INDIAN JOURNAL OF MALARIOLOGY

Volume 28 Number 3

September 1991

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 the individual subscriber on annual basis.

Payments in respect of subscription may be sent by **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

Volume 28
Number 3
September 1991

CONTENTS

Note: The editor assumes no responsibility for the statements and opinions expressed by the contributors. This issue has been delayed for reasons beyond our control.

Indian Journal of Malariology
Vol. 28, September 1991, pp. 141-145.

Tralomethrin (OMS-3048), a New Synthetic Pyrethroid against Vector Mosquitoes

D. DOMINIC AMALRAJ*, V. VASUKI* and R. VELAYUDHAN*

Tralomethrin (OMS-3048) a synthetic pyrethroid, was tested for its insecticidal properties in the laboratory against normal strains of *Culex quinquefasciatus*, *Cx. tritaeniorhynchus*, *Cx. sitiens*, *Anopheles stephensi*, *An. culicifacies*, *Aedes aegypti* and *Armigeres subalbatus* and against strains of *Cx. quinquefasciatus* resistant to fenthion and malathion. Tralomethrin showed good larvicidal activity against all the species tested. LC50 ranged between 7.00×10^{-6} and 9.10×10^{-3} mg (ai)/l. Resistant strains of *Cx. quinquefasciatus* showed higher tolerance than the normal strain. Tralomethrin was more effective against adults of *An. culicifacies* (LD50 0.18 $\mu g/cm^2$) than the other species. Residual activity of this compound lasted for 15 weeks on thatch surface at a dosage of 50 mg $(ai)/m^2$ against all the mosquito species tested. In the field, this compound was effective for a period of 1-2 days in polluted water viz., cesspits and drains and 10-24 days in less polluted water as in cement tanks, when applied at the rate of 0.002-2.0 mg (ai)/l against immatures of *Cx. quinquefasciatus*.

INTRODUCTION

Synthetic pyrethroids show a broad-spectrum biological activity against mosquitoes and many other pests and vectors. Some of these compounds were found to be highly active against mosquitoes as larvicides, pupicides, adulticides and repellents both in laboratory and under field conditions[1-7]. Effective control of organophosphorus resistant mosquito species by synthetic pyrethroids has been achieved[8]. As this group of compounds is effective at low dosage against target species and is non-toxic to non-target organisms they are found to be highly promising[1-4,8-9]. The present study reports the efficacy of Tralomethrin, a new synthetic pyrethroid, against various species of mosquito vectors. Comparative efficacy of this compound with other synthetic pyrethroids and its effect on non-target organisms are also reported.

Accepted for publication : 25 June 1991.
* Vector Control Research Centre
Indira Nagar, Medical Complex
Pondicherry-605 006, India.

MATERIAL AND METHODS

Tralomethrin (OMS-3048), (IR, 3S) 3[(I[1] RS) (I[1],2[1],2[1],2[1]-tetrabromoethyl)]-2,2-dimethylcyclopropanecarboxylic acid (S)-alpha-cyano-3-phenoxy benzyl ester, (1.5% EC), a synthetic pyrethroid manufactured by Roussel UCLAF, France, was obtained for evaluation under WHOPES through the World Health Organization. The efficacy of this compound was evaluated against the adults and larvae of normal strains of *Culex quinquefasciatus*, *Cx. tritaeniorhynchus*, *Cx. sitiens*,

Table 1. Susceptibility of vector mosquitoes to Tralomethrin

Species	LC50(mg/l)	LC90(mg/l)	Regression equation
		Larvae	
Cx. quinquefasciatus (Lab)	1.50×10^{-5}	6.40×10^{-5}	Y = 15.11 + 0.91 log X
Cx. quinquefasciatus (Field)	1.80×10^{-3}	5.02×10^{-3}	Y = 12.86 + 1.25 log X
Cx. quinquefasciatus (F-Res.)	9.10×10^{-3}	1.07×10^{-2}	Y = 8.60 + 0.76 log X
Cx. quinquefasciatus (M-Res.)	2.61×10^{-3}	8.09×10^{-3}	Y = 11.72 + 1.13 log X
Cx. tritaeniorhynchus	4.96×10^{-5}	1.23×10^{-3}	Y = 18.95 + 1.41 log X
Cx. sitiens	2.69×10^{-4}	9.03×10^{-4}	Y = 13.70 + 1.06 log X
An. stephensi	7.67×10^{-5}	1.03×10^{-4}	Y = 46.12 + 4.34 log X
An. culicifacies	3.27×10^{-4}	6.80×10^{-5}	Y = 19.05 + 1.75 log X
Ae. aegypti	7.00×10^{-6}	2.43×10^{-5}	Y = 17.22 + 1.03 log X
Ar. subalbatus	1.47×10^{-5}	4.12×10^{-5}	Y = 21.15 + 1.47 log X
Species	LD50($\mu g/cm^2$)	LD90($\mu g/cm^2$)	Regression equation
		Adults	
Cx. quinquefasciatus (Lab)	1.5463	3.4924	Y = 4.32 + 1.57 log X
Cx. quinquefasciatus (Field)	0.4853	1.8623	Y = 5.69 + 0.95 log X
Cx. tritaeniorhynchus	0.3663	1.7618	Y = 5.82 + 0.81 log X
Cx. sitiens	0.1931	0.9731	Y = 6.30 + 0.79 log X
An. stephensi	1.0119	4.1148	Y = 4.99 + 0.91 log X
An. culicifacies	0.1829	0.4775	Y = 7.27 + 1.33 log X
Ae. aegypti	0.3649	3.4135	Y = 5.58 + 0.57 log X
Ar. subalbatus	0.4402	1.1192	Y = 6.13 + 1.37 log X

F-Res. — Fenthion resistant; M-Res. — Malathion resistant.

Anopheles stephensi, An. culicifacies, Armigeres subalbatus and *Aedes aegypti*, as well as larvae of fenthion and malathion resistant strains of *Cx. quinquefasciatus* maintained at VCRC. The effectiveness of the compound against *Cx. quinquefasciatus* was also evaluated in the field.

Laboratory evaluation

Biological activity of Tralomethrin was determined by following the standard procedure[10]. One per cent stock solution was prepared in ethanol from which other stock solutions of required concentrations were made. To get the desired concentration, one ml of appropriate stock solution was added to 249 ml of tap water in 500 ml beakers to which 25 early fourth instar larvae were introduced. Mortality counts were taken after an exposure period of 24 hrs. Percentage mortality at each dose was corrected using Abbott's formula. LC50 and LC90 values were calculated by Probit analysis[11].

Lethal doses LC50 and LC90 were calculated for fenthion and malathion resistant strains of *Cx. quinquefasciatus* to find out cross-resistance if any, by following the standard procedure[12].

Acute toxicity to non-target organisms, i.e. *Toxorhynchites splendens* and *Gambusia affinis* was determined by exposing them to various concentrations of Tralomethrin[12]. Gravid females of the latter were brought from their natural habitats and kept in the VCRC aquarium to obtain the same cohort of fish. Ten fingerlings, 25-30 days old, were introduced into plastic tubs (diameter, 20 cm; depth, 9 cm) with 1.5 lit tap water, which was aerated throughout the test period. The fingerlings were then treated at four dosage levels, viz., 0.001, 0.005, 0.01 and 0.05 mg/l, and eight replicates for each dose were maintained along with a control. Mortality was recorded at 24 hrs intervals up to 72 hrs and was corrected with control mortality. Toxicity of Tralomethrin on *Tx. splendens* was studied by following the standard procedure[12].

Residual activity of Tralomethrin was tested on different surfaces, viz. cement, mud and thatch, at the application rate of 50 mg (ai)/m^2 against the adults of *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, *An. stephensi*, *An. culicifacies* and *Ae. aegypti* following the modified method developed at the VCRC[13].

Field evaluation

To test the effect of this compound under field conditions, various breeding habitats of *Cx. quinquefasciatus* were treated at the rate of 0.002, 0.02 and 2.00 kg (ai)/ha. For this the required amount of EC formulation at each rate of application was mixed with 1000 ml of tap water and applied with the help of a Ganesh sprayer (1 litre capacity). Two habitats of each type were left untreated as control. Three replicates at each application rate were maintained. Impact evaluation for the compound was done by taking two dips in small-sized habitats and four dips in large habitats prior to treatment and every two days after treatment. On each day of sampling, bioassay tests were conducted. Effective duration was calculated on the basis of 100% mortality in the bioassay tests.

RESULTS AND DISCUSSION

Dosage response lines established against fourth instar larvae of seven species of mosquitoes revealed that Tralomethrin was effective against all the species and strains tested. LC50 ranged from 7.00×10^{-6} to 9.10×10^{-3} mg (ai)/l (Table 1). Tralomethrin was highly effective against immatures of *Ae. aegypti* with LC50 value of 7.00×10^{-6} mg/l, which was 1300 times less than that of the least susceptible strain of *Cx. quinquefasciatus* (fenthion resistant strain). Comparison of LC50 values of Tralomethrin with those of other synthetic pyrethroids revealed that its larvicidal efficacy against *Cx. quinquefasciatus* is superior to that of Cyfluthrin, Fenfluthrin and Permethrin and is as good as that of Alphamethrin and Decamethrin (Table 2)[13-17]. The difference in the LC50 values of Tralomethrin against the normal

Table 2. Comparison of larvicidal and adulticidal efficacy of Tralomethrin with other synthetic pyrethroids

Compound	LC50(mg/l)/LD50(µg/cm^2)		
	Cx. quinquefasciatus	*An. stephensi*	*Ae. aegypti*
Tralomethrin	1.5×10^{-5} /1.55	7.7×10^{-4} /1.01	7.0×10^{-5} /0.37
Alphamethrin	1.2×10^{-4} /0.37	4.0×10^{-3} /0.34	3.3×10^{-4} /0.68
Cyfluthrin	7.0×10^{-4} /0.45	2.3×10^{-2} /1.85	1.0×10^{-3} /0.51
Fenfluthrin	7.0×10^{-4} /8.31	1.3×10^{-2} /0.32	1.6×10^{-3} /0.60
Permethrin	1.0×10^{-3} /12.3	5.8×10^{-3} /3.21	3.2×10^{-4} /3.78
Decamethrin	7.0×10^{-5} /0.14	2.2×10^{-3} /0.66	1.0×10^{-4} /0.11

and resistant strains of *Cx. quinquefasciatus* indicates that fenthion and malathion selected strains could tolerate high doses of Tralomethrin in comparison to the normal strain.

Among the seven mosquito species tested, adults of *An. culicifacies* were found to be the most susceptible and *Cx. quinquefasciatus* least (Table 1). A comparison of the adulticidal activity of this compound with those of other pyrethroids shows that Tralomethrin is more effective than other pyrethroids except Decamethrin against *Ae. aegypti*, whereas it is more effective than Cyfluthrin and Permethrin against *An. stephensi* and Fenfluthrin and Permethrin against *Cx. quinquefasciatus* (Table 2)[13-17].

When residual activity of Tralomethrin was tested on three surfaces, more than 50% mortality was obtained on thatch surface for about 15 weeks against *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus* and *An. stephensi* and for 21 and 31 weeks against *Ae. aegypti* and *An. culicifacies* respectively (Table 3). The effect of Tralomethrin on thatch surface at the rate of 50 mg (ai)/m^2 is comparable with that of another synthetic pyrethroid Cyfluthrin[13].

On comparing the LC50 value of Tralomethrin against the larvae of *Tx. splendens* (7.59×10^{-4}) with that of other vector mosquito species it was found that the former is equally susceptible to this compound. However, at a higher dose [16 µg(ai)/cm^2], only 20% mortality was observed. Lethal effect of this compound on the fingerlings of *G. affinis* was shown by 90% mortality when exposed to 0.05 mg(ai)/l for 72 hrs.

The biological activity of Tralomethrin against immatures of *Cx. quinquefasciatus* was investigated in the field conditions at various application rates and the results are given in Table 4. The residual effect of Tralomethrin at the application rate of 0.2-2.0 kg(ai)/ha lasted for 1-2 days in cesspits and drains respectively, while in cement tanks it was effective for 11 days at a lower dosage

Table 3. Residual efficacy of Tralomethrin treated on various surfaces against different species of vector mosquitoes

Species	Effective duration (in weeks) @ 50 mg(ai)/m^2		
	Thatch	Mud	Cement
Cx. quinquefasciatus	14.4	0	0
Cx. tritaeniorhynchus	14.0	0	0
Ae. aegypti	21.4	0	0
An. stephensi	14.4	0	0
An. culicifacies	30.9	0	0

Table 4. Field evaluation of Tralomethrin for larvicidal activity against *Cx. quinquefasciatus*

Breeding habitat	Rate of application kg(ai)/ha	Surface area (m^2)	Residual effect in days (Replicates)			Average
			1	2	3	
Cesspit	0.02	1.63	0	0	0	0
Cesspit	0.20	5.71	2	2	0	1
Cesspit	2.00	2.02	1	2	2	2
Cement tank	0.002	0.56	12	9	—	10
Cement tank	0.02	0.56	12	10	—	11
Cement tank	0.20	0.56	27	22	—	24
Drain	0.20	2.40	1	2	2	2

[0.02 kg(ai)/ha]. Further increase in the duration of the residual effect of this compound (24 days) in cement tanks was obtained when applied at 0.2 kg(ai)/ha. Owing to faster degradation of the compound in polluted water bodies, the residual effect was more pronounced only in less polluted water bodies like cement tanks.

The results point to the conclusion that Tralomethrin kills mosquito larvae and adults of susceptible and resistant strains at very low dosages. Owing to rapid degradation in polluted water, this pyrethroid cannot be recommended as larvicide against *Cx. quinquefasciatus*. However, it can be effectively used in controlling larvae of *Anopheles* and *Aedes* species.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. P.K. Rajagopalan, Director, and Dr. P.K. Das, Deputy Director, Vector Control Research Centre, Pondicherry, for encouragement and guidance. The authors are thankful to the Division of Vector Biology and Control, WHO, Geneva, for providing the chemical.

REFERENCES

1. Mulla, M.S., H.A. Navvab-Gojrati and H.A. Darwazeh (1978a). Biological activity and longevity of new synthetic pyrethroids against mosquitoes and some non-target insects. *J. Amer. Mosq. Contr. Assoc.*, **38** : 90-96.
2. Mulla, M.S., H.A. Navvab-Gojrati and H.A. Darwazeh (1978b). Toxicity of mosquito larvicidal pyrethroids to four species of freshwater fishes. *Environ. Entomol.*, **7** : 428-430.
3. Mulla, M.S., H.A. Darwazeh and M.S. Dhillon (1980). New pyrethroids on mosquito larvicides and their effects on nontarget organisms. *J. Amer. Mosq. Contr. Assoc.*, **40** : 6-12.
4. Mulla, M.S., H.A. Darwazeh and L. Ede (1982). Evaluation of new pyrethroids against immature mosquitoes and their effects on nontarget organisms. *J. Amer. Mosq. Contr. Assoc.*, **42** : 583-590.
5. Taylor, R.N., M.N. Hill, D.C. Stewart, R. Slatter and M. Gichanga (1981). A field evaluation of permethrin (OMS-1821) and NRDC-161 (OMS-1998) for residual control of mosquitoes. *J. Amer. Mosq. Contr. Assoc.*, **41** : 423-434.
6. Helson, B.V. and G.A. Surgeoner (1983). Permethrin as a residual lawn spray for adult mosquito control. *J. Amer. Mosq. Contr. Assoc.*, **43** : 164-169.
7. Roberts, R.H. (1983). Evaluation of cypermethrin as ULV cold aerosol against caged mosquitoes. *J. Amer. Mosq. Contr. Assoc.*, **43** : 156-158.
8. Darwazeh, H.A., M.S. Mulla and B.T. Witwash (1978). Synthetic pyrethroids for the control of resistant mosquitoes in irrigated pastures. *Proc. Calif. Mosq. Vect. Contr. Assoc.*, **46** : 121-122.
9. Mulla, M.S., H.A. Darwazeh and M.S. Dhillon (1981). Impact and joint action of pyrethroids and freshwater fishes on mosquitoes. *Bull. Environ. Contr. Toxicol.*, **26** : 689-695.
10. WHO (1982). Meeting of Directors of WHO collaborating centres on the evaluation and testing of new insecticides. (Mimeographed document). WHO/VBC/82, 846.
11. Finney, D.J. (1971). Probit analysis. (S. Chand & Company Ltd., New Delhi) : 328.
12. WHO (1981). Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides. Establishment of base line. (Mimeographed document). WHO/VBC/81, 805.
13. Rajavel, A.R., V. Vasuki, K.P. Paily, K.D. Ramaiah, T. Mariappan, M. Kalyanasundaram, B.K. Tyagi and P.K. Das (1987). Evaluation of a synthetic pyrethroid (Cyfluthrin) for insecticidal activity against different mosquito species. *Indian J. Med. Res.*, **85** : 168-175.
14. Das, P.K., T. Mariappan and C.B.S. Reddy (1982). Suceptibility of *Culex quinquefasciatus*, *Aedes aegypti*, *Anopheles culicifacies* and *Anopheles stephensi* against insecticides. *Indian J. Med. Res.*, **75** : 529-533.
15. Das, P.K. and M. Kalyanasundaram (1984). Evaluation of K-Othrine®, a synthetic pyrethroid, for insecticidal efficacy against mosquito vectors. *Indian. J. Med. Res.*, **80** : 74-77.
16. Mariappan, T., M. Kalyanasundaram, K.N. Panicker, N. Balakrishnan, B.K. Tyagi and P.K. Das (1985). Evaluation of fenfluthrin (OMS 2013), a synthetic pyrethroid, for insecticidal efficacy against mosquito vectors. *Indian J. Med. Res.*, **82** : 1-8.
17. Amalraj, D., K.D. Ramaiah, A.R. Rajavel, T. Mariappan, V. Vasuki, K.P. Paily, B.K. Tyagi, M.K. Kalyanasundaram and P.K. Das (1987). Evaluation of alphamethrin, a synthetic pyrethroid for insecticidal activity against mosquitoes. *Indidn J. Med. Res.*, **86** : 601-609.

Indian Journal of Malariology
Vol. 28, September 1991, pp. 147–150.

Isolation and Laboratory Evaluation of an Indigenous Strain of *Bacillus sphaericus* (9001)

D.K. GUPTA*, R.C. SHARMA*, R.M. BHATT* and A.S. GAUTAM*

An indigenous strain of *Bacillus sphaericus* H5a (9001), possessing high insecticidal properties, was isolated from diseased larvae of *Culex* species. This strain in comparison with the known strains of *B. sphaericus*, i.e., 1593 and 2362, was found to be promising against the fourth instar larvae of *Anopheles culicifacies*, *An. stephensi*, *An. subpictus*, *Aedes aegypti* and *Culex quinquefasciatus*. *B. sphaericus* 9001 is highly stable and virulent through 25 successive transfers and thus can be effectively used as a biocontrol agent against immature stages of mosquitoes.

