INDIAN JOURNAL OF MALARIOLOGY

Volume 30 **Number 4**

December 1993

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

INDIAN J. MALARIOL.

Quarterly

Year of revival: 1981

SUBSCRIPTION RATE

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

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

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

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

INDIAN JOURNAL OF MALARIOLOGY

INDIAN JOURNAL OF MALARIOLOGY

CONTENTS

Volume 30
Number 4
December 1993

Short Note

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

Indian Journal of Malariology
Vol. 30, December 1993, pp. 183-185.

Changes in Brain Neurotransmitters in Rodent Malaria

S. ROY*, R.N. CHATTOPADHYAY+ and S.K. MAITRA+

Changes in brain neurotransmitters [5-hydroxytryptamine (5-HT), norepinephrine, histamine and dopamine] were studied in *Plasmodium berghei*-infected mice and rats. 5-HT and norepinephrine contents of brain decreased significantly in *Plasmodium berghei*-infected mice and rats, but histamine and dopamine contents remained unaltered. Decreased 5-HT and norepinephrine contents of brain may play a role in cerebral vasodilatation in malaria.

Keywords: Dopamine, Histamine, 5-hydroxytryptamine, Malaria, Norepinephrine

INTRODUCTION

In the pathophysiology of cerebral malaria, the well-known features are vasodilatation, inflammatory stasis in blood vessels, increased capillary permeability with local loss of proteins and fluids, and blockage of the small vessels (capillaries and post-capillary venules by aggregated parasitised RBC due to selective adhesion of these cells to the vascular endothelium). These changes affect mainly the brain (cerebral malaria) or the lungs[1]. The mechanism of vasodilatation and allied changes is yet to be fully ascertained. Bhattacharyya *et al.*[2] reported a significant decrease in 5-hydroxy-tryptamine (5-HT) level with a significant increase in monoamine oxidase (MAO) in brain of *Plasmodium berghei*-infected rats. But reports regarding the changes in other vasoactive amines like norepinephrine, histamine and dopamine in brain are not available. Our study was undertaken to observe any possible changes in

*Department of Physiology with Community Development, Vidyasagar University, Midnapore - 721 102, India.
+Department of Pharmacology, Medical College, Calcutta - 700 073, India.

the endo-genous amines (5-HT, norepinephrine, histamine and dopamine) in the brain of *P. berghei*-infected mice and rats.

MATERIALS AND METHODS

Albino mice (20-25 g) and albino rats (80-100 g) of Wistar strain and of either sex were given standardised diet and water *ad libitum*. The animals were infected with *P. berghei* by intraperitoneal injection of infected blood, and the infection was maintained by successive passages in mice and rats. Blood smears were taken on alternate days, stained with Leishman stain and seen under microscope to determine the percentage of parasitised RBC. Saline-treated animals served as control. Animals with parasitaemia between 40 and 50% were sacrificed. Whole brains of the animals were collected and wet weights taken. 5-HT, norepinephrine, histamine and dopamine contents of whole brain were measured fluorometrically by the method of Sadavongvivad[3]. The results were statistically analysed by students's '*t*' test.

RESULTS AND DISCUSSION

5-HT and norepinephrine contents of whole brain in *P. berghei*-infected mice and rats decreased significantly as compared to those of the control group. No significant changes were observed in histamine and dopamine contents of whole brain (Table 1).

Maegraith and Alexander[4] reported that vascular changes of malaria may vary from one regional circulation to another and that in cerebral vessels vasodilatation is the main feature. So any alteration of the vasoactive substances such as 5-HT, norepinephrine, histamine and dopamine is of special interest in the pathophysiology of malaria.

It is well known tht 5-HT and norepinephrine are metabolised by MAO. MAO activity is reported to increase in the brain of *P. berghei*-infected mice and rats[2]. So it may be conjec-

Table 1. 5-HT, NE, Histamine, DA contents of brain in *P. berghei*-infected mice and rats

Neurotransmitter µg/g	Mice		Rats	
	Control	Infected	Control	Infected
5-HT	0.69±0.02	0.61±0.01*	0.44±0.02	0.37±0.01*
NE	0.69±0.03	0.50±0.02**	0.39±0.03	0.27±0.02*
Histamine	0.53±0.02	0.51±0.01	0.24±0.03	0.23±0.01
DA	0.34±0.03	0.35±0.02	0.18±0.02	0.14±0.02

n = 10; each value represents mean ± SEM; *$p < 0.05$; **$p < 0.001$; 5-HT — 5-Hydroxytryptamine; NE — Norepinephrine; DA — Dopamine.

tured that increased MAO activity is probably responsible for the significant decrease in 5-HT and norepinephrine contents of brain in the infected mice and rats.

The normal tone of blood vessels is maintained mainly by adrenergic system. Norepinephrine causes vasoconstriction by activation of alpha receptors. 5-HT also tends to increase vasomotor tone through 5-HT receptors. So decreased contents of 5-HT and norepinephrine, as observed in our study, may possibly play a role in lowering the normal vasomotor tone and may be responsible for cerebral vasodilatation in the pathophysiology of malaria.

REFERENCES

1. Maegraith, B.G. (1985). In *Clinical Tropical Diseases*, 8th edn. (ELBS): 262-265.
2. Bhattacharyya, U., P.K. Kar, S. Roy and S.C. Lahiri (1987). 5-HT, 5-HIAA and related enzymes in *P. berghei*-infected rats. *Indian J. Malariol.*, **24**: 45-49.
3. Sadavongvivad, C. (1970). Determination of 5-HT, histamine, NE and DA in brain tissue. *British J. Pharmacol.*, **38**: 353-365.
4. Maegraith, B.G. and F. Alexander (1972). The pathogenesis of mammalian malaria. In *Advances in Parasitology*, v. 10. Ed. Ben Dawes (Academic Press, London and New York): 49-75.

Accepted: 30 September 1993

Indian Journal of Malariology
Vol. 30, December 1993, pp. 187-192.

Biocontrol Efficacy of *Gerris (A) spinolae, Laccotrephes griseus* and *Gambusia affinis* on Larval Mosquitoes

T. AMBROSE*, T. MANI+, S. VINCENT*, L. CYRIL ARUN KUMAR* and K. THRESIA MATHEWS*

Predation experiments using *Gerris (A) spinolae, Laccotrephes griseus* and *Gambusia affinis* were conducted against IV stage culicine larvae with varying prey densities. Ranking of individual predatory efficiency showed the sequence: large *Gambusia* > medium *Gambusia* > small *Gambusia* > female *Laccotrephes* > male *Laccotrephes* > *Gerris*. Predation under coexistence reveals the significance of predatory efficiency of different predator combinations with reference to prey density and exposure period.

Keywords: *Culex, Gambusia, Gerris, Laccotrephes*, Predator efficiency

INTRODUCTION

Mosquitoes, chiefly *Anopheles* and *Culex*, have been incriminated as respective vectors of malaria and filariasis that lead to the concept of mosquito control as a strategy for the control of these communicable diseases[1].

Resistance to insecticides has necessitated utilization of biological agents to control larval mosquitoes[2,3]. Members of the predatory aquatic bugs[4,5] and larvivorous fishes[6] have been seriously considered as potent biocontrol agents. Hence it is essential to understand the predator-prey response, so that maximum level of control could be achieved.

*P.G. and Research Department of Zoology, Loyola College, Madras - 600 034, India.
+Entomology Research Institute, Loyola College, Madras - 600 034, India.

Predator-prey interaction has received much attention in the recent past[7]. Optimal foraging theory[8] predicts inclusion of the most profitable prey items in predators' diet[9,10]. Predatory efficiency of a water bug gets altered under the stress of prey densities to which it gets exposed[11]. Our investigation was aimed at analysing the efficacy of coexisting bioagents of larval mosquitoes under inter- as well as intra-specific competition.

MATERIALS AND METHODS

Hemipteran predatory bugs - *Gerris (A) spinolae* (Leth and Serv.), a surface feeder, *Laccotrephes griseus* (geur.), a bottom feeder, and *Gambusia affinis* (Baird and Girard), a column feeder - were collected from Chetput pond, Madras, and were acclimatized. *Gambusia* were classified, for practical convenience, into large, medium and small size groups based on weight as an index of growth as they were collected from their natural habitats. Larval *Culex quinque-fasciatus* were collected fresh from local habitats. Newly emerged III and IV stage larvae were used as prey species. Abrupt changes in the quality of holding water during rearing and experimentation were avoided.

Predation experiments were conducted in 1 litre of aged tap water in glass aquaria (9 x 9 x 20 cm) at 29 $\pm$ 1°C. Male and female *L. griseus* (0.35 $\pm$ 0.081 g), male and non-gestating females of *G. affinis* of three size classes, namely large (0.32 $\pm$ 0.02 g), medium (0.22 $\pm$ 0.03 g) and small (0.17 $\pm$ 0.02 g), and *G. (A) spinolae* (0.04 $\pm$ 0.005 g) were used as individual predators with III and IV instar larvae as prey. Six possible predator combinations were experimented with two varying prey densities of 50 and 100 of the preferential IV instar larvae for 1 h, 3 h and 24 h exposure periods and the number of prey killed was recorded. The predator combinations experimented include:

Combination A: Adult *gerris* + *Gambusia* (large) + male *Laccotrephes*;

Combination B: Adult *gerris* + *Gambusia* (medium) + male *Laccotrephes*;

Combination C: Adult *gerris* + *Gambusia* (small) + male *Laccotrephes*;

Combination D: Adult *gerris* + *Gambusia* (large) + female *Laccotrephes*;

Combination E: Adult *gerris* + *Gambusia* (medium) + female *Laccotrephes*;

Combination F: Adult *gerris* + *Gambusia* (small) + female *Laccotrephes*.

Ten replicates were tested on each occasion. Statistical significance of predation was analysed by the application of ANOVA (nested design) and superiority of the values by studentized range test. Prey death rate recorded was subjected to the random predator equation of Rogers[12].

RESULTS AND DISCUSSION

Intraspecific predation of III and IV instar culicine larvae are shown in Figs. 1 and 2 respectively. Predators were found to prefer IV instar larvae to III instar larvae. No pupation was observed during the exposure

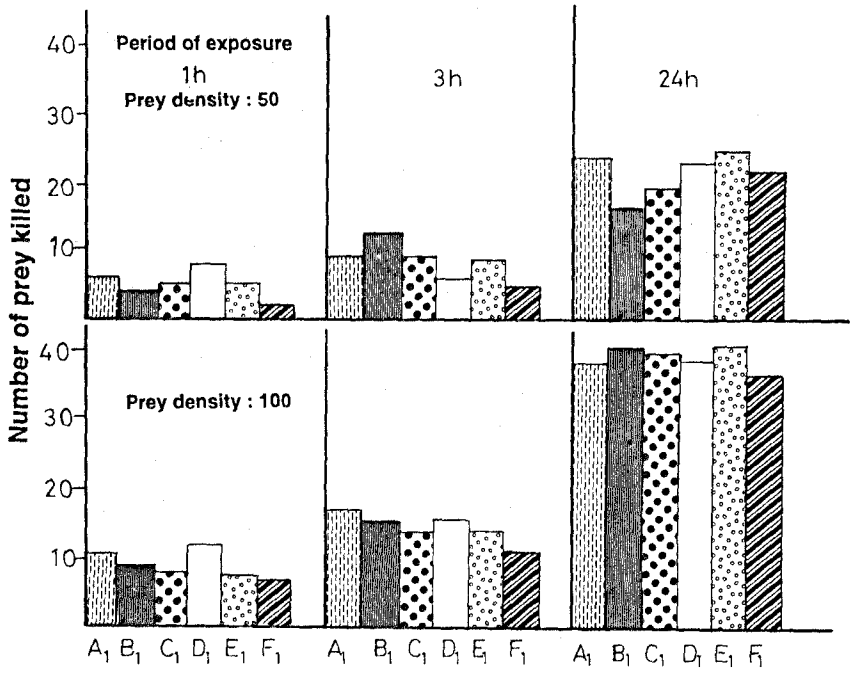


Fig. 1: Number of III instar culicine larvae killed in 1 h, 3 h and 24 h for varying prey densities. [A_1 - *Gerris (A) spinolae*; B_1 - *G. affinis* (large); C_1 - *G. affinis* (medium); D_1 - *G. affinis* (small); E_1 - *Laccotrephes griseus* (male); F_1 - *Laccotrephes griseus* (female). ($P < 0.05$)]

period. The observed preferential feeding of IV instar culicine larvae by the predators could be explained as an adaptation to reduce energy costs of prey capture by an opportunistic predator. An opportunistic predator would select a prey that could be approached and captured with less expenditure of energy[13].

Gambusia was found to kill more number of larval mosquitoes, followed by *Laccotrephes* and *Gerris*. Increased predation rate of *G. affinis* irrespective of size classes may be an attribute of a column feeder. A comparatively enhanced predation rate at higher prey density at all exposure periods was seen with *Laccotrephes* and *Gerris*. Predation by *Gambusia* was not consistent in 1 h exposure period. Prey density had no significance for 1 h exposure; however, it was significant at 3 and 24 h predations[9]. This may be due to diurnal behavioural variations of larval mosquitoes. Ranking of predatory efficiency and significance illustrates the following sequence: large *Gambusia* > medium *Gambusia* > small *Gambusia* > female *Laccotrephes* > male *Laccotrephes* > *Gerris*. With 3 h preda-

tion, prey density had a significant effect: *Gerris* was elevated to 5th rank and male *Laccotrephes* was shifted to 6th rank. At a predation period of 24 h female *Laccotrephes* was the least efficient predator. Increased predation irrespective of prey densities in *G. (A) spinolae* for 24 h is an interesting observation. This bug being a surface feeder, with an increase in predation period, the chances of encountering the prey would have been more.

Predatory efficiency of *Laccotrephes* differed between sexes. Enhanced predation by female *Laccotrephes* when compared to that of males may be due to higher energy demand[14]. Further, the males are passive and spend less energy for their maintenance by having a 'free-ride' over the female[15]. Decline in predation rate by males was further attested by lower attack rate.

