INDIAN JOURNAL OF MALARIOLOGY

Volume 28 **Number 1**

March 1991

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

INDIAN J. MALARIOL.

Quarterly

Year of revival: 1981

SUBSCRIPTION RATE

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

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

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

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

INDIAN JOURNAL OF MALARIOLOGY

INDIAN JOURNAL OF MALARIOLOGY

CONTENTS

Volume 28
Number 1
March 1991

Short Notes

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

Indian Journal of Malariology
Vol. 28, March 1991, pp. 1-8.

Antimalarial Effect of Cyclosporin-A on Murine *P. berghei* and Human *P. falciparum*

S. BISWAS*, Q.B. SAXENA* and M. UPENDER*

The effects of Cyclosporin-A (CsA) on the growth of *Plasmodia* were investigated in an experimental murine model *in vivo* and on human malaria *in vitro*. Mice were inoculated with *Plasmodium berghei* and then treated with different doses of CsA at the patent period. The development and course of this normally lethal parasitaemia in mice was affected by treatment with CsA which is a known immunosuppressant. The drug showed complete protection at a dose of 20 mg/kg wt/day without any recrudescence. Antibody level was at the detection limit after first bout of drug-cured infection. CsA was found to be an inhibitor of *P. falciparum* growth in a dose dependent fashion, as the concentrations of drug in culture medium increased, a significant reduction in parasitaemia was observed.

INTRODUCTION

Cyclosporin-A (CsA) is one of the metabolic products of a fungus named *Tolypocladium inflatum*. The active component of CsA is a cyclic undecapeptide with a distinct antilymphoidal activity. The immunosuppressive effect of this chemical moiety was first experimented in a number of mammalian species including human[1]. The antimalarial activity was established by sheer serendipity[2]. It has been shown by earlier studies[3] that in malaria, protective immunity may be evolved with close associations of T and B lymphocytes, so the idea was to establish the course of infection in murine malaria by depressing the immune system with CsA, surprisingly the workers[2] noticed the opposite effect of the component by delaying or preventing the parasitaemia. This observation threw light on the new role of CsA.

Present studies were conducted *in vivo* in a group of mice infected with *P. berghei*, and in a few isolates of *P. falciparum in vitro* with various doses of CsA. Mice were treated with CsA during patency and varying results were obtained for controlling the parasitaemia. *P. falciparum* was grown *in vitro* in presence of the drug, and the rate of proliferation of parasite was monitored. The aim of this study was to evaluate the efficacy of CsA at different concentrations for treating lethal *P. berghei* infection in mice during patent period, to establish the curative dose of the drug in murine model without impairing the immune system, and to determine the level of inhibitory effect of CsA in a group of Indian *P. falciparum* isolates *in vitro*.

Accepted for publication: 23 November 1990.

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

MATERIAL AND METHODS

Mice: Balb/c mice, 6 to 8 weeks of age were taken for the study. Animals were given regular pellet diet from Hindustan Levers Ltd. and water was provided *ad libitum*.

Plasmodia: *P. berghei* (K 175) was obtained from NICD, Delhi and maintained in our laboratory by regular *in vivo* passage in mice. *P. falciparum* isolates were collected from persons naturally infected with malaria. These isolates were grown, adapted and maintained *in vitro* by the method described earlier[4].

Parasitaemia: Parasitaemia levels were determined from JSB-stained blood smears. The number of parasitized erythrocytes in 1 x 10^3 RBC was taken for calculation of per cent parasitaemia.

Table 1. Course of parasitaemia in mice infected with *P. berghei* and treated with cyclosporin-A (CsA)

No. of batches & drug doses	Days of infection, % Parasitaemia ± SD												
	Initial parasitaemia	D-1	D-2	D-3	D-4	D-5	D-7	D-8	D-9	D-11	D-12	D-14	D-16
I 1 mg/kg wt/D	0.35 ± 0.20	3.24 ± 2.15	0.37 ± 0.25	0.32 ± 0.18	0.52 ± 0.08	0.92 ± 0.71	10.6 ± 6.46	28.0 ± 8.37	All died				
II 5 mg/kg wt/D	0.74 ± 0.42	2.16 ± 0.90	N	N	N	N	N	N	N	1.0 ± 2.24	3.08 ± 6.66	10.0 ± 5.54	0.28* ± 0.17
III 10 mg/kg wt/D	0.26 ± 0.10	2.94 ± 1.37	N	N	N	N	N	N	N	6.0 ± 13.42	2.0 ± 4.47	2.5 ± 4.45	N*
IV 20 mg/kg wt/D	0.35 ± 0.14	3.26 ± 1.42	N	N	N	N	N	N	N	N	N	N	N
V 50 mg/kg wt/D	0.41 ± 0.32	3.0 ± 1.17	N	N	N	N	N	N	N	N	N	N	N
VI 100 mg/kg wt/D	0.31 ± 0.15	3.0 ± 1.58	N	N	N	N	N	N	N	N	N	N	N
Control group. No drug given	0.73 ± 0.34	2.9 ± 0.74	7.0 ± 2.55	8.2 ± 2.86	11.2 ± 2.4	13.4 ± 4.2	29.2 ± 12.7	36 ± 13.8	45 ± 15.3	52 ± 10.56	All died		

N = Negative; * = 1 mouse died on 16th day; Parasite inoculated on "0" day — 1 x 10^6 *P. berghei* infected erythrocytes; Drug administered on 5th day at parasitaemia — 2 to 5%(This was taken to be D-1).

Cyclosporin-A: The drug was obtained by the courtesy of Dr J.F. Borel of Sandoz Ltd., Basel, Switzerland. To administer the drug *in vivo* and *in vitro*, it was dissolved in 0.5 ml ethanol, 0.2 ml Tween-80 and rest of the volume was made up by plain RPMI-1640 medium.

Infection and CsA treatment in mice: Groups of 5 mice each in 7 batches were infected by intraperitoneal injection of 1 x 10^6 *P. berghei* parasitized RBCs. When parasitaemia reached about 2-5%, mice were cured intraperitoneally with doses of 1 mg, 5 mg, 10 mg, 20 mg, 50 mg and 100 mg drug/kg wt/day for 5 days, consecutively. Parasitaemia was monitored daily by JSB stained blood smears. One group of mice was kept as control for checking the lethality of infection.

Detection of antimalarial antibody level: Sera from CsA treated mice were tested for antibody detection by ELISA test. Soluble extract of *P. berghei* was used as antigen at a concentration of 40 µg/ml. The antigen-antibody complex was trapped with anti-mouse-Ig conjugated with peroxidase. Normal and immunized sera were taken as negative and positive control.

Effect of CsA on *P. falciparum* growth *in vitro*: Ten *P. falciparum* isolates collected from Distt. Ghaziabad, Shahjahanpur and Jabalpur, were grown in presence of different doses of CsA ranging from 0.125-10 µg/ml, at ring stage. Schizont maturation was monitored for each isolate after 24-26 hours. Per cent growth inhibition and ID 50 level was determined after 44-48 hours.

RESULTS

Effect of CsA on development and course of parasitaemia in mice: Among 7 batches of mice one group of 5 mice was taken as control to monitor the course of *P. berghei*. In this group diluent (ethanol+Tween-80+RPMI-1640) was injected for 5 days in lieu of drug. The other 6 batches of mice were treated with varying doses of drug as mentioned above.

All mice developed parasitaemia of 2-5% on 5th day after inoculation with 1 x 10^6 *P. berghei*. First dose of drug was given on 5th day and this was counted as 1st day after drug. The course of parasitaemia in control and CsA treated group is shown in Table 1. Mice treated with 1 mg/kg wt/day dose initially showed a fall in parasitaemia but drug at this concentration was not effective for controlling parasite growth. Animals died on 9th day due to lethal infection. Apparently 5 and 10 mg/kg wt/day doses were also curative, since parasitaemia was not observed in peripheral blood for 9 consecutive days. Later on parasites started appearing in blood smears and 4 mice died in the group with 5 mg dose and 1 mouse died in 10 mg dose group. Mice treated with 20, 50 and 100 mg/kg wt/day doses were completely cured, no recrudescence was observed till 40 days. The control group of mice died within 10-12 days. The rate of survival observed was 20% and 80% with 5 and 10 mg/kg wt/day doses and 100% with 20, 50 and 100 mg/kg wt/day doses (Table 2). In the drug treated groups there was a rapid fall in asexual parasite stages within 12 hours of receiving a single dose of CsA. Selective reduction of ring forms and schizonts was observed within 12-18 hours and trophozoites disappeared between 21 and 24 hours.

Effects of CsA on humoral immune response: All cured mice were tested for antimalarial antibody after 35 days. Normal and *P. berghei* immunized mice sera were taken for determining positive and negative value. No significant levels of antibody were detected in experimental mice sera after 1:128 dilution. Most of the sera samples had detectable antibody except in the highest dose (100mg/kg wt/day) treated group (Fig. 1), also the antibody level in other groups was at the detection limit after first bout of drug-cured infection.

***In vitro* growth inhibition of *P. falciparum* with CsA:** Aim of this experiment was to establish whether CsA may interrupt completely or partially the growth of *P. falciparum* isolates collected from different parts of India (Table 3).

Table 2. Mortality and parasitaemia of *P. berghei* infected mice after CsA treatment

No. of batches	I	II	III	IV	V	VI	Control
CsA doses for 5 days (mg/kg wt/day)	1	5	10	20	50	100	No drug only diluent injected
Response to drug	Parasit-aemia not controlled	Parasit-aemia controlled upto 10 days	Parasitaemia controlled in 4 out of 5 mice	Parasitaemia controlled No recrudescence upto 40 days.			Fatal infection
Parasitaemia after drug	5/5	5/5	1/5	0/5	0/5	0/5	5/5
Survival	0/5	1/5	4/5	5/5	5/5	5/5	0/5

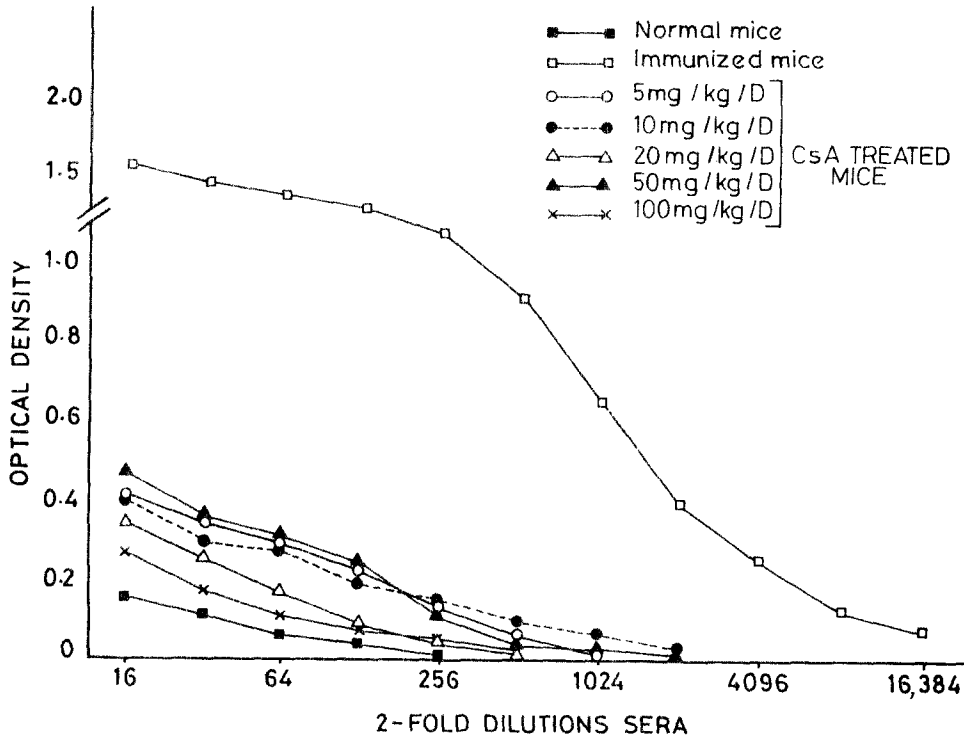


Fig. 1: Antibody profile of CsA treated mice after 35 days.

Table 3. *P. falciparum* isolates taken for the assay (10 isolates)

Sl. No.	Name	Locality
1.	FSJ - A1	From Shahjahanpur
2.	FSJ - A2	
3.	FSJ - A3	
4.	FSJ - A4	
5.	FSJ - A5	
6.	FSJ - A6	
7.	FJB - D2	From Jabalpur
8.	KC 3/13	
9.	PLI - A	From Ghaziabad
10.	G2 - N	

Synchronized culture was started with 1-1.5% ring stage. Eight sets of culture in triplicate were put up for 10 isolates. Seven sets were with varying concentrations of drug (mentioned in methodology) in complete medium and the control set was with complete medium containing drug diluent.

Rate of schizont maturation varied at different concentrations (Figs. 2&3) and per cent growth inhibition profile was also variable. With gradual increase in doses of drug, elimination of parasites by reducing the numbers of healthy stages took place. In all isolates, cultures containing 10 mg of CsA/ml, exhibited an absolute inhibition of growth. Though the maximum inhibitory concentration has been observed at 10 μg of CsA per ml, 50% growth inhibitory dose (ID 50) varied from 0.6-3 μg/ml in 10 *P. falciparum* isolates (Figs. 4 & 5).

DISCUSSION

Malaria and its transmission are sought to be controlled mainly by bioenvironmental control measures[5], by spraying insecticides, and also by chemotherapy. Chloroquine, is used everywhere

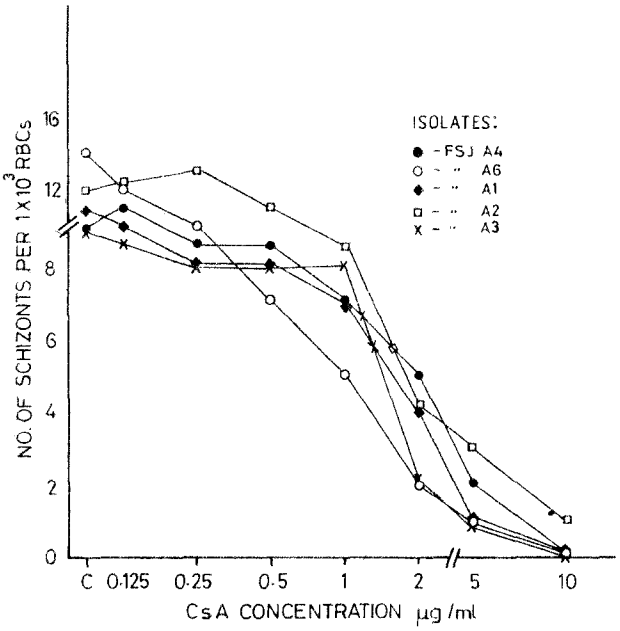


Fig. 2: Number of schizonts present in culture afer 24-26 hours.

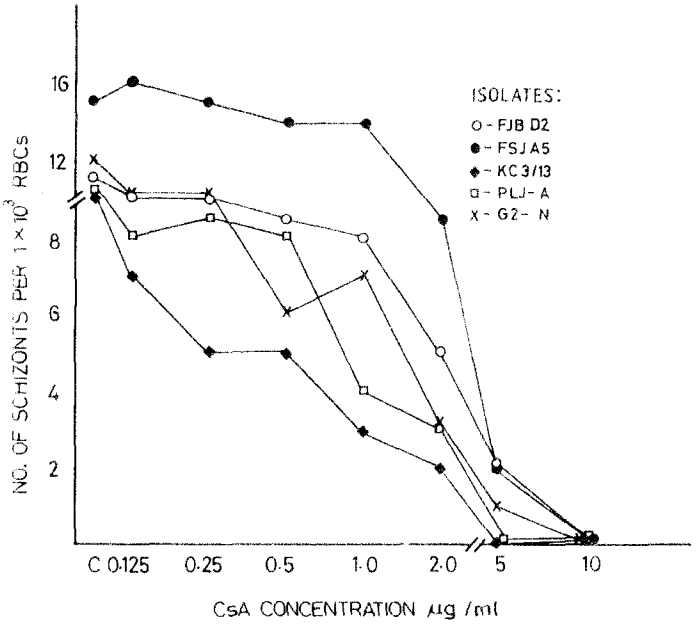


Fig. 3: Number of schizonts present in culture after 24-26 hours.

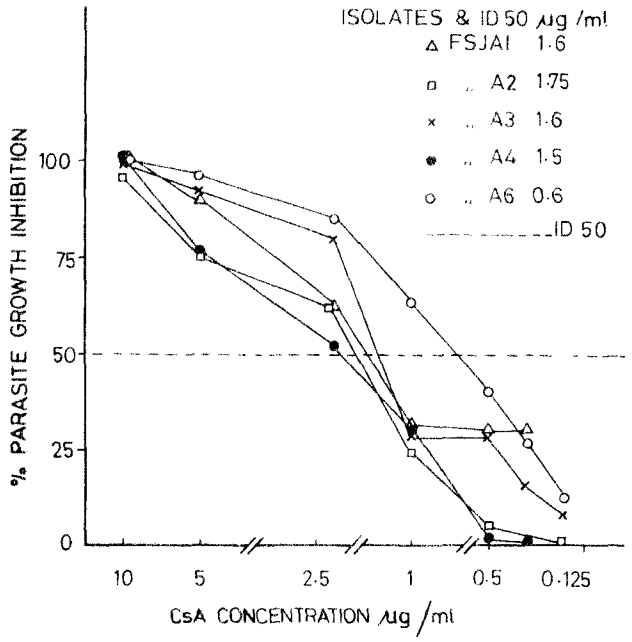


Fig. 4: Cyclosporin-A concentration response and 50 per cent inhibitory dose (ID 50) after 44-48 hours.

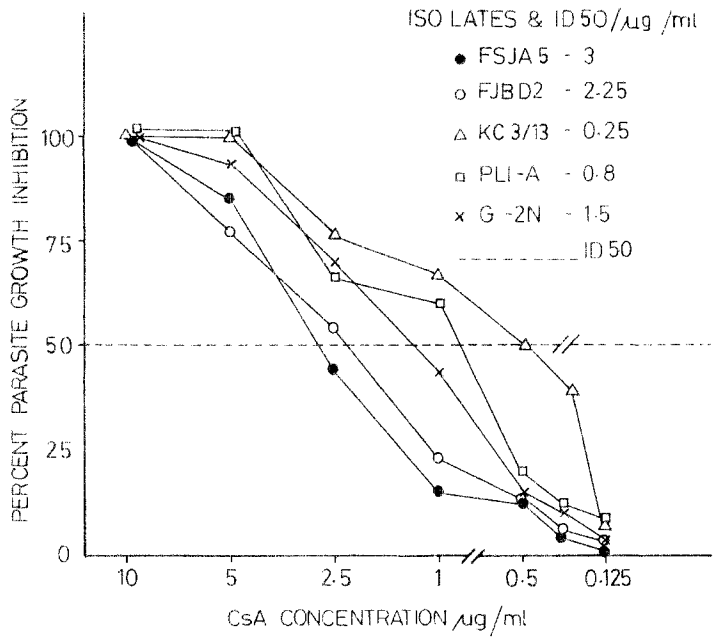


Fig. 5: Cyclosporin-A concentration response and 50 per cent inhibitory dose (ID 50) after 44-48 hours.

for treating vivax and falciparum malaria. Because of the increase in chloroquine resistant cases in *P. falciparum*, new drugs and their mode of action are presently under study.

This study was taken up as a result of the astonishing observation[2] that the immunosuppressive drug CsA caused inhibition rather than potentiation of plasmodial infection in mice. The observations made by the study demonstrated that the antimalarial activity of CsA is a dose restricted phenomenon which does not interfere with antilymphoidal activity during antiparasite episodes.

The antiplasmodial effect of CsA could be a stepwise reduction of ring stages *in vivo* within 12-18 hours of drug administration as observed earlier[6], as a result the number of healthy trophozoite and schizont stages become less or nil, which in turn interrupts the growth cycle. This establishes, the schizontocidal effect of CsA.

Several doses of CsA had no detectable effect on the T-cell dependent polyclonal B-cell activation. In addition, the only mouse that survived after 5 mg/kg wt/day for 5 days showed detectable anti-*P. berghei* antibodies, hence the cytotoxic nature of the drug to the parasite probably exists without disturbing the immune system.

Inhibition of *P. falciparum* growth was observed in a dose dependent fashion, a noticeable effect on schizont maturation was seen in synchronised *P. falciparum* culture with different doses of CsA. Surprisingly, the variable ID 50 level was also noticed in the present study with 10 isolates. Few isolates were susceptible even at a low concentration. *P. falciparum* infection in humans is malignant and develops more complications, if delayed. This also occurs in case of chloroquine resistance. The results of this study have some implications for treatment of malaria patients, especially those with intractable complications.

Though the exact mechanism of parasite-drug interaction and effects on the parasite's metabolism are not yet known, further investigations may be required to study the nature and action of this drug and its derivatives in various experimental models.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. J.F. Borel for his valuable suggestions and also for providing the drug CsA. We wish to thank Mr. A.R. Kotnala for maintenance and monitoring *P. berghei*, Mrs. N.K. Ammini, P. Singhal and Anandi Sharma for technical assistance and Shri Ravikant for blood smear preparation.

REFERENCES

1. Borel, J.F., C. Feurer, C. Magnee and H. Stahelin (1977). Effects of the new antilymphocytic peptide cyclosporin-A in animals. *Immunol.*, **32:** 1017-1025.

2. Nickell, S.P., L.W. Scheibell and G.A. Cole (1982). Inhibition by cyclosporin A of rodent malaria *in vivo* and human malaria *in vitro*. *Infect. Immunol.*, **37** (3): 1093-1100.

3. Jayawardena, A.N., G.A. Targett, R.J. Carter, F. Leuchars and A.J. Davies (1977). The immunological responses of CBS mice to *P. yeolii* I. General characteristics, the effects of the T-cell deprivation and reconstitution with thymus grafts. *Immunol.*, **32:** 849-859.

4. Trager, W. and J.B. Jensen (1976). Human malaria parasites in continuous culture. *Science*, **193:** 673-675.

5. Sharma, V.P., R.C. Sharma and A.S. Gautam (1986). Bio-environmental control of malaria in Nadiad, Kheda District (Gujarat). *Indian J. Malariol.*, **23:** 95-117.

6. Murphy, J.R., S. Baqar, R.H. Baker, E. Roberts, S.P. Nickell and G.A. Cole (1988). Stage-selective inhibition of rodent malaria by cyclosporin-A. *Antimicrob. Agents Chemother.*, **32** (3): 462-466.

Indian Journal of Malariology
Vol. 28, March 1991, pp. 9-18.

Seasonal Prevalence of Anophelines in Kheda District, Gujarat

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

Seasonal changes in the population structure of anophelines were studied in Kheda district, Gujarat. Mosquito collections were made from the villages of Nadiad taluka, representing 3 different physiographic zones. Maximum number of anopheline species (13) were recorded from canal irrigated area followed by 11 species from non-canal irrigated and 8 species from riverine areas. Four anophelines namely *An. subpictus, An. culicifacies, An. annularis* and *An. stephensi* were predominant. High densities of *An. culicifacies* and *An. subpictus* were recorded in March and August. Results of a four year study (1985 to 1988) on the population of anophelines from 3 different physiographic entities showed marked differences in the seasonal abundance of different species.

