved now by prompt and early detection of *P. falciparum* cases and their treatment with chloroquine. The alternate drugs should be administered to only those cases where parasite clearance does not occur after 48 h of drug administration and shows a tendency to in-

Table 1. Response of *P. falciparum* to chloroquine in 66 patients of Car Nicobar Island according to WHO *in-vivo* test

Sl. No.	Locality	No. tested	Tribal and Non-tribal	Status of chloroquine resistance				Remarks
				S	RI	RII	RIII	
1.	Malacca	2	Tribal	–	–	1	1	
2.	IAF	25	Non-tribal	23	2*+	–	–	1* Early and 1+ Late
3.	Kenyuka	2	Tribal	2	–	–	–	
4.	Chukchucha	1	Tribal	1	–	–	–	
5.	Tapoiming	2	Tribal	2	–	–	–	
6.	Jayanthi	1	Tribal	–	1+	–	–	Late+
7.	Kinmai	2	Tribal	2	–	–	–	
8.	Teetop	11	Tribal	10	1+	–	–	Late+
9.	Passa	6	Tribal	6	–	–	–	
10.	Sawai	2	Tribal	2	–	–	–	
11.	Arong	10	Tribal	9	1+	–	–	Late+
12.	Aukchung	2	Non-tribal	2	–	–	–	
	Total	66		59	5	1	1	

*Early recrudescence; +Late recrudescence.

crease. The situation, of course, needs constant surveillance against the appearence of RIII level of resistance.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. D.S. Choudhury, ex-Deputy Director, Malaria Research Centre, Delhi, for his suggestions and guidance.

REFERENCES

1. WHO (1986). World malaria situation 1984. *World Hlth Stat.*, **39**(2): 171-172
2. WHO (1986). Data from computerised global malaria drug resistance monitoring programme, supplied by Malaria Action Programme (WHO, Geneva).
3. Sehgal, P.N., M.I.D. Sharma, S.L. Sharma and S. Gogai (1973). Resistance to chloroquine in falciparum malaria in Assam state, India. *J. Com. Dis.*, **5**: 175-180.
4. Guha, A.K., J.R. Roy, Silpi Das, R.G. Roy and S. Pattanayak (1979). Results of chloroquine sensitivity test in *Plasmodium falciparum* in Orissa state. *Indian J. Med. Res.*, **70** (Suppl.): 40-47.

5. Das, Silpi, B.N. Barkakaty, R.G. Roy and S. Pattanayak (1981). Response of *P. falciparum* to choroquine in Andaman-Nicobar Islands, India. *Indian J. Malariol.*, **18**: 47-50.

6. De, C.M., S.C. Dutta, B. Chowdhury, R.G. Roy and S. Pattanayak (1979). Results of chloroquine sensitivity tests in *P. falciparum* in some districts of Gujarat and Maharashtra states. *Indian J. Med. Res.*, **70** (Suppl.): 23-26.

7. Choudhury, D.S., M.S. Malhotra, R.P. Shukla, S.K. Ghosh and V.P. Sharma (1983). Resurgence of malaria in Gadarpur PHC, District Nainital, Uttar Pradesh. *Indian J. Malariol.*, **20**: 49-58.

8. Sharma, V.P. (1983). Drug resistant *Plasmodium falciparum* malaria in India. In *Proceedings of the Indo-UK workshop on malaria* edited by V.P. Sharma (Malaria Research Centre, ICMR, Delhi): 169-184.

9. WHO (1973). Chemotherapy of malaria and resistance to antimalarials. *WHO Tech. Rep. Ser.* No. **529**: 32-37.

10. Lelijveld, J. and H. Kortmann (1970). The eosin colour test of Dill and Glazko, a simple field test to detect chloroquine in urine. *Bull. WHO*, **42**: 477-479.

11. Rieckmann, K.H., G.H. Campbell, L.J. Sax and J.E. Mrema (1978). Drug sensitivity of *Plasmodium falciparum*: An *in-vitro* microtechnique. *Lancet*, **1**: 22-23.

SHORT NOTES

Indian Journal of Malariology
Vol. 31, March 1994, pp. 31-34.

Breeding Habitats of Anopheline Mosquitoes in Assam

V. DEV

Keywords: Anophelines, Larval habitats, Paddy cultivation, Vector

As mosquitoes are known to breed in a wide variety of habitats, it is essential to determine species-specific breeding habitats for species sanitation[1,2]. In Assam, mosquito fauna is rich and breeding sites are innumerable owing to heavy rainfall and high humidity, rendering the environment conducive for mosquito survival and proliferation. More than 20 anopheline species have been recorded, comprising a few malaria vector species, namely *An. minimus* (the principal vector), *An. fluviatilis* and *An. culicifacies*. For vector control, residual insecticides have been used since the commencement of National Malaria Eradication Programme. There was comparative freedom from malaria and increase in acreage under cultivation coupled with other socio-economic developoment. Consequently, there have been changes in bioecological characteristics of certain vector species in areas of their influence[3].

In Southeast Asia, the rice agroecosystem seems to support a good number of vectors of malaria and other

Malaria Research Centre (Field Station), Sonapur-782 402, India.

