INDIAN JOURNAL OF MALARIOLOGY

Volume 27   Number 3

September 1990

INDIAN J. MALARIOL.

SUBSCRIPTION RATE

INDIAN JOURNAL OF MALARIOLOGY

.

The Natural Occurrence of Circulating Antibodies in Populations of Endemic Malarious Areas

S. BISWAS[1], Q.B. SAXENA[1] and A. ROY[1]

The magnitude of humoral response to soluble antigen extracted from *Plasmodium falciparum* schizonts and merozoites was assessed in 744 blood samples collected from different parts of India. In this study, parasitological and immunological data were considered for assessment of antibody during natural infections at various seasons. The antibody response has been measured by enzyme-immuno assay. Survey was done in all age groups. Overall antimalarial IgG level had started increasing after five years which shows that the rate of infection was high in the small age groups. Elevated level of IgG in populations indicates that the study area is undergoing a period prevalence of the disease. In most of the individuals with active infection, IgG and IgM levels were high. Positivity in IgM denoted the active transmission of malaria. Elevated levels of antigen specific IgA was observed in some cases but the mechanism is not yet understood. Presence of circulating immune complexes during acute infection shows the failure of detection of circulating free antibodies in some individuals. The significance of findings in relation to serological status in individuals exposed naturally to malaria has been discussed.

INTRODUCTION

Following repeated malaria infection individuals living in a malaria endemic zone develop immunity against the pathogenic asexual stages of the parasite; such immunity reduces parasite load and the severity of the clinical illness. During the development of resistance to the disease, antibodies specific for different stages involved in the life cycle are produced. Partial protection has been observed in acutely infected children, when purified gamma globulins (IgG) from immune adult was transferred during illness

(Cohen *et al.*, 1961). The cellular interactions which occur during the induction of immunity to malaria have been the subject of intensive research. The involvement of both cellular and humoral immune responses in malaria immunity has been well established. During malarial infections there is a marked increase in serum immunoglobulin levels, part of which is specific to malarial parasites, however, there is a poor correlation between immune status of the host and the levels of antimalarial antibody. Much of this antimalarial antibody is non-protective (McGregor, 1981). It is already established that specific malaria antibody activity can be demonstrated in IgG, IgM and IgA fractions of immune human sera (Tobie *et al.*, 1966; Collins *et al.*, 1971) and IgG is predominant in established infections.

In order to differentiate the natural immune response to the asexual blood stage antigens, we tested blood samples for presence of antigen-specific IgG, IgM and IgA and also a number of sera for antigen-antibody complexes. For the study, antigen was prepared from *P. falciparum* schizonts and merozoites and was semipurified by reacting with antisera to human erythrocytes and plasma. Circulating antibody complexed with antigen was assessed by simple polyethylene glycol assay.

The present cross-sectional study on malaria was carried out in four areas of the country having seasonal malaria transmission. Blood samples for parasitological and serological examination were collected from persons with acute infection and repeated attacks, after chemotherapy and also from healthy individuals. It therefore, provided a good opportunity to demonstrate the level of three antibodies in various groups and also the, appearance of antigen bound antibody at different phases. This type of study may help

to obtain some baseline information about the humoral immune status of individuals of different age groups prior to any vaccine action programme and any new drug and drug combinations trial.

MATERIAL AND METHODS

Study area

The study includes the inhabitants (n = 744) of villages in Uttar Pradesh (Shahjahanpur, Ghaziabad), villages in Jabalpur, District Mandla and of Delhi city, where malaria is seasonal; early and prolonged monsoons are responsible for intensive transmission of both *P. vivax* and *P. falciparum*.

Sample collection

Blood samples were collected by venepuncture or by finger prick on Whatman No. 3 filter paper during cross-sectional survey in the study areas

Table 1. Malaria profile of samples collected from four study areas

Areas	Blood smears +ve for MP	Blood smears –ve for MP			No. of samples in each area	Season
		Given treatment for malaria	Persons with repeated attack	Malaria history not known		
Delhi	67 (Pv)	23	30	40	160	Jul. & Aug. 1985
Ghaziabad	39 (Pf)	4	21	7	71	Nov. & Dec. 1988
Shahjahanpur	146 128 (Pv) 18 (Pf)	0	12	0	158	Jul. & Sep. 1986
Jabalpur	58 53 (Pf) 5 (Pv)	22	225	50	355	Feb. 1988

Total samples n = 744

from February to December (Table 1). One hundred and sixty specimens were collected from Delhi malaria clinics from the patients with symptoms of high fever and also from blood donors from Blood Bank. Twenty malaria negative sera which were collected from tourists belong to non-endemic, non-malarious areas. Rest of the samples are from villages of Ghaziabad, Shahjahanpur and Jabalpur. Sera and filter paper eluates were stored at -70°C until processed. Thick and thin blood films were prepared for microscopic examination after standard JSB staining.

Parasites for antigen preparation

The Indian *P. falciparum* isolate FSJ from Shahjahanpur was used for antigen preparation. Parasites were maintained in routine culture using O+ RBCs and AB+ serum (10%) by candle-jar technique (Trager and Jensen, 1976). Antigen chosen for the test was an extract of schizont and merozoite stages. The soluble portion was fractionated at 50 per cent saturation of ammonium sulphate. Isolated antigen was purified after absorption with rabbit anti-human erythrocytes and plasma antibodies by the method of Avrameas and Ternynck (1969).

Detection of circulating free antibody by ELISA

Assay was performed in 96-well round bottom microtitre plates. *P. falciparum* antigen was absorbed on solid phase at a concentration of 40 micrograms protein per ml. Samples, as source of antibodies, were treated individually with AB+ human RBCs to remove the non-specific reactants. Three types of antibodies, IgG, IgM and IgA were trapped by using specific antihuman globulin conjugates. For IgG detection serum dilution at 1:1024 was chosen and for IgM and IgA 1:128 dilution was selected.

Total immunoglobulin level estimation by single radial immunodiffusion

One hundred and sixty sera collected from Delhi and twenty negative sera from American tourists

were estimated for IgG, IgM and IgA by single radial immunodiffusion (Mancini *et al.*, 1965) in antihuman IgG, IgM and IgA coated agarose plates. These plates were obtained from Hoechst Pharmaceuticals. Concentration of immunoglobulins in sera was calculated from the conversion table supplied by the company.

Detection of antigen-antibody complex

Non-specific circulating immune complexes were detected by polyethylene glycol (PEG) assay as described by Haskova *et al.* (1978). It was done by estimation of turbidity developed in sera after addition of polyethylene glycol (MW 6000).

RESULTS

Details of the samples collected from study areas are given in Table 1. Total number of parasite positive and species positive blood samples varied due to seasonal transmission of the disease.

Table 2 shows the malaria specific antibodies (IgG, IgM and IgA) detected by ELISA in sera collected from Delhi. Parasite positive samples showed 76 per cent IgG positivity and 90 per cent IgM positivity, whereas sera after repeated attack and drug cure were 85 per cent IgG positive and 60 per cent IgM positive. Positivity for IgM denotes the active and very recent infection. About 6 per cent samples in both parasite positive and negative groups were positive for antigen specific IgA. Twenty negative sera from American tourists were tested for sero-reactivity with malarial antigen. Values (OD at 490 nm, mean ± SD) obtained in negative sera by ELISA at 1:1024 dilution for IgG and 1:128 dilution for IgM and IgA were taken as cut-off point (IgG 0.30 ± 0.13; IgM 0.24 ± 0.08; IgA 0.12 ± 0.09).

Serum immunoglobulins (IgG, IgM and IgA) level determined by single radial immunodiffusion in parasite positive and negative sera are shown in Table 3. Increased level of IgG and IgM in endemic sera was observed, IgA level

Table 2. Antigen-specific serum immunoglobulins (IgG, IgM and IgA) detected by ELISA in samples collected from Delhi and in 20 negative sera

Total no. of samples		Parasite detection by microscopy	Antibody detected by ELISA					
			IgG (Mean + 3SD)		IgM (Mean + 3SD)		IgA (Mean + 3SD)	
			No. +ve (% +ve)	No. -ve	No. +ve (% +ve)	No. -ve	No. +ve (% +ve)	No. -ve
Endemic 160	Clinic 120	67 +ve	51 (76.12)	16	60 (89.55)	7	4 (5.97)	63
		53 -ve	45 (84.91)	8	32 (60.38)	21	3 (5.66)	50
	Blood bank 40	40 -ve	17 (42.5)	23	4 (10)	36	0 (0)	40
Non-endemic		20 -ve	0 (0)	20	0 (0)	20	0 (0)	20

Table 3. Serum immunoglobulins (IgG, IgM and IgA in mg/ml) level in human samples from malaria endemic (Delhi) and non-endemic (USA) areas

Groups and no. of samples	Parasite detection by microscopy	IgG mg/ml Mean ± SD (range)	IgM mg/ml Mean ± SD (range)	IgA mg/ml Mean ± SD (range)
Endemic 120	67 +ve	16.64 ± 4.87 (5.62-27)	2.0 ± 0.88 (0.7-4.4)	1.9 ± 0.85 (0.3-4.2)
From clinic	53 -ve	14.77 ± 4.97 (6-24)	1.86 ± 0.89 (0.65-4.4)	1.68 ± 0.67 (0.25-3.1)
Non-endemic 20	20 -ve	9.6 ± 2.8 (7-16)	1.4 ± 0.3 (0.8-1.8)	1.6 ± 0.5 (0.4-2.5)

was elevated in a few sera with present and past history of malaria. Immunoglobulins concentrations in 20 negative sera from non-endemic areas were at normal range.

Venepunctured blood samples were collected from individuals, from the age group of 15 years and above, with acute P. vivax and P. falciparum infection from villages of Shahjahanpur. Out of 158 individuals who reported with high fever, 146 were positive for malaria, among them 128 persons were P. vivax infected (88%), and 18 were P. falciparum infected (12%). Twelve persons with high fever were negative for parasite
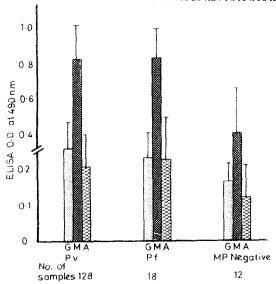
Fig. 1: Profile of antigen specific IgG, IgM and IgA in parasite +ve and -ve cases from Shahjahanpur.

but they have suffered repeatedly from malaria. ELISA IgG, IgM and IgA profiles of these samples are shown in Fig. 1, level of IgG in both infected and parasite negative groups was low, though they had multiple attacks of the disease. High level of IgM was found in infected sera and few negative sera were also positive for IgM denoting the active transmission of the disease during that season. Certain amount of antigen specific IgA was seen in both parasite positive and negative samples. In disease positive samples 71% were positive for IgG, 90% for IgM and 23% for IgA and for blood smear negative

samples 42% were positive for IgG, 58% for IgM and 33% for IgA (Table 4).

Seventy one venepunctured blood samples from adults were obtained from the villages of Ghaziabad in the months of November and December during P. falciparum transmission. Level of IgG in both positive and negative groups was more or less alike. Individuals with repeated attacks of malaria and also after treatment had an elevation in IgG level. An increased level of IgM was measured in acute infection and also in samples taken after antimalarial treatment. Antigen specific IgA was detected in few cases (Fig. 2).
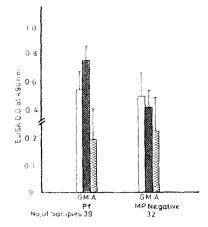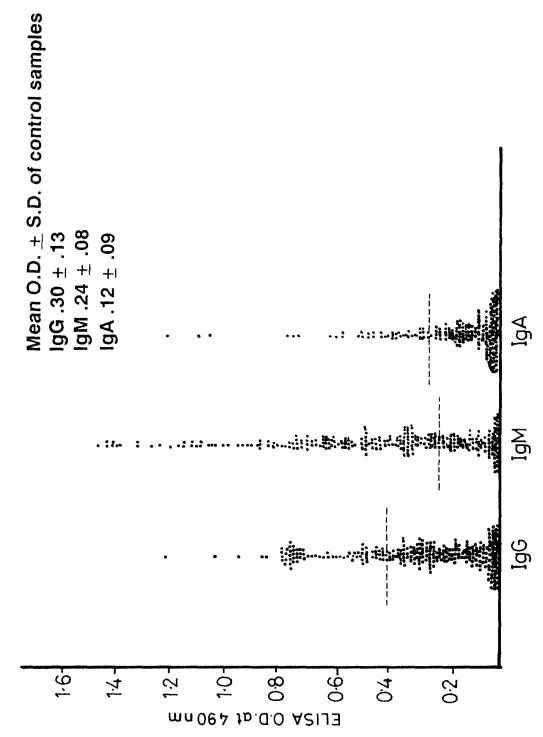
Fig. 2: Profile of antigen specific IgG, IgM and IgA in Pf cases and parasite -ve cases from Ghaziabad.

Table 4. Seropositivity in samples of different age groups collected from Shahjahanpur

Age in years	No. of samples in each age group	Blood smears +ve				Blood smears -ve			
		IgG	IgM	IgA	No. of samples	IgG	IgM	IgA	No. of samples
<10	9	8	9	0	9	-	-	-	0
<15	10	8	10	3	10	-	-	-	0
<20	38	21	36	5	36	2	1	0	2
<25	47	31	38	12	42	1	3	3	5
<30	30	19	20	8	25	2	3	1	5
>30	24	17	19	5	24	-	-	-	-

Mean O.D. ± S.D. of control samples

IgG .30 ± .13
IgM .24 ± .08
IgA .12 ± .09

Fig. 3: Scatter diagram of antimalarial IgG, IgM and IgA level in 355 samples collected from Jabalpur, Dist., Mandla. IgG was estimated at 1:1024 dilution, IgM and IgA at 1:128 dilution. Graph is plotted after subtracting the value of control from test. (——) cut-off point of 3SD after subtracting the control Mean O.D.

Blood samples on filter paper collected from individuals of villages of District Mandla, Jabalpur were tested for 3 antibodies (Fig. 3). About 28 per cent samples were detected positive for IgG, whereas 57% showed a positive indication of disease transmission by an elevated level of IgM. In most of the samples collected from parasite positive cases and from those that had been given treatment showed a high level of IgM which proves the endemicity of the areas. In a few cases antigen-specific IgA has also been detected (Table 5). The survey was done in almost all age groups (2 years and above). Overall antimalarial IgG level had started increasing after 5 years which shows that the rate of infec-

tion was high in the younger age groups but the level of IgG later was not high enough in older groups (Fig. 4). In most of the individuals with active infection, IgM was detected but in persons with a recent attack of the disease both IgG and IgM levels were elevated (Table 6).

Along with the detection of circulating free antibodies, presence of antibody complexed with antigen has been done by PEG assay in 357 sera collected from both endemic and non-endemic areas (Fig. 5). This assay is sensitive and has the limitation of detecting antigen non-specific immune-complexes (Ravindran *et al.*, 1982). Sera from endemic areas were categorised in 3

Table 5. Intensity of seropositivity in blood samples (n= 355) from Mandla for three types of antibody

Degree positivity	Samples positive for antimalarial antibody					
	IgG		IgM		IgA	
	No. of samples	% (+ ve)	No. of samples	% (+ ve)	No. of samples	% (+ ve)
+	44	12.4	101	28.45	23	6.48
+ +	49	13.8	58	16.34	7	1.97
+ + +	3	0.84	18	5.07	3	0.84
+ + + +	2	0.6	24	6.76	3	0.84
Total positive samples	98	27.6	201	56.62	36	10.14

(+) — Weakly +ve; (+ +) —+ve; (+ + +) — Medium +ve; (+ + + +) — Highly +ve.

Table 6. Seropositivity in different age groups

Age in years	No. of samples in each age group	Blood smears +ve				Blood smears -ve			
		IgG	IgM	IgA	No. of samples	IgG	IgM	IgA	No. of samples
<5	41	0	5	0	7	3	33	0	34
<10	54	0	3	0	5	12	26	2	49
<15	59	3	17	1	18	16	15	4	41
<20	53	2	5	1	7	15	30	2	46
<25	117	3	14	1	18	35	59	20	99
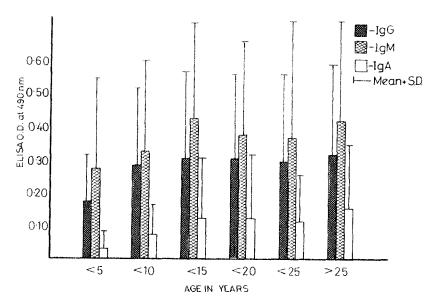
Fig. 4: Agewise distribution of IgG, IgM and IgA. Graph is plotted with mean + SD.
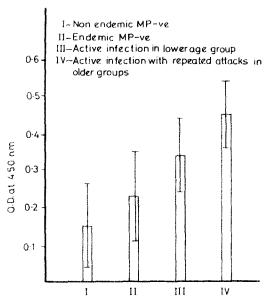
Fig. 5: Antigen bound antibody (CICs) detected by PEG assay in 357 sera collected from four areas.

groups; parasite negative, active infection in lower age groups (15-29 years) after single attack and active infection in older age groups after repeated attacks (>30 years). In healthy control sera from endemic areas (II) mean PEG

assay value was higher than that of non-endemic sera (I). Significantly high levels of circulating immune-complexes were detected in malaria infected sera after single infection (III). These individuals had no past infection with any species of plasmodia. Individuals with acute infection and also with repeated exposures (IV) showed a higher level of CICs, while there were few sera whose PEG assay value was as per control values.