For 1 h predation, prey density has no significant effect, irrespective of predator type. At 3 h exposure in a prey density of 50, predator combination D killed more prey (15.0%), but for the same exposure at a prey density of 100, combination A killed more prey (20.2 $\pm$ 0.87%). With interspecific predatory combinations prolonged for 24 h,

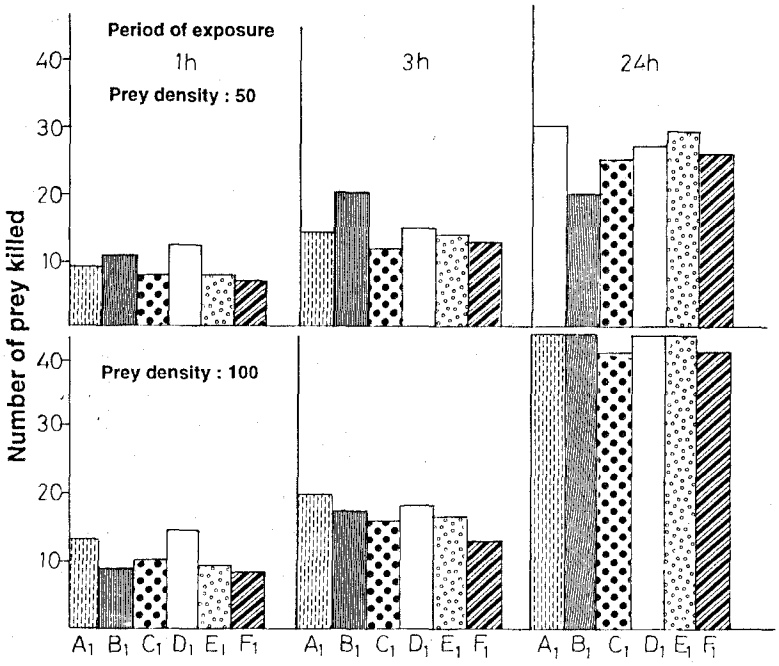


Fig. 2: Number of IV instar culicine larvae killed in 1 h, 3 h and 24 h for varying prey densities. [A_1 - *Gerris (A) spinolae*; B_1 - *Gambusia affinis* (large); C_1 - *G. affinis* (medium); D_1 - *G. affinis* (small); E_1 - *Laccotrephes griseus* (male); F_1 - *Laccotrephes griseus* (female). ($P < 0.05$)]

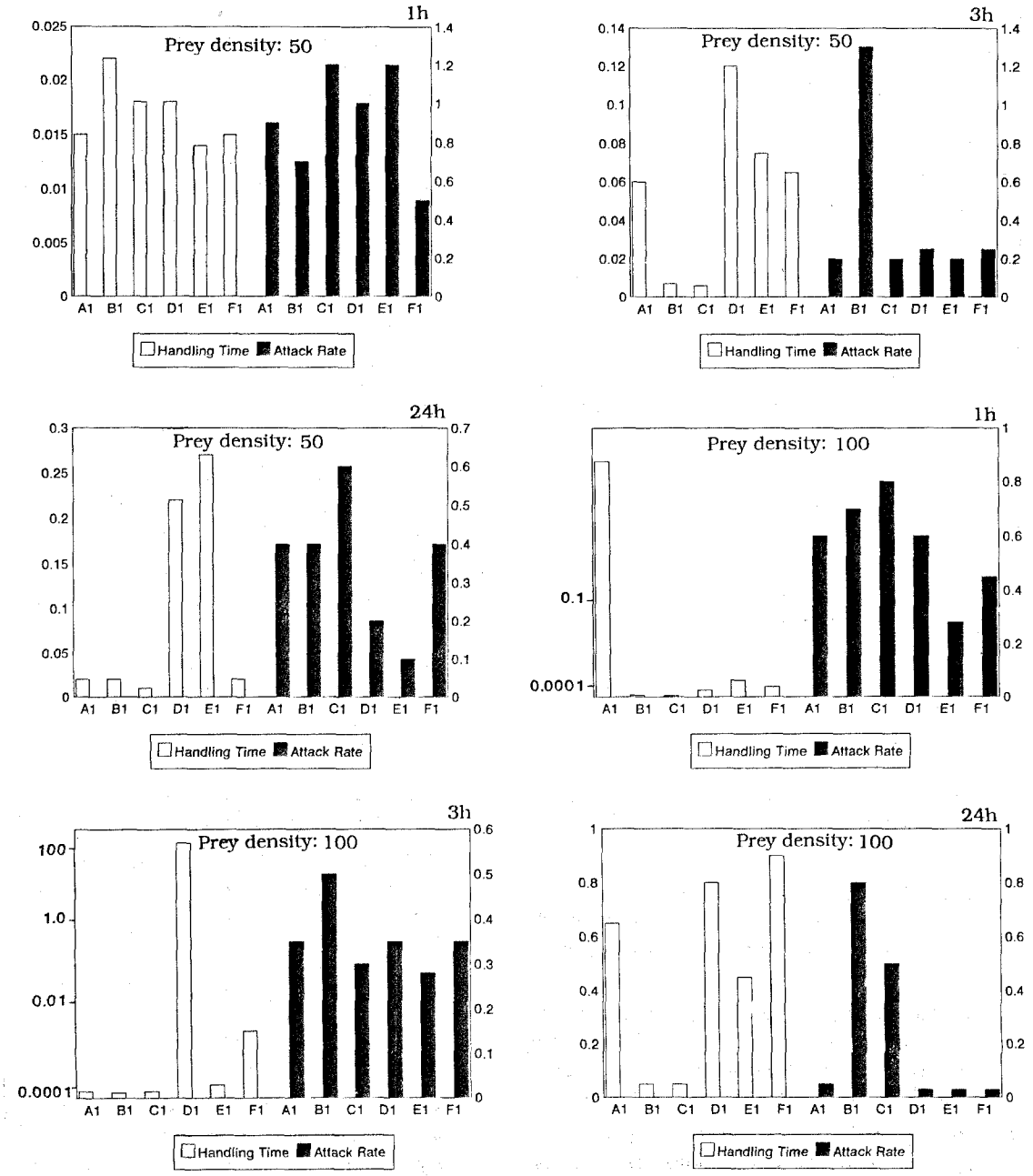


Fig. 3: Predatory response as attack rate and handling time to various prey densities. [A_1 - *Gerris (A) spinolae*; B_1 - *G. affinis* (large); C_1 - *G. affinis* (medium); D_1 - *G. affinis* (small); E_1 - *Laccotrephes griseus* (male); F_1 - *Laccotrephes griseus* (female). ($P < 0.01$)]

combination A killed significantly more prey (30.0 $\pm$ 0.99 and 45.4 $\pm$ 0.797%) ($p < 0.05$) irrespective of prey density.

Predatory responses as attack rate and handling time are shown in Fig. 3. The lowest attack rate of 0.0002 was observed in male *Laccotrephes* at 1 h in a prey density of 100. The highest attack rate (0.5292) was observed in large *Gambusia* for 3 h in a prey density of 100. The lowest handling time was shown by large *Gambusia* (- 0.02295) at 3 h predation in a prey density of 100.

ACKNOWLEDGEMENTS

The authors are thankful to Rev. Dr. Xavier Alphonse, S.J., Principal, Loyola College, Madras, for providing facilities and to Prof. Dr. V.A. Murthy for encouragement.

REFERENCES

1. ICMR (1984). Report of the WHO expert committee on vector biology, control and chemistry and specification of pesticides. *Tech. Rep. Ser.*, No. 699.
2. Vittal, M., R.B. Deobhankar and M.M. Hasan (1984). Experiments on feeding behaviour of some common larvivorous fish. *Pestology*, **8**(5): 28-31.
3. Sankaralingam, A. and P. Venkatesan (1989). Larvicidal properties of waterbug *Diplonychus indicus* (Venk. and Rao) and its use in mosquito control. *Indian J. Exp. Biol.*, **27**: 174-176.
4. Menke, A.S. (1979). The semiaquatic and aquatic Hemiptera of California (Heteroptera: Hemiptera). *Bull. Calif. Inst. Surv.*, **21**: 1-186.
5. Venkatesan, P. and C.M. Jeyachandra (1985). Estimation of mosquito predation by the water bug *Diplonychus indicus* (Venk. and Rao). *Indian J. Exp. Biol.*, **23**: 227-229.
6. Menon, P.K.B. and P.R. Rajagopalan (1977). Mosquito control potential of some species of indigenous fishes in Pondicherry. *Indian J. Med. Res.*, **66**: 765.
7. Hassel, M.P. (1978). The dynamics of Arthropod predator - prey systems. *Monogr. Pop. Biol.*, **13**: 1-237.
8. Krebs, J.R. (1978). Optimal foraging : Decision rules for predators. In *Behavioural Ecology : An evolutionary approach.* Eds. J.R. Krebs and N.B. Davis (Sinauer): 23-63.
9. Hassel, M.P. and R.M. May (1974). Aggregation of predators and insect parasites and its effect on stability. *J. Anim. Ecol.*, **43**: 547-594.
10. Crawley, K.W. (1975). The numerical responses of insect predators to changes in prey density. *J. Anim. Ecol.*, **44**: 877-892.
11. Blois, C. and A. Cloarec (1983). Density dependent prey selection in the water stick insect *Ranatra lineans* (Heteroptera). *J. Anim. Ecol.*, **52**: 849-866.
12. Rogers, D.J. (1972). Random search and insect population models. *J. Anim. Ecol.*, **41**: 369-383.
13. Gittleman, S.H. (1978). Optimum diet and body size in back-swimmers (Heteroptera: Notonectidae, Plecidae). *Ann. Entomol. Soc. Amer.*, **71**(5): 737-747.
14. Muthukrishnan, J. and T.J. Pandian (1987). Relation between feeding and egg production in some insects. *Proc. Indian Acad. Sci. (Anim. Sci.)*, **3**(96): 171-179.
15. Ravishankar, S. (1985). Studies on aquatic insects. [with special reference to bioecological aspects of *Laccotrephes griseus* (Hemiptera: Nepidae)]. Ph.D. Thesis, University of Madras.

Accepted: 22 October 1993

Indian Journal of Malariology
Vol. 30, December 1993, pp.193-199.

Mosquitoes of Sagar Island

MIHIR K. PRAMANIK*, P.M.R. ACHARY* and SAGARTIRTHA SARKAR*

Twenty mosquito species belonging to five genera, viz. *Aedes, Anopheles, Armigeres, Culex* and *Mansonia*, were collected during 1992-93 from the northern, middle and southern regions of Sagar Island. This is the first record of mosquito fauna of this island.

Keywords: Mosquitoes, Sagar Island, Fauna

INTRODUCTION

In the southernmost part of West Bengal lies a geographically isolated island, known as Sagar Deep or Sagar Island, surrounded on its three sides by rivers and on the southern side by the Bay of Bengal. Though isolated geographically from the mainland, this is the most populous island in the deltaic Bengal basin. The region is one of the most malaria-prone areas in West Bengal. We intended to look into the causative vector of malaria throughout the year in different seasons. To our surprise we came across a huge variety of vector mosquitoes in different proportions throughout the length and breadth of this island. Information on the mosquito fauna of Sagar Island had not been recorded earlier. It was therefore decided to study the mosquito fauna of Sagar Island. The results of this study are reported in this paper.

Physico-geographical features of Sagar Island

The extreme western sector of Sundarbans, consisting of Sagar Island and Lower Long Sands, falls between latitudes 21° and 21°.53'N and longitudes 88°.02' and 88°.15' E. It has a triangular outline with a length of 30 km north-south and a maximum width of 10 km east-west towards south. The northern extremity of the island is only 5 km south-west of Kakdwip point of the

*Genetics Laboratory, Department of Zoology, Calcutta University, Calcutta - 700 019, India.

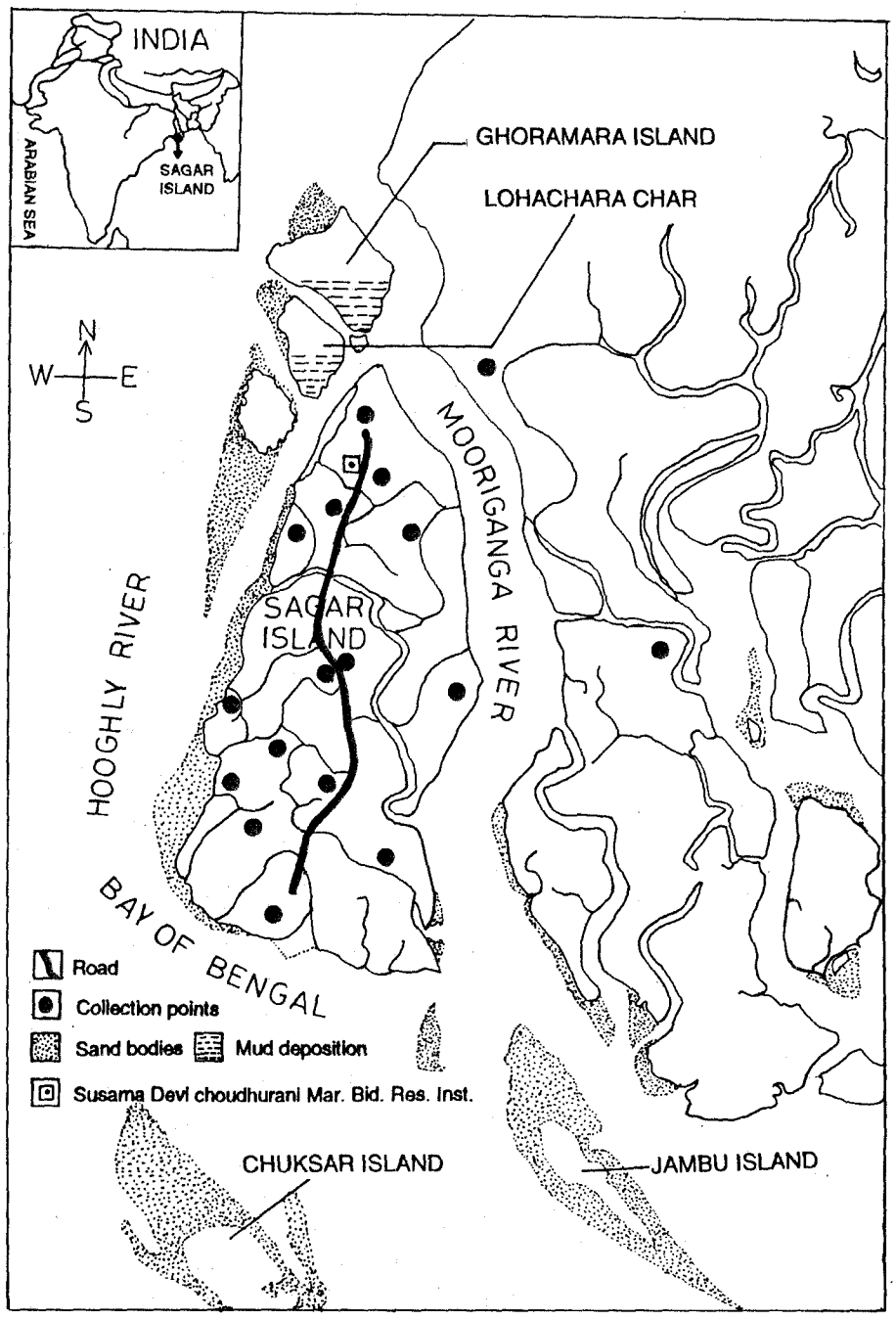


Fig. 1: Map of Sagar Island with 15 of its villages where surveys were carried out.

mainland and is separated from the mainland by the Hooghly river. On the east-west of the island it is separated by Mooriganga and Hooghly tidal rivers (Fig. 1). The southern margin of the island faces the tidal action of the Bay of Bengal. The wind velocity in the Sagar Island is known to be maximum during the months of April to June (30-50 km/h), and minimum during the winter months of December to February (1-4 km/h). The annual rainfall of the island ranges from 1483.1 to 2209.4 mm (data for 1981 to 1985 from India Meteorological Department, Calcutta), which signifies a high rainfall. The maximum rainfall takes place during June to September and minimum during December to February.