Table 1. Breeding habitats of anopheline species in Assam

Species	Larval habitats						Paddy fields					
	Ponds	Wells	Ditch-es	Strea-ms	Pits	Bam-boo holes	Barren	Sapl-ings	30 cm	730 cm	Ready to harvest	After harvest
An. aconitus	+	–	–	+	+	–	–	–	–	–	+	+
An. annularis	+	–	+	+	–	–	+	–	+	–	–	–
An. barbirostris	+	+	+	+	–	–	+	+	+	+	–	+
An. culicifacies	–	–	–	+	–	–	–	–	–	–	–	–
An. jamesii	+	–	–	+	–	–	–	–	–	–	–	+
An. jeyporiensis	–	–	–	+	–	–	–	–	–	–	–	+
An. karwari	–	–	–	–	–	–	–	–	+	–	–	–
An. kochi	+	–	+	+	–	–	+	+	+	–	+	+
An. maculatus	+	–	–	+	+	–	–	–	+	–	–	+
An. minimus	–	–	–	+	–	–	–	–	+	–	+	+
An. nigerrimus	+	+	+	+	–	–	+	+	+	+	+	+
An. pallidus	+	–	–	–	–	–	–	–	–	–	–	–
An. philippinensis	+	–	–	+	+	–	+	+	+	+	+	+
An. splendidus	+	–	+	+	–	–	–	–	+	–	+	+
An. tessellatus	–	–	–	–	–	–	–	–	+	–	–	+
An. vagus	+	+	+	+	+	–	+	+	+	+	+	+
An. varuna	+	–	+	+	+	–	+	–	+	–	+	+
Aedes	–	–	–	–	–	+	–	–	–	–	–	–
Armigeres	–	–	–	–	–	+	–	–	–	–	–	–
Toxorhynchites	–	–	+	–	–	+	–	–	–	–	–	–

arboviral diseases[4]. In Assam valley, paddy is the major agricultural produce and the staple food for subsistence. In many areas of the State, particularly those located along foothills, paddy is grown at the risk of acquiring malaria infection transmitted by the *An. minimus*. Sonapur PHC (District Kamrup) is one such area which is endemic for *Pf* and the transmission is perennial[5]. Most of its malaria-endemic villages are located along the foothills, and there are paddy fields in the plains interspersed with perennial streams. To ascertain the mosquito breeding habitats and to envisage anti-larval operations, breeding surveys were conducted in this malaria-ridden PHC. Larval samples were taken from ponds, wells, ditches,

streams, pits, bamboo holes and paddy fields (at various stages of plant growth) during the period from April 1988 to March 1989. Immatures were reared in laboratory till emergence and were identified by the taxonomic keys of Wattal and Kalra[6].

In the breeding habitats surveyed, 17 anopheline species were recorded (Table 1). Paddy fields and perennial streams accounted for the bulk of these species which served as a major source for mosquito breeding. In concurrence with the observations of Muirhead Thomson[1], *An. minimus* breeding as recorded in slow-flowing streams/ streamlets with grassy margins. Streams were also found positive for *An. culicifacies*. The most common species, namely *An. philippinensis* (*nivipes*), *An. vagus* and *An. nigerrimus* (*hyrcanus* group), were found breeding in a wide variety of habitats but most predominantly in rice fields. Larval breeding of these species were recorded at all stages of paddy growth. However, most other species had access to paddy fields up to 60 cm of plant growth. All these species were largely zoophilic. Of these, *An. philippinensis*, though incriminated in the past[7], was perhaps a poor vector owing to its zoophilic nature. Of the remaining habitats, ponds were the major site for a good number of species for oviposition, while wells, ditches, and roadside pits also supported breeding of some species. It was observed that the selection of breeding habitat was not absolute for most species. However, for some the habitat was species-specific. For example, *An. culicifacies* and *An. pallidus* were found breeding only in streams and ponds respectively. Similarly, *An. minimus* breeding was recorded primarily in slow-flowing streams but occasionally its breeding was also found in rice fields with a perceptible flow. Anophelines were not recorded in bamboo holes, but these were found positive for *Aedes*, *Armigeres* and *Toxorhynchites* species.

Although the control of vectorborne diseases through the management of the rice agroecosystem is a global concern[4], there is no serious problem in this terrain as it lends to the breeding of zoophilic species. Hence, antilarval operations should be limited to slow-flowing streams only, the preferred habitat for *An. minimus*. However, in view of the vast area, personal protection methods would be the ideal approach to reduce man/vector contact, thereby disrupting transmission. Field trials with insecticide-impregnated bednets have shown promising results in this area[8]. This strategy is simple, cost-effective, easy to implement and, produces collateral benefits[9].

ACKNOWLEDGEMENTS

The author is thankful to Drs. Wajihullah, B. Jana and B. Shahi for their cooperation. Technical assistance provided by the field staff is acknowledged.