DISCUSSION

The overall slide positivity rates in study samples confirm the indication of active transmission of malaria during this season. There was a marked areawise and seasonwise variation in seropositivity.

Serology is an important epidemiological index along with blood slide examination. Detection of antibody to malaria parasites can be useful in screening for disease carriers, mainly in non-endemic areas. However, these tests sometimes are inconclusive in identifying the active trans-

mission pocket in endemic areas, since a high prevalence of raised antibody titres would be expected in majority of the individuals. In the present study, IgG positivity is not showing any positive correlation with slide positivity for *Plasmodium*. A number of blood samples were collected after giving antimalarial treatment and also after repeated episodes of the disease. No marked elevation in IgG level has been observed in these groups. Rate of IgM positivity in the study groups showed a positive correlation with infected individuals and also in persons after treatment.

A number of voluntary blood donors were positive for IgG (42%) and IgM (10%). In earlier studies (Mahajan *et al.*, 1982), the presence of antimalarial antibodies have been detected in 692 blood donors by IHA and IFA tests. Seropositivity in these samples was 6.4 and 12 per cent, respectively. Therefore, the positivity in blood donors for IgG and IgM confirms earlier observations, since they belong to endemic malarious areas.

In a few cases antigen specific IgA has also been detected which showed that plasmodial infection may cause an elevation of IgA level in populations. About 15 per cent samples with a high amount of IgA indicate the stimulation of immune response but the mechanism is not yet understood.

The presence of circulating immune complexes, during active infection, in antigen excess have been suspected to be responsible for the chronicity encountered in many plasmodial infections. The presence of IgG, IgM and IgA in immune complexes has been demonstrated in human malaria (Houba, 1981).

For the present study the soluble extract of *P. falciparum* has been used which contains a wide range of antigens. A procedure employing a homologous (*P. falciparum/P. vivax*) antigen is likely to give more reliable information (Kumar *et al.*, 1986).

In the study of malaria at present there is no reason to use serological tests for the diagnosis of acute malaria. However, the main serological applications in malarious areas are epidemiological and serology should be used along with parasitological investigation. The test system should be the method of choice to supplement the parasitological data when malaria parasite rates fall to low levels in an area of low malaria transmission. Therefore, collection of blood samples should be an important exercise, which include collections during the non-transmission period and also from the low and non-malarious zones.

Overall data confirm that the presence of antibodies in higher age groups is not showing any marked difference to that in lower age groups. Perhaps, finer distinctions can be made if the studies are carried out in younger age groups (0-5 yrs) as suggested by Kumar *et al.*, 1986.

From the study it is concluded that alongwith IgG detection, IgM antibody detection by ELISA can also be done in endemic populations for determining the pockets of high and low transmission and of recent and active infections. This type of study would not only help to evaluate the immune status on the basis of antibody level at various periods of disease transmission but would also enable us to isolate antigens with the help of polyvalent antibodies against natural infection with malaria in populations.

the staff of MRC Field Stations at Shahjahanpur and Jabalpur. Authors still remember the kind co-operation of Late Dr. Amar Nath during the survey.

REFERENCES

1. Avrameas, S. and T. Ternynck (1969). The cross-linking of proteins with glutaraldehyde and its use for the preparation of immmunosorbents. *Immun. Chem.*, **6**: 53.

2. Cohen, S., I.A. McGregor and S. Carrington (1961). Gammaglobulin and acquired immunity to human malaria. *Nature* (London), **192**: 733-737.

3. Collins, W.E., P.G. Contacos, J.C. Skinner, A.J. Harrison and L.S. Gill (1971). Patterns of antibody and serum protein in experimentally induced human malaria. *Trans. R. Soc. Trop. Med. Hyg.*, **65**: 43-58.

4. Haskova, V., J. Kaslik, I. Riha, I. Matt and J. Rovensky (1978). Simple method of circulating immune-complex detection in human serum by polyethylene glycol precipitation. *A. Immun. Forsch.*, **154**: 399.

5. Houba, V. (1981). Immune-complexes in malaria and their immuno-pathological significance. WHO/MAL./81: 963.

6. Kumar, Ramesh, S.N. Rao, M.A. Ansari, R.K. Razdan, A. Srivastava and V.P. Sharma (1986). Feasibility of IHA and ELISA in seroepidemiology of malaria. *Indian J. Malariol.*, **23**: 75-80.

7. Mahajan, R.C., R.E. Chandnani, M. Thadani, R.N. Prasad and N.K. Ganguly (1982). Malaria antibodies in voluntary blood donors, *Indian J. Med. Res.*, **75**: 661-666.

8. Mancini, G., A.O. Garbenera and J.F. Hereman (1965). Immunochemical quantitation of antigens by single radial immunodiffusion. *Immun. Chem.*, **2**: 235-242.

9. McGregor, I.A. (1981). Immune phenomena in naturally acquired human malaria. WHO/MAL/81: 966.

10. Ravindran, B., R.R. Sharma, B.K. Sharma and Q.Z. Hussain (1982). Circulating immune-complexes in rodent and simian malaria. *J. Biosci.*, **4**: 491-498.

11. Tobie, J.E., D.C. Abele, G.J. Hill, P.G. Contacos and C.B. Evans (1966). Fluorescent antibody studies on immune response in sporozoite and blood induced vivax malaria. *Am. J. Trop. Med. Hyg.*, **15**: 676.

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

Economic Loss due to Malaria in Kheda District, Gujarat

R.C. SHARMA[1], V.S. MALVIYA[1] and P.G. BHATT[1]

A survey of nine experimental and four control villages of Kheda district, Gujarat, revealed that economic loss due to malaria was higher in the control villages where integrated vector control is not practised. Significant differences were observed between the two groups in the number of man days lost and the expenditure incurred in treatment.

INTRODUCTION

The Government of India is spending about Rs. 200 crores annually in order to keep malaria in check, still there are many reports of malaria outbreaks and even of deaths. An alternative approach to malaria control through bioenvironmental measures with a strong health education component was experimentally attempted in Kheda district of Gujarat (Sharma et al., 1986; Sharma and Sharma, 1989), with remarkable success.

A survey was carried out in December 1988 to estimate and compare the impact of the bioenvironmental control strategy on economic loss with that in the adjoining area. Observations made from the survey data have been reported in this paper.

MATERIAL AND METHODS

In November/December 1988 a survey was conducted in five villages i.e., Mahisa, Nizampur, Surashamal, Sodhpur and Tundel of Nadiad taluka and four villages i.e., Lakshmanpura, Ladvel, Kathana and Anara of Kapadwanj taluka. All the above named villages belonged to the area where bioenvironmental control of malaria was implemented and henceforth will be referred to as experimental villages. Four villages i.e., Saiyat, Ravalia, Bordi and Visnagar were selected as control villages where insecticides were used for controlling malaria. These villages belonged to Thasra taluka. Criteria for selection of the villages was possibility of encountering sufficient number of persons during the survey who had experienced malaria. Selection was on the basis of prevalence of malaria in previous three months namely August, September and October. Survey was completed with in one month (22 November—19 December 1988). Four proformas were designed to collect the information on, (1) Village, (2) Head of the family, (3) Patient and (4) Expenditure incurred.

Table 1. Basic data on study villages

		Experimental villages									Control villages			
		Mahisa	Nizam-pur	Surash-amal	Sodh-pur	Tundel	Lakshman-pura	Ladvel	Kath-ana	Anara	Saiyat	Rava-lia	Bordi	Vis-nagar
Population		5336	1420	5457	7232	3888	692	2263	2225	6396	2700	2800	2167	1500
No. of houses {	Village	717	235	326	444	518	74	309	275	457	465	300	400	210
	Field	283	7	715	905	537	49	126	177	653	105	175	150	80
Bus station		+	+	+	+	+	+	+	+	+	+	+	+	+
Post office		+	+	+	+	+		+	+	+	+	+	+	+
Bank		+		+	+					+				
Chemist shop														
Private doctor		+		+	+					+				
Allopathic														
Ayuryedic		+		+	+					+				
Homeopathic														
Bhuva (Witch doctor)		+	+	+	+	+	+	+	+	+	+	+	+	+
Electricity		+	+	+	+	+	+	+	+	+	+	+	+	+
School		+	+	+	+	+	+	+	+	+	+	+	+	+
Riverine				+	+			+						+
Canal irrigated		+	+	+	+	+	+		+	+	+	+	+	+
Water logged		+	+	+	+	+			+	+	+	+	+	+

Data was collected only from families/houses reporting malaria and was processed on computer using standard software.

RESULTS AND DISCUSSION

Village profile, socio-economic status and exposure to media for heads of families reporting malaria

Nine experimental villages (pop. 34,909) and four control villages are a good sample size for comparison purposes between the said two areas. Facilities and services available in the villages of both areas have been listed in Table 1. The villages in this region are comparatively better off because bus services, electricity supply, schools, post offices and banks are present almost in every village.

There were 2 riverine villages in experimental and one in control area. All villages were canal irrigated except one in the experimental area. Services of private doctors, all ayurvedic, were available only in experimental area (4 villages) but Bhuva (witch doctors) were practising in all the villages. None of the villages had a chemist shop.

Further information on village profile based on all houses reporting malaria positive cases is given in Table 2. As regards the location of houses, type of the houses, with or without sunlight and ventilation, location of kitchen, water logging and location of cattlesheds; villages both of experimental and control area were found to have similar habitations.

Mud houses represented 86% of the total structures. These are often mentioned as a drawback in the conventional malaria control programme using insecticides because of the practice of mud plastering soon after spray.

A total of 88% of the houses had cattlesheds either in or close to the house. Forty per cent houses were situated close to water logged ar-

eas. Majority (86.6%) of the houses had a kitchen in the living room.

Table 3 gives the basic profile of socio-economic status and exposure to media for the heads of the families reporting malaria. About 60% of the head persons had primary or higher education. Their main occupation was either agriculture or they were labourers. Occupations like business, service, housework and others constituted about 30%. Majority (86%) of the head persons were apparently poor. This view is independently supported by the other estimates as 86.4% of head persons living in mud houses, 80.1% of the head persons earning only upto Rs. 25 a day (more than half of them < Rs. 10 per day) and 79.1% of head persons having no exposure to media at all.

Economic loss

Losses in terms of mean man days lost and expenditure incurred at each episode of malaria on treatment, travel and food etc. are given in Table 4 for patients both from experimental and control villages. Average total expenditure for each malaria case comes to Rs. 73 in experimental villages and Rs. 151 in control villages. This does not include the expenditure incurred by the government agencies in both the areas through its existing services.

As regards the mean daily income of the patients who suffered from malaria in both the areas there was no significant difference. However, in terms of man days lost difference was observed. Malaria patients lost on an average 8 man days in experimental villages compared to 16 in control villages. Due to this difference mean loss in wages in experimental area comes to Rs. 27.2 against Rs. 60.8 in control area. Mean expenditure on quacks, travel and special diet was similar (about Rs. 8) in both the areas but mean expenditure on treatment differed significantly. It was Rs. 37.4 and Rs. 82 in experimental and control areas, respectively. Another important observation was that 29% patients

Table 2. Village profile based on all houses

Area	Location		Type			Sunlight	
	Village	Field	Pucca	Semi Pucca	Mud	Suffi-cient	Insuffi-cient
Experimental	269 (51.0)	258 (49.0)	14 (2.6)	60 (11.4)	453 (86.0)	437 (82.9)	90 (17.1)
Control	61 (57.5)	45 (42.5)	1 (0.9)	11 (10.4)	94 (88.7)	94 (88.7)	12 (11.3)
Total	330 (52.1)	303 (47.9)	15 (2.4)	71 (11.2)	547 (86.4)	531 (83.9)	102 (16.1)

Sample size: 527 (88.3%) from experimental and 106 (16.7%) from control villages.

Table 3. Basic profile on socio-economic status and

Area	Education				Occupation						Economic	
	Illite-rate	Pri-mary	Secon-dary	Gradu-ate	Agri-culture	Lab-our	Ser-vice	Busi-ness	House work	other	Poor	Average
Experi-mental	218 (41.4)	251 (47.6)	55 (10.4)	3 (0.6)	220 (41.7)	136 (25.8)	19 (3.6)	13 (2.5)	4 (0.8)	135 (25.6)	449 (85.2)	72 (13.7)
Control	49 (46.2)	43 (40.6)	14 (13.2)	0 (0.0)	23 (21.7)	54 (50.9)	5 (4.7)	5 (4.7)	1 (0.9)	18 (17.0)	98 (92.5)	8 (7.5)
Total	267 (42.2)	294 (46.4)	69 (10.9)	3 (0.5)	243 (38.4)	190 (30.0)	24 (3.8)	18 (2.8)	5 (0.8)	153 (24.2)	547 (86.4)	80 (12.6)

Sample size: 527 (83.3%) from experimental and 106 (16.7%) from control villages.

Table 4. Economic

A. Break up of loss

No. of cases	Mean daily income (Rs.)	Mean man days	Mean loss in wages (Rs.)	Mean expenses on (Rs.)				Mean* total expendi-ture per case (Rs.)
				(1) Quacks	(2) Travel	(3) Special diet	(4) Treatment	
Experimental area (766)	3.4	8	27.2	2.5	4.1	1.3	37.4	73
Control area (174)	3.8	16	60.8	0.1	5.8	2.0	82.0	151

* does not include expenditure incurred by government on those patients who received treatment through government dispensary or malaria worker.

reporting malaria positive cases

Ventilation		Kitchen			Water logging		Cattleshed		
Suffi-cient	Insuffi-cient	In liv-ing room	Sep-arate	In open	Yes	No	In house	Near house	Away from house
454 (86.1)	73 (13.9)	449 (85.2)	66 (12.5)	12 (2.3)	211 (40.0)	316 (60.0)	24 (4.6)	444 (84.2)	59 (11.2)
93 (87.7)	13 (12.3)	99 (93.4)	7 (6.6)	0 (0.0)	32 (30.2)	74 (69.8)	6 (5.7)	84 (79.2)	16 (15.1)
547 (86.4)	86 (13.6)	548 (86.6)	73 (11.5)	12 (1.9)	243 (38.4)	390 (61.6)	30 (4.7)	528 (83.4)	75 (11.9)

exposure to media for the heads of the families

status		Exposure to Media					Daily Income (Rs.)			
Good	Exce-llent	News-paper	TV	Radio	More than one	None	None	1-10	11-25	26-50
5 (0.9)	1 (0.2)	26 (4.9)	2 (0.4)	41 (7.8)	46 (8.7)	412 (78.2)	30 (5.7)	228 (43.3)	191 (36.2)	65 (12.3)
0 (0.0)	0 (0.0)	3 (2.8)	0 (0.0)	6 (5.7)	8 (7.5)	89 (84.0)	7 (6.6)	62 (58.5)	26 (24.5)	8 (7.5)
5 (0.8)	1 (0.2)	29 (4.6)	2 (0.3)	47 (7.4)	54 (8.5)	501 (79.1)	37 (5.8)	290 (45.8)	217 (34.3)	73 (11.5)

loss due to malaria

B. Estimated loss due to malaria in Kheda district (1988)

Strategy	Loss in (Rs.)		
	Per episode	In whole	Population/1000
A. Bioenvironmental control	73	13,36,630	500
B. Insecticidal control	151	27,64,810	1000

Total population (rural) : 27,00,000; Confirmed malaria cases: 18,310

visited quacks and 73% did not spend any money on treatment in IDVC villages. In control villages 44% patients visited quacks and only 57% did not spend any money on treatment. This difference could be due to the impact of health education as a result of which more people took services of the malaria workers.

From the study it appears that malaria is associated with people of poor socio-economic status having no or very low exposure to media (Table 3). Differences in mean man days lost and expenditure incurred on treatment by patients in experimental and control villages are notable. The less number of mean man days lost in experimental villages are perhaps due to prompt and better services available to the people.

Differences in treatment expenses can be explained using survey data on treatment agencies. A total of 345 patients out of 766 in experimental villages and 60 out of 174 in control villages took self medication or treatment from a government dispensary, malaria worker or private doctor and the remaining 421 from experimental and 114 from control villages took treatment from multiple agencies. The break up of patients who took treatment from single agency and multiple agencies is given in Table 5.

Among the patients receiving treatment from single agency in experimental area, 98.8% of the patients took treatment from malaria workers and the remaining 1.2% from private doctors or government dispensary. Contrary to the above only 30% of patients in experimental villages took treatment from malaria workers and 6.7% from government dispensaries. A big proportion of the patients (60%) went to private doctors and 3.3% took self treatment. Since the services of malaria workers paid for by the government and government dispensaries are free and only 36.7% of the patients in control area utilized them against 99.4% of the patients in experimental area, therefore expenditure on treatment prescribed by private doctors is likely

Table 5. Patients receiving treatment from different agencies

A. Treatment from single agency

Agency		Experimental	Control
1. Government dispensary	(A)	2 (0.6)	4 (6.7)
2. Malaria worker	(B)	341 (98.8)	18 (30.0)
3. Private doctor	(C)	2 (0.6)	36 (60.0)
4. Self	(D)	0	2 (3.3)
Sub total		345	60

B. Treatment from multiple agencies

		Experimental	Control
5.	A + B	46 (10.9)	4 (3.5)
6.	A + C	0	15 (13.2)
7.	B + C	329 (78.1)	89 (78.1)
8.	B + D	2 (0.5)	0
9.	C + D	1 (0.2)	0
10.	A + B + C	41 (9.7)	6 (5.3)
11.	B + C + D	2 (0.5)	0
Sub total		421	114
Grand total		766	174

pvt. doctor 62·1%   malaria worker 98·8%

govt. disp. 6·9%

malaria worker 31·0%
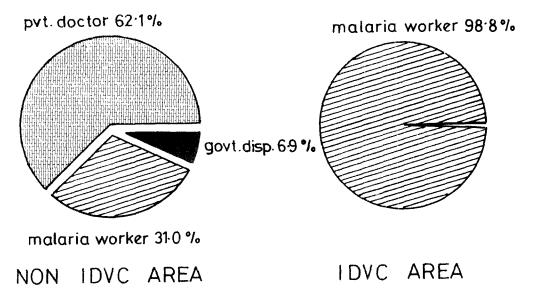
NON IDVC AREA   IDVC AREA

Fig. 1: Treatment agencies in the study area

to be higher. This was actually observed in terms of mean expenditure incurred per case in experimental (Rs. 37.4) and in control villages (Rs. 82). Among patients receiving treatment from multiple agencies the proportion of those going to government dispensaries and private doctors was the same (78.1%) in both the areas. However 10.9% of the patients in experimental area received treatment from government dispensary and malaria workers against only 3.5% in control area (Fig. 1).

In terms of loss due to malaria in Kheda district in 1988 for the total 18,310 confirmed malaria cases the total comes to Rs. 27,64,810 at the estimated rate of Rs. 151 per case per year. The said loss is according to the estimates of control area. This loss comes to Rs. 13,36,630 at a rate of Rs. 73 per case according to the estimates of experimental area. Thus had the bioenvironmental control strategy been implemented in the entire district the net saving by the population would have been Rs. 14,28,180 for the year 1988. Therefore the need for implementation of IDVC strategy under primary health care system should be considered seriously. Cost effectiveness of bioenvironmental control of malaria has

already been demonstrated by Sharma and Sharma (1986) and was subsequently confirmed by the "In-depth Evaluation Report" of the project (Anonymous, 1987).

REFERENCES

1.   Anonymous (1987). In-depth evaluation of the Community based Integrated Vector Control of Malaria project in Kheda (Gujarat).

2.   Sharma, V.P. and R.C. Sharma (1986). Cost effectiveness of bio-environmental control of malaria in Kheda district, Gujarat. Indian J. Malariol., 23(2):141-145.

3.   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(2): 95-117.

4.   Sharma, V.P. and R.C. Sharma (1989). Community based bioenvironmental control of malaria in Kheda district, Gujarat, India. J. Am. Mosq. Cont. Assoc., 5(4): 514-521.

Studies on Outbreak of Malaria in Muliad Village of Kheda District, Gujarat

R.C. SHARMA[1] and A.S. GAUTAM[1]

Irregular and inadequate surveillance of fever cases, misdiagnosis of positive cases and the near nonexistence of an appropriate monitoring mechanism culminated in an outbreak of malaria in a village of Kheda district, Gujarat. *Plasmodium falciparum (Pf)* resistance to chloroquine must have aided in the increase of *Pf* cases. DDT still appears to be effective against the vector, *A. culicifacies* in the area. Amodiaquine was successful in clearing asexual parasites in 83% cases which were resistant to chloroquine. Bioenvironmental control of malaria is suggested in order to delay the development of insecticidal resistance in vector mosquitoes.

INTRODUCTION

In December 1988, an outbreak of malaria occurred in the village Muliad of Kheda district, Gujarat. Till September not a single malaria case was reported from the village. But in October, 9 and in November, 12 malaria cases were reported, then in December, from a population of 1669, 646 malaria positive cases were detected in a mass blood survey of 1098 fever cases. In all 95% (616) cases were due to *Pf*.

The village Muliad, situated on the banks of the river Shedi, is a small village with a population of 1669 (NMEP census, 1988) (Figs. 1 and 2). Canal irrigation was started in the village in 1987. The main canal flows near the village and

its distributary passing through the village is very poorly maintained. River bed pools and numerous water bodies of all shapes and sizes around the village provide extensive breeding grounds for vector proliferation . Since 1986, no insecticide was sprayed in the village or in Chetarsumba PHC to which the village belongs.

MATERIAL AND METHODS

Under intervention measures undertaken by state health department: DDT (50%, WDP) was sprayed in Muliad and the adjoining villages from 6th December 1988 onwards with a coverage of 97%. The spray round was completed within 2 days. Fever Radical Treatment (FRT) was given from 4th December 1988 to 15th February 1989. FRT included 600 mg chloroquine and 45 mg primaquine as single adult dose for presumptive treatment of fever cases. No further treatment was given to the confirmed positive cases. Larvivorous fishes (Guppy) were introduced in all water bodies in Muliad.
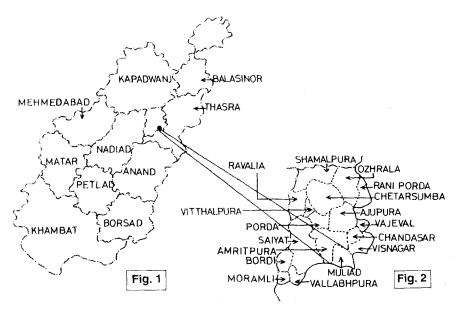
Fig. 1: Showing talukas of Kheda district and Chetarsumba PHC in Thasra taluka.

Fig. 2: Showing villages of Chetarsumba PHC.

RESULTS AND DISCUSSION

Investigations revealed that during 1988, in Chetarsumba PHC to which the village Muliad belongs, surveillance workers made 749 (78%) visits out of 960 scheduled visits in all the villages of the PHC. However, in Muliad, only 34 (47%) visits out of 72 scheduled were made. Thus, the village was poorly covered under active surveillance. But it is interesting to note that blood smear collection showed marked increase in June and then a continuously significant increasing trend was observed from August onwards (Table 1). There was no backlog of slides as per the PHC record. A sudden and large spurt in malaria cases within a month is however unlikely. The reason for the sudden spurt in malaria cases in December was due to a mass survey carried out by the district health authorities on receiving a public complaint. Laboratory technicians might have missed the detection of positive cases earlier which is evident from the data from Muliad and the rest of Chetarsumba PHC as given in Table 1.

Results of the weekly monitoring of total mosquitoes, total anophelines and A. culicifacies by hand collection method from Muliad and unsprayed neighbouring village Bordi (Figs. 1 and 2) are given in Table 2. In Muliad, the observed total per man hour mosquito density of 61.5 in the 1st week of December was reduced to and remained at very low levels for 6 weeks after the spraying of DDT; no A. culicifacies specimen was encountered for six weeks. In the control village Bordi total mosquito and total anopheline densities including that of A. culicifacies remained persistently high from December to March 1989. Mosquito densities here were similar to those in the pre-DDT spray period in Muliad and adjoining villages. It is important to note that Chetarsumba PHC received 50 rounds of DDT, 18 of HCH and 1 of malathion during the period between 1953 and 1985. This insecticidal pressure has most likely led to the selection of a resistant strain.

Bio-assay tests in Muliad were carried out on 10th January 1989. A. culicifacies were collected

from the neighbouring unsprayed village Pali of Sevalia PHC and exposed on sprayed mud plaster, wooden surface and cement walls. Results showed 9.0%, 14.7% and 12.0% mortality, respectively. Though bio-assay tests showed low mortalities, epidemiological impact of DDT spray was achieved due to excito-repellent action of DDT coupled with adverse climatic conditions which cause natural mortality. This was also observed by Kalra,1989 (Personal communication). In view of the above, studies on pro-

longed exposure to DDT beyond the standard exposure period of one hour in the area and elsewhere would be very informative. Fever radical treatment in absence of the vector is expected to break the transmission, but continued occurrence of falciparum malaria cases beyond December was probably due to inadequate dose of chloroquine administered in FRT.

The study of positive cases in Muliad which were given FRT, revealed that out of 648 *Pf* cases

Table 1. Parasitological data of Chetarsumba PHC for 1988-89

Month	Muliad			Rest of the PHC		
	BSE	Total positive	Pf	BSE	Total positive	Pf
Jan 88	3	-	-	226	1	1
Feb	13	-	-	363	1	1
Mar	8	-	-	306	4	2
Apr	9	-	-	369	2	0
May	13	-	-	450	0	0
Jun	34	-	-	395	1	0
Jul	5	-	-	227	1	0
Aug	34	-	-	711	2	0
Sep	71	-	-	535	10	4
Oct	240	9	4	482	45	32
Nov	69	12	12	211	4	4
Dec*	1098	646	616	3827	433	377
Sub total	1597	667	632	8102	504	421
Jan 89	546	309	302	303	49	49
Feb	233	56	53	266	6	4
Mar	154	40	18	207	0	0
Sub total	933	405	373	766	55	53

Note : (-) denotes Nil; * results include mass survey by district health authorities.

Table 2. Results of monitoring of mosquito densities from Muliad and the control village Bordi

	December 1988				January 1989					February				March			
	I	II	III	IV	I	II	III	IV	V	I	II	III	IV	I	II	III	IV
Muliad : Study village																	
A. culicifacies	35.0	-	-	-	-	-	-	1.5	0.5	1.0	4.5	4.5	11.5	10.5	12.0	23.5	21.0
Total anophelines	59.5	-	0.5	5.0	1.5	2.0	1.0	3.5	3.5	5.5	11.0	11.0	17.0	23.5	23.5	93.0	74.0
Total mosquitoes	61.5	-	0.5	5.5	2.5	2.0	1.0	7.5	9.0	9.5	15.0	16.0	29.0	26.5	27.0	114.0	92.5
Bordi: Control village																	
A. culicifacies	8.5	25.0	7.0	5.5	12.0	8.0	4.5	6.0	3.5	2.5	4.0	14.5	3.0	29.5	39.0	6.5	20.0
Total anophelines	102.5	177.5	87.5	122.5	93.5	80.5	46.0	82.5	56.5	73.5	91.5	111.0	59.5	279.0	259.0	368.0	340.5
Total mosquitoes	105.0	183.0	92.0	126.5	94.5	83.5	46.5	83.0	56.5	74.0	100.0	113.5	64.5	282.0	264.5	386.0	385.0

treated, 93 were relapses. In absence of vector, this was suggestive of chloroquine resistance in *Pf*. Consequently, studies on chloroquine resistance were initiated from 10th January 1989 which lasted till April. *In vivo P. falciparum* resistance was monitored following WHO (1973) extended field test for 28 days. Patients found positive for *Pf* were given adult dose of 1500 mg chloroquine (600 mg chloroquine on Day 0 and 1 and 300 mg on Day 2). Children were given proportionately low doses. Out of 296 *Pf* cases studied, 53 (17.9%) became positive after initial clearance of asexual parasitaemia within 28 days after administration of the drug. Out of these 53 cases, 42 became positive between Day 15 and Day 28. These 42 cases were administered amodiaquine 1500 mg as per chloroquine regimen given above on the assumption that chloroquine was completely excreted/metabolised within 14 days of its administration. Seven cases (16.7%) did not respond to amodiaquine in 28 days follow-up. These cases were given 3 tablets of malocide (adult dose) each containing 500 mg sulfadoxine and 25 mg pyrimethamine. From the foregoing it may be inferred that in the study area parasite has developed RI resistance to chloroquine with average of 17.9% cases which is higher than the commonly encountered average of 12.3% in neighbouring Nadiad taluka (Sharma and Sharma, 1988). Of

the cases which did not respond to chloroquine 11.8% responded to amodiaquine and 7 cases (2.4%) were found resistant to both the drugs (Table 3). Amodiaquine has been reported to be marginally more active than chloroquine on strains of *P. falciparum* resistant to chloroquine (Bruce Chwatt, 1985; 1986). Use of amodiaquine in place of chloroquine has been suggested in cases resistant to chloroquine (Park and Park, 1983). A study done by Watt *et al.* (1987), reported amodiaquine to be substantially less effective than chloroquine for treatment of *P. falciparum* infections in Filipino patients.

P. falciparum resistance to chloroquine has been reported from various parts of India (Sharma, 1984b) including Kheda district (Sharma and Sharma, 1988). Therefore, widespread monitoring of *Pf* resistance in the country is required urgently. Once *Pf* resistance is delimited it needs to be tackled for the elimination of its foci. Several outbreaks and epidemics of malaria have been investigated earlier by several authors. A more lasting solution to the problem of malaria control by implementation of the integrated disease vector control methodology on the Nadiad pattern was suggested by Sharma *et al.* (1985) and subsequently supported by Singh *et al.* (1988). Successful control of malaria by bioenvironmental methods in Kheda district of

Table 3. Study of drug resistance in Muliad

Drug administered (adult dose)	No. of cases treated	No. of cases recrudesced	% Resistance
Chloroquine (600 mg)	648	·93*	14.3
Chloroquine (1500 mg)	296	53	17.9
Amodiaquine (1500 mg)	42	7	16.7
			(2.4)**

*Repeat cases without follow-up, **Percentage out of 296 cases.

Gujarat has been reported by Sharma (1984a), Sharma et al. (1986) and Sharma and Sharma (1986). The value of good surveillance was highlighted by Chandrahas and Sharma (1983) and Ansari et al. (1984).

ACKNOWLEDGEMENTS

Authors are thankful to Shri V.S. Malviya, Health Educator and Shri S.M. Banerjee, Technical Assistant for their help during the course of these studies.

REFERENCES

1. Ansari, M.A., C.P. Batra and V.P. Sharma (1984). Outbreak of malaria in villages of Bareilly district, U.P. *Indian J. Malariol.*, **21**: 121-123.

2. Bruce Chwatt, L.J. (1985). *Essential Malariology.* ELBS edition. (William Heinemann Medical Books): 224.

3. Bruce Chwatt, L.J. (1986). *Chemotherapy of Malaria.* 2nd ed. (WHO, Geneva): 40

4. Chandrahas, R.K. and V.P. Sharma (1983). Malaria epidemic in Shahjahanpur (U.P.). *Indian J. Malariol.*, **20**: 163-166.

5. Park, J.E. and K. Park (1983).*Text Book of Preventive and Social Medicine.* (Banarasidass Bhanot, Jabalpur): 365.

6. Sharma, R.C. (1984a). *Feasibility of mosquito control by non-insecticidal methods in malaria endemic area of Gujarat, India.* XVII International Congress of Entomology, Hamburg. (Abstract).

7. Sharma, R.C. and V.P. Sharma (1988). Epidemiological implications of population migration; Part I: Imported malaria in Kheda district, Gujarat. *Indian J. Malariol.*, **25**: 113-116.

8. Sharma, V.P. (1984b). Drug resistant *Plasmodium falciparum* malaria in India. *Proceedings of the Indo-U.K. Workshop on Malaria* edited by Dr. V.P. Sharma. (Malaria Research Centre, ICMR, Delhi): 169-184.