MATERIALS AND METHODS

Mosquito fauna surveys were carried out in 15 villages (Fig. 1) from northern, middle and southern regions of this island during April 1992 to February 1993. Adult mosquitoes resting indoors and outdoors were collected by suction tubes in the mornings (0500-1030 hrs) and evenings (1700-2230 hrs). Adult collections were made from cattlesheds, human dwellings, shrubs and other man-made structures. At least five cattlesheds and three human dwellings were searched thoroughly in each village for adult collection. Larval surveys were also made in these villages. Immatures were collected from ponds, pools, pits, and wells, etc. and kept in cages until adult emergence. All mosquitoes were killed in ether and packed in cellophane paper. Field-collected mosquitoes were brought to the laboratory for identification and preservation. Mosquitoes were identified using the keys of Christophers[1] and Barraud[2], the catalogue by Knight and Stone[3] and Das *et al*[4]. Identifications were cross-checked at MRC, Delhi.

RESULTS AND DISCUSSION

Twenty species of mosquitoes belonging to five genera, viz. *Aedes, Anopheles, Armigeres, Culex* and *Mansonia*, were collected. A total of 33 specimens of genus *Aedes* comprising only a single species, 5968 specimens of genus *Anopheles* comprising 7 species, 666 specimens of genus *Armigeres* comprising 3 species, 2787 specimens of genus *Culex* comprising 6 species, and 450 specimens of genus *Mansonia* comprising 3 species were collected. The distribution of species in northern, middle and southern Sagar Island is given in Table 1.

Amongst seven anophelines collected from the island, the most prevalent species was *An. barbirostris* (34.39%), followed by *An. vagus* (17.44%) and *An. nigerrimus* (16.49%).

Among culicines, the most dominant genus was *Culex* and in this genus the most prevalent species was *Cx. quinquefasciatus* (32.50%), followed by *Cx. whitmorei* (28.96%) and *Cx. sitiens* (26.91%). The populations of genera *Aedes, Armigeres* and *Mansonia* were found in very low numbers.

A study of the seasonal variation of the mosquito population throughout the year showed the predominance of not only a particular species but a whole genus, and on other hand, complete absence of the other

Table 1. Distribution of mosquito species in Sagar Island region-wise and season-wise

Species	Summer (Apr-May'92)				Monsoon (Jul-Aug'92)				Post-monsoon (Oct-Nov'92)				Winter (Dec'92-Feb'93)				Group total	% in Genus	% in total
	N	M	S	Total	N	M	S	Total	N	M	S	Total	N	M	S	Total			
Aedes																			
Ae. aegypti	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33	33	33	100	0.33
Total																	33		0.33
Anopheles																			
An. aconitus	0	0	0	0	15	0	3	18	111	69	30	210	24	27	39	90	318	5.32	3.21
An. annularis	0	0	0	0	0	0	0	0	57	66	58	181	12	36	66	114	295	4.94	2.98
An. barbirostris	6	12	9	27	3	9	12	24	861	180	666	1707	93	102	99	294	2052	34.39	20.71
An. culicifacies	30	39	45	114	6	9	30	45	159	81	216	456	30	36	45	111	726	12.17	7.33
An. nigerrimus	6	12	6	24	0	24	30	54	366	51	120	537	147	123	99	369	984	16.49	9.93
An. subpictus	54	33	15	102	6	15	72	93	51	27	114	192	45	54	66	165	552	9.24	5.58
An. vagus	51	24	18	93	3	75	126	204	36	15	57	108	189	204	243	636	1041	17.44	10.51
Total																	5968		60.25
Armigeres																			
Ar. kuchingensis	6	9	3	18	0	0	3	3	0	0	0	0	0	0	12	12	33	4.96	0.33
Ar. subalbatus	3	9	15	27	3	6	57	66	24	6	243	273	36	27	33	96	462	69.37	4.67
Ar. theobaldi	6	15	24	45	0	0	12	12	0	0	30	30	12	18	54	84	171	25.68	1.72
Total																	666		6.72

contd...

Table 1. (*contd.*)

Species	Summer (Apr-May'92)				Monsoon (Jul-Aug'92)				Post-monsoon (Oct-Nov'92)				Winter (Dec'92-Feb'93)				Group total	% in genus	% in total
	N	M	S	Total	N	M	S	Total	N	M	S	Total	N	M	S	Total			
Culex																			
Cx. bitaeniorhynchus	12	18	30	60	0	0	6	6	6	6	15	27	24	33	51	108	201	7.21	2.02
Cx. gelidus	0	6	3	9	0	0	0	0	0	0	0	0	0	0	0	0	9	0.32	0.09
Cx. quinquefasciatus	9	78	123	210	6	15	60	81	69	36	282	387	39	81	108	228	906	32.50	9.14
Cx. sitiens	45	93	150	288	6	0	27	33	39	30	84	153	78	87	111	276	750	26.91	7.58
Cx. tritaeniorhynchus	6	12	30	48	0	0	3	3	0	0	15	15	9	15	24	48	114	4.09	1.15
Cx. whitmorei	60	72	90	222	9	3	18	30	36	30	111	177	99	123	156	378	807	28.96	8.14
Total																	2787		28.12
Mansonia																			
Ma. annulifera	3	9	51	63	3	3	6	12	0	0	0	0	9	0	9	18	93	20.66	0.93
Ma. indiana	12	18	45	75	6	6	15	27	33	48	138	219	3	9	6	18	339	75.33	3.42
Ma. longipalpis	0	6	3	9	0	0	0	0	0	0	0	0	3	0	6	9	18	4.00	0.18
Total																	450		4.53
Grand total																	9904		

N — Northern part; M — Middle part; S — Southern part.

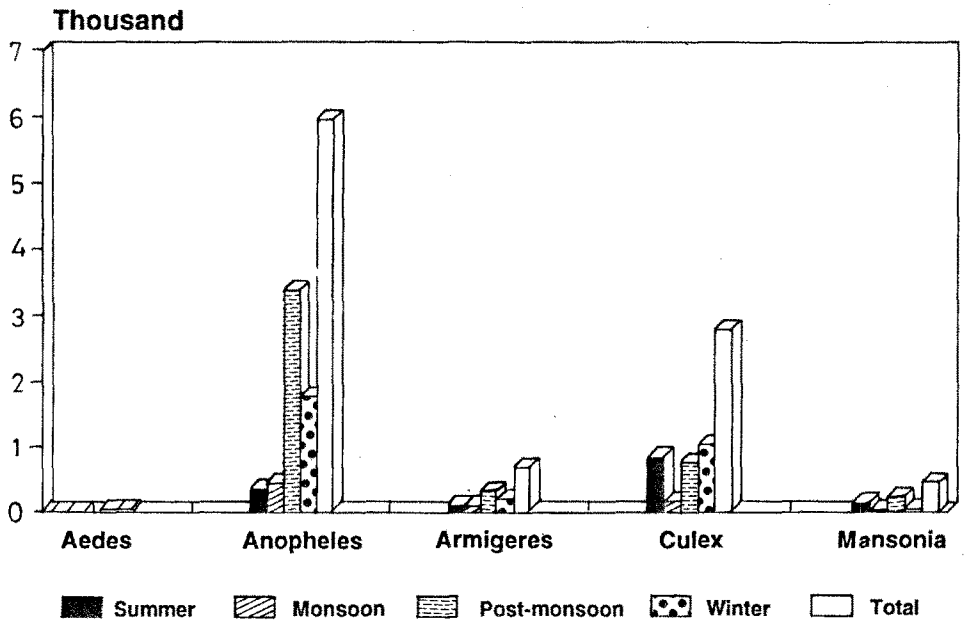


Fig. 2: Seasonal variation of five mosquito genera of Sagar Island during 1992-93.

(Fig. 2). *Anopheles* was found to be the most dominant mosquito fauna during monsoon (61.60%), post-monsoon (72.42%) and winter season (57.62%), whereas *Culex* was predominant only in summer (58.36%). *Aedes* was totally absent in our surveys during summer, monsoon and post-monsoon seasons, and 33 specimens of a single species were recorded only in winter (December to February).

The mosquito fauna of Sagar Island comprises the known malaria vector *An. culicifacies*. Although no vector incrimination studies have been done so far, it is known that this vector may play an important role in malaria transmission in this island if the density becomes significant. It was reported that *An. sundaicus* was responsible for malaria epidemics in Chilka lake in Orissa[5]. Recent surveys have revealed that *An. sundaicus* has disappeared from coastal Orissa[6]. During our surveys in the coastal island we came across some mosquito specimens which resemble *An. sundaicus*, but this is not confirmed as yet. Recently, it was reported that *An. annularis* is an important vector of malaria in rural West Bengal[7]. Mosquito fauna of Sagar Island, though, has revealed representatives of *An. annularis* but not in an alarming frequency (only 4.9%). As a few mosquito species of the island are established malaria vectors, it is important to monitor the densities of these vector species in different structures, their breeding sites and feeding preferences through-

out the year to provide baseline data for studies on the epidemiology of malaria.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. B.N. Nagpal and Dr. V.P. Sharma of Malaria Research Centre, Delhi, for helping in the identification of mosquitoes. They also extend thanks to Dr. A.P. Das and RMRC, Bhubaneswar. Thanks are due to Prof. A. Choudhury, Marine Science Department, Calcutta University, for his help and cooperation at Sagar Island. We are indebted to Prof. A.K. Duttagupta, Department of Zoology, Calcutta University, for extending laboratory facilities for this work.

REFERENCES

1. Christophers, S.R. (1933). *The Fauna of British India including Ceylon and Burma*, v. 4 (Taylor and Francis, London): 1-360.
2. Barraud, P.J. (1934). *The Fauna of British India including Ceylon and Burma*, v. 5 (Taylor and Francis, London): 1-463.
3. Knight, K. and A. Stone (1977). *A catalog of the mosquitoes of the world* (Diptera: Culicidae) (Entomological Society of America, Maryland): 1-558.
4. Das, Bina Pani, R. Rajagopal and J. Akiyama (1990). Pictoral key to Indian anopheline mosquitoes. *J. Pure and Appl. Zool.*, 2(3): 131-162.
5. Covell, G. and Pritam Singh (1942). Malaria in the coastal belt of Orissa. *J. Mal. Inst. India*, 4: 457-593.
6. Nagpal, B.N. and V.P. Sharma (1983). Mosquitoes of coastal Orissa. *Indian J. Malariol.*, 20: 141-145.
7. Ghosh, K.K., S. Chakraborty, S. Bhattacharya, A. Palit, Neelam Tandon and A.K. Hati (1985). *Anopheles annularis* as a vector of malaria in rural West Bengal. *Indian J. Malariol.*, 22: 65-69.

Accepted: 3 November 1993

Indian Journal of Malariology
Vol. 30, December 1993, pp. 201-206.

Feeding Preferences of *Anopheles sundaicus* in Car Nicobar Island

ROOP KUMARI*, HEMA JOSHI+, A. GIRI++ and V.P. SHARMA+

Host feeding behaviour of *An. sundaicus* population in Car Nicobar Island was studied by bloodmeal analysis and bait collection. Results indicated the zoophagic nature of the species in different resting sites. However, a human blood index of 0.18 was observed in specimens collected from human dwellings. The highest proportion of females was found positive for porcine antigen. Bait collections also indicated a similar feeding pattern.

Keywords: *An. sundaicus*, Car Nicobar Island, HBI

INTRODUCTION

In India, *An. sundaicus* has been reported as a primary vector of malaria throughout its range of distribution by Christophers[1]. The species has been incriminated from Bengal[2,3], Orissa[4] and Andamans[5]. However, during DDT era the species disappeared from the eastern coast of the mainland and is now limited to the deltaic region of Bangladesh and Andaman and Nicobar Islands[6].

Mosquito host preference studies are important to understand the epidemiology of malaria. Reports on the feeding behaviour of *An. sundaicus* in India are scanty. Senior-White[7] had reported an anthropophilic index of 5.6 per cent in Visakhapatnam. However, its prefernce to man and cattle differs considerably. Precipitin test of 160 abdomen bloodmeals of *An. sundaicus* was reported by Bruce-Chwatt *et al.*[8], who used primate blood instead of human blood, and in a total of 127 positive tests, only 9 were

*Malaria Research Centre (Field Station), BHEL Complex, Ranipur, Hardwar - 249 403, India.
+Malaria Research Centre, 22-Sham Nath Marg, Delhi - 110 054, India.
++Malaria Research Centre (Field Station), Car Nicobar - 744 301, India.

positive for primate blood. Our study reports the bloodmeal analysis of *An. sundaicus* population in the Car Nicobar Island.

Study area

Car Nicobar is a small island among the Nicobar group of islands, which is separated from the Andaman group of islands by 10° channel, located between latitudes 6°-10° N and between longitudes 92°- 94° E in the Bay of Bengal. The island has an area of 127 sq km with about 20,287 tribals of Mongoloid origin. About 70% of the population of Nicobar group of islands is located here. The humidity ranges from 70 to 90% and temperature from 23 to 33°C throughout the year.

MATERIALS AND METHODS

Bloodmeal analysis

Bloodmeal analysis was based upon day collection of freshly, full-fed females made with suction tube from human dwellings, cattlesheds, copra *machans* (huts for growing endocarp of coconut), hen cottages and other outdoor structures in Car Nicobar during 1990-91. The specimens were identified in the laboratory for determining the species composition. Stomach contents of the female *An. sundaicus* were dissected with a pair of needles, smeared on a Whatman filter paper no. 1 and sent to Malaria Research Centre Headquarters for bloodmeal analysis. Bloodmeal samples were analyzed by countercurrent immunoelectrophoresis of Bray *et al.*[9] with modifications as described by Joshi *et al*[10]. Each sample was tested against human, bovine, porcine and goat antisera (obtained from Serologist and Chemical Examiner, Government of West Bengal).

To check the results of bloodmeal analysis, bait collections were made. Eleven whole-night outdoor bait collections were made by using human, pig, bovine and goat baits in three selected villages, viz. Kimus, Kakana and Sawai. At a distance of about a metre, each bait was kept inside a mosquito net trap, which was tied 12 inches above the ground level. Mosquitoes were collected from each bait at hourly intervals for 15 min and were kept separately in test tubes to identify and count the number of female *An. sundaicus* caught.

The forage ratio technique was used to study the host feeding preference of *An. sundiacus*[11], and Fiseher's chi-square test was applied to test the statistical significance of the data.

RESULTS AND DISCUSSION

A total of 872 bloodmeal smears were assayed. *An. sundaicus* was found to be predominantly zoophagic. In the overall analysis, percentages positive for porcine, goat, bovine and humans blood were 57.97, 4.33, 3.38 and 2.57 respectively. About 12.97% females were found to feed on two or more hosts, which shows that some of them were apparently driven out while feeding and later came to feed again. 18.78 per cent of samples showed negative reaction to all the four antisera. Population proportions of different host species in Car

Nicobar Island are as: humans (0.317), pigs (0.256), goats (0.121), bovine animals (0.006), poultry (0.284) and ducks (0.016). Table 1 gives the data on the bloodmeal analysis of *An. sundaicus* collected from different resting sites in Car Nicobar.