INTRODUCTION

Information on the population dynamics of mosquitoes and particularly vectors of malaria is necessary for developing control measures. In any given area common mosquito species occur throughout the year but the abundance of any species depends more upon the availability of preferential breeding habitats and survival rates.

The anopheline fauna of Gujarat has been studied by various workers[1,2,3] in Kutch, Kheda, Ahmedabad and Panch Mahals.

There have been major ecological changes in Kheda district after the sixties with the onset of canal irrigation for intensive agriculture in seven out of ten talukas. Several factors associated with canal irrigation like, continuous irrigation, multiple cropping pattern, increased water-logging due to seepage from canals and lack of proper and adequate drainage have resulted in the creation of extensive mosquitogenic conditions. Further, deforestation, rapid urbanization, industrialization and extensive use of insecticides in agriculture and public health may also have contributed towards changes in the ecosystem and in the prevalence of anophelines in the district.

For implementation of any vector control programme it is essential to study the population dynamics of mosquitoes, particularly of vectors in different physiographic zones. Therefore, an attempt was made to evaluate the impact of irrigation projects, physiographic conditions and climate on the population dynamics of anophelines in Nadiad taluka of Kheda district, Gujarat.

Accepted for publication: 11 December 1990.

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

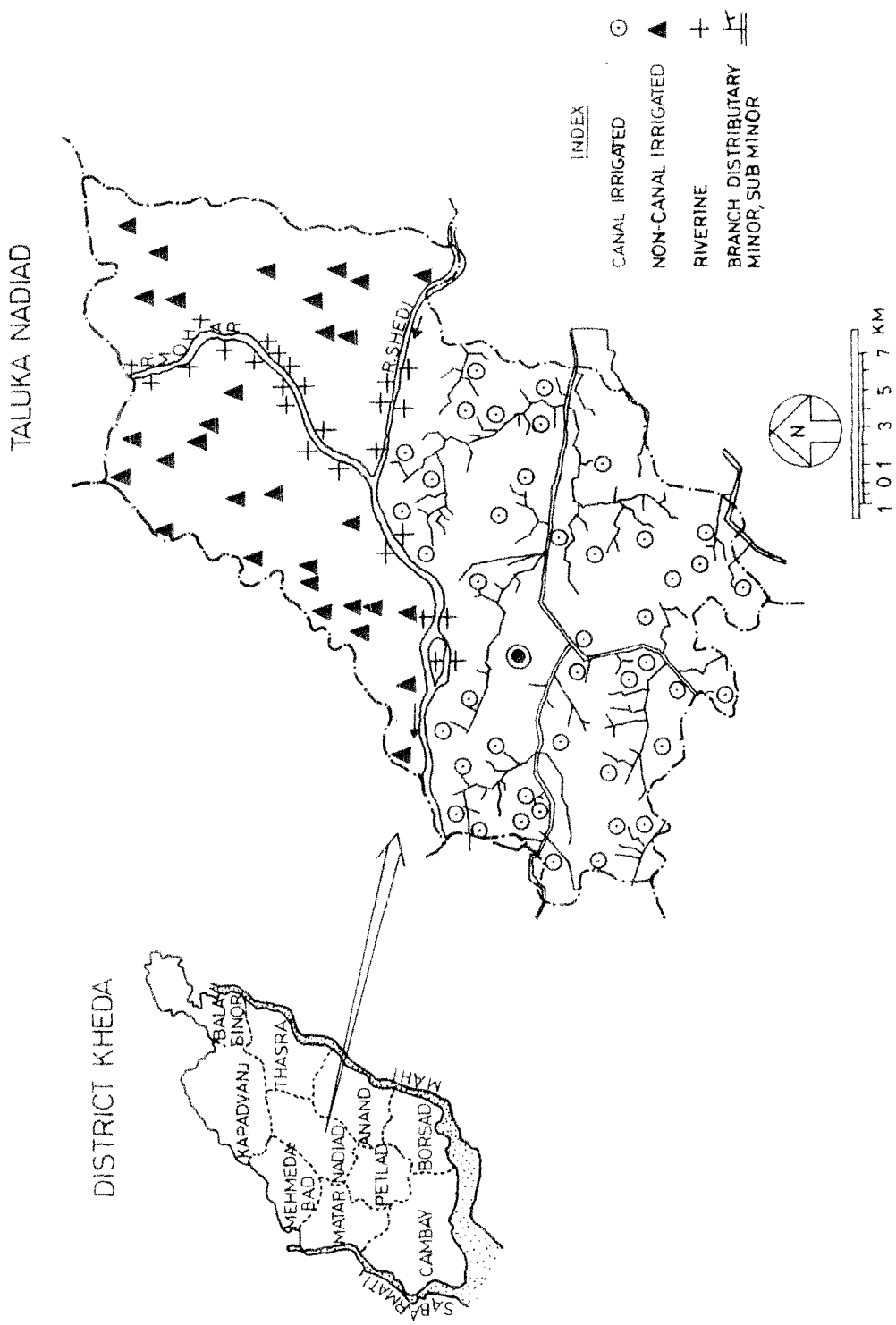


Fig. 1: Location of study area

MATERIAL AND METHODS

Study area

Nadiad taluka of Kheda district in Gujarat state with a population of 3,50,000 living in 100 villages lies between 22° 35' and 22° 54' North latitudes and 72° 46' and 73° 10' East longitudes. Total area of the taluka is 622.32 sq km which is not homogenous in the sense that about half of the area is extensively canal irrigated and the villages of this area are surrounded by a network of irrigation canals distributaries and drainage systems (Fig. 1). Water-logging and seepage from canals create ideal mosquitogenic conditions. In the remaining area agriculture is dependent on wells/tubewells and/or monsoon. In villages of the latter area mosquito breeding sources are scarce in summer. There are two seasonal rivers in Nadiad taluka viz., Shedhi and Mohar. During summer, river Mohar dries up completely whereas excess water through escape irrigation canals is released frequently in Shedhi river.

Forty-three villages in which irrigation was through an artificial channel using the stored water of rivers and lakes were grouped under the canal irrigated area. Thirty-one villages were grouped under non-canal irrigated area where only subsurface water from wells and/or tubewells was used to promote agriculture. Twenty-six villages situated on the river bank or its vicinity from which rain water flows directly in to a nearby river or vice versa were grouped under riverine area.

Breeding sources in canal irrigated area include irrigation canals and channels, drains, seepage water pools, ponds and paddy fields. Water-table is considerably high (up to 2 to 3 meters) due to continuous irrigation. In non-canal irrigated and riverine areas ponds, small pools, river and riverbed pools are the major breeding sources. Wells and intradomestic water storage containers also provide breeding opportunities throughout the year in all the three areas. In the canal irrigated area ponds, pools and seepage water collections are often found infested with aquatic vegetation like *Eichhornia, Hydrilla, Trapa* and *Ipomea.* Major crops include paddy, wheat, millet and tobacco. In canal irrigated villages two crops of paddy are cultivated every year during February-May and July-October. Thus each physiographic area offers characteristic breeding sources to mosquitoes. None of the villages received insecticidal spray during the course of the study.

Climate

Daily observations on maximum and minimum temperature, first and second hour relative humidity were obtained from the meteorological station, Gujarat Agriculture University, Anand. Daily rainfall data were obtained from the district agriculture office, Nadiad. Average of four years monthly mean temperature (°C), relative humidity (%) and rainfall (mm) are plotted in Fig. 2. The normal climate of the area can be divided into three main seasons: the monsoon (mid June to mid October), the fair weather season (mid October to February) and the hot weather season (March to mid June). Mean temperature ranges from 20.41°C in January to 32.76°C in May. Mean relative humidity varies from 43.87% in March to 78.75% in August. Average rainfall of Nadiad taluka is 792 mm with an average of 37 rainy days during a year. The rainfall was maximum (907.5 mm) in 1988 whereas in 1985, 1986 and 1987 rainfall recorded was 533.3, 304.1 and 210.1 mm, respectively.

Mosquito collection

Regular adult mosquito collections commenced from January 1985 from 24 villages (12 canal irrigated, 10 non-canal irrigated and 2 riverine). In 1986 mosquito collections were made from 80 villages (38 canal irrigated, 24 non-canal irrigated and 18 riverine). Collections from 76

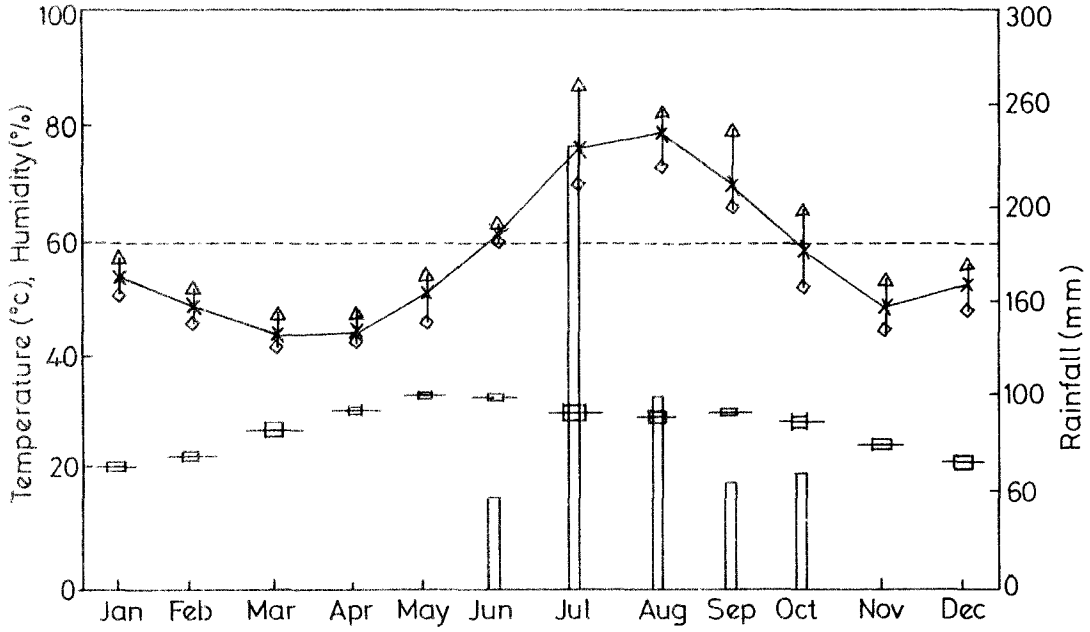


Fig. 2: Showing meteorological data (Average of four years 1985 to 1988).

villages each were made during 1987 (38 canal irrigated, 23 non-canal irrigated and 15 riverine) and 1988 (35 canal irrigated, 23 non-canal irrigated and 18 riverine).

Mosquitoes were collected by hand collection method using an aspirator in the morning hours from 4 human dwellings and 4 cattlesheds for 15 minutes each on a fortnightly basis. Mosquitoes thus collected were brought to the laboratory, anaesthetised with ether and identified using the key of Christophers[4]. Collections of four years from January 1985 to December 1988 were pooled for each month and man hour densities were calculated.

RESULTS

Table 1 gives the per cent composition of anophelines in three physiographic areas. A total of 2,47,453 anophelines comprising of 13 species viz., *An. culicifacies, An. stephensi, An. annularis, An. subpictus, An. barbirostris, An. aconitus, An. varuna, An. tessellatus, An. turkhudi, An. theobaldi, An. fluviatilis, An. nigerrimus* and *An. vagus* were collected during the study period. Canal irrigated area yielded the maximum number of species (13) followed by 11 from non-canal irrigated and 8 from riverine areas. Composition of vector anophelines, *An. culicifacies* and *An. stephensi* was highest in riverine area (10.66 and 0.65%) followed by canal irrigated (5.51 and 0.32%) and non-canal irrigated (1.70 and 0.19%) areas, respectively. Composition of *An. annularis* was highest in canal irrigated area (3.11%) followed by riverine (0.79%) and non-canal irrigated (0.47%) areas. *An. subpictus* was represented highest in non-canal irrigated area (97.62%) followed by canal irrigated (90.99%) and riverine (87.81%) areas.

Table 2 shows the seasonal variations in the densities of anophelines. As only four species i.e., *An. culicifacies, An. stephensi, An. annularis* and *An. subpictus* were encountered during all seasons of the study and other species were

occasionally found, the former have been discussed in detail under the following heads.

Canal irrigated area

Wide fluctuations were observed in the density of anophelines throughout the year with the lowest density (MHD 15.49) recorded in January and highest (MHD 439.16) in August. Density of *An. culicifacies* started to increase from February and reached a peak in March (MHD 22.18) and thereafter started to decline till July (MHD 0.42). The second rise in the density though less pronounced, was associated with the onset of monsoon and it almost stabilized from August to October (MHD 4.27-5.12) and thereafter it further declined to 1.40 per man hour in December. Density of *An. stephensi* remained below 1 per man hour throughout the year with minor fluctuations although it showed a trend similar to that of *An. culicifacies* density. *An. annularis* was abundant during winter and early summer (November to April) with the highest density recorded in

Table 1. Per cent composition of different anopheline species

Species	Canal irrigated area		Non-canal irrigated area		Riverine area	
	No. collected	Per cent	No. collected	Per cent	No. collected	Per cent
1. *An. culicifacies,* Giles, 1901	7519	5.51	1186	1.70	4399	10.66
2. *An. stephensi,* Liston, 1901	434	0.32	130	0.19	270	0.65
3. *An. annularis,* Van der Wulp, 1884	4244	3.11	326	0.47	326	0.79
4. *An. subpictus,* Grassi, 1889	124208	90.99	68022	97.62	36248	87.81
5. *An. barbirostris,* Van der Wulp, 1884	5	0.0036	1	0.0014	—	—
6. *An. nigerrimus,* Giles, 1900	1	0.0007	2	0.0028	—	—
7. *An. aconitus,* Donitz, 1902	36	0.0263	2	0.0028	1	0.0024
8. *An. varuna,* Iyenger, 1924	17	0.0124	—	—	—	—
9. *An. tessellatus,* Theobald, 1901	18	0.0131	4	0.0057	3	0.0072
10. *An. turkhudi,* Liston, 1901	3	0.0021	3	0.0043	31	0.0751
11. *An. theobaldi,* Giles, 1901	1	0.0007	1	0.0014	—	—
12. *An. fluviatilis,* James, 1902	4	0.0029	—	—	—	—
13. *An. vagus,* Donitz, 1902	5	0.0036	1	0.0014	2	0.0048
Total	13,6495	100	69,678	100	41,280	100

(—) denotes not found.

Table 2. Man hour density of anophelines (Average of four years, 1985 to 1988)

Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
A. **Canal irrigated area**												
An. culicifacies	0.84	6.93	22.18	8.64	3.69	0.72	0.42	5.12	4.27	5.00	2.11	1.40
An. stephensi	0.14	0.33	0.39	0.51	0.13	0.11	0.57	0.78	0.16	0.09	0.13	0.27
An. annularis	4.47	3.04	5.33	3.54	1.82	2.07	1.13	1.23	1.81	1.48	5.11	4.05
An. subpictus	9.65	18.23	91.42	64.27	40.72	24.06	46.94	432.01	236.87	80.50	56.35	15.04
Other anophelines*	0.11	0.13	0.04	0.05	0.04	0.03	0.007	0.02	0.02	0.09	0.13	0.04
Total	15.49	28.67	119.37	77.02	46.42	26.99	49.08	439.16	243.13	87.16	63.84	20.79
B. **Non-canal irrigated area**												
An. culicifacies	0.28	0.95	1.85	1.10	0.17	0.05	0.26	2.54	1.90	1.81	1.03	1.04
An. stephensi	0.02	0.09	0.14	0.23	0.12	0.05	0.19	0.36	0.10	0.06	0.04	0.03
An. annularis	0.02	0.06	0.07	0.12	0.02	0.18	0.16	0.51	0.44	0.25	0.10	1.95
An. subpictus	5.83	4.56	16.28	24.34	18.16	8.37	34.68	297.82	202.42	77.56	54.51	15.01
Other anophelines*	0.02	0.02	—	—	—	—	—	0.011	—	0.07	0.011	—
Total	6.17	5.69	18.34	25.79	18.48	8.65	35.29	301.25	204.87	79.77	55.68	15.26
C. **Riverine area**												
An. culicifacies	20.79	34.57	34.55	10.56	2.07	1.84	3.62	6.36	7.02	2.47	4.63	7.27
An. stephensi	1.33	1.43	1.76	1.00	0.30	0.21	0.66	0.77	0.14	0.11	0.08	0.58
An. annularis	0.25	0.13	0.35	1.76	0.23	1.41	0.34	0.23	0.16	0.11	0.66	3.18
An. subpictus	8.50	8.73	20.74	35.84	16.99	14.19	98.25	374.84	185.55	64.94	37.31	14.75
Other anophelines*	0.04	0.70	0.15	0.04	—	—	—	0.02	0.06	0.02	—	—
Total	30.92	45.57	57.57	49.20	19.59	17.65	102.87	382.23	192.94	67.67	42.68	25.78

* Includes *An. barbirostris, An. nigerrimus, An. aconitus, An. varuna, An. tessellatus, An. turkhudi, An. theobaldi, An. fluviatilis* and *An. vagus*; (—) denotes not found.

March (MHD 5.33), and lowest in July (MHD 1.13) *An. subpictus* showed wide fluctuations with the lowest density (MHD 9.65) recorded in January and the highest (MHD 432.01) in August. Though 13 species were recorded, from canal irrigated area the contribution of remaining species to the total anopheline density remained below 1 per man hour throughout the year.

Non-canal irrigated area

In this area the anopheline density followed a trend similar to that of canal irrigated area. The first peak was observed during April (MHD 25.79) of which 95% were *An. subpictus*. Highest anopheline density (MHD 301.25) was recorded in August and lowest (MHD 6.17) in January. Density of *An. culicifacies* remained low throughout the year with less wider fluctuations. It showed an increasing trend from February, attained a peak in March (MHD 1.85) and thereafter started to decline till June (MHD 0.05). With the onset of monsoon its density increased during August (MHD 2.54) and thereafter showed slow declining trend. *An. stephensi* and *An. annularis* were collected throughout the year and their densities remained below 1 per man hour except *An. annularis* density in December (MHD 1.95). *An. subpictus* was the predominant species and constituted 80 to 98% of the collection. Increase in its density was observed from March which attained its first peak in April (MHD 24.34) and second in August (MHD 297.82). *An. culicifacies* and *An. subpictus* accounted for 1.70 and 97.62% respectively and rest of the 9 species for only 0.68% of the collection.

Riverine area

In this area high anopheline density was recorded from January to March unlike in the other two physiographic areas. *An. culicifacies* contributed between 67 to 75% of the total anopheline population. The monsoon peak was observed in September (MHD 7.02). Decline after the September peak was not gradual, per man hour density crashed to 2.47 in October but showed an increase thereafter. Density of *An. stephensi* remained less than 2 per man hour throughout the year and between January and August it followed the trend shown by *An. culicifacies*. Highest density (MHD 1.76) was recorded in March and lowest (MHD 0.08) in November. Its density remained stable during the first four months of the year.

Whereas no clear pattern was observed in *An. annularis* density, the changes in *An. subpictus* densities were similar to those observed in the other two areas, with the highest density (MHD 374.84) recorded in August and lowest (MHD 8.50) in January. Among total anophelines the composition of *An. culicifacies* and *An. subpictus* was 10.66% and 87.81%, respectively.

DISCUSSION

Two clear peaks were observed in the densities of vectors and non-vectors (Fig. 3). The first peak in March in all the three physiographic areas was most likely due to the increase in temperature which plays an important role in the persistence and growth of larvae. The speed of growth is accelerated in warm waters because higher temperatures stimulate growth of aquatic plankton and this provides more food to the growing larvae[5].

Highest MHD of *An. culicifacies* was recorded from riverine area followed by canal irrigated area and lowest density was recorded from non-canal irrigated area. In canal irrigated area the first peak in *An. culicifacies* density was perhaps due to the commencement of canal irrigation for premonsoon rice crop which also resulted in percolation of water to low-lying borrow pits on both sides of canal giving rise to numerous fresh water pools. Freshly filled borrow pits and fallow rice fields are preferred by *An. culicifacies*.

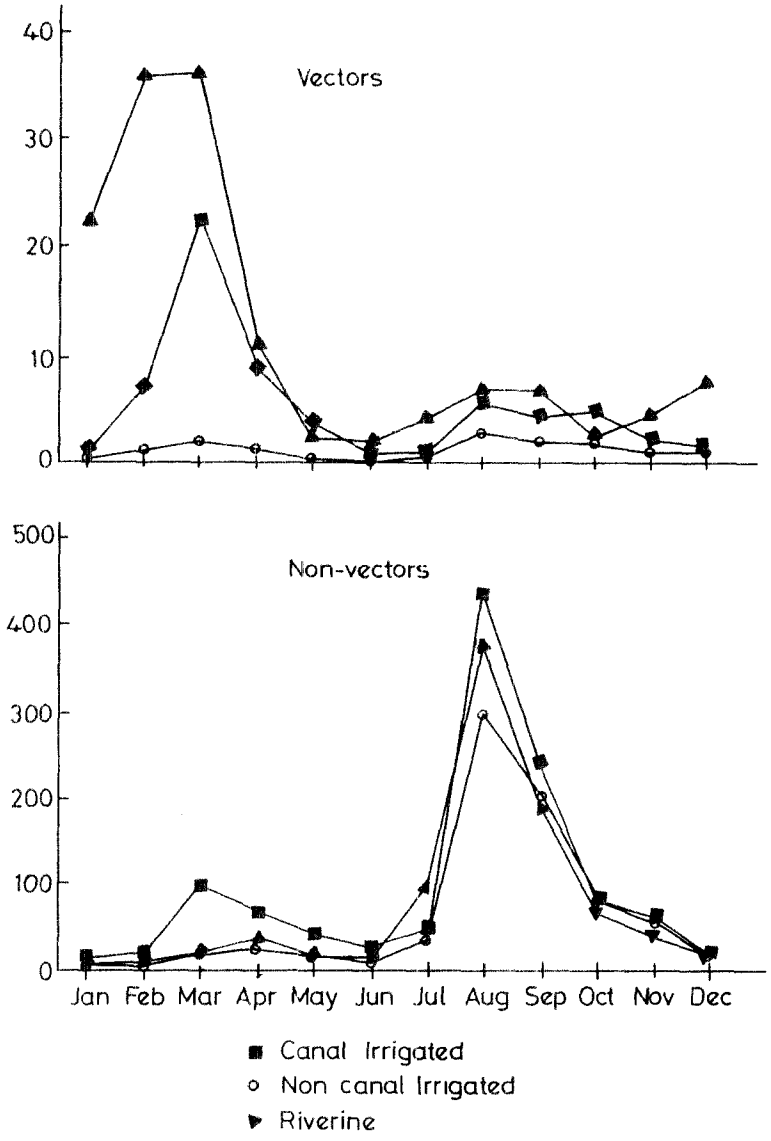


Fig. 3: Man hour densities of anophelines in three physiographic areas of Nadiad taluka (Average of four years 1985 to 1988).

During their study of the anophelines of rice fields in South Eastern Madras[6] observed that the density of *An. culicifacies* was highest in newly wetted fields prior to ploughing and it was not abundant in nurseries. After the rice stood 12 inches (30 cms) or more above the water surface *An. culicifacies* breeding was effectively checked. In another study on the ecology of *An. culicifacies* larvae in seepage waters in borrow pits, Russell and Rao[7] observed the largest number of eggs and larvae soon after water entered the newly dug pits. Larval density

declined as the water in pits became stale. In the riverine area the peak in March may be associated with drying up of river which leads to the formation of numerous riverbed pools which have been observed to breed profusely for *An. culicifacies*[8]. In non-canal irrigated area slow drying up of the breeding sources resulted in fewer habitats hence the small peak in March can only be attributed to favourable water temperature. In the summer months of May and June there was overall reduction in densities of all the mosquitoes due to reduced availability of breeding habitats and unfavourably high temperatures which adversely affect longevity. In canal irrigated area low level breeding takes place in wells, seepage water pools, irrigation canal and ponds, whereas in non-canal irrigated area, only ponds and wells are available to maintain the breeding. In riverine area breeding is restricted to river and riverbed pools.

July onwards densities of vector and non-vector mosquitoes started building up and a second peak was reached in August. Compared to 1st peak in March, *An. culicifacies* densities in August were less prominent in canal irrigated and riverine areas but were at the same level in non-canal irrigated area. Riverbed pools eliminated due to rivers flowing high in the monsoons does not allow *An. culicifacies* a chance to establish river breeding. In canal irrigated area due to continuous flow of canals, their distributaries and drains, breeding of *An. culicifacies* in these habitats is almost eliminated. Rain water collections initially support *An. culicifacies* breeding and later it is replaced by *An. subpictus*.

An. stephensi densities remained low in all the three physiographic areas throughout the year. This may be attributed to breeding behaviour as its breeding has been observed mainly in wells and intradomestic water storage containers. Yadav *et al.*[8] in their studies on the species specific breeding sources of anophelines in Kheda district observed the breeding preference of *An. stephensi* for wells and intradomestic water storage containers. Out of the total samples found positive for mosquitoes, *An. stephensi* was present in 46.99 and 80.15% samples from wells and intradomestic containers, respectively. Except wells it showed little preference for other peridomestic breeding sources. Since these two habitats are common in all three physiographic areas the changes observed in the density of *An. stephensi* may be due to prevailing local conditions.

Density of *An. annularis* remained consistently high in canal irrigated villages with less fluctuations as compared to *An. culicifacies*. Abundance of this species is generally associated with high concentration of aquatic vegetation and plankton which provide optimum opportunities for intense breeding[5]. High persistence of *An. annularis* in canal irrigated area may be because of the availability of favourable breeding habitats throughout the year. However, its prevalence was affected by environmental factors in different seasons.

In all the three areas, *An. subpictus* remained the predominant species except during the early months in riverine area. First peak in *An. subpictus* density during March-April was small while the second peak in August was much larger in all the three areas. Though in canal irrigated villages, there was no significant difference in area under paddy cultivation during both the seasons, rain water collections during monsoon supported extensive breeding in all the three areas resulting in a larger peak during August. Though it is an ubiquitous species and breeds prolifically in all the available breeding sources, climatic factors i.e., temperature and humidity affected its prevalence in all the three areas equally. However, due to its availability in large numbers in comparison to other species throughout the year it remains a major nuisance.

This study clearly shows that there were marked differences in the density patterns of anophe-

lines in three physiographic areas. Canal irrigated and riverine areas show high *An. culicifacies* densities during February and March which can support low level malaria transmission which in turn can help in building up the parasite load in the community. Also there was an increase in species diversity due to canal irrigation. Though the seasonal pattern was not affected by irrigation, there was some quantitative change.

It is hoped that this study on population dynamics of vectors would help in suitably organizing the antimosquito operations in Kheda district.

ACKNOWLEDGEMENTS

Authors are thankful to Dr. V.P. Sharma, Director, Malaria Research Centre, Delhi and Dr. N.L. Kalra, Consultant Entomologist, NMEP, Delhi for previewing the manuscript and their valuable suggestions. Technical assistance of Mr. V.P. Singh and secretarial assistance of Miss Rama Shah is gratefully acknowledged.

REFERENCES

1. Afridi, M.K., S.A. Majid and Jaswant Singh (1938). Malaria in Kutch State. *J. Mal. Inst. Ind.*, **1:** 187-213.
2. Singh, Neeru, B.N. Nagpal and V.P. Sharma (1985). Mosquitoes of Kutch, Gujarat. *Indian J. Malariol.*, **22:** 17-29.
3. Vishwanathan, D.K. (1950). *Malaria and its control in Bombay State.* (Aryabhusan Press, Poona).
4. Christophers, S.R. (1933). *The fauna of British India including Ceylon and Burma.* V. 4. (Today and Tomorrow's Printers and Publishers, New Delhi).
5. Rao, T. Ramachandra (1984). *The Anophelines of India.* Rev. ed. (Malaria Research Centre, ICMR, Delhi).
6. Russell, P.F. and H. Ramanatha Rao (1940). The *Anopheles* of rice fields in South Eastern Madras. *J. Mal. Inst. Ind.*, **3:** 427-446.
7. Russell, P.F. and T. Ramachandra Rao (1942). On the ecology of larvae of *Anopheles culicifacies* Giles, in borrowpits. *Bull. Ent. Res.*, **32:** 341-361.
8. Yadav, R.S., R.C. Sharma, R.M. Bhatt and V.P. Sharma (1989). Studies on the anopheline fauna of Kheda district and species specific breeding habitats. *Indian J. Malariol.*, **26:** 65-74.

Indian Journal of Malariology
Vol. 28, March 1991, pp. 19-28.

Bionomics of *Anopheles culicifacies* Giles in Riverine Tract Rural Areas of District Shahjahanpur, Uttar Pradesh

S.N. SHARMA* and R.N. PRASAD*

Studies on seasonal prevalence, resting behaviour, gonotropic conditions and breeding habits of *Anopheles culicifacies* were carried out from January 1987 to December 1989 in riverine villages of Dadraul PHC in Shahjahanpur district, Uttar Pradesh. Man hour densities of *An. culicifacies* varied from 11.3 to 125.1 during the study period. The species showed two peaks of high prevalence: one during March to June and the other during October to December. The first peak was higher than the second. The collection revealed a higher proportion of freshly fed to semi-gravid females thus indicating the outdoor resting behaviour of the species. From the immatures collected from riverbed pools, 7 anopheline species were identified. Of the 955 specimens identified, *An. culicifacies* constituted the highest number (62.1%). The study indicates that low rainfall and drought did not affect *An. culicifacies* densities or malaria transmission in riverine areas.

INTRODUCTION

Studies carried out earlier[1,2] revealed serious malaria problem in Shahjahanpur district, of Uttar Pradesh. The state unit of the National Malaria Eradication Programme (NMEP) reported high malaria incidence in Dadraul PHC of this district in 1985. A mission oriented Integrated Disease Vector Control (IDVC) programme was launched in Dadraul PHC in January 1986. The main objective of the programme is to control vector populations using biological control agents and by improving the environment to eliminate mosquito breeding sites. To achieve the above objective it was essential to study the main vector *Anopheles culicifacies*. The results of investigations carried out in three riverine villages of Dadraul PHC are reported in this paper.

MATERIAL AND METHODS

Shahjahanpur is situated between two rivers, viz., Khannaut in the east and Garrah on the west. The two rivers meet at a point approximately 10 kms south of Shahjahanpur. Three villages viz., Bijlapur, Udhopara and Beheta located on the banks of Garrah river were selected for the study. Bijlapur is situated near the confluence of these rivers. Both Udhopara and Beheta are situated 25 kms north of Bijlapur (Fig. 1).

The study villages consist of 362 human dwellings with a population of 1308 (711 males + 597 females) and 123 cattlesheds with 724 animals (mainly buffaloes, cows, goats, horses and pigs). Majority of the houses are built of mud walls with thatched roofs and 22% houses

Accepted for publication: 14 December 1990.

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

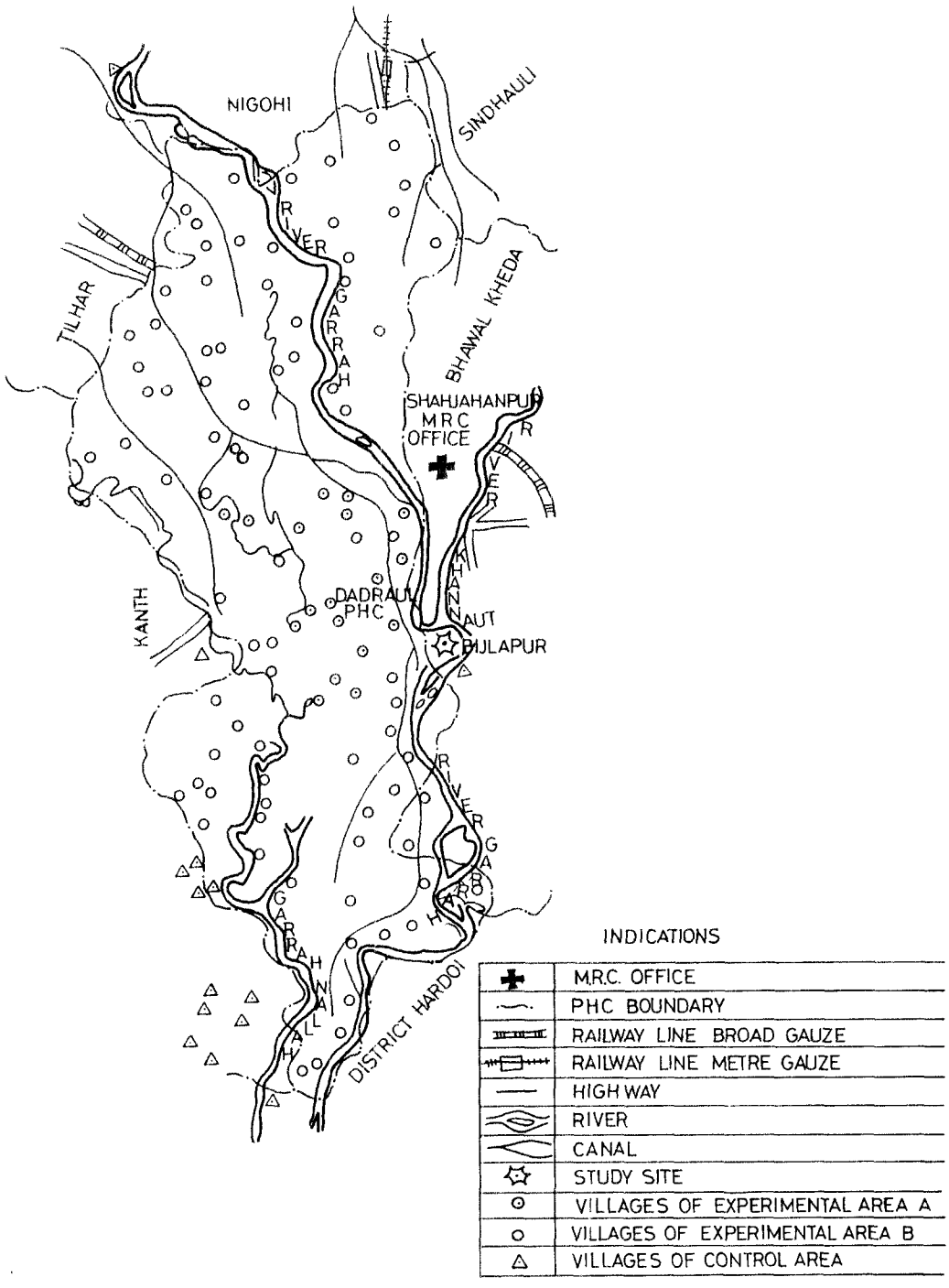


Fig.1 : Map showing sites of study villages in Dadraul PHC, Shahjahanpur.

are mixed dwellings (cattle were found tethered inside).

Investigations on adult density, gonotropic conditions, resting and breeding habits of *Anopheles culicifacies* were carried out during January 1987 to December 1989. Mosquitoes were collected using the following methods.

(a) *Hand collection:* Hand collections of indoor resting mosquitoes were carried out by suction tube method for 15 minutes in a room. For this six catching stations (3 human dwellings and 3 cattlesheds) were fixed in each study village. All hand collections were carried out at fortnightly intervals in the early morning hours. Mosquito species collected from human dwellings (HD) and cattlesheds (CS) were recorded separately, man hour densities were calculated using standard procedure.

(b) *Total catch:* The indoor resting mosquitoes were knocked down by pyrethrum space spray. Total catch was carried out once a month from the same fixed catching station selected for hand collection. Species identification of knocked down mosquitoes was done. They were further classified into unfed (UF), freshly fed (FF), semi-gravid (SG) and gravid (G) on the basis of their abdominal condition.

(c) *Larval surveys:* Intensive larval surveys were carried out to detect breeding of *An. culicifacies* in the study area using a standard dipper of 500 ml capacity, well surveys were done with the help of a bucket. In addition monthly collections of larvae and pupae were made from the riverbed pools. All larvae and pupae collected from the field were brought to the laboratory and held for adult emergence and species identification.

To assess the epidemiological situation active fever surveillance was carried out at weekly intervals. All blood smears were examined for malaria parasites after staining with JSB stain[3,4].

RESULTS AND DISCUSSION

In surveys carried out during January 1987 to December 1989, 12,393 (49.7%) *An. culicifacies* were collected alongwith 12,554 (50.3%) of 9 other species viz., *An. aconitus* 117 (0.46%), *An. annularis* 5026 (20.1%), *An. pallidus* 71 (0.3%), *An. subpictus* 6627 (26.6%), *An. vagus* 2 (0.008%), *An. stephensi* 687 (2.75%), *An. splendidus* 1 (0.004%), *An. barbirostris* 13 (0.05%) and *An. nigerrimus* 10 (0.04%). Man hour densities (MHD) of *An. culicifacies* varied from 11.3 to 125.1 during the study period (Table 1). Densities of *An. annularis* and *An. subpictus* varied from 3.2 to 111.3 and 0.3 to 146.3, respectively. The results showed that *An. stephensi* was not found till October but afterwards the species was found with MHD ranging from 0.2 to 71.8. Other anophelines were also found in low densities.

Seasonal prevalence

Results of the study showed that there was considerable seasonal variation in the density of *An. culicifacies*. Every year there are two peaks, the first one commencing March/April reaches high density levels by May/June (average density was 37.2 during January and February). Average temperature and relative humidity during these months varied from 14.5 to 17.6°C and 62.7 to 73.8%, respectively. A sudden spurt in density occurred in March which continued upto June with a more or less similar pattern each year. Average densities varied from 73.7 to 87.2 during March to June. Average temperature and relative humidity varied from 22.7 to 31.6°C and 32.3 to 54.5%, respectively during these months. Per man hour densities declined considerably during July and the trend continued upto September each year. Average temperature and relative humidity during the above months varied from 25.5 to 33.3°C and 72.3 to 80%, respectively. This indicated that densities were low in spite of suitable temperature and humidity. The reason for this decline may be due to heavy rainfall which would have had a flushing effect in the river.

Density started increasing again in October and the species prevailed with higher densities upto December. Average temperature and relative

Table 1. Per man hour density of *Anopheles culicifacies* (Jan. 1987 to Dec. 1989)

Month	Year	Per man hour density					
		1987		1988		1989	
		No. collected	Density	No. collected	Density	No. collected	Density
Jan.		34	11.3	294	49	147	24.5
Feb.		53	17.7	440	73.3	124	20.7
Mar.		338	84.5	655	109.2	407	67.8
Apr.		302	100.7	429	71.5	393	65.5
May		418	104.5	357	67	297	49.5
Jun.		1149	114.9	355	59.2	442	73.7
Jul.		796	88.4	223	37.2	258	43
Aug.		377	62.8	172	28.7	204	34
Sep.		296	37	119	19.8	118	19.7
Oct.		237	47.8	230	38.3	127	21.2
Nov.		876	125.1	334	55.7	187	62.3
Dec.		612	102	283	47.2	260	43.3

humidity during these months varied from 12.8 to 28.4°C and 67.6 to 73.5%, respectively. The two peaks of high prevalence of *An. culicifacies* were also observed in an earlier study[5] in Basantpur village situated on the bank of Jumna.

Results of total catch are given in Table 2. Number of *An. culicifacies* per structure varied from 26 to 558.5. Results showed that number of *An. culicifacies* per structure were relatively higher during October to December, 1987 as compared to that of the corresponding period of 1988 and 1989. Data obtained for both hand collection and total catch showed similar pattern for the seasonal prevalence of *An. culicifacies* in riverine areas.

Density of *An. culicifacies* found in human dwelling (HD) and cattlesheds (CS) is given in Table 3. The density in human dwellings varied from 1.3 to 48.4. Per man hour densities in cattlesheds varied from 10 to 92. Data collected shelterwise clearly indicated that cattlesheds were preferred resting sites to human dwellings as was observed earlier [6,7,8].

Results of gonotropic conditions of females are given in Table 4. There was wide variation in the percentage of unfed (UF), freshly fed (FF), semi-gravid (SG) and gravid (G) females from month to month during the same year and also as compared with the same month of the previous year. Overall results of the study revealed 2.86% unfed, 48.3% freshly fed, 36.6% semigravid and 11.96% gravid mosquitoes. Some specimens (43-0.3%) could not be classified due to their abnormal gonotropic conditions. This showed that females in all stages of gonotropic conditions rested inside the houses during day time. The results further revealed that the proportion of freshly fed mosquitoes was higher than that of semi-gravid females.

Table 2. Number of *Anopheles culicifacies* collected per structure in pyrethrum space spray (Oct. 1987 to Dec. 1989)

Month	Number collected		
	1987	1988	1989
Jan.		168.5	43
Feb.		155.5	44.5
Mar.		558.5	339.5
Apr.		348	132
May		431.5	58.5
Jun.		99	116.5
Jul.		195.5	48
Aug.		131	75
Sep.		44.5	26
Oct.	130	112.5	42.5
Nov.	176	130	28.5
Dec.	91.5	76	78

Breeding source surveys

Local breeding sites contributing mosquitoes in the study area and surrounding areas may broadly be classified into domestic and peridomestic ones.

Domestic

A common domestic mosquito breeding place is the "Nandh", a wide mouthed earthen pot which is dug into one corner of the house for storing waste water. Such troughs and old pitchers are found in almost all houses of the area. The other domestic breeding places are wells, cisterns and waste water collections near hand pumps and community wells. Such breeding sites did not support *An. culicifacies* but other species such as *An. subpictus, An. annularis* and *Culex quinquefasciatus* were observed breeding. Breeding of *An. stephensi* was encountered in wells.

Peridomestic

Ponds, pits, roadside water collections, riverbed pools, water collections in paddy fields the canal and its seepage all contribute to the breeding of

Table 3. Per man hour density in different structures

Month	Human dwelling			Cattleshed		
	1987	1988	1989	1987	1988	1989
Jan.	1.3	13	4.2	10	36	20.3
Feb	3.3	19.8	4.3	14.3	53.5	16.3
Mar.	17	27.2	8.8	67.5	82	59
Apr.	19.3	19.2	5.5	81.3	52.3	60
May	35.7	18.4	7.3	68.7	48.6	42.2
Jun.	48.4	17	13.5	66.5	42.2	60
Jul.	42.2	13.2	11.3	46.2	24	31.7
Aug.	23.5	8.8	7	39.3	19.8	27
Sep.	19.6	5	5.8	17.4	14.8	13.8
Oct.	22.2	6.3	6	25.6	32	15.2
Nov.	33.1	11.3	9	92	44.3	53.3
Dec.	37.6	11.3	5.5	64.3	35.8	37.8

Table 4. Abdominal condition of indoor resting *Anopheles culicifacies*

Month	Abdominal condition											
	Unfed			Freshly fed			Semi-gravid			Gravid		
	1987	1988	1989	1987	1988	1989	1987	1988	1989	1987	1988	1989
Jan		4 (1.05)	25 (26.04)		110 (29.1)	38 (39.6)		141 (37.3)	29 (30.2)		123 (32.5)	4 (4.2)
Feb		8 (2.04)	2 (5.3)		234 (59.8)	17 (44.7)		103 (26.3)	14 (36.8)		46 (11.8)	5 (13.2)
Mar		8 (0.86)	6 (1.7)		324 (35)	188 (55.1)		375 (40.5)	104 (30.5)		218 (23.6)	43 (12.6)
Apr		3 (0.55)	9 (3.8)		154 (28.2)	171 (71.5)		284 (51.9)	54 (22.6)		106 (19.4)	5 (2.1)
May		68 (6.54)	5 (2.5)		286 (27.5)	135 (68.9)		534 (51.3)	51 (26)		152 (14.6)	5 (2.5)
Jun		9 (2.3)	5 (1.4)		256 (65)	228 (62.1)		103 (26.1)	115 (31.3)		26 (6.6)	19 (5.2)
Jul		10 (2.2)	9 (5)		120 (26.5)	97 (53.6)		283 (62.5)	46 (25.4)		40 (8.8)	29 (16)
Aug		2 (1.5)	17 (12.6)		68 (51.5)	57 (42.2)		46 (34.8)	44 (32.6)		16 (12.1)	17 (12.6)
Sep		0 (0)			43 (60.6)			20 (28.2)			8 (11.3)	
Oct	8 (3)	5 (2.7)		47 (17.8)	104 (56.8)		162 (61.4)	64 (35)		47 (17.8)	10 (5.5)	
Nov	13 (2.1)	8 (3.6)		98 (16.1)	137 (61.4)		372 (61.3)	65 (29.1)		124 (20.4)	13 (5.8)	
Dec	8 (1.54)	3 (1.7)		100 (19.2)	105 (59.3)		279 (53.7)	55 (31.1)		133 (25.6)	14 (7.9)	
Total	29 (2.1)	128 (2.6)	78 (4.9)	245 (17.6)	1941 (39.5)	931 (58.4)	813 (58.4)	2073 (42.2)	457 (28.7)	304 (22)	772 (15.7)	127 (8)

Note: Figures in parentheses indicate %

Table 5. Adult emergence from river bed pools of Garrah

Month/Year		Species													
		A. cul.		*A. aco.*		*A. ann.*		*A. sub.*		*A. steph.*		*A. barb.*		*A. nig.*	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
	1987	—	—					Larval							
Jan.	1988	2	15.4	3	23.0	3	23.0	Stage						5	38.5
	1989	—	—												
	1987	—	—												
Feb.	1988	16	69.5	1	4.3	2	8.7							4	17.4
	1989	37	82.2			7	15.5			1	2.2				
	1987	—	—												
Mar.	1988	3	10	0		26	86.7							1	3.3
	1989	40	90.9			4	9.1								
	1987														
Apr.	1988	19	79.2	1	4.1	1	4.1							3	12.5
	1989	21	56.8			16	43.2								
	1987														
May	1988	13	48.1	0		2	7.4	12	44.4						
	1989	12	52.2			11	47.8								
	1987														
Jun.	1988	7	21.2			26	78.8								
	1989	38	100												

(Contd.)

Table 5. Adult emergence from river bed pools of Garrah *(Contd.)*

Month/Year		Species													
		A. cul.		*A.aco.*		*A. ann.*		*A. sub.*		*A. steph.*		*A. barb.*		*A. nig.*	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
	1987	—	—												
Jul.	1988	—	—												
	1989	2	100												
	1987	—	—												
Aug.	1988	—	—												
	1989	6	100												
	1987	—	—												
Sep.	1988	—	—												
	1989	—	—												
	1987	25	78.1												
Oct.	1988	20	90.9			1	4.5			1	4.5				
	1989	10	100												
	1987	8	4.2	1	0.5			179	94.7					1	0.5
Nov.	1988	68	93.2	0				4	5.5	1	1.3			1	2.2
	1989	6	100												
	1987	21	46.7	4	8.9			18	40.0			1	2.2	1	2.2
Dec.	1988	124	97.6			2	1.6	1	0.8						
	1989	95	96.9												
	Total	457	57	10	1.2	101	12.6	214	26.7	3	0.4	1	0.1	16	2

— = Not recorded.

An. culicifacies. Since the area has rich production of rice and sugarcane, many rice mills, cardboard factories, sugarcane crushers and mills are located here. Effluents from factories are discharged in the open due to improper drainage system. All these effluents accumulate in low-lying areas forming big tanks of polluted water where heavy breeding of *An. subpictus* and culicine mosquitoes was encountered. The favourable breeding sites for *An. culicifacies* were the river and riverbed pools; along the margins of canal and its seepage water, ponds and water collection in paddy fields.

Larvae and pupae were regularly collected from Garrah river and its bed pools (Table 5). *An. culicifacies* was found breeding in large numbers in association with 6 other anophelines viz., *An. aconitus* 10(1%), *An. annularis* 109 (11.4%), *An. subpictus* 223 (23.4%), *An. stephensi* 3(0.3%), *An. barbirostris* 1(0.1%) and *An. nigerrimus* 16 (1.7%). There was no exclusive breeding habitat for any single species as was also observed earlier[9]. Results revealed that breeding of *An. culicifacies* occurred in the river throughout the year. However, it would be important to point out that during the monsoons bulk of breeding was washed away due to flood in the river, but after the flood receded extensive breeding of *An. culicifacies* was observed in the river bed pools and all along the weedy margins of the river. The study further indicated that due to low rainfall during 1987 (Table 6) water level in the river declined considerably as a result of which it stagnated at several places. Submerged vegetation also sprang up. Intense breeding of *An. culicifacies* was observed in the above situations. This resulted in higher densities during drought period in comparison with the low densities during period of heavy rainfall. Similar observations were made in Sri Lanka[10] (Quoted by Rao)[11].

Results of parasitological investigations are given in Table 7. Slide positivity rates (SPR) recorded during 1987, 1988 and 1989 were 32.1,

Table 6. Rainfall in Distt. Shahjahanpur during 1987 to 1989*

Month/Year	1987		1988		1989	
	Rainfall mm	No. of rainy days	Rainfall mm	No. of rainy days	Rainfall mm	No. of rainy days
Jan	4.2	6	0	0	44.4	3
Feb	5.0	3	6.3	2	3.9	2
Mar	5.5	3	12.6	3	19.4	4
Apr	1.0	1	41.6	6	0.6	1
May	88.6	8	3.6	1	7.8	2
Jun	78.8	3	102.4	5	87.8	11
Jul	150.1	8	428.9	16	267.2	14
Aug	86.3	8	309.9	20		
Sep	118.5	10	137.2	9		
Oct	14.4	3	8.3	2		
Nov	0	0	0	0		
Dec	5.0	1	50.4	2		

* Data obtained from U.P. Sugar Cane Research Council, Shahjahanpur.

Table 7. Parasitological data of 3 riverine villages (1987 to 1989)

Year	B.S. examined	Results		Total (+)ve	SPR	SFR	ABER	API
		Pv	Pf					
1987	856	189	86	275	32.1	10	65.4	210.2
1988	806	119	49	168	20.8	6.1	61.6	128.4
1989	699	74	9	83	11.9	1.3	53.4	63.5

20.8 and 11.9, respectively. Slide falciparum rates (SfR) were found to be 10, 6.1 and 1.3, respectively during the same study period. The annual parasite incidence (API) recorded during the above period of study were found to be 210.2, 128.4 and 63.5 respectively. The annual blood examination rate (ABER) recorded during the 3 years of study was 65.4, 61.6 and 53.4, respectively. The above malariometric indices revealed higher malaria incidence during 1987 as compared to the subsequent study period.

Overall results of the present study indicated that low rainfall and drought did not affect either *An. culicifacies* densities or malaria transmission in riverine areas. However, decline in incidence of the disease could be obtained with the help of thorough surveillance and prompt treatment of malaria cases. Based on these results it can be concluded that riverine villages are more prone to malaria because of high mosquitogenic conditions prevalent in these areas. Therefore, malaria control operations should be carried out in such areas on priority basis.

ACKNOWLEDGEMENTS

The study received financial supports from S&T Project under IDVC mission. The field assistance given by Insect Collectors is gratefully acknowledged. The authors are grateful to Miss Anjali Gupta for her assistance in typing the manuscript.

REFERENCES

1. Chandrahas, R.K. and V.P. Sharma (1983). Malaria epidemic in Shahjahanpur (U.P.). *Indian J. Malariol.*, **20:** 163-166.
2. Sharma, V.P., R.K. Chandrahas, B.N. Nagpal and P.K. Srivastava (1985). Follow-up studies of malaria in villages of Shahjahanpur District, U.P. *Indian J. Malariol.*, **22:** 119-122.
3. Singh, J (1956). JSB stain—A review. *Indian J. Malariol.*, **10:** 117-129.
4. Singh, J. and L.M. Bhattacharji (1944). Rapid staining of malarial parasites by a water soluble stain. *Indian Med. Gaz.*, **79**(3).
5. Ansari, M.A., R.K. Razdan, V.P. Sharma and T.R. Mani (1982). Ecology of anophelines in Basantpur village situated on the bank of Jumna. *Indian J. Malariol.*, **19**(1): 65-68.
6. Afridi, M.K., Jaswant Singh and Harwant Singh (1939). Food preference of *Anopheles* mosquitoes in Delhi urban area. *J. Mal. Inst. Ind.*, **2:** 219-228.
7. Pal, Rajinder (1945). On the bionomics of *A. culicifacies*. Giles Part III. The behaviour of adults. *J. Mal. Inst. Ind.*, 6(2): 217-238.
8. Sen, R.N., R. Rajagopal and P.K. Chakraborty (1960). Observations on the seasonal prevalence of adult anophelines near Dhanbad. *Indian J. Malariol.*, **14:** 23-54.
9. Russell, P.F. and T.R. Rao (1940). On habitat and association of species of *Anopheles* larvae in South eastern Madras. *J. Mal. Inst. Ind.*, **3:** 153-178.
10. Rajendram, S. and S.H. Jayawickrame (1951). Malaria in Ceylon. Part I. The control and prevalence of epidemic malaria by the residual spraying of houses with DDT. *Indian J. Malariol.*, **5:** 1-73.
11. Rao, T. Ramachandra (1984). *The Anophelines of India*. Rev. ed. (Malaria Research Centre, ICMR, Delhi): 317-357.

Indian Journal of Malariology
Vol. 28, March 1991, pp. 29-37.

Living Conditions and Occurrence of Malaria in a Rural Community

S. SUBRAMANIAN*, A. MANOHARAN*, S. SAHU*, P. JAMBULINGAM*, P. GOVARDHINI*, S.S.S. MOHAPATRA* and P.K. DAS*

Results of a prospective study of the association between environmental factors and malaria occurrence among 1461 individuals in an Indian rural community are reported. A multiplicative model was fitted by relating malaria positivity with different environmental risk factors as categorical variables. The risk of acquiring malaria infections varied significantly within a village stratified by proximity of breeding sources to human dwellings (X^2 = 19.87, df = 5, p = 0.0000) and different types of houses (X^2 = 11.32, df = 2, p = 0.0035). People residing in thatched houses with [Relative Risk (RR) = 6.72] and without false ceiling (RR = 11.27) are exposed to greater risk of contracting malaria infections when compared to tiled houses (RR = 1.00). However, malaria infection was not significantly associated with proximity of cattlesheds to human dwellings (X^2 = 1.54, df = 2, p = 0.46). A significantly high risk was observed in zones where the cattle to man ratio was very low and vice versa (X^2 = 15.32, df = 6, p = 0.018). These data suggest and corroborate with earlier studies that transmission is a local problem and it varies within a village according to the microepidemiological factors.

INTRODUCTION

Knowledge of the natural course of a disease and reliable information on the main factors involved in the transmission process are a prerequisite for planning preventive measures against any communicable disease. In malaria, the man/parasite/vector complex remains fully stable only under natural evolutionary conditions. Any intervention affecting one of these components or the environment in which they have been evolving naturally introduces an element of imbalance, which may have adverse effects if the activity in question is broken off[1].

The factors responsible for the persistence of malaria in an area can be broadly classified as direct or indirect[2,3]. The direct factors influencing the transmission process are entomological, parasitological and immunological variables. The indirect factors, namely the meteorological and environmental can create a potential situation for increased malaria transmission by acting singly or in combination. Environmental factors at macro level (mountain, forest, river etc.) and micro level (house type, living conditions and association with other animals etc.) are known to influence disease transmission. Burkot *et al.*[4] examined the influence of these factors on some of the

Accepted for publication: 17 December 1990.

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

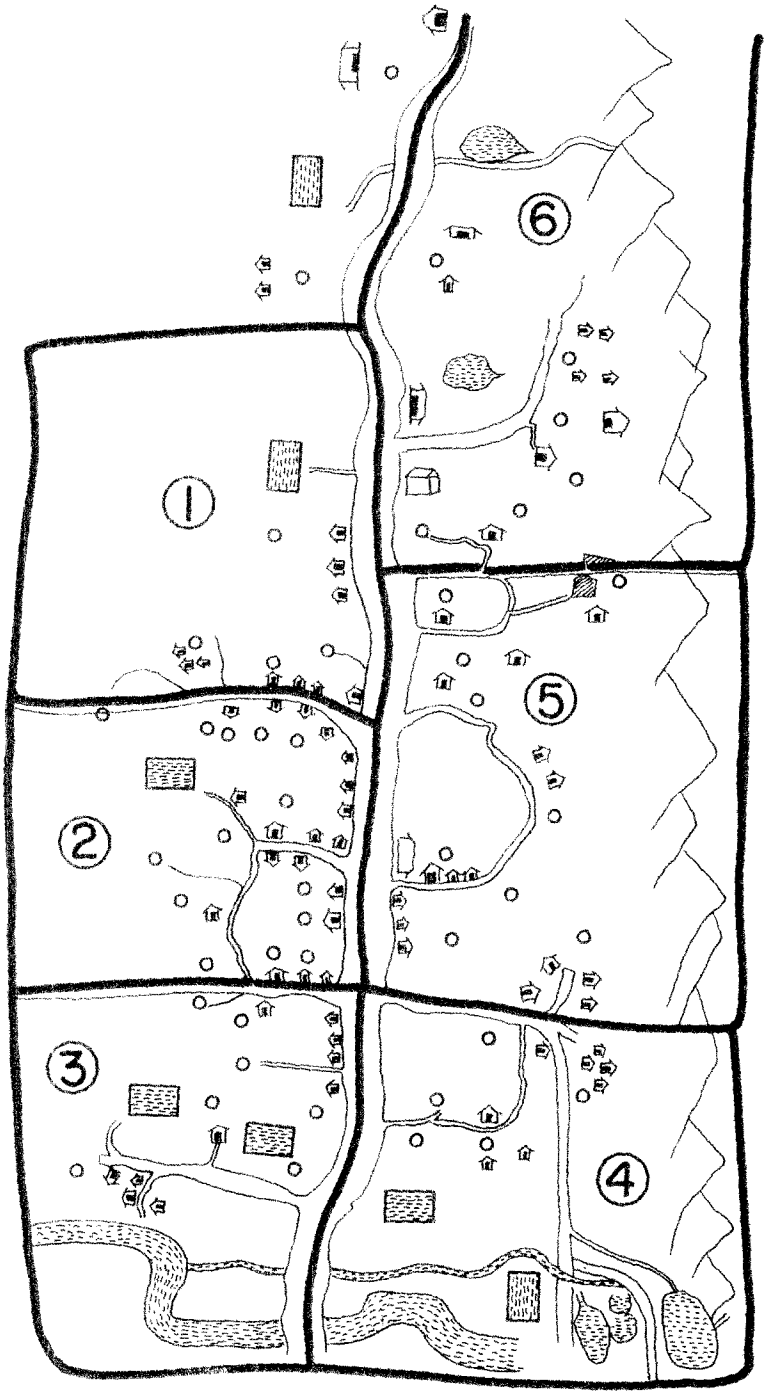


Fig. 1: Map showing the study area.

entomological variables and concluded that in a village the human biting rate of *Anopheles punctulatus* was affected by the type of house and location of domestic animals.

While the qualitative impact of factors like type of dwellings[5], social organization, economic activities[6,7] and population movement[8,9] on malaria persistence has been well documented, the role of these factors on malaria transmission has not been quantified mainly due to the complexity of factors involved. In this paper, an attempt has been made to quantify the effects of some of these micro level epidemiological factors on the persistence of malaria infection stratified by geographical location of houses and to define their role in malaria transmission.

MATERIAL AND METHODS

Study area

B. Singpur, a hyperendemic area in Boriguma PHC of Koraput district, Orissa, is the study area. This village, situated at the base of hills, was characterized by the presence of ponds, paddy fields and wells in addition to streams and rivulets. Terrace cultivation which is common in this village facilitates perennial breeding due to water stagnation. It has been reported that the main vector is *An. fluviatilis,* which is highly anthropophilic and other vectors include *An. culicifacies, An. annularis, An. varuna* and *An. aconitus*[10]. The study area was divided into six zones based on the geographical location of the houses (Fig. 1).

Baseline data

Fortnightly fever surveillance carried out during March 87—February 88 forms the data base for this study. Complete enumeration of the village was carried out prior to the malariometric survey. Information about the type of house, size of the family, education, income, occupation, number of cattle, proximity of cattlesheds was obtained from all the households. The environmental factors were categorized and coded for the analysis, e.g. geographical location of houses (1-6) (GL), cattle to man ratio (CAT), house type (HT): (1-Tiled or asbestos sheeted, 2-Thatched without false ceiling and 3-Thatched with false ceiling) and proximity of cattleshed to human dwelling (HDCS): (1-no cattleshed inside the campus, 2-both are in the same campus but separated, and 3-both are under the same roof).

Statistical analysis

Examination of parasite incidence within households is complicated by the variability of the geographical location of houses, type of house, proximity of cattlesheds to human dwelling and cattle population. The number of slide positive malaria cases is assumed to follow a binomial distribution with population exposed in each category as the denominator of the parasite incidence. The multiplicative model with all possible interactions was chosen to see the effects of different risk factors stratified by geographical location of houses. Since individuals positive more than once may be relapses or recrudescences, the multiplicative model was fitted to data in which the individuals were positive only once during the study period. The coefficients are the parameter estimates of the corresponding risk factors. Method of fitting the log-linear models by maximum likelihood using the computer program GLIM are given by Baker and Nelder[11]. This program uses a modification of the Newton-Raphson algorithm to solve the non-linear likelihood equation; standard errors of the parameter estimates arise as a by-product of this calculation. An over all evaluation of the goodness of fit was done using the G^2 or X^2 statistics. Further, the standardized residuals and the fundamental property of *hat* matrix 'H' were used as a diagnostic measure to see if there is any systematic departure from the fitted model[12]. The log-odds ratio interaction test from the fitted log-linear model[11] was used for comparing the malaria incidence at different levels of these factors.

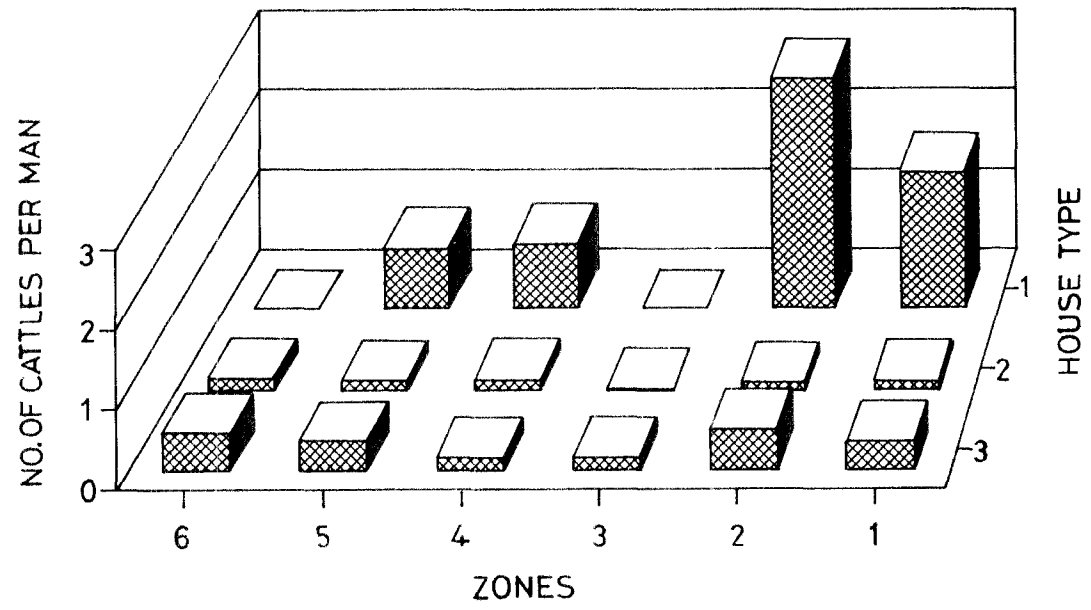


Fig. 2: Number of cattle per man in different types of houses and zones.

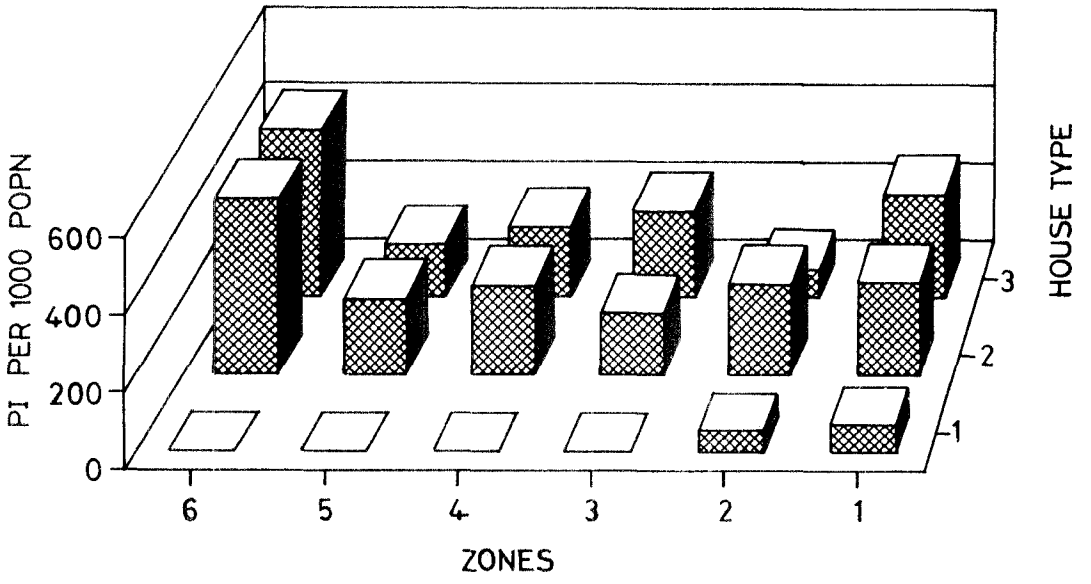


Fig. 3: Malaria incidence in different types of houses and zones.

RESULTS

Social and demographic information about the study area

The people of B. Singpur are relatively homogeneous with regard to education, occupation or economic factors. The population of the village is 1,476 inhabiting 316 houses. The total population of the village is distributed in 6 different geographical locations of houses with a maximum 34.0% in zone 5, followed by 16.2%, 15.2%, 13.8%, 12.9% and 7.9%, respectively in zones 2, 6, 4, 3 and 1. The average number of persons per family is 5. About 90% of the houses are thatched with/without false ceiling and 94% of the people are living in this type of houses. The rest are living in houses with tin/asbestos sheet roofs. Most of the houses are adjoined by a cattleshed with a cattle man ratio of 1:3. The cattle to man ratio in different zones classified by type of house is shown in Fig. 2. It varied from 1:1 in zone 2 to 1:10 in zone 3. While in tiled houses the human and cattle population are equal in ratio, it was 1:10 and 1:3, respectively, in thatched houses without and with false roof.

Malaria situation

A total of 740 blood smears were collected and examined over a period of one year (March 1987 to February 1988) on 24 occasions. A total of 296 malaria positive slides (40.0%) were collected from 253 individuals. Of these, 234 (92.5%) were positive only once and the rest were positive more than once. The annual parasite incidence (API) for the village was 201 per 1000 population. Of all positives *P. falciparum* was the major species (81.8%) followed by *P. vivax* (14.8%), and mixed infection of *P. falciparum* and *P. vivax* (3.4%). Among the 296 parasite carriers 32 had gametocytes in their blood. The infant parasite rate was found to be 10.0% with an API of 25 per 1000 infants. Children of the 5-9 years age group recorded the highest parasite incidence (API = 245 per 1000 population of that age class).

Parasite incidence classified by risk factors

Amongst the three types of houses, parasite incidence was highest in thatched houses without false ceiling (235 per 1000 population), followed by thatched with false ceiling (API = 177/1000) and tin/asbestos sheeted roof (API=23/1000). Fig. 3 shows the parasite incidence in different types of houses classified by zones. Comparison of Figs. 2 and 3 show that a high parasite incidence was observed in thatched houses of zone 6 where the cattle to man ratio is relatively low. While parasite incidence is high in thatched houses of all the zones, it is low in tiled houses of all the zones. Similarly cattle to man ratio is low in thatched houses in comparison to tiled houses of all the zones.

Model parameter estimates and their significance

The parameter estimates and their significance (based on Z-test), standard errors, relative risks (RR) and the G^2 and X^2 statistics are given in Tables 1 and 2. An evaluation of the goodness of fit of the multiplicative model was made by examination of the residuals and the associated G^2 statistic. The observed G^2 value (38.1, df=34, p=0.29) was less than that expected under chi-square sampling. No systematic pattern or trend in the residuals was observed and none of the residuals showed significant deviations when referred to tables of standard normal distribution. The Filliben's correlation coefficient was 0.9765. The grand total of the elements of the *hat* matrix (16.19) was approximately equal to the number of independent parameters in the model (16) indicating the validity of the binomial assumption used in the model.

The overall incidence within six geographical zones was highest (379.4 per 1000 population) in zone 6, followed by 239.3, 193.7, 187.1, 151.3 and 96.2, respectively for zones 1, 3, 4, 5 and 2 and it

Table 1. Analysis of variance based on the multiplicative model

Source of variation	Df	Chi-square	P
Geographical location (GL)	5	19.87	0.001
House Type (HT)	2	11.32	0.003
Proximity of Cattleshed to Human Dwelling (HDCS)	2	1.54	0.460
GL x CAT	6	15.32	0.018

Table 2. Regression coefficients, standard errors and associated relative risks for the multiplicative model

Factor	Level	Estimate	S.E.	Relative risk	Z	P
Constant term		−3.76	1.50			
GL	1	—	—	1.00		
	2	−0.42	0.51	0.65	0.83	0.410
	3	−0.18	0.45	0.83	0.41	0.680
	4	0.43	0.48	1.53	0.88	0.380
	5	−0.27	0.44	0.76	0.62	0.540
	6	0.77	0.44	2.16	1.73	0.080
HT	1	—	—	1.00		
	2	2.42	1.23	11.27	1.97	0.049
	3	1.91	1.32	6.72	1.45	0.150
HDCS	1	—	—	1.00		
	2	0.17	0.26	1.19	0.67	0.500
	3	0.16	0.22	1.17	0.71	0.480
GL x CAT	1	0.34	1.00	1.40	0.34	0.730
	2	-0.45	0.67	0.64	0.67	0.500
	3	1.90	1.00	6.68	1.89	0.058
	4	−4.59	2.11	0.01	2.17	0.030
	5	-0.77	0.75	0.47	1.02	0.310
	6	0.95	0.62	2.59	1.53	0.130

Deviance: G^2 = 38.133 on 34 degrees of freedom; P = 0.2869.

differs significantly among zones (X^2 = 19.87, df = 5, p = 0.0000). Though the risk in zone 1 is higher than that of zones 2, 3 and 5, their differences were not statistically significant ($p>0.05$). However, a relatively lower risk was observed in zone 1, when compared to zones 4 and 6 (Table 2). As the zonal differences could be due to the population distribution of non-immunes (<15 years) and immunes (≥15 years) or variations in microepidemiological factors the data were further analyzed for their effects. The parasite incidence in age class below 15 and above 15 in the different geographical zones are given in Fig. 4. It was observed that the population distribution of the two age classes between zones ($X^2=4.996$, df=5, p=0.42) and the parasite incidence (all zones combined) between the two age classes ($X^2=0.82$, df=1, p=0.36) do not differ significantly. However, the parasite incidence between zones in age class less than 15 years ($X^2=19.94$, df=5, p=0.0013) and above 15 years ($X^2=59.28$, df=5, p=0.0000) differed significantly.

The model parameter estimates showed that the effect of cattle population among zones ($X^2=15.32$, df=6, p=0.018) (Table 1) varied significantly. There was an increased risk of acquiring malaria infections in zones 1, 3 and 6 (Table 2). While a relatively lower risk was observed in zones 2, 4 and 5, where the cattle man ratio was high, a significantly lower parasite incidence was observed only in zone 4 (Table 2).

Analysis of variance based on the log-linear model showed that malaria incidence was significantly associated with the type of houses ($X^2=11.32$, df=2, p=0.0035). However, the proximity of cattlesheds to human dwelling was not significantly associated with the risk of acquiring malaria infections ($X^2=1.543$, df=2, p=0.46). The two factor interaction effects of proximity of cattleshed to human dwelling and the type of house are not significant. The risk is 1.19 and 1.17, respectively for houses with cattle-sheds in the same campus and for houses where the cattlesheds are under the same roof,

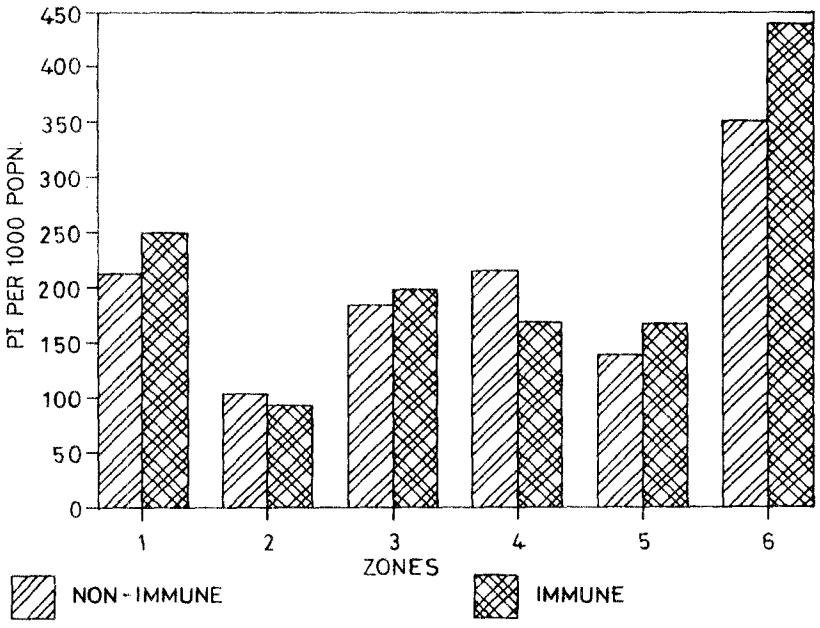


Fig. 4: Malaria incidence in children (<15 years) and adults (≥15 years) in different zones.

when compared to houses without cattlesheds. Though the relative risk of staying in houses where cattlesheds are absent is lower than that of houses in which both are under the same roof and are in the same campus, it is not significantly different (Table 2). The relative risk of staying in thatched houses with and without false roofing is 6.7 and 11.3, times higher than that of tiled houses. The log odds ratio test shows that the relative risk of acquiring malaria infection in thatched houses (with and without false ceiling) was at the limit of probability of significance when compared to tiled houses ($Z=1.97$, $p=0.049$).

DISCUSSION

El Samani[6] had reported the importance of socio-economic and environmental factors in relation to malaria transmission based on a cross sectional study in a rural Sudan community. Their study is based on a retrospective method of disease detection (cases were classified based on the clinical history and not based on blood smear examination) and is subject to misclassification of both cases and non-cases and would have affected the measures of association. The present study however is a prospective one and cases were classified based on blood smear examination and hence the estimates are more reliable. Analysis of parallel entomological data could have enhanced the evidences for the risk factors. Since in the present study no such data are available, the conclusions drawn are subject to this limitation. However, published results have shown that human biting rates were significantly related to housing pattern and the relative abundance and distribution of humans and domestic animals[3,13,14,15].

A recent paper, based on a theoretical model suggests that introduction of domestic animals may lower malaria endemicity, if the vector population has attained its maximum level prior to introduction of domestic animals, or if a large number of domestic animals are introduced for which the vector has greater preference[16] Though the present study shows an associated decline in risk of acquiring malaria infection with an increase in cattle population (in at least some geographical locations), it is not known whether there is an optimum level of cattle population at which the man vector contact is reduced due to change in feeding habits of the vector. This is probably due to the fact that the major vector *An. fluviatilis* being anthropophilic the relative abundance of cattle altered the risk of transmission. However, this could have affected the risk of malaria transmitted by other zoophilic species. The high risk of malaria incidence in thatched houses and in houses where cattlesheds are absent could be due to the human host selection behaviour of the mosquitoes, as this is influenced by a variety of cultural factors (bednet usage, type of house, animal husbandry practices etc.) and intrinsic mosquito preferences for blood meals[2,17]. It should also be noted that the cattle to man ratios in thatched houses with and without false roof are lower than that of tiled houses.

In a recent review of the National Malaria Eradication Programme in India, many references were made to the role played by human factors and it was recommended that long term studies should concern themselves with the effects of human and socio-economic factors on malaria epidemiology[18] Though the general classifications based on malaria endemicity may help in the planning of global malaria control programmes, they are of less value when malaria control is considered at a more local level[19] Hence recognition of local variations in the epidemiology of malaria must also be taken into account when any kind of malaria intervention trial is planned.

ACKNOWLEDGEMENT

The authors are grateful to Dr. P.K. Rajagopalan, Director of Vector Control Research Centre, Pondicherry for his constant guidance and encouragement.

REFERENCES

1. Julvez, J., J. Galtier and J. Mouchet (1989). Integrated malaria control achieves results in Mayotte. *World Health Forum,* **10**(2): 213-218.
2. Onori, E. and B. Grab (1980). Indicators for the forecasting of malaria epidemics. *Bull. WHO,* **58**(1): 91-98.
3. White, G.B. (1969). Factors influencing densities of mosquitoes resting indoors. *Annual Report of the East African Institute of Malaria and Vector-Borne Diseases.*
4. Burkot, T.R., C. Dye and P.M. Graves (1989). An analysis of some factors determining the sporozoite rates, human blood indexes, and biting rates of members of the *Anopheles punctulatus* complex in Papua New Guinea. *Amer. J. Trop. Med. Hyg.,* **40**(3): 229-234.
5. Schofield, C.J., R. Briceno-Lenon, N. Kolstrup, D.J.T. Webb and G.B. White (1990). The role of house design in limiting vector borne disease. *In Appropriate Technology in Vector Control.* Ed. C.F. Curtis. (CRC Press, Inc., Boca Raton, Florida): 187-212.
6. El Samani, F.Z., W.C. Willett and J.H. Ware (1987). Nutritional and socio-demographic risk indicators of malaria in children under five: a cross-sectional study in Sudanese rural community. *J. Trop. Med. Hyg.,* **90:** 69-78.
7. Fungladda, W., S. Sornmani, K. Klongkamnuankarn and T. Hungsapruek (1987). Sociodemographic and behavioural factors associated with hospital malaria patients in Kanchanaburi, Thailand. *J. Trop. Med. Hyg.,* **90:** 233-237.
8. Rajagopalan, P.K. and P. Jambulingam (1986). Population movement and malaria persistence in Rameswaram Island. *Soc. Sci. Med.,* **22**(8): 879-886.
9. Butraporn, P., S. Sornmani and T. Hungsapruek (1986). Social, behavioural, housing factors and their interactive effects associated with malaria occurrence in east Thailand. *Southeast Asian J. Trop. Med. Pub. Hlth.,* **17:** 386-392.
10. Gunasekaran, K., S.S. Sahu, S.K. Parida, C. Sadanandane, P. Jambulingam and P.K. Das (1989). Anopheline fauna of Koraput district, Orissa state, with particular reference to transmission of malaria. *Indian J. Med. Res.,* **89:** 340-343.
11. Baker, D. and J.A. Nelder (1978). *Numerical Algorithms Group: GLIM Version 3.77: User Manual.* (Oxford).
12. Aitkin, M., D. Anderson, B. Francis and J. Hinde (1989). Statistical Modelling in GLIM. *Oxford Statistical Science Series* 4. (Clarendon Press, Oxford).
13. MacCormack, C.P. (1984). Human ecology and behaviour in malaria control in tropical Africa. *Bull. WHO,* **62** (suppl.): 81-87.
14. Coluzzi, M. (1984). Heterogeneities of malaria vectoria' system in tropical Africa and their significance in malaria epidemiology and control. *Bull. WHO,* **62** (suppl.): 107-113.
15. Kirnowordoyo, S. and Supalin (1986). Zooprophylaxis as a useful tool for control of *A. aconitus* transmitted malaria in Central Java, Indonesia. *J. Com. Dis.,* **18:** 90-94.
16. Sota, T. and M. Mogi (1989). Effectiveness of zooprophylaxis in malaria control: a theoretical inquiry, with a model for mosquito populations with two blood meal hosts. *Med. Vet. Entomol.,* **3:** 337-345.
17. Burkot, T.R. (1988). Non-random host selection by anopheline mosquitoes. *Parasitol. Today,* **4:** 156-162.
18. Kalra, N.L. (1978). National Malaria Eradication Programme, India — its problems, management and research needs. *J. Com. Dis.,* **10:** 1-20.
19. Greenwood, B.M. (1989). Impact of culture and environmental changes on epidemiology and control of malaria and babesiosis: the microepidemiology of malaria and its importance to malaria control. *Trans. R. Soc. Trop. Med. Hyg.,* **83** (suppl.): 25-29.

Indian Journal of Malariology
Vol. 28, March 1991, pp. 39-43.

Evaluation of Juvenile Hormone Analogue JHM/S-31183 against Immature Stages of Mosquitoes in Natural Habitats

M.A. ANSARI*, V.P. SHARMA*, P.K. MITTAL* and R.K. RAZDAN*

A study was carried out to evaluate the potentiality of Juvenile hormone compound JHM/S-31183 against immatures of mosquitoes in natural habitats. Of two formulations tested 1% emulsifiable formulation was marginally superior than the granule formulation. Adult emergence of *An. stephensi* was completely inhibited upto 12 weeks when 0.5% granule formulation was applied @ 0.04 ppm in wells as against 50% inhibition upto 8 weeks in pools. However, in *Culex quinquefasciatus* the per cent inhibition of adult emergence varied from 52 to 90 per cent. 100% inhibition in pools upto one week at 0.04 ppm in *An. stephensi* was also obtained with 1% emulsifiable formulation but the effect was diluted in successive weeks. The impact of this formulation was not much pronounced against *Culex quinquefasciatus.*

INTRODUCTION

Insect growth regulators are fourth generation insecticides and are quite specific and selective in action. These are mainly of two types viz., Ecdysoids and Juvenoids. Ecdysoids inhibit chitin synthesis in embryonic stages particularly larvae and Juvenoids or Juvenile Hormone mimics, inhibit pupation[1]. These compounds are highly effective against mosquito immatures and do not produce harmful effects on non-target organisms[2]. In view of this characteristic they are considered potential alternatives for the control of mosquito breeding[3,4]. Recently a new Juvenile Hormone mimic compound JHM/S-31183 has been developed and its exogenous application interferes with the development sequence of metamorphosis, particularly against late instars and results into larval-pupal mosaics and over pupal moulting[5]. Studies were therefore initiated to evaluate this compound in the laboratory and field. Results of this study are reported in this paper.

Accepted for publication: 20 December 1990.

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

MATERIAL AND METHODS

Formulations of JHM/S-31183 (2-[1-methyl-2-(4-Phenoxy Phenoxy) ethoxy] pyridine) were obtained through the courtesy of Sumitomo Chemical Company Ltd. of Japan. The active ingredient was 1 and 0.5 per cent in emulsifiable concentrate (E.C.) and granule formulation, respectively. Since it was not possible to dilute the granule formulation therefore only 1% emulsifiable concentrate was tested in the laboratory by making serial dilutions with distilled water. Tests were conducted in plastic bowls containing 200 ml water. Twenty larvae (IV stage) of colonized strains of *An. stephensi*

and *Culex quinquefasciatus* were introduced in each bowl and observations were recorded at an interval of 24 hours till the emergence of adults. Control with plain water was run concurrently. Four replicates of each concentration were made. Routine food schedule to larvae was followed both in treated and untreated bowls[6]. Different stages such as larval pupal mosaics and incomplete emergence of adults were taken as dead pupae. Lc50 and Lc90 values were determined by plotting the dosage mortality curve on log-probit paper.

Field evaluation was carried out in pools and unused and abandoned wells against immatures of culicines and anophelines. Granule formulation (0.5%) was tested both in pools and wells while E.C. formulation (1%) was tested only in pools. The compound was applied in these habitats on the basis of volume of water in the natural habitat. The granule formulation was broadcast over the water surface while E.C. formulation was sprayed with the help of stirrup pumps. As per instructions of the manufacturers two dosages viz., 0.04 mg/l and 0.1 mg/l almost equal to 2 and 5 g (ai) per 100 square meter (Average depth approx. 50 cms) were tested under field conditions. Standard procedure as described by Hemmingway *et al.*[7] was used to evaluate the impact of the compound on adult emergence. Water samples from treated and untreated habitats were collected and laboratory reared IV instar larvae of *An. stephensi* and *Culex quinquefasciatus* were introduced for bioassay. Observations were made till the emergence of adults. Initial samples from the field were collected at intervals of one week for a month and later at intervals of 1 month till the pools were completely dried or the impact was diluted. Per cent inhibition was calculated on the basis of untreated control run concurrently by using the following formula:

$$\% \text{ inhibition} = \frac{\text{No. of larvae kept for emergence} - \text{No. of adults emerged}}{\text{No. of larvae kept for emergence}} \times 100$$

RESULTS AND DISCUSSION

Results of laboratory tests revealed that the compound JHM/S-31183 was highly effective against IV instar larvae of *An. stephensi* and

Table 1. Evaluation of S-31183 (0.5% granule) against *Anopheles stephensi* and *Culex quinquefasciatus* in unused abandoned wells

Days Post-treatment	% inhibition of adult emergence					
	A. stephensi			*Culex quinquefasciatus*		
	Untreated	Treated		Untreated	Treated	
		0.04 ppm	0.1 ppm		0.04 ppm	0.1 ppm
1	20	100	100	17	87.5	100
7	20	100	100	20	62	96
14	19	100	100	19	80	100
21	23	100	100	17	90	84
28	27	100	100	15	68	76
56	37	100	98.6	11	74	68
84	36	100	100	19	52	69.3

Culex quinquefasciatus. *An. stephensi* was more susceptible than *Culex quinquefasciatus*. The estimated Lc50 and Lc90 values were 0.046 ppb (0.000046 mg/l) and 0.1 ppb (0.0001 mg/l) for *An. stephensi* and 0.098 ppb (0.000098 mg/l) and 0.5 ppb (0.0005 mg/l) for *Culex quinquefasciatus*. This conforms to the earlier findings of Mulla *et al.*[4] who obtained 90% inhibition of emergence in *Culex quinquefasciatus* at a concentration of 0.0004 mg/l with 10% E.C. formulation.

Results of field evaluation are presented in Tables 1-3.

Table 1 shows the impact of granule formulation against larvae of *An. stephensi* and *Culex quinquefasciatus* in water collected from unused wells. It appears from the table that impact of the compound was variable from species to species. Anophelines were more susceptible than culicines. Complete inhibition of adult emergence was observed at doses as low as 0.04 ppm in *An. stephensi* in unused wells for 12 weeks. However in *Culex quinquefasciatus* the per cent inhibition varied from 52 to 90%. Higher dosages i.e., 0.1 ppm produced almost complete inhibition of adult emergence (96-100%) for 2 weeks but the impact was diluted in successive weeks. Nevertheless 60-70% inhibition of adult emergence was observed throughout the study period.

It is interesting to note that similar results were not reproduced when the same formulation was evaluated in pools which were directly exposed to sunlight and frequently disturbed by cattle. The per cent inhibition varied from 84 to 89% at 0.04 and 0.1 ppm, respectively just after 24 hrs. of treatment. It was further reduced to 52 and 60% after 4 weeks of treatment. As pointed out earlier the larvae of *Culex quinquefasciatus* were not as susceptible as anophelines. This was further substantiated when the results of per cent inhibition were compared with anophelines in pools. The per cent inhibition was 40.0 in *Culex quinquefasciatus* in pools after 48 hours as against 84-89% in *An. stephensi*. Similarly, after 4 weeks it was reduced to 12-16% at 0.04 and 0.1 mg/l, respectively as against 52 and 60% in *An. stephensi* (Table 2). This clearly indicates that the compound is not equally effective against culicines and anophelines and to obtain similar levels of inhibition of adult emergence higher concentrations are required.

Table 3 shows the efficacy of 1% E.C. formulation against *An. stephensi* and *Culex quinquefasciatus* in water collected from treated pools. The formulation at 0.04 and 0.01 ppm produced 100% inhibition of adult emergence in *An. stephensi* upto one week as compared to 98 and 100% against *Culex quinquefasciatus* for just one day. After one week the per cent inhibition against *Culex quinquefasciatus* was only 26 and 46 at 0.04 and 0.01 ppm, respectively. The impact was not much pronounced in successive weeks of observations. However, against *An. stephensi* 89-92 % inhibition was observed even after 4 weeks of the treatment. It is interesting to note that enhanced doses of E.C. formulation (0.1 ppm) did not produce proportionate increase in inhibition of adult emergence under field conditions.

The persistence of the compound was also variable from species to species and habitat to habitat. The activity of this compound in unused abandoned wells was observed >84 days against *An. stephensi* at 0.04 ppm with granule formulation as against 21 days in *Culex quinquefasciatus*. The persistence in pools with similar dosages was <1 week against *An. stephensi* and *Culex quinquefasciatus*. Similar results were obtained with E.C. formulation in pools. Though factors responsible for low persistence in pools were not investigated, it may be possible that the compound degrades fast under direct sunlight or disturbance of water by cattle. Earlier studies by Hemmingway *et al.*[7] revealed about 70% chemical degradation of S-31183 within a day after its application. However, further degradation was slowed down and it took about 70 days for complete degradation of the compound. During

Table 2. Evaluation of S-31183 (0.5% granule) against *Anopheles stephensi* and *Culex quinquefasciatus* in pools

Days Post-treatment	% inhibition of adult emergence					
	A. stephensi			*Culex quinquefasciatus*		
	Untreated	Treated		Untreated	Treated	
		0.04 ppm	0.1 ppm		0.04 ppm	0.1 ppm
1	17	84	89	4	40	40
7	14	62	68	12	38	59
14	12	62	78	4	28	37
21	16	54	76	6	52	56
28	24	52	60	6	12	16
56	26	46	48	8	24	18

Table 3. Evaluation of S-31183 (1% EC) against *Anopheles stephensi* and *Culex quinquefasciatus* in pools

Days Post-treatment	% inhibition of adult emergence					
	A. stephensi			*Culex quinquefasciatus*		
	Untreated	Treated		Untreated	Treated	
		0.04 ppm	0.1 ppm		0.04 ppm	0.1 ppm
1	16	100	100	8	98	100
7	36	100	100	8	26	46
14	20	54	84	8	22	44
21	12	86	62	4	32	16
28	36	92	89	4	10	13
56	30	96	82	2	86	28

the present study indirect observations revealed no degradation of the compound in wells which were not exposed to direct sunlight. This may be one of the reasons why the compound was effective for a longer duration in unused wells in comparison to pools which are exposed to direct sunlight. The study has also revealed that of two formulations viz., 0.5% granule and 1% E.C. formulation tested in pools, the latter one was more effective initially at the time of application. However, the delayed impact was more or less the same. It may be pointed out that these compounds are highly specific in their action and are quite unlikely to interfere in the development of other aquatic organisms. Earlier studies[4] revealed that the compound is safe to fish and other non-target aquatic organisms.

Though the compound S-31183 was found to be highly effective particularly against anophelines at very low rates of application (0.04 ppm) in unused wells, it did not produce the same results when it was applied in shallow water bodies which support heavy breeding of *An. culicifacies* a principal vector of rural malaria in Northern India. Similarly the low activity of these formulations against pest mosquito *Culex quinquefasciatus* that breeds profusely in urban areas limits its use in large-scale control operations. Further studies are therefore indicated to evaluate the impact of the compound on vector density and incidence of malaria and also its impact on non-target organisms.

ACKNOWLEDGEMENTS

Technical assistance rendered by Mr. R.K. Razdan, Mr. C.S. Sahota, Mr. S.N.S. Kuchwaha and Mr. K.C. Pushap is gratefully acknowledged.

REFERENCES

1. Mouchet, J. (1980). Lutte contre les vecteurs et nuisances en santil publique, *Paris Maladies Infectieuses,* 8120 B-10, 3-1980: 1-16.

2. Mulla, M.S., H.A. Darwazeh, L. Ede and B. Kennedy (1985). Laboratory and field evaluation of the IGR fenoxycarb against mosquitoes. *J. Am. Mosq. Cont. Assoc.*, **1:** 442-448.

3. Schaefer, C.H., T. Miura, E.F. Dupras Jr., R.J. Stewart, W.H. Wilder and L. Jurd (1984). Biological activity of J-2931 against mosquitoes (Diptera: Culicidae) and selected non-target organisms and assessments of potential environment impact. *J. Econ. Entomol.*, **77:** 425-429.

4. Mulla, M.S., H.A. Darwazeh, B. Kennedy and D.M Dawson (1986). Evaluation of new insect growth regulators against mosquitoes with notes on non-target organism. *J. Am. Mosq. Cont. Assoc.*, **2:** 314-320.

5. SCC Ltd. (1988). A guide for field evaluation. Juvenile Hormone Mimic S-31183. (Sumitomo Chemicals Co. Ltd., Osaka, Japan) : 1-36.

6. Ansari, M.A., V.P. Sharma and R.K. Razdan (1978). Mass rearing procedures for *Anopheles stephensi* Liston. *J. Com. Dis.*, **10**(2) : 131-135.

7. Hemmingway, J., B.C. Boning, K.G.I. Jayawardana, I.S. Weerasinghe, P.R.J Herath and H. Oouchi (1988). Possible selective advantage of *Anopheles* spp. (Diptera: Culicidae) with the oxidase and acetylcholinesterase based insecticide resistance genes after exposure to organophosphates or an insect growth regulator in Sri Lankan rice fields. *Bull. Ent. Res.*, **78:** 471-478.

Indian Journal of Malariology
Vol. 28, March 1991, pp. 45-50.

Mosquito Fauna of Certain Areas of Deltaic West Bengal—A Preliminary Report

MITA S. MAHAPATRA*, SUKUMAR DAS* and NEELAM TANDON**

Survey of mosquito fauna in certain areas of deltaic Bengal was conducted between September 1989 to February 1990. *Anopheles vagus* predominated in Canning study area and *Anopheles hyrcanus* in Budge Budge. Comparatively low densities of *Anopheles annularis* were recorded from Canning (6.05% of the total anophelines) and Budge Budge (7.36% of the total anophelines), respectively. *C. vishnui, C. pseudovishnui* and *C. tritaeniorhynchus* were collected from both Canning and Budge Budge study areas. *C. pseudovishnui* predominated in Canning and *C. vishnui* in Budge Budge. Amongst the three species of *C. vishnui* complex, *C. tritaeniorhynchus* ranked third in order of preponderance at both the study areas.

INTRODUCTION

Mosquito fauna of deltaic West Bengal was explored in the early decades of the century [1,2,3,4,5] and studies on the distribution, relative prevalence, breeding habits, taxonomy and bionomics of vector species of malaria were made [6,2,7,8,9]. *Anopheles sundaicus* and *Anopheles philippinensis* were incriminated as the principal vectors of malaria in the estuarine and deltaic areas of West Bengal, respectively [3,4,5,10,11]. Claims of disappearance of the two species were made [12,13] but the presence of *An. philippinesis* in certain pockets of rural Bengal in Birbhum district was reported a decade later [14].

Accepted for publication : 27 December 1990.

* MUC Womens College
Burdwan-713 101, India.
** Department of Medical Entomology
School of Tropical Medicine
Calcutta-700 073, India.

An increase in the incidence of malaria has been reported from certain areas of deltaic Bengal (South 24-Parganas), in spite of implementation of chemical and biological methods of vector control. (Data obtained from Dy. Chief Medical Officer of Health-II, South 24-Parganas, Govt. of West Bengal).

Perusal of literature reveals that mosquito survey in the above areas has not been conducted in the recent past and our knowledge of the mosquito fauna of 24-Parganas dates back to almost half a century ago.

Against this background, a preliminary survey of the mosquito fauna was conducted in certain areas of deltaic Bengal, and the results are presented in this communication.

MATERIAL AND METHODS

Adult mosquitoes were collected from 32 randomly selected villages, 11 in block Canning-I

Table 1. Female mosquito collection during September 1989-February 1990 in Block Canning-I of District South 24-Parganas, West Bengal

Sl. No.	Species	Collection spots											No. of mosquitoes/No. of mosquitoes per man hour	Percentage of total anopheline catch
		Mahamaya pur	Dighirpar	Kumarsha	Herobhanga	Paramura	Grosthingroy	Rajarhat	Nikari ghata	Himachakhali	Mayapur	Chakhimatpur		
1.	*Anopheles vagus*	22	44	22	44	42	4	6	26	18	62	20	310/14.09	44.66
2.	*An. hyrcanus*	4	14	36	4	20	10	6	66	8	6	6	180/8.18	25.93
3.	*An. barbirostris*	—	2	—	—	8	—	—	12	64	62	—	148/6.72	21.32
4.	*An. aconitus*	—	6	—	—	2	—	—	—	—	—	—	8/0.36	1.15
5.	*An. annularis*	1	—	18	4	—	—	—	4	10	4	1	42/1.90	6.05
6.	*An. subpictus*	—	—	6	—	—	—	—	—	—	—	—	6/0.27	0.86
	Total												694/31.54	
														Percentage of total culicine catch
7.	*Culex quinquefasciatus*	4	22	8	8	32	4	54	22	30	—	—	184/8.36	42.99
8.	*Cx. vishnui*	—	6	2	2	20	22	7	4	—	3	—	66/3	15.42
9.	*Cx. pseudovishnui*	2	6	10	42	8	3	21	6	10	8	—	116/5.27	27.10
10.	*Cx. tritaeniorhynchus*	8	—	—	4	—	9	6	—	3	—	4	34/1.54	7.94
11.	*Cx. gelidus*	—	—	—	—	—	—	—	—	—	—	—	—	—
12.	*Armigeres* sp.	—	—	—	4	4	—	12	—	—	—	—	20/0.90	4.67
13.	*Mansonia annulifera*	—	—	—	—	—	—	2	—	—	6	—	8/0.36	1.86
	Total												428/19.43	

Total no. of manhours = 22.

(district South 24-Parganas) and 21 in Budge Budge Block (district North 24-Parganas) respectively. Altogether 32 survey tours were carried out (eleven in Canning-I and twenty one in Budge Budge), between September 1989 to February 1990. Collection of mosquitoes was done in the early morning between 0600 hrs to 0800 hrs with the help of aspirators and test tubes, from human dwellings and cattlesheds.

RESULTS

A total of 1122 and 1292 adult mosquitoes were collected from 11 and 21 villages in Canning-I and Budge Budge, respectively. The mosquito fauna of Canning-I comprised of 12 species belonging to 4 genera. Only 11 species of mosquitoes distributed over two genera were recorded from Budge Budge area. Anophelines comprised 61.76% and 88.09% of the total mosquito collection made at Canning-I and Budge Budge areas, respectively. Six species of anophelines, namely *An. vagus, An. hyrcanus, An. barbirostris, An. aconitus, An. annularis* and *An. subpictus* were recorded from both the study areas. *An. annularis,* the reported vector of malaria in rural Bengal, ranked fourth in order of dominance (amongst anophelines) at both the study sites. The species was collected from 7/11 villages in Canning and 9/21 villages in Budge Budge area. *An. vagus* was the most dominant species (44.66%) in Canning-I followed by *An. hyrcanus* (25.93%) and *An. barbirostris* (21.32%) [Tables 1 and 2].

In Budge Budge area, however, *An. hyrcanus* group constituted 46.49% of the total anopheline collection, followed by *An. barbirostris* (32.45%) and *An. vagus* (12.63%), respectively (Table 2.) *An. subpictus* and *An. aconitus* were encountered in scanty numbers both at Canning and Budge Budge study areas.

Four species of *Culex* mosquitoes were recorded from Canning and five from Budge Budge area, respectively. All the three species of *Culex vishnui* complex were encountered at both the study sites. In Canning, of the 11 villages surveyed, *Culex vishnui* (3/man hour) *Culex pseudovishnui* (5.27/man hour) and *Culex tritaeniorhynchus* (1.54/man hour) were recorded from 8, 10 and 6 villages, respectively. *Culex pseudovishnui* (27.1%) was the most dominant amongst the three species in Canning followed by *C. vishnui* (15.42%) and *C. tritaeniorhynchus* (7.94%), respectively. Of the 21 villages surveyed in Budge Budge, *Culex vishnui* (1.38/man hour) and *Culex pseudovishnui* (0.76/man hour) were recorded from 8 villages each, while *Culex tritaeniorhynchus* (0.28%) was encountered in three villages only. *C. vishnui, C. pseudovishnui* and *C. tritaeniorhynchus* constituted 38.15%, 21.05% and 7.89% of the total culicine population.

Culex quinquefasciatus (42.99%) was the most dominant species in Canning. In Budge Budge study area however, *Culex vishnui* (38.15%) predominated, and *C. quinquefasciatus* ranked second (27.63%) in order of predominance.

A few *Armigeres* species (4.67%) and *Mansonia annulifera* (1.86%) were encountered in Canning only, while *Culex gelidus* (5.26%) was recorded from 3 villages in Budge Budge only (Tables 1 and 2).

DISCUSSION

During mosquito surveys of deltaic West Bengal, *Anopheles philippinensis* and *An. sundaicus* were incriminated as vectors in the plains and estuarine areas, respectively[1,3,5]. Subsequently, after the disappearance of these two species[12,13], *An. annularis* was reported to be the vector of malaria in rural West Bengal[15].

During the present survey, except *An. annularis,* no other species of *Anopheles,* known or suspected to be the vector of malaria, was encountered in the study areas. In view of the high prevalence of malaria in some areas of

Table 2. Female mosquito collection during September 1989 - February 1990 in

Sl. No.	Species	Collection										
		Nauda-khali	Dhancha beria	Hauri	Banarai-pur	Malan-gadar	Jana-para	Don-garia	Raniya	Pukur-shita	Kulto-kari	Telari
1.	*Anopheles vagus*	2	—	4	—	2	12	4	12	28	6	—
2.	*An. hyrcanus*	—	28	38	42	38	2	8	16	34	60	4
3.	*An. barbirostris*	—	42	22	26	20	6	16	10	4	20	4
4.	*An. aconitus*	—	2	—	—	—	—	—	—	2	—	—
5.	*An. annularis*	—	—	—	—	2	12	—	—	26	—	—
6.	*An. subpictus*	—	—	—	—	—	—	—	—	—	—	—
	Total	—	—	—	—	—	—	—	—	—	—	—
7.	*Culex quinque-fasciatus*	10	—	—	2	—	—	—	—	4	—	2
8.	*Cx. vishnui*	4	—	—	—	4	—	—	—	6	6	—
9.	*Cx. pseudovishnui*	—	—	—	2	—	—	4	—	8	—	—
10.	*Cx. tritaeni-orhynchus*	—	—	—	—	—	—	—	—	—	—	—
11.	*Cx. gelidus*	—	—	—	—	—	—	—	—	—	—	—
12.	*Armigeres* sp.	—	—	—	—	—	—	—	—	—	—	—
13.	*Mansonia annulifera*	—	—	—	—	—	—	—	—	—	—	—
	Total											

Total no. of man hours = 42.

deltaic Bengal, and also due to the doubtful position of *An. annularis* in maintaining endemicity in the rural areas[13], the role of secondary vectors and new vectors is worth considering[16], and needs to be investigated.

It is worth pointing out that *C. vishnui* has been incriminated as the vector of Japanese encephalitis in West Bengal and northern India, and *C. tritaeniorhynchus* in southern part of the country and other areas[17,18].

Budge Budge block of District North 24-Parganas, West Bengal

Spots										No. of Mos./ No. of Mos. per man hour	Percentage of total anopheline catch
Bawali	Malgadar(S)	Janapara	Raipur	Naldanri	Talmaria	Chalkkashipur	Banaraipur	Paulpara	Sk. para		
—	2	16	10	2	2	6	—	14	28	144/3.42	12.63
42	38	22	—	28	18	16	42	4	50	530/12.61	46.49
6	20	14	—	30	16	24	26	6	18	370/8.80	32.45
—	—	—	—	—	—	—	—	—	2	6/0.14	0.52
—	2	14	14	2	8	—	—	—	4	84/2	7.36
—	—	—	2	4	—	—	—	—	—	6/0.14	0.52
										1140/27.14	
											Percentage of total culicine catch
—	—	—	—	6	—	—	2	—	16	42/1	27.63
—	4	—	—	28	2	—	—	—	4	58/1.38	36.15
—	—	—	—	10	4	4	2	—	6	32/0.76	21.05
—	—	—	—	14	—	4	—	—	8	12/0.28	7.89
—	—	—	—	4	—	—	—	—	—	8/0.19	5.26
—	—	—	—	—	—	—	—	—	—	0	—
—	—	—	—	—	—	—	—	—	—	0	—
										152/3.61	

It is however, interesting to mention that in mosquito surveys conducted for three consecutive years (1980-1983) in JE affected areas of Burdwan district, *Cx. tritaeniorhynchus* was not recorded[17]. Prevalence of *Culex tritaeniorhynchus* in JE affected areas of Burdwan and Bankura was however reported earlier [19,20].

It is worth pointing out that 24-Parganas is one of the three districts in West Bengal which have not been affected by Japanese encephalitis till now[18]. This however presents an enigmatic situation, more so when the density of three species of *Culex vishnui* complex in the study area are almost comparable to those recorded

from Asansol subdivision of Burdwan district, West Bengal[19]. In addition two anopheline species, i.e., *Anopheles hyrcanus* and *An. barbirostris*, from which JE virus was isolated during 1973 epidemic from Burdwan district, were also recorded in the study areas. It is likely that the virus is probably in the quiescent form in the unaffected districts, and as soon as the biological and ecological factors become favourable, there might be an outbreak, more so since the ecological conditions here do not substantially differ from those in areas where the disease has been prevalent[21].

With the aforesaid backdrop, systematic entomological surveys, along with investigations on related biological and ecological parameters are likely to solve the intriguing problems pertaining to vector borne diseases.

ACKNOWLEDGEMENT

The authors are grateful to the Director, School of Tropical Medicine, Calcutta for permission to publish this paper.

REFERENCES

1. Iyengar, M.O.T. (1931). The distribution of *Anopheles ludlowii* in Bengal and its importance in malarial epidemiology. *Indian J. Med. Res.*, **19:** 499.
2. Iyengar, M.O.T. (1932). *Anopheles* breeding in relation to season. *Indian J. Med. Res.*, **19** (3): 917.
3. Iyengar, M.O.T. (1939). A years work on dissection of *Anopheles* for natural malarial infection. *J. Mal. Inst. Ind.*, **2**(1): 105.
4. Iyengar, M.O.T. (1940). Further observations on vectors of malaria in Bengal and notes on the seasonal infectivity of *Anopheles*. *J. Mal. Inst. Ind.*, **3:** 115.
5. Iyengar, M.O.T. (1942). Studies on malaria in the deltaic region of Bengal. *J. Mal. Inst. Ind.*, **4**: 435.
6. Iyengar, M.O.T. (1928). *Report on the malaria survey of the environs of Calcutta.* (Bengal Secretariat Book Depot).
7. Sen, P. (1937). The relative prevalence of anophelines in houses and cattle sheds in deltatic Bengal. *Rec. Mal. Surv. Ind.*, 7 (2 & 3): 147.
8. Harry, G. and Timbers (1935). Studies on malaria in villages in Western Bengal. *Rec. Mal. Surv. Ind.*, **5**: 345.
9. Tandon, N., A.K. Hati and A.K. Mukhopadhyaya (1978). On taxonomy of malaria vectors of India. *Indian Med. Gaz.*, **112** (9): 315.
10. Sen, P. (1938). *Anopheles sundaicus* (Ludlowii) and malaria in Calcutta. *J. Mal. Inst. Ind.*, **1**: 83-98.
11. Sur, P. (1928). *Anopheles philippinensis* as a natural carrier of the malaria parasite in Bengal. *Indian J. Med. Res.*, **16** (1): 45.
12. Anon (1970). Report of the evaluation in depth of the NMEP of India.
13. Basu Mallik, K.C. (1989). *Malaria in West Bengal.* 1st ed. (Srikanta Basu Mallik, C.I.T. Scheme VIM, Calcutta): 185.
14. Hati, A.K. and N.C. Mukhopadhyay (1980). Distribution of *Plasmodium falciparum* in West Bengal. *Trans. R. Soc. Trop. Med. Hyg.*, **74** (3): 480.
15. Ghosh, K.K., N. De, N. Tandon and A.K. Hati (1985). *Anopheles annularis* as the vector of *Plasmodium vivax* malaria in rural West Bengal. *J. Beng. Nat. Hist. Soc. New series*, **4**(2): 93.
16. Arora, R.R. (1961). An outbreak of malaria in Durgapur Steel Project area (West Bengal) during 1960. *Bull. Nat. Soc. Ind. Mal. Com. Dis.*, **9**: 189.
17. Hati, A.K. (1986). Studies on *Culex vishnui* and other related vectors of Japanese encephalitis in rural West Bengal. (Prof. A.K. Hati, Deptt. of Medical Entomology, School of Tropical Medicine, Calcutta).
18. Sehgal, S., R. Bhatia and M. Bhardwaj (1988). *Proceedings of the workshop on Japanese encephalitis.* National Institute of Communicable Diseases, 18-22 Jan.
19. Soman, R.S., H.N. Kaul, P.Y. Guru, G.B. Modi, S.N. Guttikar and V. Dhanda (1976). A report on the mosquitoes collected during an epidemic of encephalitis in Burdwan and Bankura districts, West Bengal. *Indian J. Med. Res.*, **64**: 808-813.
20. Rajagopalan, P.K. and K.N. Panicker (1978). A note on the 1976 epidemic of Japanese encephalitis in Burdwan District, West Bengal. *Indian J. Med. Res.*, **68**: 393-398.
21. Chakraborty, S.K., J.K. Sarkar, M.S., Chakraborty, M.K. Mukherjee, K.K. Mukherjee, B.C. Das and A.K. Hati (1975). The first epidemic of Japanese encephalitis studies in India—Virological studies. *Indian J. Med. Res.*, **63** (1): 77-82.

Indian Journal of Malariology
Vol. 28, March 1991, pp. 51-54.

Role of *Azolla* in Controlling Mosquito Breeding in Ghaziabad District Villages (U.P.)

M.A. ANSARI* and V.P. SHARMA*

A survey was carried out during post-monsoon period (1988-89) in villages of Dhaulana Primary Health Centre, Distt. Ghaziabad (U.P.) to evaluate the utility of *Azolla pinnata* for the control of mosquito breeding in different habitats. Results of the survey revealed that pools, ponds, wells, rice fields and drains were infested with *Azolla*. Infestation rate and intensity of infestation varied from habitat to habitat. Maximum infestation (36.5%) was observed in pools and minimum (3.7%) in rice fields. Anopheline breeding was almost completely suppressed (0-1.6/dip) in pools, wells and ponds completely covered with *Azolla*. The breeding of *Culex* spp. was not completely inhibited in any habitat, though reduction in immature density was observed in comparison to control. The role of *Azolla* in controlling mosquito breeding and its association with the blue green algae which fixes nitrogen is discussed.

INTRODUCTION

The presence or absence of aquatic plants is one of the ecological factors that determine the suitability of a habitat for mosquito breeding. There are certain plants which provide shade, shelter, food, resting places, refuge from predation and higher oxygen. However, certain free floating ferns belonging to the family Azollacae may prove deterimental for mosquito breeding. The possibility of utilizing this biomass for mosquito control and simultaneously as a manure for paddy cultivation is attracting attention. Of the six species of *Azolla,* only *A. pinnata* is found in India with a blue green algal symbiont *Anabaena azollae* which fixes atmospheric nitrogen for rapid growth of the plant. In view of its potential as a biological control agent a survey was carried out in 1988-89 during post-monsoon period in certain villages of Distt. Ghaziabad (U.P.) to evaluate its potential as a biological control agent for the control of mosquito breeding. Results of this study are reported in this paper.

MATERIAL AND METHODS

Surveys were carried out in post-monsoon period in certain villages of Dhaulana PHC, Distt. Ghaziabad (U.P.) to assess the density of immatures of mosquitoes in *Azolla* infested habitats. Dipping method was used to monitor the density of mosquitoes in places covered completely or partially with *Azolla*. Habitats which were not covered with *Azolla* were taken as control. Wells, pools, drains and paddy fields were checked for the presence of *Azolla pinnata*.

Accepted for publication : 1 January 1991.

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

Samples of *Azolla* from both partially and completely covered habitats were brought to the laboratory and sent to the National Facility for Blue Green Algal Collections, Indian Agriculture Research Institute (IARI), New Delhi for confirmation of the fern species. The pH of each habitat was also recorded to observe any correlation with the growth of *Azolla*. Density of all mosquito immatures was pooled together and per dip density was calculated for each habitat.

RESULTS AND DISCUSSION

Results of the survey revealed that natural infestation of *Azolla* species was observed in pools, ponds, wells, rice fields and drains which are important breeding places for *An. culicifacies* during post-monsoon period. However, the infestation rate and intensity of infestation vary from habitat to habitat. The highest infestation was observed in pools and lowest in paddy fields. Of the 178 pools checked, 65(36.5%), were completely covered with fern growth while 62(34.8%) were partially covered. No infestation was observed in 51(28.6%) pools during the entire period of the survey. Similarly 5(6.5%) wells were completely and 7(9.2%) were partly covered with *Azolla*. The remaining wells 64(84.2%) had no *Azolla* (Table 1). It may be pointed out that infested wells were located in fields and had access to sunlight. It was also interesting to note that large water bodies viz., ponds, drains, rice fields were not found completely covered with *Azolla* although par-

Table 1. Natural infestation of *Azolla* in different habitats (Sept.-Oct. 1980-89)

Habitat	Total checked	Completely covered	Partially covered	Without *Azolla*
Pools	178	65	62	51
Wells	76	5	7	64
Paddy fields	27	1	2	24
Ponds	30	0	22	8
Drains	34	0	19	15

Table 2. Cumulative record of immature density in different habitats infested with *Azolla*

Habitats	Completely covered		Partially covered		Without *Azolla*	
	Culex sp.	Anophelines	*Culex* sp.	Anophelines	*Culex* sp.	Anophelines
Pools	5.7	1.6	31.0	23.0	91.8	25.0
Wells	6.6	0.0	13.1	0.8	52.7	34.9
Paddy fields	8.8	0.0	8.4	1.7	11.6	1.4
Ponds	—	—	8.4	1.4	73.3	13.9
Drains	—	—	21.0	11.2	153.0	25.4

tially covered habitats were quite common. This suggests that the growth of *Azolla* under natural conditions is not uniform and this may be the reason that except one paddy field none of the other habitats were found completely covered with *Azolla*.

The impact of *Azolla* on density of mosquito immatures is shown in Table 2. It is clear from the table that mere presence of *Azolla* did not completely eliminate the breeding of mosquitoes.

The anopheline immature density/dip was 1.6, 0.0 and 0.0 in pools, wells, and paddy fields respectively in completely covered habitats as against 25.0, 34.9 and 1.4 in *Azolla* free habitats. The partial coverage of habitats also suppressed the breeding of anophelines in wells, ponds and drains but not in pools and paddy fields. It was interesting to note that the breeding of *Culex* spp. was not completely inhibited even in those habitats which were found completely covered with *Azolla*. However the reduction in density/dip was evident in most of the *Azolla* infested habitats. The immature *Culex* density/dip was 5.7, 6.6 and 8.8 in pools, wells and paddy fields, respectively in completely covered habitats as against 91.8, 52.7 and 3.0, respectively in *Azolla* free habitats. Similarly in partially covered habitats the density/dip was 31.0, 13.1, 8.4, 0.4 and 21.0 in pools, wells, paddy fields, ponds and drains, respectively as against 91.8, 52.7, 3.0, 73.3 and 153.0 in *Azolla* free habitats. This clearly indicates that natural infestation of *Azolla* in temporary and permanent water bodies does suppress the breeding of mosquitoes. These observations confirm the earlier findings of Lu Bao-lin[1] who observed an inhibitory effect of *Azolla filiculoides* on the breeding of *Culex tritaeniorhynchus* under controlled conditions. Similar inhibitory effect on anophelines was observed[2] with another aquatic fern *Salvinia auriculata* in Guatemala. The degree of suppression was proportionate to the intensity of infestation in each habitat. The high inhibition of anophelines as compared to *Culex* may be due to the fact that anopheline larvae are surface feeders and *Azolla* acts as a physical barrier for both oviposition and development of the larvae.

It was also observed that there was no correlation of pH with the infestation rate of *Azolla*. In completely covered habitats pH ranged from 6.5 to 8.5 and the same range was observed in partially covered and *Azolla* free habitats.

The possibility of using *Azolla* for the control of mosquito breeding particularly the vectors of malaria does exist provided the technology is developed to produce *Azolla* for dissemination in water bodies of the area. To optimize its effect in rice fields, early application of *Azolla* is suggested so that it can cover the entire surface of water before the breeding starts.

Azolla has an additional advantage of fertilizing the rice paddy as it lives in association with blue green alga *Anabaena azollae* which fixes nitrogen. The association yields substantial amount of nitrogen ranging from 15-40 kg/hac.[3]. In addition *Azolla* is also used as feed for livestock, weed control agent and can be converted to compost. Chinese and Vietnamese consider the *Azolla* plant as a miniature nitrogen factory and it is being used as biofertilizer in rice cultivation[1]. Studies are therefore needed on *Azolla* propagation and its role in mosquito control.

ACKNOWLEDGEMENTS

Technical assistance rendered by Mr. R.K. Razdan, Mr. C.S. Sahota, Mr. S.N.S. Kuchwaha and Mr. K.C. Pushap is gratefully acknowledged.

REFERENCES

1. Lu Bao-lin (1988). The Effect of *Azolla* on Mosquito Breeding. *Parasitol. Today*, **4**(11): 328-329.

2. Hobbs, J.H. and P.A. Molina (1983). The influence of the aquatic fern *Salvinia auriculata* on the breeding of *Anopheles albimanus* in Coastal Guatemala. *Mosq. News*, **43**(4): 456-459.

3. Lumpkin, T.A. and D.L. Plucknett (1982). Azolla as a Green Manure: Use and Management in Crop Production. *Tropical Agriculture Series* No. 5. (Westview Press, USA): 1-230.

Indian Journal of Malariology
Vol. 28, March 1991, pp. 55-62.

Symptomatic Diagnosis of *Plasmodium falciparum* Malaria in Field Conditions

P. GOVARDHINI*, A. MANOHARAN*, S. SUBRAMANIAN*, S.S.S. MOHAPATRA*, P. JAMBULINGAM* and P.K. DAS*

This paper examines the relationship between clinical manifestations and parasitaemia in relation to malaria endemicity. Discriminant analysis, showed that fever alone can detect 74.4% of the parasite positive cases and the inclusion of other symptoms like headache, vomiting, nausea, bodyache and diarrhoea marginally increases the efficiency of discrimination (i.e., from 74.4% to 74.7%). It was observed that the association of symptoms with parasitaemia varies with the degree of malaria endemicity. The percentage of correct classification of parasite carriers varied from 45.7% in the immune population to 80.6% in the non-immune population. A significant difference was observed in the density grades between symptom positive and symptom negative cases. Slide examination in hyperendemic area does not give any advantage over the clinical examination and the data obtained from the slides collected during fever surveys tend to overestimate the malaria incidence in hyperendemic area.

INTRODUCTION

Integration of malaria control with the primary health care system lead to decentralization of slide examination from regional or district level laboratories to primary health centres. Since then the quality of slide examination deteriorated and the main cause of this deterioration has been attributed to increased workload especially in hyperendemic areas[1]. Since the objective of the programme is to control morbidity and mortality and not the eradication of infection, the utility of large scale slide collection and examination has been the subject of much discussion. This paper examines the possibility of malaria diagnosis without resorting to slide examination and utility of this data for epidemiological surveillance.

The foothill and hilly tracts of India represent the core areas of stable malaria with hyperendemicity and with predominance of *Plasmodium falciparum*. These areas are also inaccessible and slide collection and examination causes considerable problems. This communication specially addresses to hyperendemic hilly areas of Koraput District, Orissa. It is well known that *Plasmodium falciparum* infection in human

Accepted for publication: 17 January 1991.

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

beings is characterized by its severity and its irregular pattern of clinical manifestation. Though most *Plasmodium* infections cause chills and fever, *P. falciparum* infection does not follow any of the regular patterns[2]. Therefore, there is a necessity to establish a cause and effect relationship between the parasite infection and the clinical symptoms commonly observed in the field, so that clinical diagnosis of malaria can be more accurate.

MATERIAL AND METHODS

The study has been undertaken in the Borigumma villages of Koraput district comprising hill top and foothill villages of high intensity of transmission (hyperendemic area) and plain and riverine villages with low rates of transmission (hypoendemic area). The earlier analysis of age prevalence data indicated that the immunity is dependent on degree of transmission and that peak prevalence is reached at different age classes in different villages. In hill top villages the non-immune population comprises of persons of age <2, in foothills age <9 and age <15 years in plain and riverine villages (VCRC, unpublished data). Blood samples were collected fortnightly from people complaining of fever. Any other symptoms or complaints made by the patients were recorded. Blood smears were collected from afebrile persons for comparison and clinical symptoms/ complaints were also noted. The density of parasite in thick smears was graded according to standard method used in field conditions[3]. The data collected for the period March 88 to February 89 serve as the database for this analysis. Since this analysis was restricted only to *P. falciparum* infection, persons with mixed infection and infections other than *P. falciparum* were excluded for analysis. Chi-square and log odds ratio tests were used to test the association between fever and parasitaemia. The multivariate linear discriminant function,

$$D = L_1X_1 + L_2X_2 + L_3X_3 + L_4X_4 + L_5X_5 + L_6X_6$$

(Where X_1, X_2, X_3, X_4, X_5, X_6 represent the symptoms fever, headache, vomiting, nausea,

Table 1. Parasitaemia and different clinical symptoms

Symptoms		Parasite status			
		+ve	%	—ve	%
Fever	+ve	726	74.39	1647	38.22
	—ve	250	25.61	2662	61.78
Headache	+ve	524	53.69	1251	29.03
	—ve	452	46.31	3058	70.97
Vomiting	+ve	138	14.14	179	4.15
	—ve	838	85.86	4130	95.85
Nausea	+ve	70	7.17	187	4.34
	—ve	906	92.83	4122	95.66
Bodyache	+ve	346	35.45	867	20.12
	—ve	630	64.55	3442	79.88
Diarrhoea	+ve	46	4.71	166	3.85
	—ve	930	95.29	4143	96.15

bodyache and diarrhoea respectively) was used to identify the symptoms that separate the *P. falciparum* infection from the normal population. Discriminant analysis refers to various multivariate statistical methods that use a set of observed variables to characterize differences between groups and to devise "optimal" rules for classifying units into one category or another. For each individual, symptoms were coded as "0" for the absence and "1" for the presence of any given symptom. The analyses were carried out using the SPSS PC$^+$ advanced statistics package considering Wilk's lambda criterion in stepwise procedure.

RESULTS

A total of 5,285 blood smears were collected and examined during the study period. The number of cases positive and negative for malaria for each symptom is shown in Table 1. *P. falciparum* infection was observed in 976 slides out of which 726 had fever. The log odds ratio test (LOR)[4] showed that the proportion of fever cases among parasite carriers (74.4%) was significantly higher than that of the non-fever cases (25.6%) (LOR = 1.54; SE=0.006359, p=0.00). Individually other symptoms namely headache, vomiting, nausea, bodyache and diarrhoea do not show any significant association with parasitaemia.

Discrimination based on entire data

The results of disciminant functional analysis of the entire data are shown in Table 2. A significant discrimination was observed between the parasite positive and negative groups. Though fever is found to be the major symptom among the parasite carriers, discriminant analysis showed that the combination of other symptoms were also important in identifying the *P. falciparum* infection. However, significance test based on the coefficients of DF for individual symptom reveals that diarrhoea is independent of *P. falciparum* infection. This DF classifies 63.84% of the population correctly.

Table 2. Coefficients of the discriminant function and their significance

Symptoms	Coefficients	Wilk's lambda
Fever	0.8089	0.921
Headache	0.2135	0.910
Vomiting	0.3398	0.911
Nausea	−0.0498	0.909
Bodyache	−0.1280	0.909
Diarrhoea	*	*

Overall correct classification = 63.84%; Correct classification of positives = 74.70%; Correct classification of negatives = 61.40%; * Symptom not significant in the DF.

The correct classification was found to be more among positives (74.70%) than negatives (61.40%). This would imply that some positives will always be missed and also some negatives would be classified as positives.

Discrimination of parasite carriers in the immune and non-immune population

Though the DF based on this data as a whole gives better discrimination, the spatial and age dependent variations in parasite incidence based on the previous studies necessitates further analysis by considering the above factors. Hence, the discriminant analysis was extended to immune and non-immune populations of different groups of villages.

Table 3 shows the discriminant coefficients of significant clinical symptoms for immune and non-immune populations of different types of villages. The probability values of DF for different groups of villages show the significance of the discriminant function. From the DF analysis only fever was found to be the predominant symptom in all types of villages. The significance of other symptoms varies with degree of endemicity. Even though, vomiting and diar-

Table 3. Discriminant coefficients of significant symptoms for two groups of population

Variables	Non-immune group				Immune group			
	HT	FH	PL	RR	HT	FH	PL	RR
Fever	0.5429	0.7523	0.6089	0.2615	0.8149	0.6848	0.6554	0.8989
Headache	*	0.4473	0.5344	0.5944	−0.2569	0.3838	*	*
Vomiting	0.7068	0.1478	*	−0.4449	0.6126	0.2914	0.5047	*
Nausea	*	0.1301	*	*	−0.3226	−0.1201	*	−0.3355
Bodyache	*	−0.1602	*	0.3344	−0.2975	0.0843	*	0.1644
Diarrhoea	−0.2713	−0.1200	*	0.2328	*	*	0.2989	0.2099
Wilk's lambda	0.8152	0.7973	0.9487	0.9486	0.9631	0.8579	0.9338	0.9465
Prob.	0.0006	0.0000	0.0003	0.0000	0.0001	0.0000	0.0000	0.0000

HT = Hill top ; FH = Foothill; PL = Plain; RR = Riverine; * Indicates the symptoms that are not significant for discrimination; Wilk's lambda = ratio of within groups sum of squares to total s.s.

Table 4. Classification details based on DF analysis

Variables	Non-immune group				Immune group			
	HT	FH	PL	RR	HT	FH	PL	RR
% of CC (1)	68.18	70.95	65.29	79.06	59.86	66.38	66.87	67.56
% of CCP (2)	76.00	80.56	73.70	51.50	45.70	76.70	75.00	68.80
% of CCN (3)	57.90	68.18	64.70	80.80	67.50	62.60	66.20	67.40

(1) = % of correct classifcation (overall); (2) = % of correct classification of positives; (3) = % of correct classification of negatives; HT = Hill top; FH = Foothill; PL = Plain; RR = Riverine.

rhoea in combination with fever were found to discriminate malaria parasite positives from the negatives more efficiently, the actual role of these symptoms individually in discrimination is highly variable from village to village. The percentage correct classification of the positive cases is given in Table 4. Since the symptoms other than fever have lower discriminant power, inclusion of these symptoms marginally improves the classification.

Since the density of the parasite plays an important role in the manifestation of clinical symptoms, the mean parasite densities for these

symptoms were analysed. It was observed that the parasite density was not significantly higher in symptom positive cases than symptom negative cases in non-immune population, whereas in immune population significantly higher rate of parasite density was observed among symptom positive cases than the symptom negatives (Tables 5a-5d).

Seasonwise discriminant analysis showed that in non-immune population most of the symptoms were associated with parasitaemia during hot and rainy seasons. Whereas during the cold season, fever and headache were the only two symptoms that showed significant association. Though most of the symptoms in the immune group were significantly associated with parasitaemia in the winter season, only fever and vomiting are the most common symptoms in all the seasons (Table 6).

DISCUSSION

In hyperendemic areas, the population develops a certain degree of immunity due to repeated exposure to infection which is known to influ-

Table 5(a). 95 per cent confidence intervals of *P. falciparum* density (Hill top)

Symptoms	Hill top					
	Non-immune group			Immune group		
	Sym. (–)ves	Sym. (+)ves	P	Sym. (–)ves	Sym. (+)ves	P
Fever	2.22 ± 0.70	2.83 ± 0.34	0.14	1.39 ± 0.15	2.36 ± 0.19	0.00
Headache	2.76 ± 0.34	2.50 ± 0.84	0.54	1.96 ± 0.19	2.07 ± 0.22	0.25
Vomiting	2.55 ± 0.36	3.25 ± 0.59	0.06	1.90 ± 0.15	2.61 ± 0.38	0.00
Nausea	2.72 ± 0.31	—	—	2.00 ± 0.15	2.33 ± 1.16	0.49
Bodyache	2.77 ± 0.32	2.00 ± 2.03	0.25	1.98 ± 0.17	2.09 ± 0.28	0.25
Diarrhoea	2.71 ± 0.35	2.75 ± 0.68	0.93	2.00 ± 0.15	2.14 ± 1.15	0.76

Table 5(b). 95 per cent confidence intervals of *P. falciparum* density (Foothills)

Symptoms	Foothills					
	Non-immune group			Immune group		
	Sym. (–)ves	Sym. (+)ves	P	Sym. (–)ves	Sym. (+)ves	P
Fever	1.67 ± 0.60	2.95 ± 0.24	0.00	1.52 ± 0.20	2.59 ± 0.14	0.00
Headache	2.59 ± 0.31	3.02 ± 0.34	0.07	1.81 ± 0.19	2.61 ± 0.15	0.00
Vomiting	2.71 ± 0.25	3.14 ± 0.69	0.23	2.22 ± 0.13	3.05 ± 0.28	0.00
Nausea	2.77 ± 0.25	2.20 ± 1.75	0.33	2.28 ± 0.13	2.85 ± 0.37	0.00
Bodyache	2.68 ± 0.27	3.09 ± 0.49	0.17	2.02 ± 0.16	2.75 ± 0.18	0.00
Diarrhoea	2.80 ± 0.25	2.14 ± 0.92	0.19	2.35 ± 0.12	2.38 ± 1.10	0.95

Table 5(c). 95 per cent confidence intervals of *P. falciparum* density (Plains)

Symptoms	Plains					
	Non-immune group			Immune group		
	Sym. (—)ves	Sym. (+)ves	P	Sym. (—)ves	Sym. (+)ves	P
Fever	1.00 ± 0.00	1.36 ± 0.35	0.22	1.08 ± 0.16	2.29 ± 0.38	0.00
Headache	1.30 ± 0.46	1.22 ± 0.32	0.76	1.53 ± 0.42	2.24 ± 0.45	0.03
Vomiting	1.24 ± 0.28	1.50 ± 4.49	0.52	1.90 ± 0.35	2.25 ± 1.00	0.43
Nausea	1.28 ± 0.28	1.00 ± 0.00	0.63	1.90 ± 0.34	2.33 ± 1.31	0.39
Bodyache	1.23 ± 0.35	1.33 ± 0.49	0.71	1.58 ± 0.36	2.33 ± 0.53	0.02
Diarrhoea	1.28 ± 0.30	1.00 ± 0.00	0.66	2.02 ± 0.34	1.25 ± 0.69	0.20

Table 5(d). 95 per cent confidence intervals of *P. falciparum* density (Riverine)

Symptoms	Riverine					
	Non-immune group			Immune group		
	Sym. (—)ves	Sym. (+)ves	P	Sym. (—)ves	Sym. (+)ves	P
Fever	1.00 ± 0.00	1.95 ± 0.45	0.00	1.27 ± 0.31	2.40 ± 0.29	0.00
Headache	1.35 ± 0.39	1.94 ± 0.55	0.07	1.74 ± 0.35	2.33 ± 0.33	0.01
Vomiting	1.63 ± 0.33	2.00 ± 0.00	0.70	2.00 ± 0.25	3.13 ± 1.06	0.01
Nausea	1.70 ± 0.35	1.00 ± 0.00	0.23	2.05 ± 0.25	2.80 ± 1.45	0.19
Bodyache	1.39 ± 0.33	2.20 ± 0.77	0.02	1.79 ± 0.32	2.45 ± 0.36	0.00
Diarrhoea	1.66 ± 0.36	1.50 ± 1.38	0.76	2.09 ± 0.26	2.14 ± 1.25	0.91

ence clinical outcome[5] and hence many parasite carriers remain asymptomatic. It has been reported recently that the cases identified through mass screening programmes in WHO regions of Asia, in part reflect the prevalence as opposed to clinical infections[6]. In such a situation, getting a correlation with any symptom is possible even if the symptoms are unrelated. Therefore, interpretation of data alone will not be enough to establish a cause and effect relationship between parasitaemia and symptoms. The seasonwise analysis showed that most of the diarrhoea cases occurred during rainy season which was also the peak season for malaria. Therefore, the association of diarrhoea with *P. falciparum* infections may be circumstantial[7].

The significant relationship between the density grades and symptoms like fever and vomiting indicate that these symptoms mostly appear over parasite density grade 2 in hill top and foothill areas where transmission is more intense. In

Table 6. Seasonwise discriminant coefficients for significant symptoms

Symptoms	Non-immune group			Immune group		
	S1	S2	S3	S1	S2	S3
Fever	0.56	0.73	0.83	1.02	0.70	0.63
Headache	*	0.41	0.34	*	0.20	0.34
Vomiting	0.19	0.31	*	0.33	0.41	0.34
Nausea	−0.28	*	*	*	*	0.23
Bodyache	0.61	−0.24	*	−0.28	*	−0.21
Diarrhoea	0.17	−0.18	*	*	*	*

* indicates the symptoms that are not significant in DF; S1 = Hot summer (March - June); S2 = Rainy (July - October); S3 = Cold winter (November - February).

plain and riverine areas, where transmission is much lower, the symptoms appear over parasite density grade I. The density grade for manifestation of symptoms is considerably higher for immune population than the non-immune population. This means that in hyperendemic areas (hill top and foothill) people with low grade parasitaemia will never be detected in fever surveys. Thus, if the objective of the programme is to prevent morbidity and mortality alone and not the elimination of infection, slide examination does not provide any advantage. In hyperendemic areas the identification of *P. falciparum* based on clinical symptoms differs according to the immunity of the population. In non-immune group the presence of fever, vomiting and diarrhoea can safely be considered as a clear indication of *P. falciparum* infection. Slide examination can be restricted only to doubtful cases which would reduce the workload of PHC technicians.

Since it was observed that only fever was closely associated with parasitaemia in the <15 years age group, it is suggested that slide examination and radical treatment could be intensified for the below 15 years age class. In hyperendemic areas a large number of fever cases had parasite density lower than the threshold level of 2.17, indicating that the morbidity due to malaria is minimal in adult age class.

Since more than 80% of the population are parasite carriers and all fever cases in the adult age class are not entirely due to malaria, the assessment of malaria situation based on slide examination has little role. The slide collection is generally biased towards adult age class which gives an overestimate of malaria situation.

Since the objective of the programme is to reduce morbidity and mortality keeping in mind the resource constraints, slide examination above 15 years age class can be restricted to only doubtful cases. The present practice of utilising the data collected during case detection for malaria assessment is highly variable and of little epidemiological significance. The integration of malaria control with primary health care system is expected to increase the workload of the technicians and consequently the quality of slide examination will deteriorate further. Hence, it is suggested that independent epidemiological survey units should be established to monitor malaria situation by adopting properly designed sampling techniques.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. P.K. Rajagopalan, Director, VCRC, Pondicherry for his constant guidance and support. The sincere work carried out by technical staff in Jeypore field station is also acknowledged.

REFERENCES

1. Beljaev, A.E., J.A. Brohult, G.K. Sharma, M.A. Haque and K.C. Samantaray (1985). Studies on the detection of malaria at Primary Health Centres. Part I. reliability of parasitological diagnosis by decentralized laboratories. *Indian J. Malariol.*, **22:** 85-103.

2. Kondrashin, A.V., W. Rooney and Nihal Singh (1987). Dynamics of *P. falciparum* ratio—an indication of malaria resistance or a result of control measures? *Indian J. Malariol.*, **24:** 89-94.

3. Bruce-Chwatt, L.J. (1985). *Essential Malariology.* (William Heinemann Medical Books Ltd., London).

4. Kahn, Harold A. and Christopher T. Sempos (1989). *Statistical Methods in Epidemiology.* (Oxford University Press): 109-117.

5. Viswanathan, D.K. (1951). A review of immunity and endemicity in malaria and a discussion on their relationship with malaria control. *Indian J. Malariol.*, 5: 251-285.

6. Philips-Howard, P.A. and E.B. Doberstyn (1990). *Malaria, present situation of the disease.* WHO report CTD/CPI2/90.6 Geneva, 12-13 June 1990.

7. Greenwood, B.M., P. Byass, A.M. Greenwood, R.J. Hayes, A. Menon, F.C. Shenton, J. Stephens and R.W. Snow (1989). Lack of an association between acute gastroenteritis, acute respiratory infections and malaria in young Gambian children. *Trans. R. Soc. Trop. Med. Hyg.*, **83:** 595-598.

SHORT NOTES

Indian Journal of Malariology
Vol. 28, March 1991, pp. 65-68.

Insecticide Susceptibility of Mosquito Vectors in Sundargarh District, Orissa

S.K. CHAND* and R.S. YADAV*

Enormous quantities of insecticides are being used in India under the countrywide malaria eradication programme, besides their use in agriculture. During recent years vector resistance to DDT in areas of 18 states, double resistance to DDT and HCH in areas of 17 states and triple resistance to DDT, HCH and malathion in areas of 4 states have been recorded[1,2]. Stratified maps of India showing areas with DDT, HCH and malathion resistance to *An. culicifacies*, the major vector of rural malaria in India, have been presented by Sharma[3].

In Orissa, Raghavan *et al.*[4] reported that *An. culicifacies* was susceptible to DDT. In areas of Cuttack and Koraput districts *An. annularis* was reported to be susceptible to DDT but *An. subpictus* and *An. hyrcanus* (= *nigerrimus*) were resistant[5]. He also observed that *An. hyrcanus* and *An. aconitus* were resistant to dieldrin and gamma-HCH *An. culicifacies* resistance to DDT was reported in northern Orissa, however, there was no information on the susceptibility of mosquitoes to malathion and dieldrin in Orissa State[3]. In 1988 Dash *et al.*[6] recorded that *Culex quinquefasciatus* adults from inbred colonies maintained from the mosquitoes of Bhubaneswar, Puri and Patrapada towns were resistant to DDT but susceptible to malathion and K-othrine and the larvae were susceptible to malathion, fenitrothion (baytex), fenitrothion and temephos (abate). Recently in Koraput, the southern most district of Orissa, *An. fluviatilis* was reported to be susceptible to DDT, HCH, malathion and deltamethrin, whereas, *An. culicifacies* and *An. annularis* were found to be resistant to DDT and HCH[7].

In district Sundargarh which is a part of the hard core area of the Garhjat hills in northern Orissa, malaria has existed since long in hyperendemic form. The area is also endemic for lymphatic filariasis and a Japanese encephalitis epidemic occurred in Rourkela city in 1989. Since the adoption of NMEP's strategy of intradomiciliary spraying of residual insecticides in 1958, the area received two rounds of DDT spray every year. Besides, more than 48 tonnes of HCH was used every year in agriculture in the district (Source: District Agriculture Officer, Rourkela). Despite this massive use of insecticides, susceptibility status of mosquitoes, except for *An. culicifacies* resistance to DDT, was not known. The susceptibility of *An. culicifacies, An. annularis,* (malaria vectors), *Culex quinquefasciatus* (lymphatic filariasis vector) and *Culex tritaeniorhynchus*

Accepted for publication. 18 December 1990.
Malaria Research Centre (Field Station)
Civil Township
Rourkela - 769 004, India

Table 1. Results of insecticide susceptibility tests on mosquitoes

Species	Chemicals	Diagnostic conc. (%)	Standard exposure time (hrs)	Control			Test			Susceptibility status
				No. exposed	No. dead	Per cent mortality	No. exposed	No. dead	Per cent mortality	
An. culicifacies	DDT	4.0	1	160	2	1.2	160	8	5.0	R
	Dieldrin	0.4	1	160	8	5.0	160	23	14.4 (9.9)	R
	Malathion	5.0	1	160	3	1.9	160	160	100.0	S
	Fenitrothion	1.0	2	120	4	3.3	120	120	100.0	S
An. annularis	DDT	4.0	1	160	4	2.5	160	7	4.4	R
	Dieldrin	0.4	1	120	4	3.3	120	82	68.3	R
	Malathion	5.0	1	160	2	1.2	160	160	100.0	S
	Fenitrothion	1.0	2	120	5	4.2	120	120	100.0	S
Cx. quinquefasciatus	DDT	4.0	4	200	8	4.0	240	20	8.3	R
	Dieldrin	0.4	1	160	4	2.5	160	71	44.4	R
	Malathion	5.0	1	160	4	2.5	160	160	100.0	S
	Fenitrothion	1.0	2	120	9	7.5	120	114	95.0 (94.6)	V
Cx. tritaeniorhynchus	DDT	4.0	4	80	3	3.7	120	61	50.8	R
	Dieldrin	0.4	1	80	2	2.5	120	40	33.3	R
	Malathion	5.0	1	60	2	3.3	100	100	100.0	S
	Fenitrothion	1.0	2	80	3	3.7	140	140	100.0	S

R = Resistant; S = Susceptible; V = Verification required; Figures in parentheses are percentage corrected mortality.

(JE vector) to DDT, dieldrin, malathion and fenitrothion are reported for the first time.

Susceptibility tests were conducted between April 1989 and July 1990 with the diagnostic concentrations of DDT (4%), dieldrin (0.4%), malathion (5%) and fenitrothion (1%) using WHO kit. Blood fed females of *An. culicifacies, An. annularis, Culex quinquefasciatus* and *Culex tritaeniorhynchus* were collected in the morning hours in small mosquito cages from unsprayed surfaces in the villages around Rourkela city in District Sundargarh, (map coordinates: 22.25° N Lat. and 85.00° E Long.). The tests were conducted as per standard procedure[1,8] in the insectary of the field station under optimum conditions of temperature (22 to 30°C), relative humidity (65-80%) and diffused illumination.

Sixty to 100 females, 3 to 5 replicates of 20 females each, were used in each test and control and the experiment was repeated after some time. No insecticide paper was used after three weeks of removal from the original packing or beyond ten times of use.

Mortalities in mosquitoes were observed at the end of standard exposure periods and after 24 hours. In two instances when control mortalities were 5 and 7.5%, percentage mortalities in tests were corrected using Abbott's formula.

Results of the tests are given in Table 1. It is apparent that *An. culicifacies* and *An. annularis* were found resistant to DDT and dieldrin, (organochlorines) but fully susceptible to malathion and fenitrothion (organophosphates). Development of resistance in mosquitoes to DDT in several parts of India is well known[3]. In Sundargarh dieldrin has never been used, however, its resistance to the above vectors could safely be taken equivalent to that of HCH, as the two cyclodienes involve equivalent resistance mechanisms. The fact, that HCH is being used in agriculture in the district, indicates that vector resistance has either been developed due to the exposure of immatures in agricultural fields or is due to cross resistance by long use of DDT, or a product of both. Organophosphates are minimally used in agriculture in Sundargarh and as expected, malathion and fenitrothion were found fully affective.

Until the report by Das[5], *An. annularis* was susceptible to DDT in Cuttack and Koraput. However, *An. annularis* and *An. culicifacies* are now resistant to DDT and HCH but susceptible to malathion in Koraput in southern Orissa[7] and the same is confirmed by the present report for Sundargarh district in northern Orissa.

Culex quinquefasciatus, was also found to be resistant to DDT and dieldrin and susceptible to malathion and fenitrothion in our tests. Similar results were recorded earlier in tests with DDT and malathion in the coastal area of Orissa by other workers[6]. Though, there is no report on the susceptibility of *Culex tritaeniorhynchus* in Orissa, this species was reported to be susceptible to organochlorines and organophosphates elsewhere in India[9]. In our study, however, it has been found resistant to DDT and dieldrin and susceptible to malathion.

Earlier Sharma *et al.*[10] demonstrated that by correct insecticide dosage and increasing the insecticide coverage malaria incidence could still be brought down in villages with resistant *An. culicifacies* as vector. In practice these conditions are hardly fulfilled nowadays and there are other incipient problems of socio-cultural and behavioural resistance in the people against insecticide spray. Since DDT still remains the mainstay of malaria control operations in the state, there is an urgent need for adoption of an integrated approach for the control of mosquito-borne diseases.

REFERENCES

1. WHO (1986). Resistance of vectors and reservoirs of disease to pesticides; tenth report of the WHO Expert Committee on Vector Biology and Control. *Tech. Rep. Ser.* No. 737.
2. Kondrashin, A.V. and K.M. Rashid (1987). Epidemiological considerations for planning malaria control in the WHO South-East Asia Region. (WHO Regional Office for South-East Asia, New Delhi): 411.
3. Sharma, G.K. (1983). Review of Malaria and its Control in India. In *Proceedings of the Indo-UK workshop on malaria,* edited by V.P. Sharma. (Malaria Research Centre, ICMR, Delhi):13-40.
4. Raghavan, N.G.S, B.L. Wattal, V.N. Bhatnagar, D.S. Choudhury, G.C. Joshi and K.S. Krishnan (1966). Present status of susceptibility of arthropods of public health importance to insecticides in India. *Bull. Ind. Soc. Mal. Com. Dis.*, **3** : 209-245.
5. Das, M. (1966). A note on susceptibility status of some *Anopheles* to chlorinated hydrocarbon insecticides in Orissa. *Bull. Ind. Soc. Mal. Com. Dis.*, **3:** 323-329.
6. Dash, A.P., N. Tripathy and R.K. Hazra (1988). Bionomics and vectorial capacity of mosquitoes in Puri district, Orissa. *Proc. 2nd Sym. vectors and vector-borne Diseases,* 90-100.

7. Sahu, S.S., K. Gunasekaran, P. Jambulingam and P.K. Das (1990). Susceptibility status of *Anopheles fluviatilis, A. annularis* and *A. culicifacies* to insecticides in Koraput district, Orissa. *Indian J. Malariol.,* **27:** 51-53.

8. WHO (1981). Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides-diagnostic test. WHO unpublished document-VBC/81: 806.

9. WHO (1980). Resistance of vectors of disease to pesticides: Fifth report of the WHO Expert Committee on Vector Biology and Control. *Tech. Rep. Ser.* No. 655.

10. Sharma, V.P., R.K. Chandrahas, M.A. Ansari, P.K. Srivastava, R.K. Razdan, C.P. Batra, K. Raghavendra, B.N. Nagpal, S.C. Bhalla and G.K. Sharma (1986). Impact of DDT and HCH spraying on malaria transmission in villages with DDT and HCH resistant *Anopheles culicifacies. Indian J. Malariol.,* **23:** 27-38.

Indian Journal of Malariology
Vol. 28, March 1991, pp. 69-71.

Impact of Monitoring on Malaria Control Activities of PHC Workers

R.C. SHARMA* and A.S. GAUTAM*

Monitoring of a programme is as important as the programme itself. Monitoring should ensure that staff engaged in the programme works to the expected level, all services rendered are properly recorded and data should qualify both quantitatively and qualitatively, so that timely corrective measures can be taken if required. With this objective, and as per the recommendation of the in-depth evaluation committee[1] a monitoring system was introduced from July to December 1989 in Palana PHC of Kheda district.

In the system Multipurpose Health Workers (MPWs) were required to record daily the number of hours they worked in the field on each working day, number of houses visited and blood smears collected and submit their report fortnightly. Data supplied by the MPWs of the period before introduction of the monitoring system upto June and of the period when monitoring system was in operation is given in Table 1 for both male and female multipurpose health workers. In Palana PHC, where the study was conducted, on an average male MPWs worked 81% of the total working days (252) in the year whereas female MPWs worked only 63% of the total working days. There was no apparent difference between the per cent days worked by male MPWs in pre-monitoring (Jan-June, 77.8%) and monitoring period (July-Dec, 83.3%). However, 63% increase was observed in case of female MPWs. Similarly there was no difference in average number of hours worked by each male MPW in pre-study and study period but in case of female MPWs on an average there was an increase of 1.3 hours on each working day during the monitoring period. In control PHC Pij no such improvement was observed.

Female MPWs also showed an improvement in the average number of houses visited per hour from 0.5 in pre-monitoring period to 0.6 in monitoring period. Improvement was also reflected in the population coverage by female MPWs who covered 18.2 and 48.1% of their targeted population in pre-monitoring and monitoring period, respectively with an average of 33.2%. It was much below the average of

Accepted for publication: 26 December 1990.

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

male MPWs (74.3%) with no apparent difference in both the periods, respectively.

As regards blood smear collection there was a definite improvement in performance of both male and female MPWs which is reflected in blood examination rate (BER). In case of male MPWs there was an improvement in BER from 0.9 to 3.1 (244%) and from 0.1 to 1.2 (1100%) in case of female MPWs in pre-monitoring and monitoring period, respectively. It is opined that this improvement was definitely due to introduction of monitoring and also to some extent due to transmission season falling during the monitoring period.

This hypothesis finds support when we compare the data of PHC Palana with the data from its neighbouring PHC Pij (Table 1). In PHC Pij neither the male nor the female workers showed any improvement during the second half of the year (Jul-Dec) which corresponds with the monitoring period in PHC Palana, in any of the first four parameters listed in Table 1. In case of fifth parameter i.e., blood examination rate there was an improvement of 45% in male workers and 106% in female workers. This improvement can be attributed to high fever rate during malaria transmission season beginning July in the area.

During the discussions with the MPWs on their performance below the expected levels it came out that they had various reasons to explain their shortcomings. These problems were discussed in the project committee meeting held on 17.11.89. The reasons given were mainly related to Family Welfare, Immunisation and Mother and Child Health Programmes. The project committee suggested inclusion of all the health programmes in monitoring system as it was impossible to work in isolation for malaria alone under primary health care system[2]. In view of this there is a need to develop a comprehensive health

Table 1. Impact of monitoring

	Parameter	MPW (Sex)	Experimental PHC (Palana)			Control PHC (Pij)		
			Pre-monitoring period (Jan-Jun)	Monitoring period (Jul-Dec)	Total	Jan-Jun	Jul-Dec	Total
1.	Average days worked by each worker (%)	M	77.8	83.3	80.6	77.0	79.7	78.3
		F	47.6	77.8	63.1	79.1	81.7	80.5
2.	Average no. of hours worked per day by each worker	M	7.0	7.3	7.2	6.0	5.7	5.8
		F	5.0	6.3	5.8	6.2	6.4	6.3
3.	Average no. of houses visited per hour by each worker	M	3.9	3.4	3.6	14.7	15.6	15.1
		F	0.5	0.6	0.6	0.6	0.5	0.6
4.	Population covered (%)	M	75.8	72.8	74.3	97.5	97.3	97.4
		F	18.2	48.1	33.2	79.8	73.8	75.7
5.	Blood examination rate	M	0.9	3.1	4.0	1.1	1.6	2.7
		F	0.1	1.2	1.3	1.5	3.1	4.6

monitoring system which would cover all the health programmes under primary health care system.

ACKNOWLEDGEMENTS

Authors are thankful to Dr. V.P. Sharma, Director, Malaria Research Centre, Delhi for his guidance and to Dr. R.N. Shah, Chief Medical Officer and Dr. H.M. Thakkar, Medical Officer of PHC Palana of Kheda district for their kind help and cooperation.

REFERENCES

1. Anonymous (1987). In-depth evaluation of the community based integrated vector control of malaria project in Kheda (Gujarat) October, 1987.
2. Anonymous (1989). Annual report of S & T Project in mission mode. (Malaria Research Centre, Delhi): 449.

Corrigenda

IJM 27(4) December 1990

Indian Journals on Malariology
by B.K. Sen *et al.*

Page No. 201—Table 4—S.No. 14

Please read Indian J. Malariol.*
for Indian J. Malariol.

Plasmodium falciparum—Chloroquine *In vivo* Test in Northeast India: Reclassification and Extended Follow-up till Day 14
by A.P. Pandya *et al.*

Page 227—Fig. 1

Please read Y-axis 10^3 Asexual Parasitaema/μl of blood
for 10 Asexual Parasitaemia/μl of blood

Page 228—Table 7

Please read 75 per cent on day 4
for 75 per cent on day 14

INDIAN JOURNAL OF MALARIOLOGY

Instructions to Authors

Editorial Policy

The Indian Journal of Malariology is devoted to the publication of original research which contributes significantly to the knowledge of any field of malariology. Papers of routine and repetitive nature dealing with gross observations may not be included. Articles will be published at the editor's discretion in the order accepted. Date of acceptance will be that date on which copy is accepted in final form for publication. Manuscripts should be submitted in triplicate to:

The Editor
Indian Journal of Malariology
Malaria Research Centre (ICMR)
22-Sham Nath Marg
Delhi-110054, India.

Classes of Items Published

In addition to regular papers the journal publishes short notes and invites review articles. Book reviews may be published at the end of the journal.

Format

The matter should be arranged in the following order: Title, Name(s) of the author(s) with address of the Institute/University (as footnotes and numbered serially in superscript). Abstract, Introduction, Material and Methods, Results, Discussion, Acknowledgement and References. Authors should provide a short title to be used as running title, of not more than 5 words in case the title of the paper is long.

Preparation of Copy

Manuscripts should be typewritten in English on one side of the paper leaving 1½ inch left-hand margins. The entire matter should be typed double space including references, tables and captions. Abstract, tables, references and legends for illustrations should be typed on separate sheets of paper. Pages are to be numbered consecutively.

Tables should be placed singly on sheets of paper, along with relevant headings and footnotes. Tables should be self-explanatory and referred to in the text. Designation of tables must be with arabic numerals (e.g. 1, 2), avoid roman numerals (e.g., I, II). Do not use any horizontal or vertical lines in the body of the table.

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

Illustrations should be sent in duplicate. All illustrations including figures, photographs, graphs and maps should be numbered consecutively in order of appearance in the text. Captions and legends should be typed separately and must not appear on the face of illustrations. Authors should identify each illustration on the reverse side with author's name, fig. no. and abbreviated captions. Line drawings should be clear and letters and numerals should be planned for legibility following reduction. Labelling should be neat and accurate. Photographs should be sharp, glossy, black and white prints, preferably mounted and covered with a transparent overlay for protection. Photographs should have allowance for reduction to 1/3 size. Size of art work for quarter page should be 24 x 21 cms; half page 45 x 24 cms and full page 57 x 45 cms approximately.

Data for tables, graphs etc., should be carefully verified. All statistics, percentages and other calculations should be checked thoroughly before submission of a communication. Once a paper is

accepted for publication, data in it should be treated as final.

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

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

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

References should include only published references and papers in press. References in the text may be cited as Sharma *et al.*(1976) or (Mount *et al.,* 1972; Coombes *et al.* 1973; Mount *et al.,* 1974). For references to a paper accepted for publication, the word 'in Press' should appear after the title of the periodical. Citations of unpublished work should be incorporated in the text itself (e.g., R.G. Roy, unpublished data or S. Pattanayak, personal communication). If the reference is to an article published without any authorship in a periodical, in place of author's name the term "Anonymous" should be used. Titles of Periodicals cited in the references are to be abbreviated as in the *World List of Scientific Periodicals.*

References to the literature, arranged alphabetically and numbered consecutively, must be placed at the end of the paper. The following style is acceptable for this journal:

Research Paper

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

Book/Monographs

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

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

Paper presented at Symposium/Conference

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

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

Killick-Kendrick, R. and W. Peters (1978). Ed. *Rodent Malaria.* (Academic Press Inc., London): 406. **(Incorrect).**
Landau, I. and Y. Boulard (1978). In *Rodent Malaria,* edited by R. Killick-Kendrick and W. Peters. (Academic Press Inc., London): 53–84. **(Correct).**

Correct references are the responsibility of the author.

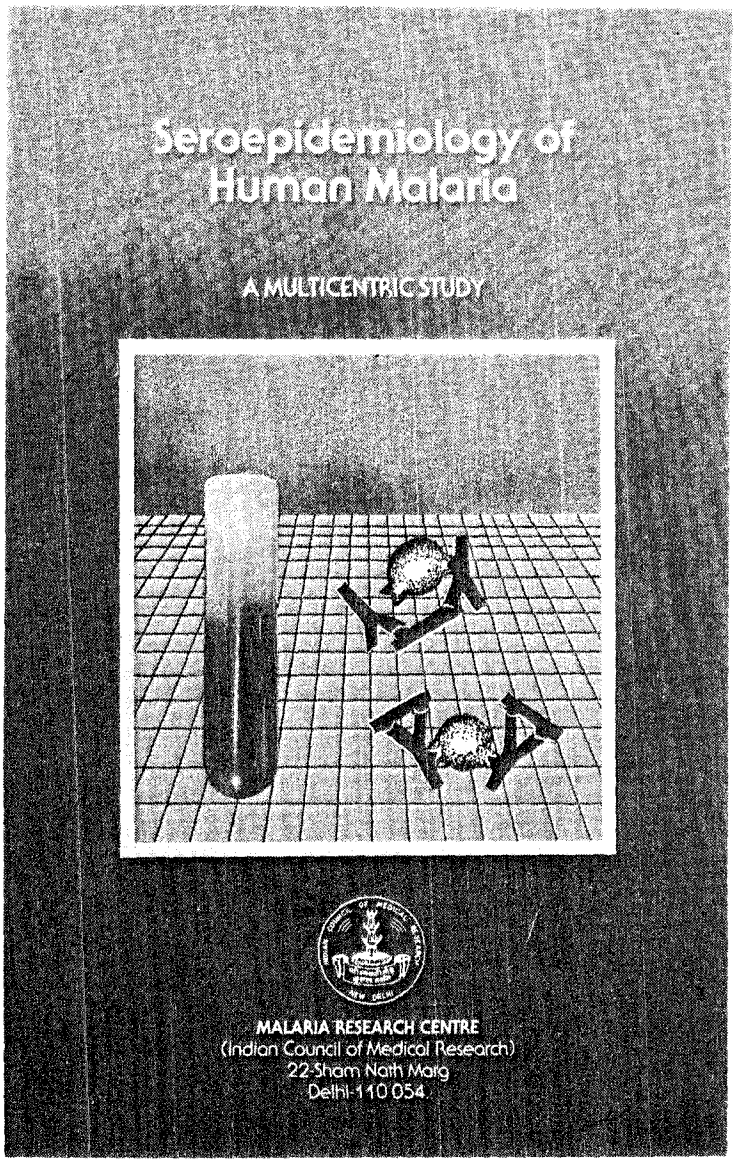
Short notes should be prepared in a manner similar to the research papers and will have title, name(s) of author(s) with address of Institute/University as footnotes, acknowledgement and references.

Proofs

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

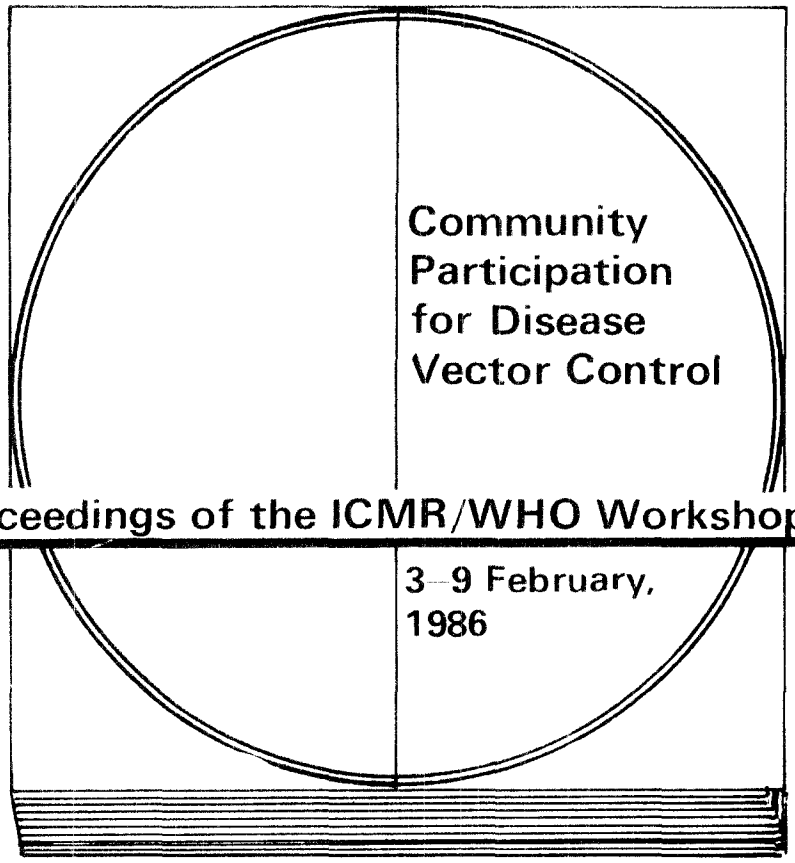
Reprints

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



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

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



Proceedings of the ICMR/WHO Workshop on

3–9 February,
1986

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

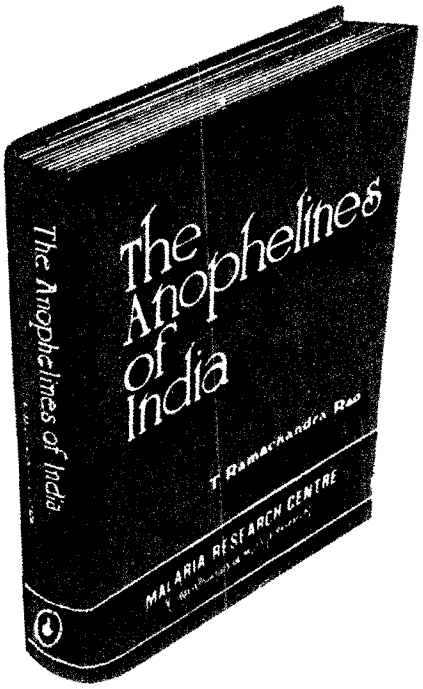
A review of the process, role and outcome of people's involvement in vector control. Invaluable for workers and decision makers in Primary Health Care and health-related projects.

THE ANOPHELINES OF INDIA

Revised Edition 1984

by

T. Ramachandra Rao



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

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

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

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

Statement about ownership and other particulars of Indian Journal of Malariology

FORM IV

(See Rule 8)

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

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

15 March 1991

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

MALARIA RESEARCH CENTRE

PRICED PUBLICATIONS

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

Price { India Rs. 150/- / Foreign US $ 45/- } 25% Discount

Indian Journal of Malariology

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

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

(+To be released in June, September and December 1991)

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

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

Sir,

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

.....

.....

.....

.....