REFERENCES

1. Muirhead Thomson, R.C. (1940). Studies on the behaviour of *Anopheles minimus*: Pt I – The selection of the breeding place and the influence of light and shade. *J. Mal. Inst. India*, **3:** 265-294.
2. Covell, G. (1944). Notes on the distribution, breeding places, adult habits and relation to malaria of the anopheline mosquitoes of India and the Far East. *J. Mal. Inst. India*, **5:** 399-433.
3. Dutta, P., V. Dev and D.R. Bhattacharrya (1993). Anopheline fauna and malaria incidence in Changlang district (Arunachal Pradesh). *Indian J. Malariol.*, **30:** 135-143.
4. Anon. (1988). Vectorborne disease control in humans through rice-agroecosystem management. In *Proceedings of workshop on research and training needs in the field of integrated vectorborne diseases control in riceland agroecosystem of developing countries*, 9-14 March, 1987 (IRRI, Philippines): 237
5. Dev, V. and B. Shahi (1990). Epidemiology of malaria in Sonapur PHC, District Kamrup, Assam. In *Tenth Natl. Cong. of Parasitol.*, Dehra Dun: 42 (Abstract).
6. Wattal, B.L. and N.L. Kalra (1961). Region-wise pictorial keys to the female Indian *Anopheles*. *Bull. Natl. Soc. Ind. Mal. Mosq.-borne Dis.*, **9:** 85-138.
7. Rajagopal, R. (1976). Studies on persistent transmission of malaria in Burnihat, Meghalaya. *J. Com. Dis.*, **8:** 235-245.
8. Dev, V., B. Shahi and V.P. Sharma (1994). Field trials with insecticide-impregnated bednets for malaria control in Assam. In *Proceedings of CSIR golden jubilee symposium* (Central Drug Research Institute, Lucknow): 387-396.
9. Dev, V., H.K. Nayak, K. Baruah and B. Jana (1993). Promoting insecticide-impregnated bednets for malaria control in Assam. In *Community participation in malaria control* edited by V.P. Sharma (Malaria Research Centre, Delhi): 247-257.

Indian Journal of Malariology
Vol. 31, March 1994, pp. 35-38.

Incrimination of *Anopheles vagus* Donitz, 1902 as an Epidemic Malaria Vector in Bangladesh

N.P. MAHESWARY, S. MAJUMDAR[a], A.R. CHOWDHURY[b], M.S. FARUQUE[c] and R.M. MONTANARI[d]

Keywords: *An. vagus*, Bangladesh, Malaria, *P. vivax*

Among the 34 *Anopheles* species recorded from Bangladesh[1], four are considered relatively important vectors: *An. dirus* Peyton and Harrison, *An. minimus* Theobald, *An. philippinensis* Ludlow, and *An. sundaicus* (Rodenwaldt)[2]. Of these, only *An. philippinensis* occurs in the flood plain area of the country, where most of the population lives[3]. Historically transmission in this lowland area has been sporadic and weak. During the malaria eradication campaign (1961-1976) the flood plain was classified as a maintenance zone and indoor residual spraying with DDT was done only in the event of epidemics. In 1992, a routine surveillance mechanism recorded a sharp rise in *Plasmodium vivax* cases in Sachail village (pop. 6608) of Tarail thana (Kishoreganj district), about 100 km northeast of Dhaka city. On investigating the cause of the epidemic, parasite prevalence rate was found to be substantial in all age groups (Table 1) and the case history of individual patients who had never left the village for many years indicated local transmission. Our entomological investigations demonstrated the absence of *An.*

Malaria and Parasitic Disease Control Division, Directorate General of Health Services, Mahakhali, Dhaka-1212, Bangladesh.
[a]Civil Surgeon Office, Sylhet; [b]Dhaka; [c]Kishorganj; [d]WHO Medical Officer, Mahakhali, Dhaka, Bangladesh.

Table 1. Age distribution of *Plasmodium vivax* cases at Sachail village (pop. 6608), Tarail Thana, Kishoreganj Dist., Bangladesh, Jan to Dec 1992

Age group (Yrs.)	Slides examined (No.)	Slides* positive (No.)	Frequency positive %
0-1	-	1	0.2
1-4	-	29	5.7
5-9	-	61	11.9
9-14	-	92	18.0
0≥ 14	-	327	64.1
Total	1212	510	99.9

*All are *P. vivax*.

philippinensis (last detected in very low density in 1989 in Sachail village) and also the scarcity of *An. aconitus* and *An. annularis*. Both the vectors were recently incriminated in plain areas of Bangladesh[4] but a high proportion of *An. vagus* specimens was collected, out of which 1.6% were found to be positive for malaria sporozoite following dissection of salivary glands (Table 2). During the last few years some of the malaria outbreaks, sustained exclusively by *P. vivax* malaria parasites, have occurred in the flood plain areas of Bangladesh.

Environmental factors, such as frequent floods, change in the rain pattern and siltation of rivers or canals and concomitant creation of new breeding sources, may have caused the disappearance of common vector species and increased the density of others (here *An. vagus*). Use of pesticides and fertilizers for agricultural purposes might have also influenced the situation.

Up till now *An. vagus*, mainly zoophilic, exophilic and exophagic mosquito, was neither found positive for oocysts or sporozoite nor suspected as a malaria vector in Bangladesh. Following the parasitological incrimination of *An. vagus* in the flood plain area of Sachail village it is postulated that *An. vagus* has had a significant role in the transmission of *P. vivax* malaria and in the genesis of the present *P. vivax* malaria outbreak in Sachail village.