INTRODUCTION

Many microbes used as biological control agents, e.g., *Bacillus thuringiensis* var. *israelensis* serotype H-14 and *B. sphaericus* Neide and other serotype strains, are reported to possess a high level of insecticidal activity against the larvae of various mosquito species under laboratory and field conditions[1-4]. This property of *Bacillus sphaericus* has attracted greater attention in recent years, with potent strains being isolated and tested against mosquito larvae. The strains of *B. sphaericus* have been reported to have a potential to recycle[5]. Out of 48 serotypes known, the most active strains belong to serotypes H5a, H5b, H6 and H25[6,7].

In our study, one indigenous strain of *B. sphaericus* H5a (9001) was isolated from District Kheda, Gujarat and evaluated in the laboratory to determine its efficacy against various species of mosquitoes and compared with the known *B. sphaericus* strains 1593 and 2362.

Accepted for publication : 10 July 1991.

* Malaria Research Centre (Field Station)
Civil Hospital
Nadiad-387 001, India.

MATERIAL AND METHODS

Seventy-two bacterial isolates were isolated from diseased mosquito larvae and soil samples of Kheda distt., Gujarat. Fourteen of them were sent to Institut Pasteur, Paris for identification. Out of these, one bacterial strain, *B. sphaericus* 9001, was selected for further study.

This strain was maintained in laboratory at 30°C in Poly medium (Peptone 5g/l; Glycerol 10g/l; Yeast extract 10g/l; Meat extract 5g/l; NaCl 3g/l; pH 7.2) in liquid culture in conical flasks. The bacterial cells were harvested after 48 hrs by centrifuging the broth of 3000 rpm for 10 min. The strain was tested against fourth instar larvae of five species of mosquitoes, viz., *Ae. aegypti*, *An. stephensi*, *An. subpictus*, *An. culicifacies* and *Cx. quinquefas-*

ciatus, for pathogenicity as described by Gupta[8]. Serial dilutions of whole culture were made to determine the lethal concentration required to kill 50% (LC50) of mosquito larvae. Mortality (cells/ml) was observed after 24, 48 and 72 hrs. Twenty-four hours period was taken as the cut-off period of observation for determining LC50 value in order to provide better approximation of total mortality response. The strain was passed through 25 successive transfers on artificial media at 3-4 days' interval for testing its stability and virulence against *Ae. aegypti*.

RESULTS AND DISCUSSION

Table 1 shows the list of strains of *Bacillus* species isolated from Kheda distt. The phenotypic characteristics of *B. sphaericus* are given in Table 2. The strain was identified as *B. sphaericus* H5a (9001), and was registered in Culture Collection of WHO Collaborating Centre for Entomopathogenic *Bacillus* of Institut Pasteur, Paris. The colonies are irregular, smooth, opaque and creamish in colour, and spores are round with bulging sporangium. The size of vegetative cell is 2.2-4.8 µm X 1.0-1.5 µm and of spores, 0.70-0.96 µm.

Table 1. Bacterial isolates from diseased mosquito larvae and soil samples from Kheda district, Gujarat

	MRC Code	Strain
1.	8801	*Bacillus subtilis*
2.	8802	*B. cereus*
3.	8803	*B. subtilis*
4.	8901	*B. subtilis*
5.	8902	*B. subtilis*
6.	8903	*B. licheniformis*
7.	8904	*B. cereus*
8.	8905	*B. cereus*
9.	8907	*B. cereus*
10.	8907	*B. thuringiensis neoleonesis* H24
11.	8908	*B. sphaericus* H2
12.	8909	*B. cereus*
13.	9001	*B. sphaericus* H5a*
14.	9002	*B. sphaericus* H1a*

*Highly pathogenic against mosquito species and registered at Institut Pasteur, Paris, as 9001 and 9002.

Table 2. Phenotypic characters of *Bacillus sphaericus* H5a (9001)

Spore R Def	Hugh-Leifson I
Corps para. +	Gram +
Gel. Nut. Sm	Taille <0.9 µ
Gel. pH6 +	Mobilite +
Anaerobiose —	Gel Amidon—
BN+Glu. 1% ana. —	Gel. Gelatine +
Gaz <—BNO3 ana.	Xylose —
AMC —	Glucose —
Lecithinase —	Mannitol —
Christensen + 24h	Amidon —
Uree <	Lactose —
Fergysson + 24h	Raffinose —
Nitrate reductase —	Inuline —
ADH To	Tox. B-exo.
B-glactosidase —	(% de mortalite)
Rouge de methyl —	Srotype 5a
Indole —	

Table 3 summarizes the comparison of LC50 values and confidence limits of the isolated strain 9001 with known strains 1593 and 2362 against *Ae. aegypti*, *An. stephensi*, *An. subpictus*, *An. culicifacies* and *Cx. quinquefasciatus*. The table shows the susceptibility of all the five mosquito species to the above-mentioned strains. Among the anophelines, *An. subpictus* was most susceptible. However, *An. culicifacies* required a higher concentration for medium lethal dose response. The results show that the indigenous strain 9001 is superior to 1593 against *Ae. aegypti*, *An. stephensi* and *An. culicifa-*

Table 3. Efficacy of *Bacillus sphaericus* strains

Mosquito species	Concentration (cells/ml)					
	9001		1593		2362	
	LC50	CL	LC50	CL	LC50	CL
Ae. aegypti	$1.1x10^3$	$9.7x10^2$–$1.2x10^3$	$5.3x10^4$	$3.3x10^4$–$8.5x10^4$	$4.3x10^3$	$3.4x10^3$–$5.5x10^3$
An. stephensi	$5.2x10^3$	$4.6x10^3$–$6.0x10^3$	$2.4x10^4$	$2.0x10^4$–$2.9x10^4$	$1.9x10^3$	$1.2x10^3$–$3.1x10^3$
An. culicifacies	$1.8x10^4$	$1.6x10^4$–$2.0x10^4$	$1.0x10^5$	$8.3x10^4$–$1.3x10^6$	$4.3x10^3$	$3.9x10^3$–$4.7x10^3$
An. subpictus	$4.8x10^2$	$4.4x10^2$–$5.3x10^2$	$0.5x10^1$	$0.5x10^1$–$0.6x10^1$	$2.2x10^1$	$1.9x10^1$–$2.5x10^1$
Cx. quinquefasciatus	$1.6x10^3$	$1.5x10^3$–$1.8x10^3$	$1.4x10^3$	$1.2x10^3$–$1.6x10^3$	$3.2x10^2$	$3.0x10^2$–$3.5x10^2$

LC50 = Lethal concentration; CL = Confidence limit.

cies. Among these three, it is most effective against *Aedes aegypti. B. sphaericus* strains belonging to H serotype have been assayed on *Culex pipiens*, *Anopheles stephensi* and *Aedes aegypti* under standard conditions. The most potent strains are distributed in serotypes H5a and H5b, generally toxic to three mosquito species[7]. The phenotypic characteristics of 9001 show that this strain belongs to the highly pathogenic group of serotype H5. *B. sphaericus* (9001) was found to be quite stable as it retained its virulence through 25 successive transfers. The LC50 values were 1.2×10^3, 1.6×10^3 and 1.4×10^3 for subculturing at F_7, F_{16} and F_{24} generations respectively.

Some strains of *B. sphaericus* which were isolated from caterpillars were highly toxic to mosquito larvae but not to the insect from which they were isolated[9]. Our study shows that the strain 9001 isolated from *Culex* species is highly effective against *Culex* and other mosquito species. Menon *et al.*[10] had reported an LC50 value of 1.7×10^4, CFU/ml for the indigenous strain *B. sphaericus* IPSC-5 against the second instar larvae of *Culex fatigans* after 24 hrs of exposure. The strain 9001 is more effective in comparison with *B. sphaericus* (IPSC-5) as the LC50 value is 1.6×10^3 cells/ml. The study shows that this indigenous strain, *B. sphaericus* 9001, could be a very promising biocontrol agent against the vector mosquitoes.

ACKNOWLEDGEMENTS

The authors are grateful to Prof. H. de Barjac and Dr. J.F. Charles, Institut Pasteur, Paris, for identification of bacterial strains and their valuable suggestions. They are thankful to Mr. K.C. Pramanik, Mr. Vijay Bahadur and Mr. Alok Kulshreshtha for technical assistance. Thanks are also due to Mr. Sanjay Jain, Department of Computer Sciences, DDIT, Nadiad, for processing the data. We also thank Mr. T.R. Babu for secretarial assistance.

REFERENCES

1. Kellen, W.R., T.B. Clark, J.E. Lindergren, B.C. HO, M.H. Rogoff and S. Singer (1965). *Bacillus sphaericus* Neide as a pathogen of mosquitoes. *J. Invert. Pathol.*, 7: 442-448.
2. Singer, S. (1973). Insecticidal activity of recent bacterial isolates and their toxins against mosquito larvae. *Nature*, 224 (5410): 110-111.
3. Goldberg, L.J. and J. Margalit (1977). A bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii, Uranotaenia unquiculata, Culex univitattus, Aedes aegypti and Culex pipiens. Mosq. News*, 37 (3): 355-358.
4. Yousten, A.A. (1984). Bacteriophage typing of mosquito pathogenic strains of *Bacillus sphaericus. J. Invert. Pathol.*, 43 (1): 124-125.
5. Hertlein, B.C., R. Levy and T.W. Miller Jr. (1979). Recycling potential and selective retrieval of *Bacillus*

sphaericus from soil in a mosquito habitat. *J. Invert. Pathol.*, **33** (2): 217-221.

6. de Barjac, H., I.L. Thiery, V.C. Dumanoir and H. Ripouteau (1985). Serological classification of *Bacillus sphaericus* strains on the basis of toxicity to mosquito larvae. *Appl. Microb. Biotech.*, **21**: 85-90.

7. Thiery, I.L. and H. de Barjac (1989). Selection of the most potent *Bacillus sphaericus* strains based on activity ratios determined on three mosquito species. *Appl. Microb. Biotech.*, **31**: 577-581.

8. Gupta, D.K. (1983). Studies on the microbiological control of mosquito causing malaria. Ph.D. thesis. Dr. H.S. Gaur: Vishwavidhyalaya, Sagar, 133.

9. Lysenko, O., E.W. Davidson, L.A. Lacey and A.A. Yousten (1985). Five new mosquito larvicidal strains of *Bacillus sphaericus* from non-mosquito origins. *J. Amer. Mosq. Contr. Assoc.*, **1** (3): 369-371.

10. Menon, K.K.R., A.S. Rao and S.V. Amonkar (1982). Isolation, identification and toxicity of a spore-bearing *Bacillus* (ISPC-5) from diseased mosquito larvae. *Indian J. Exp. Biol.*, **20**: 312-315.

Indian Journal of Malariology
Vol. 28, September 1991, pp. 151-155.

Aquatic Macrophytes and the Associated Mosquitoes in and around Madurai City (Tamil Nadu)

T. JOHN VICTOR*, S. MARIMUTHU* and K.G. SIVARAMAKRISHNAN*

A survey of mosquitoes was carried out in 7 weeded astatic ponds in and around Madurai from October 1990 to January 1991 to qualitatively determine the macrophyte-mosquito larvae association. Members of *Culex vishnui* subgroup were associated with most of the macrophytes. *Eichhornia crassipes, Marsilea quadrifolia* and *Spirodella polyrhiza* showed a high potential for mosquito breeding and *Azolla* sp., a very low potential for both anophelines and culicines.

INTRODUCTION

Many species of mosquitoes prefer habitats with well-developed beds of submergent, floating-leaf or emergent aquatic macrophytes[1-3]. The macro-invertebrate fauna of water bodies with dense aquatic vegetation are mostly associated with macrophytes. Owing to their greater leaf dispersion aquatic macrophytes provide a lot of space for periphytic organisms and thus, better feeding grounds for macro-invertebrates[4]. Aquatic macrophytes can enhance the survival of immature stages of mosquitoes by providing favourable microhabitat and refuge from predation[5-7]. The vegetated regions of aquatic ecosystem provide protection from physical disturbance, mechanical support, in other words, and also favourable conditions for oviposition[1,8-11]. The macrophyte surface canopy may reduce the effectiveness of natural predators and thus enhance survival of mosquito larvae by harbouring them.

This study aims at updating the knowledge of the distribution and abundance of mosquito species in different ponds with dense macrophyte vegetation and at determining the potentiality of different macrophytes to provide mosquito larvae refuge from natural predation. Since larval survival is strongly influenced by vegetation type[12] we have studied the mosquito larva-macrophyte association qualitatively in the study area.

MATERIAL AND METHODS

Description of study site

The collections were confined to seven weeded astatic ponds in and around Madurai City (11°N and 77°50'E), Tamil Nadu. The annual rainfall in the region ranges from 68 to 372 mm, and is mainly influenced by the northeast monsoon (October to January).

Accepted for publication : 10 July 1991.

* Centre for Research in Aquatic Entomology
Department of Zoology
Madura College
Madurai - 625 011, India.

Immature stages of mosquitoes were collected from different ponds with different species of macrophytes, namely *Eichhornia crassipes, Marsilea quadrifolia, Spirodella polyrhiza, Typha angustata, Ottelia alismoides, Lemna paucicostata* and *Azolla* sp. They were reared in the laboratory to adult stage and were identified on the basis of adult characters. Macrophytes were collected from respective ponds and identified.

RESULTS AND DISCUSSION

A total of 9 species of mosquitoes were collected from different ponds vegetated with various types of aquatic macrophytes (Table 1). Among the species collected, members of *Culex vishnui* subgroup, vectors of Japanese encephalitis, were found to be more in number (53.57%). They were associated with the macrophytes, namely *E. crassipes, L. paucicostata, M. quadrifolia, O. alismoides* and *S. polyrhiza* and among the three species of *Cx. vishnui* subgroup, *Cx. tritaeniorhynchus* was associated with all these five species of macrophytes. *Cx. gelidus*, which is also a vector of JE, was collected (14.91%) only from ponds with *E. crassipes* vegetation.

Among the anophelines collected, *An. subpictus* (21.27% of the total collection) was found to have a wide distribution in different ponds. The ponds vegetated with *Azolla* spp. and *T. angustata* were

Table 1. List of mosquito species and predators in association with different macrophytes

Mosquito species collected	Macrophyte and the natural predators present in the pond							
	Azolla sp. (Hemipteran bugs) Dissolved O_2 4.5 mg/l	*Eichhornia crassipes* (*Anisops* sp., *Sphaerodema* sp., *Ranatra* sp., Odonates and Coleopteran beetles) Dissolved O_2 4.9 mg/l	*Lemna paucicostata* (*Sphaerodema* sp., *Anisops* sp., and Odonates) Dissolved O_2 5.3 mg/l	*Marsilea quadrifolia* (Hemipteran bugs) Dissolved O_2 5.1 mg/l	*Ottelia alismoides* (*Laccotrephes* sp. and Odonates) Dissolved O_2 4.5 mg/l	*Spirodella polyrhiza* (*Ranatra* sp., and *Anisops* sp.) Dissolved O_2 5.1 mg/l	*Typha angustata* (Predators were not found during collection) Dissolved O_2 5.4 mg/l	% in total collection
An. (Ano.) barbirostris Van der Wulp	6	–	5	–	–	–	6	4.69
An. (Ano.) peditaeniatus (Leicester)	–	4	–	–	–	–	–	1.10
An. (Cel.) subpictus Grassi	8	–	4	43	–	18	4	21.27
An. (Cel.) vagus Doenity	–	–	–	–	–	2	–	0.55
Cx. (Cux.) bitaeniorhynchus Oviles	–	–	14	–	–	–	–	3.86
Cx. (Cux.) gelidus Theobald	–	54	–	–	–	–	–	14.91
Cx. (Cux.) pseudovishnui Colless	–	4	–	10	19	9	–	11.60
Cx. (Cux.) tritaeniorhynchus Giles	–	21	13	22	17	63	–	37.56
Cx. (Cux.) vishnui Theobald	–	–	–	16	–	–	–	4.41

found to have less potential for mosquito fauna and from these two ponds no culicine larva was collected. *An. vagus* was present only in the pond with *S. polyrhiza* vegetation. The natural predators present in ponds during collection are given in Table 1. The distributions of anophelines and culicines in association with different types of macrophytes are given in Figs. 1 and 2 respectively.

In this study, *An. subpictus* was collected in large numbers in the pond vegetated with *M. quadrifolia* and no anopheline larva was collected in the pond vegetated with *O. alismoides*. The preference of the pond with *M. quadrifolia* for *An. subpictus* may be due to the greater leaf dispersion of the plant in the water surface. Since *O. alismoides* is a submerged plant, it could not provide much leaf dispersion in the water surface and thus the microhabitat for anophelines was unfavourable. Singh[4] has also emphasized that greater leaf dispersion provides better feeding grounds for macro-invertebrates. Ponds vegetated with *Azolla* sp. showed a very low potential for mosquito breeding. Rajendran[13] has also reported that if more than 80% water surface is covered with *Azolla* sp., a significant reduction in larval densities of both anophelines and culicines is observed. In Singapore, Colless[14] found the members of *Cx. vishnui* group breeding in hyacinth ponds. Reuben[15] has also found a large number of *Cx. tritaeniorhynchus* breeding in ponds in North Arcot district, Tamil Nadu, India. Zaim[16] has found that vegetation in ponds provides favourable conditions for mosquito larvae; he has also found a greater number of *Cx. tritaeniorhynchus* breeding in transient water. The presence of a large number of mosquito larvae in the pond with *E. crassipes* vegetation may be due to the influence of structural complexity of

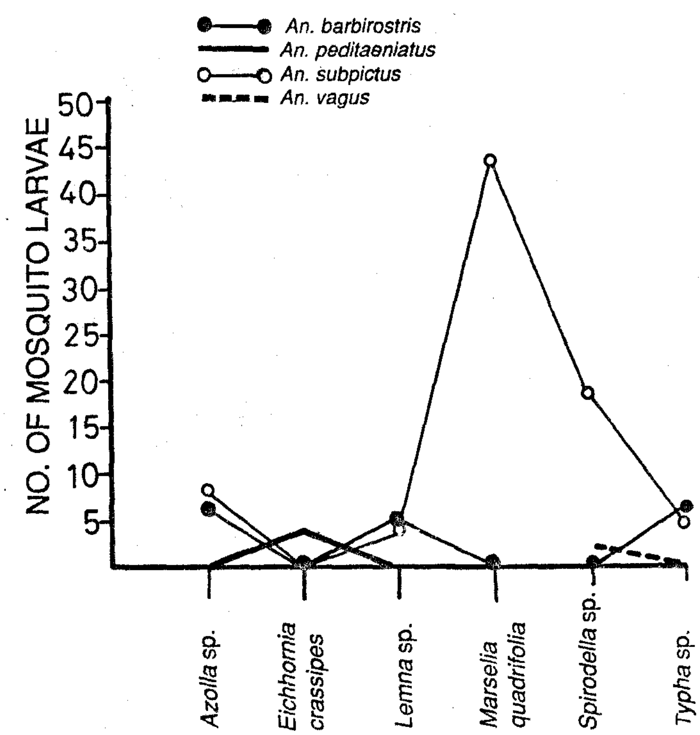


Fig. 1: Distribution of anophelines in association with some common aquatic macrophytes in ponds in and around Madurai (Oct. 1990-Jan. 1991).