9. Sharma, V.P., R.K. Chandrahas, B.N. Nagpal and P.K. Srivastava (1985). Follow-up studies of malaria epidemic in villages of Shahjahanpur district, U.P. *Indian J. Malariol.*, **22**:119-121.

10. Sharma, V.P. and R.C. Sharma (1986). Review of integrated control of malaria in Kheda district, Gujarat, India. In *Community Participation for Disease Vector Control,* edited by Dr. V.P. Sharma. (Malaria Research Centre, ICMR, Delhi): 59-84.

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

12. Singh, Neeru, V.P. Sharma, M.M. Shukla and Gyan Chand (1988). Malaria outbreak in Kundam block, District Jabalpur (M.P). *Indian J. Malariol.*, **25**: 41-49.

13. Watt, George, G.W. Long, L. Padre, P. Alban, R. Sangalang, C.P. Ranoa and L.W Laughlin (1987). Amodiaquine less effective than chloroquine in the treatment of falciparum malaria in the Philippines. *Am. J. Trop. Med. Hyg.,* **36**: 3-8.

14. WHO (1973). Chemotherapy of malaria and resistance to antimalarials. *Tech. Rep. Ser.*, 529.

Antimalarial Activity of some 4-Alkylamino 2/3 methoxy-4-aminodiphenyl Sulphones

SUBHASH CHANDRA[1], RITA RAINA[1], A.B. SEN[2], MRIDULA SAXENA[3] and A.K. SAXENA[3]

From a series of thirty six 2,3, N-substituted 4,4'-diaminodiphenyl sulphones studied for their suppressive activity in mice against blood induced erythrocytic stage of *Plasmodium berghei* infection, six sulphones (1-6) showed 100% suppressive and curative activity at an intraperitoneal dose of 1 mg/kg x 4 days. These sulphones have been studied for their suppressive activity in still lower doses ranging from 1.0-0.25 mg/kg i.p. x 4 days and for their curative activity at 1 mg/kg i.p. x 4 days in comparison to DDS as standard drug. The maximum tolerated dose of these compounds and DDS has also been determined. These sulphones have better therapeutic efficacy for their suppressive and curative action than DDS.

INTRODUCTION

Sulphones are comparatively slow acting and weak antimalarial compounds acting as dihydropteroate synthetase inhibitors. Their action is potentiated synergistically by DHFA inhibitors or antibiotics (Clyde *et al.*, 1971). The most commonly used drug of this class is 4,4'-diaminodiphenyl sulphone (DDS) which has shown some causal prophylactic, gametocytocidal or sporontocidal action (Ramakrishnan *et al.*, 1962) but it acts mainly as blood schizontocide in

avian, simian and rodent malaria. It is also effective against chloroquine resistant strains. Further DDS has been used against *P. falciparum* malaria in man and it does not show cross resistance with chloroquine. In continuation of our effort to develop better understanding of structure activity relationship among the substituted 4-4'-diaminodiphenyl sulphones, thirty six compounds of this series have been screened against *Plasmodium berghei* blood induced infection in mice. The promising compounds showing activity in mice have been investigated in detail and results are described in this paper.

MATERIAL AND METHODS

Parasite

The strain of *P. berghei* is being maintained by serial syringe passage in the Division of Parasitology, CDRI. The strain was sensitive to chloroquine, primaquine and DDS, the ED $_{100}$

being 5,7 and 0.15 mg/kg, respectively. On rechecking it was found that this strain had the same level of sensitivity to these standard anti-malarials.

Drug

Compounds were synthesized according to the procedure described earlier (Saxena *et al.,* 1989). Suspensions were made in 0.1% Tween 80. The dose levels tried were 1, 0.5 and 0.25 mg/kg in mice infected with *P. berghei.* The standard drug DDS was also used for comparison. For each dose level study, 5-10 animals were used in 2-3 replicates. All compounds were administered by i.p. route for 4 consecutive days. Identical infected untreated animals served as control group.

Assessment of antimalarial activity

Suppressive activity according to Peters (1965) has been followed. Male mice (Park strain) weighing 20 $\pm$ 2 g were infected with standard inoculum of one million parasitized cells (10^6 parasitized red blood cells). The animals were treated for the test compound for 2-3 hrs post-infection. For treatment the inoculated animals were divided into four groups of 5 animals each. All five animals of I, II and III group received the same dose of compounds and IV group served as untreated control. The treatment was given for 4 consecutive days.

Blood films of both treated and untreated animals were examined for malarial parasites on IV day post-commencement of treatment and thereafter every alternate day till the 16th day and subsequently every 3rd day till the death of the animals or upto 30 days whichever was earlier

Curative method

Mice were infected with an inoculum containing 10^6 parasitized erythrocytes. Treatment was commenced and continued for 4 days when the parasitaemia reached about 2% (usually reached on V day post-inoculation). Untreated infected animals served as control.

RESULTS AND DISCUSSION

Compound nos. 1 to 6 showed 100% inhibition of parasitaemia at an initial dose of 1 mg/kg x 4 days i.p. to mice infected with *P. berghei* as compared to untreated control animals. These compunds did not produce 100% inhibition at 0.25 mg/kg i.p. x 4 days (Table 1). The minimum effective doses (MEDs) for the com-

Table 1. Suppressive activity of the 4-alkylamino 2/3 methoxy-4'-aminodiphenyl sulphones

Compd. No.	Dose mg/kg	% Inhibition of parasitaemia on Day						
		5	7	9	11	13	15	17
3	0.25	100	100	80	60	40	35	25
4		90	80	75	65	30	20	15
1		95	90	75	65	30	25	20
2	0.5	85	70		30	10		
5		90	85	70	50	30	8	
6		89	70	63	38	20	10	-

	R	R'
1	2-OCH$_3$	C$_3$H$_7$(n)
2	2-OCH$_3$	C$_4$H$_9$ (n)
3	H	CH$_3$
4	H	C$_2$H$_5$
5	H	C$_4$H$_9$(n)
6	H	H (Suspeded on starch)

Chemical structures of sulphones (1-6)

pounds were determined using different doses ranging form 1.0-0.25 mg/kg i.p. x 4 days and are described in Table 2. When compound 3 and 4 were given at 0.25 mg/kg i.p. x 4 days, there was suppression of parasitaemia initially but soon it attained a steady level as in control animals. The same pattern of activity was observed for the compounds 1,2,5 and 6 even at 0.5 mg/kg. Based on these findings the MED values for these sulphones have been determined and described in Table 2 in comparison to DDS.

The greater safety range of these compounds indicates that the substitution of an alkyl group on 4-amino group of DDS greatly decreases the

Table 2. Comparative chart showing minimum effective doses (MED), maximum tolerated dose (MTD) & therapeutic index (TI) of DDS and test compounds

Compd. No.	MED[a] (mg/kg)	MTD[b] (mg/kg)	TI[c]
DDS	0.15	100	667
1.	1	500	500
2.	1	500	500
3.	0.5	500	1000
4.	0.5	500	1000
5.	1.0	500	500
6.	1.0	500	500

a. Minimum effective dose; b. Maximum tolerated dose; c. Therapeutic index.

toxicity without having any adverse effect on their antimalarial activity. Compound 3 and 4 are the most efficient of the six, and have methylamino and ethylamino substitution respectively at the position 4 of the diphenyl ring. Substitution of a butyl group at the same position as in 5, however, decreases the antimalarial activity. Addition of a methoxy group at position 2 in compounds 5 and 1, does not help to improve the antimalarial activity; unlike in the cell free system isolated from P. berghei or from other micro-organisms such as M. lufu, M. smegmetice (Hopfinger et al., 1987; Saxena et al., 1989). This may be due to complexity of the whole animal system where pharmacokinetics and metabolism play an important role. The 4-methylamino and 4-ethylamino substitution in DDS structures are the best combinations for having good activity with less toxicity.

All these compounds and the standard drug DDS were administered i.p. at dose level of 1 mg/kg x 4 days in mice for curative action. There was 100% suppression of parasitaemia till day 30th in all the cases while DDS suppressed the parasitaemia till 25th day. Thus the above sulphones have better curative action than DDS.

The maximum tolerated dose (MTD) for all these compounds was determined and found to be 500 mg/kg while the MTD for DDS under identical conditions was found to be 100 mg/kg. As these compounds have higher MTD in comparison to DDS (Table 2) they are safer and better tolerated. The order of therapeutic efficacy in suppressing parasitaemia based on Therapeutic Index (TI) is in the present order 3=4>DDS> 1=2=5=6 suggesting that the substitution of amino group by alkylamino group at 4-position reduces the toxicity and increases the curative action while substitution by methoxy group at ortho-position to sulphone group decreases the suppressive activity without affecting the toxicity in comparison to corresponding unsubstituted compounds. Further work on these compounds is under progress.

REFERENCES

1. Clyde, D.R., R.M. Miller, A.R. Schwartz and M.M. Levine (1971). Treatment of *falciparum* malaria with sulfalene and trimethoprim. *Am. J. Trop. Med. Hyg.,* **20**: 805-810.

2. Hopfinger, A.J., R.L. Lopez de Compadre, M.G. Koshler and S. Emery (1987). Quantitative structure-activity relationship, **6**: 111-117.

3. Peters, W. (1965). Drug resistance in *Plasmodium berghei* Vincke and Lips, 1948. II. Triazine resistance. *Exp. Parasitol.,* **17**: 80-89.

4. Ramakrishnan, S.P., P.C. Basu, H. Singh and H. Singh (1962). Studies on the toxicity and action of Diaminodiphenyl sulfone (DDS) in avian and simian malaria. *Bull. WHO,* **27**: 213-221.

5. Saxena, M., A.K. Saxena, R. Raina, S. Chandra, A.B. Sen, N. Anand. J.K. Seydel and M. Wiese (1989). Studies on 2,3,N,N'-substituted 4, 4'-Diaminodiphenyl sulphones as potential agents. *Drug Res.,* **39**: 1081-1084.

Interspecific Associations among Anophelines in Different Breeding Habitats of Kheda District, Gujarat. Part I: Canal Irrigated Area

R.M. BHATT[1], R.C. SHARMA[1] and V.K. KOHLI[1]

In a study covering two years, 1306 samples of immatures were collected from 7 different breeding habitats of canal irrigated villages of Kheda district in Gujarat. Out of the samples belonging to 8 anopheline species, 14396 adults emerged. Breeding of *An. culicifacies* was associated with *An. annularis* in ponds, paddy fields, small pools and with *An. stephensi* in paddy fields. Maximum association was observed between *An. barbirostris* and *An. nigerrimus* in irrigation canals and *An. culicifacies* and *An. subpictus* in intradomestic containers. *An. annularis*, *An. stephensi* and *An. subpictus* were strongly associated in paddy fields. Strong interspecific repulsion was observed between *An. subpictus* and *An. stephensi* in intradomestic containers and *An. barbirostris* and *An. subpictus* in ponds.

INTRODUCTION

It is important to know the habitatwise species specific breeding preferences and interspecific associations of malaria vectors in order to optimize species sanitation. In case of stenogamous mosquitoes which either prefer to breed in intradomestic breeding sites or tree holes it is relatively simple to study the interspecific competition whereas, this is relatively complex and difficult in eurygamous species whose range of breeding habitats varies widely. Among larvae which inhabit small containers, intra and interspecific competition have been inferred and demonstrated, albeit primarily from laboratory studies (Moore and Fisher, 1969). Earlier stud-

ies on associated breeding of different species under field conditions were based mainly on the frequency of co-occurrence of immature stages and were not analysed statistically to know the strength of association or repulsion between them (Bhat, 1975a; 1975b; Malhotra *et al.*, 1987). Lounibos (1981) quantified the interspecific associations among tree hole mosquito community of Kenya coast. The present study is the first attempt to know the interspecific associations among anophelines in different breeding habitats in the canal irrigated villages of Kheda district.

MATERIAL AND METHODS

Study area

Studies were conducted for two years from January 1987 to December 1988 in four talukas of Kheda district. District covers an area of 7194 sq

kms which is mainly plain except small hilly regions in Kapadwanj and Balasinor talukas. There are two perennial rivers, Mahi and Sabarmati, whereas rivers Vatrak, Shedi, Meshow and Khari are seasonal, flowing only during the monsoons. Average annual rainfall of the district is between 600 to 750 mm. Except for occasional showers in winter, annual rainfall is received between June and October which shows variation in time and duration, necessitating irrigation for certain crops. The district has a good network of canals and except for Mahemdabad, Kapadwanj and Balasinor talukas, villages of rest of the seven talukas are interspersed by a network of canals and distributaries. As the canal irrigated area represents a stable ecosystem and the non-canal irrigated and riverine areas show seasonality in the availability of breeding sources, the present study was undertaken in the canal irrigated villages of four talukas namely Nadiad, Anand, Matar and Petlad.

Methodology

Larval and pupal collections were made from the breeding sites which included ponds, small pools, paddy fields, irrigation canals and chan-

Table 1. Per cent composition of anophelines identified from different habitats

| Species | Habitats | | | | | | |
	Irrigation canals	Irrigation channels	Paddy fields	Ponds	Small pools	Wells	Intra-domestic containers
1. *An. culicifacies*	68.55 (71.49)	34.41 (31.93)	15.78 (36.09)	7.64 (14.11)	2.15 (7.94)	9.74 (21.79)	1.56 (5.83)
2. *An. stephensi*	0.72 (3.07)	0.66 (2.41)	2.11 (8.27)	0.46 (2.90)	0.49 (2.65)	33.80 (52.56)	76.13 (89.17)
3. *An. annularis*	12.90 (33.77)	4.27 (8.43)	10.03 (30.07)	11.85 (17.43)	2.24 (5.59)	1.39 (5.13)	-
4. *An. subpictus*	15.17 (27.19)	54.34 (63.85)	70.18 (78.19)	76.21 (69.71)	93.90 (87.35)	52.88 (58.97)	22.30 (21.67)
5. *An. aconitus*	0.19 (1.31)	0.09 (0.60)		0.06 (0.83)	0.02 (0.29)	-	-
6. *An. barbirostris*	1.79 (3.95)	5.60 (15.66)	1.67 (3.76)	3.07 (12.45)	1.04 (4.41)	1.79 (6.41)	-
7. *An. nigerrimus*	0.68 (1.31)	0.43 (2.41)	0.22 (0.75)	0.62 (5.39)	0.15 (0.88)	0.40 (2.56)	-
8. *An. tessellatus*		0.19 (1.81)	-	0.09 (0.41)	-	-	-
Total adults	2070	2107	1375	3064	4506	503	771
Total samples	228	166	133	241	340	78	120

Figures in parentheses represent sample positivity.

nels, wells and intradomestic water storage containers like earthen pots, tanks, barrels etc. The immatures were collected by using either a dipper (9.5 cm diameter and 300 ml capacity), well net (25 cm diameter) or a teated glass dropper. The samples were brought to the laboratory in specimen tubes and transferred to disposable plastic containers in the insectary. Culicine larvae were removed from the samples to avoid any predation of anopheline larvae. Larvae were reared in dechlorinated tap water and were fed larval food containing powdered dog biscuits and yeast tablets. Interspecific competition (and other factors) leads to reports of lack of association if the criterion is merely measure of abundance (Southwood, 1978). Hence the mortality of the immatures while rearing was ignored. All adult anophelines that emerged from the collections were anaesthetized with ether and identified using the key of Christophers (1933). The species which emerged from each sample from different habitats were recorded separately. Sampling efforts varied from month to month and the monthly collections do not represent a complete survey of all the breeding habitats. Therefore for quantifying the associations habitatwise emergence of different species occurring together or alone were pooled and C_8 index of association of Hurlbert (1969) which ranges from +1 to –1 was used. Statistical significance was assessed with the corrected X^2 formula indicated by Pielou (1977) for approximating a discrete distribution. When any expected cell value was less than or equal to five, Fisher's exact test was applied. Only samples from which one or more adults emerged were included in calculations.

RESULTS AND DISCUSSION

In all, 14,396 anophelines were identified from 1306 samples of immatures from different habitats. Table 1 shows per cent composition and sample positivity of anophelines identified from different habitats. Eight species namely *An. culicifacies, An. stephensi, An. annularis, An. subpictus, An. aconitus, An. barbirostris, An. niger-*

rimus and *An. tessellatus* emerged out of 241 samples from ponds and 166 samples from irrigation channels. All anophelines mentioned above except *An. tessellatus* were recorded from canals and small pools. *An. aconitus* and *An. tessellatus* were not recorded from wells and paddy fields, intradomestic containers were positive for only three anopheline species i.e., *An. stephensi, An. subpictus* and *An. culicifacies*. The frequency of distributon of different species in various types of aquatic habitats show that *An. culicifacies* preferred to breed mostly in irrigation canals and channels and *An. stephensi* in intradomestic containers and wells. *An. annularis* showed preference for irrigation canals, ponds and paddy fields. *An. subpictus* appeared to be an ubiquitous species as it was observed breeding prolifically in all the breeding habitats searched. Present observations are in conformity with the findings of Yadav *et al.* (1989).

Interspecific associations among anophelines in different habitats (Table 2a-g) are as follows:

a. Irrigation canal: The samples from this habitat produced fourteen pairings with ten positive C_8 values. Highest C_8 values were recorded between *An. barbirostris* and *An. nigerrimus, An. subpictus* and *An. annularis* (p<0.001), and *An. subpictus* and *An. stephensi* (p<0.05).

b. Irrigation channels: Fourteen pairings from the samples produced six positive and two negative associations. Highest C_8 value was recorded between *An. annularis* and *An. barbirostris* (p<0.01). *An. barbirostris* was also positively associated with *An. culicifacies* (p<0.01) and *An. nigerrimus* (p<0.05). Both the known malaria vectors, *An. culicifacies* and *An. stephensi* were also positively associated but produced low C_8 value significant at 1% level.

c. Paddy fields: The samples of immatures collected from this habitat produced eleven pairings of which eight yielded positive C_8 values. Positive association of *An. annularis* with *An. stephensi* (p<0.001), *An. subpictus* (p<0.001)

Table 2. Coefficient of association (C_8) of anophelines in different habitats

a. IRRIGATION CANALS
(n = 228)

Anopheles	culicifacies	stephensi	annularis	subpictus	barbirostris	nigerrimus	aconitus
culicifacies							
stephensi	0.021						
annularis	0.012	0.0					
subpictus	0.0	0.593*	0.363***				
barbirostris	0.03**		0.067**	0.07**			
nigerrimus	0.0				1.00***		
aconitus	0.014	0.0		0.07**			

b. IRRIGATION CHANNELS
(n = 166)

Anopheles	culicifacies	stephensi	annularis	subpictus	nigerrimus	tessellatus
culicifacies						
stephensi	0.127**					
annularis	0.077					
subpictus	-0.180	0.0	0.0			
barbirostris	0.161**		0.30**	-0.183		
nigerrimus	0.054				0.125*	
tessellatus	0.0			0.0	0.0	0.0

c. PADDY FIELDS
(n = 133)

Anopheles	culicifacies	stephensi	annularis	subpictus	barbirostris	nigerrimus
culicifacies						
stephensi	0.265***					
annularis	0.060***	0.846***				
subpictus	0.273	0.717*	0.797***			
barbirostris	0.130*	0.0	0.344**			
nigerrimus			0.0	0.0		

d. PONDS
(n = 241)

Anopheles	culicifacies	stephensi	annularis	subpictus	aconitus	barbirostris	nigerrimus	tessellatus
culicifacies								
stephensi	0.0							
annularis	0.322***	0.0						
subpictus	0.151	0.0	0.112					
aconitus	0.0			0.0				
barbirostris			0.069	-0.787***				
nigerrimus	0.090		0.083	0.409	0.0	0.051		
tessellatus		0.0						

e. SMALL POOLS
(n = 340)

Anopheles	culicifacies	stephensi	annularis	subpictus	barbirostris	nigerrimus
culicifacies						
stephensi	0.0					
annularis	0.394***	0.0				
subpictus	-0.143	-0.271	-0.243*			
barbirostris	0.182*	0.0	0.303**	-0.482**		
nigerrimus	0.0		0.0	0.372		

f. WELLS
(n = 78)

Anopheles	culicifacies	stephensi	annularis	subpictus	barbirostris	nigerrimus
culicifacies						
stephensi	0.0					
annularis						
subpictus	0.149	0.0	0.0			
barbirostris	0.0	0.0		0.0		
nigerrimus	0.0	0.077		0.0		

g. INTRADOMESTIC CONTAINERS
(n = 120)

Anopheles	culicifacies	stephensi	subpictus
culicifacies			
stephensi	1.00		
subpictus	1.00***	-0.628***	

Levels of significance for $x^2_{(1)}$: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

and *An. barbirostris* (p<0.01), produced highest C_8 values. Similarly *An. stephensi* and *An. subpictus* were also strongly associated at 5% level. Like in irrigation channels, *An. culicifacies* and *An. stephensi* were positively associated here also with low C_8 value but produced highly significant x^2 value.

d. Ponds: Sixteen pairings from the samples revealed five positive and four negative associations. Highest positive C_8 value was recorded between *An. culicifacies* and *An. annularis* and produced highly significant x^2 value. Strong negative association was observed between *An. subpictus* and *An. barbirostris* (p<0.001).

e. Small pools: Thirteen pairings from the samples yielded three positive and five negative associations. Highest positive C_8 values were recorded between *An. culicifacies* and *An. annularis* (p<0.001) and *An. annularis* and *An. barbirostris* (p<0.01). *An. culicifacies* was also observed breeding with *An. barbirostris* at a low level (p<0.05). *An. subpictus* was negatively associated with *An. annularis* (p<0.05) and *An. barbirostris* (p<0.01).

f. Wells: Samples of immatures revealed ten pairings out of which only two yielded low C_8 positive values which were non-significant. From Table 1 it can be inferred that though *An. stephensi* breeds prolifically in wells, its breeding is not significantly associated with other species.

g. Intradomestic containers: Three pairings from this habitat produced two positive and one negative C_8 values. Maximum positive association (C_8 = 1) was observed between *An. culicifacies* and *An. subpictus* which produced highly significant x^2. *An. stephensi* was negatively associated with *An. subpictus* (p<0.001). Though *An. culicifacies* and *An. stephensi* showed maximum positive association, it was non-significant.

Positive associations between species may result from a common preference for a particular habitat or from interspecific attraction, negative

associations may be caused by interspecific repulsion. This study clearly shows that maximum significant interspecific associations among anophelines are encountered in paddy fields followed by irrigation canals and channels. *An. culicifacies* preferred to breed in association with *An. annularis* in ponds, paddy fields and small pools and with *An. stephensi* in paddy fields. Complete positive association was observed between *An. barbirostris* and *An. nigerrimus* in irrigation canals and between *An. culicifacies* and *An. subpictus* in intradomestic containers. *An. subpictus* showed strong interspecific repulsion with *An. stephensi* in intradomestic containers and with *An. barbirostris* in ponds.

This study is suggestive of the role of competition in shaping the mosquito populations but it cannot be considered the only factor influencing the population structure. Further studies are required to elucidate interspecific associations in different physiographic areas in order to understand the factors influencing breeding preferences of any particular species.

REFERENCES

1. Bhat, H.R. (1975a). A survey of haematophagous arthropods in Western Himalayas, Sikkim and hill districts of West Bengal: Records of mosquitoes collected from Himalayan region of West Bengal and Sikkim with ecological notes. *Indian. J. Med. Res.*, **63**: 232-241.

2. Bhat, H.R (1975b). A survey of haematophagous arthropods in Western Himalayas, Sikkim and hill districts of West Bengal: Records of mosquitoes

collected from Himalayan region of Uttar Pradesh with ecological notes. *Indian J. Med. Res.,* **63:** 1584-1608.

3.   Christophers, S.R. (1933). *The fauna of British India including Ceylon and Burma.* V. IV.(Today and Tomorrow's Printers and Publishers, New Delhi): 1-359.

4.   Hurlbert, S.H. (1969). A coefficient of interspecific association. *Ecology,* **50:** 1-9.

5.   Lounibos, L.P. (1981). Habitat segregation among African tree hole mosquitoes. *Ecol. Entomol.,* **6:** 129-154.

6.   Malhotra, P.R., P.K. Sarkar, N.G. Das, S. Hazarika and V.M. John (1987). Mosquito survey in Tirap and Subansiri districts of Arunachal Pradesh. *Indian J.*

Malariol., **24:** 151-158.

7.   Moore, G.G. and B.R. Fisher (1969). Competition in mosquitoes. Density and species ratio effects, on growth, mortality, fecundity and production of growth retardant. *Ann. Entomol. Soc. Amer.,* **62:** 1325-1331.

8.   Pielou, E.C. (1977). *Mathematical Ecology.* (John Wiley and Sons, New York).

9.   Southwood, T.R.E. (1978). *Ecological Methods with particular reference to the study of insect populations.* (Chapman and Hall, London).

10. Yadav, R.S., R.C. Sharma, R.M. Bhatt and V.P. Sharma (1989). Studies on the anopheline fauna of Kheda district and species specific breeding habitats. *Indian J. Malariol.,* **26:** 65-74.

Seasonal Prevalence and Resting Behaviour of Malaria Vectors in Koraput District, Orissa

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

Among the seven vector species studied *A. fluviatilis*, *A. jeyporiensis* and *A. maculatus* were exophilic whereas *A. aconitus*, *A. annularis*, *A. culicifacies* and *A. varuna* were endophilic. All the species collected indoors preferred cattlesheds to human dwellings for daytime resting and 71.3% of the total indoor collection was obtained from cattlesheds. Among the outdoor shelters pit shelters were found to be most attractive for all anophelines resting outdoors. In human dwellings anophelines generally preferred to rest on walls during winter and summer but on ceilings during the rainy season. While *A. culicifacies* has two peaks in density, during June-July and March all the other species had only one peak. There was a general decline in the density of vectors observed earlier in this area.

INTRODUCTION

The hilly tracts of the Indian subcontinent are generally malarious. Efforts to control malaria vectors in such situations pose a formidable task. The bionomics and behaviour of vectors are stated to be an important obstacle to the interruption of malaria transmission by intradomiciliary application of residual insecticides (WHO Steering Committee Report, 1983). Vector bionomics change due to insecticidal pressure and environmental degradation and these changes have impact on the effectiveness of residual spray, thus information on these aspects is an important prerequisite for developing suitable control strategy.

Koraput district of Orissa state has a hilly, forested terrain. The reasons for selecting this district are that this has been one of the worst malaria affected areas in India (Perry, 1914; Senior White, 1937; Rajagopalan et al., 1990) and all the four species of human Plasmodia are prevalent (Jambulingam et al., 1989) with the predominance of *Plasmodium falciparum*. Though *Anopheles fluviatilis* has been incriminated as the main vector *A. annularis*, *A. aconitus*, *A. culicifacies*, *A. jeyporiensis*, *A. maculatus* and *A. varuna* were also considered to play some role in malaria transmission by different workers (Weeks, 1951; Stephens and Christophers, 1902; Gunasekaran et al., 1989). Even though the major vector is still susceptible to DDT and HCH (Sahu et al., 1990), domiciliary residual spray carried out by the state government has not been able to interrupt transmission in this area. Finally information on the bionomics and behaviour of the vector species in this area are available only for pre-DDT era. Since

Table 1. Number of anophelines collected from resting sites from May 1988 to Nov. 1989

S. No.	Species	Number of females collected			
		Human dwellings	Cattle-sheds	Total	
				Indoors	Outdoors
	Man hours spent	449	448.5	897.5	299.0
1.	A. aconitus	26	111	137	25
2.	A. annularis	61	185	246	11
	A. annularis var. adiei	0	5	5	0
3.	A. culicifacies	989	5928	6917	114
4.	A. fluviatilis	62	86	148	312
5.	A jeyporiensis	98	594	692	125
6.	A. maculatus	2	5	7	11
7.	A. philippinensis	2	9	11	0
8.	A. varuna	11	35	46	10
9.	A. subpictus	1341	2991	4332	57
10.	A. vagus	2738	3049	5787	179
11.	A. nigerrimus	16	100	116	6
12.	A. barbirostris	2	75	77	3
13.	A. splendidus	20	127	147	74
14.	A. pallidus	10	98	108	3
15.	A. jamesi	1	2	3	4
16.	A. tessellatus	6	8	14	121
17.	A. karwari	0	1	1	0
18.	A. theobaldi	1	4	5	3
	Total	5386	13413	18799	1058

information on the resting behaviour, density and seasonal prevalence of these vectors is not available for the post-DDT era, a systematic study on these aspects was undertaken from May 1988 to November 1989 and the results are presented.

MATERIAL AND METHODS

The study was carried out in four villages of Borigumma PHC (600 mts altitude) representing four different ecotypes viz., plains (Benasur), riverine tract (Seamalaguda), foothills (B.

Singpur), and hill top (Champapodar). Hill top and foothill ecosystem is chracterised by presence of stream, rivulet and terraced ricefield as the major breeding habitat and covered with thick vegetation. Whereas plain and riverine villages are devoid of thick vegetation and stream. Characteristics of hill top and foothill ecosystem are described in detail by Kondrashin and Kalra, 1989. Daytime indoor resting collections were made using aspirators and torch lights in 18 fixed catching stations (9 human dwellings and 9 cattlesheds) for 10 mins in each holding. Equal time was spent for collecting mosquitoes from eaves, walls and roofs. Outdoor collections were made by aspirators from selected natural resting sites like pit shelters, tree holes, bushes, culverts etc., and also from artificial pit and pot shelters. The anophelines collected were identified, classified according to abdominal stages and age graded. Meteorological data were obtained from the Block Development Office, Borigumma.

RESULTS AND DISCUSSION

The number of different anopheline species captured from human dwellings, cattlesheds and outdoor shelters are presented in Table 1. *A. vagus* was the predominant species among those resting in human dwellings, followed by *A. subpictus*. In cattlesheds, *A. culicifacies* was the most abundant species constituting 44.2%, followed by *A. vagus* and *A. subpictus*. All the species collected indoors preferred cattlesheds to human dwellings for daytime resting. About 71.3% of the total indoor collections were obtained from cattlesheds.

Except *A. philippinensis* and *A. karwari* other anophelines were found resting in outdoor shelters also in varying numbers. Among these, *A. fluviatilis* was the predominant species forming 28.8% of the total outdoor collection. The number collected from outdoors was significantly higher than indoors. This pattern was also followed by *A. maculatus* and *A. tessellatus*.

Since the abdominal condition of daytime resting females, provide additional evidence of resting behaviour, the same was analysed for the vector species collected indoors and outdoors separately (Table 2). If there is no outdoor resting habit the proportion of freshly fed and semigravid is expected to be roughly equal in indoor resting mosquitoes (Rao, 1984). The proportion of semigravid to fully fed specimens in indoor collection was less for *A. maculatus* and *A. jeyporiensis* which indicates a relatively higher degree of exophily in these species. In *A. fluviatilis* though direct collections yielded higher numbers from outdoors, the proportion of semigravids in indoors was equal to fully fed specimens which apparently indicates an endophilic nature. However, a markedly lower proportion of gravid females collected indoors indicates that the majority of females leave indoor shelters immediately after feeding and those which fail to leave immediately, do so after partially completing the gonotropic cycle. Such behaviour has been reported in *A. gambiae* (Davidson and Draper, 1953) and in *A. fluviatilis*, (Viswanathan *et al.*, 1944). In *A. aconitus*, *A. annularis*, *A. culicifacies* and *A. varuna* the proportion of semigravid to fully fed specimens was significantly higher indicating a high degree of endophily.

The abdominal condition of outdoor resting population shows that in *A. fluviatilis*, *A. jeyporiensis* and *A. maculatus* the proportion of fully fed specimens was more than the semigravids which further confirms the exophilic nature of these species. On the other hand, in *A. aconitus*, *A. annularis*, *A. culicifacies* and *A. varuna* the proportion of fully fed specimens was less in comparison to semigravids indicating that the majority of these species remain indoors after feeding.

Though, *A. fluviatilis* has been known to prefer outdoor resting places in many parts of India (Rao, 1984), in an earlier study in Jeypore hills, this species was collected in greater numbers

Table 2. Percentage of females with different abdominal condition

Species		No. dissected	UF	FF	SG	G
A. aconitus	In	121	8.3	23.1	62.8	5.8
	Out	21	19.0	14.3	57.1	9.5
A. annularis	In	211	2.4	27.0	52.6	18.0
	Out	10	20.0	0	50.0	30.0
A. culicifacies	In	5490	2.1	19.5	56.4	22.0
	Out	99	48.5	15.2	16.2	20.2
A. fluviatilis	In	139	2.2	46.8	47.5	3.6
	Out	274	13.9	37.6	23.7	24.8
A. jeyporiensis	In	469	1.7	45.8	40.7	11.7
	Out	117	10.3	56.4	22.2	11.1
A. maculatus	In	5	20.0	60.0	20.0	0
	Out	9	11.1	44.4	22.2	22.2
A. varuna	In	41	4.9	41.5	48.8	4.9
	Out	10	0	30.0	60.0	10.0

In - Indoors; Out - Outdoors; UF - Unfed; FF - Fully fed; SG - Semigravid; G - Gravid.

Table 3. Seasonality and preference in the resting behaviour of seven anopheline species

Seasons		A. aco-nitus	A. annu-laris	A. culici-facies	A. fluvia-tilis	A. jeypori-ensis	A. macu-latus	A. varuna
Rainy	HD	3.45	26.67	14.88	6.17	9.90	0	11.11
(Jul-Oct)	CS	55.17	60.00	84.30	6.17	49.50	22.22	55.56
	OD	41.38	13.33	0.83	87.65	40.59	77.78	33.33
Winter	HD	17.24	37.5	6.57	18.69	11.2	33.33	30.00
(Nov-Feb)	CS	77.59	62.5	90.90	28.04	76.8	66.67	70.00
	OD	5.17	0	2.53	53.27	12.0	0	0
Summer	HD	0	20.41	18.82	6.45	23.26	23.08	23.08
(Mar-Jun)	CS	23.52	71.43	77.58	1.61	23.26	0	0
	OD	76.47	8.16	3.59	91.94	53.49	76.92	76.92

HD - Human dwelling; CS - Cattleshed; OD - Outdoor shelter.

from indoor shelters (Senior White, 1937) in comparison to that in the present study. While A. jeyporiensis type form is shown to be an indoor rester (Rao, 1984). In this study this species was collected in considerable number from outdoor shelters. The degree of exophily observed in this area in A. aconitus, A. annularis, A. culicifacies and A. varuna was less than that reported from other areas (Mani et al., 1984; Reisen et al., 1976; Shalaby, 1971).

Seasonwise analysis of indoor and outdoor collections shows that in all seasons, density of *A. fluviatilis* was higher in outdoors and that of *A. annularis* and *A. culicifacies* was more in indoors (Table 3) whereas, *A. jeyporiensis, A. varuna* and *A. aconitus* were found more in indoor shelters during rainy and winter months and in outdoor shelters during summer. The data shows that *A. maculatus* tends to rest more in outdoor shelters during rainy and summer season but in winter season not a single specimen could be collected from outdoor shelters.

Among the outdoor resting shelters, pit shelters are the most preferred resting habitat for all the vectors (Table 4). However, during summer *A. fluviatilis* rested more in tree holes.

To determine the preferential resting places inside human dwellings and cattlesheds, collections from different areas such as walls, ceilings and eaves were analysed. The preferential resting places inside houses varied with seasons (Table 5). In human dwellings anophelines generally preferred to rest on walls during the win-

ter and summer months and on ceilings during the rainy season. In cattlesheds, they prefered walls in winter and ceilings in the summer and rainy seasons. Since cattlesheds are open and more exposed to sunlight and rains, mosquitoes do not prefer to rest on the walls during these seasons. The compactness of human dwellings and frequent mud plastering of walls seem to provide ideal (cool and humid) conditions for mosquitoes to rest on walls during summer. Preference of resting places in case of individual species could not be analysed since the samples were too small.

The density of vector species in different ecotypes indicate that maximum numbers of *A. fluviatilis, A. jeyporiensis* and *A. maculatus* were recorded from hill top and foothill villages. Riverine villages contribute more specimens of *A. culicifacies* and the maximum number of *A. aconitus, A. annularis* and *A. varuna* was collected from plains villages (Table 6). This difference is mainly due to the variation in the availability and type of breeding habitats (VCRC, unpublished data).

Table 4. Percentage of female anophelines collected from different outdoor resting shelters

Species	Number collected				
	Bushes	Tree holes	Pit shelters	Pot shelters	Others
No. of habitats	4	24	18	10	7
No. of times searched	74	209	209	77	79
A. aconitus	0	4.0	68.0	0	28.0
A. annularis	0	26.3	52.6	0.9	20.2
A. culicifacies	0	0	45.5	9.1	45.5
A. fluviatilis	2.9	11.5	73.1	6.4	6.1
A. jeyporiensis	0	13.6	62.4	13.6	10.4
A. maculatus	0	9.1	72.7	0	20.0
A. varuna	0	0	80.0	0	20.0
Total	1.48	13.98	66.45	6.4	11.68

Table 5. Percentage of female anophelines collected from different resting places in human dwellings and cattlesheds

| Seasons | Proportion of anopheline resting | | | | | |
| | Human dwellings | | | Cattlesheds | | |
	Wall	Eaves	Roof	Wall	Eaves	Roof
Winter	67.4	21.7	10.9	48.0	25.8	26.2
Summer	49.9	19.2	30.9	34.3	23.9	41.8
Rainy	24.0	23.5	52.5	14.2	37.1	48.7

Table 6. Per man hour density of anopheline species in different ecotypes

| S. No. | Species | Number/Man/Hour | | | |
		Hill tops	Foothills	Riverine tracts	Plains
1.	A. aconitus	0.006	0.13	0.08	0.36
2.	A. annularis	0.03	0.28	0.12	0.50
3.	A. culicifacies	3.96	7.74	7.84	4.41
4.	A. fluviatilis	1.17	0.09	0.05	0.01
5.	A. jeyporiensis	2.1	0.13	0.05	0.047
6.	A. maculatus	0.04	0.007	0.004	0
7.	A. varuna	0.03	0.075	0.004	0.08

Monthwise analysis shows that there is no variation in the seasonal prevalence of the vectors among the four villages of different ecotypes and between indoor and outdoor collections within the village. The data of four villages was combined and analysed to calculate the per man hour density of all vectors for different months along with rainfall and temperature and is shown in Fig. 1.

The per man hour density of A. fluviatilis and A. jeyporiensis started increasing in the rainy season and reached a peak during October-November i.e., the post-monsoon period. The increase in breeding during rainy and winter season was contributed by terraced paddy fields along stream beds.

The density of A. culicifacies showed two seasonal peaks. The first peak in June-July was due to the initial shower (rains) which increased the breeding surface area. The decline observed in October was the result of flooding of habitats due to heavy and continuous rains and the paddy fields becoming unconducive to mosquito breeding because of thick growth of plants. A lower density was maintained throughout the winter. During February-March, with the onset of summer the density increased to a peak probably due to receding water in rivers, with numerous pool formations favouring intense breeding.
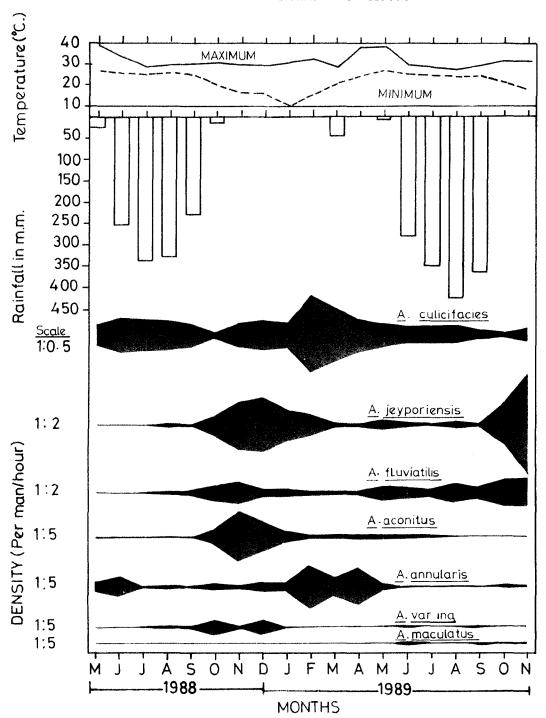
Fig. 1: Seasonal variation in density of anopheline vectors.

A. annularis, A. aconitus and *A. varuna* density was higher in winter and early summer (January-February and March-April) when thick growth of vegetation in ponds favoured heavy breeding.

In the pre-DDT period, the indoor resting density (per man hour) of *A. funestus* group (which included *A. fluviatilis, A. minimus* and *A. varuna*) ranged from 1.7-15.8 in human dwellings and 0-5.8 in cattlesheds, which was much higher than that observed in the present study (Senior White, 1937). *A. minimus* population has reduced to such a low level that it was not possible to collect this species from the resting sites by standard methods. *A. varuna* and *A. maculatus* were also found in very low numbers.

Such drastic reduction in vector population in the area is due to reduced breeding sources and changes in other environmental conditions because of deforestation over the years. There is evidence for the reduction in the amount of rainfall received by Jeypore hills (Statistical Abstract of Orissa, 1981) resulting in drying up of many perennial streams in summer. Another reason for the decline in indoor resting population could be attributed to prolonged use of DDT. However, the observation that *A. fluviatilis* is still susceptible to DDT (Sahu *et al.,* 1990) but that it does not come in contact with the insecticide, is probably due to inadequate spray coverage because of operational difficulties or mud plastering by the community or due to avoidance of insecticide treated surface by the vector or a combination of all these factors.

While, this study clearly indicates high degree of exophily in *A. fluviatilis*, certain degree of endophily was also shown by this species. Whether, there is any change in the vector behaviour due to insecticide pressure is not yet clear since earlier studies did not carry out outdoor collection. It is possible that there are two races and the population of the endophilic vec-

tor was suppressed due to insecticidal pressure, as we could collect fewer specimens from indoors than outdoors. However, *A. fluviatilis* is now resting more in cattlesheds than in human dwellings which is just reverse of the results of earlier study.

In Koraput district two peaks of malaria transmission (one during July-August and the other during November-December) have been observed and two rounds of indoor residual spraying are in practice. In this area, though *A. annularis* and *A. culicifacies* have developed resistance, the major malaria vector, *A. fluviatilis* is still susceptible to both DDT and HCH (Sahu *et al.,* 1990). Since a majority of *A. fluviatilis* rest outdoors during the period when the first round of spray (May-July) is done, the degree of contact of vectors with the insecticides is greatly minimised even if the sprayed surfaces are not mud plastered. The second round of spray starts from August, whereas, malaria incidence starts rising from October onwards and reaches a peak during December when resting of *A. fluviatilis* is also more indoors. Even though exophilic behaviour of main vector appears to be the major cause for inefficacy of indoor residual spray, there are indications that the possibility of contact with residual spray is substantial since many vectors do rest for completing gonotropic cycle. Therefore, a proper indoor residual spray can still interrupt the transmission. However, the real impact of DDT can be assessed only after studies on biting behaviour of this vector are carried out.

REFERENCES

1. Anonymous (1981). *Statistical Abstract of Orissa.* (Bureau of Statistics and Economics, Orissa, Bhubaneswar): 7.

2. Davidson, G. and C.C. Draper (1953). Field studies of some of the basic factors concerned in the transmission of malaria. *Trans. R. Soc. Trop. Med. Hyg.*, 47(6): 522-535.

3. 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.

4. Jambulingam, P., S.S.S. Mohapatra, L.K. Das, P.K. Das and P.K. Rajagopalan (1989). Detection of *Plasmodium ovale* in Koraput District, Orissa State. *Indian J. Med. Res.*, 89: 115-116.

5. Kondrashin, A.V. and N.L. Kalra (1989). Malaria as anthropo-ecosystem. Part-III. Diversity of MAES. *J. Com. Dis.*, 21:62-70.

6. Mani, T.R., S.C. Tewari, R. Reuben and M. Devaputra (1984). Resting behaviour of anophelines and sporozoite rates in vectors of malaria along the river Thenpennai (Tamil Nadu). *Indian J. Med. Res.*, 80:11-17.

7. Perry, E.L. (1914). Endemic malaria of the Jeypore hill tracts of the Madras Presidency. *Indian J. Med. Res.*, 2:456-491.

8. Rajagopalan, P.K., P.K. Das, S.P. Pani, P. Jambulingam, S.S.S. Mohapatra, K. Gunasekaran and L.K. Das (1990). Parasitological aspects of malaria persistence in Koraput district of Orissa state. *Indian. J. Med. Res.*, 91(A): 44-51.

9. Rao, T. Ramachandra (1984). *The Anophelines of India.* Rev. ed. (Malaria Research Centre, ICMR, Delhi):282-285.

10. Reisen, W.K., M. Aslam Khan and Z.H. Naqvi (1976). Observation on the resting habits on diel changes in the ovarian conditions of some Punjab mosquitoes. *Biologia*, 22:67-77.

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

12. Senior White, R. (1937). On malaria transmission in the Jeypore hills. Part I. A year's dissection results. *Rec. Mal. Surv. Ind.*, 7:47-75.

13. Shalaby, A.M. (1971). Sampling of outdoor resting populations of *Anopheles culicifacies* and *Anopheles fluviatilis* in Gujarat state, India. *Mosq. News*, 31(1):68-73.

14. Stephens, J.W.W. and S.R. Christophers (1902). An investigation into the factors which determine malaria endemicity. *R. Soc. Rep. Mal. Com.* 7th Series: 23-45.

15. UNDP/World Bank/WHO (1983). Report of the steering committee of the scientific working group on malaria. June 1980-June 1983. *(Mimeographed Document).* TDR/MAL/SC-SWG (80-83)/83.3.

16. Viswanathan, D.K., T. Ramachandra Rao and T.S. Rama Rao (1944). The behaviour of *A. fluviatilis.* Part-II. Experiments on the behaviour of gravid females. *J. Mal. Inst. Ind.*, 6:243-245.

17. Weeks, E.B. (1951). In *Final report on WHO/UNICEF malaria control demonstration project, Jeypore hill tract, Orissa, May 1949-May 1951.* (WHO/SEARO, New Delhi).

Response of *Plasmodium falciparum* to Chloroquine in a Tribal Area of Madhya Pradesh

NEERU SINGH[1] and M.M. SHUKLA[1]

In vivo tests for chloroquine sensitivity of *P. falciparum* malaria parasites were carried out on a sample of 130 patients (mean age, 21 years) from two PHCs during October-November 1988. During this period 20 cases (5.2%) were found positive for all seven days suggesting the presence of ·RII/RIII chloroquine resistance. All the resistant cases were treated with metakelfin successfully without any recrudescence.

INTRODUCTION

A study on malaria control was undertaken in January 1987 in a tribal block (Bizadandi) through 4 mobile teams in a rural population of approximately 40,000 (Singh *et al.,* 1989a). Malaria is endemic in this area with a high level of transmission throughout the year. During surveillance and treatment, decrease in the level of sensitivity of *P. falciparum* to chloroquine was evaluated in this block in 1987 (Singh *et al.,* 1989b) and results were made available to Director, Health Services, Madhya Pradesh. Based on this report, Government of M.P. recommended 25 mg/kg chloroquine as first line treatment for fever/malaria in their national control plan for Jabalpur and Mandla districts.

Bizadandi PHC is bordered by Kundam and Niwas PHCs. *P. falciparum* malaria is endemic

throughout the tribal belt and accounts for over 65% (range 30-92%) of all malaria infections (Singh *et al.,* 1989a). These studies were performed during October-November 1988·in these two PHCs (Niwas and Kundam), to obtain information for determining appropriate malaria treatment policy in these areas and to assess the changes in patients' responses to chloroquine; the results are presented here.

MATERIAL AND METHODS

A standard WHO *in vivo* test (WHO, 1973) was used for screening children and adults in villages. One PHC from Jabalpur district and another from Mandla district consisting of predominantly tribal population (75%) were selected for *in vivo* test. Criteria for the test included the following — no reported history of antimalarial drug ingestion in the previous 7 days, negative Dill-Glazko urine test (Lelijveld and Kortmann, 1970) for chloroquine and the presence of high *P. falciparum* asexual parasitaemia. Serious patients, those with mixed infections and patients with scanty infections were excluded from the study.

All doses were administered under supervision of team members. Children receiving chloroquine, were observed for 30 mins and those who spat or vomited the drug were eliminated from the study. No adverse effects of chloroquine were observed, vomiting occurred rarely. Patients were given chloroquine* orally @ 25 mg/kg (600 mg on D0; 600 mg on D1; 300 mg on D2). Children were given proportionately low doses. The course of asexual parasitaemia of each patient was evaluated over 7 days/28 days.

One hundred fields of thick blood smears were examined for asexual parasites. If the parasite density on Day 2 was > 25% of the initial value (0 day, before treatment) the patient was designated as resistant (RII/RIII). All drug-resistant cases were treated with 3 tablets of metakelfin (1500 mg sulfalene + 75 mg pyrimethamine) adult dose.

RESULTS

Five hundred cases of above mentioned 2 PHCs were screened out of which 383 were found parasitaemic (Pf) and 130 (34% mean age 21 years) met all study criteria and completed the 7 days/28 days follow-up (Table 1).

Among the 30 cases, 6 (20%) in Niwas PHC and 14 out of 100 in Kundam (14%) were found resistant at RII/RIII level. Although 20 out of

130 cases had resistant parasites, only one adult who was initially febrile remained so for 7 days. Fever after chloroquine treatment was not predictive of drug failure in either PHC. Twenty four cases in Niwas and 86 in Kundam showed complete clearance of asexual parasitaemia after treatment with 1500 mg chloroquine, however, 9 and 38 cases again became positive within 28 days in both the PHCs respectively (delayed RI).

No significant difference was found between the overall failure rate for children and adults. After chloroquine administration to children 2 out of 9 (22%) from Niwas and 4 of 34 (12%) from Kundam did not show complete clearance of parasites (Table 1).

The pattern of decline in parasite density was found similar for both the sites. Similarly reduction in parasite density from Day 0 to Day 7 among children and adults did not differ significantly (Table 2).

DISCUSSION

Chloroquine resistant strains of P. falciparum are prevalent in the area. A surveillance system has been established to monitor in vivo drug effectiveness in endemic areas. Till 1987 10 mg/kg of chloroquine was advised for the study area. In vivo testing in Bizadandi (Singh et al., 1989b) and in vitro testing in Kundam (Ghosh et al., 1989) revealed the presence of

Table 1. Results of WHO in vivo test for chloroquine resistance in Jabalpur and Mandla districts

District/PHC	Pf cases detected	Follow-up	Adults	Children	Resistant adults	Resistant children
Mandla/Niwas	51	30	21	9	4	2
Jabalpur/Kundam	332	100	66	34	10	4

*NMEP supply, manufactured by Govt. Medical Stores Depot, Factory, Bombay; each tablet containing 250 mg of chloroquine phosphate equivalent to 150 mg of chloroquine base.

Table 2. Chloroquine sensitivity of *P. falciparum* (WHO *in vivo* test) in Kundam and Niwas PHCs Distt. Jabalpur and Mandla**

Age/Sex	Drug given	Parasitaemia per mm^{3***}							
		D0	D1	D2	D3	D4	D5	D6	D7
Kundam									
22/F	I-600 mg II-600 mg III-300 mg	12025	2275	2000	2225	1500	825	950	1550
30/M	I-600 mg II-600 mg III-300 mg	10700	2000	2200	5625	675	625	1000	*
35/M	I-600 mg II-600 mg III-300 mg	1775	700	1725	600	975	1025	625	825
15/M	I-600 mg II-600 mg III-300 mg	12250	3375	15700	3325	1500	1475	1400	975
20/F	I-600 mg II-600 mg III-300 mg	1975	650	2400	550	-	625	975	1025
6/F	I-300 mg II-300 mg III-150 mg	1300	1700	1800	2400	10125	3125	825	675
10/M	I-450 mg II-450 mg III-225 mg	14200	7025	13225	2800	2225	1250	950	825
21/F	I-600 mg II-600 mg III-300 mg	2475	650	2700	1950	1300	550	775	825
Niwas									
30/M	I-300 mg II-300 mg III-150 mg	2050	600	2450	1500	1375	850	1650	1900
5/M	I-600 mg II-600 mg III-300 mg	1925	1025	850	625	925	825	625	500
30/M	I-300 mg II-300 mg III-150 mg	1225	825	925	625	925	825	625	925

*Blood smear not available; **Only few cases are given here for brevity; ***Cured with metakelfin.

chloroquine resistant *P. falciparum* strains. The State Health Department was informed and National Malaria Plan has been modified accordingly and now recommends 25 mg/kg chloroquine for all areas in and around Jabalpur as first line treatment. It is, therefore, desirable to determine the approximate prevalence of malaria parasites exhibiting reduced sensitivity to revised dose of chloroquine.

The failure rate of chloroquine to clear *P. falciparum* by *in vivo* testing was 14% and 20% in Kundam and Niwas PHCs, respectively. After chloroquine treatment at both sites 80-86% of the patients were non-parasitaemic out of which 38% (Niwas) and 44% (Kundam) again showed the symptoms of parasitaemia in 28 days (delayed RI). A number of cases (15%) did not clear parasites by Day 7 (RII/RIII).

It will not be out of place to mention that in 1987 and 1988, NMEP surveillance recorded 5194 and 3612 cases of falciparum malaria as against 763 in 1986 in Mandla district and 5263 and 5984 in 1987 and 1988 in Jabalpur as against 294 cases in 1986. Vital statistics of malaria in M.P. showed that during 1987 in 44 districts there was a 67.25% average rate of increase of malaria and a 39.14% increase in falciparum malaria (source NMEP, Bhopal). Houghton (1983) in Bastar recorded that resistance to chloroquine varied from RI to RIII level.

The high proportion of chloroquine resistance and predominance of RI level of resistance indicates the immune competence of the host population. This is further confirmed by low parasite density (Table 2). Similar studies should be carried out in other districts of M.P. as it is necessary to know how chloroquine is working on *P. falciparum* after so many years of drug pressure.

REFERENCES

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

2. Houghton, D.L. (1983). Assignment report, *Plasmodium falciparum* containment programme, Zone III (A), Madhya Pradesh, Bhopal :33-34.

3. Lelijveld, J. and H. Kortmann (1970). The eosin colour test of Dill and Glazko. A simple field test to detect chloroquine in urine. *Bull. WHO*, 4 : 477-479.

4. Singh, Neeru, V.P. Sharma, A.K. Mishra and O.P. Singh (1989a). Bio-environmental control of malaria in a tribal area of Mandla district, Madhya Pradesh, India. *Indian J. Malariol.*, 26 (2):103-120.

5. Singh, Neeru, M.M. Shukla, V.P. Sharma and B.N. Saxena (1989b). A focus of high degree chloroquine resistant *P. falciparum* in Mandla district (M.P.). *Indian J. Malariol.*, 26 (1): 45-51.

6. WHO (1973). Chemotherapy of malaria and resistance to antimalarials. *Tech. Rep. Ser. No.* 529: 30-39

Density Patterns of Anophelines and their Relation to Malaria in Bastar District, Madhya Pradesh

S.M. KULKARNI[1]*

Density patterns of 17 species of anopheline mosquitoes collected between 1980 and 1981 from indoor and outdoor resting places of a highly malarious area of Bastar district, Madhya Pradesh are given. *A. culicifacies* and *A. fluviatilis* are the important vector species in the region. The former was highly endophilic and found throughout the year and its man hour density (MHD) ranged from 7.6 to 78 with peaks in February and July. The latter was highly exophilic, also found throughout the year. Its density ranged from 1.6 to 7.0 per 10 man hours with a peak in July/August. *A. subpictus* was considered as potential vector species, found throughout the year and was endophilic, having MHD ranging from 0.9 to 148 with peaks in February-March and July-August. The MHD variations of other species were; *A. aconitus* (0.02-1.04), *A. annularis* (0.02-10.4), *A. barbirostris* (0.04-1.07), *A. hyrcanus* (1.30-1.86), *A. jamesii* (0.01-0.46), *A. jeyporiensis* (0.01-1.06), *A. karwari* (0.01-0.04), *A. maculatus* (0.01-0.03), *A. pallidus* (0.02-2.4), *A. splendidus* (0.02-0.32), *A. tessellatus* (0.02-0.12), *A. theobaldi* (0.01-0.03), *A. vagus* (0.01-2.3) and *A. varuna* (0.03-0.31). Results of gut and gland dissections showed correlation of infection with the occurrence of peak vector densities. The density levels and seasonal activities of vectors as well as potential vector species are favourable for perennial transmission of malaria in Bastar district.

INTRODUCTION

Bastar district in Madhya Pradesh is highly endemic for malaria and its transmission is perennial. This has been attributed to the increasing annual parasite incidence, number of malaria cases and natural infection in anopheline vector species viz., *A. culicifacies* and *A. fluviatilis* (Kalra, 1978; Vaid and Nagendra, 1964; PfCP Report of Madhya Pradesh, 1980; Kulkarni, 1987). Considering the transmission of malaria

parasite by vectors as a density-dependent phenomenon, attempts were made to study the density patterns of indoor and outdoor resting anophelines and to correlate the results with the malaria transmission in south Bastar district. The results of the investigations carried out between January 1980 and May 1981 are presented in this paper.

MATERIAL AND METHODS

Study area

The topographical and ecological features of Bastar district have been described by Agarwal (1968). It has an area of 39,086 sq km and a

*Present address: National Institute of Virology, Pune-411 001, India.

population of 15,15,956 (1971 census). The district comprises of south Bastar with a population of over 9 lakhs and north Bastar with a population of about 6 lakhs. The population is mainly tribal.

Bastar lies in the southeast of Madhya Pradesh. The climate is moderate to hot throughout the year. There are roughly four seasons — monsoon (July to September), winter (October to December), spring (January to March) and summer (April to June). The area is drained by many perennial rivers and streams. It receives an annual rainfall of about 1500 mm both from the southwest and northwest monsoons. The vegetation is largely tropical with moist deciduous forests intermixed with paddy cultivation.

Five ecologically and topographically different areas were selected within a radius of 40 kms from Jagdalpur township of south Bastar viz., (i) Darbha area has a thick forest surrounded by hill streams with decaying humus soil and mosaic paddy cultivation, (ii) Kurandhi area has a moderate forest cover with grasslands and shrubs surrounded by large fresh-water ponds having water hyacinth and other vegetation, (iii) Tokapal area is rocky surrounded by grass lands intermixed with paddy cultivation, (iv) Bastar area has plains with paddy cultivation having some irrigation facilities, and (v) Kumrawand area has plains with paddy cultivation but without any irrigation facilities. In each area four localities about 3-4 kms away from each other were selected randomly for mosquito collection.

Mosquito collection

Weekly collections of adult mosquitoes resting in indoor and in outdoor habitats were made from four localities in each area between 0700 hrs and 1030 hrs.

Indoor collection

Two insect collectors were allotted the work of indoor collection from four cattlesheds/mixed
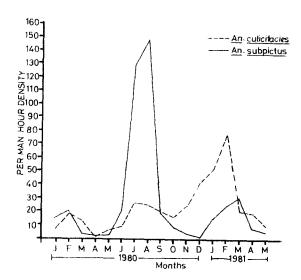
Fig. 1: Density pattern of *A. culicifacies* and *A. subpictus* in Bastar district.

dwellings. They were allowed to enter the structure one at a time to catch the resting adult anophelines for 10 mins each from each locality of the study area, using an aspirator and a torch-light. The collected mosquitoes were transferred into Barraud cages (30 cm³), and kept separately localitywise.

Outdoor collection

Two insect collectors were allowed to collect outdoor resting mosquitoes mainly from the bushes, fences, pit-shelters, tree holes etc., surrounding domestic/peridomestic areas in each locality for 30 mins. The mosquitoes were held in glass tubes separately and in case of larger numbers they were transferred into Barraud cages.

All the collected mosquitoes were brought to the laboratory and were kept for 2-3 hrs, at 80% humidity by means of a wet lint cloth. They were then anesthetised and identified with the help of keys (Christophers, 1933; Puri, 1937). Majority of the females were then dissected in normal saline and examined for oocyst and sporozoite infections in gut and salivary glands.

RESULTS AND DISCUSSION

A total of 74,395 adult   female anophelines (73,574 from indoor and 821 from outdoor) comprising of 17 species were collected. For convenience, the data of the density patterns of *A. culicifacies* and *A. subpictus* which were found in large numbers during indoor collections from January 1980 to May 1981, are given in Fig. 1. The data for remaining 15 species, for 12 months (January to December) are presented in Tables 1-2. The data on the density patterns of all the 17 species collected from outdoors for only six months (July to December 1980) are presented. The density pattern and seasonal activity of each species is discussed below with special reference to malaria transmission in the area.

1. *A. culicifacies:* Mostly endophilic and found throughout the year. It showed two density peaks one in the month of February (20 MHD) and another in the month of July (28 MHD). The per man hour density of this species was 78 in February 1981. In outdoor collections, MHD (0.84) was maximum in the month of July. The population increases from January, reaches its first peak in February and then declines during April-May. It showed a second peak during July and then remained stable till October. Earlier, this species was shown to be responsible for spring as well as monsoon transmission of malaria (Kulkarni, 1987). Thus, the peak densities of this species correspond to the period of active transmission of malaria in south Bastar.

During the study period, one out of 18,042 guts (in September) and 3 out of 18,236 glands (2 in March and 1 in September) were found positive for oocyst and sporozoite infections, respectively. The period in which they showed *Plasmodium* infections also corresponds to the peak densities of this species.

2. *A. subpictus:* Mainly endophilic and found throughout the year. This species showed two density peaks, a low peak during February-March and a high peak during July-August. The highest MHD was 148 in August and the lowest (0.9) was in December. These peaks corresponded with the increase in turbidity of the rain-water. A total of 12,107 specimens were examined for gut and gland infections. Of these, 3 glands (one in February and two in July) were found positive for sporozoite infections. These periods of infection corresponded with the peak activities of the species when its anthropophilic index (35%) was also high (Kulkarni, 1983). Earlier, Panicker *et al.* (1981) incriminated this species in coastal villages of southeast India. Hence this species could be contributing to the malaria transmission in this region.

3. *A. aconitus:* Found more in indoors than in outdoors. The highest MHD, 1.04 was in November. It is more prevalent during latter half of the year. To check the gut and gland infections 88 specimens were dissected and all were found negative. Earlier this species was found biting man/cattle during October to December (Kulkarni, 1987; Husainy, 1978). It is reported to be a malaria vector in some parts of southeast Asian countries (Rao, 1984).

4. *A. annularis:* More prevalent in indoors than in outdoors. The highest MHD (10.4) was in February and the lowest (0.02) was in September. *A. annularis* is found throughout the year but is more prevalent during January to April. All 2337 specimens examined for gut and gland infection were found negative. This species is regarded as a malaria vector of some local importance particularly in coastal Orissa, Bihar, Bengal and Assam (Rao, 1984).

5. *A. barbirostris:* Found indoors as well as outdoors. The highest MHD   (1.07) was observed in October, and the species showed higher prevalence during September-October. Absent during April to June. A total of 168 specimens were examined for gut and gland infections with negative results.

Table 1. Per man hour densities of indoor resting anophelines in Bastar district

Months of the year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Total
No. of female mosquitoes collected	6118	5339	3157	3453	1434	3677	13489	13308	3993	2308	2899	4016	63191*
No. of man hours spent	81	47	52	97	75	115	85	75	92	71	76	80	946
Species						Man hour density							
A. aconitus	0.05	-	-	-	-	-	-	-	-	0.30	1.04	0.18	118
A. annularis	6.80	10.40	7.50	4.70	1.90	0.07	0.15	0.06	0.02	1.30	3.00	3.60	2671
A. barbirostris	0.10	0.04	0.04	-	-	-	0.10	0.10	1.03	1.07	0.60	0.30	256
A. fluviatilis	0.10	0.10	-	0.02	0.10	0.08	0.20	0.40	0.20	-	-	0.07	97
A. hyrcanus 'group'	0.10	0.30	0.15	0.05	-	-	-	0.02	1.10	1.20	1.30	0.40	352
A. jamesii	0.10	0.01	-	-	-	-	-	-	-	-	0.01	0.19	23
A. jeyporiensis	1.06	0.30	0.01	-	-	-	-	-	-	-	0.30	0.80	163
A. karwari	-	-	-	-	-	-	-	-	0.01	-	-	0.02	3
A. maculatus	-	-	-	-	-	-	-	0.01	-	0.01	-	-	2
A. pallidus	0.13	0.04	0.11	0.70	0.46	1.02	2.40	0.65	0.06	0.30	0.40	0.13	560
A. splendidus	0.29	0.32	0.02	-	-	-	-	-	-	-	0.01	0.06	46
A. tessellatus	-	-	-	-	-	-	-	-	-	0.04	0.03	-	5
A. theobaldi	0.01	-	-	-	-	-	-	-	0.01	-	-	-	2
A. vagus	0.20	0.66	2.30	2.16	1.22	-	0.20	0.40	1.10	1.80	2.30	0.77	955
A. varuna	0.22	-	-	-	-	-	-	-	-	-	0.03	0.31	45

*includes : A. culicifacies = 25,569 and A. subpictus = 32,324.

Table 2. Per man hour densities of outdoor resting anophelines in Bastar district - July to December 1980

Months of the year	Jul	Aug	Sept	Oct	Nov	Dec	Total
No. of female mosquitoes collected	96	117	123	205	118	162	821
No. of man hours spent	25	29	111	52	47	93	357
Species	Man hour density						
A. aconitus	-	0.03	-	-	0.02	0.12	13
A. annularis	-	-	-	0.04	0.02	0.06	9
A. barbirostris	-	-	0.04	0.54	0.15	0.05	44
A. culicifacies	0.84	0.60	0.24	0.20	0.08	0.04	84
A. fluviatilis	0.50	0.70	0.16	0.60	0.40	0.23	120
A. hyrcanus 'group'	-	-	0.32	1.86	0.95	0.17	194
A. jamesii	0.04	0.03	0.05	0.36	0.46	0.29	75
A. jeyporiensis	-	-	-	0.04	0.15	0.32	39
A. karwari	-	-	-	0.04	0.02	0.02	5
A. maculatus	-	0.03	0.02	-	0.02	0.01	5
A. pallidus	0.08	0.06	0.02	0.10	0.04	-	13
A. splendidus	-	-	-	0.02	-	0.04	5
A. subpictus	2.00	2.20	0.09	0.57	0.02	-	128
A. tessellatus	-	0.03	0.03	0.02	0.10	0.12	21
A. theobaldi	-	0.03	-	-	-	-	1
A. vagus	0.36	0.02	0.14	0.07	0.06	0.02	41
A. varuna	0.04	0.06	-	-	-	0.22	24

6. *A. fluviatilis:* More exophilic than endophilic. The MHD in outdoor collections varied from 0.16 to 0.70 and the highest density was in July; but in indoor collections, it varied from 0.02 to 0.4 with highest density in August. Found almost throughout the year either outdoors or indoors with highest prevalence during July to December. A total of 230 specimens were examined for gut and gland infection and all were found negative. However one specimen each was found positive for sporozoite and oocyst infections from biting collections (Kulkarni and Wattal, 1982). Earlier, it was shown that this species feeds throughout the year on man and is responsible for transmission of malaria from April to December (Kulkarni, 1987).

7. *A. hyrcanus* 'group': Endophilic as well as exophilic in habitat, highest MHD was observed in October-November (range 1.30-1.86). Density remained low from January to April, became nil in July and again started building up from August onwards, with a peak in October-November. A total of 496 guts and 520 glands were examined for oocyst and sporozoite infections with negative results. This species does not play any role in the transmission of malaria (Rao, 1984).

8. *A. jamesii*: More abundant outdoors than indoors, prevalent during October to December with highest MHD of 0.46 in outdoor collection. Even during biting collections it was found in more numbers during the same period (Kulkarni, 1987). In south India, it is reported to be prevalent during January to May with a MHD of 0.2 (Rao, 1984). 215 specimens were examined for gut and gland infection and all gave negative results.

9. *A. jeyporiensis*: Found indoors and outdoors from October to March with a higher prevalence during December-January. Highest MHD was 1.06 in January. No infection of *Plasmodium* was found in 812 specimens examined. However, this species is considered to be a secondary vector of local importance in Orissa state (Wattal, 1961).

10. *A. karwari*: This species was found only during September to December in resting collections with a very low MHD range (0.01 - 0.04). Earlier, it has been shown that this species was highly prevalent during July to September and could be collected in large numbers while biting on man/cattle with a per night density of 1.5 and 7.5, respectively peak activity was after dusk and midnight (Kulkarni, 1987). A total of 115 specimens were dissected for gut and gland infections with negative results. This species does not play any role in the transmission of malaria (Rao, 1984).

11. *A. maculatus*: A very few specimens (2 from indoor and 5 from outdoor) were collected during latter half of the year, MHD was very low (0.01 to 0.03). However, one adult collected from outdoor pit-shelter around a human dwelling was found positive for sporozoite infection. Earlier, this species was reported to be found in large numbers during biting collections on man and cattle with a peak around dusk in the months of July, August and September (Kulkarni, 1987).

12. *A. pallidus*: Found throughout the year with a preference of indoor resting. Highest MHD

(2.4) was in July, which was also observed by Husainy (1986). It is more prevalent from April to August. Of 211 adults dissected all were found negative for *Plasmodium* infection. However, many workers have found this species positive for sporozoite infections especially from Orissa and east central India (Rao, 1984). Hence this species could be regarded as malaria vector of some significance in Bastar district.

13. *A. splendidus*: Predominance during the first and last quarters of the year. Highest MHD was 0.32 in indoor resting collection during February. A total of 192 adults were dissected and examined but all with negative results.

14. *A. tessellatus*: More exophilic than endophilic. Prevalent during August to December with highest MHD (0.12) in December. Out of 33 specimens examined for *Plasmodium*, all were found negative.

15. *A. theobaldi*: Only three specimens one each during January and September (indoors) and one in August (outdoors) were collected. They were negative for any infection. Earlier, this species was collected in large numbers while biting on man or cattle during July to September (Kulkarni, 1987).

16. *A. vagus*: Found throughout the year with a preference of indoor resting. Prevalent during March-May and again in September to November. Highest MHD was 2.30 in March as well as in November indoors, while it was 0.36 outdoors during July. For gut and gland infections, 444 adults were examined with negative results.

17. *A. varuna*: Found indoors and outdoors, prevalent during December and January. Highest MHD was 0.31 and 0.22 in December indoors and outdoors, respectively. All the 69 specimens collected were found negative for gut and gland infection. However, it has been reported earlier that one out of 44 specimens collected during biting collections was found

positive for oocyst infection (Kulkarni, 1987). This species is considered to be a secondary vector in east central and northern peninsular India (Kalra, 1978).

The data shows that all the 17 species were found in indoor as well as in outdoor resting habitats. Five species viz., *A. culicifacies*, *A. subpictus*, *A. annularis*, *A. pallidus* and *A. vagus* were found throughout the year indoors while four species, viz., *A. culicifacies*, *A. fluviatilis*, *A. jamesii* and *A. vagus* were found throughout the period of July to December in outdoor resting habitats. Earlier, Kulkarni, 1987, while reporting the feeding behaviour of anophelines of Bastar district has shown that *A. fluviatilis* is responsible for transmission of malaria in summer through winter while *A. culicifacies* for spring as well as monsoon transmission. In addition *A. subpictus*, *A. maculatus* and *A. varuna* could be the potential vectors in the region. Even an analysis of the epidemiological data on SPR rate (Fig. 2) obtained from NMEP unit, Jagdalpur showed that the malaria transmission is perennial (SPR > 17%) with a small peak in June-July and a high peak in November-December. How-
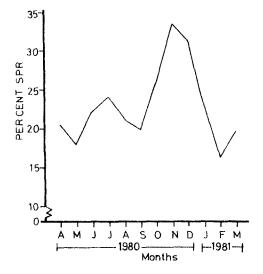
ever, according to Russel and Rao (1942) and Jaswant Singh *et al.* (1957) the malaria transmission depends on the density levels of vector species. They have reported that the critical density of *A. culicifacies* as a vector would be 5 per man hour while that of *A. fluviatilis* would be 4 per 10 man hours. In the present study, these densities varied from 7.6 to 78 throughout the year except for the month of April (2.3) for *A. culicifacies* in indoor collection and that of *A. fluviatilis* which varied between 1.6 and 7.0 for 10 man hours from July to December in outdoor collection.

Hence, both these vector species remain active within the specified limits of critical densities of active malaria transmission in the region. In addition, the per man hour density of *A. subpictus* was high during February-March (24-30) and again in July-August (128-148). Sporozoite infections were also found in this species during the month of February and July. Hence, this species could also support malaria transmission especially during summer season. The other two species, viz., *A. maculatus* and *A. varuna* may act as potential vectors in the area. Therefore, the density levels and seasonal activities of vectors as well as potential vector species are favourable for perennial malaria transmission in Bastar district.

Fig. 2: Epidemiological data of NMEP Unit, Jagdalpur, South Bastar, 1974-1978. Monthwise average slide positivity rate (SPR) for malaria.

REFERENCES

1. Agarwal, P.C. (1968). *Human Geography of Bastar District.* (Garg Brothers, Allahabad).

2. Anon. (1980). *A report on the PfCP in Madhya Pradesh,* Quarterly review meeting of PfCP-Zone-

III held at Bhopal, Madhya Pradesh from 2-3 May 1980. (The Directorate of Health Services, Madhya Pradesh).

3. Christophers, S.R. (1933). *The fauna of British India including Ceylon and Burma*. V. IV. (Today & Tomorrow's Printers and Publishers, New Delhi):1-359.

4. Husainy, Z.H. (1978). Observations on *Anopheles (Cellia) aconitus* Donitz, 1902 (Diptera: Culicidae) in Bastar district, Madhya Pradesh. *J. Bom. Nat. Hist. Soc.*, 75: 304-311.

5. Husainy, Z.H. (1986). Observations on the bionomics and epidemiological significance of *Anopheles (Cellia) pallidus* Theobald, 1901 (Diptera: Culicidae) in Bastar district, Madhya Pradesh. *Entomon*, 11: 319-327.

6. Jaswant Singh, B.A. Rao and S.P. Ramakrishnan (1957). National Malaria control programme in India, 1953-1956. *Indian J. Malariol.*, 11:419.

7. Kalra, N.L. (1978). National Malaria Eradication Programme, India - its problems, management and research needs. *J. Com. Dis.*, 10: 1-20.

8. Kulkarni, S.M. (1983). Detection of sporozoites in *Anopheles subpictus* in Bastar district, Madhya Pradesh. *Indian J. Malariol.*, 20:159-160.

9. Kulkarni, S.M. (1987). Feeding behaviour of anopheline mosquitoes in an area endemic for malaria in Bastar district, Madhya Pradesh. *Indian J. Malariol.*, 24:163-171.

10. Kulkarni, S.M. and B.L. Wattal (1982). A report on the natural infection of *Anopheles fluviatilis* James

1902, (Diptera: Culicidae) with malaria parasites in Bastar district, Madhya Pradesh. *Proceedings of the Symposium on Vectors and Vector-Borne Diseases*. Eds. R.S. Prasad and K.M. Alexander.(Trivandrum Unit of the Association of Microbiologists of India, Deptt. of Zoology, University of Kerala, Trivandrum) : 85-86.

11. Panicker, K.N., M. Geetha Bai, U.S. Bheema Rao, K. Viswam and U. Surya Narayan Murthy (1981). *Anopheles subpictus*, vector of malaria in coastal villages of south-east India. *Curr. Sci.*, 50: 694-695.

12. Puri, I.M. (1937). *Synoptic table for identification of the Anopheline mosquitoes of India*. (The National Society of India for Malaria and other Mosquito-borne Diseases, Delhi).

13. Rao, T. Ramachandra (1984). *The Anophelines of India*. Rev. ed. (Malaria Research Centre, ICMR, Delhi).

14. Russel, P.S. and T.R. Rao (1942). A study of the density of *Anopheles culicifacies* in relation to malaria endemicity. *Am. J. Trop. Med. Hyg.*, 22: 535-558.

15. Vaid, B.K. and S. Nagendra (1964). *Malaria eradication in Madhya Pradesh. A report*. (Directorate of Health Services, Malaria, Indore).

16. Wattal, B.L. (1961). Vectors of Malaria—*A. jeyporiensis* var. *candidiensis* Koidzumi, 1924. In *Vectors of Malaria in India*. (The National Society of India for Malaria and other Mosquito-borne Diseases, Delhi).

Letter to the Editor

Three Case Reports of Behavioural Problems in Malaria Treatment

Sir—Malaria patients recover rapidly with proper treatment but at times behavioural indifference or ignorance may pose difficult problems as far as treatment is concerned. During malaria work in 1989 we encountered 3 such cases and a brief account is given below.

Case No. 1

A 50-year old person belonging to Banjara tribe was suffering from fever for 2 months. Initially, he was treated by quacks. Since his condition did not improve his relatives engaged a *tantrik* to free him from the influence of evil spirits. When his condition deteriorated further he was advised by the *tantrik* to go to a hospital. A mobile team of MRC responsible for bioenvironmental control interventions detected this case when he was on his way to hospital. This patient had high fever and was in a semi-conscious condition. The team collected a blood smear of the patient and examined it for the malaria parasite. He was found positive for *P. falciparum* with very high parasitaemia (40%). An immediate inquiry was made about the condition of the patient and we were informed that the patient had already succumbed to malaria.

Case No. 2

A 59-year old lady was suffering from vivax malaria for about 3 months. She was being detected positive for *P. vivax* through surveillance and given chloroquine for treatment. Since she complained of fever on every visit of the surveillance worker when she was found positive for *P. vivax* on the fourth visit, there was suspicion of either drug failure or that the patient was not taking chloroquine. This patient was interrogated and she confessed that she had no faith in malaria workers or the free medicines distributed. When she was assured of the efficacy of chloroquine and the government policy of providing treatment free through the primary health care system she agreed to take chloroquine. She recovered completely from malaria after taking the drug.

Case No. 3

This is a case of a mentally retarded malaria patient of a poor and uneducated rural family. When this patient had fallen ill, members of his family did not bother much because of his mental condition. When his condition deteriorated, they informed the surveillance worker for medical help. Blood smear of the patient was prepared with great difficulty. The patient was advised to take presumptive treatment for which he did not agree. The blood smear was positive for *P. falciparum*
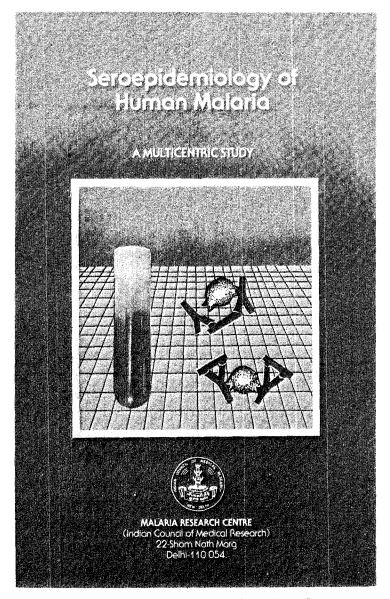
with 10% parasitaemia. Next day the patient was approached for radical treatment and he reacted violently. Even his family members ignored the advice of treatment. Later a team of officers visited the village and held a meeting with the family members of the patient. They were informed of the likely consequences of not taking the treatment and also the fact that the patient would spread the disease to others in the village. At that time patient was very weak and he agreed to take the anti-malarial treatment. He fully recovered from the infection after taking the treatment.

Such instances as reported above have been observed in the past as well (Aggarwal and Sharma, 1983). There is need to educate people on the importance of correct diagnosis and radical treatment of malaria, particularly in remote, inaccessible and tribal areas. Health education should therefore become an integral part of anti-malaria work.

Malaria Research Centre (Field Station)   R.N. PRASAD
Khirni Bagh, Sadar Bazar   H. PRASAD
Shahjahanpur-242 001, India.   S. HAQ

REFERENCE
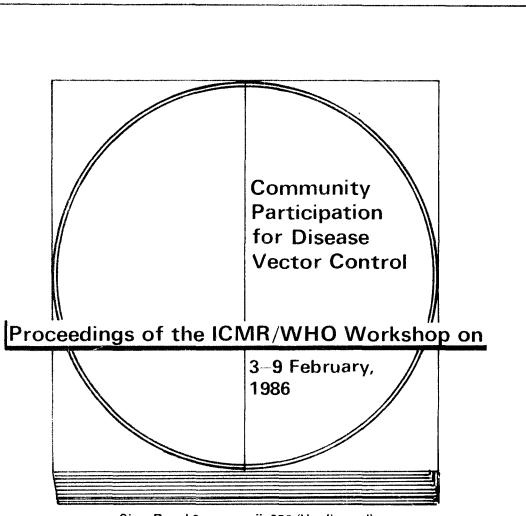
1.   Aggarwal, V.K. and G.K. Sharma (1983). A report on case of *P. vivax* infection apparently refractory to antimalarials. *J. Com. Dis.*, 15(4): 284-286.

Seroepidemiology of Human Malaria

A MULTICENTRIC STUDY

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

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.

Community Participation for Disease Vector Control

Proceedings of the ICMR/WHO Workshop on

3–9 February, 1986

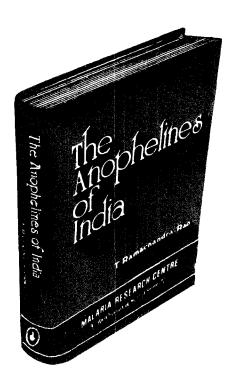Size: Royal 8vo pp. xvii, 256 (Hardbound)

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

MALARIA RESEARCH CENTRE

PRICED PUBLICATIONS

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

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

Indian Journal of Malariology

Volume 18 Nos. 1&2 (1981)*
Volume 19 Nos. 1&2 (1982)*
Volume 20 Nos. 1&2 (1983)*   Annual Subscription   { India Rs. 75.00 ●
Volume 21 Nos. 1&2 (1984)*   { Foreign US $ 20.00
Volume 22 Nos. 1&2 (1985)*   { ● 25% discount for individuals.
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. 1-3 (1990)
Volume 27 No. 4[+] (1990)
([+] To be released in December 1990).

*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:

....

....

....