Possible reasons for this unusual role of *An. vagus* as a vector may be related to the very low cattle density in the area, and the absence of a primate population or of any suitable alternative blood meal source except for humans.

In addition, low dispersal and the observed adequate longevity of the *An. vagus* population (parous rate, 51.3%) are considered as plausible contributing factors leading to the active *P. vivax* transmission role of *An. vagus*. Information on laboratory-confirmed malaria cases was obtained from the laboratory records of Tarail Thana Health Complex, Kishoreganj Civil Surgeon's Office and the Central Malaria Reference Laboratory of Malaria and Parasitic Disease Control Unit (M&PDC) of the Directorate General of Health Services, Dhaka (Table 1). All these malaria case records were obtained from Giemsa-stained thick blood smears collected from fever cases and suspected cases found through monthly house visits and special surveys (active case detection) as well as cases attending the Thana Health Complex located close to the village (passive case detection).

An. vagus was the most common of the five species collected (Table 2). On dissection, four out of 245 parous *An. vagus* (1.6%) had sporozoite-positive salivary glands. *An. vagus* can breed in all types of still water such as pools and puddles, rice fields and dead rivers, and can tolerate organic contamination. It is widely distributed in the oriental region of Asia[5,6].

This is the third uncommon vector we have incriminated during our field epidemiological investigations of the *P.*

Table 2. Anopheles collection and dissection at Sachail, Tarail Thana, Kishoreganj District

Anopheles species	No. collected	No. dissected	No. parous	% parous	No. infective (sporozoite)	% infective
13-16 Feb 1993						
vagus	583	253	130	51.3	2	1.5
annularis	157	124	67	54.0	–	–
hyrcanus group	21	21	7	33.3	–	–
barbirostris group	1	1	1	100	–	–
6-10 Apr 1993						
vagus	287	142	115	80.9	2	1.7
annularis	41	32	22	68.7	–	–
aconitus	1	1	1	100	–	–
hyrcanus group	5	5	2	40	–	–

vivax focal epidemic outbreaks which have affected plain areas of central Bangladesh during the last three years[4]. Additional information on the vector bionomics of *An. vagus* needs to be collected and analyzed in order to better understand the disease and vector dynamics of the present *P. vivax* epidemic outbreak in Sachail village. A new situation has arisen in plain areas of Bangladesh where it seems that virtually any anopheline species, under right conditions, can become an epidemic force.

A field operational research project to control the spread of the epidemic with insecticide-treated mosquito nets while strengthening epidemiological and entomological surveillance is under way in Sachail village.

ACKNOWLEDGEMENTS

We are indebted to F.R. Molla, Chief Entomological Technician, T.A. Howladar, S.U. Ahmed, Z.A. Khandakar, H.B. Ray, S.R. Ray, A.A. Khan and M. Rahman, Entomological Technicians, for their hard and sincere work in the field. Dr. M.R. Chowdhury (Director/PHCDC), Dr. M.I. Haq (Dy. Director/M&PDC), Dr. M.L. Ali (Civil Surgeon) and Dr. M.S.H. Talukdar, generously aided the investigation. We also thank Dr. A.N.A. Abeyesundere, WHO Representative in Bangladesh, and Dr. A.M. Bangali (Assistant Director) for their support and encouragement. Secretarial help by Ms. Shahida Akhtar is greatly appreciated.

REFERENCES

1. Ahmed, T.U. (1987). Checklist of mosquitoes of Bangladesh. *Mosq. Sys.*, **19:** 187-200.
2. Elias, M., Z. Dewan and R. Ahmed (1982). Vectors of malaria in Bangladesh. *J. Prevent. Social Med.*, **1:** 20.
3. Elias, M., A.J.M.M. Rahman, M.M. Ali, J. Begum and A.R. Chowdhury (1989). The ecology of malaria-carrying mosquito *Anopheles philippinensis* Ludlow and its relation to malaria in Bangladesh. *Bangladesh Med. Res. Coun. Bull.*, **13:** 15-28.
4. Maheswary, N.P., M.A. Habib and M. Elias (1992). Incrimination of *Anopheles aconitus* Donitz as a vector of epidemic malaria in Bangladesh. *Southeast Asian J. Trop. Med. Public Hlth.*, **23**(4): 798-801.
5. Horsfall, W.R. (1955). *Mosquitoes: Their bionomics and relation to disease* (The Ronald Press, New York): 723.
6. Rao, T.R. (1984). *The Anophelines of India.* Rev. ed. (Malaria Research Centre, ICMR, Delhi): 518.

Indian Journal of Malariology
Vol. 31, March 1994, pp. 39-41.

In-vitro Chloroquine Resistance *of P. falciparum* in Vellore, Tamil Nadu

E.A. VENKATESAN, E. MATHAI[a], A. SUDARSANAM[b], U. SITARAM[b], MOLLY THOMAS[c], A. GANESH, DILIP MATHAI and T. JACOB JOHN[a]

Keywords: Chloroquine resistance, Drug resistance, *Plasmodium falciparum*

Transmission of *Plasmodium falciparum* occurs in many parts of India. Resistance of *P. falciparum* to chloroquine has also been documented in many places including some areas of Tamil Nadu[1,2]. In this paper we document the recent transmission of chloroquine-resistant *P. falciparum* (CRPf) in Vellore where malaria itself was not endemic for many years.

Patient 1, a 23-year-old female resident of Old Town, Vellore, presented on 27 October 1993 with a 2-day history of high spiking fever, chills, headache and myalgia. A diagnosis of falciparum malaria was made on the basis of the presence of characteristic ring forms in the peripheral blood. No other asexual stages were seen. Infected erythrocytes were not enlarged. Multiple rings, accole forms and rings with double chromatin were seen. The parasitic index was 2%.

Patient 2, the 20-year-old sister of Patient 1, living in the same household, presented on 29 October with high fever of one day's duration and mild hepatosplenomegaly. Falciparum

Departments of General Medicine, [a]Microbiology, [b]Clinical Pathology, and [c]Clinical Pharmacology, Christian Medical College and Hospital, Vellore-632 004, India.

malaria was diagnosed on blood smear examination with a parasitic index of 3%.

The first patient had travelled to Tirupattur 39 days prior to the onset of fever, and had returned after 4 days. Her sister had not travelled outside Vellore for several months.

Prior to starting on quinine and tetracycline therapy, blood was collected for *in-vitro* chloroquine sensitivity testing[3] and also a urine sample for screening for the presence of chloro-quine by the Wilson Edeson test[4]. For the former test, 0.5 ml of blood, collected in a heparinised tube, was added to 4.5 ml of sterile complete RPMI-1640 medium (Hi-Media, Bombay, India) with bicarbonate, Hepes buffer and group AB human serum. Chloroquine sulphate (Rhone-Poulenc, Zurich, Switzerland) was diluted serially in the same medium in 50 µl volumes in sterile flat-bottom wells in cell culture grade plates so as to obtain a range of 1 to 64 pmol per well. To each well, 50 µl of the diluted blood of the patient was added. Each patient's blood was thus tested in duplicate, along with a control test without the drug. The plate was incubated at 37°C in a candle-extinguishing jar for 36 h. At the end of 36 h, thick smears were made from each well, stained with Leishmann's stain and examined for malarial parasites. A parasite showing more than 3 chromatin dots was considered a schizont[3]. Strains showing schizont maturation at a chloroquine level of 8 pmol or more were considered resistant[3]. This test showed no inhibition of maturation of the plasmodium even at the 64 pmol level in both cases.

For screening of chloroquine in the urine, 5 drops of Mayer's reagent (6.8 g of mercuric chloride, 24.9 g of potassium iodide, and 500 ml of distilled water) were added to 3 ml of urine[4]. No turbidity appeared, indicating the absence of chloroquine in the urine[4].

The usual incubation or prepatent period of falciparum malaria is 5-7 days. Since neither patient had travelled out of Vellore for over a month, the infection must have been acquired in Vellore. Since the two sisters living in one household had developed symptoms within 2 days of each other, they would have got the infection almost simultaneously. For many years there has been no transmission of malaria in the town (unpublished). Recently we have seen many patients in Vellore with vivax malaria, suggesting that it might have become endemic here (unpublished). However, the local transmission of falciparum malaria is an important observation, which has serious implications to the local population who are naive to plasmodial infections.

The two strains exhibited a high degree of *in-vitro* resistance to chloroquine, the minimum inhibitory concentraion (MIC)

values exceeding 64 pmol. *In-vivo* response to chloroquine and *in-vitro* resistance data cannot be directly correlated because strains with high MICs *in vitro* may manifest a relatively lesser degree of resistance clinically, particularly in individuals with good immunity[5]. However, MIC of 64 pmol indicates a very high level of resistance[2,3,6]. Since we have seen patients coming from Madras and Chittoor with RII and RIII levels of resistance of *P. falciparum*, we anticipated chloroquine resistance and thus did not give our patients this drug. We believe that RIII level resistance would have been observed, had we given these patients chloroquine[2,6,7]. The resistant strains of *P. falciparum* have probably been introduced to Vellore from nearby areas with prevalence of resistant malaria, rather than local strains developing resistance which increased over time to high-level resistance. We recommend that falciparum malaria in southern India be treated with quinine and tetracycline rather than with chloroquine.

REFERENCES

1. Directorate of NMEP (1990). *Malaria and its control in India*, v. III (Director General of Health Services, Government of India): 545-546.

2. Ghosh, S.K., D.S. Choudhury, R.K. Chandrahas, Neeru Singh, T.V. Ramanaiah and V.P. Sharma (1989). Drug resistant *Plasmodium falciparum* in Madras (Tamil Nadu) and District Jabalpur (Madhya Pradesh). *Indian J. Malariol.*, **26:** 87-90.

3. Reickmann, K.H., G.H. Campbell, L.J. Sax and J.E. Mrema (1978). Drug sensitivity of *Plasmodium falciparum*: an *in-vitro* microtechnique. *Lancet*, **i:** 22-23.

4. Manson-Bahr, P.E.C. and D.R. Bell (1987). *Manson's tropical diseases*, 19th Edn. (ELBS, Bailliere Tindal): 3-47.

5. Spencer, H.C., D.M. Kariuki and D.K. Koech (1983). Chloroquine resistance in *Plasmodium falciparum* from Kenyan infants. *Amer. J. Trop. Med. Hyg.*, **32:** 922-925.