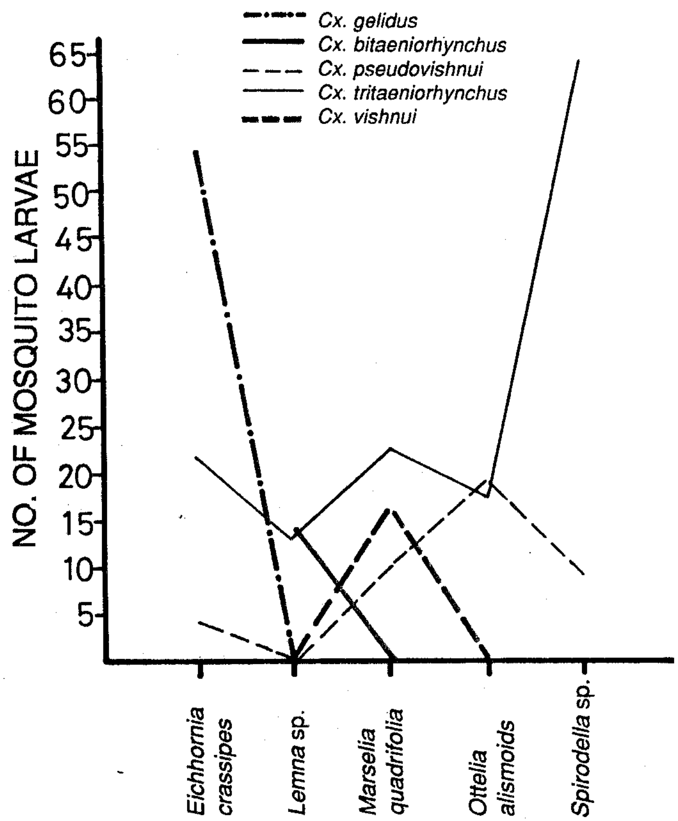


Fig. 2: Distribution of *Culex* spp. in association with some common aquatic macrophytes in ponds in and around Madurai (Oct. 1990-Jan. 1991).

the plant canopy, which in turn may reduce the effectiveness of natural predators of mosquito larvae present in the pond. Similar results have been obtained by Orr and Resh[17], who have also determined the increased survival of mosquito larvae with increasing plant cover.

ACKNOWLEDGEMENTS

We are thankful to Dr. R. Reuben, Director, Centre for Research in Medical Entomology (ICMR), Madurai, for giving us permission to utilize the laboratory facilities of the Centre. Our thanks are due to staff members of Faunistic Survey Division of CRME (ICMR), Madurai, who confirmed the identifications of our mosquito specimens. We are grateful to Dr. K. Venkataraman for his valuable suggestions.

REFERENCES

1. Hess, A.D. and T.F. Hall (1943). The intersection line as a factor in anopheline ecology. *J. Nat. Malar. Soc.*, 2:93-98.

2. Aitken, T.H.G. (1945). Studies on the anopheline complex of Western America. *Univ. Calif. Pub. Entomol.*, 7:273-364.

3. Service, M.W. (1980). *A Guide to Medical Entomology.* (Macmillan Press Ltd., London).

4. Singh, D.N. (1989). Studies on weed associated macrofauna of an oxbow lake. *Proc. Nat. Acad. Sci. Ind.*, 59(B)III: 271-277.

5. Bailey, S.F. and D.C. Baerg (1966). Ecological studies of California anophelines — a preliminary report. *Proc. Calif. Mosq. Vect. Contr. Assoc.*, 34:54-59.

6. Bay, E.C. (1967). Potential for naturalistic control of mosquitoes. *Proc. Calif. Mosq. Vect. Contr. Assoc.*, 35: 34-37.

7. Collins, J.N., S.S. Balling and V.H. Resh (1983). The Coyote Hills Marsh Model: calibration of interactions among floating vegetation, waterfowl, invertebrate predators, alternate prey, and *Anopheles* mosquitoes. *Proc. Calif. Mosq. Vect. Contr. Assoc.*, 51:69-73.

8. Collins, J.N., K.D. Gallagher and V.H. Resh (1985). Thermal characteristics of aquatic habitats at Coyote Hills Marsh: implications for simulation and control of *Anopheles* mosquitoes. *Proc. Calif. Mosq. Vect. Contr. Assoc.*, 53:83-86.

9. Orr, B.K. and V.H. Resh (1987). Interactions among mosquito fish (*Gambusia affinis*), Sago pondweed (*Potamogeton pectinatus*), and the survivorship of *Anopheles* mosquito larvae. *Proc. Calif. Mosq. Vect. Contr. Assoc.*, 55:94-97.

10. Walker, E.D., R.W. Merritt and R.S. Wotton (1988). Analysis of the distribution and abundance of *Anopheles quadrimaculatus* (Diptera:Culicidae) larvae in a marsh. *Environ. Entomol.*, 17:992-999.

11. Collins, J.N. and V.H. Resh (1989). Guidelines for ecological control of mosquitoes in non-tidal wetlands of the San Francisco Bay Area. California Mosquito and Vector Control Association, Sacramento. *Chem. Abstr.*: 93.

12. Orr, B.K. and V.H. Resh (1990). Influence of aquatic macrophytes on the distribution and abundance of anopheline mosquitoes in a California freshwater marsh. *Bull. North Amer. Bentholog. Soc.*, 7(1):70 (abstr.).

13. Rajendran, R. (1988). Studies with *Azolla* in rice fields. Centre for Research in Medical Entomology (ICMR), Madurai, India. Annual Report (1988-1989):41-44.

14. Colless, D.H. (1957). Notes on the culicine mosquitoes of Singapore: Part III–Larval breeding places. *Ann. Trop. Med. Parasitol.*, 51:102-116.

15. Reuben, R. (1971). Studies on the mosquitoes of North Arcot District, Madras State, India: Part 5—Breeding places of the *Culex vishnui* group of species. *J. Med. Entomol.*, 8(4):363-366.

16. Zaim, M. (1987). The distribution and larval habitat characteristics of Iranian Culicinae. *J. Amer. Mosq. Contr. Assoc.*, 3(4):568-573.

17. Orr, B.K. and V.H. Resh (1989). Experimental test of the influence of aquatic macrophyte cover on the survival of *Anopheles* larvae. *J. Amer. Mosq. Contr. Assoc.*, 5:579-585.

Indian Journal of Malariology
Vol. 28, September 1991, pp. 157-159.

Gel Diffusion Analysis of Host Preference Pattern of *Anopheles subpictus* in West Bengal, India

P.K. BANERJEE*, K.N. GHOSH* and R.N. CHATTERJEE*

Host preference pattern of *Anopheles subpictus* was studied from cattlesheds and human dwellings of Uttarpara, Hooghly, West Bengal using a modified gel diffusion technique. Our results reveal that *An subpictus* is becoming less zoophagic in this area.

INTRODUCTION

Previous studies[1-5] have shown that the species *Anopheles subpictus* acts as malaria vector in different parts of southeast Asia. As there is resurgence of malaria in eastern India particularly in West Bengal recently, it has necessitated the blood meal study of the anopheline mosquitoes of this area, as suggested by Weitz[6]. Since, *An. subpictus* is abundantly distributed throughout West Bengal[7,8] and since no attempt has been made so far to analyse the host preference pattern of the species, the present investigation was undertaken to analyse the blood meals of the species.

MATERIAL AND METHODS

The investigation was carried out in Uttarpara, Hooghly (West Bengal). The freshly engorged (blood fed) females were collected in the morning (0600 to 0900 hours) from cattleshed and human dwellings (close to cattleshed) by suction tube. The average distance between cattleshed and human dwellings ranged from 15 to 20 m. Mosquito identification was made according to Roy and Brown[9]. Modified gel diffusion method[10] was used in the study. The mosquito blood meal was eluted into 200 µl of phosphate buffer (0.01 *M*, pH 7.2) at room temperature. Three antisera, viz. human, buffalo and cow, were raised and/or purchased from Serologist and Chemical Examiners, Government of India, Calcutta. The antisera and mosquito blood meal were put in the peripheral and central well respectively and kept at room temperature as described by Collins *et al.*[10] (Fig. 1).

Contingency chi-squre test was used for the analysis of level of significance.

RESULTS AND DISCUSSION

Table 1 gives the data on blood meal analysis of *An. subpictus* by gel diffusion precipitation test. Data reveal that in the population collected from

Accepted for publication : 16 July 1991.

* Department of Zoology
University of Calcutta
35, Ballygunge Circular Road
Calcutta-700 019, India.

Fig. 1: Gel diffusion of two test patterns showing positive precipitate reaction between wells.

Central wells (M) represent the mosquito extract of unknown antigen; top well contains buffalo antisera (B), left well human antisera (H) and right well cow antisera (C).

Table 1. Data on blood meal indices determined by gel diffusion technique

Habitat	No. of mosquitoes examined	Positive smear			χ^2 value
		Human only O	Mixed blood (Human + Bovine) HB	Bovine (B) (Cow + Buffalo)	
Cattleshed	160	0	12 (7.5%)	148 (92.5%)	20.85
Human dwelling	161	0	43 (26.71%)*	118 (73.29%)	

* Significantly different ($p < 0.001$).

cattleshed, the number of blood meal positives for human and bovine (HB) and only bovine (B) were 7.5% and 92.5% respectively. On the other hand, in human dwellings they were 26.7% and 73.29% respectively. Analysis of the data reveal that human dwellings contributed a three-fold higher rate of human fed specimen than cattlesheds (Table 1). This observation leads us to conclude that there is a direct influence of the host availability on biting habit of mosquitoes. A similar situation was also noted earlier by Bruce-Chwatt *et al.*[11]. However, Collins *et al.*[12] observed that in Orissa, *An. subpictus* population was poorly anthropophagic but strongly zoophagic. We believe that the difference in the behaviour is due to the different ecological conditions of the two states. This view is greatly strengthened by the finding of Burkot *et al.*[13], who also noted that in anopheline mosquitoes the host feeding pattern is directly related to the ecological condition of that area.

ACKNOWLEDGEMENTS

Authors wish to thank Dr. S.K. Subbarao and her colleagues for their help during the investigation and preparation of the manuscript. We are also grateful to Prof. T.N. Ghosh for his help and advice.

REFERENCES

1. Rahman, M. and A. Muttalib (1967). Determination of malaria transmission in central part of Karachi city and incrimination of *An. stephensi* as vector. *Pakistan J. Health*, 17 : 73-84.

2. Lien, J.C., A.U. Usfinit, S. Atmosoedjono and B.F. Gundelfinger (1975). Observation on natural plas-

modial infections in mosquitoes and a brief survey of mosquito fauna in Belu Regency, Indonesian Timor. *J. Med. Entomol.,* **12** : 333-337.

3. Panicker, K.N., M. Geetha Bai, U.S. Bheema Rao, K. Viswam and U. Suryanarayana Murthy (1981). *Anopheles subpictus,* a vector of malaria in coastal villages of southeast India. *Curr. Sci.,* **50** : 694-695.

4. Suguna, S.G. (1982). Cytological and morphological evidences for sibling species in *Anopheles subpictus* Grassi. *J. Com. Dis.,* **14** (1) : 1-9.

5. Kulkarni, S.M. (1983). Detection of sporozoites in *Anopheles subpictus* in Bastar District, Madhya Pradesh. *Indian J. Malariol.,* **20** : 151-160.

6. Weitz, B. (1956). Identification of blood meals of blood sucking arthropods. *Bull. WHO,* **15** : 473-490.

7. Iyengar, M.O.T. (1931). The distribution of *Anopheles ludlowii* in Bengal and importance in malarial epidemiology. *Indian J. Med. Res.,* **19** : 499-522.

8. Anon. (1984). Annual Report 1983-84. Malaria Research Centre (ICMR), Delhi.

9. Roy, D.N. and A.W.A. Brown (1956). *Entomology* (Medical and Veterinary). 2nd edn. (Excelsior Press) : 44-69.

10. Collins, R.T., B.K. Dash, R.S. Agarwala and K.B. Dhal (1986). An adaption of the gel diffusion technique for identifying the source of mosquito blood meals. *Indian J. Malariol.,* **23** : 81-89.

11. Bruce-Chwatt, L.J., C. Garret-Jones and B. Weitz (1966). Ten years study (1955-1964) of hosts selection by anopheline mosquitoes. *Bull. WHO,* **35** : 405-435.

12. Collins, R.T., G.K. Sharma and K.B. Dhal (1989). Gel diffusion determinations on feeding behaviour patterns of Anophelines in Orissa State, India. *J. Com. Dis.,* **21** (1) : 1-23.

13. Burkot, T.R., A. Narara, R. Peru, P.M. Graves and O. Gerner (1989). Human host selection by Anophelines : no evidence for preferential selection of malaria of microfilariae-infected individuals in hyperendemic area. *Parasitol.,* **98** : 337-342.

Indian Journal of Malariology
Vol. 28, September 1991, pp. 161-165.

Genetic Markers in Refractory and Susceptible Malaria Patients in Village Bhanera, Distt. Ghaziabad, U.P.

HEMA JOSHI*, K. RAGHAVENDRA*, SARALA K. SUBBARAO*, M.A. ANSARI*, R.K. RAZDAN* and C.P. BATRA*

Malaria 'susceptible' and 'refractory' subjects from village Bhanera in District Ghaziabad (Uttar Pradesh) were examined for various genetic markers, viz., ABO, haptoglobin, haemoglobin and glucose-6-phosphate dehydrogenase polymorphism. One hundred and nine susceptible and 36 refractory subjects were studied. No significant differences with respect to distribution patterns of the genetic markers were observed in the two groups except for AB blood group. In general, a high incidence of ahaptoglobinaemia was observed in this population and incidence increased with the increase in malaria attacks, suggesting that repeated malaria attacks cause ahaptoglobinaemia.

INTRODUCTION

The selective advantage of glucose-6-phosphate dehydrogenase deficiency and sickle cell haemoglobin against *Plasmodium falciparum* infections[1] and Duffy negative antigen against *P. vivax* infection[2,3] has been well established in African populations. In Central Sudan, Bayoumi *et al.*[4] have shown a significantly higher prevalence of HbS, 6-phosphogluconate dehydrogenase A (PGD-A) and phosphoglucomutase (locus 1) among individuals "resistant" to *P. falciparum* infection. In our regular epidemiological surveys, it has been observed that in village Bhanera (Loni PHC, Distt. Ghaziabad, U.P.) during the malaria transmission period, some of the residents suffered multiple attacks of *Plasmodium vivax* as well as *P. falciparum* malaria while others did not (Malaria Research Centre, Annual Report, 1983-1984). The "refractoriness" to malaria was observed between the families and within the members of the family.

Therefore, a study was initiated to examine genetic markers, viz., ABO, haptoglobin, haemoglobin and G-6-PD polymorphism, in refractory and susceptible individuals from Bhanera. The results of analysis of data on the relationship between malaria and genetic markers are reported in this study.

MATERIAL AND METHODS

Study areas

The study was conducted during 1984-1985 in village Bhanera (Loni PHC, Distt. Ghaziabad,

Accepted for publication: 22 July 1991.

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

U.P.) located on the bank of river Hindon. Farming and dairying are the villagers' main occupations. Because of low economic status all the family members (minimum 5 to 6 persons in a family) live in one or two rooms.

Malaria in Bhanera was hyperendemic during 1983-1984 (Malaria Research Centre, Annual Report, 1983-84). Slide positivity rate (SPR) and slide falciparum rate (SfR) ranged between 74.02 and 91.04 and 63 and 98.03, respectively. Peak transmission of *P. vivax* malaria was in August-September and that of *P. falciparum* in September-October.

Study samples

Blood (0.3-0.4 ml) was collected in a heparinized vial by pricking a finger and a smear was also made for parasite examination. At the time of blood collection the case history was also noted. Subjects selected had resided for at least 3 years in the study village.

Susceptible subjects (Malaria patients)

Blood samples from 109 susceptible subjects were collected, of which 46 were males and 63 females. Of these, nine samples were positive for malaria at the time of blood collection. Based on the number of malaria attacks the subjects had during their stay in Bhanera, they were subgrouped as follows: (i) one or two attacks, and (ii) three or more attacks.

Refractory subjects

Subjects with no history of malaria or malaria-like symptoms who never took treatment for malaria or never took prophylaxis were considered "refractory" subjects. Thirty-three subjects, 17 males and 16 females, were identified as "refractory".

Sample analysis

Methods used for the collection, storage and analysis of samples were the same as described by Joshi *et al.*[5,6]. ABO grouping was performed according to the standard tube technique of Bhatia[7]. Haptoglobin typing was carried out by electrophoresis using 5 per cent polyacrylamide horizontal slab gel at 25°C for 2 hrs at a constant current of 40 mÅ[5]. Gels were stained with benzidine hydrochloride. Haemoglobin electromorphs were typed using cellulose acetate membrane and for glucose-6-phosphate dehydrogenase deficiency fluorescent spot test was used[8]. Electrophoretic phenotypes of G-6-PD were identified on 7.5 per cent polyacrylamide horizontal slab gel with 0.1*M* phosphate buffer (pH 7.0) and also by the staining procedure described by Mathai *et al.*[9] Blood smears were stained with JSB and examined under a bright field Leitz microscope (1000 x) for malaria parasites[10].

Fisher's chi-square test was used to find out if the groups differed significantly as far as these genetic parameters are concerned. However, to determine the association of malaria susceptibility or refractoriness to the polymorphs, Woolf's method as used by Bayoumi *et al.*[4] was adopted.

RESULTS AND DISCUSSION

Distribution of phenotypes and gene frequencies of polymorphic genetic traits in malaria patients and subjects refractory to malaria are shown in Table 1.

Frequencies observed for various ABO groups were 24.4% for group 'A', 37.8% for group 'B', 11.1% for group 'AB' and 26.7% for 'O' blood group among susceptible groups. Among the malaria refractory group, 23.4% were of group 'A', 33.3% of group 'B' and 'AB' each, and 10.0% of group 'O'. Allelic frequencies were 0.1979 for p, 0.2865 for q, 0.5156 for r among malaria susceptible group and 0.32, 0.40 and 0.28 respectively among malaria refractory group.

Observed frequencies were within the reported range for Indian populations[11]. Frequencies of p and q alleles were higher in the refractory group while the frequency of r was higher in the malaria-

Table 1. Gene frequencies among malaria susceptible and refractory individuals

Phenotypes	Susceptible		Refractory	
	No. obs. (%)	Gene freq.	No. obs. (%)	Gene freq.
ABO				
A	22 (24.4)	p = 0.1979	7 (23.4)	p = 0.32
B	34 (37.8)	q = 0.2865	10 (33.3)	q = 0.40
AB	10 (11.1)	r = 0.5156	10 (33.3)	r = 0.28
O	24 (26.7)		3 (10.0)	
Total	90		30	
Haptoglobin				
Hp 1-1	5 (4.76)		3 (11.11)	
Hp 2-1	24 (22.86)	$Hp^1 = 0.2537$	10 (37.04)	$Hp^1 = 0.4211$
Hp 2-2	38 (36.19)		6 (22.22)	
Hp 0-0	38 (36.19)	$Hp^2 = 0.7463$	8 (29.63)	$Hp^2 = 0.5789$
Total	105		27	

susceptible group. Fisher's comparative chi-square analysis has shown that the two groups differed significantly with respect to ABO polymorphism ($\chi^2 = 9.57$, df = 3, $p<0.05$).