Table 1 shows that the proportion positive for different antisera varies from resting place to resting place of *An. sundaicus*. Comparative chi-square values of the observed numbers showing human, bovine, pig and goat antigen among different habitats (Table 1) showed that the proportion positive for human, bovine, pig and goat antisera differed significantly in different ecotypes except between cattlesheds and outdoor structures and between copra *machan* and outdoor structures. Statistical comparison of different biotopes with regard to human blood index (HBI) ($\chi^2 = 104.92$; $p < 0.001$) showed that proportions of human positive samples are significantly higher in human dwellings than in other habitats. Overall human positivity in our study sample was 2.91 (HBI = 0.03). Porcine antigen-positive mosquitoes were highest in proportion in all the resting sites, whereas bovine antigen-positive mosquitoes were predominant in human dwellings (0.25) (Table 1). The highest proportion of mosquitoes with goat blood was found in outdoor structures (0.23). *An. sundaicus* occurred in high densities during monsoon and post-monsoon months. The HBI was highest (0.10) during July, followed by 0.045 during September-October, and 0.02 during November.

The results of bait collection (Table 2) showed that *An. sundaicus* preferred to feed on pig and bovine blood followed by goat and human blood. The density per night per bait was 96.54 for calf, 95.27 for pig, 69.18 for goat and 12.81 for man. The ratio

Table 1. Proportion of bloodmeal analysis of *Anopheles sundaicus* collected from different resting sites in Car Nicobar

Resting sites	Human dwelling (HD)	Cattleshed (CS)	Copra *machan* (CM)	Hen cottage (HC)	Outdoor structure (OS)
No. tested	99	377	265	75	56
Human	0.15	0.00	0.01	0.00	0.01
Bovine	0.25	0.09	0.10	0.10	0.15
Porcine	0.41	0.57	0.68	0.50	0.66
Goat	0.17	0.11	0.07	0.23	0.13
Not reactive	0.12	0.22	0.14	0.16	0.04

Chi-square values: HD vs CS : 78.43, $p < 0.0002$; HD vs CM : 53.67, $p < 0.0002$; HD vs OS : 14.92, $p < 0.002$; HD vs HC : 15.63, $p < 0.002$; CS vs CM : 11.91, $p < 0.01$; CS vs OS : 4.93, Not significant; CS vs HC : 19.50, $p < 0.0005$; CM vs OS : 7.28, Not significant; CM vs HC : 10.48, $p < 0.02$; OS vs HC : 60.51, $p < 0.002$.

Table 2. Number of female *An. sundaicus* collected on different baits

Time of collection (hrs)	Bait				Total
	Pig	Calf	Goat	Man	
1800-1900	77	92	77	1	247
1900-2000	115	116	101	10	342
2000-2100	134	162	106	11	413
2100-2200	143	112	80	18	353
2200-2300	111	86	79	8	284
2300-2400	82	75	62	12	231
0000-0100	87	56	51	12	206
0100-0200	76	98	41	13	228
0200-0300	111	102	81	28	322
0300-0400	83	94	59	18	254
0400-0500	22	49	22	10	103
0500-0600	7	20	2	0	29
Total no. collected	1048	1062	761	141	3012
Density per night per bait	95.27	96.54	69.18	12.81	273.81

of human to pigs/calf was 1:7 in the island and almost the same figure was reported by Reid[12]. The feeding behaviour reflects the same trend as in bloodmeal analysis.

Forage ratios of greater than one for bovine animals and pigs indicated selective preference for these hosts (Table 3). A significantly higher proportion of bloodmeal samples positive for porcine antigen may be due to higher proportion of pigs in the island as compared to cows and buffaloes (Kalra, personal communication, 1993).

The predominantly zoophagic nature of *An. sundaicus* was observed in Car Nicobar Island in our study. The same was reported in Malaysia by Wharton *et al.*[13] and in Kampuchea by Eyles *et al*[14]. In Indonesia the species shows a very high anthropophilic

Table 3. Forage ratios of *An. sundaicus*

Host	% in bloodmeals A	% in population B	Forage ratio A/B
Human/Pig	62.24	25.64	2.42
Human/Goat	4.51	12.08	0.37
Human/Bovine	3.64	0.63	5.77
Human/Domestic animal	2.91	31.65	0.09

index (AI) ranging up to 98 per cent[15,16]; in South Java, AI was 51 per cent in human dwellings and 22 per cent outdoors. But in Malaysia and Kampuchea, cattle have a better attraction of *An. sundaicus* than man, as observed by Wharton *et al.*[13] and Eyles *et al*[14]. Indiscriminate feeding behaviour of *An. sundaicus* in cattle and man was also reported from Myanmar. Differential behaviour of *An. sundaicus* in different geographical areas has been observed. In West Java (Chilachap) the species is exophagic, endophilic, anthrophagic, susceptible to malathion and was responsible for malaria epidemic in the area in 1988 (Kalra, personal communication, 1993), while in Central Java the species is exophilic, exophagic, anthropophagic and resistant to DDT, and malaria in the area is endemic[17]. However, in East Java (Yoggakata) the species is exophilic, exophagic and zoophagic, and the area is totally free from malaria (Kalra, personal communication, 1993).

Senior-White reported an HBI of 0.05 among 90 specimens of *An. sundaicus* in Visakhapatnam, while in our study, it was very low (0.03). However, an HBI of 0.18 observed in human dwellings in our study suggests that a good proportion feeds on human hosts and can maintain effective transmission of malaria.

However, the most interesting part of the study relates to high feeding preference of the species on pigs, not reported elsewhere over the distribution of *An. sundaicus*. Since pigs are the acknowledged amplyfying host of Japanese encephalitis, *An. sundaicus* pose high potential for transmission of JE, if this species is susceptible to the virus.

ACKNOWLEDGEMENTS

The authors wish to thank Shri N.L. Kalra, Honorary Consultant, Malaria Research Centre, Delhi, for his constant encouragement and critical review of the manuscript. We are thankful to *pradhans* of Car Nicobar villages for their help. The technical assistance of Mrs. Alka Kapoor and Mrs. Lalita Gupta in the analysis of bloodmeal samples is duly acknowledged.

REFERENCES

1. Christophers, S.R. (1933). *The fauna of British India including Ceylon and Burma,* v. IV. (Taylor and Francis, London): 249-250.
2. Iyengar, M.O.T. (1931). The distribution of *Anopheles ludlowii* in Bengal and its importance in malaria epidemiology. *Indian J. Med. Res.*, **19**: 499-524.
3. Sen, P. (1938). *Anopheles sundaicus* and malaria in Calcutta. *J. Mal. Inst. India*, **1**: 83-97.
4. Senior-White, R. and A.K. Adhikari (1939). On malaria transmission around the Chilka Lake. *J. Mal. Inst. India*, **2**: 395-423.
5. Covell, G. (1927). Report of an enquiry into malaria conditions in the Andamans (Government Press, Delhi).
6. Kalra, N.L. (1991). Forest malaria vectors in India: Ecological characteristics and epidemiological implications. Forest Malaria in Southeast Asia. In *Proceedings of Informal Consultative Meeting WHO/MRC,* 18-22 Feb.: 93-114.
7. Senior-White, R. (1947). On the anthrophilic indices of some *Anopheles* found in east central India. *Indian J. Malariol.*, **1**: 111-122.

8. Bruce-Chwatt, L.J., C.J. Garrett and B. Weitz (1966). Ten years' study (1955-64) of host selection by anopheline mosquitoes. *Bull. WHO,* **35:** 405-435.

9. Bray, R.S., G.S. Gill and Killick-Kendrick (1984). Current and possible future technique for the identification of bloodmeals of vector haematophagous arthropods. *WHO/VBC/*84. 905 (Unpublished documents).

10. Joshi, H., K. Vasantha, S.K. Subbarao and V.P. Sharma (1988). Host feeding patterns of *Anopheles culicifacies* species A and B. *J. Amer. Mosq. Contr. Assoc.,* **4**(3): 248-251.

11. Hess, A.D., Haves Richardo and C.H. Tempelis (1968). The use of the forage ratio technique in mosquito host preference studies. *Mosq. News,* **28**(3): 386-389.

12. Reid, J.A. (1961). The attraction of mosquitoes by human or animal baits and in relation to the transmission of disease. *Bull. Entomol. Res.*, **52:** 43-62.

13. Wharton, R.H., D.E. Eyles, M. Warren and W.H. Cheong (1964). Studies to determine the vectors of monkey malaria in Malaya. *Ann. Trop. Med. Parasitol.*, **58:** 56-57.

14. Eyles, D.E., R.H. Wharton, W.H. Cheong and W.M. Warren (1964). Studies on malaria and *Anopheles balabacensis* in Cambodia. *Bull. WHO,* **30:** 7-21.

15. Toumanoff, C. (1936). Anophelism on extreme Orient (Anophelism in the Far East) Collin. Masson and cic., Paris. *Soc. Path. Exot. Monog.*, **4:** 434 (ONS).

16. Walch, E.W. (1932). Het Verband tusschen de voorkeur van Nederlandsch Indische Anophelism voor menschan of dierne bloed haar gevaarligkheid (Tweede Meeled eiling). *Geneesk. Tijdschr. Ned. India*, **72:** 682-709 (ONS).

17. Sundararaman, S., R.M. Soeroto and M. Siram (1957). Vector of malaria in mid-Java. *Indian J. Malariol.*, **11:** 321-338.

Accepted: 17 November 1993

Indian Journal of Malariology
Vol. 30, December 1993, pp. 207-214.

Effects of Malaria Infection on Pregnancy

LATIKA S. NAIR* and A.S. NAIR*

A study of the effects of malaria infection on the progress and outcome of pregnancy was carried out during 1987-88 in the Medical College Hospital, Surat, Gujarat. Pregnant women were highly susceptible to the infection (SPR, 57.7) compared to the general population (SPR,18.6). *P. falciparum* infection was predominant (62.4%). The infection rate was also found to be higher (SPR, 72.2%) in second trimester compared to first and third semesters. Primigravidae seemed to be at a greater risk as the mean parasitaemia level was higher (39%) and the outcome poor as compared to multigravidae (29%). Infection during pregnancy caused severe maternal complications like abortion (9.7%), premature labour (59.6%), and still-births (5.7%), which were higher in *P. falciparum* infection. Microcytic anaemia combined with dimorphic anaemia was predominant in the infected group (89.5%). Cord blood in 4 cases and on baby's blood were found positive for malaria parasite, showing transplacental passage of malaria parasites, which is rare. The infection was found to have a definite bearing on the low birth weight of babies. Chemoprophylaxis could obviate much of the complications.

Keywords: Malaria, Pregnancy, Congenital Malaria, Chemoprophylaxis

INTRODUCTION

In the countries which took up malaria eradication the most vulnerable group of population, viz. pregnant women and infants, has been exposed to high risk, after two decades of successful eradication compaigns, bringing down the level of acquired immunity[1]. Even though hazards of this disease have been estimated and recorded, no specific data have been maintained in respect of the morbidity and mortality due to malaria in pregnant women. Some studies have been carried out in African countries but very little information is available with regard to India.

Surat District and Surat City had the distinction of bringing down the incidence of the disease to low levels by 1965 (API,

*85-86, Krishnakunj Nagar, Bhestan, Surat-395 023, India.

0.26). But thereafter extensive irrigation without proper water management, rapid industrialisation without proper antimalaria measures at sites and mass migration of population for work, made the area highly receptive and vulnerable. The extent of re-appearance of malaria could be judged from the fact that the Annual Parasite Incidence (API) recorded in the district for 1987-88 was as high as 32.50.

Hence this study was undertaken in the Medical College (Gynaecology Section), Surat, with the collaboration of the District Malaria Eradication Programme Office, for a period of 14 months from June 1987 to July 1988.

MATERIALS AND METHODS

Pregnant women who attended the antenatal clinic with history of fever were considered for the study. Initially, blood smears (thick and thin) were collected from them in accordance with the standard prescribed in "Instructions for Microscopists" under the National Malaria Eradication Programme and these were examined for malaria parasites. The number of parasites and WBCs were counted for 100 fields of each thick film and parasite density was calculated against 7000 WBC. This is expressed in terms of percentage (against 100 WBC). Those cases who could be persuaded were admitted as indoor patients and others were followed up in the OPD. Thus, out of a total of 8960 pregnant women who attended antenatal clinic during the study period, blood smears of 322 with history of fever could be examined. From the positive cases detected, 104 cases could be followed up. Another 104 cases without infection were also followed up for comparison. Distribution of cases in relation to gestation period and parity, in control as well as infected group, with species-wise distribution, is given in Table 1.

Detailed history of fever, urinary tract infections, respiratory tract infection, prema-

Table 1. Distribution of cases in relation to gestation period and parity in infected and control group with type of infection in infected group

Parity	Trimester						Total control	Total infected
	I		II		III			
	Control	Infected	Control	Infected	Control	Infected		
Primipara	2	1 (1F)	2	3 (3F)	31	20 (12V+8F)	35	24 (12V+12F)
Multipara	3	2 (1V+1F)	4	11 (3V+8F)	62	67 (32V+35F)	69	80 (36V+44F)
Total	5	3 (1V+2F)	6	14 (3V+11F)	93	87 (44V+43F)	104	104 (48V+56F)

V — *P. vivax* infection; F — *P. falciparum* infection.

ture labour, hypertension in past pregnancy, and parity were elicited and recorded in a prescribed pro forma. A temperature chart was maintained. Obstetrical examination and examination of respiratory, cardiovascular and alimentary system were done and observations recorded. Signs of threatened abortion and preterm labour were looked for and foetal heart sounds were recorded.

Routine laboratory investigations including complete haemogram, blood group, urinalysis and investigation for anaemia were done. In complicated cases, other investigations like blood urea, blood sugar, liver function tests, and lumber puncture were also carried out.

All the fever cases were given initially a single dose of chloroquine 600 mg (4 tablets of 150 mg base each) and those found positive were given a complete course of treatment with chloroquine[2]. Those patients who could not tolerate oral administration were given intramuscular injection of chloroquine. In those cases where the patients did not show improvement within 48 h symptomatically, a further blood smear examination was done. If the blood smear was found positive for *P. falciparum* it was considered as unresponsive to chloroquine and was given quinine in tablet form. In complicated cases, i.e. where patients were admitted in an unconscious state or with high-grade fever, disorientation and poor general condition, intravenous infusion of quinine was done[2]. Earlier, the patients' base-line temperature, pulse, blood pressure and, in some cases, ECG were taken for further comparison. All the cases were followed up till delivery. Placental weight and baby's weight were recorded. Also, cord blood smears and baby's blood smears were taken and examined for malaria parasites. Placental biopsy was taken in 20 cases.

Weekly chemoprophylaxis with a single dose of 300 mg chloroquine administration was done on 53 pregnant women and the mean weight of the babies delivered was compared with that of 51 who did not have the prophylatic dose.

Statistical significance of the data was determined by applying t and z tests.

RESULTS

From the 322 blood smears examined, 186 (57.7%) were found positive for malaria parasites. Out of this, 116 (62.4%) were of *P. falciparum* infection and 70 (37.6%) of *P. vivax* infection. 4 cases showed mixed infection (included in falciparum cases). The slide positivity rates and slide falciparum rates for the district as a whole,

Table 2. Slide positivity rate and slide falciparum rate in district, Surat City and study group during study period June 1987 to July 1988

Area	Slide positivity rate	Slide falciparum rate
Surat District	18.60	8.50
Surat City	27.20	10.50
Study group (pregnant women)	57.70	36.00

Table 3. Distribution of cases in relation to Hb level in control and infected groups and species-wise

Hb level	Control group	Infected group	Species of infection	
			Pf	*Pv*
< 6	2	8	7	1
6 - 8	24	35	22	13
8 - 10	58	50	23	27
> 10	20	11	4	7
Total	104	104	56	48

Surat City and the study group for the study period are given in Table 2.

All the patients had fever and 79.8% had associated rigour. Other major complaints were nausea and/or vomiting (66.8%), pain in abdomen (86.2%) and general malaise (23.5%).

The slide positivity rates for 1st, 2nd and 3rd trimesters were 38, 72.2 and 47 respectively. The differences in SPR between 2nd trimester and 1st as well as 3rd trimester, viz. 34.2% and 25.2%, are statistically significant ($z = 3.1$ and 4.6, $p < 0.001$). The mean parasite densities in primipara and multipara were 39% and 29% respectively. The difference of 10% in mean parasite densities is statistically significant ($z = 3.7$, $p < 0.001$).

Comparative Hb levels in control and infected group and species-wise in the case of infected group are shown in Table 3. The mean Hb levels in control and infected group were 8.3 g% and 7.9 g% respectively. The difference in mean Hb levels in control and infected group is 0.4 g%, which is statistically significant ($z = 2$, $p < 0.05$), indicating a lower level of Hb in the infected group. 41.3% of the infected cases had less than 8 g% Hb level as compared to 25% in the control group. The mean Hb levels in falciparum cases and vivax cases were 7.6 g% and 8.2 g%, the difference in mean Hb being 0.6 g%, which is significant (z=2.3, $p < 0.05$). 51.8% of falciparum cases had Hb levels less than 8 g%, corresponding to 29.2% of vivax cases. Distribution of cases according to the type of anaemia present is given in Table 4.

Table 4. Distribution of cases according to type of anaemia present

Type of anaemia	Control group	Infected group
Normocytic	20 (19.3)	11 (10.5)
Dimorphic	7 (6.7)	22 (21.3)
Microcytic	77 (74.0)	71 (68.2)

Figures in parantheses are percentages.

Microcytic and dimorphic anaemia accounted for 89.5% of the infected cases while it was 80.7% in the control group.

Foetal outcome in control and infected group and species-wise in infected group is shown in Table 5. During the study period 12 maternal deaths occurred out of which 4 (33.3%) were with confirmed falciparum infection and symptoms of cerebral malaria.

The mean weights of the babies in the control group and infected group with different types of infection in mothers as well as those with placental infection and placenta without such pathology are given in Table 6. The mean birth weight of the babies of the infected group was found to be significantly lower than that of the control group. The difference in mean weights (600 g) is statistically significant ($z = 6$, $p < 0.001$). Similarly, the mean birth weight of babies of the falciparum-infected group was significantly lower than that of vivax-infected group, the difference being 390 g ($z = 2.8$, $p < 0.05$). The percentage of babies having less than or equal to 2.5 kg weight in the infected group was 81.7 as compared to 40.3 in the control group. The mean weight of the placenta in the infected group was 412.4 g as com-

Table 5. Foetal outcome in control and infected groups and species-wise distribution

Outcome	Control group	Total	Infected	
			P. vivax	*P. falciparum*
Full-term normal delivery	86 (82.7)	22 (21.2)	17 (35.4)	5 (8.9)
Preterm	16 (15.4)	62 (59.6)	27 (56.2)	35 (62.6)
Abortion	2 (1.9)	10 (9.7)	4 (8.4)	6 (10.7)
Still-births	0 (0.0)	6 (5.7)	0 (0)	6 (10.7)
Deaths	(0)	4 (3.8)	(0)	4 (7.1)
Total	104 (100)	104 (100)	48 (100)	56 (100)

Figures in parentheses are percentages.

Table 6. Mean weight of the baby and difference in control and different types of infected groups

Particulars	Control	Infected	*P. vivax*	*P. falciparum*	Without placental infection	With placental infection
Mean weight of baby, g	2590	1990	2200	1810	2300	1700
Difference, g		600		390		600

pared to 455.0 g in the control group. The mean placental weight of the infected group was found to be lower by 42.6 g than that of the control group, which is significant ($z = 2.3$, $p < 0.05$). Also, the mean birth weight of the babies of the infected placenta was significantly lower by 0.6 kg than of those without placental infection ($t = 2.31$, $p < 0.05$).

All vivax cases responded to chloroquine while 87.5% (49 cases) of the falciparum cases responded to this drug. In two patients, chloroquine resistance was observed and hence quinine was administered orally. In five complicated cases, quinine had to be administered by intravenous infusion.

The mean weight of babies in relation to infection and parity and its comparison with those under chemoprophylaxis are shown in Table 7. The difference in mean weights of babies of primigravidae is 1253 g and that of multigravidae is 391 g, which are statistically significant ($t = 3.9$, $p < 0.001$ and $t = 2.1$, $p < 0.05$). The difference in mean birth weight of babies of mothers with falciparum infection is significantly lower by 771 g ($z = 6.2$, $p < 0.001$) than the mean weight of babies of the protected group.

DISCUSSION

During the study period the slide positivity rate in pregnant women was found much higher than in general population, indicating higher susceptibility to infection. Falciparum infection was predominant. The mixed cases indicated a high level of transmission of the disease in this area. The intensity of infection was higher in primigravidae as this group had shown a higher mean parasite density. Also, the positivity rate was much higher in 2nd trimester. Similar observations were also made by other workers in other countries[3-5].

Because of perennial and high level of transmission of the disease in this area, repeated infection resulted in the development of partial immunity to malaria. Pregnancy tends to interfere with the immune mechanism thereby lowering the level of acquired immunity. This seems to be particularly so in primigravidae. The higher incidence of falciparum infection may be attributed to appearance of resistant strains to 4 aminoquinoline drugs in

Table 7. Mean weight (g) of baby in relation to protection (by chemoprophylaxis), parity and infection

Prophylaxis	Primipara			Multipara		
	P. falciparum	*P. vivax*	Mean	*P. falciparum*	*P. vivax*	Mean
No	1120 (10)	1500 (15)	1247 (15)	1160 (22)	1800 (14)	1409 (36)
Yes			2500 (9)			1800 (44)

Figures in parentheses are the number of cases.

vogue, among other reasons.

Pyrexial illness and anaemia were the predominant clinical features. In view of the high prevalence of the disease in this area it is advisable to exclude malaria in fever cases by blood examination before thinking of any other infection/disease, especially in pregnant women.

The predominance of microcytic anaemia with dimorphic variety in the infected group could be explained by the fact that women here are generally iron-deficient and the added folate deficiency[4] caused by malaria resulted in the iron deficiency supervening the latter.

The mortality directly due to malaria (33.3%) was found to be very high. Also, the percentage of preterm deliveries, stillbirths, and abortions were much higher in the infected group than in uninfected cases. The outcome was poorer in falciparum cases than in vivax cases. Pyrexia produced by malaria infection (maximum recorded temperature 106°F) caused abortion and premature labour probably by stimulating the prostaglandin metabolism.

When compared with other studies[6-12] carried out elsewhere the difference in birth weight was maximum in our study. This may be attributed to certain other factors like anaemia, intercurrent infection and nutritional deficiencies prevalent in women of this area.

The study has revealed that placenta is an effective barrier for the passage of malaria parasites. A majority of the placental infection was due to *P. falciparum*. Histopathologically all changes like (a) parasites in the intervillous space, (b) malaria pigment deposits, and (c) fibrinoid necrosis were present. The foetal involvement in the majority of cases could be explained by mechanisms other than direct placental transfer of the organisms, viz. (i) maternal fever per se with its effect on the foetus, (ii) possible toxins liberated during haemolytic crisis crossing the placental barrier and exerting their direct effect on the foetus, (iii) a heavily infected placenta being functionally inefficient in the transfer of oxygen and nutrients[13] and (iv) theoretically the foetus could be directly infected by the malarial parasites if maternal blood gained access to the foetus, such access being possible if there are microscopic or gross breaks in the placental barrier.

From the study it is also evident that malaria parasites can cross the placental barrier and enter foetal circulation causing congenital malaria. This was observed in the case of mothers who showed a high density of malaria parasites with falciparum infection. Hence it can be inferred that the occurrence of such cases depends upon the severity of infection, high level of susceptibility of the individual concerned and high grade transmission of the disease. The foetus was asymptomatic as it was well protected by the antibodies, etc[1].

The mean weight of the babies of mothers with chemoprophylaxis was more than that of those without such protection. The difference in weight found in this study is

more significant in comparison to those of similar studies[10,13-15] undertaken in other countries.

Our study confirmed that chloroquine is still the drug of choice against malaria in pregnant women even though quinine was effective in complicated cases. Hence early diagnosis and proper initiation of therapy can reduce the risks of serious maternal complications. Because of the chances of getting infected due to the existence of high level transmission of the disease in these parts and the existing anaemia in partially immune women, chemoprophylaxis becomes a necessity during the period of pregnancy. Chloroquine 300 mg base per week has proved less harmful for protecting pregnant women from hazards of malaria infection.

ACKNOWLEDGEMENTS

The authors are grateful to the authorities of the Medical College, especially of the Department of Obstetrics and Gynaecology, for the encouragement, interest and guidance. The District Malaria Laboratory staff also deserve all praise and gratitude from the authors for their active participation and help in carrying out the study.

REFERENCES

1. Pampanna, E.J. (1969). *A Textbook of Malaria Eradication*, 2nd edn., Oxford University Press London:69.
2. WHO (1979). *Chemotherapy of Malaria* (Rev. edn. 1986), **27**: 123-129.
3. Bray, R.S. and M.J. Anderson (1979). Falciparum malaria and pregnancy. *Trans. R. Soc. Trop. Med. Hyg.*, **73**: 427.
4. Hamilton, P.J.S. (1972). The role of malaria, folic acid deficiency and haemoglobin as in pregnancy at Mulago Hospital. *Trans. R. Soc. Trop. Med. Hyg.*, **66**: 594.
5. Mcgregor, I.A. (1984). Epidemiology, malaria and pregnancy. *J. Trop. Med. Hyg.*, **33**(4): 517.
6. Archibald, H.M. (1958). Influence of maternal malaria on newborn infants. *British Med. J.*, **2**: 15-12.
7. Bruce-Chwatt, L.J. (1983). Malaria and pregnancy. *British Med. J.*, **286**: 1457.
8. Cannon, D.S.H. (1958). Malaria and prematurity in the western region of Nigeria. *British Med. J.*, **2**: 877.
9. Gelliffe, E.F.P. (1968). Low birth weight and malaria infection of the placenta. *Bull. WHO*, **38**: 69.
10. Macgregor, J.D. and J.G. Avery (1974). Malaria transmission and foetal growth. *British Med. J.*, **3**: 433.
11. Mclaren, D.S. and P.G. Ward (1962). Malaria infection of placenta and foetal nutrition. *East. Afr. Med. J.*, **39**: 182.
12. Spitz, A.J.W. (1959). Malaria infection of the placenta and its influence on the incidence of prematurity in Eastern Nigeria. *Bull. WHO*, **21**: 242.
13. Giles, H.M. (1969). Malaria, anaemia and pregnancy. *Ann. Trop. Med. Parasite*, **63**: 245.
14. Mcgregor, I.A., M.E. Wilson and W.Z. Billeweicz (1983). Malaria infection of placenta in the Zambia, West Africa, its incidence and relationship to still-birth, weight and placental weight. *Trans. R. Soc. Trop. Med. Hyg.*, **77**: 237.
15. Morley, D., M. Woodland and W.F.J. Cuthbertson (1964). Controlled trial of pyremethamine in pregnant women in an African village. *British Med. J.*, **1**: 667.

Accepted: 7 December 1993

Indian Journal of Malariology
Vol. 30, December 1993, pp. 215-220.

Observations on Mosquito Breeding in Wells and its Control

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

Studies on mosquito breeding in wells revealed the dominance of *An. stephensi* among the malaria vectors, whereas *Cx. quinquefasciatus* was most abundant in disused wells and was present in wells of all depths. None of the anopheline species was encountered when well depth up to water level exceeded 12 m. Larval breeding was effectively controlled through the introduction of larvivorous fish *Poecilia reticulata* and expanded polystyrene (EPS) beads in comparison to untreated control wells.

Keywords: Mosquito breeding, Mosquito control, Larvivorous fish

INTRODUCTION

Mosquitoes are known to breed in a variety of breeding sources. The occurrence and prevalence of each species depend on the type and prevailing ecological conditions of the habitat. Wells are the main source of mosquito production in many parts of India. In villages around Delhi, profuse breeding of *Cx. quinquefasciatus* has been reported to occur in wells, and the species is occasionally replaced by *An. culicifacies* and *An. stephensi*[1]. In other parts of the country, *An. stephensi* breeds mainly in wells and maintains malaria transmission[2,3].

An innovative approach to control malaria was launched in 1984 in rural Nadiad taluka of Kheda district, Gujarat, with em-

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

phasis on source reduction, community participation and biological control methods[4-6]. Besides other breeding sources, wells were found to support prolific mosquito breeding. In our study we attempted to determine the mosquito composition in wells and to control the breeding through non-insecticidal methods.

MATERIALS AND METHODS

All types of wells in 100 villages of the study area (Nadiad taluka) were surveyed for mosquito breeding. A total of 5354 wells were located which were numbered and classified into four categories, viz. draw wells (22.40%), disused wells (21.60%), irrigation wells (fitted with electric pumpset) (44.10%) and dry wells (11.90%), which occasionally supported mosquito breeding during monsoon season only. The density of wells in the study area in 1989 was 8 per sq km and their location with respect to compact village and adjoining field area was in the ratio of 1:5. Wells were surveyed on fortnightly basis for checking mosquito breeding in all the study villages, whereas a quarterly survey of wells (539) was done in the villages belonging to neighbouring talukas for comparison. However, to study the composition of different mosquito species and their relationship to varying water-table in different categories of wells, a random survey of wells (180) was done in both the areas, particularly where fishes could not be introduced or could not survive. Larval samples from the positive wells were collected using a well net (25 cm dia.), and brought to laboratory for rearing and adult emergence. Adults that emerged were identified using the keys of Christophers[7] and Barraud[8]. The depth of water-table in the wells was measured with the help of a rope.

Mosquito breeding in the wells of experimental villages was controlled largely by introducing the larvivorous fish *Poecilia reticulata* (Guppy) at the rate of 5-10 fishes/m^2, as documented by Sharma and Sharma[5], whereas in most of the disused wells (889), expanded polystyrene (EPS) beads were applied manually at the rate of 50 g/1000 cm^2. To collect fishes, small rearing ponds were developed in almost all the villages to avoid distant transportation. Fishes were collected from the ponds and introduced as and when required. EPS-treated wells did not require repeated application, except occasionally when beads were disturbed or were accidentally removed. The results were statistically analysed by applying student *t* test and analysis of variance (ANOVA) as described by Zar[9].