6. Barkakaty, B.N. and M.V.V.L. Narasimham (1992). A longitudinal study to monitor chloroquine-resistant *Plasmodium falciparum* in Bokajan and Manja PHC areas of Karbi-Anglong District, Assam. *Indian J. Malariol.*, **29:** 173-183.

7. Ibrahim, A.M., F.E.R.A. Ali and M.E.M. Ali (1992). Assessment of chloroquine resistance of *Plasmodium falciparum* in children of Wad Medani (Central Sudan). *J. Trop. Paediatr.*, **38:** 1626.

INDIAN JOURNAL OF MALARIOLOGY

Instructions to Authors

Editorial Policy

The 'Indian Journal of Malariology' is devoted to the publication of original research papers which contribute significantly to any field of malariology. Papers of routine and repetitive nature dealing with gross observations may not be included. Articles will be published at the Editor's discretion in the order accepted. Date of acceptance will be the date on which copy is accepted in final form for publication. The authors should also submit names of three experts in the field of research on which the paper has been submitted. If there is no expert in India, experts from outside the country may be suggested. Manuscripts in triplicate along with the undertaking form duly filled by author(s) should be submitted to:

The Editor
Indian Journal of Malariology
20-Madhuvan
Delhi-110 092, India.

Classes of Items Published

In addition to full papers the Journal publishes short note. Review articles are also invited. Book reviews may also be published at the end of the journal.

Format

The matter should be arranged in the following order: Title, Name(s) of the author(s) with address of the Institute/ University (as footnotes and indicated serially in superscript), Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements and References. Authors should provide keywords and a short title to be used as running title, of not more than five words.

Preparation of Copy

Manuscript should be typewritten in English on one side of the paper leaving $1^1/_2$ inch left-hand margin. The entire matter should be typed double space including references, tables and captions. Abstract, tables, references and legends for illustrations should be typed on separate sheets of paper. Pages are to be numbered consecutively.

Tables should be placed singly on sheets of paper, along with relevant headings and footnotes. Table width should not be more than 80 characters including column space and should be self-explanatory and referred to in the text. Tables should be numbered in arabic numerals (e.g. 1, 2); avoid roman numerals (e.g. I, II). Do not use any horizontal or vertical lines in the body of the table.

Footnotes to the text should be avoided as far as possible parenthetical insertions are preferable.

Illustrations should be sent in triplicate. All illustrations including figures, pho-

We accept MSS on floppy. Please specify software used.

tographs, graphs and maps should be numbered consecutively in the order in which they appear in the text. Captions and legends should be typed separately and must not appear on the face of illustrations. Authors should identify each illustration on the reverse side with author's name, fig. no. and abbreviated captions. Line drawings should be clear, and letters and numerals should be planned for legibility after reduction. Labelling should be neat and accurate. Photographs should be sharp, glossy, black and white prints, preferably mounted and covered with a transparent overlay for protection. Photographs should have allowance for reduction to 1/3 size. The approximate sizes of art work should be : 24 x 21 cm for quarter page, 45 x 24 cm for half page and 57 x 45 for full page.

Data for tables, graphs, etc. should be carefully verified. All statistics, percentages and other calculations should be checked thoroughly before submission of a paper. Once a paper is accepted for publication, data in it would be treated as final.

Nomenclature. Authors of scientific names of insects should be omitted in abstract and title, but should be included at first instance in the body of the text.

Numbers less than one should have a zero set before the decimal point, e.g. 0.1.

Measurements should follow the International System (SI) of units. Kindly see WHO publication *The SI for the Health Professional*, WHO, Geneva, 1977. Use of the 24-hour time system (e.g. 0830 hrs, not 8:30 A.M.) is preferable.

References should include only published references and papers in press. References to literature cited should be numbered consecutively and placed at the end of the manuscript. In the text they should be indicated above the line as a superscript number. As far as possible mentioning names of author(s) under references should be avoided in the text. For references to a paper accepted for publication, the words 'in press' should appear after the title of the periodical. Citations of unpublished work should be incorporated in the text itself (e.g. R.G. Roy, unpublished data; or S. Pattanayak, personal communication). If the references is to an article published without any authorship in a periodical, in place of author's name the word "Anonymous" (Anon.) should be used. Titles of periodicals cited in the references are to be abbreviated as in the *World List of Scientific Periodicals*. The following style is accepted for this journal:

Research Paper

Sharma, V.P. (1976). Elimination of aziridine resi-dues from chemosterilised mosquitoes. *Nature*, **261**: 135.

Book/Monograph

Rao, T. Ramachandra (1981). *The Anophelines of India* (WQ, Judge Press, Bangalore).

Landau, I. and Y. Boulard (1978). In *Rodent malaria*, edited by R. Killick-Kendrick and W. Peters (Academic Press Inc., London): 53-84.

Paper presented at Symposium/Conference

Subbarao, S.K. (1981). *Cytoplasmic incompatibility in mosquitoes.* Paper presented at the International symposium on recent developments in the genetics of insect disease vectors. Bellagio, Italy, 20-24 April.

Authors are requested to verify spelling, punctuation, titles and dates of all references. The address of the publisher should be given for books. References are attributable to authors, not to editors in the case of compilations or contributory texts e.g.:

Killick-Kendrick, R. and W. Peters (1978). Ed. *Rodent Malaria.* (Academic Press Inc., London): 406. **(Incorrect).**

Landau, I. and Y. Boulard (1978). In *Rodent Malaria,* edited by R. Killick-Kendrick and W. Peters (Academic Press Inc., London): 53-84. **(Correct).**

Providing correct and complete references is the sole responsibility of the author.

Short notes should be prepared in a manner similar to the research papers and should contain Title, Name(s) of author(s) with Address of Institute/University as footnotes, Acknowledge-ments and References.

Proofs

Page proofs of the articles will be sent to the authors for correction. Corrected proofs must be returned promptly to the editor or else the article may not be printed in the stated issue, or may be printed as it stands. Only minimal changes, i.e. those that do not substantially alter the page make-up, are permissible at the proof stage and only in exceptional cases. Alterations which are disallowed by the Editor shall be deleted or charged to author.

Reprints

Reprints will be mailed to the senior author. Twenty-five reprints will be supplied free of charge in the case of single-author papers and 30 in the case of multiple-author papers.

Check-list

1. Manuscript to be checked as per the format of IJM.
2. Three copies of the manuscript in double space with a covering letter.
3. Short title of the research paper (max. 5 words).
4. Keywords.
5. Undertaking by the author(s).
6. Names of at least three experts on the subject of paper submitted for publication.
7. Set of figures with legends and captions in triplicate on a separate page.

Statement about ownership and other particulars of the Indian Journal of Malariology

FORM 1V
(See Rule 8)

1.	Place of publication	Malaria Research Centre (ICMR) 22-Sham Nath Marg Delhi-110 054.
2.	Periodicity of its publication	Quarterly
3.	Printer's Name Nationality Address	Dr. V.P. Sharma Indian Malaria Research Centre (ICMR) 22-Sham Nath Marg Delhi-110 054.
4.	Publisher's Name Nationality Address	Dr. V.P. Sharma Indian Malaria Research Centre (ICMR) 22-Sham Nath Marg Delhi-110 054.
5.	Editor's Name Nationality Address	Dr. V.P. Sharma Indian Malaria Research Centre (ICMR) 22-Sham Nath Marg Delhi-110 054.
6.	Name and addresses of individual who own the newspaper and partners or shareholders holding more than one per cent of the total capital	Malaria Research Centre (ICMR) 22-Sham Nath Marg Delhi-110 054.

I, V.P. Sharma, hereby declare that the particulars given above are true to the best of my knowledge and belief.

Sd/
(V.P. SHARMA)
(Signature of publisher)

15 March 1994

Announcement

INDIAN ASSOCIATION OF MEDICAL MICROBIOLOGISTS

XVIII National Congress of the Indian Association of Medical Microbiologists

Venue	:	Armed Forces Medical College, Pune
Dates	:	12-14 November 1994
CME	:	11 November 1994 on
CME Topic	:	*Vaccines: The state of the art* and *perspectives for India*
For details contact	:	Col. V.C. Ohri Organising Secretary Prof. and Head Department of Microbiology Armed Forces Medical College Pune - 411 040, India.

Announcement

We prefer submission of manuscripts on electronic media.

— Acceptable medium is $^1/_2$" or $5\,^1/_4$" disk in MSDOS compatible format with file name, software/hardware used.

— The contents on the disk should exactly match with the manuscript and should be submitted with the hard copy (printed copy). The disk would be sent back in case of revision; the same should be returned to editor along with the revised copy of the manuscript. The file on the disk and printout should be identical. 'R' should be marked with red ink with the file name for revised manuscript.

— Package used for graphs should be mentioned.

— Floppies will be sent back to the authors after a final decision on the manuscript only on request.

— Editors

Book Release September 1994

INDIAN ANOPHELINES

(Authors: *B.N. Nagpal* and *V.P. Sharma*)

Malaria Research Centre,
22 Sham Nath Marg,
Delhi-110 054

Book embodies results of studies on Indian anophelines spread over a decade and spanning both laboratory studies and field investigations. The book contains the following:

* Distribution of all anophelines in the world's 12 epidemiological zones of malaria
* Key to the identification of Indian Anopheles mosquitoes (*58 full mosquito drawings and 209 drawings of body parts*)
* Brief biology
* Distribution in India and world (*with maps*)
* Vector status

A useful manual to the students, researchers and those associated in malaria control.

Published by Oxford & IBH Publishing Co. Pvt.Ltd. New Delhi.

ISBN 81-204-0929-9 c 440 Pages Rs. 750/-

INDIAN SOCIETY OF PARASITOLOGY

Announces

Twelfth National Congress of Parasitology
23-25 January 1995

Venue: Panaji, Goa

For further information contact

Dr. V.P. Sharma
President
Indian Society of Parasitology
Malaria Research Centre
20, Madhuban
Delhi-110 092

THE NATIONAL ENVIRONMENTAL SCIENCE ACADEMY

Announces

Tenth Annual Congress

on

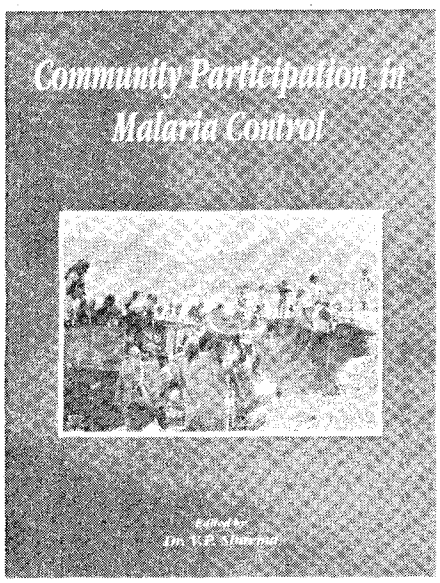
Man and Environment

8-10, March 1995

Venue: National Institute of Oceanography (NIO)
Dona Paula, Panaji,Goa

For further information contact

Dr. V.P. Sharma
President
Xth Annual Congress (NESA)
Malaria Research Centre
20, Madhuban
Delhi-110 092



A complete review of the experience on community participation from various areas in the country in relation to vector/malaria control. A commendable document for future management of vector control through community participation.

Size: Crown 4To
pp. vii, 295 (Hardbound)
1993

OTHER PUBLICATIONS OF MALARIA RESEARCH CENTRE

(1) *The Anophelines of India* (Rev. edn.) 1984, pp. 538; Rs. 150.00, US$ 45.00, 25% discount per copy
T.Ramachandra Rao

(2) Proceedings of the ICMR/WHO Workshop on *Community Participation for Disease Vector Control* (1986) pp. 256
Edited by ***V.P. Sharma***

(3) *Seroepidemiology of Human Malaria* - A multicentric study (1989), pp. 206
Edited by ***V.P. Sharma***

(4) *Indigenous Larvivorous Fishes of India* (1991), pp. 66
A.G.K. Menon

(5) Proceedings of an Informal Consultative meeting WHO/MRC on *Forest Malaria in Southeast Asia* (1991), pp. 206
Editors ***V.P. Sharma*** *and* ***A.V. Kondrashin***

(6) *Malaria Patrika* quaterly (Hindi) 1993 onwards.

INDIAN JOURNAL OF MALARIOLOGY
MALARIA RESEARCH CENTRE (ICMR)

UNDERTAKING BY AUTHORS

We, the undersigned, give an undertaking to the following effect with regard to our article entitled, "_____

_____

submitted for publication in the **Indian Journal of Malariology** :—

1*. Ethical committee has approved the research as presented in this research paper/this piece of research does not require ethical committee clearance.

2. The article mentioned above has not been submitted for publication in any form to any other journal.

3. We also agree to the authorship of the article in the following sequence :—

	Authors' names (in sequence)	Signature of Authors
1.	_____	_____
2.	_____	_____
3.	_____	_____
4.	_____	_____
5.	_____	_____
6.	_____	_____
7.	_____	_____
8.	_____	_____

IMPORTANT

1. All authors are required to sign independently in the form and in the sequence given above. A photocopy of this form may also be used.
2. No addition/deletion/ or any change in the sequence of the authorship will be permissible at a later stage, without valid reasons. If change is valid, then all the authors involved should attest to the change. The decision however, rests with the Editor.
3. If the authorship is contested at any stage, the article will be either returned or will not be processed for publication till the dispute is dissolved.

* Please write the applicable statement below:

MALARIA RESEARCH CENTRE

PRICED PUBLICATIONS

The Anophelines of India (Rev. edn.) 1984 by T. Ramachandra Rao

Price India Rs. 150.00 / Foreign US $ 45.00 } 25% Discount

Indian Journal of Malariology

Volume 18 Nos. 1-2 (1981)*
Volume 19 Nos. 1-2 (1982)*
Volume 20 Nos. 1-2 (1983)*
Volume 21 Nos. 1-2 (1984)*
Volume 22 Nos. 1-2 (1985)*
Volume 23 Nos. 1-2 (1986)*
Volume 24 Nos. 1-2 (1987)
Volume 25 Nos. 1-2 (1988)
Volume 26 Nos. 1-4 (1989)
Volume 27 Nos. 1-4 (1990)
Volume 28 Nos. 1-4 (1991)
Volume 29 Nos. 1-4 (1992)
Volume 30 Nos. 1-4 (1993)
Volume 31 Nos. 1-4 (1994)

Annual Subscription { India Rs. 75.00++ / Foreign US $ 20.00 / ++25% discount for individuals

*Back issues are available at old rates, i.e. Rs. 30.00 or $ 10.00

The Editor
Indian Journal of Malariology
Malaria Research Centre
20-Madhuvan
Delhi-110 092 (India)

Sir,

I enclose herewith a bank draft or postal order(s) No.(s) ..... for $/Rs. ..... (in favour of the Director, Malaria Research Centre, Delhi-110 054) towards subscription/payment for **Indian Journal of Malariology** for the year(s) .....(2/4 Nos.)/**The Anophelines of India** (Revised edition) 1984. The journals/publication should be mailed to me/my client at the following address:

.....
.....
.....
.....