Analysis of data by Woolf's method has shown that the proportion of AB phenotype was significantly higher among the refractory subjects than among the malaria patients ($\chi^2 = 7.27$, df = 1, $p<0.01$), suggesting that subjects with AB phenotype are less susceptible to malaria. However, no correlation between ABO blood groups and malaria was observed in African populations[12], and in various Indian populations, viz., tribals of Dadra and Nagar Haveli[13], population of Delhi[6] and Buksa tribe of Distt. Nainital, U.P., Gond tribe of Distt. Mandla, M.P., and tribes of N.E. states (unpublished data).

Frequencies observed for common polymorphic forms of haptoglobin were 4.76% for Hp 1-1, 22.86% for Hp 2-1 and 36.19% for Hp 2-2 among malaria susceptible subjects while among malaria refractory subjects, frequencies observed were 11.11, 37.04 and 22.22% respectively. Gene frequencies calculated were 0.2537 and 0.4211 for Hp^1 and 0.7463 and 0.5789 for Hp^2 among malaria susceptible and refractory subjects respectively.

Frequencies of Hp^1 and Hp^2 alleles observed in the study were within the reported range for Indian populations[14]. Among the refractory subjects, the frequency of Hp^1 was 0.42 whereas among the malaria patients, it was 0.25. However, the analyses of data by Fisher's and Woolf's methods have shown that the differences were not statistically significant.

The incidence of ahaptoglobinaemia (Hp^o) in various Indian populations ranges between 0 and 7%[14]. In this population, incidence of Hp^o (34.85%) is very high and does not fall in the reported range. Further, it is observed that subjects with frequent attacks of malaria (3 or more) had a higher occurrence of Hp^o (42.2%) than those who did not have any (29.6%) (Table 2).

Table 2. Incidence of ahaptoglobinaemmia Hp°

Groups	Samples tested	Hp°	
		Numbers	Percentage
Refractory (No attack)	27	8	29.6
One or two attacks	60	19	31.7
Three or more attacks	45	19	42.2

In our earlier study on Delhi population, a higher incidence of Hp° was observed among the malaria patients (19.6%) than in the normal controls (3.9%). Trape *et al.*[15] reported malaria to be the only significant cause of ahaptoglobinaemia in tropical African populations where ahaptoglobinaemia was suppressed within a few weeks of treatment with antimalarials. Studies by Hill *et al.*[16] have also reported similar findings in Melanesia population.

It was observed that 3.6% of the individuals belonging to susceptible group were deficient for G-6-PD enzyme in comparison to 9.1% in the refractory group. The higher value observed in refractory group supports the view that G-6-PD deficiency has a protective role against malaria. The incidence of G-6-PD deficient allele varied from 0 to 20% within the Indian subcontinent with a few exceptions[17]. In the present study, all the subjects had the common electrophoretic form G-6-PD 'B'.

All the subjects in this study were of haemoglobin A type. Absence of HbAS in our study samples is not unexpected. The incidence of sickle cell haemoglobin in Indian populations varies from 0 to 30%, and northern Indian populations are generally characterized by a very low incidence of HbS allele[18].

Though a significantly higher frequency of 'AB' blood group was found among the refractory subjects, it is not possible to draw a definitive conclusion because of the generally low frequencies of 'AB' blood group in the populations and also of low sample size of the study population. However, the study is indicative of further work on the relationship between malaria incidence and blood group phenotypes.

ACKNOWLEDGEMENTS

We wish to thank Mr. Y.P. Chawla, Mr. Surinder Kumar, Mrs. Alka Kapoor, Mrs. Lalita Gupta and Mrs. Poonam Gupta for technical assistance.

REFERENCES

1. Luzzatto, L., O. Sodeinde and G. Martin (1983). Genetic variation in the host and adaptive phenomenon in *Plasmodium falciparum* infection. In *Malaria and red cells*. Ciba Foundation Symposium 94. (Pitman, London) : 159.

2. Miller, L.H., S.J. Mason, J.A. Dvorak, M.H. McGinniss and I.K. Rothman (1975). Erythrocyte receptors for (*Plasmodium knowlesi*) malaria : Duffy blood group determinants. *Science*, **189** : 561-562.

3. Miller, L.H., S.J. Mason, D.F. Clyde and M.H. McGinniss (1976). Resistance factor to *Plasmodium vivax* in Blacks—The Duffy-Blood-Group genotype, FyFy. *New Eng. J. Medicine*, **295**: 302-304.

4. Bayoumi, R.A., A.H. Bashir and N.H. Abdulhadi (1986). Resistance to *Falciparum* malaria among adults in Central Sudan. *American J. Trop. Med. Hyg.*, **35** (1) : 45-55.

5. Joshi, Hema, K. Raghavendra, Sarala K. Subbarao and V.P. Sharma (1985). Distribution of human blood polymorphic systems in two Haryana villages. *Indian J. Med. Res.*, **81** : 180-185.

6. Joshi, Hema, K. Raghavendra, Sarala K. Subbarao and V.P. Sharma (1987). Genetic markers in malaria patients of Delhi. *Indian J. Malariol.*, **24** : 33-38.

7. Bhatia, H.M. (1977). *Procedures in blood banking and immunohaematology*. (Indian Council of Medical Research, New Delhi): 13.

8. Schmidt, Robert M. and Effie M. Brosious (1978). *Basic Laboratory methods of haemoglobinopathy detection*. (US Deptt. of Health, Education and Welfare, Atlanta, Georgia, USA).

9. Mathai, C.K., S. Ohno, and Earnest Beutler (1966). Sex linkage of the G-6-PD gene in equidae. *Nature*, **210** : 115.

10. Singh, Jaswant, A.P. Ray and C.P. Nair (1953). J.S.B. Stain — Its preparation in the powder form and the staining technique. *Indian J. Malariol.*, **7** : 267-270.

11. Majumdar, P.P. and J. Roy (1982). Distribution of ABO blood groups in the Indian sub-continent : A cluster analytic approach. *Curr. Anthropol.,* **23** : 538.

12. Molineaux, L. and G. Gramiccia (1980). *The Garki Project*: Research on the epidemiology and control of Malaria in the Sudan Savanna of West Africa. (WHO, Geneva): 1-311.

13. Vasantha, K., A.C. Gorakhshaker, R.S. Pavri and H.M. Bhatia (1982). Genetic markers in malaria endemic tribal populations of Dadra and Nagar Haveli. In *Proc. Recent Trends in Immunohaematology*. (Institute of Haematology, ICMR, Bombay) : 162-168.

14. Seth, S., I.J.S. Bansal and P.K. Seth (1971). Studies on haptoglobin and transferrin types in Khatris and Aroras of Punjab, India. *Humangenetik.,* **14** : 44.

15. Trape, J.F., A. Fribourg-Blanc, M.F. Bosseno, M. Lallemant, R. Engler and J. Mouchet (1985). Malaria, cause of ahaptoglobinaemia in Africans. *Trans. R. Soc. Trop. Med. Hyg.,* **79** : 430-434.

16. Hill, A.V.S., D.B. Whitehouse, D.K. Bowden, D.A. Hopkinson, C.C. Draper, T.E.A. Peto, J.B. Clegg and D.J. Wealtherall (1987). Ahaptoglobinaemia in Melanesia DNA and malarial antibody studies. *Trans. R. Soc. Trop. Med. Hyg.,* **81** : 573-577.

17. Ghosh, T.N. (1983). Factors of human genetics in malaria. In *Proceedings of the Indo-UK Workshop on Malaria*, edited by V.P. Sharma. (Malaria Research Centre, ICMR, Delhi) : 251-267.

18. Sharma, A. (1983). Haemoglobinopathies in India. In *Peoples of India, some genetical aspects*. (Indian Council of Medical Research, New Delhi) : 31-49.

Indian Journal of Malariology
Vol. 28, September 1991, pp. 167-170.

Acute Toxicity of Certain Organochlorine, Organophosphorus, Synthetic Pyrethroid and Microbial Insecticides to the Mosquito Fish *Gambusia affinis* (Baird and Girard)

P.K. MITTAL*, T. ADAK* and V.P. SHARMA*

Acute toxicity of certain organochlorine, organophosphorus, synthetic pyrethroid and microbial insecticides to the mosquito fish *Gambusia affinis* were determined to collect baseline data for selecting the resistant strains of the fish The synthetic pyrethroid, Lambdacyhalothrin was most toxic to the fish (LC50 = 0.0022 ppm), followed by deltamethrin, cypermethrin and fenvalerate. Organochlorine insecticides, DDT and γ-HCH, were less toxic than the pyrethroids, and these were followed by organophosphorus insecticides, malathion, fenthion, monocrotophos and temephos. The last two insecticides were least toxic among the different chemical insecticides (LC50 > 80 ppm ai). The microbial insecticide ABG-6262 (Vectolex 2.5 AS), a *Bacillus sphaericus* preparation, was totally harmless to the fish at 2500 μl/l up to one week.

INTRODUCTION

Larvivorous fishes had been utilized in the control of malaria vectors even before the discovery of DDT[1]. Later, with the application of DDT and other residual insecticides in malaria control, the role of larvivorous fishes in malaria control became restricted to certain urban areas. Extensive use of insecticides in vector control resulted in the development of resistance in the vectors as well as in the contamination of environment[2]. To overcome the dual problem of insecticide resistance in the vectors and environmental contamination, the WHO expert committee on insecticides[3] noted the importance of integrated control and evaluation of new compounds against major vector species with particular emphasis on larvivorous fish *Gambusia affinis*, which has been extensively used in the control of mosquitoes. With increasing use of insecticides in agriculture and public health, it is inevitable that larvivorous fishes and other beneficial non-target organisms will also be exposed to insecticides due to contamination of their habitats, and may therefore be eliminated. Hence, it is advisable to select resistant stocks of the fishes, which may be utilized effectively in the control of mosquito breeding. Therefore, a laboratory study was carried out to determine the acute toxicity of certain insecticides to the mosquito fish *Gambusia affinis* to collect baseline data for selecting resistant lines.

Accepted for publication : 5 August 1991.

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

MATERIAL AND METHODS

The mosquito fish used in this study were obtained from MRC fish farms at Seelampur, Delhi, and were kept outdoors in an artificial tin tank. The fish were provided with fish food and also mosquito larvae as source of food and were given routine care required for their healthy maintenance. During this period the fish reproduced and multiplied in the tank. The insecticides, recrystallized *p/p*-DDT and γ-HCH, were of analytical grade, deltamethrin (technical 99.7%), cypermethrin (technical 71%), temephos (technical 90%), fenthion (technical 97%), K-othrine 2.5% flow and 'karate' (Lambdacyhalothrin 5% EC), were obtained directly from the manufacturers and malathion 50% EC, Fen-Fen (fenvalerate 20% EC), Monocil (monocrotophos 32% SC) and Decis (deltamethrin 2.5% EC) were purchased from the market. ABG-6262 (Vectolex 2.5 AS flow), a *Bacillus sphaericus* 2362 formulation, was supplied by Abbott Laboratories (USA) through WHO. The technical-grade insecticides were dissolved in absolute alcohol to prepare 0.1% and 1% stock solutions (10% stock for temephos and fenthion), while water-based flow and emulsifiable concentrate formulations were dissolved in water to obtain the stock suspensions.

The study was carried out during July–December 1990. The susceptibility tests were conducted in tarsons beakers of 5-l. capacity. Ten fish, 25 to 30 mm in length, were placed in each beaker containing 2 l. of stored tap water and at least four replicates were used for the same concentration. Thus a minimum of 40 fish were tested at each concentration. The test concentrations were prepared by dissolving 1 ml of the serially diluted stock solution in 2 l. of water. A pair of controls was run concurrently to check the control mortality and the tests were repeated if more than 20% control mortality was observed. The per cent mortality at different concentrations of insecticides was corrected from the control mortality by using Abbott's formula[4]. Lethal concentrations for 50% and 90% mortality were determined from the regression line plotted on a log probit paper and confidence limits for LC50 with probability of 95% were determined by the method of Litchfield and Wilcoxon[5].

RESULTS AND DISCUSSION

Table 1 shows the acute toxicity of different insecticides' formulations to the mosquito fish *Gambusia affinis*. The results revealed that the mosquito fish is highly susceptible to synthetic pyrethroids. Karate (Lambdacyhalothrin 5% EC) was most toxic (LC50 = 0.0022 ppm, ai) followed by deltamethrin, cypermethrin and fenvalerate. However, K-othrine 2.5% flowable formulation of deltamethrin was an exception (LC50 = 5 ppm ai), but Decis, the emulsifiable concentrate of deltamethrin was more toxic than the technical deltamethrin (LC50 = 0.0075 ppm ai). The low toxicity of K-othrine 2.5% flow is probably due to incomplete solubility of the insecticide in water, which may be due to the adsorption of the active ingredients to particulate material in the formulation. Organochlorine insecticides, DDT and γ-HCH were comparatively less toxic than the synthetic pyrethroids. Of the two organochlorine compounds, DDT was found more toxic (LC50 = 0.095 ppm) than γ-HCH (LC50 = 0.145 ppm). Pillai *et al.*[6] found an LC50 = 0.06 ppm of DDT to the mosquito fish *Gambusia affinis* collected from a pond in Delhi, while Joshi and Rege[7] reported an LC50 = 0.07 ppm to DDT in the mosquito fish collected from Masunda lake in Thana, Bombay. A higher LC50 in this study may be due to higher tolerance to DDT in the fish used in the study or may be due to development of resistance to DDT. Resistance to DDT and cross-resistance to other organochlorine insecticides in *Gambusia* has been reported from USA[8,9]. The toxicity of the organophosphorus insecticides tested in this study against *Gambusia affinis* was even less than that of the organochlorine insecticides. Both temephos and monocrotophos (Monocil) were least toxic (LC50 > 80 ppm ai), while malathion was most toxic (LC50 = 1.65 ppm) among the different organophosphorus insecticides. Fenthion was less toxic (LC50 =

Table 1. Acute toxicity of certain organochlorine, organophosphorus, synthetic pyrethroid and microbial insecticides to the mosquito fish *Gambusia affinis*

Insecticide/ Formulation	Active ingredients	LC50 (confidence limits) (ppm, ai)		LC90 (ppm, ai)	Regression equation	χ^2 (df)
DDT	pp-DDT	0.095	(0.078-0.114)	0.205	$Y=1.329+3.757\ x$	1.54(3)
HCH	γ-HCH	0.145	(0.123-0.169)	0.27	$Y=-0.154+4.439\ x$	7.78(3)
Fenthion (technical)	Fenthion	8.0	(6.89-6.64)	15.0	$Y=0.692+4.771\ x$	0.68(2)
Temephos* (technical)	Temephos	>80	—	—	—	
Malathion 50% EC	Malathion	1.65	(1.323-2.057)	4.0	$Y=1.055+3.241\ x$	6.49(3)
Monocil* 32% SL	Monocrotophos	>80	—	—	—	
Fen-Fen 20% EC	Fenvalerate	0.033	(0.029-0.038)	0.06	$Y=3.6+5.076\ x$	0.41(2)
Cypermethrin (technical)	Cypermethrin	0.0175	(0.015-0.02)	0.034	$Y=3.86+4.69\ x$	2.21(2)
Deltamethrin (technical)	Deltamethrin	0.0075	(0.006-0.009)	0.017	$Y=1.96+3.473\ x$	0.88(2)
K-othrine 2.5% flow	Deltamethrin	5.0	(3.76-6.64)	16.5	$Y=3.26+2.487\ x$	1.15(3)
Decis 2.8% EC	Deltamethrin	0.0066	(0.0056-0.0078)	0.01	$Y=0.42+5.586\ x$	1.98(2)
Karate 5% EC	Lambdacyhalothrin	0.0022	(0.0019-0.00025)	0.0037	$Y=3.0+5.847\ x$	0.784(2)
ABG-6262 Vectolex 2.5 AS flow	*Bacillus sphaericus*	>2500 µl/l (No mortality was observed up to one week)				

*Higher concentrations were not tested because the application dosage in the field is 1ppm or less.

8.0 ppm) than malathion but more toxic than temephos and monocrotophos. Darwazeh and Mulla[10] reported nil and 40% mortality after 48 hrs of exposure to *Gambusia affinis* against Abate (temephos EC) and fenthion EC at 5 ppm, respectively. They also noted that EC formulations of these compounds were more toxic to the fish than the technical-grade insecticides. Since the toxicity of monocrotophos and temephos to the fish is very low, these insecticides may be considered safe for use in conjuction with the fish; however, a prologed observation will be more useful than the acute toxicity of these insecticide. The microbial insecticide ABG-6262 (Vectolex 2.5 AS), a *Bacillus sphaericus* 2362 formulation, was highly safe to the fish: up to a concentration of 2500 ppm it was totally harmless up to one week of observation.

Almost all the chemical insecticides tested in the study were able to produce abortion in the Gravid females of the mosquito fish. A similar observation was also reported by Joshi and Rege[7].

REFERENCES

1. Hora, S.L. (1927). The use of fishes for the control of mosquitoes. *Indian Med. Gaz.,* **42**: 187-188.
2. WHO (1970). Insecticide resistance and vector control. *WHO Tech. Rep. Ser.,* No. 443.
3. WHO (1976). Resistance of vectors and reservoirs of disease to pesticides. Twenty-second report of the WHO expert committee on insecticides. *WHO Tech. Rep. Ser.* No. 585.
4. WHO (1963). Thirteenth report of WHO expert committee on insecticides. *WHO Tech. Rep. Ser.* 265.
5. Litchfield, J.T. (Jr.) and F. Wilcoxon (1949). A simplified method of evaluating of dose-effect experiments. *J. Pharmacol. Exp. Ther.,* **96**: 99-113.
6. Pillai, M.K.K., H.C. Agarwal and D.V. Yadav (1977). Tolerance uptake and metabolism of DDT in *Gambusia affinis. Indian J. Exp. Biol.,* **15** (1): 40-41.
7. Joshi, A.G. and M.S. Rege (1980). Acute toxicity of some pesticides and a few inorganic salts to the mosquito fish *Gambusia affinis* (Baird and Girard). *Indian J. Exp. Biol.,* **18** (4): 435-437.
8. Vinson, S.B., C.E. Boyd and D.E. Ferguson (1963). Resistance to DDT in the mosquito fish *Gambusia affinis. Science,* **139** : 217-218.
9. Boyd, C.E. and D.E. Ferguson (1964). Spectrum of cross-resistance to insecticides in the mosquito fish *Gambusia affinis. Mosq. News,* **24** (1): 19-21.
10. Darwazeh, H.A. and M.S. Mulla (1974). Toxicity of herbicides and mosquito larvicides to the mosquito fish *Gambusia affinis. Mosq. News,* **34** (2): 214-219.