RESULTS AND DISCUSSION

Mosquito composition in different types of wells and their relative abundance in relation to depth of water-table in the wells are given in Table 1. A total of eight anopheline species were found breeding in the wells. *An. barbirostris* (45.38%) was the most predominant species followed by *An. stephensi* (36.12%), *An. subpictus* (13.54%) and *An. culicifacies* (3.45%) and these four species contributed about 98% of the total adults that emerged. The rest of the spe-

Table 1. Per cent composition of mosquitoes with respect to type and depth of wells

Species	Type of wells				Depth up to water level (m)				
	Irrigation	Draw	Disused	Total	0-3.0	3.1-6.0	6.1-9.0	9.1-12.0	>12
Anophelines									
An. barbirostris	47.89	40.95	32.14	45.38	60.60	43.54	35.46	62.50	—
An. stephensi	33.13	45.7	35.71	36.12	9.09	36.36	56.73	18.75	—
An. subpictus	12.65	13.34	25.00	13.54	27.27*	14.35*	3.54*	6.25*	—
An. culicifacies	4.81	—	—	3.45	1.01	4.78	2.12	12.50	—
An. annularis	0.30	—	—	0.21	1.01	—	—	—	—
An. nigerrimus	0.90	—	—	0.65	—	0.95	0.70	—	—
An. fluviatilis	—	—	7.14	0.44	—	—	1.41	—	—
An. theobaldi	0.30	—	—	0.21	1.01	—	—	—	—
Total adults emerged	332	105	28	465	99	209	141	16	—
Culicines									
Cx. quinquefasciatus	71.62	60.13	85.71	72.00	73.15	54.38	76.08	100.0	84.14
Cx. vishnui	7.58	—	—	4.28	0.67	13.45	2.17	—	—
Cx. fuscanus	4.78	2.79	0.75	3.49	6.71	2.33	1.44	—	3.65
Cx. seniori	—	13.98	6.76	3.16	—	—	—	—	12.19
Cx. gelidus	0.28	—	—	1.58	—	—	7.24	—	—
Ae. aegypti	0.56	—	—	0.31	1.34	—	—	—	—
Other culicines	15.16	23.07	6.77	15.18	18.12	29.82	13.04	—	—
Total adults emerged	356	143	133	632	149	171	138	10	164
No. of wells checked	127	34	19	180	61	69	36	5	9

*Composition at different depths varied significantly ($F = 6.12$; $df = 3,8$; $p < 0.05$) by applying ANOVA.

cies were present in low proportions.

Breeding of *An. subpictus, An. stephensi* and *An. barbirostris* was found in all types of wells with varying proportions but they did not exhibit significant differences in their composition when tested through ANOVA. Except for *An. fluviatilis,* most of the anopheline species were present in irrigation wells. *Cx. quinquefasciatus* (72%) was the predominant species among the culicines and was found to breed mostly in disused wells. The contribution of other culicines was comparatively less.

An. barbirostris was found to be the predominant species in irrigation wells, which were mainly located in agricultural fields,

owing to limited outdoor breeding potential. *An. stephensi*, a known malaria vector, was found to be the second predominant species inhabiting wells, particularly draw wells, as the species prefers to breed in clean water, whereas *An. subpictus* is the ubiquitous species and breeds in all types of waters. The prolific breeding of *An. stephensi* in wells has been reported by Batra and Reuben[2], Rao[3], and Yadav *et al*[10]. However, the breeding of *An. culicifacies* and *An. fluviatilis* in wells may be due to certain ecological necessity.

When the correlation of different mosquito species with the depth of the wells up to water level was studied, 6 out of 8 anopheline species were present up to 9 m from the ground level and none of the species were found breeding as the depth reached beyond 12 m. *An. barbirostris* was prevalent at all depths and its maximum breeding occurred when the well water-table exceeded 9 m. *Ae. aegypti* was not found breeding below 3 m. The composition of *An. subpictus* at different depths showed a significant difference ($p < 0.05$) and the species preferred to breed mostly in shallow wells.

Larval positivity in different categories of wells of both the areas is given in Table 2. With the introduction of larvivorous fish and application of EPS beads, well positivity was kept under control (6.9%) in the experimental area during the study period, whereas in the control area it was as high as 29.3%, and the same trend was evident in different periods as well (Fig. 1). Statistical analysis also showed a significant difference between two areas ($p < 0.05$). Poor survival of larvivorous fishes was observed in disused wells, which could not be treated with EPS beads because of refusal by the villagers. This was mainly due to polluted water and hence control measures were less effective in these wells.

Control of mosquito breeding in wells was successfully demonstrated through the introduction of larvivorous fish *Gambusia* in Hyderabad[11]. There are many other reports on the role of *Gambusia, Poecilia* and other fishes in the control of mosquito

Table 2. Larval positivity in different types of wells

Type of well	Experimental			Control		
	No. surveyed	+ve	%	No. surveyed	+ve	%
Draw wells	20969	931	4.4	153	57	37.2
Irrigation wells	41155	2884	7.0	266	60	22.8
Disused wells	5941	934	15.7	120	41	34.1
Total	68065	4749	6.9	539	158	29.3

($t = 4.02$; $df = 4$; $P < 0.05$). Disused (889) wells in experimental area were capped with EPS beads, *P. reticulata* (Guppy) was introduced in the rest of the wells. All results are for the year 1989.

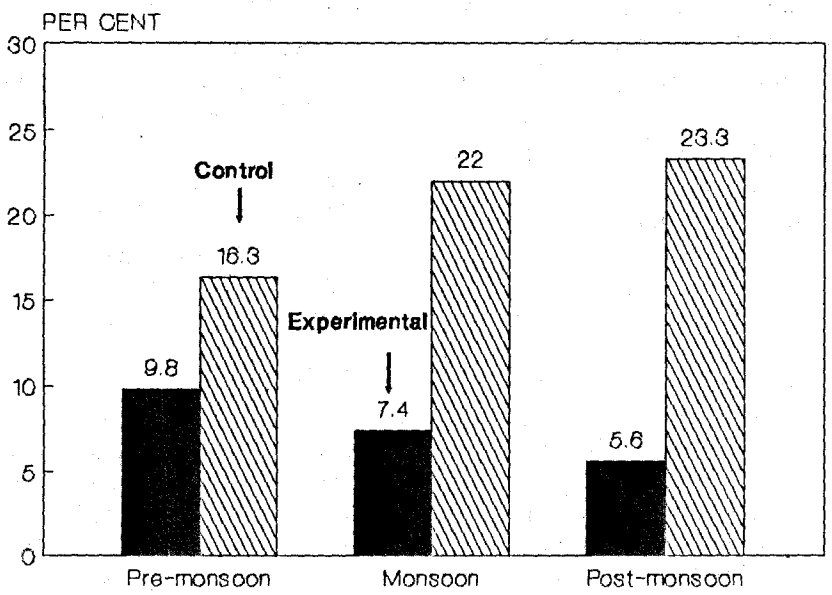


Fig 1: Periodic well positivity in experimental and control areas.

breeding in wells[12-14]. However, chlorination of wells sometimes poses problems by causing fish mortality and subsequent increase in mosquito breeding. Application of EPS beads in disused wells was found to be effective. Since EPS beads are safe and one application lasts for a long time[5,15], they can greatly reduce mosquito breeding in wells. Initial field trials with EPS beads in biogas plants and wells by Sharma *et al.*[15] revealed that larval density reached zero by the 5th week in all the sites. EPS beads need reapplication only when the layer is disturbed, creating patches or is removed by the villagers for reusing the wells for irrigation especially during drought conditions.

Wells remain the important source of breeding during unfavourable conditions of non-monsoon period when the other sources become scarce, particularly in non-canal irrigated areas, owing to increase in temperature, which, in turn, results in the reduction of temporary mosquito breeding places. If the breeding in wells is controlled during this period it is quite likely that there would be an impact on population build-up during a favourable season. As the vector mosquitoes of malaria, filariasis and certain viral infections breed in wells, it is a prerequisite to keep a regular check on such habitats, which may ultimately be helpful in the ongoing national malaria control programme.

ACKNOWLEDGEMENTS

The authors are highly thankful to Dr. V.P. Sharma, Director, Malaria Research Centre,

Delhi, for constant encouragement and unstinted support during the entire course of the study. We are also thankful to village heads and members of village panchayats for their cooperation in the programme. Thanks are also due to Mrs. Bina Srivastava for secretarial assistance.

REFERENCES

1. Sharma, V.P. (1985). Field experience with thiotepa sterilized *Culex quinquefasciatus* in India. In *Integrated Mosquito Control Methodologies*, v. 2, edited by M. Liard and J.W. Miles (Academic Press, New York): 117-140.
2. Batra, C.P. and R. Reuben (1979). Breeding of *Anopheles stephensi* (Liston) in wells and cisterns in Salem, Tamil Nadu. *Indian J. Med Res.*, **70**: 114-122.
3. Rao, Ramachandra T. (1984). *The Anophelines of India* (Malaria Research Centre, ICMR, New Delhi).
4. Sharma, V.P., R.C. Sharma and A.S. Gautam (1986). Bioenvironmental control of malaria in Nadiad, Kheda, Gujarat. *Indian J. Malariol.*, **23**: 95-117.
5. Sharma, V.P. and R.C. Sharma (1989). Community based bioenvironmental control of malaria in Kheda, Gujarat. *J. Amer. Mosq. Contr. Assoc.* **5**: 514-521.
6. Sharma, R.C., A.S. Gautam, R.M. Bhatt, D.K. Gupta and V.P. Sharma (1991). The Kheda malaria project: the case for environmental control. *Health Policy and Planning*, **6**: 262-270.
7. Christophers, S.R. (1933). *The Fauna of British India including Ceylon and Burma*, v. 4 (Today and Tomorrow's Printers and Publishers, New Delhi).
8. Barraud, P.J. (1934). *The Fauna of British India including Ceylon and Burma*, v. 5 (Today and Tomorrow's Printers and Publishers, New Delhi).
9. Zar, J.H. (1984). *Biostatisitcal Analysis* (Prentice-Hall Inc., Englewood Cliffs, N.J.).
10. Yadav, R.S., R.C. Sharma, R.M. Bhatt and V.P. Sharma (1989). Studies on the anopheline fauna of Kheda district and species-specific breeding habitats. *Indian J. Malariol.*, **26**: 65-74.
11. Sitaraman, N.L., M.A. Karim and G. Venkat Reddy (1975). Observations on the use of *Gambusia affinis* Holbrooki to control *An. stephensi* breeding in wells. Results of two years' study in Greater Hyderabad city, India. *Indian J. Med. Res.*, **63**: 1509-1516.
12. Rao, Bhaskar R. and H. Ramoo (1942). Observation on the relative utility of *Gambusia affinis* and *Panchax parvus* in the control of mosquito breeding in wells and tanks. *J. Mal. Inst. India*, **4**: 633-634.
13. Sitaraman, N.L., S. Mahadevan and S. Swamidas (1976). Biological control of *An. stephensi* larvae in wells by *Poecilia reticulata* in Greater Hyderabad city, India. *J. Com. Dis.*, **8**: 315-319.
14. Menon, P.K.B. and P.K. Rajagopalan (1978). Control of mosquito breeding in wells by using *Gambusia affinis* and *Aplocheilus blochi* in Pondicherry town. *Indian J. Med. Res.*, **68**: 927-933.
15. Sharma, R.C., R.S. Yadav and V.P. Sharma (1985). Field application of expanded polystyrene (EPS) beads in mosquito control. *Indian J. Malariol.*, **22**: 107-109.

Accepted: 10 December 1993

Indian Journal of Malariology
Vol. 30, December 1993, pp. 221-225.

Influence of Soil Moisture on Survival and Oviposition of *Romanomermis iyengari* a Mermithid Nematode Parasite of Mosquitoes

K.P. PAILY* and K. BALARAMAN*

The oviposition pattern of *Romanomermis iyengari* (Nematoda : Mermithidae) in relation to the moisture level in the soil was studied by seeding postparasites into two sets of 12 cm soil columns. While one set of the soil column was allowed to dry over a period of 60 days, the other set was maintained at a constant level of 15-20% moisture throughout the soil column. In the soil column maintained at constant moisture level, the postparasites oviposited primarily in the upper 3 cm layer (80-97%). In the soil column which was allowed to dry the percentages of eggs laid in the upper 3 cm layer were 96, 24 and 6 respectively on D20, D30 and D40 after seeding and the moisture levels on respective days were 12, 5 and 3%. On corresponding days the postparasites laid 1, 56 and 42% of the eggs in the lower most layer (9-12 cm) and the moisture levels were respectively 15, 10 and 9%. The results indicate that the adult nematodes migrated towards the bottom layer of the soil and laid eggs as the moisture of the upper layers decreased.

Keywords: Biological control, Mermithid nematode, Mosquito larvae, Oviposition, *Romanomermis iyengari*, Soil moisture

INTRODUCTION

Romanomermis iyengari (Welch), the tropical species of mermithid nematode, has been reported to recycle in rice fields and infect anopheline and culicine larvae season after season[1-2]. This species was evaluated recently for its biocontrol potential against *Anopheles subpictus* and *Culex tritaeniorhynchus* breeding in rice fields and

*Vector Control Research Centre (ICMR), Indira Nagar, Pondicherry-605 006, India.

was found to be effective[3]. The moisture level of soil was considered an important factor determining the survival of mermithid nematodes[4]. The eggs of *Octomyomermis muspratti* (Obiamiwe and Macdonald) are known to survive through long periods of drought[5]. *Romanomermis culicivorax* (Ross and Smith) was found to establish itself in rice fields and oviposit in the top 4-6 cm layer of soil[6-8]. But no information is available on the mechanism of survival of *R. iyengari* during fallow periods (dry spell when rice crops are not raised). Hence our study was aimed at uncovering the mechanism of survival of *R. iyengari*.

MATERIALS AND METHODS

Twenty plastic containers, 14 cm height and 11 cm diameter, were filled with autoclaved rice-field soil (clayey loam) up to a height of 12 cm. The soil was flooded with chlorine-free tap water. Newly emerged postparasitic stages of *R. iyengari*, obtained from a cycling colony of the Vector Control Research Centre, were added to the containers at the rate of 100 males and 100 females per container. The water level in the containers was maintained at a constant level of 1 cm above the soil surface. After 7 days the excess water was decanted, and the containers were incubated at 30±2°C. The first sampling was done after dividing the containers into two sets on D20 and two containers per set were used for assessing the depth of oviposition by the nematodes as per the standard method[8]. For this, the soil column was removed from the plastic container, cut transversely into 4 equal sections (3 cm each), placed in separate enamel trays, broken into smaller pieces and flooded with 1 litre of water. After 24 h, laboratory-reared II instar larvae (500) of *Culex quinquefasciatus* were added to each tray and reared until IV stage. Later they were transferred to another set of trays containing 1 litre of water and the emerging postparasites were collected and counted. The number of postparasites thus obtained was considered as equivalent to the number of viable eggs (eggs laid minus the number of eggs perished) in a particular soil layer.

Four sections of soil columns from two other containers maintained at similar conditions without postparasites were used to determine the moisture content of the soil. For this, the soil sections were weighed individually and dried in a hot air oven at 105°C to a constant weight. The moisture content was calculated from the difference in weight before and after drying[9].

After the first sampling the moisture level in one set of containers was maintained at 15-20% by spraying water over the soil, while in the other set the soil was allowed to dry. On D30, D40 and D60 the oviposition by *R. iyengari* and moisture level in different layers of the soil columns were determined using two containers per set as detailed above. The experiment was replicated four times and the results are presented as average of the replicates.

RESULTS

When the soil columns (filled in plastic con-

tainers) were added with postparasites of *R. iyengari* and different layers examined on different days for the presence of viable eggs, the following observations were made. In the containers in which the soil moisture was not maintained at constant level and allowed to dry, the topmost layer (0-3cm) had 12% moisture on D20. In this soil layer, 96% of the total viable eggs were present. Although the bottommost soil layer (9-12 cm) had 15% moisture, no viable eggs were seen (Table 1). On D30 and D40 the 9-12 cm layer had 9-10% moisture and 42-56% of the total viable eggs. When the entire soil column was considered, 129 eggs were present on D20, 370 on D30 and 262 on D40. On D60 no viable eggs were seen in the soil column when the soil moisture was 2-5%.

In contrast, in the containers in which the soil moisture was maintained at a constant level of 15-20%, 80-97% of the total viable eggs were present in the upper 0-3 cm layer throughout the study period (Table 2).

Table 1. Postparasites of *Romanomermis iyengari* emerged from larvae released into the four sections (3 cm) of the soil after the soil column was allowed to dry

Days after seeding	Postparasites emerged (eggs laid/viable) in the indicated section (depth) of the soil column								Moisture level (%) in the indicated section (depth) of the soil column			
	0-3 cm		3-6 cm		6-9 cm		9-12 cm		0-3 cm	3-6 cm	6-9 cm	9-12 cm
	No.	%	No.	%	No.	%	No.	%				
20	124	96.1	3	2.3	1	0.8	1	0.8	11.5	13.3	4.6	15.2
30	90	24.3	35	9.5	38	10.3	207	55.9	5.2	8.9	9.5	10.0
40	15	5.7	66	25.2	72	27.5	109	41.6	3.0	7.7	8.0	8.5
60	0	0	0	0	0	0	0	0	1.9	3.6	4.7	4.9

Table 2. Postparasites of *Romanomermis iyengari* emerged from larvae released into the four sections (3 cm) of the soil when the soil column was maintained at a constant moisture level of 15-20 per cent

Days after seeding	Postparasites emerged (eggs laid/viable) in the indicated section (depth) of the soil column							
	0-3 cm		3-6 cm		6-9 cm		9-12 cm	
	No.	%	No.	%	No.	%	No.	%
20	150	94.9	5	3.2	3	1.9	0	0
30	142	97.3	4	2.7	0	0	0	0
40	159	85.9	18	9.7	4	2.2	4	2.2
60	622	80.3	124	16.0	29	3.7	0	0

In the bottom most soil layer (9-12 cm), viable eggs were either absent or lesser than in containers in which the soil column was allowed to dry. When the entire soil column was considered, the number of eggs present on D20, D30, D40 and D60 respectively were 158, 146, 185 and 775.

DISCUSSION

With an adequate level of moisture throughout the soil column, most females of *R. iyengari* remained in the topmost soil layer (0-3 cm) and laid eggs. As the moisture content of the upper layers of the soil decreased to levels which are unsuitable for survival, they migrated to the lower layers of the soil. The eggs which were laid earlier in the upper soil layers perished partially owing to desiccation. This was evident from the reduction in the total number of viable eggs present on D40 compared to that on D30. When the moisture content of the entire soil column reached 2-5% on D60, the female nematodes were killed and/or the eggs laid earlier were damaged owing to desiccation and this was evidenced by the absence of viable eggs on that day.

In the soil column where the moisture level was kept at constant level (15-20%), a higher percentage (80-97%) of the eggs was encountered in the uppermost 0-3 cm layer, throughout the study period. *R. iyengari* must have therefore oviposited primarily in the upper 3 cm soil layer when the moisture level was adequate for its survival. This observation is in conformity with the observations of Walker *et al.*[8] that *R. culicivorax* oviposited primarily in the upper 2-6 cm soil layer. However, in no case were eggs of *R. culicivorax* found in the soil strata below 6 cm. Our data show that at a constant moisture level of 15-20%, some of the *R. iyengari* females burrowed up to 12 cm of soil and oviposited. This might be due to either the difference in the texture of the soil used in the study or behavioural difference between the two nematode species. On D60 no viable eggs could be obtained from the soil columns which were allowed to dry, whereas in the soil columns maintained at constant moisture level, on D60 not only viable eggs were obtained but they were obtained in high numbers. In laboratory studies *R. iyengari* was found to complete oviposition in 50-52 days[3], and in our study also we infer that this nematode might have completed oviposition around the same time.

On D30 and D40 the total number of eggs present in the entire soil column which was allowed to dry was higher than that in the soil columns maintained at a constant moisture level. We find it probable that owing to the stress induced by the low moisture level the females might have laid eggs at an enhanced rate.

The results of our study suggest that in its natural habitats like rice fields, *R. iyengari* withstands dry spells by burrowing deep into the soil where an adequate level of moisture is present. When the dry habitat gets flooded and mosquito breeding appears, at least a part of the eggs laid by the females in the deeper soil strata con-

tributes to the recycling of the species.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. P.K. Rajagopalan, former Director, for guidance and suggestions and Dr. Vijai Dhanda, Director, for reviewing the manuscript. This investigation received financial support from the UNDP/WHO/Special Programme for Research and Training in Tropical Diseases.

REFERENCES

1. Chandrahas, R.K. and P.K. Rajagopalan (1979). Mosquito breeding and the natural parasitism of larvae by a fungus *Coelomomyces* and a mer-mithid nematode *Romanomermis* in paddy fields in Pondicherry. *Indian J. Med.Res.*, **69**: 63-70.
2. WHO (1980). Data Sheet on the Biological Control Agent WHO/VBC/80.765.
3. Paily, K.P. (1990). Studies on the biology and biological control potential of *Romanomermis iyengari* (Welch), a mermithid nematode parasite of mosquitoes. Ph.D. Thesis, Pondicherry University, India.
4. Petersen, J.J. (1984). Nematode parasites of mosquitoes. In *Plant and Insect Nematodes,* edited by W. R. Nickle (Marcel Dekker, Inc., New York): 797-820.
5. Petersen, J.J. (1981). Observations on the biology of *Octomyomermis muspratti*, a nematode parasite of mosquitoes. *J. Invert. Pathol.*, 37: 290-294.
6. Petersen, J.J. and O.R. Willis (1975). Establishment and recycling of a mermithid nematode for the control of mosquito larvae. *Mosq. News*, 35: 526-532.
7. Kerwin, J.L. and R.K. Washino (1985). Recycling of *Romanomermis culicivorax* (Mermithidae: Nematoda) in rice fields in California, USA. *J. Med. Entomol.*,6: 637-643.
8. Walker, T.W., C.L. Meek, V.L. Wright and J.S. Billodeaux (1985). Susceptibility of *Romanomermis culicivorax* postparasites to agrichemicals used in Louisiana rice production. *J. Amer. Mosq. Contr. Assoc.*, 1: 477-481.
9. Head, K.H. (1980). *Manual of Soil Laboratoy Testing,* v. I. *Soil Classification and Compaction Tests* (Pentech Press, London): 339.

Accepted: 13 December 1993

SHORT NOTE

Indian Journal of Malariology
Vol. 30, December 1993, pp. 229-233.

Three Genetic Markers and Malaria in Upper Caste Hindus of Kheda District of Gujarat State

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

Keywords: ABO group, G-6-PD deficiency, HbAS

Some genetic abnormalities of human erythrocytes have been found to decrease their susceptibility to malaria parasite, viz. the selective advantage of G-6-PD deficiency and sickle cell haemoglobin against *P. falciparum*[1] and Duffy negative antigen against *P. vivax* infection[2]. Some work on the genetic markers and malaria in Indian populations has been done[3-7]. We report the distribution of ABO blood grouping, sickle cell haemoglobin and G-6-PD deficiency and their relationship with malaria among five population groups of upper caste Hindus from Kheda district of Gujarat state.

As many as 1659 individuals from five caste groups (Brahmin, Patel, Rajput, Vania and Sindhi) were screened for three genetic markers and malaria. Blood samples (0.3-0.4 ml) were collected in heparinized vials from persons of all age groups and both sexes. Thick and thin blood smears were also prepared for the detection of malaria cases. The sex ratio in the sample size was 55 males to 45 females. Of the sample, 1440 were apparently normal, healthy individuals and 219 were infected with malaria (*P. vivax*: 97; *P. falciparum*: 122). Methods used for the detection and analysis of samples were the same as described by Pant *et al*[6].

The frequency of occurrence of three genetic markers in five caste groups is given in Table 1. The table shows that blood group B was dominant in Brahmins (34.1%), Patels (41.3%) and Rajputs (35.5%), whereas in

*Malaria Research Centre (Field Station), BHEL Complex, Ranipur, Hardwar - 249 403, India.
+Malaria Research Centre (Field Station), Civil Hospital, Nadiad - 387 001, India.

Table 1. Frequency of occurrence of ABO blood groups, sickle cell haemoglobin and G-6-PD deficiency

Subcaste	Sample size	Blood group				Sickle cell trait	G-6-PD	
		A	B	AB	O		Male sample	Deficient
Brahmin	135	33 (24.4)	46 (34.1)	14 (10.4)	42 (31.1)	—	80	4 (5.0)
Patel	223	47 (21.1)	92 (41.3)	17 (7.6)	67 (30.0)	—	151	9 (5.9)
Rajput	1134	316 (27.9)	403 (35.5)	114 (10.1)	301 (26.5)	15 (1.3)	593	17 (2.9)
Vania	92	26 (28.3)	29 (31.5)	6 (6.5)	31 (33.7)	—	55	5 (9.1)
Sindhi	75	6 (8.0)	23 (30.7)	12 (16.0)	34 (45.3)	—	41	—
Total	1659	428 (25.9)	593 (35.7)	163 (9.8)	475 (26.6)	15 (0.9)	920	35 (3.8)

Figures in parentheses are the percentages.

Vanias (33.7%) and Sindhis (45.3%), blood group O was dominant. Chi-square test revealed a significant difference in the association of ABO groups in different caste groups ($\chi^2 = 31.944$, $p < 0.001$).

Of the 1659 individuals screened, 15 were found to have sickle cell trait. All the 15 sicklers were Rajputs. Other groups did not show any sickling. However, chi-square test did not reveal any significant difference in the association of sickle cell trait in different cast groups ($\chi^2 = 7.014$, $p > 0.05$). A high frequency of sickle cell trait in Scheduled Tribes (14.9%) and a low frequency in Scheduled Castes and Muslims (1.5%) have been reported from the same area[6,7]. Review of the Indian studies shows a high frequency among tribals throughout India except north-west and extreme south. Occurrence of other populations is sporadic[8].

Only 920 males were screened for G-6-PD deficiency. Females were excluded as fluorescence spot test invariably misses heterozygous females. 35 individuals exhibited G-6-PD enzyme deficiency. Highest deficiency was observed in Vanias (9.1%), followed by Patels (5.9%), Brahmins (5.0%) and Rajputs (2.9%). However, there was no de-

ficiency in the Sindhi community. Significant association of G-6-PD deficiency in caste groups has also been observed (χ^2 = 9.554, $p < 0.05$). Earlier studies showed 5.9% deficiency in Scheduled Castes and Christians, 4.2% in Scheduled Tribes and 1.87% in Muslims from the same study area[6,7]. Baxi *et al.*[9] have also reported 3.54 and 0.78% deficiencies in Brahmins and Lad Vanias respectively from Gujarat.

The distribution of three genetic markers in healthy and malaria-infected individuals is given in Table 2. Malaria incidence was almost the same in the blood groups A (15.2%) and B (14.8%), followed by AB (11.7%) and O (9.9%). Chi-square test revealed a significant difference in the distribution of ABO blood groups among malaria and normal subjects (χ^2 = 7.893, $p < 0.05$). Similar observations were also made in a previous study[7]. Gupta and Raichowdhuri[3] reported that malaria parasite shares group A antigen and hence is better tolerated by the host immune system. Artheya and Coriell[10] reported that blood group B may have an advantage in malarious regions. However, no correlation has been found between ABO blood groups and malaria from Delhi and African populations[4,11].

In our study, no *P. falciparum* case was detected from the sickler samples, which suggests that the presence of sickle cell gene

Table 2. Distribution of sickle cell trait, G-6-PD deficiency and ABO blood grouping in malaria patients and healthy individuals

Genetic marker	Healthy individual	Malaria cases		
		Pv	*Pf*	Total
Sickle cell trait	14 (93.3)	1 (6.7)	—	1 (6.7)
G-6-PD deficiency	33 (94.3)	1 (2.8)	1 (2.8)	2 (5.7)
Blood group A	363 (84.8)	28 (6.6)	37 (8.6)	65 (15.2)
Blood group B	505 (85.2)	41 (6.9)	47 (7.9)	88 (14.8)
Blood group AB	144 (88.3)	5 (3.1)	14 (8.6)	19 (11.7)
Blood group O	428 (90.1)	23 (4.8)	24 (5.1)	47 (9.9)

Figures in parentheses are the percentages.

in the population may have an advantage against falciparum infection. Evidences are also available that HbS mutation confers protection against *P. falciparum* infection[12]. Allison[13] also reports that HbAS has a survival advantage over homozygous HbA in the malarious region.

In the absence of results from heterozygous females, it is not possible to correlate the protective role of G-6-PD deficiency against malaria. The hypothesis that female heterozygotes for G-6-PD deficiency are protected from lethal malaria infection has been developed on the basis of parasite-encoded G-6-PD[14]. Subsequent reports on the distribution of enzyme deficiency and malaria throughout the world have generally confirmed this hypothesis[15].

ACKNOWLEDGEMENTS

The authors are thankful to Mrs. Bina Srivastava, Miss Pratiksha Desai, Mr. Vijay Bahadur and Mr. Alok Kulshreshtha for their 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 Cell.* Ciba Foundation Symposium 94 (Pitman, London): 159.
2. Miller, L.H., S.J. Mason, J.A. Davorak, M. McGinniss and I.K. Rothman (1975). Erythrocyte receptors for (*Plasmodium knowlesi*) malaria : Duffy blood group determinant. *Science*, **189**: 561-562.
3. Gupta, M. and A.N. Raichowdhuri (1980). Relationship between ABO blood groups and malaria. *Bull. WHO*, **56**(6): 913-915.
4. Joshi, Hema, K. Raghavendra, S.K. Subbarao and V.P. Sharma (1987). Genetic markers in malaria patients of Delhi. *Indian J. Malariol.*, **24**: 33-38.
5. Mathews, S.T., P.R. Kumaresan and R. Selvam (1991). Glucose-6-phosphate dehydrogenase deficiency and malaria A study on north Madras population. *J. Com. Dis.*, **23**(3): 178-181.
6. Pant, C.S., D.K. Gupta, R.C. Sharma, A.S. Gautam and R.M. Bhatt (1992). Frequency of ABO blood groups, sickle cell haemoglobin and G-6-PD deficiency and their relation with malaria in Scheduled Castes and Scheduled Tribes of Kheda district, Gujarat. *Indian J. Malariol.*, **29**: 235-239.
7. Pant, C.S., D.K. Gupta, R.M. Bhatt, A.S. Gautam and R.C. Sharma. An epidemiological study of G-6-PD deficiency, sickle cell haemoglobin and ABO blood groups in relation to malaria incidence in Muslim and Christian communities of Kheda district, Gujarat, India. *J. Com. Dis.*, **24**(4): 199-205.
8. Sharma, A. (1983). Haemoglobinopathies in India. In *Peoples of India. XV International Congress of Genetics,* Ed. G.V. Satyavati (Indian Council of Medical Research, New Delhi): 31-49.
9. Baxi, A.J. N.P. Parikh and H.I. Jhala (1969). Incidence of G-6-PD deficiency in three Gujarati populations. *Human Genet.*, **8**: 62-63.
10. Athreya, B.H. and L.L. Coriell (1967). Relation of blood groups to infection: I - A survey and review of data suggesting possible relationship between malaria and blood groups. *American J. Epidemiol.*, **86**: 292-304.
11. Molineaux, L. and G. Gramiccia (1980). *The Garki Project* (WHO, Geneva): 172.
12. Ghosh, T.N. (1983). Factors of human genetics in malaria. In *Proceedings of Indo-UK Workshop on Malaria.* Ed. V.P. Sharma (Ma-

laria Research Centre, ICMR, Delhi): 251-267.

13. Allison, A.C. (1954). Protection afforded by sickle cell trait against subtertian malaria infection. *British Med. J.*, **1**: 290-294.
14. Luzzatto, L., E.A. Usango and S. Reddy (1969). G-6-PD deficient red cells: Resistance to infection by malarial parasites. *Science*, **164**: 939.
15. Hetzeroth, H.W. and K. Bender (1980). Erythrocyte G-6-PD and 6-PGD genetic polymorphism in southern African Negroes with a note on G-6-PD and malaria hypothesis. *Human Genet.*, **54**: 233-242.

Accepted: 11 October 1993

INDIAN JOURNAL OF MALARIOLOGY

Editorial Acknowledgement

The Editor gratefully acknowledges the help of the following scientists who have kindly reviewed the papers for the 1993 issues of *Indian Journal of Malariology:*

Dr. S.S. Agarwal, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow; Dr. J. Akiyama, World Health Organization, Delhi; Dr. M.A. Ansari, Malaria Research Centre, Delhi; Dr. S.K. Basu, National Institute of Health and Family Welfare, Delhi; Dr. R.K. Chandrahas, Malaria Research Centre, Madras; Dr. S.K. Chawla, National Malaria Eradication Programme, Delhi; Dr. D.S. Choudhury, Malaria Research Centre, Delhi; Dr. V.S. Chauhan, National Institute of Immunology, Delhi; Dr. V. Dhanda, Vector Control Research Centre, Pondicherry; Dr. B.N. Dhawan, Central Drug Research Institute, Lucknow; Dr. G.P. Dutta, Central Drug Research Institute, Lucknow; Shri N.L. Kalra, Malaria Research Centre, Delhi; Dr. R.C. Mahajan, Postgraduate Institute of Medical Education and Research, Chandigarh; Dr. K.N. Mehrotra, Banaras Hindu University, Varanasi; Dr. A.G.K. Menon, Zoological Survey of India, Madras; Dr. M.V.V.L. Narasimham, National Malaria Eradication Programme, Delhi; Dr. V. Orlov, Martisinivsky Institute of Medical Parasitology and Tropical Medicine, Moscow; Dr. K.N. Paniker, Vector Control Research Centre, Pondicherry; Dr. M.K.K. Pillai, University of Delhi, Delhi; Dr. R.N. Prasad, Malaria Research Centre, Shahjahanpur; Dr. A.P. Ray, 240- Kailash Colony, Delhi; Dr. R. Reuben, Centre for Research in Medical Entomology, Madurai; Dr. R.G. Roy, Salt Lake, Calcutta; Dr. G.V. Satyavati, Indian Council of Medical Research, Delhi; Dr. S.K. Saxena, Government of India/WHO National Task Force on Revised Strategy for Control of Malaria, Delhi; Dr. P. Sen, Unerversity College of Medical Sciences, GTB Hospital, Delhi; Dr. S.D. Seth, All India Institute of Medical Sciences, Delhi; Dr. Shantanu, ASTRA Research Centre, Bangalore; Dr. G.K. Sharma, Janakpuri, Delhi; Dr. R.C. Sharma, Malaria Research Centre, Nadiad; Dr. S.K. Sharma, National Institute of Communicable Diseases, Delhi; Dr. Padam Singh, Institute for Research in Medical Statistics, Delhi; Dr. Santok Singh, St. John's College, Agra; Dr. S.K. Subbarao, Malaria Research Centre, Delhi; Dr. G.P. Talwar, National Institute of Immunology, Delhi; Dr. Neena Valecha, Malaria Research Centre, Delhi; Dr. R.L. Yadav, National Malaria Eradication Programme, Delhi; Dr. R.S. Yadav, Malaria Research Centre, Rourkela.

INDIAN JOURNAL OF MALARIOLOGY

Author Index

*Directorate of National Malaria Eradication Programme

INDIAN JOURNAL OF MALARIOLOGY

Instructions to Authors

Editorial Policy

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

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

Classes of Items Published

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

Format

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

Preparation of Copy

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

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

We accept MSS on floppy. Please specify software used.

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

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

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

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

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

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

The following style is accepted for this journal:

Research Paper

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

Book/Monograph

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

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

Paper presented at Symposium/Conference

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

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

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

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

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

Short notes should be prepared in a manner similar to the research papers and should contain Title, Name(s) of Author(s) with Address of Institute/University as footnotes, Acknowledgements 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 slated issue, or may be printed as it stands. Only minimal changes, i.e. those that do not substantially alter the page make-up, are permissible at the proof stage and only in exceptional cases. Alterations which are disallowed by theEditor shall be deleted or charged to author.

Reprints

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

Check-list

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

Announcement

We prefer submission of manuscripts on electronic media.

- Acceptable media is 3 1/2"or 5 1/4" disk in MS-DOS or MSDOS compatible format with file name, software/hardware used.

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

- Package used for graphs should be mentioned.

- The floppies would be sent back to the authors after a final decision on the manuscript only on request.

EDITORS

ANNOUNCEMENT

HEALTH MEDIA CENTRE - INDIA

There is no dearth of organisations in our country and why one more? This is a legitimate question that may come up in one's mind.

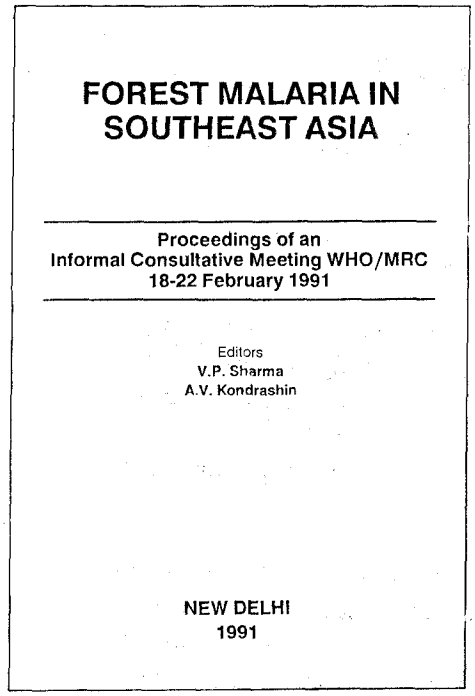
What makes the Health Media Centre - India different is that it is not a body of persons belonging to one profession alone as is normally the case, but two - media and/medical/health - who have all along been considered to be poles apart in their attitude and approach to men and matters. The only thing common between them is that both are concerned with people and their betterment.

The objectives of the Health Media Centre - India are to: (a) disseminate information on various health subjects; (b) prepare educational materials for use in educating the public and orienting the media; (c) issue features on various health-related themes; (d) provide an interface between the media and medical experts; and (e) undertake all necessary activities for promotion of health education.

During its first two years, the HMC-I had organised programmes on AIDS, Diabetes, Malaria, Dangers of tobacco use, Coping with Natural Disasters and Tuberculosis. These helped the media to have a better understanding of the issues involved in tackling these problems.

The HIC-I represents the first ever attempt to bring together the media and medical/health professionals. If you are connected with the media or medical profession, no matter at what level, and interested in promoting the concept of health for all, this is the right forum for you to join.

For membership and details please contact:
Secretary General
Health Media Centre - India
B-15, Swasthya Vihar, Vikas Marg
Delhi - 110 092, India

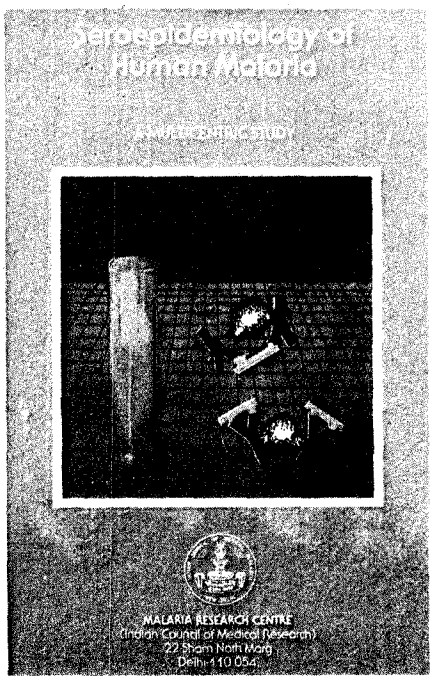


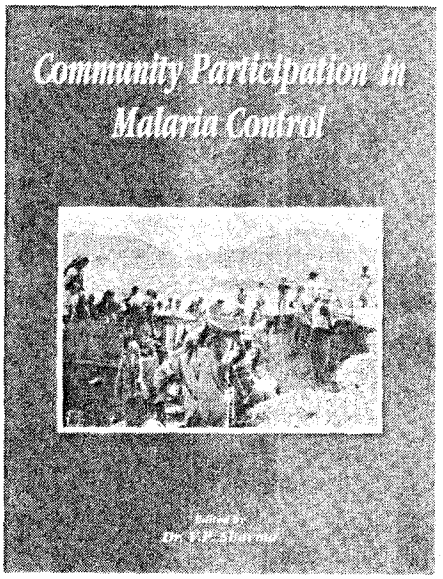
A consolidated volume on various facets of forest related malaria problem in Southeast Asia. An important source material for malariologists and researchers for achieving cost-effective, rational and lasting control of the problem and provides direction for future management.

Size : Royal 8VO
pp. viii, 234 (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.

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



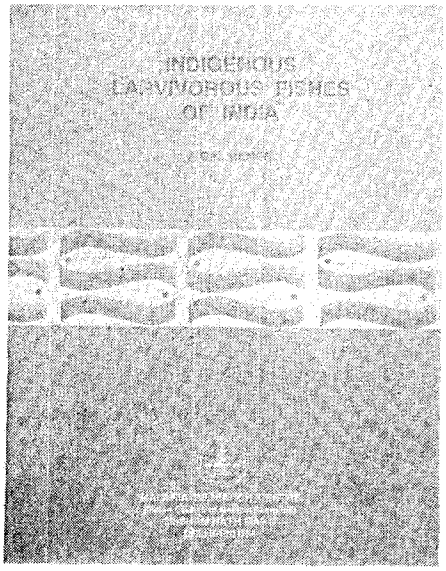


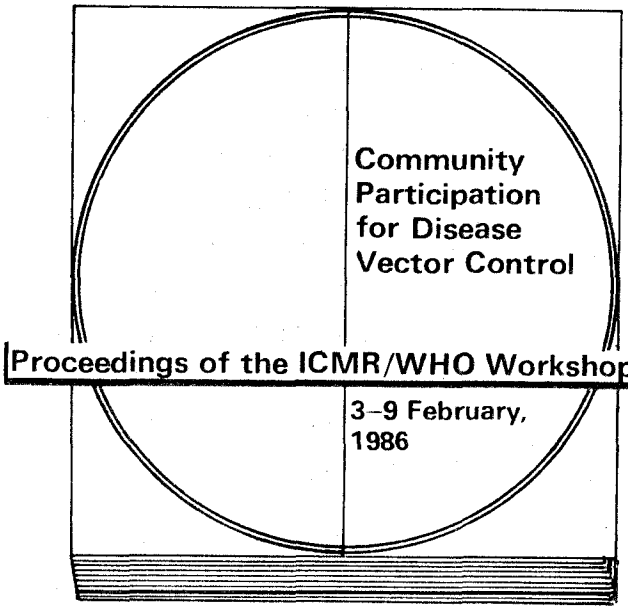
A complete review of the experience on community participation from various areas in the country in relation to vector/malaria control. A commendable document for future management of vector control through community participation.

Size: Crown 4TO
pp. viii, 295 (Hardbound)

A detailed and exhaustive key on the identification of larvivorous fishes of Indian origin for vector control specialists to combat public health problem like Malaria.

Size: Crown 4TO
pp. x, 66 (Paperback)





A review of the process, role and outcome of people's involvement in vector control. Invaluable for workers and decision makers in Primary Health Care and health-related projects.

Size: Royal 8VO
pp. xvii, 256 (Hardbound)

THE ANOPHELINES OF INDIA

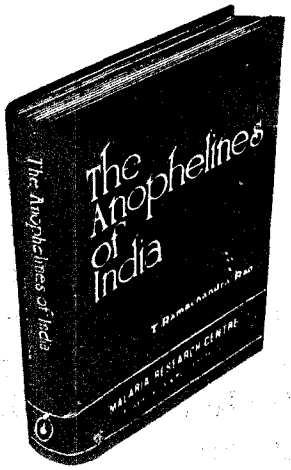
Revised Edition 1984

by

T. Ramachandra Rao

An authoritative and comprehensive publication which brings together all that is known about distribution, bionomics, ecology, relation to disease and basic principles of control of anopheline mosquitoes.

Size Crown 4TO pp. 538 (Hardbound)
Rs. 150.00 (India) US $ 45.00 (Other countries)
25% Discount per copy



The monograph can be obtained by sending **bank draft** or **postal order** only, payable at Delhi to

The Director
Malaria Research Centre (ICMR)
22-Sham Nath Marg
Delhi-110 054, INDIA.

INDIAN JOURNAL OF MALARIOLOGY
MALARIA RESEARCH CENTRE (ICMR)

UNDERTAKING BY AUTHORS

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

_____

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

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

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

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

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

IMPORTANT

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

* Please write the applicable statement below: