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Indian Journals on Malariology

B.K. SEN1, ARUNA KARANJAI1 and USHA MUJOO MUNSHI!

INTRODUCTION

The history of malaria research in India in modern times can be traced back to the last decade of the last century when in 1897 Dr. Ronald Ross, a British Army physician, stationed in India, proved that bird malaria is transmitted by Culex mosquitoes and narrated in detail the development of the parasite in the mosquito. In November 1898, three Italian investigators, viz., A. Bignami, G.B. Grassi and G. Bastianelli showed that malaria was transmitted in man in the same way as in birds, and probably the transmission took place through anopheline mosquitoes. Needless to say, the observations of the Italian scientists were found to be correct.

In view of the innumerable cases of malaria in the country, causing millions of deaths and untold miseries to the people, the Government of India took a positive step in 1909 for establishing a permanent organisation in the country for dealing with the problems of malaria, which gave rise to Central Malaria Bureau at Kasauli.

In order to have a vehicle of dissemination of research results on malaria and its associated areas, the aforesaid committee started in 1910, a half yearly periodical called Paludism, as its Transactions. This periodical, most probably the first periodical on malaria in the world, was published from Simla and contained original contributions devoted to morphology, anatomy and physiology of the mosquito. Five issues of the periodical were published till September 1912, when the periodical became the official organ of the Indian Research Fund Association and the title was changed to Indian Journal of Medical Research, the first issue of which appeared in July 1913, Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India, publishing since 1885 [Vol. 1 (1885) to 12(1902) of the Memoirs were titled as Scientific Memoirs by Medical Officers of the Army of India also got merged in Indian Journal of Medical Research in 1913 itself resulting in the change of the scope of the aforesaid journal. Hence, from 1913 to 1928 there was no journal from India exclusively devoted to malariology.

Records of the Malaria Survey of India started in 1929 by the Indian Research Fund Association (now Indian Council of Medical Research) filled up this gap. In December 1937, the Government of India took over the public health and advisory functions of Malaria Survey of India and the

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name of the Survey was changed to Malaria Institute of India. In order to have conformity with the name of the Institute. Records of the Malaria Survey of India was rechristened as Journal of the Malaria Institute of India in March 1938. The scope of the journal gradually widened to include articles not only from the workers of the Institute, but also from outside. With the changed scope, the title of the journal was once again changed to Indian Journal of Malariology (IJM) in 1947. Col. Jaswant Singh, the first Indian Director of the Malaria Institute of India, was the editor of the journal. The Journal maintained a high standard and served as the main vehicle for the dissemination of information on almost all aspects of malaria (Neelameghan, 1963; Kumar, 1985; Sharma, 1985).

The National Malaria Control Programme (from 1958 onwards known as National Malaria Eradication Programme) achieved remarkable success in its objective, and by 1965, the number of malaria cases in India dropped down from about 10 crores in 1950/51 to about 100,000 with no cases of death in 1965. The euphoria generated by the success gradually brought about a natural decline in the malaria research in the country resulting thereby in the discontinuation of the Indian Journal of Malariology from December 1963 (Vol. 17. No. 4). Till 1963, in all 30 volumes were published (Records of the Malaria Survey of India-7 vols., Journal of the Malaria Institute of India-6 vols., and Indian Journal of Malariology-17 volumes) containing 1112 articles from 475 contributors (122 foreign). The articles covered "all aspects of malaria (human, avian, simian and rodentsphysiology of malaria parasite, pathology, chemotherapy, immunology, epidemiology, control and eradication) and studies on mosquitoes both Culex and Anopheles (bionomics, distribution, vectorial capacity, relation to malaria, destruction by use of insecticides, resistance to insecticides), the Journal has covered valuable knowledge on rat-fleas, house-flies, bed-bugs, trypanosomes, blackwater fever, kala azar, filariasis, plague, etc."

In 1953, when Indian Journal of Malariology was running in its 7th volume, another journal partially devoted to malaria entitled Bulletin of the National Society of India for Malaria and other Mosquito Borne Diseases sprang up from Delhi. Till 1963, 11 volumes of the journal were published when the title of the journal was changed to Bulletin of the Indian Society of Malaria and other Communicable Diseases in order to have conformity with the changed title of the society. The reason, that is, paucity of articles on malaria, which caused the discontinuation of Indian Journal of Malariology in 1963 might have also occasioned the change of the scope and thereby the title of the aforesaid Bulletin to Journal of Communicable Diseases in 1969. From 1969 to 1980, again there was no journal from India exclusively on malariology.

The euphoria which engulfed the malaria eradicators of the country in mid-1960s with the dwindling down of malaria cases below one lakh and fatalities to zero in the whole country gradually vanished as malaria staged a steady comeback in subsequent years with much more resistive power in its arsenal, and the number of malaria cases touched the figure 64.67 lakhs in 1976.

INDIAN JOURNAL OF MALARIOLOGY

As malaria cases grew more numerous the literature on malaria also gradually picked up momentum and in 1981, when the revival of the *Indian Journal of Malariology* after a dormancy of 17 years was announced, it received a warm welcome from all parts of the world (IJM,1981). This year (1990), the Journal is completing 10 years of its revival. Perhaps it is time to look back and take stock as to how the journal has fared in the decade that it has traversed.

I'm bliochteiriceadad e Moude o interest alle clementos el centro (el centro	1981	1982	1983	1984	1985	1986	1987	1988	1989
Editorials	er egren for a restauteauxencerrotes	0	0	0	0	0	0	0	0
Papers	18	19	19	14	9	14	22	14	22
Short Notes	14	11	6	4	9	7	8	8	7
Obituaries	2	0	e	3	0	0	0	1	0
Book Reviews	0	0	7	1	19.0	0	0	0	0
Letter to Editor	0	0	9	0	0	0	0	0	1
Total	26	30	26	22	19	21	30	23	30

Table 1. Yearwise distribution of various types of articles in LIM

Literature Output

During the period under review, i.e., 1981 to 1989, the journal has published 227 articles, comprising research papers (151), short notes (65), obituaries (6) book reviews (3), editorial (1), and letter to the editor (1) [Table 1]. On an average 25 articles have appeared each year. The articles dealt with malaria in men and animals, malaria control, anti-malarials, malarial entomology, malarial parasites, drug response, animal studies, etc.

Quality and International Standing

Since 1960s attempts are being made to determine the quality of journals using bibliometric methods. These methods are based on statistical analysis of citations received by journals devoted to a subject over a certain period of time. The method developed by Bradford (1948) helps us to identify the topmost journals, i.e., core journals, in a subject. The method developed by Garfield (1972) also helps us to identify the best journals in a subject, as well as their international standing.

The Journal Citation Report (JCR), a companion publication of Science Citation Index provides the list of journals devoted to a field according to their impact factors. The Subject Category Table (Table 2) reproduced below from the Journal Citation Report 1988 lists the journals on Tropical Medicine in the descending order of their impact factors.

Looking at the Table 2 one can easily identify the topmost journals in the subject and also the standing of any journal on the subject covered by SCI database.

The impact factor of a journal is basically a ratio of citations received by a journal in a particular calendar year on its citable items published during the previous two calendar years. The impact factor is not constant and usually undergoes change every year. The impact factor of a journal for a particular calendar year is determined as described below:

Indian Journal of Medical Research (IJMR) published 243 and 281 source items in 1986 and 1987, respectively. These source items received 51 and 56 citations, respectively in 1988. Now, the 1988 impact factor of IJMR will be

$$l_{\rm f} = \frac{51 + 56}{243 + 281} = \frac{107}{524} = 0.204$$

The Journal Citation Report provides impact factors of some 4000 journals every year selected

Table 2. Journals on Tropical Medicine ranked by Impact Factor

Table 3. Impact Factors of some indian Medicai Journals (1986)

3.No.	Journal	Impact Factor
7	Leprosy Rev.	1.921
ž.,	Am. J. Trop. Med. Hyg.	1.751
.5.	Trans, R. Soc. Trop. Med. Hyg.	1.278
ŧ.	int. J. Leprosy	1.104
۶.	Trop. Med. Parasuol.	0.968
6.	Аста Тгор.	0.818
7.	Ann. Trop. Med. Parasitol.	0.614
3.	J. Frop. Med. Hyg.	0.286
9.	Rev. I. Med. Trop.	0.254
10.	Ann. Soc. Belg. Med. Tr.	0.231
11.	J. Trop. Paediatr.	0.217
12.	Ann. Trop. Paediatr.	0.211
13.	Trop. Geogr. Med.	0.190
14.	Trop. Doct.	0.141
15,	Papua New Guinea Med.	0.093
16.	m. J. Zoonoses	0.83
17.	Cent. Afr. J. Med.	0.060

on the basis of peer suggestions and several other parameters. The impact factor of journals not figuring in the Journal Citation Report can also be determined using the method developed by us (Sen et al., 1989). Using our method we determined the 1986 impact factors of quite a few Indian medical journals as shown in Table 3 (Munshi et al., 1990).

It can be seen from the Table 3 that the impact factor of IJM is the highest among Indian medical journals, whose impact factors we have determined and shown in our paper. IJM would have ranked 5th amongst the journals of tropical medicine of the world in 1987, when its impact factor touched 0.897. It is worth mentioning here that ever since the publication of impact factor in JCR (i.e., from 1975), not many Indian S&T journals have reached the 1987 impact factor of IJM.

S.No.	Journal	Impact Factor
1.	Adv. Biol.	0.069
2.	Arogya	0.057
3.	Indian Drug	0.207
4.	Indian J. Envir. Hlth.	0.189
5.	Indian J. Chest Dis.	0.096
6.	Indian J. Leprosy	0.245
7.	Indian J. Malariol,	0.5 28
8.	Indian J. Parasitol.	0.100
9.	Indian J. Paediatr.	9.0 30
10.	Indian J. Phar. Sci.	9.077

Taole 4 depicts the impact factors of all Indian journals covered by SCI during 1986 to 1988. Even from this list, it is quite clear that the impact factor of IJM is higher than most of the Indian periodicals. It has been normally seen that impact factor has got positive correlation with the quality of journals. As the quality of a journal moves up or down so does its impact factor. So it can be safely concluded that the quality of IJM is definitely comparable with other Indian medical or S&T journals. We can very safely state that as far as impact factor and quality are concerned IJM is good enough to be covered by SCI, or any other international secondary service devoted to medicine.

Coverage by International Abstracting and Indexing Services

IJM is being covered by international indexing and abstracting services like Biological Abstracts (i.e., BIOSIS database), Index Medicus (Medline database), Review of Applied Entomology, Protozoological Abstracts, Quarterly Bibliography of Major Tropical Diseases, Tropical Diseases Bulletin. In 1960s, it used to be selectively covered also by Excerpta Medica. It is strongly

Table 4. Impact Factor of Indian Journals covered by SCI

S.No.	Journal		Impact Factor	
		1986	1987	1988
\$	Ann. Arid. Zone	0.028	0.000	0.000
2.	B. Mater, Sci.	A) electric		0.265
3.	Colourage	0.007	0.012	0.006
4.	Comp. Physiol. Ecol.	0.065	0.051	0.060
5.	Curr. Sci.	0.117	0.150	0.164
6 .	Entomon.	0.053	0.021	0.025
7.	Indian J. Agric, Sci.	9.015	0.024	0.011
3.	Indian J. Agron.	800,0	0.004	0
9.	Indian J. Anim, Sci.	0.019	0.013	0.014
10.	Indian I. Biochem. Biophys.	p. ====	0.242	0.214
11.	Indian J. Chem. A	0.301	0.245	0.314
12.	Indian J. Chem. B	0.371	0.222	0.281
13.	Indian J. Expl. Biol.	NA AMPLY	0.687	0.151
14.	Indian J. Malariol.	0.528	0.897	0.392
ŧ3.	inuan I. Marine Sci.	0.069	0.115	0.072
16.	Indian J. Med. Res.	0.287	0.265	0.204
17.	Indian J. Pure App. Mat.	0.082	0.069	0.064
:3.	Indian I. Pure App. Phys.		0.124	0.116
19.	ındian J. Radio Space Phys.	(5-0 M)	9.056	9.047
20.	indian J. Technol.	0.084	0.084	0.130
21	Indian Vet. J.	0.022	0.010	0.017
22.	int. J. Trop. Agric.	Allered	0.047	0.023
23.	J. Adv. Zool.	0.000	0.037	0.017
24.	1. Astrophys. Astron.	1 311	0.453	0.585
25.	1. Biosci.	0.347	0.387	0.282
26.	J. Environ. Biol.	0.150	0.026	0.259
27.	J. Food Sci. Tech.	0.071	0.098	0.044
28.	J. Genet.	ALL STATES	age	0.588

Contd...