Indian Journal of Malariology
Vol. 28, September 1991, pp. 171-177.

Evaluation of Mosquito Fish *Gambusia affinis* in the Control of Mosquito Breeding in Rice Fields

M.K. DAS* and R.N. PRASAD*

The mosquito control potential of *Gambusia affinis*, a mosquito fish, was evaluated in rice fields in the Shahjahanpur district of Uttar Pradesh. This fish, at a stocking rate of 5 fishes/sq m, significantly reduced the larval and pupal densities in experimental fields as compared to control fields during the entire observation period of 42 days. Control of mosquito breeding in rice fields through this fish seems to be promising.

INTRODUCTION

Rice cultivation contributes to the production of many mosquito vectors. In India, rice fields serve as extensive breeding places for several species of Anopheles, Culex, Aedes, Mansonia and Psorophora[1], and many of them are the vectors of several dreadful diseases. The control of the mosquitoes breeding in rice fields poses a most difficult problem in public health programmes. In the rice cultivating zone of Shahjahanpur dist., Uttar Pradesh, many deaths occurred owing to malaria in 1983 (August to October)[2], which may have been due to extensive breeding of the malaria vectors in rice fields, resistance of these vectors to insecticide, high falciparum infection etc. In view of the seriousness of the malaria situation, an alternative method to chemical control method, namely integrated disease vector control methodology, has been launched by the Malaria Research Centre (Field Station) in Shahjahanpur distt. This field station had also taken up research on the control of filariasis and Japanese encephalitis vectors, since both these diseases are endemic in this state. In this strategy, biological control of the disease vectors through the mosquito fish *Gambusia affinis* is becoming an important component.

The role of larvivorous fishes in the control of mosquito breeding in rice fields in India is poorly understood. The two indigenous fishes *Danio* and *Oryzias* tried in rice fields[3] have a common drawback, i.e. difficulty in their mass-scale production for field use. On the other hand the exotic fish *G. affinis* poses no such difficulties and can be cultivated on a large scale as the unusual plasticity of *G. affinis* makes it suitable for mass production, long distance transportation and application to the vector breeding sites. A large-scale production of *G. affinis* has been achieved by us in this area and the present stock is about 35 million[4]. Although rice fields are potential and major breed-

Accepted for publication: 8 August 1991.

* Malaria Research Centre (Field Station)
Khirni Bagh,
Shahjahanpur-242 001, India.

Table 1. Larvivorous potential of *Gambusia affinis* in experimental plots in rice fields

Days	Control*					Experimental*					
	I & II instars	III instar	IV instar	Pupae	Egg-raft	I & II instars	III instar	IV instar	Pupae	Egg-raft	%R‡
0	154	67	59	24	—	92	79	73	23	—	—
2	115	87	60	12	—	51	27	9	6	—	77
4	92	71	51	9	—	22	19	4	2	—	84
6	10	70	52	6	—	13	9	2	0	1	93
12	112	73	62	13	—	15	2	0	0	—	99
18	89	81	73	12	1	8	0	0	0	—	100
24	86	79	65	16	—	4	0	0	0	—	100
30	75	61	70	15	—	3	0	0	0	-	100
36	81	55	68	17	—	2	0.3	0	0	-	100

*Density per five dips; †Corrected per cent reduction in density of III and IV instar and pupae (after Mulla[7]).
Calculated "t" value at 5% level of significance is 6.11.

ing sites in this area[5] as well as in a major part of India, no information on the role of *G. affinis* in the control of mosquito breeding in rice fields in relation to the geographical reconnaissance is available. This study was therefore designed to evaluate the effectiveness of *G. affinis* in the control of mosquito breeding in alluvial rice fields in Shahjahanpur district.

MATERIAL AND METHODS

Study area

Chaknow village was selected for the trial of *G. affinis* for the control of mosquito breeding in the rice fields. The village is at a distance of 15 km from Shahjahanpur town, and is situated in a low-lying plain. Human dwellings are very close to the rice fields.

A field trial on the mosquito control efficacy in the rice field by *G. affinis* was carried out with the established protocol as described by Ungureanu *et al*[6]. A particular corner of a rice field with a high larval density was selected. Six quadrates of a square metre surface each were prepared. The walls of the quadrates were made of wood/bricks. The movement of mosquito larvae and fish was restricted to each quadrate. Rice plants were of 50-60 cms size in the quadrates. The water depth was maintained between 10 and 16 cm. The larval and pupal density was monitored by a standard dipper, measuring 250 ml. Out of the six quadrates, three were experimental and three control. Field trials were carried out from the last week of August to mid-October 1989.

G. affinis were seined from the rearing ponds of the village Satwan and transported in plastic drums to the experimental site. The fishes were released in experimental quadrates at the rate of five fishes per quadrate and no fish was introduced in the three remaining control quadrates. Immature mosquito populations were monitored by a dipper, restricted to 5 dips per quadrate, on days 2, 4, 6, 12, 18, 24, 30 and 36. The percentage reduction in the density of larvae and pupae was calculated by using the formula described by Mulla *et al*[7]. In addition to a small-scale trial, a large-scale trial in the rice field in the village Chaknow of Sindhauli PHC was also carried out. The fishes, which included fry and adults of both sexes, were released at the rate of 5 fishes sq m area with an area of 689 sq m. Another rice field with an area of 742 sq m served as control. The immature stages and pupal density were monitored for 42 days as described earlier. But here 16 dips were taken around the perimeter and 4 dips through the interior of the rice field. The results of both the experiments were statistically analysed by applying students 't' test to test the significance of difference in the mean of two densities.

Ten fishes were collected from the rice field weekly for gut content analysis to observe the occurrence frequency of mosquito larvae. All dip samples were immediately counted near the rice field and recorded. The average temperature and rainfall were also recorded. The experiments were conducted during the high mosquito breeding period. Immature stages were brought from the rice fields to the vivarium for adult emergence and identification.

RESULTS

During the experimental period the average rainfall was 115.7 mm and the number of rainy days was 13. The water depth in the rice field ranged between 12 and 16 cm and pH, between 7 and 8. There were, on an average, 30 rice plants/sq m area.

The results obtained during the trials in rice field quadrates (Table 1 and Fig. 1) show that *G. affinis* were effective in lowering the immature mosquito population in the experimental field. Overall reduction was observed from the beginning. *G. affinis* reduced the larval densities by 77% on second day. Gradually the reduction was more prominent and the percentage reduction ranged between 84 and 100 during the experimental pe-

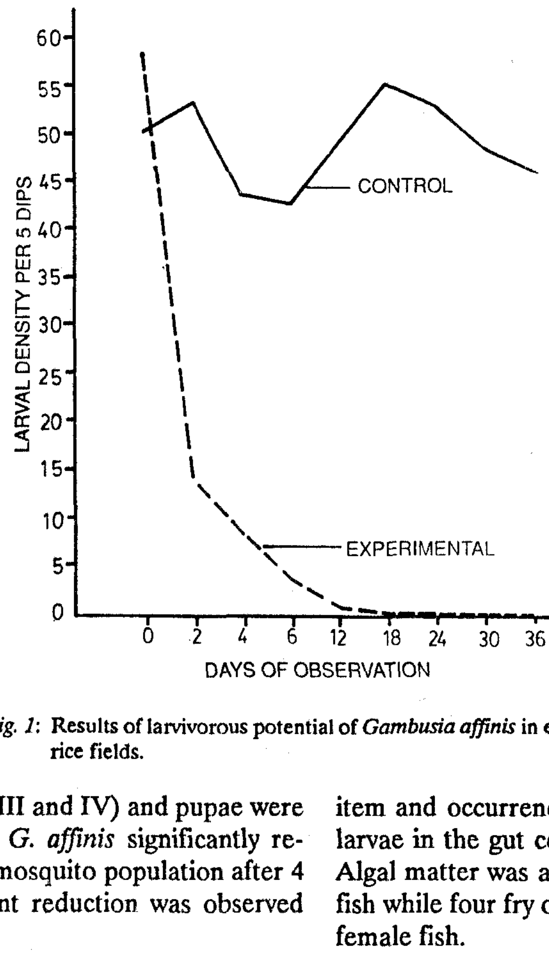


Fig. 1: Results of larvivorous potential of *Gambusia affinis* in experimental rice fields.

riod. The late instar (III and IV) and pupae were separately evaluated. *G. affinis* significantly reduced the immature mosquito population after 4 days and 100 per cent reduction was observed after 12 days.

In the large-scale trial in the rice field (Table 2 and Fig. 2) the immature mosquito population in experimental fields was significantly lower in 6 days. The density of IV instar and pupae was reduced in 12 days, it was nil up to the last day of the observation, except that there was only one-fourth instar on 36 days of observation. The appearance of one-fourth instar larvae was negligible. Statistical analysis of the results of both the experiments showed significant differences in densities.

Fifty *G. affinis* were dissected for gut content analysis. The fish were found to feed mainly on insect larvae, phytoplanktons and zooplanktons. Mosquito larvae constituted the bulk of the food item and occurrence frequency of the mosquito larvae in the gut content of *G. affinis* was 80%. Algal matter was also observed in the gut of the fish while four fry of *G. affinis* were found in one female fish.

Four Anopheles spp. (*An. subpictus, An. culicifacies, An. annularis, An. nigerrimus*), three Culex spp. (*Cx. quinquefasciatus, Cx. tritaeniorhynchus, Cx. minutissimus*) and one Aedes spp. (*Aedes aegypti*) emerged from the immature sample brought to the vivarium.

DISCUSSION

G. affinis was quite effective in controlling the mosquito breeding in the rice field. However, it was more effective in smaller quadrates than in big rice fields. This may be due to the fact that the access of the fish for the predation towards mosquito larvae in quadrates is better than that in big

Table 2. Results of trials with *Gambusia affinis* in control of mosquito breeding in rice fields

Days	Density of immatures per 20 dips										Corrected per cent reduction in density of III & IV instars and pupae
	Control*					Experimental†					
	I & II instars	III instar	IV instar	Pupae	Egg-raft	I & II instars	III instar	IV instar	Pupae	Egg-raft	
0	556	300	71	10	2	594	161	83	7	2	—
2	621	124	89	11	2	201	30	17	1	0	68
4	648	105	50	9	—	141	26	11	0	0	66
6	258	69	67	8	—	132	15	2	0	0	83
12	240	42	60	8	—	78	7	0	0	0	91
18	243	53	63	7	—	30	2	0	0	0	98
24	305	33	65	10	—	29	—	0	0	0	100
30	289	38	59	8	—	25	—	0	0	0	100
36	291	42	60	11	—	26	3	1	0	0	95
42	272	35	59	7	—	3	1	0	0	0	99

*Surface area of control rice field was 742 sq m.
†Surface area of experimental rice field was 689 sq m; water pH 7-8.
Calculated "t" value at 5% level of significance is 3.07.

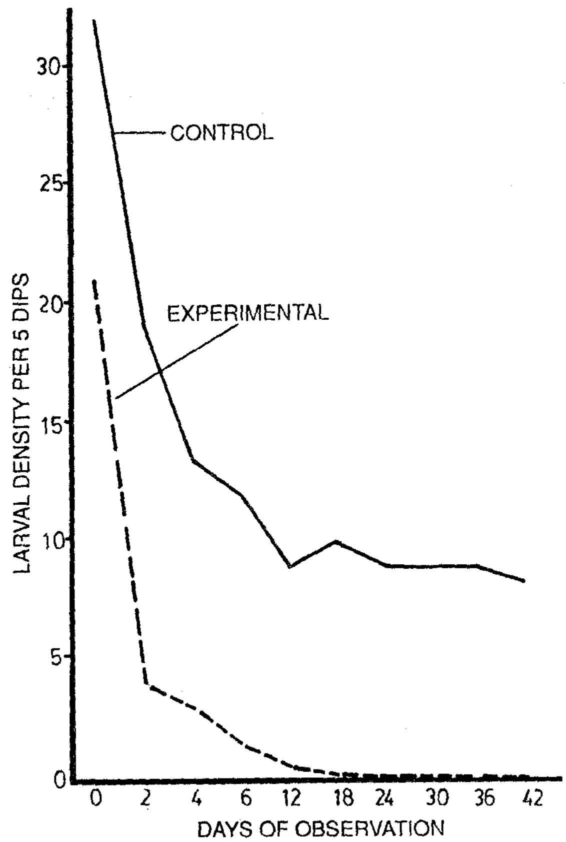


Fig. 2: Results of trial with *Gambusia affinis* in control of mosquito breeding in experimental and control rice fields.

rice fields. The availability of an alternative food item was also limited. Whereas in the big rice fields the movement of the fish is not restricted, the growth of algae and other vegetation in close association with rice plants obstructs the easy access of the fish and provides shelter for the mosquito larvae. The water level of the big rice fields was not constant throughout. The vegetation and algal cover provide the habitat which support heavy mosquito breeding.

The gut content analysis indicates the omnivorous feeding behaviour of *G. affinis*. Such a feeding behaviour has also been reported by Farley[8]. However, the availability of mosquito larvae in the bulk of food of *G. affinis* may be due to the abundance of mosquito larvae in the rice fields and the easy access of the fish for predation.

Our study corroborates the findings of Sokolov and Chvaliova (cited by Hora[9]), who had observed the nutrition of *G. affinis* in the rice field of Turkestan. According to them, Anopheles larvae form the bulk of the food of the young fish amounting to 64-68% and the fish exterminates Anopheles larvae to the extent of 80-90%.

We have observed that *G. affinis* significantly reduces the immature mosquito population in rice fields at a stocking rate of 5 fish/sq m. A similar observation has been reported by Rafatjah and Arata[10] in the rice field of Kunduz Valley, where

G. affinis 'satisfactorily' reduced anopheline larval densities at a stocking rate of 4-6 fish/sq m of water surface. The effective control achieved in rice fields by *G. affinis* is due to their feeding habits, dorsal mouth plus their frequenting the surface of water and its ability to penetrate the rice plants in shoals.

The study shows that the use of *G. affinis* could be a promising approach to control mosquito breeding in rice fields.

REFERENCES

1. Bang, Y.H. and C.P. Pant (1983). A review on disease vectors breeding in rice fields in tropical Asia and research needs. *J. Com. Dis.*, **15**(4):268-279.
2. Chandrahas, R.K. and V.P. Sharma (1983). Malaria epidemic in Shahjahanpur (U.P.). *Indian J. Malariol.*, **20**(4) : 163-166.
3. Yadav, R.S. and M.K. Das (1990). Role of *Danio* and *Oryzias* fishes in the control of mosquito breeding in rice fields. In *Proceedings of the MRC-CICFRI workshop on larvivorous fishes of inland ecosystem.* (In Press).
4. Prasad, R.N., M.K. Das, K.J. Virk and S. Haq (1990a). Mass culture of *Gambusia affinis* for mosquito control – an overview. In *Proceedings of the MRC-CICFRI workshop on larvivorous fishes of inland ecosystem.* (In Press).
5. Prasad, R.N., S.N. Sharma, K.J. Virk and V.P. Sharma (1990b). Anopheline breeding in paddy fields and its relationship with the growth of plant. *Mosq. Born. Dis. Bull.,* **7**: 104-106
6. Ungureanu, E., J.H. Pull and R. Pal (1981). Detailed study design for field studies regarding the evaluation of the efficacy of the larvivorous fish for the control of malaria. WHO/VBC/81, 816. (WHO mimeographed document).
7. Mulla, M.S., R.L. Norland, D.M. Fanara, H.A. Darwazeh and D.W. Mckean (1971). Control of Chironomid midges in recreational lakes. *J. Econ. Entomol.*, **64**(1): 300-307.
8. Farley, D.G. (1980). Prey selection by the mosquito fish *Gambusia affinis* in Fres-no country rice fields. *Pro. Calif. Mosq. Vect. Contr. Assoc.*, **48**: 51-54.
9. Hora, S.L. (1937). Fish and mosquito control. *Curr. Sci.*, **5**(10): 554-555.
10. Rafatjah, H.A. and A.A. Arata (1975). The use of larvivorous fish in antimalaria programme. (Unpublished document). MAL/WP/75.6. Rev. 1. (WHO, Geneva).

Indian Journal of Malariology
Vol. 28, September 1991, pp. 179-182.

Donor Screening for Malaria by Antibody and Antigen Detection in Endemic Area

NABAJYOTI CHOUDHURY*, J.G. JOLLY*, RAMESH C. MAHAJAN†, MOHAN LAL DUBEY†, NIRMAL K. GANGULY‡ and S.K. AGNIHOTRI#

Transfusion-associated malaria is one of the dreaded threats to the safety of transfusion services in this malaria endemic world. The main drawback in preventing this menace is that routine blood donor screening techniques are not very satisfactory. In this study, blood donors were screened for antimalarial antibody by micro ELISA and Indirect Fluorescence Antibody (IFA) test and malarial antigen by monoclonal antibody (MAB) technique. A total of 19.37% and 12.39% blood donors showed significantly high antibody by ELISA and IFA test respectively, and 0.35% donors showed the presence of antigen by the MAB technique. Solitary dependence on malarial antibody detection as a screening test might have led to rejection of 19.02% blood donors without any apparent infection. So antigen detection in blood donors with the help of the MAB technique seems to be more sensitive and a practically feasible screening test to prevent transfusion malaria.

INTRODUCTION

Post-transfusion malaria (PTM) cases are grossly under-reported[1]. One of the main reasons is that there is not a single, practical and sensitive technique for screening blood donors to diagnose the disease, because blood smear examination shows poor results due to low parasite concentration[2]. Malaria antibody detection is one of the sensitive diagnostic tools for blood donor screening. The most widely used techniques are Indirect Fluorescence Antibody (IFA) test and micro ELISA test. The latest trend in blood donor screening is the detection of malaria antigen by specific monoclonal antibody (MAB), which seems to give promising results. In this study, malaria antibody detection by IFA and micro ELISA was used along with antigen detection by MAB to find a suitable blood donor screening test.

MATERIAL AND METHODS

This study was carried out to test the antimalarial antibody status and presence of malaria antigen simultaneously in voluntary blood donors who

Accepted for publication: 8 August 1991.

* Department of Transfusion Medicine
Sanjay Gandhi Post Graduate Institute of Medical Sciences
Post Box 375
Lucknow-226 001, India.
† Department of Parasitology
‡ Department of Experimental Medicine
# Department of Blood Transfusion
Post Graduate Institute of Medical Education & Research
Chandigarh-160 012, India.

visited the Blood Donation Centre of the Nehru Hospital of PGIMER, Chandigarh. A total of 4542 randomly selected blood donors were screened for antimalarial antibody by micro ELISA test. Among the donors, 232 were chosen randomly for screening by IFA test. In the case of blood donors who gave the suggested history of malaria infection, antigen detection was carried out with the help of MAB. Owing to the shortage of MAB reagents, antigen detection was done only in 52 suspected donors. All antigen-positive donors were treated with the standard regimen of chloroquine base and also with primaquine tablets in the case of *Plasmodium vivax*. From each blood donor, 5 ml of blood was collected each in EDTA and empty vials. Serum was separated from the empty vial for antibody detection and EDTA blood was used for antigen detection, wherever necessary.

Malarial antibody detection : (1) *IFA technique* : *P. knowlesi* mature schizonts were used as antigen, and antibody titre of 1:20 dilution or above was accepted as significant level[2,4]. (2) *Micro ELISA technique* : Soluble *P. knowlesi* antigen was used for coating the Dynatech® disposable microtitre plates, and for reading the tests, Dynatech ELISA reader was used[5,6]. To find the cut-off point for significant antibody level, sera of 200 blood donors who showed negative result by smear examination and IFA technique were tested first. The cut-off point was calculated by adding 2 to standard deviation (SD) to the mean optical density (OD) reading of the negative samples. An optical density of 0.604 or more was found to be significant by ELISA test.

Antigen detection by MAB : Monofluo kit—*P. falciparum* was obtained from Diagnostic Pastuer, France, and was used for malaria antigen detection. Five ml of blood in EDTA was collected from subjects and packed cells were obtained after centrifugation at 400 g for 10 min. Next, 500 µl of packed cell was washed with 5 ml of normal saline and 100 µl of washed packed cell was taken to make a suspension in 400 µl Phosphate Buffer Saline (PBS) – Lactose. With 20 µl of this suspension a thin smear on an immunofluorescence slide was prepared. 20 µl of monoclonal antibody (MAB) was added to the dried smear and incubated at 37°C for 30 min in a humid chamber. After three washings with PBS, 20 µl of fluorescein-isothiocyanide (FITC) conjugate was added and incubated again for 30 min at 37°C. The slide was washed three times with PBS, dried and covered with buffered glycerine and examined under a fluorescent microscope (400 X). Slides showing apple green fluorescence bodies were reported as positive.

RESULTS

A total of 4532 blood donor samples were tested by ELISA technique and 880 (19.37%) samples showed significant anti-malaria antibody level but none of the samples showed zero optical density. Owing to technical difficulties, 242 blood donors were randomly screened from 4532 donors by IFA test and 30 (12.39%) donors demonstrated significantly high antibody level. A total of 52 blood donors were suspected for malaria infection due to suggestive medical history and they were tested

Table 1. Screening of voluntary blood donors

Micro ELISA		IFA test		Antigen detection		Total +ve donors
Tested	Sig.*	Tested	Sig.*	Tested	+ve	
4542	880 (19.37%)	242	30 (12.39%)	52	13 (26.0%)	16 (0.35%)

*Sig. = Significant.

for antigen and 13(26.0%) of them showed positive results (Table 1). In another 3 cases, peripheral smears were positive but samples were not available for antigen detection and thus the number of blood donors who showed active infection was 16. The 30 donors who showed significantly high antibody by IFA were all screened for malaria antigen.

The antigen-positive blood donors were followed up fortnightly after chloroquine treatment until their antibody level became non-significant by ELISA test, wherever possible. Antigen detection by MAB was also carried out at the same time until the donor showed negative result. In 16 cases, the antibody level became non-significant after a variable period with an average of 61.15 days after completion of full treatment. But the antigen was negative after 15 days of full treatment.

DISCUSSION

Transfusion-induced malaria is the second most common problem in transfusion therapy in India. This problem is being encountered increasingly in the non-endemic countries also due to increased international travel, migration as well as greater use of blood and blood products[7]. Detection of malaria in blood donors is a difficult task. The commonly used smear examination technique is labour-intensive and unreliable due to low concentration of parasites in many donors[2,8]. Screening of blood donors by antigen detection was found to be superior to that by antibody detection. IFA and ELISA demonstrated significantly high antibody levels in 12.39% and 19.37% of healthy population respectively.

None of them gave the history of malaria or any type of fever during the previous three months. Among 4542 donors screened by ELISA, only 16(0.35%) were malaria-positive by antigen detection technique. The positivity in the antigen detection group seems to be high (26.0%), which might be due to screening of only high-risk persons and the small number of samples. A total of about 880 screened donors would had been deferred if the antimalarial antibody had been taken as the screening method. A poor country like India, where there is acute shortage of blood in blood banks, can ill afford the luxury of donor screening by antibody level, a technique which is not reliable.

In this study, there was one subject in whom the antibody level was not significant in spite of the presence of the antigen. Similar results were observed in other studies in which 3 out of 15 patients with parasitaemia failed to reveal any antibody level, which was difficult to explain[9]. It could be that the person was a non-responder to malaria antigen. On the other hand, most of the donors with significant antibody levels did not show evidence of malaria infection by the sensitive antigen detection technique.

When we compared the screening of blood donors for malaria infection by IFA and ELISA tests, the latter showed better results in terms of specificity and sensitivity. Micro ELISA offers the advantage of objectivity, precision, and reproducibility; also, the test can be done in a large number of samples in a short span of time[8]. If antigen and antibody detection techniques are compared, the former is much superior to the latter. The presence of antigen in blood donors is a direct evidence of infection. Monoclonal antibody is very useful to detect antigenaemia when detection of parasites is difficult by conventional smear examination. It recognises all intra-erythrocytic forms of plasmodia. However, *P. falciparum* and *P. vivax* could not be differentiated. Polyclonal plasma antiplasmodial antibodies do not interfere with this test. So it can be used in persons living in endemic areas. A large number of samples can also be examined in a short span of time with greater sensitivity (50-30 plasmodia/μl)[10].

Therefore, we recommend that blood donor screening should be done by direct demonstration of antigen rather than the indirect evidence of infection by antibody detection. It may be adopted as a routine donor screening procedure in both endemic and non-endemic countries.

ACKNOWLEDGEMENT

We acknowledge the help of Prof. D. Richard, CNTS Foundation, Paris, for supplying the Monofluo kit–*P. falciparum*, free of cost.

REFERENCES

1. Bruce-Chwatt, L.J. (1972). Blood transfusion and tropical disease. *Trop. Dis. Bull.*, 69(9) : 825-862.
2. Bruce-Chwatt, L.J. (1987). International forum: Which are the appropriate modifications of existing regulations designed to prevent the transmission of malaria by blood transfusion, in view of the increasing frequency of travel to endemic areas? *Vox. Sang.*, **52** : 138-148.
3. Voller, A. (1964). Fluorescent antibody methods and their use in malaria research. *Bull. WHO*, **30**(3): 343-354.
4. Mahajan, R.C., R.E. Chandnani, M. Thadani, R.N. Prasad and N.K. Ganguly (1982). Malaria antibodies in voluntary blood donors. *Indian J. Med. Res.*, **75** : 661-666.
5. Voller, A. and C.C. Draper (1982). Immunodiagnosis and seroepidemiology of malaria. *British Med. Bull.*, 38(2) : 173-177.
6. Mahajan, R.C., N.K. Ganguly, M. Thadani, Navanjit Gill and R.E. Chandnani (1981). Comparison of ELISA with indirect haemagglutination and indirect fluorescent antibody tests in malaria. *Indian J. Med. Res.*, 73 : 874-879.
7. Bruce-Chwatt, L.J. (1982). Transfusion malaria revisited. *Trop. Dis. Bull.*, 79(10) : 827-840.
8. Wells, L. and F.A. Ala (1985). Malaria and blood transfusion. *Lancet*, 1 : 1317-1319.
9. Spencer, H.C., W.E. Collins and J.C. Skinner (1979). The enzyme-linked immunosorbent assay (ELISA) for malaria. Part II—Comparison with the malaria indirect fluorescent antibody test (IFA). *American J. Trop. Med. Hyg.*, 28(6) : 933-936.
10. Hassig, A., P. Ambroise-Thomas, L.J. Bruce-Chwatt, J.C. Emmanuel, R. Masure, L.H. Perrin and J.P. Soulier (1987). International forum : Which are the appropriate modifications of existing regulations designed to prevent the transmission of malaria by blood transfusion in view of the increasing frequency of travel to endemic areas ? *Vox. Sang.*, 52 : 138-148.

Indian Journal of Malariology
Vol. 28, September 1991, pp. 183-187.

Importance of Clinical Diagnosis of Malaria in National Malaria Control Programme

A.S. GAUTAM*, R.C. SHARMA*, V.P. SHARMA† and G.K. SHARMA†

The study conducted at Kheda district, Gujarat, revealed that judgement of patient on the basis of symptoms and diagnosis of the doctor were correct in 50 and 27% of the suspected malaria cases respectively. In malaria control programme, emphasis on health education and passive case detection is indicated.

INTRODUCTION

Under the Modified Plan of Operation (MPO) of National Malaria Eradication Programme (NMEP) of the Government of India, an average of 72 million blood smears are collected annually and examined to detect around 2 million malaria cases (Fig. 1). For a number of years, it has been recognized that the system of case detection designed for identifying malaria cases during the consolidation phase of an eradication campaign is not required in control programmes[1]. The strategy of malaria control approved by the World Health Assembly in 1978[2] and further developed by the 18th expert committee in 1985[3] is based on two major principles: (i) the accessibility to diagnosis and treatment facilities for the whole population of endemic areas; and (ii) selective use of measures aimed at checking transmission for the control and/or prevention of epidemics or for reducing malaria incidence in areas of high endemicity or of particular socio-economic value. The first proposition has to be established on a total coverage basis while the second has to be guided by appropriate epidemiological consideration and services. An in-depth evaluation by the NMEP during 1985 recognised that the system of active and passive case detection is adequate neither for treatment of malaria nor for the epidemiological guidance of operations[4]. Collection of a large number of blood smears and the conditions in which blood is taken have been recognized as highly dangerous for transmission of blood-borne diseases, particularly Hepatitis B and AIDS[1]. Besides, the present system of case detection also results in presumptive treatment with antimalarials of over 72 million people annually (Fig. 1) and among them 97% do not have malaria. This unnecessary drug pressure can only contribute to further selection of drug-resistant *P. falciparum*.

Accepted for publication : 12 August 1991.

* Malaria Research Centre (Field Station)
Civil Hospital
Nadiad-387 001, India.

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

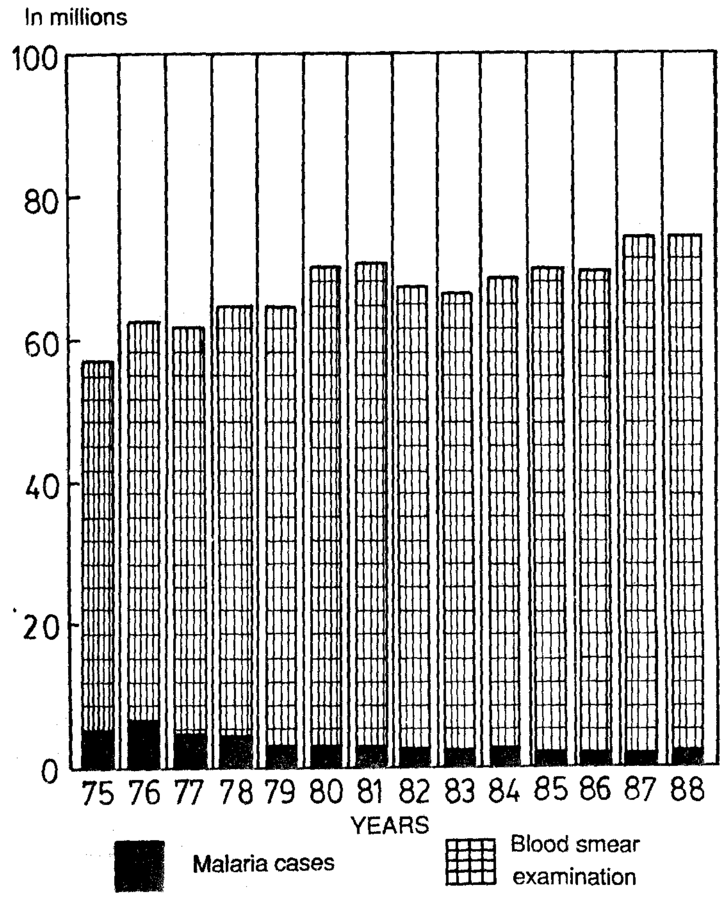


Fig. 1: Annual blood smear examination and malaria in India.

Accurate clinical diagnosis of malaria can help in reducing (i) the number of blood smears collected and examined, (ii) the drug pressure, and (iii) in minimizing the risk of accidental transmission of blood-borne diseases. Against this background, the current study set out to examine the clinical characteristics of those individuals being treated as suspected malaria cases with a view to identifying clinical characteristics most closely associated with confirmed malaria status.

MATERIAL AND METHODS

The study on 211 patients was conducted during April to June 1989 in Kheda district of Gujarat at five places, namely two malaria clinics of MRC and NMEP at Nadiad, two PHCs, Karamsad and Bakrol in Anand Taluka, and the Civil Hospital at Nadiad. At the malaria clinics, patients presented themselves for blood tests for malaria, whereas at the PHCs and the Civil Hospital, medical officers or the physician referred suspected malaria cases for blood examination. A blood smear, with thick and thin films, was prepared, stained with JSB stain and examined for malaria parasites. Two hundred fields of thick smear and 200 fields of thin smear were examined in each case at the MRC laboratory.

A case sheet with personal information, history and physical examination was filled at the time of examination and results of microscopic examination of blood smear were recorded for collation and interpretation.

RESULTS AND DISCUSSION

Of the 211 patients suspected for malaria, 116 (55%) were males and 95 (45%) females. Thirty-five per cent of males and 31% of females were found to be positive for malaria of which 81% and 19% of infections were due to *P. vivax* and *P. falciparum* respectively. The results of microscopic examination of 211 clinically suspected malaria cases representing various age groups are given in Table 1. Samples covered all the age groups and both *P. vivax* and *P. falciparum* infections.

Table 1. Results of microscopic examination of clinically suspected malaria cases

Age group	Sample size	Pv	Pf	Total positive
< 1	2	0	0	0
2 – 5	19	4	0	4
6 – 15	49	21	1	22
16 – 30	62	15	7	22
31 – 45	51	13	3	16
46 +	28	3	2	5
Total	211	56	13	69

The number of clinically suspected malaria cases by each agency and the results of microscopically confirmed cases are listed in Table 2. At malaria clinics of MRC and NMEP, slide positivity rates were very similar (50% and 52% respectively). Judgements of two clinicians were comparable in detecting 27 and 28% of microscopically confirmed malaria cases respectively. This can be explained by the fact that patients who go to malaria clinics for examination suspect malaria infection themselves whereas patients going to hospitals/dispensaries may include those who suspect some other disease or have no idea at all with regard to their illness. This would explain the high SPR (52%) at malaria clinics and the lower one (24%) at hospitals and dispensaries (Table 2). Another important observation is that at malaria clinics, 94% of positive cases were of *P. vivax* against 69% among those suspected by medical doctors. Whether *P. vivax* infections are easier to suspect or recognize by the patients themselves

Table 2. Results of suspected malaria cases

A. *Malaria Clinic*

Agency	Total suspected cases	Positive			SPR(%)
		Total	Pv	Pf	
(i) MRC	8	4	3	1	50
(ii) NMEP	56	29	28	1	52
Total	64	33	31	2	52

B. *Hospital OPD*

Agency	Total suspected cases	Total	Pv	Pf	SPR(%)
(i) Physician	86	23	14	9	27
(ii) Medical Officer*	47	13	11	2	28
(iii) Medical Officer**	14	0	0	0	0
Total	147	36	25	11	24

* Karamsad PHC; ** Bakrol PHC.

remains to be proved. Thus it becomes important to know what symptoms lead to suspect malaria infection and whether symptoms for *P. vivax* and *P. falciparum* infections differ significantly for easy recognition.

Table 3 gives symptomwise classification of clinically suspected and microscopically confirmed malaria cases. Absence or presence of symptoms, namely fever, bodyache, headache, cough, vomiting, nausea, abdominal pain, retrosternal burning, giddiness and rigours singly, did not have a significant difference on positivity rate of malaria. Fever on alternate days was found to be associated with *vivax* and chills with *falciparum* malaria.

An attempt to correlate a group of symptoms with malaria was also made. The results are given in Table 4. 211 cases represented a total of 61 different combinations of symptoms of which 86 cases fell in 54 different combinations. Therefore, no clue could be drawn from these cases because of the very small sample sizes in each group. Among 7 other groups of symptoms, only fever with rigours

Table 3. Symptomwise classification of clinically suspected and microscopically confirmed malaria cases

Symptom	Present(+) or Absent(–)	No. of cases(%)		Z test for Pv		Z test for Pf	
		Pv	Pf	Value	Significance	Value	Significance
Fever	+ (206)*	55	13	0.3352		0.5798	
	– (5)	1	0				
Bodyache	+ (61)	13	4	1.0969		0.1526	
	– (150)	43	9				
Headache	+ (64)	20	4	1.0223		0.0354	
	– (147)	36	9				
Cough	+ (14)	3	0	0.4482		0.9922	
	– (197)	53	13				
Vomiting	+ (32)	11	3	1.236		0.8209	
	– (179)	45	10				
Nausea	+ (9)	1	0	1.0714		0.7856	
	– (202)	55	13				
Pain in abdomen	+ (5)	0	0	1.3602		0.5798	
	– (206)	56	13				
Retrosternal burning	+ (4)	·1	0	0.0704		0.5173	
	– (207)	55	13				
Giddiness	+ (12)	5	0	1.2219		0.9139	
	– (199)	51	13				
Rigours	+ (121)	36	10	1.2251		1.4733	
	– (90)	20	3				
Chills	+ (58)	10	9	1.8834	Significant	3.4800	Significant
	– (153)	46	4				
Alternate day fever	+ (11)	7	1	2.8620	Significant	0.4150	
	– (200)	49	12				

*Figures in parentheses are the actual number of cases.

Table 4. Different groups of symptoms associated with malaria cases

	Symptoms	Total cases	Positive	% Positive
1.	Fever alone	32	10	31
2.	Fever + Rigours	30	11	37
3.	Fever + Rigours + Chills	17	7	41
4.	Fever + Rigours + Vomiting	6	4	67
5.	Fever + Headache	13	5	38
6.	Fever + Bodyache	16	2	12
7.	Fever + Headache + Rigours + Bodyache	11	4	36
8.	Other combinations (54)	86	16	19
	Total	211	69	32.7

and vomiting was associated with malaria in 67% cases. Among the cases of fever, fever with rigours, fever with rigours and chills, fever with headache, and rigours and bodyache, malaria was associated in 31-41% cases and only in 12% cases of fever with bodyache. It has been reported that among the patients from Koraput district of Orissa, fever status was highly associated with *P. falciparum* infection, and headache and diarrhoea along with fever were closely associated with the presence of *falciparum* parasite[5].

As improved diagnosis of malaria can help the National Malaria Control Programme, what is called for is an extensive study covering a large sample through all the seasons of the year for more useful correlation of symptoms or group of symptoms. And the fact that slide positivity rate among patients presenting themselves for diagnosis at malaria clinics was as high as 52% points to the suggestion that health education should be emphasized and intensified so as to improve passive case detection with the objective to reduce parasite load in the community.