Table 4. Impact Factor of Indian Journals covered by SCI (Conid.)

5.No.	Journal		Impact Factor	outlike de prime an Floure 1900 to Flouresholder (18 June 1904 to 1907 to
		1986	1987	1988
29.	I. Geol. Soc. India	A Marie prime problem from vide curve in model in 1 en inger frange dry public commence transfer models and de Marie prime problem in the curve in model in 1 en inger france dry public commence transfer models and design	9.130	0.144
30.	I. Indian Chem. Soc.	no m	0.097	0.108
31.	1. Optics	0.635	0.313	0.138
12.	J. Scs. Ind. Res.	0.194	0.188	0.248
33.	Natl. Acad. Sci. Lett.	0.029	0.013	0.033
14.	P. Indian Acad. Sci-Anim. Sci.	0.028	0.07)	9.048
35.	₽. [ndian Acad. Sci-Chem. Sci.	0.399	0.328	0.300
36.	P. Indian Acad. Sci-Earth Sci.	0.386	0.725	0.106
37.	P. Indian Acad. Sci-Math. Sci.	0.000	0.034	0.019
38.	P. Indian Acad. Sci-Plant Sci.	0.096	9. 098	0.036
39.	Prumana	0.428	0.339	0.521
¥0.	Res. Ind.	0.032	0.044	0.019
ŧİ.	Sodhana	0.027	****	9.055
12.	Sankinya-A	er Manie	0.143	0.208
13.	Sankhya-B	waw.	0.086	0.159
44.	F. Indian Inst. Metals	9.017	0.084	0.020

^{*} Not covered by SCI_If calculated by method of Sen et al. (1989).

felt that IJM should be covered by such services also as Chemical Abstracts, Current Contents, Excerpta Medica and Science Citation Index.

It is eather strange that the most commonly used directory of periodicals called Ulrich's International Periodicals Directory which includes details of about 1,11,950 serials (1989/90 edition) from all over the world is not aware of the existence of IJM. Similar is the case with the Serials Directory. Despite its covering of 1,23,000 serials from all over the world in 1990, IJM could not find a place in it. This may be brought to the notice of the publishers of the aforesaid directories for its inclusion in their next edition.

Internationality Index

As to the inclusion of a journal in Science Citation Index (SCI) the factors considered are: citation data, journal standard and expert judgement. For deciding the journal standard, peer review submission, editorial board membership and reputation of the publisher are taken into account. In addition, timeliness of publication and internationality of the journal are also considered important for the inclusion of a journal in SCI. As to internationality, Garfield observes "The geographical representation of a journal is another consideration. Unless a journal of interest to only a small region of the world is exceptional in some way,

Yable S. Editorial Board membership of LJM

t gagerate have the device of any constraint and the properties and the												
Members	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990		
indian(a)	13	13	11	10	10	10	10	10	10	10		
Foreign(b)	0	0	0	0	0	ð	0	0	0	0		
Internationality Index (100b/a)	0	0	0	9	0	9	0	0	0	0		

l'able 6. Nationality of articles published in iJM

Members	1981	1982	1983	1984	1985	1986	1987	1988	1989
Indian (a)	22	30	24	17	18	18	29	22	28
Foreign or multinational (b)	# · · ·	0	ž	1	;)	3	a de	0	1
Internationality Index (100b/a)	4.55	0	4.17	\$.85	9	16.67	3.45	0	3.57

we are less likely to cover it" (Garfield, 1990). Keeping the above observation in view, we shall now check the internationality index of IJM taking into consideration the members of its editorial board as well as the nationality of the articles published in the journal during the last decade (Tables 5 and 6).

CONCLUSION

From the internationality indexes depicted in Tables 5 and 6 it is evident that IJM has been and still today is only a local journal, and we strongly feel that the image of the journal has to be radically changed from local to international for the following reasons:

The incidence of malaria is not restricted to India alone. It encompasses the entire South and southeast Asia, parts of Africa, and many other parts of the Third World.

Compared to its widespread incidence, the journals solely devoted to malaria are practically

non-existent. To our knowledge, only one journal devoted to malaria i.e., Rivista de Malariologia appeared from Rome since 1922 and ceased publication in 1967.

It is to be noted in this context that there is no international journal exclusively devoted to malaria till today. It is time that we take a lead in this direction, and transform the Indian Journal of Malariology into an international journal. India has all the infrastructure i.e., a running journal on malariology as well as refereeing experts besides editorial, printing and marketing facilities. Possibly, our contribution on malariology in terms of the number of papers is largest compared to any other country's contribution. It is also likely that no other country has so many research workers in the field as we have. With all these facilities in our hand, it should not be difficult to raise the status of the journal to an international one.

In order to internationalize the IJM the following may be done:

- (a) The Editorial Board should have a sizable number of internationally well-known experts drawn from various countries of the world.
- (b) The transformation of the journal into an international one should be given world-wide publicity through correspondence and other means to ensure regular flow of good articles from inside and outside the country, and also a good number of subscribers or even advertisements from multinational concerns abroad.
- (c) Rigorous refereeing of each contribution should be ensured by having a three referee system.
- (d) Timely publication of a journal ensures regular flow of articles, coverage by international abstracting and indexing services, and also subscription by various libraries and individuals. Hence, the time schedule of the periodical should be strictly adhered to.
- (e) The international abstracting and indexing services including the Science Citation Index should be kept informed. If some of these services are not willing to subscribe, provision should be made for mailing free copies to them.
- (f) The quality of paper, printing and editing of IJM is tolerably good. However, it would be better, if these areas are also taken good care of.

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In vivo Test for Sensitivity of Plasmodium falciparum to Chloroquine in Faridabad Complex of Haryana (India)

USHA ARORA[‡], R.S. SHARMA², GURCHARAN SINGH², R.K. DASGUPTA[‡] and M.V.V.L. NARASIMHAM¹

A study was conducted to know the chloroquine sensitivity of *Plasmodium falcaparum* in Faridabad complex of Haryana state where indigenous resistant cases of *P. falcaparum* to chloroquine have been detected for the first time in the district. Out of 13 cases tested, 3 cases showed resistance at RIII level, 5 cases at RII level and 5 cases at RI level.

INTRODUCTION

Chloroquine resistance in *Plasmodium faici-parum* malaria has been reported from different parts of the world (WHO, 1986a; 1986b). The problem becomes acute when drug resistance coexists with vector resistance to insecticides. In India, the problem of drug resistance has been reported from many parts (Choudhury *et al.*, 1983; Das *et al.*, 1979; De *et al.*, 1979; Dwivedi *et al.*, 1981; Guha *et al.*, 1979; Sehgal *et al.*, 1973 and Verma *et al.*, 1986). In Haryana chloroquine sensitivity studies were carried out in Hissar, Sirsa and Gurgaon (NMEP Report, 1986 to

1988). In these areas, no resistant case at RIII level was found. The present study of drug sensitivity was undertaken at Faridabad complex of Haryana in January 1989 to know the drug sensitivity status in the district.

MATERIAL AND METHODS

On the basis of epidemiological data (Table 1) on malaria incidence in Faridabad complex, 5 villages reported to have adequate number of P. falciparum cases, were selected for the study. Blood surveys of the active fever cases in these villages were carried out. Only these P. falciparum cases showing asexual parasite density of 1,000 or more per µl of blood were selected for the test. Cases with history of 4-aminoquinoline administration were dropped from the study. Urine was examined by Dill and Glazko test (Lelijveld and Kortmann, 1970). Mixed infection cases (P. vivax and P. falciparum) and seriously all patients were excluded from the study. WHO recommended standard extended field test

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National Malaria Eradication Programme 22-Sham Nath Marg Delhi-110 054, India.

Regional Office for Health and Family Welfare Kothi No. 3281
 Sector-21/D
 Chandigarh-160 022, India.

Table I. Epidemiological data for Faridahad district of Haryana state

Year	Population	B.S. exam.	ABER	No. of +ve cases	No. of Pf cases	SPR	API	P(
3984	1328,000	201441	15.2	12623	3055	6.3	9.5	24.2
1985	1,358,000	214948	15.8	9314	2174	4.3	5.9	23.3
:986	1395,000	221444	15.9	5394	390	2.4	3.9	7.2
1987	1429,000	221052	15.5	2020	95	0.9	1.4	4.7
1988	1484,000	262951	17.3	2056	434	8.0	1.4	21.1

Table 2. Chlorogoine sensitivity of P. Jalciparum in Faridabad complex

Case No.	Age	Sex	D-0	D-1	D-2	D-3	D-4	D-5	D-6	D-7	D-14	D-21	D-28	Level of resis-
1	40	F	6427	4507	1120	Pfg	Pfg	Pfg	Pfg	Pfg	Pfg	Pfg	Pſg	S
?.	35	Ţ.	7413	2373	693	213	Pfg	Pfg	Pfg	Pfg	Pfg	Pfg	Pig	s
ā,	1.5	M	2160	373	Pfg	Pfg	Pig	Pfg	Pfg	Pfg	Pfg	Pfg	Ptg	S
-\$.	15	*vi	4187	1547	933	80	Pfg	Pfg	Pfg	Pfg	Pfg	Pfg	Pfg	S
5.	90	Ní.	2773	773	Pfg	Píg	Pfg	Pfg	Pfg	Pfg	9440	*****		RI
3.	0	ç.	2480	1200	213	160	Pfg	Pfg	Pfg	Pfg	Pfg	1627	***	RJ
7	45	14	26426	8567	5226	3680	480	Pfg	Pfg	Pfg	880	ANGLE .		RI
8.	7	.\4	14933	3493	2213	240	Pfg	Pfg	Pfg	Pfg	3733			RI
9.	iù	М	9493	5040	Pfg	Pfg	Pfg	Pfg	Pfg	Pfg	Pfg	6480	****	RI
G.	14	.W	32320	7440	5813	13653	8720	7067	2587	187	× 48.50	the fe	red.	RII
13.	36	Vá	33893	1640	13258		**************************************	****	- Appella	ar worth				RIII
.2.	Ą	4	16480	8373	5147		address	-44-44				Jan A. s	,	RIII
13.	.15	F	37653	24453	21733		91.180	J.C. 89						RIII

Note: Figures from Day-0 to Day-28 indicate parasitaemia (Pf rings) per one mm³ of blood; Pfg = Piasmodium falci-parum gametocytes.

(WHO, 1973) (28-days) was followed to study in vivo response on selected P. falciparum cases. Patients were given chloroquine base @ 25

mg/kg body weight divided over 3 days, i.e., 10 mg/kg each on first and second days and 5 mg/kg on the third day. All the study slides were



Fig. 1: Map of Haryana showing location of study area and earlier foci of resistance

stained with Giemsa 5% for 30 mins. Parasite density of asexual stages was counted against 300 leucocytes. Antimalarial drug i.e., chloroquine phosphate was supplied by IDPL.

RESULTS

A total of 169 blood smears were collected to screen out suitable *P. falciparum* cases for the test. Of those, 13 *P. falciparum* cases were found suitable for drug sensitivity by *in vivo* test. Results of the test are shown in Table 2. Out of 13

cases, in 4 (30.8%) parasitaemia was absent for the full observation period of 28 days showing them to be sensitive. In 5 cases (38.5%), though the asexual parasites were cleared by Day-4 and Day-5, there was subsequent resurgence of parasitaemia, showing RI level of resistance. In 1 (7.7%) case parasitaemia did not clear, but was markedly reduced showing RII level of resistance. In 3 (23.1%) cases the reduction of parasitaemia was less than 75% during the first 48 hrs and these cases were classified as high degree of chloroquine resistance i.e., RIII level.

DISCUSSION

In Haryana resistance at RI and RII level has already been reported from Hissar and Sirsa districts during 1986-87 and 1988 (Fig. 1). Verma et al. (1980) have reported chloroquine resistance of P. falciparum in the neighbouring state, i.e., Punjab. All the cases tested were indigenous. Although the sample size is small, the results clearly indicate that a high degree of chloroquine resistance in P. falciparum, (i.e., up to RIII level) is present in Faridabad district. The results have provided much needed attention for gearing up surveillance and anti-vector measures. Further studies are warranted with larger sample size for change of drug regimen if necessary.

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Breeding Habitats of Malaria Vectors: A. fluviatilis, A. annularis and A. culicifacies, in Koraput district, Orissa

S.S. SAHU 1 , S.K. PARIDA 1 , C. SADANANDANE 1 , K. GUNASEKARAN 1 , P. JAMBULINGAM and P.K. DAS 1

Anopheline breeding habitats were studied in two different geochmatic zones. The vectors A. fluviatilis, A annularis and A. culicifacies breed in all types of habitats. Streams were most productive for the main vector, A. fluviatilis and breeding was intense during winter and early summer. In the rainy season, breeding occurred in terraced paddy fields. A. culicifacies were produced in large numbers in rivers in summer and in paddy fields in rainy and cold seasons. Considering the vastness of these breeding habitats, vector control measures by larviciding or any other bioenvironmental means will not be a feasible alternative even though house spraying poses serious problems in this area due to several reasons. The use of personal protection methods is suggested.

INTRODUCTION

The hilly and forested tracts of central, northern and eastern parts of India represent a core of stable malaria, with high endemicity and predominance of *Plasmodium falciparum*, which is refractory to present control strategy. Recent studies in hilly and forested tract of Koraput district Orissa showed that A. fluviatilis is the major malaria vector and A. culicifacies and A. annularis play a minor role in malaria transmission (Gunasekaran et al., 1989). Inadequacy of available techniques is due to the development of insecticide resistance in A. annularis and

A. culterfactes (Sahu et al., 1990), exophilic behaviour of A. fluviatilis (Das et al., 1990), parasite resistance to chloroquine (Mohapatra et al., 1989), the people's resistance to residual spray and operational inefficiency due to resource constraints (Rajagopalan and Das, 1990). Similar situations in other parts of the country are not uncommon. In such situations some alternative control measures need to be established.

Integrated vector control strategy with major emphasis on bioenvironmental measures and reduction of man vector contact with community participation has been attempted in many areas and the results were encouraging (Rajagopalan and Panicker, 1984; Sharma et al., 1986; Dua et al., 1988). Therefore it would be desirable to review the feasibility of undertaking an integrated

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¹Vector Control Research Centre Medical Complex, Indira Nagar Pondicherry - 605 006, India.

vector control strategy in this hilly and forested tract. Since information on the breeding habitats is also essential for designing such strategy, studies were undertaken from January 1988 to April 1989 in two different geoclimatic zones viz., Jeypore and Malkangiri and the results are discussed.

MATERIAL AND METHODS

Study area

Koraput district and its climate, topography, population etc., have been described earlier

(Rajagopalan et al., 1990). The study was carried out in 11 villages viz., Benasur, Seamalaguda, B. Singpur and Champapodar of Borigumma PHC (Jeypore zone) and Bondhaguda, Siadimal and MV-7 of Malkangiri PHC and Bandhaguda, Badapada, Dantipada and Bandiguda in Bonda Hills, of Khairput PHC (Malkangiri zone). Villages under Borigumma PHC are situated at an altitude of 580-680 mts above MSL and those under Malkangiri PHC at 150 mts above MSL and Bonda Hill villages at 850 mtrs. Of these villages, Champapodar of Borigumma PHC and villages in Bonda hill are situated on hill tops where streams are the major breeding habitat.

Table 1. Estimated surface area of breeding habitats available in study villages

PHC/	Village	Surface ar	ea of breeding	habitats within o	one km radius o	f the village (in	m ²)
Altitude		Ponds	Weils	Streams	River	Paddy fields (1000)	Other
Borigumma	et in der Franchisch von eine Anthonie in der Germanne in der Germanne der Germanne der Germanne der Germanne d	and the second s	All the state of t				
á 5 0	Benasur	21984	226.3	NA	NA	3970	198
	Scamalaguda	1257	364.5	NA	7850	2170	NA
	B. Singpur	50587	402.2	2100	1860	3650	100
	Champapodar	1350	37.7	6000	NΑ	400	NA
Malkangiri							
150	Bandhaguda	5600	ì.ú	5000	NA	80	200
	MV-7	5240	35.6	NA	4000	60	472
	Siadimal	1200	1.6	NA	NA.	30	300
3ond a Hills_	Кнайгриі						
350	Badapada	NA	NA	1050	NA		NA
	Oantipada	NA	NA	2100	NA	4	NA
	Bandhaguda	NA	NA	1050	NA	Ť	NA.
	Bandiguda	NA	NA	2100	NA	*	NA

⁵ Stream beds terraced into paddy fields.

B. Singpur and Bondhaguda are situated in the foot hills near streams and are surrounded by paddy fields. Seamalaguda and MV-7 are situated near the bank of a river. Other villages are in the plains surrounded by extensive low land paddy fields. There are a number of ponds and wells in all these villages.

All breeding habitats within 1 km radius of the study villages were identified, enumerated and mapped. Habitats from each type were randomly selected and surveyed fortnightly to monitor the seasonality of anopheline breeding.

Sequential sampling (Southwood, 1978) was done using a standard dipper (10 cm diameter, and 300 ml capacity) in all habitats. One dip was taken at every two meter interval in streams, rivers and ponds along the edges and at one meter interval in paddy fields along the periphery. Two to five dips were taken from each borrow pit and irrigation pool depending on the size. Three to five bucket samples (3 lit capacity) were taken from each well, two/four from the sides and one from the centre. The number/type of the breeding habitats surveyed in each village, total dips taken and number of positive dips were recorded. The immatures collected were reared to adults and identified.

RESULTS AND DISCUSSION

The perennial breeding habitats are streams, rivers, ponds and wells and the seasonal breeding habitats are paddy fields, borrow pits, hoof marks and irrigation pools. The extent of breeding habitats in villages of different altitudes is given in Table 1. In Borigumma and Malkangiri PHC areas ponds, wells, paddy fields and other transient habitats are common to all villages, streams are present in foothill and hill top villages only and rivers in riverine villages. In Bonda Hills of Khairput PHC streams and terraced paddy fields are the only breeding habitats.

A total of 20 anopheline species have been identified from all habitats (Table 2). Habitats with running water, such as streams, and rivers were found to support breeding of eight vector species viz., A. aconitus, A. annularis, A. culicifacies, A. jeyporiensis, A. maculatus, A. philippinensis and A. varuna in higher numbers as compared to stagnant water habitats like ponds, wells, irrigation pools and borrow pits where the non-vector species breed in higher numbers (Table 2). Since only three species namely A. fluviatilis, A. annularis and A. culicifacies have been incriminated as vectors in this area, further analysis is restricted only to these species.

In streams twenty species have been recorded. Breeding occurred throughout the year, with a relatively higher density during winter season, (November-February). During rainy season breeding was less due to swift water current and flushing away of larvae (Fig. 1).

Comparison of species composition in streams at different altitudes shows that at 850 mts (Bonda hills) A. fluviatilis was the most predominant breeder among the vectors and the density of A. culicifacies and A. annularis was very low. At 650 mts, both in hill tops and foothills A. culicifacies was predominant over the other two species in summer and early monsoon period, However, in winter months A. fluviatilis was predominant in hilltops and A. annularis in foothills (Fig. 1). At lower altitudes i.e., 150 mts. A. culicifacies was predominant over A. fluviatilis throughout the year and in late winter and summer A. annularis was predominant over the other two species. This cannot be attributed entirely to altitude since the stream sampled at 150 mts was dammed by the inhabitants of the nearby village in summer and late winter season thereby stagnating the water. This might have favoured breeding of A. culicifacies and A. annularis.

A total of 18 species were identified from the

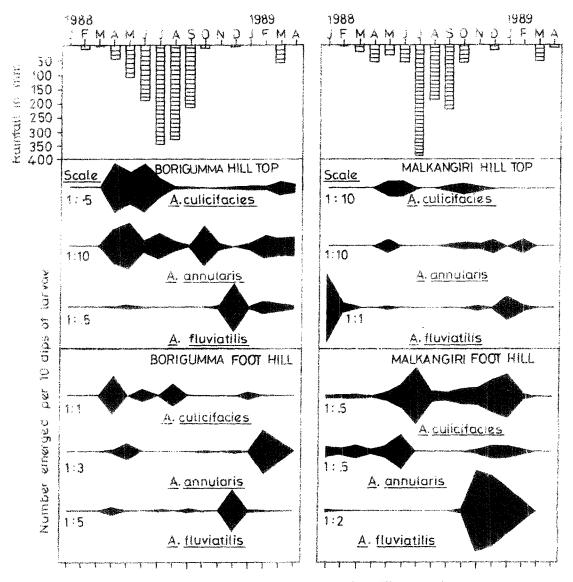


Fig. 1: Breeding of three vectors in streams from different terrain.

samples collected from rivers. About 57.1% of total anophelines was A. culicifacies. A. annularis and A. fluviatilis were found in low densities (Table 2). The breeding of vector species was higher during winter and summer months, November to June, with a peak in February, and lower during rainy months. No larvae could be collected during the months of August and September, when there was heavy

rain. During rainy seasons the river was completely flooded and the grassy margins all along the course of the river were submerged and hence anopheline breeding was very low. During winter and summer, the water level receded and the margins became grassy and many pools were formed in the river beds, favouring anopheline breeding. There was no difference in the seasonality of breeding of A.

Table 2. Number of anopheline species emerged from different breeding habitats of Borigumma, Malkangiri and Khairput (Bonda Hills) PHCs

Pit	Irrigation pool	Paddy	Well field	Pond	River	Stream	Species	SI. No.
5	2	0	Transmission and the cost resides an investment contents and an investment of the cost of the cost of the cost	73	10	5	A. aconitus	Į,
(0.24)	(0.16)		(0.04)	(0.59)	(0.2)	(0.07)		
107	60	100	16	1147	405	471	A. annularis	2.
(5.19)	(4.7)	(4.01)	(0.57)	(9.3)	(7.7)	(6.6)		
89	28	270	464	353	2993	1802	A. culicifacies	3.
(4.32)	(2.2)	(10.8)	(16.48)	(2.9)	(57.1)	(25.5)		
24	1	154	75	144	189	1234	A. fluviaulis	4.
(1.6)	(0.08)	(6.18)	(2.66)	(1.2)	(3.6)	(17.4)		
10	19	1	0	35	10	5	A. philippinensis	5.
(0.48)	(1.49)	(0.16)		(0.28)	(0.2)	(0.07)		
11	10	15	9	48	30	59	a. varana	5.
(0.53)	(0.79)	(0.6)	(0.32)	(0.39)	(0.57)	(8.0)		
0	0	12	8	19	27	238	A. maculatus	7.
		(0.48)	(0.28)	(0.15)	(0.5)	(3.4)		
20	1	414	26	154	114	823	A. jeyporiensis	8.
(0.97)	(0.08)	(16.6)	(0.92)	(1.2)	(2.2)	(11.0)		
69	62	173	137	536	129	165	A. rugerrimus	9.
95	24	87	207	705	314	121	.4. barbirostris	10.
875	994	237	742	7321	330	595	A. subpictus	1).
317	43	748	940	973	495	467	A. vagus	12.
53	13	13	3	395	22	68	A. jamesi	13.
0	0	*	9	* 3	1	2	A. ramsayi	<u>i</u> 4.
371	0	170	117	304	84	579	A. splendidus	15.
ο	0	***************************************	15	4	36	337	A. theobaldi	10.
0	0	25	()	3	\$	‡7	A. tessellatus	17.
0	0	0	0	ij	0	6	A. majidi	18.
16	16	52	55	130	49	52	A. pallidus	19.
0	0	9	0	ð	t)	5	A. sergenti	20.
1796	1152	1522	2216	10371	1461	2440	Non vectors total	
(87.09)	(90.49)	(61.1)	(78.7)	(84.0)	(27.8)	(34.47)		
2062	1273	2491	2815	12344	5239	7077	Grand total	AND PROPERTY OF

^{*} All the non-vector species combined together; Figures in parentheses denote percentages.

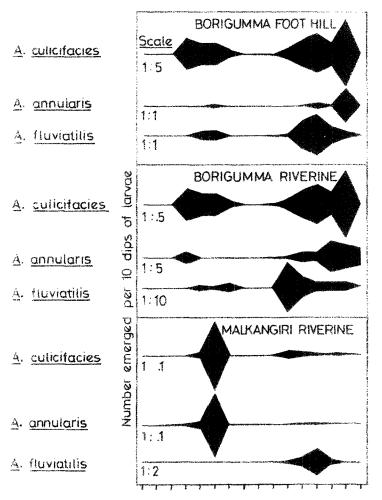


Fig. 2: Breeding of three vectors in river from different terrain.

culicifacies, A. fluviatilis at the different altitudes (Fig. 2).

There were 18 anopheline species breeding in ponds, majority of them being, non-vectors (Table 2). Among the vector species A. annularis was predominant over A. fluviatilis and A. culicifacies and accounts for 9.29% of total anophelines. Vector breeding in ponds occurred throughout the year with a peak during postmonsoon or cooler months (November to February). This was due to increase in the water level following the rains and growth of vegetation. There was no difference in seasonality in

breeding of vectors in ponds in different altitudes. As already stated, ponds are not found in higher altitudes.

Fifteen anopheline species were recorded from wells. A. culicifacies (16.5%) was the predominant vector species breeding in wells followed by A. fluviatilis (2.7%) and A. annularis (0.57%). The breeding of A. culicifacies was higher during rainy season showing a peak in the month of August-September. Similar trend of breeding in wells was observed in all types of terrain. It may be mentioned that in other habitats breeding was low in rainy months due to flushing away of

larvae while the water in wells was undisturbed and hence breeding was higher in these months.

Seventeen species were recorded from paddy fields. In this habitat also, A. culicifacies (10.8% of the total) was the predominant species next to A. vagus. A. fluviatilis and A. annularis were also found breeding (6.18% and 4.01% of the total) Since rice cultivation is mainly rainfed, contribution of paddy fields to vector breeding is restricted to the months of June to February. The larval density reached a peak during August-September (rainy months) and during cold season.

Transient breeding habitats like rain water pools, hoof marks and borrow pits also support anopheline breeding during post rainy and cold seasons. These habitats were found to predominantly support the non-vector species (90.5%). However among the vector species A. culicifacies (3.75%) dominated over the other two vectors.

The study shows that the vectors of malaria in Koraput district viz., A. fluviatilis, A. annularis and A. culicifacies breed in all types of breeding habitats in varying proportions. The preferred breeding habitats of A. fluviatilis were streams and terraced rice fields. Peak density of the species was found during cooler months in all habitats.

A. culicifacies preferred to breed in river, streams, wells and paddy fields. The higher density of this species was found during winter and summer months in river and streams while it was during monsoon in wells and rice fields. Breeding of this species also depends upon the altitude. At higher altitudes it is negligible but at lower altitudes it breeds in higher numbers.

A. annularis preferred to breed in ponds with association of vegetation with a peak density during post-monsoon period. There is no difference

in the seasonality of the breeding of A. annularis in different terrain and altitude.

The study shows that the perennial water bodies like streams, ponds and wells are the important breeding habitats supporting vector breeding throughout the year. Among these streams are the most important and wherever they are present perennial transmission is observed.

Seasonal breeding habitats, particularly terraced paddy fields increase the density during the rainy and post rainy months, the period of peak malaria incidence. Since these habitats cover vast areas, antilarval measures will be ineffective and impractical. The vectors in this area are refractory to control with residual spray. In this situation, attempts should be made to test other suitable methods of control such as use of insecticide impregnated bed nets, curtains, slow-release repellents etc., which can be implemented with active community participation.

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Influence of Some Growth Promoting Substances on Multiplication of *Plasmodium falciparum in vitro*

N.K. SUTAR1 and D.M. RENAPURKAR1

A number of growth promoting substances used in plant tissue culture were investigated for their effect on cultures of malaria parasites. In all, seven such growth promoting substances were employed. Out of these, only three substances, namely indoleacetic acid, indolebutyric acid and gibberellic acid improved *P. falciparum* growth in vitro.

INTRODUCTION

Several workers have used growth promoting substances like hypoxanthine (Zolg et al., 1982) to improve P. falciparum multiplication in cultures. Besides hypoxanthine a number of substances are being employed to improve cell multiplication in plant and animal tissue culture. The effect of these substances viz., 3-indoleacetic acid (IAA), 3-indolebutyric acid (IBA), gibberellic acid (GA) etc., on P. falciparum multiplication has not been investigated by any worker so far. As we have been striving to establish cultures of P. falciparum in vitro and improve parasite multiplication we considered it worthwhile to evaluate the effect of some of these substances on P. falciparum in vitro.

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¹Department of Zoonosis Haffkine Institute Parel Bombay 400 012, India.

MATERIAL AND METHODS

Incorporation of growth promoting substances

Except GA, the material was incorporated at the concentration of 2μg/lit in the medium. For GA the concentration used was 5μg/lit. The following plant hormones/chemicals obtained from Hi-Media Laboratories Pvt. Ltd. (India), Bombay were investigated.

- (a) 3-Indoleacetic acid (IAA)
- (b) 3-Indolebutyric acid (IBA)
- (c) Gibberellic acid (GA)
- (d) 2-4, Dichlorophenoxy-acetic acid
- (e) a-Naphthylacetic acid
- (f) Meso-inositol
- (g) 4-Nitrophenyl phosphate

Preparation of solutions

Except for 3-IBA all solutions were prepared in double distilled water (DDW). In the case of 3-

*BA two drops of dimethyl formide (DMF) were added to attain complete dissolution. These solutions were filtered through 0.22 µm millipore filter and then incorporated in the RPMI 1640 complete medium to achieve the requisite concentration.

In all the above experiments, fresh medium RPMI 1640 was added every 24 hrs by replacing the old medium. Initial parasitaemia was recorded at the time of setting the experiment. The parasite growth was observed by taking smears after 48 and 96 hrs. The experiments were repeated for three 96 hr cycles by adding fresh erythrocytes and bringing down the parasitaemia to 0.1%. Data was worked out on the basis of parasite growth during three cycles.

Erythrocyte suspension

Fresh erythrocytes of A+ group were used for the experimental work for diluting the parasitaemia. The blood was obtained from healthy donors and 5 per cent suspension was prepared in RPMI 1640 complete medium. The erythrocytes stored in citrate phosphate dextrose (CPD) were used within 3-4 weeks of collection and 0.5 ml normal cell suspension was added in each culture dish (35 mm x 10 mm).

Culture of parasites

Parasitized erythrocytes were obtained by collecting 6 mi aliquots of blood in 1 ml CPD by venepuncture from clinically diagnosed cases of *Plasmodium falcipanum* malaria from Malaria Unit, Vashi, New Bombay, Distt. Thane (National Malaria Eradication Programme Unit). The blood samples were collected and brought to our laboratory at Haffkine Institute. The samples were examined, by preparing thick and thin smears and staining the smears with 10% Giemsa. The levels of per cent parasitaemia of the samples were recorded. Five per cent cell suspension was prepared in complete

RPMI 1640 medium. Cultures were set up by dispensing 0.2 to 0.5 ml parasitized cell suspension in each sterile disposable plastic culture dish (35 mm x 10 mm). To this was added 1 ml of complete medium. These plates were then placed in a sterile desiccator (size 160 mm x 255 mm) containing a white candle. The candle was lit and lid was sealed leaving the stop-cock open until the candle extinguished. The stop-cock was then closed and the desiccator was placed in 37°C incubator. This maintained the requisite atmosphere of CO2 inside the desiccator (Jensen and Trager, 1977).

Changing the culture medium

The culture medium was changed every 24 hrs. The old medium was removed from the culture by gently tipping the petri-dish and aspirating off the medium with sterile pasteur pipette. 1.5 ml of fresh medium was added back to each culture dish; cells were resuspended by gentle swirling and plates were replaced in the desiccator. Thick and thin smears were prepared after 48 hrs and 96 hrs. Two plates were set up for each experimental medium consisting of one growth promoting substance. Likewise two plates were set up for control medium RPMI 1640 which did not have any growth promoting substance. Smears were stained with Giemsa and parasite count was determined.

Parasite multiplication rate

Parasite multiplication rate was calculated as per the following formula:

Final Parasitaemia
Initial Parasitaemia

Statistical analysis

Student's unpaired 't' test was applied for statistical analysis of the data pertaining to parasite multiplication.

RESULTS

Parasite multiplication with various growth promoting substances is presented in the following account.

- (a) 3-Indoleacetic acid: When 3-IAA was incorporated in the medium there was distinct improvement in parasite multiplication. The parasite multiplication rate was 790 and 280, where as in the control it was 130 and 170, respectively for *P. falciparum* (Table 1).
- (b) 3-Indolebutyric acid: 3-IBA also increased parasite multiplication in vitro. The parasite multiplication rate was observed to be 1370 and 920 at 48 and 96 hrs respectively (Table 1).
- (c) Gibberellic acid: The use of gibberellic acid produced higher parasite multiplication rate. This can be seen from the data presented in Table 1.
- (d) 2,4-Dichlorophenoxyacetic acid: In the case of 2,4-dichlorophenoxyacetic acid also, we observed improvement in parasite multiplication. The parasite multiplication rates were 200 and 530 for *P. falciparum* (Table 1).
- (e) a-Naphthylacetic acid: Although there was improved parasite multiplication with a-Naphthylacetic acid in comparison to GA and IAA, it was low (Table 2).
- (f) Meso-inositol: The use of meso-inositol in the RPMI 1640 medium did not produce any significant improvement in parasite multiplication as seen in Table 2.
- (g) 4-Nitrophenyl phosphate: As with meso-inositol, there was no improvement in parasite multiplication rate when 4-nitrophenyl phosphate was incorporated in the medium. None of these substances, however, produced any adverse effect on parasite growth in vitro (Table 2).

These findings are summarised in Table 2. From the data, it appears that it could be possible to use IAA, IBA and GA for enhancing in vitro multiplication of *P. falciparum*.

In Tables 3 and 4 percentages of different parasite stages seen in the smears obtained from cultures treated with different growth promoting substances are shown. These tables reveal that the percentage of schizonts was high in cultures carried out with IBA and 2,4-dichlorophen-oxyacetic acid. In case of other growth promoting substances higher percentage of schizonts was observed in the medium containing NAA.

DISCUSSION

Gustafson (1936; 1938) studied the effect of synthetic hormones on fruit formation in plant tissue culture. He tried several substances like indoleacetic acid, indolepropionic acid, indolebutyric acid, n-naphthaleneacetic acid and phenylacetic acid. In his opinion these compounds exerted beneficial effect. Howlett (1943; 1944) sprayed some of the chemicals on the flowers and obtained large parthenocarpic fruits. He tried several substances like indolebutyric acid, indoleacetic acid, napthaleneacetic acid, 2,4-dichlorophenoxyacetic acid etc. Dure and Jensen (1957) reported that gibberellic acid increased cell division and elongation in Gossypium embryos. Addicott (1943) had reported that several substances including vitamins, plant hormones, pyridine and purines are able to bring about stimulation and germination of pollen tubes. In his experiment inositol increased the germination of Milla pollen by 90 per cent while guanine increased the length of the tube upto 157 per cent over that of the control.

Gibberellins stimulate pollen germination and the growth of the pollen tubes as shown by Kato (1955) and Chandler (1957). Steward and Caplin (1951) were able to induce growth in cells of

Table 1. Influence of	some growth promotin	g substances on multiplicati	on of P. falciparum in vitro
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Species	Media	Per cent paras	itaemia <u>+</u> S.D.	Multiplic	ration rate
		48 hrs	96 hrs	48 hrs	96 hrs
P. falciparum	RP - S(M) + IAA 2μg/lit	0.79 ± 0.12*	0.28 <u>+</u> 0.07	790	280
	$RP - S(M) + IBA 2 \mu g/lit$	1.37 ± 0.19°	0.92 <u>+</u> 0.12	1370	920
	$RP - S(M) + GA.5 \mu g/lit$	0.92 <u>+</u> 0.12	0.80 + 0.14	920	800
	$RP \cdot S(M) + 2,4-Dich. 2\mu g/lit$	0.20 <u>+</u> 0.04	0.53 <u>+</u> 0.10*	200	530
	RP - S(M) Control	0.13 ± 0.04	0.17 <u>+</u> 0.05	130	170

n = 2; Initial % parasitaemia -Pf- 0.10%; IAA = 3-Indoleacetic acid; IBA = 3-Indolebutyric acid; GA = Gibberellic acid; 2,4-Dich. = 2,4-Dichlorophenoxyacetic acid.

Table 2. Influence of some growth promoting substances on multiplication of P. falciparum in vitro

Species	Media	Per cent paras	sitaemia <u>+</u> S.D.	Multiplic	eation rate
		48 hrs	96 hrs	48 hrs	96 hrs
A falciparum	RP - S (M) + a-NAA 2μg/lit	0.18 + 0.07	0.67 <u>+</u> 0.19	180	670
	RP - S (M) + Meso-ino. 2 µg/lit	0.17 <u>+</u> 0.05	0.32 ± 0.12	170	320
	RP - S (M) + 4 NPP 2µg/lit	0.19 <u>+</u> 0.12	0.27 <u>+</u> 0.05	190	270
	RP - S (M) Control	0.20 ± 0.14	0.32 + 0.12	200	320

a=2; Initial % parasitaemia . Pf - 0.10%; a-NAA = a-Naphthylacetic acid; Meso-ino. = Meso-Inositol; 4-NPP = 4hitrophenyl phosphate; Experimental values are compared with the control values; Student's unpaired 't' test.

potato tuber by providing them a mixture of 2,4-dichlorophenoxyacetic acid and coconut milk. Although these growth promoting hormones are shown to exert beneficial effect on plant tissue culture, no such data are available to measure their effect on animal cells. Considering the growth stimulating nature of these substances their effect on *P. falciparum* growth in vitro was assessed. It was observed that of the substances employed, 3-indoleacetic acid, 3-indolebutyric acid, gibberellic acid and 2,4-dichlorophenoxy-

acetic acid definitely improved parasite multiplication in vitro. There was 3 to 4 fold increase in parasitaemia in cultures maintained with IAA, IBA, GA and 2,4-dichlorophenoxyacetic acid. Other growth promoting substances viz., anaphthylacetic acid, meso-inositol, 4-nitrophenyl phosphate did not produce any significant increase in parasite multiplication. However, none of these substances produced any adverse effect on parasite growth. It may be worthwhile to recall here that hypoxanthine has been shown

Experimental values are compared with the control values; *indicate significant difference p<0.05; Student's unpaired 't' test.

Table J. Per cent stages parasitaemia at 48 and 96 hours

Media		jaker (Per cent stages parasitacmia ± S.D.	Sitacmia + 5.U.		
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	Rings	Trophozoites	Schizonts		Rings Trophozoites	Schizonts
RP-S (M) + IAA 2µg/lit	73.00 ± 1.41	27.00 + 1.41	0,00	00.00 ± 00.69	28.50 ± 0.70	2.50 ± 0.70
RP-S (M) + IBA 2 µg/lit	73.50 ± 2.12	24.50 ± 0.70	2.00 ± 1.41	72.00 ± 2.83	24.00 ± 2.83	4.00 ± 0.00
RP-S (M) + GA Sµg/lit	72.50 ± 2.12	26.50 ± 2.12	1.00 ± 0.00	68.00 ± 2.83	31.50 ± 2.12	0.50 ± 0.70
RP-S (M) + 2,4-Dich. 2 µg/lit	73.50 ± 4.95	26.50 ± 4.95	0.00	63.50 ± 0.70	34.50 ± 0.70	2.00 ± 0.00
RP-S (M) Control	64.50 + 0.70	34.50 ± 0.70	1.00 ± 0.00	71.50 ± 0.70	24.00 + 1.41	4.50 ± 2.12

Table 4, Per cent stages parasitaemia at 48 and 96 hours

Media			Per cent stages parasitaemia ± S.D.	rasitaemia ± S.D.		
		48 hrs	erenene en e	TO A	% hrs	es establishes de la seguina de la companya de la c
	Rings	Trophozoites	Schizonts	Rings	sajozoudorj	Schizonts
RP-S (M)+a-NAA 2 µg/lit	74.00 ± 5.66	25.00 ± 4.24	1.00 ± 1.41	64.50 ± 2.12	26.50 ± 2.12	9.00 ± 0.00
RP-S (M) + Meso- inositof 2 µg/lit	64.00 ± 5.66	35.00 ± 5.66	1.00 ± 0.00	74.50 ± 0.70	24.00 ± 1.41	1.50 ± 2.12
RP-S (M) +4-NPP 2 µg/lit	76.50 ± 2.12	23.00 ± 2.83	0.50 ± 0.70	66.50 ± 4.95	32.00 ± 4.24	1.50 ± 0.70
RP-S (M) Control	77.50 ± 3.54	22.50 ± 3.54	+ 00:00	65.00 ± 1.41	29.00 ± 2.83	6.00 ± 1.41

n = 2; a-NAA = a-Naphthylacetic acid; 4-NPP = 4-Nitrophenyl phosphate; Experimental values are compared with the control values.

to improve parasite multiplication in the case of P. falciparum in vitro (Zolg et al., 1982).

The growth obtained by incorporating indoleacetic acid, indolebutyric acid and gibberellic acid is comparable to the growth obtained with the incorporation of hypoxanthine by Zolg et al. (1982). It will be interesting to study the effect of these substances on long-term culture of P. falciparum.

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Plasmodium falciparum— Chloroquine In vivo Test in Northeast India: Reclassification and Extended Follow-up till Day 14

A.P. PANDYA¹, B.N. BARKAKATY¹, M.V.V.L. NARASIMHAM² and P.K. MAHAPATRA¹

In vivo test for the susceptibility of Plasmodium falciparum to chloroquine was carried out in 390 patients. Parasite density was analysed by calculating the mean of each day's parasite count and the value was expressed per μ I of blood. Majority of sensitive and resistant infections attained minimal values on day 4. Further more, this value provides a clear-cut, statistically significant difference between sensitive and RI cases (Z = 2.14, P < 0.05) RII, RIII cases (Z = 3.86, P < 0.001). It is proposed that assessment of sensitivity should be based on D-4 parasite density per μ I of blood considering D-0 as base i.e., (proportionate reduction of parasite density on D-4 as compared to that on D-0) utilising the present criterion. In endemic areas, where reinfection is possible, patency on D-14 will nearly always be due to resistance. It is therefore, suggested that a better, more economical and an epidemiologically viable result can be obtained by extension of in vivo test upto D-14.

INTRODUCTION

Delay in clinical response to chloroquine in *Plasmodium falciparum* malaria was first noticed in 1957. *P. falciparum* resistance to chloroquine was confirmed in parts of Thailand during 1961. Subsequently, reports appeared in literature from Cambodia. Malaya and Vietnam (WHO, 1973). A resistant strain of *P. falciparum* was first detected at Manja, Assam (Sehgal et al.,

1973) and subsequent studies detected several foci from northeastern and other parts of India.

Drug resistance was defined by WHO in 1965 and the *in vivo* technique has been standardized (Bruce-Chwatt, 1981) which classifies resistance in *Plasmodium falciparum* infections as follows, (i) Sensitive (S)—if no asexual parasites are found on day 6 and day 7 and parasites do not reappear by day 28 (ii) Resistant at the RI level—if asexual parasites disappear for at least two consecutive days but return and are present on day 7 or if reinfection can be excluded, asexual parasites recrudesce within 28 days (iii) Resistant at the RII level—if asexual parasitaemia does not clear, but is reduced to 25% or less of the pre-treatment level during the first 48 hrs of treatment (iv) Resistant at the RIII level—if

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¹Regional Office for Health and Family Welfare "Felli Ville", Lumsohphoh Shillong-793 014, India.

National Malaria Eradication Programme
 22-Sham Nath Marg
 Delhi-110 054, India.

Table 1. Statewise distribution of P. falciparum monitoring studies

States	No. of	Study	No. of		Sensitiv	vity status*	
	studies	period	cases	S	RI	RII	RIII
325807	10	1986-88	227	122	79	15	11
Azunachal Pradesh	1	1988	25	11	7	3	4
Meghalaya	3	1987-88	58	41	17	0	0
∀izoram	1	1988	20	9	5	3	3
West Bengai	1	1985	60	27	21	12	******
Total	16		390	210	129	33	1.8

^{*} Chloroquine administered @ 25mg/kg body weight.

Table 2. Distribution values of asexual P, falciparum parasitaemia on day θ

Test results	No. of cases	Parasita	emia/ µl of blood
	Lasus	Mean parasite count on Day 0	Standard deviation
5 (28 days)	210	23260	27623
% (early recrudescence)	129	22665	33352
RII	33	28667	31207
RIII	18	37996	18345

Note: Sensitive-MPCT = 2.86 days; RI-MPCT = 3.15 days; MPRT = 10.72 days.

Table 3. Mean asexual parasite count per \$11 of blood

Sensitivity status	No. of cases	D-0	D-1	D-2	D-3	D-4	D-5	D-6	D-7
5	210	23260	7645	1257	206	7	24		·
10	29	22665	9645	2154	533	78	78	260	2629
Mi.	13	38667	16872	6366	4069	1354	2433	3730	5488
RIII	18	37966	33637	31827	21298	12815	9127	8748	11312

reduction of the asexual parasitaemia is less than 75% during the first 48 hrs or it remains at the same level or continues to rise.

Extended in vivo test may provide a precise classification of all cases. However, RI infections are divided into early and late recrudescence without specific differentiation criteria. The present study is an attempt (i) to devise a compatible, feasible and economically viable methodology for quantification and gradation of P. falciparum sensitivity status to chloroquine; (ii) to simplify in vivo test and to suggest a basis for management of complicated P. falciparum cases.

MATERIAL AND METHODS

In vivo tests carried out in 390 patients during 1985-88 in northeast India were analysed. The distribution of test cases according to state and year has been shown in Table 1. All age groups and both sexes were included in the study. The cases were selected as per WHO standard methodology and Dill and Glazko technique (Lelijveld and Kortmann, 1970) was adopted to detect the presence of chloroquine in urine before and after drug administration. Chloroquine was administered @ 10 mg/kg body weight on day 0 (D-0) and day 1 (D-1) and subsequently @ 5 mg/kg body weight on D-2. Blood smears of all selected cases were taken consecutively on D-0 to D-7, thereafter on D-10, D-14, D-21 and D-28.

The smears were stained with 3% Giemsa for 30 mins and the parasites were counted against 300 WBC. Parasitaemia was calculated per μ 1 of blood as number of asexual parasites per 7500 WBC. In all studies, blood slides were double checked (at Shillong and NMEP Directorate, Delhi). The parasite count taken in the present study is based on results of the cross-checking laboratory at the Directorate of NMEP, Delhi.

Test results were classified according to WHO rules with the following amendments: RI cases

with patent recrudescence before or on day 14 were considered early recrudescence. Out of 41 cases with parasitaemia on day 21, 28 were not taken up as in field conditions reinfection could not be ruled out. When parasitaemia was absent from D-5 to D-28 such cases were considered as sensitive. Proportionate reduction was calculated as a percentage for every day studied, considering D-0 as base. Appropriate statistical tests were applied whenever necessary.

RESULTS

The distribution of initial parasitaemia value (D-0) for test results, classification, Malaria Parasite Clearance Time (MPCT) and Malaria Parasite Recrudescence Time (MPRT) for sensitive and RI cases is shown in Table 2. A graph of S, RI (early recrudescence), RII and RIII was constructed using the arithmetic mean of respective days (Fig. 1 and Table 3). Standard error of mean (SEX) for the distribution of parasitaemia on individual days was less than 1.96 limit suggesting firm conformity with normal distribution.

In sensitive and RI cases parasitaemia attained the lowest mean value on day 4. On this day, all sensitive cases were negative, although a few were positive on day 5 with microscopically undetectable parasitaemia. Even the RIII cases typically suffered a marked reduction of parasitaemia to around 66% of initial value on day 4 (Table 3). The comparison of S versus RI cases on D-4, showed a significant difference in clearance of parasitaemia at 5% level (Z = 2.14, P < 0.05) whereas, it was insignificant on D-2 (Z = 1.85, P > 0.05). Similarly, RII versus RIII on D-2 and D-4 showed a highly significant difference as on D-4 (Z = 3.86, P<0.001) as compared to D-2 (Z = 3.09, P < 0.01).

Table 4 shows distribution of percentile reduction of parasitaemia on D-1 to D-4, as a proportionate reduction percentage of day 0 parasitaemia for S, RI, RII and RIII cases. On day 3, 77% (162/210) of S cases were negative,

Table 4. Discribution of percentile reduction of parasitaentic on Day 3 to 4 compases to D-6 values

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	ļ	L.	210	129	33	RIII 18
		Sensi- tivity	s	X	RII	R

^{*} Reduction was more than 99 per cent; N = No. of cases.

Table S. Comparison of RI in light of D-14 (early recrudescence, ER) and D-28 (ER + late recrudescence, LR) observations

Type of	No. of	MPC	.C.I.	Σ	MPRT				Q.	arasite re	Parasite reappeared on	i on			
test	cases	а	e ga Andrea The control translated by the physician through	E	and the second s	0.5	D-5 D-6 D-7 D-10 D-14 D-17 D-21	D-7	D-10	D-14	D-17	D-21	D-5 D-6 D-7 D-10 D-14 D-17 D-21 D-28 (D-2) + D-28	D-28 (D-2) + D-28	+ D-28
D14 (ER) 129 3.15 10.72 3 6 19 48 53 1 27 13 (2) 41	129	3.15	of for another payment regions of chickens of the second s	10.72	energianje verken i kirkenderkanski	A A A A A A A A A A A A A A A A A A A	9	51	48	53	and and	TT	emparaterization on a security to	(2)	1
							58.19%			41.09%					
D28 (ER + LR) 170		3.20	2.79	10.84	23.24										

^{*} Reached 6 parasitaemia on day 5.

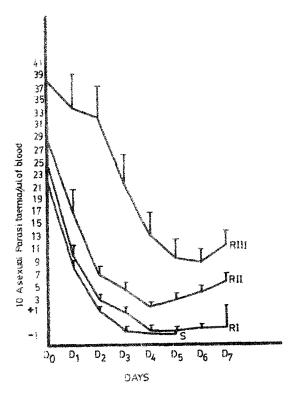


Fig. 1: Mean and Standard error of the mean of parasitaemia for days 1 to 7 of P. falciparum in vivo tests with chloroquine 25 mg/kg, grouped according to test result classification. The value —1 corresponds to parasitaemia = 0.

thereby distinguishing themselves from RII and RIII cases, but not from RI. On D-4, 99.96% of S infections were negative (only 7 cases had 0.04% parasitaemia). Similarly, 92.25% of RI cases were also negative. Distribution of parasitaemia as percentage of D-0 values on D-2, reflect that S infections distinguish themselves from RIII first on D-2, showing in 93% (196/210) cases a reduction of more than 75 per cent.

Table 5 shows comparison of D-14 (early recrudescence) and D-28 extended study data for RI cases. All 41 cases reached zero parasitaemia [on D-2 (16), D-3 (13), D-4 (10), D-5 (2)] and the parasite reappeared in 68.29% cases on D-17 ± 21 where as in 31.71% cases on D-28.

It is very clear from the data available on spray coverage, that in general less than 80% room coverage was achieved, giving less than 40% or less total sprayable surface coverage, leading to uninterrupted transmission. Hence the possibilities of reinfection are more in such situations in 28 days extended studies.

As per WHO classification RIII is present, if asexual parasitaemia remains above 25% of the pre-treatment level during the first 48 hrs of treatment. As per definition, D-2 will be 48 hrs from D-0 with only 20 mg/kg body weight chloroquine dose and D-4 will be 48 hrs after completion of chloroquine dosage @ 25 mg/kg body weight. On the contrary, if the same cases are followed upto D-4, 50% of these RIII cases (as on D-2) are found to be RII cases, based on their parasitaemia reduction on D-4 as compared to that on D-0.

When these same RIII cases (18) were followed upto D-7 then only 3 cases (16.67%) were found to be RIII, 66.67% and 16.66% came under categories of RII and RI, respectively.

DISCUSSION

The *in vivo* test has two limitations, which are inevitable in field work in endemic areas. Even when the technique is cent per cent correct, the result is found to be influenced by the host's immunity and there are difficulties in distinguishing recrudescence from reinfection. However, *in vivo* test is more helpful as compared to *in vitro* test, for gradation of resistance, which may provide a tool for formulation of treatment schedules.

Upto day 7, after standard treatment, chloroquine blood concentrations prevent multiplication of sensitive *P. falciparum* erythrocytic forms (Fadeke Aderounmo et al., 1980). Similar findings have been reported by Brohult et al., (1979) amongst non-immunes. Bruce-Chwatt (1985) stated that, *P. falciparum* usually

becomes patent in the blood stream after 2-4 days of growth. Thus, the only sensitive parasites that can become patent on day 14 are those released from the liver on days 8-12. Molineaux and Gramiccia (1980) while working on Garki project reported that, in holo-endemic areas, the parasitological conversion rate is rarely above 0.01 per day, so frequencies of patency on D-14 significantly above 5 x 0.01 must be ascribed to tesistant infections (Schapira et al., 1988).

The results presented in the present paper clearly suggest that, pre-treatment (D-0) parasite density is not an important determinant of

the in vivo test result. Similar findings have been reported from Mozambique (Schapira et al., 1988). Though Payne (1982) suggested that, different materials could be compared by the slopes of the geometric mean parasitaemia curves of the first day, definition of the slope may be difficult and the decline may not be linear.

On D-4, most infections whether sensitive or resistant, reached minimal parasitaemia as compared to D-0, suggesting that relative parasitaemia on D-4 would be a sensitive measure of the efficacy of standard chloroquine therapy.

Table 5. Gradation of RIII (on WHO criteria) on cut-off at D-2, D-4 and D-1 = D-17 (overall)

Days		Sensitivity			
	RI	RII	RII		
Day 2	in davine rings step, tills, steps i sente om i spenningstytte blev på stepse om i de sindere de op met en ste Indame	од с съд в с тод вы СПВ (СПВ ССВ). С се	18 (100%)		
Day 4	*200°C	9 (50%)	9 (50%)		
Overail D-1 - D-7	3 (16.66%)	12 (66.67%)	3 (16.67%)		

No. of cases = 18.

Table 7. Recommended grading of P. falciparum resistance

Response	Recommended symbol	Evidence
Sensitive	S	If no asexual parasites are found on day 6 and day 7 and parasites do not reappear by day 14.
Resistant	RI	If asexual parasites disappear for at least two consecutive days but are present on day 7 or if asexual parasites recrudesce before day 14.
	RII	If asexual parasitaemia does not clear upto day 7, but is reduced to 25 per cent or less of the pre-treatment level on day 4.
	RIII	If asexual parasitaemia does not clear upto day 7, but the reduction of asexual parasitaemia is less than 75 per cent on day 14, or if it remains at same level or if it continues to rise.

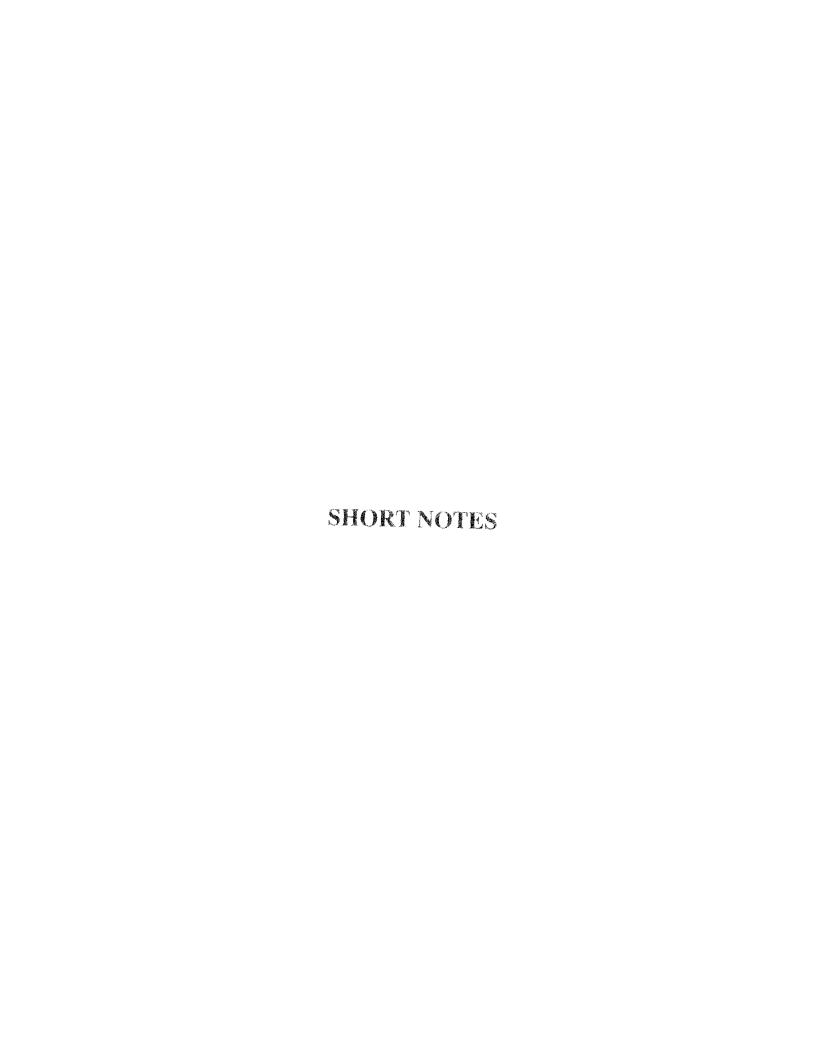
In RIII infections mean parasitaemia value (X) typically showed a reduction of 15.98% on D-2 as compared to that on D-4 i.e., 66.25% (Table 3). However, examination of individual cases reflected that 50% of RIII as on D-2 converted into RII when followed upto D-4, therefore, the D-4 parasitaemia as percentage reduction of that on D-0 would be a more sensitive means to separate resistant infections (RIII) from partially (RII) resistant ones (Table 6). Similar conclusions have also been drawn by Schapira et al. (1988), in their study. The existing criterion may be useful for differentiation of sensitive cases from resistant ones since 99% of sensitive infections showed more than 75% reduction on D-2 as compared to D-0 (Table 4). However, a change of treatment is indicated in those cases, where the patient is severely ill and continues to show a parasitaemia above 25% on day 2 of that on day 0. Similarly, it is acceptable to change treatment if a patient, has both symptoms and asexual parasites on day 4 without a marked reduction (below 75% as on D-4). Parasitaemia will be present only in 0.04% of sensitive cases. Schapira et al. (1988) reported, that at 95% confidence limits, the frequency of patency on day 4 was 0-7% for S infections.

The present findings suggest that, better, more reliable and statistically significant results can be obtained based on D-4 parasite density by adopting the above classification (Table 7). It is rather difficult to predict from parasite density data of first 4 days whether a case will prove to be sensitive or RI, as the resistant parasite in RI will be at very low levels which may remain undetected for a long time. To rule out possible reinfection in field conditions in an area where perennial transmission is maintained by vectors

like A. minimus and A. dirus, in vivo tests should be followed-up till day 14 only.

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Mosquito Fauna of Medical Importance in Kumbakkarai: a Tourist Spot near Madurai (Tamil Nadu)

T. JOHN VICTOR¹, K. VENKATARAMAN¹ and K.G. SIVARAMAKRISHNAN¹

Kumbakkarai, a part of Palani hills, lies in the Western Ghats in Tamil Nadu. People from different parts of Tamil Nadu visit this place throughout the year. The increased incidence of mosquito-borne diseases is often observed in conjunction with the migration of people from disease endemic areas. Probability of disease incidence correlates with the abundance of vector species present in the area, since the density of vectors determines the degree of contact between man and vector (Rao, 1984). Apart from the studies of Christophers (1933) and Barraud (1934), mosquito fauna of foothills of Western Ghats in Tamil Nadu were also studied by Measham and Chowdhuri (1934) in Anamalais, Russell and Jacob (1942) in Nilgiris and Tewari et al., (1987) in hill ranges such as Agastya, Varusha Nadu, Elamalai, Palani, Anamalai and Nilgiris.

The present study aims at updating the knowledge of distribution of vectors in relation to their number in this area.

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The collections were confined to 'Kumbakkarai' area (11°N and 77° 50°E), situated about 10 kms from Periyakulam Taluk, Madurai District. It lies on the eastern slopes of Palani Hills, a part of Western Ghats in Tamil Nadu, at an elevation of 450 metres above mean sea level. A perennial stream cascades down the 'Kumbakkarai falls'. The rainfall ranges trom 175 to 210 cms./year which is mainly influenced by the Northeast monsoon (November-January). There are human inhabitations on the banks of the down stream which flows through Periyakulam town. Due to extensive deforestation in this area the natural forest has been replaced by plantations and roads.

Mosquito immatures were collected from different breeding habitats such as slow flowing streams, rocky and sandy pools on the streambed, irrigation channels, tree holes and other stagnant waters and were brought to the laboratory for identification. Outdoor resting adult mosquitoes were collected by using an aspirator. Four such surveys were made from October 1989 to March 1990. The larvae were reared to the adult stage and were identified on the adult characters.

A total of 20 species, belonging to 4 genera and 10 sub-genera were collected in the present sur-

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

Lable 1. Mosquito species collected in Kumbakkatal area

S. S.	Species collected	Rocky	Sandy	(rrigation channe)	Stagnant	Stream (slow flowing)	Tree	Outdoor resting	Total	% in total collection
	Anopheles (Anopheles) aitkenii James.	antendo 	engana managing pipelan dani i nama dana s	manage of the set of t	And the Commence of the Commen	3		Frank 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	3	0.4
ći	Anopheles (Anopheles) barbirostris Van der wulp.		vulenda		Pile anno	,t	1	vilope	#mont/	6.14
ιń	Anopheles (Anopheles) pediaeniaius (Leicester)	1804-1904	Arrage	From From	;	;	i	Taken 1	17	2.5
4	Anopheles (Cellia) culicifacies Giles	2 - 20 - 20 - 20 - 20 - 20 - 20 - 20 -	91	Эv	proof.	82	;	ì	3	9.3
s,	Anopheles (Cellia) theobaldi Gites	~	*****	l	į	2 0	:	i	35	~~
9	Anopheles (Cellia) vagus Donitz	14	Š	,	;	91	1	:	8	ণ) শ্ব
7.	Anopheles (Cellia) varuna lyengar	•	1	22		Ñ	į		rod V)	7.3
တ	Aedes (Adimorphus) vinatus (Bigot)	106	ļ	3	:	de es	:	1	106	15.6
6	Aedes (Finlaya) pseudotaeniatus (Giles)	Ŗ		:	:		* * * * * * * * * * * * * * * * * * * *		¥	4.9
10.	Aedes (Stegomyia) aegypti (Linnaeus)	1.5	** **	Hones	***************************************	:	•	!	SI	7.
_ i	Aedes (Stegomyia) albopicuts (Skuse)	1 200			;	i	₹ 3°	į	4	0.5
12.	Culex (Culex) bitaeniorhynchus Giles	42	***	j	17	112 **	***	į	171	24.8
13.	Culex (Culex) mimulus Edwards	3/2	ţ	ayees	of freedy	91	•	;	95	13.6
14.	Culex (Culex) pseudovishnui Coliess	м	:	40	i	ì	!	;	x 0	1.2
15.	Culex (Culiciomyia) nigropunctatus Edwards	w.	į	i	;	ž	i	:	35	5.05
16.	Culex (Culiciomyla) pallidothorax Theobald	₹.~.	į			j	:	*	ţ.,	10.1
17.	Culex (Eumelanomyia) khazani Edwards	3	į	·	region o	and the state of t		1	14	2,02
90	Culex (Luzia) fuscanus Wiedemane	vs.			:	2 8		٠	46°3	ć. <u>.</u> 0
19.	Uranotaenia (Uranotaenia) unnandalet Barraud	;	ŧ	t 2			3	9	ð	٥.9
20.	Uranotaenia (Uranotaenia) campestris Lewestes	•		;	:	ग्रंथ	ì	ş	Ŋ	2.3
The local Alles	Total	Control of the contro		1	and the state of t				692	

*Adults captured in one occasion; **Inclusive of adults reared from egg rafts collected.

vev. Of the total collection, 25.4% were anophelines and 74.6% were culicines. An. barbirostris, An. peditaeniatus, An. culicifacies, An. theobaldi, An. vagus and An. vanina were also collected in the present survey which were not recorded in Palani Hill ranges in the previous survey done by Tewari et al., (1987) though their collections were carried out in both eastern and western slopes of Palani Hills. Non-occurrence of these 6 spp. in their survey and the absence of An. barbumbrosus. An. gigas, An. lindesayi, An. elegans, An. jeyporiensis, An. maculatus and An. moghulensis in the present survey may be due to seasonal variation. An. aitkenii was collected in low number (1.7% among the anophelines) and An. barbirostris was represented by the lowest number of specimens in the total collection (0.14%). 4n. varuna, a vector of minor importance, was also collected in sizable numbers (7.3%). In addition, other vector species such as Culex pseudovishnui, a JE vector (1.2%) and Aedes aegypti, a dengue fever vector (2.1%), were also observed. Details of mosquitoes collected are given in Table 1.

In India, An. culicifacies is the main vector of malaria in rural and peri-urban areas responsible for 60-70% malaria cases throughout the country (Subbarao, 1988). Of the total collection, An. culicifacies constitutes 9.3% and it was collected mainly in the downstream area. The number of An. culicifacies was higher in the two early surveys done during October and December 1989. This shows seasonality in its abundance.

Measham and Chowdhuri (1934) in Anamalai hills, Covell and Harbhagwan (1939) in Wynaad, Russell and Jacob (1942) in Nilgiris and Rao (1945) in North Kanara obtained 26.7%, 8.8%, 11.9%, 5.0% of An. fluviatilis in their collections, respectively in the pre-DDT era, whereas Brookeworth (1953) in Hassan District and Tewari et al., (1987) in the six hill ranges of Western Ghats in Tamil Nadu obtained only 2.1% and 0.9% of the same species, respectively

in the post-DDT period. In the present survey the total absence of An. fluviatilis may be due to extensive deforestation for transportation and agricultural purposes, it has been replaced by An. culicifacies, a rural malaria vector. Similar trends have also been reported by Sharma et. al., (1984) in the U.P. Terai, and Nagpal and Sharma (1986) in Orissa.

Though the number of An. culicifacies specimens collected from different habitats of Kumbakkarai are appreciable, the incidence of malaria has not been reported so far, according to the Tamil Nadu Public Health Authorities at Periyakulam (personal communication). As suggested by Tewari et al. (1987) vigilance could be maintained in future in this area, which is a tourist place, and hence more susceptible to the occurrence of imported malaria.

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Studies on Genetic Polymorphism in relation to Malaria in Tea Gardens of Dibrugarh district, Assam

R.S. BALGIR and L.P. DUTTA 1

With the establishment of the tea industry in Assam around 1840, many economically weak people from other states of India such as West Bengal, Bihar, Orissa, Madhya Pradesh, Andhra Pradesh and Tamil Nadu migrated to Assam in search of employment in the tea industry. They were mostly tribals who joined as tea planters or tea leaf pluckers.

This study deals with the tea garden tribal population of Barbaruah, Bokel, Dikom, Mattack, Nudwa and Singlijan Tea Estates of Dibrugarh district, Assam. An enquiry into their migratory history revealed that the forefathers of most of the peoples under study belonged to the states of Orissa and Madhya Pradesh. Further, this is a mixed population of various tribal groups that migrated from different states and can collectively be called the tea garden population.

For the present study, about 2 ml of blood was drawn by venepuncture from each individual. Blood samples of both sexes were randomly

collected from the tea garden population in Dibrugarh district, Assam during January to September 1988. The blood samples that were collected in vials containing ethylene diamine tetra acetic acid (EDTA) were transported under wet ice-cold conditions to the laboratory at Dibrugarh town and all the tests were performed within 24 hrs of collection.

Tests for A₁A₂BO, Rhesus (subtype) and MN blood groups were performed by the glass concavity technique (Bryant, 1982); Glucose-6phosphate dehydrogenase (G-6-PD) deficiency was determined by fluorescent spot screening and heterozygote females were confirmed by methemoglobin reduction. Abnormal haemoglobins were determined by performing sickling test and electrophoresis on cellulose acetate strips following the standard technique (Dacie and Lewis, 1984). As no female was found G-6-PD deficient, the gene frequency calculations were made on male subjects only. For other genetic markers, the gene frequencies were calculated as per the standard guidelines (Mourant et al., 1976).

The distribution of genetic markers in the tea garden population of Dibrugarh district is given in Table 1. The frequency of blood group B is the highest, followed by O and A_1 in the tea

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¹Regional Medical Research Centre N.E. Region (ICMR) Post Box No. 105 Dibrugarh-786 001, India.

Vable 1. Distribution of genetic markers in the tea garden population of Dibrugach district, Assam

Genetic markers	No.	%	Gene		Frequency
14A2BO blood groups			and the second s	or of the second control of the second contr	- endere i uz milio dinga disele kart
A4	26	23.6			
13	ġ.	2.7	P_{j}		0.180
AjB	10	3.1	p_2	2	0.028
1 <u>5</u> 8	3	1.8	4	=	0.249
(\$	36	32.7	Ł	22	0.543
)	33	30.0		r yns gol I ^{man} effeddiwydaid y la Mhondi ys arann w	Annual and a company of the second
Yotal	110	99.9			
Thesus blood groups					
CCDe	71	64.5			
-Cdc	1.	0.9			
CcDe	30	27.3	CDe	-24	0.790
:DE	2	1.8	cDE	27	0.018
cDe	5	4.5	eDe	===	0.113
Aic .	1	0.9	ede	ALL STATES OF THE STATES AND ADDRESS OF THE STATES AND ADDRESS AND	0.079
foral	110	99.9		na agusto "NOT FRANCISCO A III de la Seculto de la Comunidación de la	
MN blood groups					
M	30	31.6			
. V	18	18.9	ពរ	:=	0.564
MN	47	49.5	33	The second secon	0.436
Total	95	100.0			
G-6-PD deficiency					
∵ 3d +	149	93.7	Gd+	22	0.93
Э с -	10	6.3	Gd-	P for Prints	0.063
Total	159	100.0		arrayi valibles Militaryyumasa	
Haemoglobin (Hb) types			. . .		
A.A.	292	86.9			
AS	43	12.8	A	**	0.933
55	1	0.3	S		0.067
i'otai	336	100.0			

planters. Blood group A₂ (2.7%) and A₂B (1.8%) were also encountered. The genotype CCDe is most common, followed by CcDe and cDe in the tea garden population (Table 1). The frequency of M, N and MN blood groups has been observed to be 31.6%, 18.9% and 49.5% respectively.

Out of 159 subjects tested for G-6-PD deficiency, ten cases were found deficient for this enzyme (Table 1). Sickle cell haemoglobin was detected both in heterozygous (12.8%) and homozygous (0.3%) form. This is the first comprehensive genetic polymorphism study carried out in the tea garden population of Dibrugarh district, Assam. Earlier studies were sporadic and piecemeal (Flatz et al., 1972; Deka, 1984; Das and Deka, 1985).

The distribution of genetic traits studied in the tea garden population (Table 1) reveals several distinguishable features like the distribution of blood groups A₂, B, N and sickle cell haemoglobin.

The relatively higher frequency of B seen in the present study conforms to the general observations for the Indian region (Mourant et al., 1976) and also with those for the local Caucasoid populations studied from Assam (Das and Deka, 1985). Az gene is rare or absent in the northeastern region of India (Balgir and Dutta, 1990; Deka, 1984), but has been encountered in the present study with a gene frequency of 0.028.

The frequencies of CDc (0.790), cDe (0.113) and cDE (0.018) haplotypes observed in the present study are distinct from those observed by earlier workers for other sympatric populations. (Bhattacharjee, 1975; Kumar and Sastry, 1961; Deka, 1984; Bhattacharjee, 1968). Further, the observed frequency of CDe conforms to the results of Papiha et al. (1988) for tribal populations of Orissa.

In comparison to the other northeast Indian populations both Mongoloid and Caucasoid so far studied, where the frequency of m gene varies from 0.77 to 0.89 (Deka, 1984), the frequency in the present study is the lowest (0.564) in the tea garden population.

The incidence of G-6-PD deficiency (6.3%) in the present population falls within the range so far observed for the populations of Assam (Balgir, 1989).

Abnormal hacmoglobin E and S are prevalent in the northeastern region of India. The prevalence of sickle cell haemoglobin in the present study (13.1%) is lower than the values of 15% and 29% reported by earlier investigators (Dunlop and Mazumdar, 1952; Batabyal and Wilson, 1958). This is due to the fact that the previous studies were hospital based and the present one is strictly a random survey conducted carefully to know the exact prevalence of abnormal haemoglobin in the tea garden population. Further, although the prevalence of sickle cell haemoglobin (around 40%) is quite high in the home states (Orissa and Madhya Pradesh) of these migrated tribal people (Balgir and Sharma, 1988), but, keeping in mind their pathetic migratory history, it may be presumed that the persons severely afflicted with haemoglobinopathy either could not migrate or cope with the prevalent conditions in Assam and being vulnerable and unfit were naturally eliminated. Moreover, depending upon the size of migrating population the founder effect might have contributed significantly to limit the dispersal of abnormal haemoglobin.

In the present study, sickle cell haemoglobin has been encountered in the heterozygote form in all but one of the 43 cases. Persons homozygous for sickle cell haemoglobin are not viable and die early in childhood. As a result of this, a large number of genes are selectively destroyed in each generation by sickle cell anaemia (Serjeant,

1985). Genetically, the heterozygotes enjoy some selective advantage over normal persons and are thought to be differentially maintained in the population because individuals with this phenotype are less likely to succumb to *P. falciparum* malaria and consequently survive to contribute genes to the next generation (Serjeant, 1985; Bunn and Forget, 1986).

Dibrugarh district of Assam is malaria endemic with high incidence of *Plasmodium falciparum* malaria. A house to house survey done in the study area during the reported period in a population of about 3000 for the detection of fever cases revealed that out of total 546 blood slides collected, 88 cases were of malaria, of which 68 had *P. falciparum* and 20 had *P. vivax* miection, showing a slide positivity rate (SPR) and slide falciparum rate (SfR) of 16.1% and 12.5%, respectively (Dutta et al., 1989). In populations having gene for haemoglobin-S and andemic for malaria, the incidence of sickle cell heterozygotes is generally high which is also true in case of the present study.

this interesting to note that the tea garden tribat people who migrated to Assam about one and half century ago and are living in close proximity with the surrounding Mongoloid and Caucasoid populations (possessing distinct genetic markers including abnormal haemoglobin-E) are without any apparent gene flow and have retained their distinctive genetic identity. This testifies to their complete biosocial isolation from the neighbouring populations and, probably, their favourable eco-biological adaptation in the malaria endemic terrain.

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Letter to the Editor

Transfusion Malaria in Operated Neurosurgical Cases: A Report of Two Cases

Sir—Malaria is a rare complication of blood transfusion, reported in 0.2-50 per million blood transfusions (Bruce-Chwatt, 1974). The true incidence is difficult to estimate as there is little data from the endemic areas. Statistics from the United States, even with their strict blood bank rules, had indicated a rise in such incidence (Guerrero et al., 1983). Though there is no large published series from India, Mahapatra and Banerji (1989) had described their experiences of transfusion malaria in operated neurosurgical cases. In their study the serum antimalarial antibody levels were not determined and hence the diagnosis was presumptive, based on clinical picture and therapeutic response to chloroquine. In the present report transfusion malaria (TM) was diagnosed clinically and confirmed by measuring antimalarial antibody levels by enzyme immunoassay.

Case No. 1

A 6-year old boy was admitted to our neurosurgical unit with clinical evidence and radiological diagnosis of fracture edontoid, four months following a fall from a height. He had no past history of malaria. Routine investigations revealed low haemoglobin, 8.3 gm%, X-ray of cervical spine revealed fracture base of the odontoid with atlanto-axial dislocation. Twelve days after admission to our unit, the patient was operated under general anaesthesia. Posterior fixation of occiput and C2 vertebra spinous process was done using stainless steel wire and bone graft from the iliac crest. Intra-operative blood loss was about 300 ml and he received one unit of blood transfusion. Postoperatively, the patient was well for 5 days, thereafter he developed fever, ranging from 102°F to 103°F. The temperature usually started at 1400 hrs, reached a peak by 1800 hrs and then slowly subsided by 2200 hrs. It continued for 3 days and all routine investigations were normal, wound was healthy and there was no evidence of meningitis, urinary tract infection (UTI) or thrombophlebitis. Because of spiky fever the possibility of malaria was considered. A blood sample was tested for malarial antibody and the patient was prescribed chloroquine. His temperature came down to normal within 36 hours and remained normal thereafter (Fig. 1). When the sera was tested for the antibodies to circumsporozoite protein (CS) of Plasmodium falciparum and Plasmodium vivax using immunodominant repeat sequences viz., (NANP)4 and (GDRADGQPA)2 respectively, strong positive reactivity was observed (1.4 and 0.9 OD respectively) for both the antigens. A positive reaction for Pf and Pv circumsporozoite antigen is defined as an optical density (OD) exceeding the mean +5 SD of the 20 control sera $[0.4 + 0.075 \times 5 = 0.775]$ (De Virgillis et al., 1981). The patient had a quick recovery and was discharged on the 10th post-operative day.

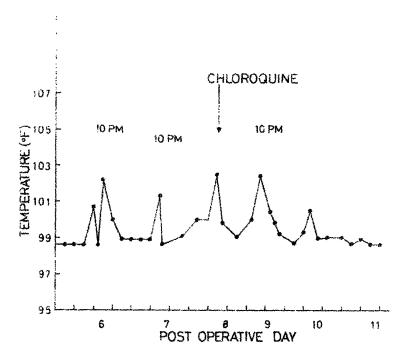


Fig. 1: Temperature chart of Case No. 1 showing spiky temperature which subsided after starting chloroquine.

Case No. 2

A 24-year old female was admitted to the neurosurgery ward on 5.12.1988 with clinical features suggestive of syringomyelia. There was no history of malaria in the recent past. Routine investigations were normal. She was operated 2 days after admission. Under general anaesthesia, plugging of the obex ventriculo-subarachnoid (IV ventricle) shunt was performed. She received 300 ml of blood transfusion during operation. Twenty four hours after surgery she developed high grade fever. The temperature ranged from 101°F to 104°F and was noticed during 1400 hrs to 1800 hrs, however throughout the day low grade temperature did persist. As she was on a indwelling urinary eatheter, UTI was suspected, which was confirmed by urine culture. Urine culture grew E. coli which was sensitive to gentamycin, Nalidixic acid and Amikacin. She received gentamycin 80 mg I.V. 3 hourly. As there was no response, she was started on Nalidixic acid 1 gm orally 6 hourly alongwith capsule cephaxin 500 mg 6 hourly. With the above combination the temperature came down to 101°F. Three days later temperature rose again and went upto 104°F. At this stage all the investigations were normal, which included total and differential leucocyte count, peripheral smear for malaria parasites and blood cultures. Chest X-ray was normal and CSF excluded the possibility of meningitis. Sera were tested for antimalarial antibody as described before. She was prescribed chloroquine 600 mg to start 300 mg after 6 hours and then 150 mg twice daily for next 2 days. The temperature came down after 48 hours of the first dose of chloroquine and within next 24 hours, she became afebrile. Her sera showed strong positivity for malarial antibodies with CS peptide antigens of both Plasmodium falciparum and Plasmodium vivax (1.04 and 1.4 OD, respectively). She was discharged from the hospital on 20th day following surgery.

Fever in the postoperative period is quite common (Ajao, 1978) and on first two days, is supposed to be due to tissue reaction to surgical trauma. Fever persisting beyond 2nd day could be the result of infection and require investigations to establish the cause of fever. In a number of patients all the investigations may be normal (Mahapatra and Banerji, 1989), and surgeons are left with no choice-but to start with empirical antibiotics, which is expensive and sometimes harmful. In such a situation awareness of TM may be helpful.

In our first case, the patient was afebrile for 5 days and he developed fever on 6th day, appearing on every afternoon. All routine investigations were normal. In patients with TM, the incubation period is relatively short and could range from 24 to 48 hours to several weeks. Our 1st case was in the hospital for 12 days prior to surgery and is unlikely to be in incubation period. His temperature pattern, response to chloroquine and strong antibody positivity to circumsporozoite antigens of Pf and Pv proved beyond doubt, the diagnosis of TM.

In the 2nd case, fever started on the first postoperative day. As the patient was on urinary catheter, it was logical to think of the possibility of UTI, which was proved by microscopic examination of urine, turther supplemented by urine culture. However, failure to respond to the appropriate antibiotics did raise the possibility of another infection. Multiple infections are well known in the postoperative period. Unless one is aware of this entity it may be overlooked, because of the presence of an overt infection. In the 2nd case, fever did persist even with combinations of proper antibiotics, and it was only a course of chloroquine therapy that brought the temperature down to normal. The antimalarial antibody study, which was obtained later, revealed strong positive reaction, both for Plasmodium falciparum and vivax CS peptide antigens which is an indicative of recent infection, thus further confirming the diagnosis of TM.

This highlights the possibility of TM in operated neurosurgical cases and an awareness of this condition among surgeons will prevent unnecessary use of antibiotics and reduce the cost of treatment.

Departments of Neurosurgery and Biochemistry All India Institute of Medical Sciences New Delhi - 110 029, India. A.K. MAHAPATRA D.N. RAO

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Announcement

International Conference on Malaria Control

29 to 31 August 1991 at the Institute of Fundamental Studies, Kandy, Sri Lanka

Organizers: Professor Ranjan Ramasamy and Dr. Lionel Samarasinghe

The Conference

This three-day regional conference on malaria control will assess the current state of research and future trends in this field and discuss possible collaborative research programmes in the region.

Date and Venue

29 to 31 August 1991 at the Institute of Fundamental Studies, Kandy.

Purpose

Malaria is a serious health problem in South and Southeast Asia with many countries spending a significant proportion of their budget on malaria control. Recent years have seen advances in understanding the immunology of malaria, vaccine development, the molecular mechanisms of drug resistance, vector physiology and vector taxonomy.

This conference will bring together local and regional scientists working on different aspects of malaria control and selected experts in the field, with the objective of assessing the current state of research and future trends. It is anticipated that the conference will lead to new collaborative research programmes within the region being established.

Programme

The conference will be divided into three eight-hour sessions on 1. Entomology, 2. Biology and Immunology of *Plasmodia*, and 3. Clinical Aspects and Drug Resistance. Besides the lectures during the plenary sessions of the morning there will be an opportunity for shorter 15-minute presentations in the afternoons of each session for the participants.

Provisional topics include:

- 1. Entomology: Sibling species of Anopheles culicifacies; Integrated vector control; Novel methods of mosquito control; Insecticide resistance
- 2. Biology and Immunology of Plasmodia: Drug resistance; Transmission-blocking immunity and immunity to Plasmodium vivax; Immunity to asexual stages of the malaria parasite; Human antibody response in malaria; Synthetic vaccines
- 3. Clinical Aspects and Drug Resistance: Drug resistance; Molecular mechanisms of drug resistance; Epidemiology of malaria; Immunopathology of malaria

Provisional Speakers

S. Subbarao—Malaria Research Centre, Delhi; M.B. Wickramasinghe—Anti-Malaria Campaign, Colombo; M.S. Ramasamy—Institute of Fundamental Studies, Kandy; P. Wilairat—Mahidol

University, Bangkok; Y. Yuthavong—Mahidoi University, Bangkok; R. Ramasamy—Insutute of Fundamental Studies, Kandy; D. Rao—All India Institute of Medical Sciences, New Delhi; L. Samarasinghe—Anti-Malaria Campaign, Colombo.

Participation

Those who wish to participate in the conference should send their applications before 30 March 1991 to:

The Coordinator
Conference Division
The Institute of Fundamental Studies
Hantana Road
Kandy (Sri Lanka)

Tel: 094-8-32001 Tix: 21700 IFS CE Cable: FUNDAMENTAL Fax: 094-8-32131

The application should include a brief curriculum vitue and the official address with telex/telephone numbers. Those who wish to present papers at the conference should send a 250-word abstract of the paper.

The Institute of Fundamental Studies may be able to arrange air tickets through sponsors and provide local accommodation for selected scientists who present papers at the conference. Applicants seeking financial support for travel, etc. should provide details of their budget.

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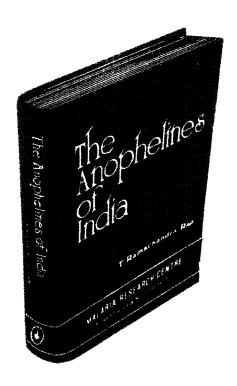
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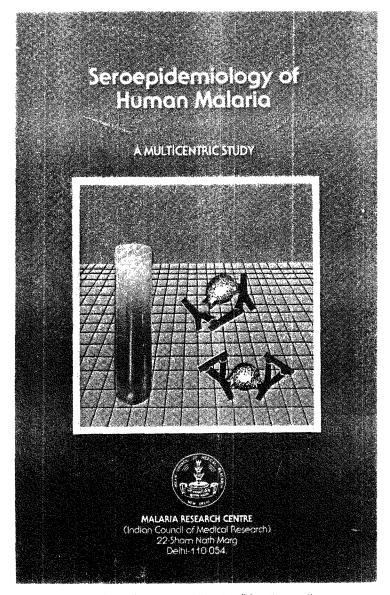
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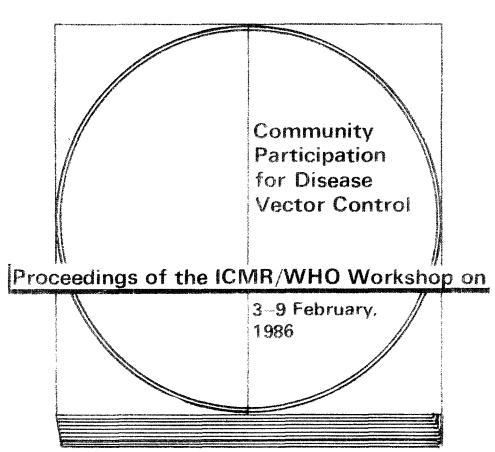
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