ACKNOWLEDGEMENTS

Authors are greatly thankful to Dr. H.K. Bhavsar, Physician, Civil Hospital, Nadiad, Dr. V.A. Desai, Medical Officer, Karamsad PHC, and Dr. D.A. Zala, Medical Officer, Bakrol PHC and Dr. R.N. Shah, Chief Medical Officer, Nadiad, for their kind cooperation in conducting the study. Thanks are due also to the technicians of parasitology laboratory of MRC, Nadiad, and to Mr. Sunil Nath of IIM, Ahmedabad, for their help during the study.

REFERENCES

1. Anon. (1987). In-Depth Evaluation of the Community based Integrated Vector Control of Malaria Project in Kheda (Gujarat): 26-27.
2. World Health Assembly (1978). Published by WHO, Geneva.
3. WHO (1986). Expert Committee on Malaria. *WHO Tech. Rep. Ser.* 735.
4. Anon. (1985). In-Depth Evaluation Report of the Modified Plan of Operation under National Malaria Eradication Programme of India, October, 1985.
5. Anon. (1989). Annual Report. Vector Control Research Centre, Pondicherry: 29-30.

Indian Journal of Malariology
Vol. 28, September 1991, pp. 189-196.

Host Immune Response to Plasmodium

LALITHA S. KABILAN*

Malaria is the most widely spread infectious disease of man affecting almost half of the world's population. Control of malaria remains one of the world's biggest health challenges. Development of vaccines has been considered a valid and necessary complement to control malaria in addition to the control measures of the vectors. The *Plasmodium* parasites that cause the disease have many stages in their cycle, each with distinct morphology and antigenicity. Understanding the activation, interaction and effector function of the different components of the immune system in relation to target antigens on different stages of malaria parasites is necessary to achieve complete protection by vaccination.

INTRODUCTION

Among the *Plasmodium* species, *P. vivax, P. falciparum, P. ovale* and *P. malariae* are pathogenic to man, each with a characteristic course of infection and pattern of disease. Of the four pathogenic species of *Plasmodium*, *P. vivax* causes a significant morbidity and *P. falciparum* causes high morbidity and mortality. The malaria parasite undergoes development in two hosts, i.e., asexual cycle in vertebrates and sexual development in mosquitoes[1]. Immunity to malaria is a complex process which is not completely understood. In endemic areas sustained clinical immunity develops under natural conditions only after repeated exposures over several years and may not be absolute, i.e., it is frequently accompanied by persistent or recurrent low level of infection and immunity wanes rapidly once the exposure ceases[2]. Immunity to malaria is both species-and stage-specific[3].

Studies on host-parasite relationship may reveal potential targets for interruption and help characterize protective immune responses which may suppress parasite growth. At present it is not known with certainty how the *Plasmodia* live in harmony with the hosts. The purpose of this review with superficial description of aspects of immunity to malaria is an attempt to create awareness in readers (other than immunologists) that knowledge of host-parasite relationship is required to develop new control measures against malaria.

Immune system – Organization

The term "immune" is derived from the Latin term "immunis" (free from burden). In classic usage, immunity is referred to the relative resistance of the host to reinfection by a given microbe.

Accepted for publication: 29 August 1991.

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

It is now evident that immune responses to a pathogen are not necessarily associated with resistance to infection. On the contrary, they can even confer unpleasant and harmful effects on the host.

In vertebrates, the lymphoreticular system has evolved to carry out functions of immunity. Its cells are housed within blood, tissues, lymph nodes, bone marrow, thymus and spleen. The origin of these cells is pluripotential hematopoietic stem cells located within the bone marrow and foetal liver. The tissues contain a variety of cell types, each performing a separate function either directly or through the liberation of cell products. The system may be activated by a variety of influences that share the common characteristic of being recognized as foreign body by the host. Following activation, a spectrum of cellular and humoral (antibody production) events occurs that comprise specific and non-specific immune responses. Specific immune responses depend upon exposure to a foreign configuration and subsequent recognition and reaction to it, whereas non-specific responses do not depend on specific recognition.

The three general characteristics of the specific immune responses that distinguish it from the non-specific responses are (1) specificity, (2) heterogeneity, and (3) memory. It is the property of the immune response which distinguishes one antigen from another. Specificity is highly selective, because of which the products of the immune response will react solely with the configuration identical with or similar to that which initiated the response. Operationally, the immune response can distinguish and differentiate antigens originating from different species (species-specific) and from different organs (organ-specific).

Heterogeneity is the second characteristic of the specific immune response in which a vast array of cell types and cell products are induced to interact with a variety of responses and give rise to heterogeneous population of cell products (antibodies). The third hallmark of the immune response is memory which results in augmentation of the specific responses through proliferation.

Cell types and effector mechanisms involved in specific immune responses

The specific immune responses are carried out by two populations of lymphocytes, namely T and B cells. Lymphocytes on activation undergo clonal expansion. A proportion of activated lymphocytes become the effector cells, B-cells into antibody producing plasma cells (humoral immunity) and T-cells producing various cytokines (cell mediated immunity) and the remainder into an expanded population of memory cells. T-cells mature under the influence of thymus and B-cells in bone marrow in mammals (bursa of fabricius in birds). Both B- and T-cells are recognized by different markers. T-cells recognize processed antigen on the surface of an antigen-presenting cell along with histocompatibility antigen, whereas B-cells recognize antigen by surface Ig receptor.

Both T and B-cells are housed in the lymph nodes occupying specific areas (the paracortical area by T-cells and the cortical area by the B-cells). The lymphoid tissue is also scattered in the subcutaneous tissue respiratory passages, the intestine and the urinogenital tract. They are particularly well developed in structures such as tonsils, Peyer's patches and appendix. Both B and T lymphocytes are present in spleen whereas thymus is responsible for the development of lymphocytes which are involved in cell-mediated immune responses (thymus-derived or T lymphocytes). There are different subsets of helper T-cells and cytotoxic T-cells. Helper T-cells on activation produce various soluble factors of variable function; some of them help B-cells in proliferation and maturation, while others help the cytotoxic cells to proliferate and become the effector cells. Cytotoxic T-cells recognize the host cells infected with the organism along with the histocompatibility antigen class 1 and kill the host cell.

Cell types and effector mechanisms involved in non-specific immune responses

Non-specific immune responses consist of phagocytosis, inflammatory responses and natural killer (NK) cell activity. Phagocytosis is carried out by granulocytes (neutrophils, eosinophils) and monocytes, and killing activity is carried out by NK-cells. The process of phagocytosis represents the host's initial encounter with foreign particles. The process of engulfment of particles is by phagocytosis and uptake of nonparticulates is by endocytosis. In some cases, subsequent digestion of these materials into smaller fragments facilitates their elimination. Several integrated functions, cell products and factors may be required to achieve these goals. These cells originate from pluripotential hematopoietic stem cells located within the bone marrow and foetal liver cells[4]. Natural Killer (NK) cells are thought to be involved in non-specific killing of viral transformed target cells, allografts and tumour rejection. The lineage of NK cells is under dispute. A large portion of these cells express Fc receptors.

Immune responses in *plasmodial* infections

It is well-known that some genetic traits confer innate resistance to malaria[5], where genes control the constituents of red blood cells which resist the invasion of merozoites or their growth in erythrocytes. The host factors play an important role in the regulation of the immune responses in malaria. Some of the immune responses to malaria antigens may be protective.

Specific immune responses

B-cells and protective antibodies

Sufficient data are available which support the existence of protective immune responses in malaria. During the acute phase of the disease in some rodents, there is a close positive correlation between antibody levels and the development of protective immunity; non-fatal *P. yoelii* infections induce higher antibody levels than do fatal *P. berghei* and *P. yoelii* infections[6].

Administration of IgG fractions of immune serum to humans and animals was shown to alleviate symptoms of malaria infection[7-9], demonstrating a protective role for specific antibodies. In humans and monkeys, antibodies to asexual blood stages correlate with clinical protection in certain cases[10,11]. Monoclonal antibodies directed against schizont, merozoite derived antigens have been shown to inhibit the *in vitro* growth of the parasite in culture[12,13]. Although these experiments indicate that serum antibodies can have a protective role, the limited effects of passively transferred immune sera indicate that immunity to malaria is unlikely to depend exclusively on humoral mechanisms (antibody-mediated protection).

T-cell mediated protective immunity to malaria

Demonstration in animal models

T lymphocytes play a crucial role in acquisition and maintenance of immunity against malaria[14,15]. There is convincing evidence from experimental infections that specific immunity against infection can be elicited and maintained in the absence of a functioning B-cell system[16,15]. Animals immunized with sporozoites normally develop a protective immune response against *P. berghei* or *P. yoelii* when challenged with parasites, while thymectomized animals failed to do so[17]. T-cell deficient hosts are more susceptible to malarial infection than intact animals[17,18]. Moreover, B-cell deficient mice spontaneously resolve some malarial infections[16]. Additional support for the role of T-cell-mediated mechanisms in malaria has come from adoptive transfer studies where T-cells were shown to be essential for the transfer of immunity in some murine model systems[19,20].

T-cell responses

T-cell proliferation

Recognition of antigen by T-cell requires presentation of the antigen by accessory cells (monocytes) along with histocompatibility antigen. Activation of T-cells may be followed by proliferation. In one particular mouse model, a positive correlation was seen between T-cell activation *in vitro* and the induction of protective immunity *in vivo*[6]. Peripheral lymphocytes from malaria-exposed donors have been shown to proliferate *in vitro* in response to homologous antigen in an antigen-specific manner[21,22].

T-cell dependent humoral responses

T-cells help B-cells and activate them to mature and secrete antibody in response to antigenic stimulation. The biological relevance of T-cell-dependent antibody production was demonstrated *in vivo* in mouse models: neonatal thymectomy[7] or treatment with anti-thymocyte serum[18] prevented the development of immunity to certain blood stage murine *plasmodia* which appeared to be susceptible to antibody-mediated mechanisms of resistance as determined by passive immunization studies[8]. This gave rise to the concept that T-cells have a helper role in the production of these antibodies and it has also been demonstrated that T-cells help B-cells to produce anti-malarial antibodies[23]. T-cell stimulation with selected constructs of the circumsporozoite protein of *P. falciparum* containing T-cell epitopes help immunized mice to produce IgG antibodies after a challenge[24]. Thus it is evident that although antibodies are important for protection, the production and secretion of these antibodies is T-cell dependent[23].

T-cell derived factors

Interferon gamma

T-cell derived factors are also believed to be important mediators of cellular effector mechanisms in the absence of humoral mechanisms. IFN-r produced by antigen activated T-cells is an important regulatory lymphokine and considered to be a good indicator of cell-mediated immunity. T-cells from *P. falciparum* patients and immune individuals living in endemic area have been reported to produce IFN-r in response to stimulation with homologous antigen. The production of IFN-r was antigen-specific[25]. IFN-r has been shown to be effective in controlling the exo-erythrocytic cycle of a mouse malaria parasite[26].

IFN-r can activate macrophages and the activated macrophages have been shown to induce crisis forms in human malaria. Oxidative killing of intra-erythrocytic parasites of *P. yoelli* by IFN-r activated macrophages has also been reported[27,28]. These observations support the concept that a major pathway of cell-mediated immunity in malarial infection involves the release of IFN-r from antigen-activated T-cells which subsequently activate monocytes to exert anti-parasitic activity.

Regulatory T-cells

Mouse T-helper cells are divided into subsets based on their pattern of lymphokine production; helper cells designated as TH1 produce interleukin-2 (IL-2) and IFN-r, whereas TH2 helper cells produce interleukin-4 and -5 (IL-4, IL-5)[29]. Functional heterogeneity of the murine T-helper cell response to *P. chabaudi chabaudi* has been reported: one subset which produces IFN-r and IL-2 (TH1) appears to be responsible for early antibody-independent mechanisms against the parasite, whilst the other subset (TH2) which produces IL-4 and IL-5 provides help for antibody production later in the course of infection[30]. The question of TH1- and TH2-like subsets in humans is controversial.

Cytotoxic T-cells

In rodent malaria models it was shown that one set of T-cells (CD8) may exert their antimalarial activity in a number of ways[26]. CD8 + cells could kill parasite infected hepatocytes by cytotoxic mechanisms or by local release of lymphokines[31]. Mice immunized with sporozoites or with recombinant vaccinia virus expressing the CS protein of *P. falciparum* contain cytotoxic T lymphocytes (CTL) that specifically kill fibroblasts transfected with the gene encoding the CS protein[32]. It was proposed from mouse model studies that protection against sporozoite infection is likely to be mediated by cytotoxic CD8+ cells, as depletion of CD8+ T-cells in a sporozoite immunized animal can completely abrogate immunity[26]. The role of human cytotoxic cells in *P. falciparum* infections remains to be explored.

Non-specific immune responses

The effector cells which may play a role in immune protection against parasite in non-specific manner include cells of macrophage-monocyte series, neutrophils, and NK cells[27,28]. Although these cells can be activated non-specifically, the major pathway of activation appears to be initiated by antigen-stimulated T-cells and controlled by soluble factors[33]. Thus, while induction of cellular immunity in malaria is usually antigen-specific, its execution is non-specific, unless there is cooperation between effector cells and antibodies[3]. Killing of parasites or parasitized erythrocytes may involve phagocytosis and/or release soluble factors from the effector cells or reactive oxygen radicals which may kill intraerythrocytic parasites[27,28]. Cell-mediated protection by macrophages, neutrophils or NK cells against the invading parasites may occur without direct T-cell participation. However, the involvement of soluble factors (cytokines) in the protection are produced by activated specific T-cells[25,34].

Despite that antibodies have been shown to be protective, the antibody production is T-cell-dependent[23] immunoglobulin isotypes and stage-specific[35]. These results underline the importance of T-cell mediated immunity for the development and maintenance of protective immunity against malarial infections[14,34].

Acquired protective immunity to malaria obtained by natural infections comprises both antibody-dependent and antibody-independent effector mechanisms. Although the relative roles of B and T-cells differ in different malaria systems and different stages of the parasite[34], T-cells are essential for the induction and maintenance of immunity against malaria while antibodies are required for the control of parasitaemia in acute infections.

Evasion of immune responses by parasites —Mechanisms

An infection by *Plasmodium* induces a variety of responses[36]. Immunosuppression and antigenic diversity may be considered among other mechanisms used by the malarial parasites to evade immune responses.

Immunosuppression

Malaria infections are reflected by either enhancement or suppression of lymphocyte responses to mitogens or unrelated antigens depending on immune status, disease severity and duration of clinical illness[37-39]. It has been shown in animal models that spleen cells from mice infected with *P. yoelii* or *P. berghei* show rapid loss of responses to mitogens[6]. Antigen-specific suppression has been reported in humans acutely infected with *P. falciparum* [22,40,41] and also with *P. vivax* [42]. Thus the immunosuppression seen in acute malaria is complex and seems to involve both antigen-specific and non-specific mechanisms. The factors and their involvement remain to be explored in detail.

Antigenic variation and diversity

Antigenic variation and diversity may play a role in immune evasion by the parasites. Antigenic diversity in natural isolates of *P. falciparum* has been demonstrated by showing variation in expression of epitopes on particular malaria proteins. Antigenic variation refers to the expression of new antigenic phenotypes in malaria parasites through genetic recombination or through mutation. Parasites can escape host responses if the parasites have the potential to express different targets to the immune system or to express new antigenic phenotypes[43].

Some immunodominant epitopes are shared by different parasite-derived proteins in the sense of cross-reactivity not in sequence identity[44]. A panel of monoclonal antibodies against *P. falciparum* merozoite protein exhibited different patterns of reactivity to different clones of *P. falciparum*[45]. Thus, the antigenic diversity among epitopes that make up an immunological cross-reactive family of molecules may also affect the development of immunity to malaria. The diversity of these molecules may activate the immune system to respond to antigens that are not relevant to protection. Cross-reactivity among malaria antigens could interfere with normal maturation of high-affinity antibody to the malaria parasites[36,43], which may affect the development of protective immunity to malaria. Thus, there are a number of mechanisms by which the parasites evade the host's immune responses.

CONCLUSION

Despite the antigenic diversity and antigenic variation in plasmodial antigens, recent advances in molecular biology and in synthetic peptide technology have provided adequate knowledge of many plasmodial antigens and epitopes involved in protective immunity against malaria. However, taking into consideration the existing complex relationship between plasmodia and the host it is extremely difficult to evaluate the host responsiveness to plasmodial antigens. An understanding of the activation, interaction and effector function of the different components of the immune system in relation to target antigens on different stages of malaria parasites is required to achieve complete protection by vaccination.

REFERENCES

1. Garnham, P.C.C. (1966). *Malaria parasites and other haemosporidia*. (Blackwell, Oxford).
2. Bjorkman, A. (1985). Holoendemic malaria and the effect of chemotherapy. Thesis. (Karolinska Institute, Stockholm, Sweden).
3. Perlmann, P. (1987). Malaria vaccines — Are they approaching reality? *Nature*, **328**: 205.
4. Paul, W.E. (1984). In *Fundamental Immunology*. (Ed.: W.E. Paul): 3-22.
5. Miller, L.H. and R.A. Carter (1976). Review: Innate resistance to malaria. *Exp. Parasitol.*, **40**: 132.
6. Jayawardena, A.N., G.A.T. Target, E. Leuchars, R.L. Carter, M.J. Doenhoff and A.J.S. Davies (1975). T-cell activation in murine malaria: *Nature*, **258**: 149-151.
7. Brown, I.N. and S.R. Phillips (1974). Immunity to *Plasmodium berghei* in rats: Passive serum transfer and role of the spleen. *Infect. Immun.*, **10**: 1213-1218.
8. Butcher, G.A., S. Cohen and P.C.C. Garnham (1970). Passive immunity in *P. knowlesi* malaria. *Trans. R. Soc. Trop. Med. Hyg.*, **64**: 850-856.
9. Cohen, S., I.A. McGregor and S. Carrington (1961). Gamma globulin and acquired immunity to human malaria. *Nature*, **192**: 733-737.
10. David, P.H. (1985). Immunization of monkeys with a 140 kilodalton merozoite surface protein of *Plasmodium knowlesi* malaria appearance of alternate form of this protein. *J. Immunol.*, **134**: 4146-4150.
11. Fandeur, T., P. Dubois, J. Gysin, J.P. Dedet and L. Pereira Da Silva (1984). *In vitro* and *in vivo* studies on protective and inhibitory antibodies against *Plasmodium falciparum* in the Saimiri monkey. *J. Immunol.*, **132**: 432-437.
12. Banyal, H.S. and J. Inselberg (1985). Isolation and characterization of parasite-inhibitory *Plasmodium falciparum* monoclonal antibodies. *American J. Trop. Med. Hyg.*, **34**: 1055-1064.
13. Miller, L.H., P.H. Davis, D.E. Hudson, T.J. Hadley, R.L. Richards and M. Aikawa (1984). Monoclonal antibodies to a 140,000 mw protein on *Plasmodium knowlesi* merozoites inhibit their invasion of Rhesus erythrocytes. *J. Immunol.*, **132**: 438-442.
14. Jayawardena, A.N. (1981). Immune responses in Malaria in Mansfield. *Parasit. Dis.*, **1**: 85-136.
15. Weidanz, W.P. (1982). Malaria and alterations in immune reactivity. *British Med. Bull.*, **38**: 167-172.
16. Grau, G.E. P.F. Piquet, H.D. Engers, J.A. Louis, P. Vasalli and P.H. Lambert (1986). L3T4+ T-Lymphocytes play a major role in the pathogenesis of murine cerebral malaria. *J. Immunol.*, **138**: 2348-2354.
17. Brown, I.N., A.C. Allison and R.B. Taylor (1968). *Plasmodium berghei* infections in thymectomized rats. *Nature*, **219**: 292-293.
18. Spira, D.T., J. Golenser, and I. Gery (1976). The reactivity of spleen cells from malarious rats to non-specific mitogens. *Clin. Exp. Immunol.*, **24**: 139-145.
19. Cavacini, L.A., C.A. Long and W.P. Weidanz (1986). T-cell immunity in murine malaria: Adoptive transfer of resistance to *Plasmodium falciparum chabaudi adami* in nude mice with splenic T-cell. *Infect. Immun.*, **52**: 637-643.

20. Jayawardena, A.N., D.B. Murphy, C.A. Janeway ano R.K. Gershon (1982). T-cell mediated immunity in malaria. The Ly phenotype of T-cells mediating resistance to *P. yoelii*. *J. Immunol.*, **129**: 377-381.

21. Kabilan, L., M. Troye-Blomberg, H. Perlmann, G. Andersson, B. Hogh, E. Petersen, A. Bjorkman and P. Perlmann. (1988). T-cell epitopes in *Pf*155/RESA, a major candidate for a *Plasmodium falciparum* malaria vaccine. *Proc. Natl. Acad. Sci. USA*, **85** (15): 5659-5663.

22. Riley, E.M., G. Andersson, L.N. Otoo, S. Jepsen and B.M. Greenwood (1988). Cellular immune responses to *Pf* antigens in Gambian children during and after an acute attack of falciparum malaria. *Clin. Exp. Immunol.*, **73**: 17-21.

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

24. Good, M.F., W.L. Maloy, M.N. Lunde, H. Margalit, J.L. Cornette, G.L. Smith, B. Moss, L.H. Miller and J.A. Berzofsky (1987). Construction of synthetic immunogen: use of new T-helper epitope on malaria circumsporozoite protein. *Science*, **235**: 1059-1062.

25. 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 *Pf*155/RESA, a malaria vaccine candidate antigen. *Infect. Immun.*, **58**: 2989-2994.

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

27. Ockenhouse, C.F., S. Schulman and H.L. Shear (1981). Induction of crisis forms in the human malaria parasite *Plasmodium falciparum* by gamma-interferon-activated, monocyte-derived macrophages. *J. Immunol.*, **133**: 1601-1608.

28. Ockenhouse, C.F. and H.L. Shear (1984). Oxidative killing of the intraerythrocytic malaria parasite *Plasmodium yoelii* by activated, macrophages. *J. Immunol.*, **132**: 424-431.

29. Mossman, T.R. and R.L. Coffman (1989). TH1 and TH2 cells: Different patterns of lymphokine secretion lead to different functional properties. *Ann. Rev. Immunol.*, **7**: 145-173.

30. 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. *Int. Immunol.*, **1**: 416-424.

31. Weiss, R.W., N. Sedegah, R.L. Beaudoin, L.H. Miller, and M.F. Good (1988). CD8+ T-cells (cytotoxic/suppressors) are required for protection in mice immunized with malaria sporozoites. *Proc. Nat. Acad. Sci. USA*, **85**: 573-576.

32. Kumar, S., L.H. Miller, J.A. Quakyi, D.B. Keister, R.A. Houghten, W.L. Maloy, B. Moss, J.A. Berzofsky and M.F. Good (1988). Cytotoxic T-cells specific for the circumsporozoite protein of *Plasmodium falciparum*. *Nature*, **334** (6179): 258-260.

33. Allison, A.C. and E.M. Euqui (1983). The role of cell-mediaed immune responses in resistance to malaria, with special reference to oxidant stress. *Ann. Rev. Immunol.*, **1**: 361-391.

34. Weidanz, W.F. (1988). The role of T-cells in immunity of malaria. *Prog. Allergy*, **41**: 215-252.

35. Jarra, W. and K.H. Brown (1985). Protective immunity to malaria: studies with cloned lines of *Plasmodium chabaudi* and *P. berghei* in CBA/Ca mice. I. The effectiveness and inter and intra-species specificity of immunity induced by infection. *Parasit. Immunol.*, **7**: 595-606.

36. Anders, R.F. (1986). Multiple cross-reactivities amongst antigens of *Plasmodium falciparum* impair the development of protective immunity against malaria. *Parasit. Immunol.*, **8**: 529-539.

37. Greenwood, B.M. (1984). Immunosuppression in malaria and trypanosomiasis; in parasites in the immunized host; mechanisms of survival. *Ciba Fdn. Symp.*, N.S., **25**: 137-146.

38. McGregor, I.A. and M. Barr (1962). Antibody response to tetanus toxoid inoculation in malarious and non-malarious Gambian children. *Trans. R. Soc. Trop. Med. Hyg.*, **56**: 364-367.

39. Williamson, W.A. and B.M. Greenwood (1978). Impairment of immune response to vaccination after acute malaria. *Lancet*, **1**: 1328-1334.

40. Brasseur, P., M. Agrapart, J.J. Ballet, P. Druilhe M.J. Warrel and S. Tharavani (1983). Impaired cell-mediated immunity in *Plasmodium falciparum* infected-patients with high-parasitaemia and cerebral malaria. *Clin. Immunol. Immunopathol.*, **27**: 38-50.

41. Ho, M., H.K. Webster, S. Looareesuwan, W. Supanaranond, R.E. Phillips, P. Chanthavanich and D.A. Warrell (1986). Antigen specific immunosuppression in human malaria due to *Plasmodium falciparum*. *J. Infect. Dis.*, **150**: 708.

42. Goonewardene, R., R. Carter, C. Gamage G.D. Giudice, P.H. David, S. Howie and Kamini N. Mendis (1991). Human T-cell proliferative responses to *Plasmodium vivax* antigens: evidence of immunosuppression following prolonged exposure to endemic malaria. *Eur. J. Immunol.*, **20**: 1387-1391.

43. McCutchan, T.F., V.F. de la Cruz, F. Good and E. Wellems (1988). Antigenic diversity in *Plasmodium falciparum*. *Prog. Allergy*, **41**: 173-192.

44. Anders, R.F., R.L. Coppel, G.V. Brown and D.J. Kemp (1988). Antigens with repeated amino acid sequences from the asexual blood stages of *Plasmodium falciparum* *Prog. Allergy*, **41**: 173-192.

45. McBride, J.S., D. Walliker and G. Morgan (1982). Antigenic diversity in the human malaria parasite *Plasmodium falciparum*. *Science*, **217**: 254-257.

SHORT NOTE

Indian Journal of Malariology
Vol. 28, September 1991, pp. 199-200.

A Study of Current Practices in the Treatment of Malaria in Industrial Complexes in India

V.K. DUA*, S.K. SHARMA* and V.P. SHARMA†

According to India's National Drug Policy, chloroquine is to be given in *Plasmodium falciparum*-sensitive areas, amodiaquine in predominantly RI areas, and long-active sulpha drugs (sulfalene or sulfadoxine with pyrimethamine) in RII and RIII areas (Source: NMEP Directorate). Our observations during the implementation of bioenvironmental control of malaria in the industrial areas have revealed that metakelfin (sulfalene + pyrimethamine) was used indiscriminately, regardless of the species of the parasite or sensitivity of *P. falciparum* to chloroquine[1]. We report the pattern of the use of metakelfin from hospitals of four major industrial complexes, viz., Bharat Heavy Electricals Limited (BHEL), Hardwar; Indian Drugs and Pharmaceuticals Ltd. (IDPL), Rishikesh; Bharat Heavy Electricals Ltd. (BHEL), Jhansi; and Indian Oil Corporation Ltd. (IOCL), Mathura.

Table 1 shows the total number of malaria cases (*P. falciparum* cases) and the number of metakelfin tablets distributed from 1984-85 to 1989-90 in the four major industrial complexes. The total *Pf* cases during 1984-85, 1985-86, 1986-87, 1987-88, 1988-89 and 1989-90 at BHEL, Hardwar, were 337, 417, 134, 21, 54 and 10 respectively. *In vivo* and *in vitro* study of *P. falciparum* cases of 1986 and 1987[2] and *in vivo* study of all cases from 1988-89 revealed that chloroquine resistance is mostly limited to imported cases, whereas all indigenous BHEL cases were sensitive to chloroquine. According to the National Drug Policy, chloroquine should be given for presumptive and radical treatment to all malaria cases except the resistant ones. However, it was noticed that metakelfin consumption in BHEL, Hardwar, was quite high, i.e., 2796, 15750, 7766, 5686 tablets for the years 1984-85, 1985-86, 1986-87 and 1987-88 respectively, which implies that the distribution of metakelfin was not restricted to chloroquine-resistant *Pf* cases but also included presumptive and radical treatment of all malaria cases. At IDPL, Rishikesh, there was no microscopical examination of blood films till July 1987 and all cases were treated on clinical diagnosis, resulting in high consumption of antimalarials. From 1987 to 1990, only 10 *P. falciparum* cases were recorded at IDPL complex, which suggests that even in the previous years there may be a few *P. falciparum* cases. *In vivo* study of all *Pf* cases during 1987-90 revealed that all cases were sensitive to chloroquine showing thus that only chloroquine was sufficient. Instead, metakelfin was used liberally. A similar pattern was found at BHEL, Jhansi, where *P. falciparum* cases were few and were sensitive to chloroquine. In IOCL,

Accepted for publication : 7 August 1991.

* Malaria Research Centre (Field Station)
BHEL, Ranipur
Hardwar-249 403, India.

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

Table 1. Malaria cases and metakelfin tablets distributed by hospitals during the years 1984-85 to 1989-90

	BHEL, Hardwar (Pop. 70,000)		IDPL, Rishikesh (Pop. 25,000)		BHEL, Jhansi (Pop. 6,000)		IOCL Mathura (Pop. 16,000)	
	Total +ve	Meta-kelfin tablets	Total +ve	Meta-kelfin tablets	Total +ve	Meta-kelfin tablets	Total +ve	Meta-kelfin tablets
1984-85	1918 (337)	2796	*	8630	*	800	4593 (1447)	4950
1985-86	3014 (417)	15750	*	6040	*	1600	1270 (151)	5020
1986-87	2557 (134)	7766	*	8000	*	1250	1028 (171)	3960
1987-88	494 (21)	5686	137 (4)	4500	207 (8)	1100	602 (232)	1540
1988-89	433 (54)	334	134 (4)	2100	216 (8)	3350	1123 (346)	1740
1989-90	425 (10)	250	101 (3)	307	106 (9)	2250	874 (201)	840

Source: Chief Medical Officer.
Metakelfin tablet = Sulfalene (500 mg) + pyrimethamine (50 mg).
* Data not available.
Note : Figures in parentheses indicate *P. falciparum* cases.

Mathura, the consumption of metakelfin was high and the number of *P. falciparum* cases was also relatively more. Discussions revealed that 20-25% *P. falciparum* cases did not respond to chloroquine therapy, but metakelfin consumption far exceeded the *P. falciparum* incidence.

Correct malaria chemotherapy in the BHEL and IDPL hospitals during implementation of bioenvironmental malaria control programme resulted in 90% reduction in metakelfin consumption during 1989-90 as compared to the figure for previous years (Table 1). Regular medical education to physicians was important in the context of malaria situation in endemic areas.

Resistance to sulfalene/sulfadoxine and pyrimethamine combinations has become well established in Thailand and neighbouring countries[3], and cases have been reported from India[4]. If corrective measures are not initiated, indiscriminate use of sulfonamides may precipitate early resistance in *P. falciparum* involving large areas.

REFERENCES

1. Dua, V.K., V.P. Sharma and S.K. Sharma (1988). Bioenvironmental control of malaria in an industrial complex at Hardwar (U.P.), India. *J. Amer. Mosq. Contr. Assoc.*, **4** : 426-430.
2. Sinha, S., V.K. Dua and V.P. Sharma (1989). Chloroquine-resistant imported *Plasmodium falciparum* in an industrial complex at Hardwar (U.P.). *Indian J. Malariol.*, **26** : 123-125.
3. Black, F., I. Bygberg, P. Effersoe, G. Gomme, S. Jensen and G.A. Jensen (1982). Fansider-resistant *falciparum* malaria acquired in Southeast Asia. *Trans. R. Soc. Trop. Med. Hyg.*, **75** : 715-716.
4. Choudhury, D.S., S. Sinha, S.K. Ghosh, C. Usha Devi and V.P. Sharma (1987). Report of a case of *P. falciparum* malaria resistant to chloroquine and combination of sulfalene and pyrimethamine in Delhi. *Indian J. Malariol.*, **24** : 95-96.

INDIAN JOURNAL OF MALARIOLOGY

Instructions to Authors

Editorial Policy

The Indian Journal of Malariology is devoted to the publication of original research which contributes significantly to the knowledge of 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 that date on which copy is accepted in final form for publication. The authors should also submit names of three experts of their field of research on which the paper has been submitted. If there is no expert in India, experts from outside India may be suggested. Manuscripts in triplicate alongwith undertaking from duly filled by author(s) should to:

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

Classes of Items Published

In addition to regular papers the journal publishes short notes and invites review articles. Book reviews may 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 numbered serially in superscript). Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgement and References. Authors should provide key words and a short title to be used as running title, of not more than five words.

Preparation of Copy

Manuscripts should be typewritten in English on one side of the paper leaving 1½ inch left-hand margins. 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. Tables should be self-explanatory and referred to in the text. Designation of tables must be with 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, photographs, graphs and maps should be numbered consecutively in order of appearance in the text. Captions and legends should be typed separately and must not appear on the face of illustrations. Authors should

We accept MSS. on floppy, please specify software used.

identify each illustration on the reverse side with author's name, fig. no. and abbreviated captions. Line drawings should be clear, letters and numerals should be planned for legibility following 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. Size of art work for quarter page should be 24 x 21 cms; half page 45 x 24 cms and full page 57 x 45 cms approximately.

Data for tables, graphs etc., should be carefully verified. All statistics, percentages and other calculations should be checked thoroughly before submission of a communication. 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 of units (SI Units). Kindly see WHO publication *The SI for the Health Professional*, WHO, Geneva, 1977. Use of the 24-hour time system (e.g. 0830 hours, 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 word '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 reference is to an article published without any authorship in a periodical, in place of author's name the term "Anonymous" 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 acceptable for journal:

Research Paper

Sharma, V.P, (1976). Elimination of aziridine residues from chemosterilised mosquitoes, *Nature*, **261** (5556): 135.

Book/Monographs

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 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).**

Correct references are the responsibility of the author.

Short notes should be prepared in a manner similar to the research papers and will have title, name(s) of author(s) with address of Institute/

University as footnotes, acknowledgement and references.

Proofs

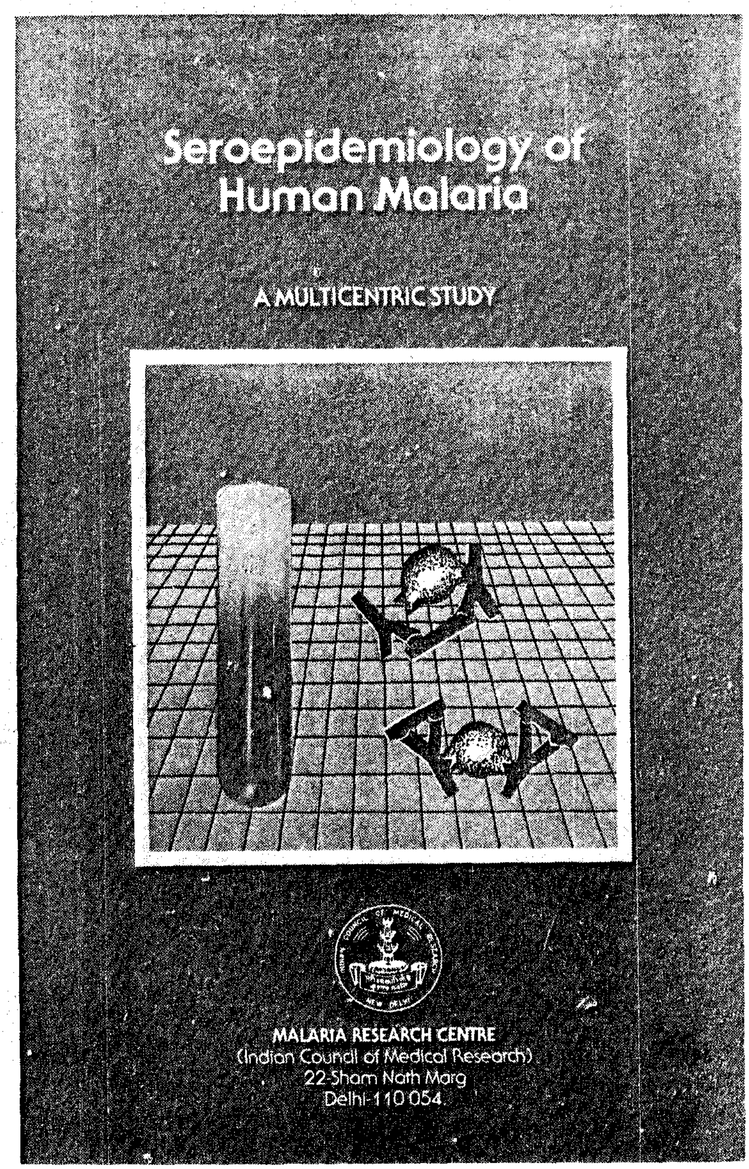
Page proofs of the articles will be sent to the authors for correction. Corrected proofs must be returned promptly to the editor or else the article may not be printed in the current issue or may be printed as it stands. Only minimal changes i.e. those that do not substantially alter the page make-up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

Reprints

Reprints will be mailed to the senior author. Twenty-five reprints will be supplied free of charge to single author and thirty for joint authors to be distributed on an equal basis. Additional reprints may be ordered on payment when the proofs are returned. A reprint order form will be supplied with the proofs.

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. five words).
4. Keywords.
5. Undertaking by the authors.
6. At least names of three experts on the subject of paper submitted for publication.
7. Set of figures with legends and captions in triplicate on a separate page.



Size : Royal 8 vo pp. viii, 206 (Hardbound)

Results of Multicentric Trials on the seroepidemiology of human malaria by different institutions under the auspices of the Indian Council of Medical Research. This volume brings out the development and field application of serology in malaria. An essential document for malariologists, epidemiologists and scientists working in the field.

MALARIA RESEARCH CENTRE

PRICED PUBLICATIONS

The Anophelines of India (Rev. ed.) 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. 4† (1991)

Annual Subscription:
India Rs. 75.00‡
Foreign US $ 20.00
‡25% discount for individuals.

(†To be released in December 1991)

*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:

....

....

....

....

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.

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: