## INDIAN JOURNAL OF MALARIOLOGY

Volume 24

Number 2

December 1987

### MALARIA RESEARCH CENTRE

Indian Council of Medical Research 22-Sham Nath Marg Delhi-110054

### INDIAN J. MALARIOL.

Half Yearly

Malaria Research Centre 1987

Year of revival: 1981

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Annual

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Rs. 75.00\* \$ 20.00

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

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## INDIAN JOURNAL OF MALARIOLOGY

CONTENTS	Volume 24 Number 2 December 1	987
Studies on the Detection of Malaria at Primary Health Centre Parasitological Profile of Population surveyed for Malaria through F Detection  A.E. Beljaev, J.A. Brohult, G.K. Sharma and K.C. Samantaray		97
In vitro Chloroquine resistant Plasmodium falciparum in Calcutta and it to Qinghaosu (Artemisitene) S. Sinha, D.S. Choudhury, S.K. Ghosh, C. Usha Devi and V.P. Sharma	ts sensitivity	107
In vitro antimalarial activity of Neem (Azadirachta indica A. Juss) le extracts  Lalita Badam, R.P. Deolankar, M.M. Kulkarni, B.A. Nagsampgi and U.		111
Immunofluorescence Test in the Seroepidemiology of Malaria around Ramesh Kumar, Y. Bharadwaj, M.A. Ansari, R.K. Razdan and V.P. Sha		119
Specific IgM and IgG antimalarial Antibody responses in paired sa Malaria patients  Krishan Gopal, I.K. Srivastava, Ragini Sinha, S.K. Das, G.P. Dutta, Madhur Kudesia and S.S. Agarwal	•	125
Comparative cost of mosquito control with Larvicidal Bacilli and In K. Balaraman and S.L. Hoti	secticides	131
Studies on the Development of Plasmodium vivax in Anopheles subpid Nutan Nanda, C.M.S. Dass, Sarala K. Subbarao, T. Adak and V.P. Sha		135

Survey of Mosquito Fauna of Northeastern Region of India  B.N. Nagpal and V.P. Sharma	143
Mosquito Survey in Tirap and Subansiri districts of Arunachal Pradesh P.R. Malhotra, P.K. Sarkar, N.G. Das, S. Hazarika and V.M. John	151
Incrimination of Anopheles minimus Theobald as a Vector of Malaria in Arunachal Pradesh P. Dutta and B.D. Baruah	159
Feeding Behaviour of Anopheline mosquitoes in an area endemic for Malaria in Bastar district. Madhya Pradesh S.M. Kulkarni	163
Short Notes	
Small-scale Field Trials with Polystyrene beads for the Control of Mosquito Breeding  R.K. Chandrahas and V.P. Sharma	175
Importation of Malaria Cases from Sri Lanka to Rameswaram Island (Tamil Nadu)  A. Ramalingeswara Rao, A. V. Sadanand and N. Jaleel	181
Incrimination of Anopheles stephensi Liston as Malaria Vector in Calcutta S.K. Choudhuri and A.K. Sen	183

Note: The editor assumes no responsibility for the statements and opinions expressed by the contributors. This issue has been delayed due to unavoidable circumstances.

### Studies on the Detection of Malaria at Primary Health Centres. Part III. Parasitological Profile of Population surveyed for Malaria through Passive Case Detection

A.E. BELJAEV<sup>1</sup>, J.A. BROHULT<sup>2</sup>, G.K. SHARMA<sup>3</sup> and K.C. SAMANTARAY<sup>4</sup>

Parasitological profile and its possible correlation with various physiographic features has been worked out for 39 PHCs of Orissa. Variations in the parasite densities (PD) and classes of parasites have also been analysed with respect to age and sex of the population surveyed. The article attempts to show that identification of high prevalence areas and high risk groups is facilitated by careful analysis of parasite densities and epidemiological indices.

#### INTRODUCTION

After the adoption of the Modified Plan of Operations (MPO), in 1977–1983 incidence of malaria in India declined steadily. This was, however, mostly due to reduction in *P. vivax*. Annual vivax incidence steadily dropped, from 9.93 per 1000 in 1976 to 2.06 in 1983. On the other hand, annual falciparum incidence (after a spectacular decline from 1.31 in 1976 to 0.79 in

1977) remained by and large stable during 1979-1983, oscillating between 0.81 and 0.92 per 1000. This difference in the behaviour of the two species might be due to an increase of the scope of drug therapy as an antimalaria measure (Ray and Beljaev, 1984). Cases of partially suppressed parasitaemia resulting from insufficient treatment probably became widespread against the background of rising immunity. This phenomenon was further exacerbated by a declining sensitivity of *P. falciparum* to chloroquine. As a result, the parasitological profile of malaria changed and proportion of *P. falciparum* increased in almost every state.

Considerable amount of parasitological information collected in the course of the study on the reliability of malaria diagnosis by Primary Health Centre (PHC) laboratories was described in Part I of this series (Beljaev et al.; 1985). Higher than usual standard of blood slide examination during this project gave us the opportunity to observe the true parasitological

Accepted for publication: 18 September 1987.

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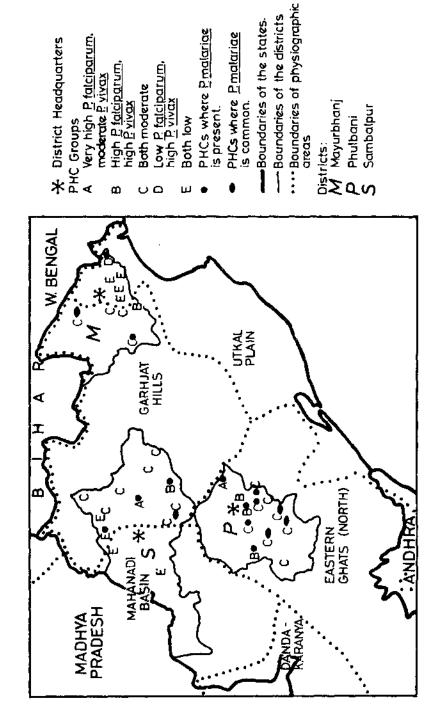


Fig. 1: Map of Otissa showing physiographic regions.

profile of the Passive Case Detection (PCD) subjects.

#### MATERIAL AND METHODS

The study was conducted in 1981-83 in Orissa state which is an area of high incidence of malaria, with predominance of *P. falciparum*. Physiographic and malariogenic conditions vary considerably in the state. As per the National Atlas of India (1977), Orissa has five physiographic provinces which are parts of three subdivisions belonging to two divisions (Fig. 1).

The areas with high malariogenic potential belong mostly to Eastern Ghats and Garhjat Hills. while Utkal Plains and Mahanadi Basin are much less malarious. Transmission of malaria which, at least in some areas, may occur throughout the year, increases soon after the onset of monsoon which usually sets in at the beginning of June. As a rule, incidence reaches its maximum in August and continues to be high till November. It declines considerably in December and January, mostly due to low temperatures, especially in hilly areas. A moderate increase of transmission occurs in January and March followed by a depression in April-May as a result of rise in temperature and drying up of breeding sources. This depression is more pronounced in the plains.

Thirty nine PHCs belonging to Sambalpur. Mayurbhanj and Phulbani districts were included in the study (Fig. 1). They cover parts of the following physiographic provinces:

- Sambalpur (Western part)-Mahanadi Basin
- Sambalpur (Eastern part) Garhjat Hills
- Mayurbhanj (Eastern and Central part) Garhjat Hills
- Mayurbhani (Western part)-Utkal Plains
- --- Phulbani--- Eastern Ghats (North)

In short, the study design was to collect slides in duplicate from patients seeking medical care at the Primary Health Centres (PHCs) and to process them independently at the PHC and at the Project laboratories. A detailed description of the methods is given in Part I of this series (Beljaev et al., 1985).

Slides were collected by the PHC laboratory technicians from 100-200 consecutive cases who were referred for blood examination for malaria. The duplicate slides were processed at the PfCP research laboratory in Bhubaneswar. The essential features of the processing were: use of Giemsa stain, examination under standard conditions (100 microscopic fields using an optical system of x100 objective and x7 eyepiece), recording of parasite densities (PD) and blind cross-checking by an independent laboratory in Delhi. Densities were recorded separately for the following species/forms: P. falciparum (asexual). P. falciparum (gametocytes), P. vivax (all forms) and P. malariae (all forms). The density grades were recorded in the following way:

Grade	Number of parasites
5	more than 100 per field
4	10 to 100 per field
3	1 to 10 per field
2	1 to 10 per 10 fields
1	less than 1 per 10 fields

The PHCs were classified on the basis of the specific prevalence of *P. falciparum* and *P. vivax*. Statistical analysis was conducted within each of the obtained classes of PHCs, sex and agewise. Ten age groups were taken, six groups at 5 year intervals from 0 to 29, three groups at 10 year intervals from 30 to 59 and a group of 60 and above.

#### RESULTS

From 39 PHCs, 4115 slides were collected between July 1981 and April 1983. Quality of some

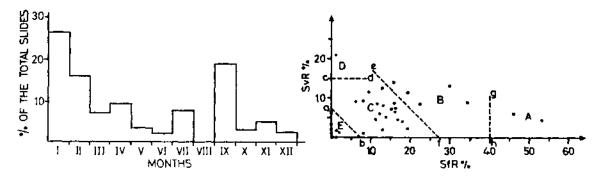


Fig. 2: Monthwise distribution of slide collection.

Total amount of slides examined by the project laboratory is taken for 100%.

of the slides was substandard and only 3506 slides from 35 PHCs were accepted for examination at the research laboratory of which 3462 were complete with age and sex data. The distribution of those slides by month of collection is shown in Fig. 2.

Prevalence of malaria varied considerably. The observed epidemiological situations were classified according to the Slide Positivity Rate (SPR) with a further differentiation on the basis of Slide falciparum Rate and Slide vivax Rate

Fig. 3: Classification of the PHCs on the basis of SPR, SfR and SvR obtained from the results of the project laboratory.

Each dot corresponds to one PHC. Definitions of the PHC categories (A to E) are given in the text.

(SIR and SvR). This was done by plotting the data on a scatter diagram and observing the patterns of clustering of the dots, each representing a PHC (Fig. 3). The following landmarks were used for classification as shown by broken lines: SPR 7% (line ab), SvR 15% (cd), SPR 27% (ef) and SIR 40% (gh). Five areas were delineated in such a way, labelled A to E, to which five types of epidemiological situations correspond. Their general characteristics are given in Table 1.

Table 1, Specific prevalence in five groups of PHCs

Types of	No.	Total		No. of cas	es (%)			
situations	of PHCs	exam- ined	P. falc	iparum	P. vivax (SVR)	P. mala- riae (SMR)		
			All (SFR)	Gamet. (%)*	(5711)	/ (S.		
A	2	252	119(47.2)	15(12.6)	13 (5.16)	2(0.79)		
В	5	628	154(24.5)	49(31.8)	73(11.6)	6(0.96)		
C	19	1799	240(13.3)	91(37.9)	119 (6.61)	24(1.33)		
D	1	142	1 (0.70)	0(NA**)	30(21.1)	2(1.41)		
E	8	641	4(0.62)	2(NA**)	9 (1.4)	1(0.16)		
Total	35	3462	518(15.0)	157(30.3)	244 (7.05)	35(1.01)		

<sup>\*</sup>Percentage of slides with gametocytes out of all P. falciparum cases.

<sup>\*\*</sup>Not applicable, number of observations too small.

In the situations belonging to the types A, B and C the prevalence of P. falciparum is high while prevalence of P. vivax may or may not be high. In type D, the prevalence of P. vivax is higher than anywhere, but the prevalence of P. falciparum is low; in type E, the prevalence of all species is low.

The geographical distribution of various epidemiological situations as shown in Fig. 1 may be described as follows:

- (a) PHCs with high SfR (i.e., groups A.B and C) never occurred in Utkal Plain and Mahanadi Basin provinces;
- (b) PHCs with low SfR (i.e., groups D and E) never occurred in Eastern Ghats (North) and were very uncommon in Garhjat Hills;
- (c) P. malariae was quite common in Eastern Ghats (North) and less common in Garhjat Hills, but was found only once in Utkal Plain and never found in Mahanadi Basin provinces. Its distribution generally coincided with the areas of high prevalence of P. falciparum.
- (d) P. vivax did not show confinement to any geographical province.

Temporal distribution in relation to the seasons of transmission in Orissa is given in Table 2.

Totals in Tables 1 and 2 differ slightly because in a few cases information about the time of collection was missing. It may be observed that with the exception of type A situations, in which slides were mostly collected during the season of maximum incidence, majority of slides were collected during December-March which is a period of moderate incidence. Number of slides collected during the peak season (July-November) was less in types D and E than in types B and C.

Age prevalence of *P. falciparum* is given in Fig. 4. In situations of type A. SIR has a marked peak at the youngest age and gradually declines afterwards. In type B peaks of prevalence are not so well marked. The prevalence is more or less

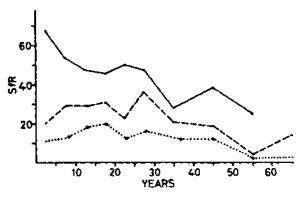


Fig. 4: Age prevalence of P. falciparum (asexual).

Table 3	Time	~6	collection	af aka	a11.dan
Jabie Z.	rime	03	collection	of the	SIMICS

Type of situations	No. of PHCs	No of stide	es collected (% of the g	roup total)	Total
×11.011.12		Dec-Mar	Apr-Jun	Jul-Nov	
A	2	0 (0.0)	0 (0.0)	252(100)	252
В	5	280(44.6)	86(13.7)	262(41.7)	628
C	19	1058(59.6)	182(10.2)	536(30.2)	1776
D	]	73(51.4)	43(30.3)	26(18.3)	J42
E	8	352(55.0)	189(29.4)	100(15.6)	641
Total	35	1763(51.3)	500(14.5)	1176(34.2)	3439

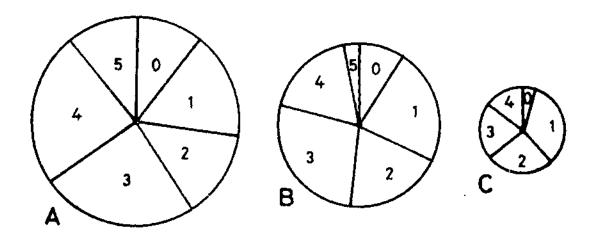


Fig. 5: Distribution of P. falciparum (asexual) cases by classes of parasite densities.

uniformly high in young adults and in children, except the youngest age group of 0-4 years. The maximum SfR of 35.8% is observed at the age of 25-29 years and after that the prevalence declines rapidly. In type C the difference between the age groups is less marked, but the trend is similar to the type B. In all the three types of situations, SfR in groups below 30 years of age is significantly higher than in the older groups (Table 3).

Table 3. Agewise comparison of SfR

Туре	SFR (0-29yrs)	SFR (30 and above	Significance of the difference
——— A	52.7%	31.8%	p>99%
В	27.4%	17.3%	p >95%
C	14.5%	10.5%	p >95%

In the situations of low *P. falciparum* prevalence (D and E combined) only five cases of *P. falciparum* could be observed among 783 patients. Four of them belonged to the age group of 30-49 years and one to 0-4 age group.

Distribution of the classes of asexual parasitaemia in P. falciparum positive cases is shown

in Fig. 5. In this and the following pie diagrams the surfaces of the circles representing each type of situation were made proportional to the absolute number of cases. Class 0 refers to the cases with gametocytes only and no asexual parasites at all. Cases of *P. falciparum* without asexual parasites were rare in type A situations (2.5%) but they occurred more often in type B and especially in type C (7.8 and 13.8% respectively).

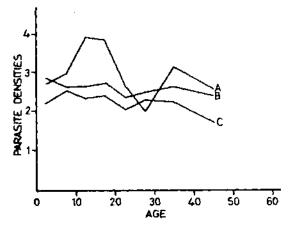


Fig. 6: Age distribution of average parasite densities of P. falciparum (asexual).

Average parasite densities (APD) of *P. falciparum* (asexual) agewise are shown in Fig. 6. In group A there is a marked peak at the age of 10-19 years. There is not much variation in the types B and C. In high *P. falciparum* prevalence situations, very high PDs which are associated with lifethreatening conditions (class 5) were unusual in age 20 and above and never occurred after 40 years. Out of all *P. falciparum* positive cases, class 5 parasitaemia was observed in 10.7% of patients of 0-19 years and only 2.24% of cases of 20-39 age group.

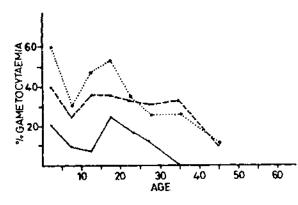


Fig. 7: Percentage of cases with patent gametocytaemia among all cases of P. falciparum.

In situations of high *P. falciparum* prevalence, percentage of *P. falciparum* cases with patent gametocytaemia (Fig. 7) showed two peaks, one in 0-4 and another in 10-14 or 15-19 years age group. In older age gametocytes occurred only rarely. In type A they never occurred after 30 years and in types B and C only few cases were found in patients older than 40 years.

Distribution of *P. falciparum* cases in relation to gametocyte densities is shown in Fig. 8. Class 0 applies to the cases with asexual parasites only. Densities of gametocytes were usually low: 76.1% of all cases of gametocytaemia were of grade 1.18.1% of grade 2 and only 5.8% of grade 3. There was no remarkable difference in the APD of gametocytes in different age groups.

Age distribution of *P. vivax* was more uniform. compared with *P. falciparum*. In high malaria prevalence situations (types A. B. C and D combined) the highest SvR was observed at the age 10-14 (12.5%). 15-19(12.3%) and 0-4(10.1%). SvR in the age below 20 was significantly higher than in older age groups (9.7 and 5.6% respectively).

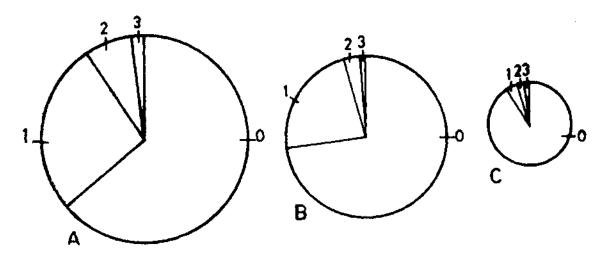


Fig. 8: Distribution of P. falciparum (gametocytes) cases by classes of parasite densities

Distribution of *P. vivax* cases by PD is shown in Fig. 9. There was not much variation of PD with age in *P. vivax* cases. APD varied from 2.40 in 0-4 age group to 2.80 in 30-39 age group. Cases with high PD (class 4) could be observed at any age.

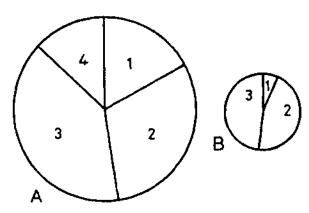


Fig. 9: Distribution of P. vivax and P. malariae cases by classes of parasite densities.

Prevalence of *P. malariae* in the A. B. C and D types combined peaked at the age of 20-24 years (2.9%). There was a significant difference in prevalence in the groups below and above 25 years of age (1.52 and 0.58% respectively). APD was 2.44. In individual cases, PD never exceeded grade 3. No correlation of density of *P. malariae* with age could be found, but this could hardly be expected in view of the small number of cases.

There was no consistent difference in the prevalence depending on sex, except in group D and E. In the former, SvR in males was significantly higher than in females (24.7 and 15.8% respectively). In group E not a single case of P. falciparum was found among women (SfR in males was 1.0%). Prevalence of P. vivax was also higher in males than in females, SvR was 1.80 and 0.79% respectively. The only case of P. malariae observed in this group was also in a male. As a result, SPR was significantly higher in males, 3.09 as against 0.79%.

In the above data the cases of double infections were included into both the species groups. The total number of mixed cases was distributed as follows:

P. falciparum +P. vivax 14 cases (including 3 cases without asexual P. falciparum)
P. falciparum +P. vivax +P. malariae 1 case

I case

P. vivax + P.malariae

Cases of mixed infections were observed only in situations **B** and C in which *P. falciparum* prevalence was high, they amounted to 4.0% of the total positive cases in **B** type and 1.9% in C type of situation (difference non-significant).

Twelve mixed cases were observed in males and 4 in females (3.3% and 1.7% of the total number of positive cases in the respective sex groups in B and C types of situations). The difference is, however, below the significance level.

Mixed cases were never observed in patients of the age of 40 and above. Prevalence of mixed cases was higher in young adults than in children. In the age group of 15-39 years (B and C situations only) mixed cases were observed in 4.0% of all positive cases, whereas in the age group of 0-14 years only 1.6% of positives had mixed infection (difference significant in one-tailed test at 95% level).

In mixed cases. *P. falciparum* usually dominated. In 10 out of 12 cases in which asexual *P. falciparum* was present, its density was higher than the density of the other species.

#### DISCUSSION

A great diversity of epidemiological situations was observed in this study. This is partly explained by the difference in time of collection. This factor probably accounts for the observed

differences between the types A, B and C. The seasonal factor alone, however, cannot explain the extremely marked differences between situations where the prevalence of *P. falciparum* is high (types A, B and C) and where it is low (types D and E).

It is well-known that various geographical areas of Orissa have different malariogenic potential. Coastal plains, for example, are presently much less affected by malaria than foothills but intensive transmission occurred in coastal areas in the past, mostly due to transmission by A. sundaicus which is now thought to be extinct in the area.

Physiographic stratification is a system describing various hierarchic territorial strata which was developed for general purposes. Since this system is a result of integration of a number of characteristics such as orography, climate, flora and fauna which are all interlinked in a million ways, physiographic stratification may be used as a framework for specific purposes, including malariologic stratification.

By and large, the results of this study are in agreement with the notion of the decreasing malariogenic potential from Eastern Ghats (North) to Garhjat Hills and further to Utkal Plain and Mahanadi Basin. This study demonstrates that boundaries of physiographic provinces are of a considerable predictive value in identifying areas with high prevalence of *P. falciparum*. Further refinement may probably be done by identifying substrata within such a physiographic framework, by adding other relevant factors.

There is no evidence of sex difference in malaria prevalence in situations of high incidence of *P. falciparum*. Only when the incidence of *P. falciparum* is low, males are more affected than females, which is probably due to greater mobility of the former.

However, such situations in Orissa generate only a fraction of the total number of malaria cases. Therefore, in areas where malaria is a major problem, predomination of males among the patients is the result of under-representation of females which was discussed in the Part II of this series (Beljaev et al., 1986).

When discussing the subject of prevalence and PD in relation to age one must bear in mind that the sample examined is by no means a random sample. It was created by a double and sometimes a triple selection:

- (a) self-selection by the patients which may depend on the distance, sex and age;
- (b) in the case of young children selection by their custodians;
- (c) selection by the physicians.

That is why many of the observations in this study differ strikingly from the usual picture of hyperendemic areas. Malariometric surveys repeatedly conducted by us in the same districts under another PfCP project demonstrated the age specific prevalence usual for hyper or mesoendemic areas with marked peaks in 5-9 or sometimes 10-14 years age groups.

Relatively low prevalence of *P. falciparum* in the youngest age group is probably because among the children subjected to PCD the causes of fever are many while among the adults excluding those of advanced age malaria becomes the most important single cause of fever. However, in A type of situations where a very active transmission was taking place on the background of hyperendemicity, the excess over the normal incidence occurred mostly among the youngest, non-immune group of children.

APD in this study culminated much later than is usually observed in the groups subjected to malariometric surveys. This is probably because only fever cases are screened by PCD and cases of

lower parasitaemia are more likely to be detected in young and less immune children in whom lower densities of parasites are sufficient to produce fever. Cases with low parasitaemia among older children and adults are simply not screened by PCD so APD remains high in those groups too.

Very high PD of *P. falciparum* is occurring not only in children but sometimes in young adults also. However, increasing immunity protects people older than 40 years from very high parasitaemia. It cannot be excluded that their relatively protected status is due to their experience of very high malaria transmission in the years before malaria control programme started i.e., 1953.

Two peaks of *P. falciparum* gametocytaemia are observed, probably because the presence of gametocytes depends both on age (they are more likely to appear in the youngest age which explains the peak at the age 0-4) and on the period of the disease (usually not observed before 12th day of the disease). Therefore, they are more likely to appear in cases where the disease develops slower as in semi-immunes which explains the second peak at the age of 10-14 or 15-19. In the intermediate group, the gametocytes are not present so often as in the youngest group but the disease is still very acute and cases are probably being screened on earlier stages of the disease.

Prevalence of *P. vivax* may be very high sometimes. Often, but not always the prevalence of *P. falciparum* is also high in such situations.

P. malariae is practically not reported from Orissa nowadays. In actuality this species is by no means very rare. As may be expected, mixed cases are mostly observed in situations of high prevalence of malaria. The second component of the

mixed infections which is usually *P. vivax* or *P. malariae* is as a rule presented in small numbers and, therefore, is usually missed in superficial examination of a slide.

It should be emphasized that the picture described above does not represent the totality of malaria cases because of a selective screening in the process of the passive case detection. Therefore, the values, age-specific prevalence and other indices differ from what is usually observed in malariometric surveys.

Nevertheless PCD data gives a good idea about the priority of malaria among other diseases and of the high risk groups in relation to clinically manifested malaria. Therefore, they need more careful analysis than is usually being done in the field, including age and sex breakdown and evaluation of parasite densities.

#### ACKNOWLEDGEMENT

This project was supported by the Swedish International Development Agency (SIDA) through WHO SFARO, as a part of Plasmodium falciparum Containment Programme (PfCP).

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# In vitro Chloroquine resistant Plasmodium falciparum in Calcutta and its sensitivity to Qinghaosu (Artemisitene)

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In vitro sensitivity of Plasmodium falciparum from Calcutta was assessed against chloroquine and qinghaosu by micro in vitro test. Six out of 28 (21%) specimens were resistant to chloroquine. All the 17 specimens tested were susceptible to qinghaosu. This is the first report of chloroquine resistance from Calcutta.

#### INTRODUCTION

Chloroquine resistant falciparum malaria was first reported by Sehgal et al. (1973) from Assam. Since then there was a rapid spread of chloroquine resistant strains in different areas of the country (Sharma, 1984). Chloroquine resistant parasites have been detected in twenty one states or union territories so far (WHS, 1986; Verma et al., 1986). In West Bengal resistance to chloroquine has been reported from Jalpaiguri and Purulia districts (WHS, 1986).

The present study was undertaken to determine the *in vitro* chloroquine sensitivity of *P. falci*parum from Calcutta metropolitan area and also to test the efficacy of the Chinese antimalarial drug qinghaosu (artemisitene).

Accepted for publication: 21 September 1987.

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#### MATERIAL AND METHODS

The study was conducted during November 1986 to January 1987 at the malaria clinic of Regional Health and Family Welfare Office in Calcutta on patients belonging to southern Calcutta area. Peripheral blood smears were collected from fever cases, stained with JSB (Jaswant Singh and Bhattacharji, 1944) and examined for malaria parasites. Patients showing adequate asexual parasitaemia were selected for *in vitro* tests. Finger prick blood samples (200 mcl) were collected from malaria patients after ensuring the absence of chloroquine in their urine samples following the Dill and Glazko test.

Micro in vitro tests for chloroquine were conducted essentially as per the WHO protocol in predosed chloroquine plates supplied by WHO. Stock solution of qinghaosu ( $10^{-3}$  M) was prepared in DMF and sterilized by filtration. Plates were predosed with  $2 \times 10^{-11}$ ,  $2 \times 10^{-10}$ .  $2 \times 10^{-9}$ ,  $2 \times 10^{-8}$  and  $2 \times 10^{-7}$ M concen-

trations of qinghaosu prior to the test in the laboratory. RPMI-1640 medium with HEPES buffer (30 mM), gentamycin (40 mg/l) and pH adjusted 7.2 to 7.4 with 5 per cent sodium bicarbonate was used as growth medium. Remaining procedures for incubation, staining of parasites from pre and post incubation smears were same as described in the WHO monograph series, 1981. A test was valid where at least 10 per cent schizont maturation was observed in control wells (Draper et al., 1985). Schizont maturation at >5.7 pmols of chloroquine was considered as an indication of resistance (WHO monograph series, 1981). The minimum inhibitory concentrations (MICs) of both the drugs were ascertained by microscopic examination from post incubation smears.

#### RESULTS AND DISCUSSION

Out of a total of 294 blood smears examined from fever cases, 84 were positive for *P. vivax* (28%) and 69 for *P. falciparum* (23%). The age of the patients selected for this study ranged from 6-53 years (mean 25) and asexual parasitaemia ranged from 550-92000 per mel blood (mean 24591).

Out of 69 falciparum cases, micro in vitro tests were carried out on 47 as the remaining cases were found unsuitable for the test. Eighty micro in vitro tests were carried out, of which 47 were against chloroquine and 33 against qinghaosu. Adequate schizont maturations in control wells were observed in 28 (59%) and 17 (51%) tests respectively.

Results of the micro in vitro tests for chloroquine (Table 1) indicated that MICs for this drug ranged from 2-32 pmols. Six specimens (21%) were resistant to this drug where schizont growth at  $\geq 5.7$  pmols was observed. The MICs for resistant parasites varied from 8-32 pmols.

Seventeen specimens including chloroquine resistant cases exhibited sensitive responses to qinghaosu (Table 1) where MICs ranged from

Table 3. Micro in vitro test: sensitivity of P. falciparum to chloroquine and qinghaosu

Sex/age	Asexual parasites/	MIC	s of
	mm3 of blood	Chloroquine pmol/well	Qinghaosu (M)
M/50	24200	16*	2 × 10-8
M/13	80000	16*	$2 \times 10^{-8}$
M/11	5000	2*	2 × 10 · 9
F/53	4800	8*	$2 \times 10^{-9}$
M/39	13000	5.7	$2 \times 10^{-9}$
F/6	38000	16*	2 × [0·9
F/6	3100	2	NT
M/14	22500	2	$2 \times 10^{-8}$
F/20	10000	4	$2 \times 10^{-6}$
F/7	4000	4	$2 \times 10^{-8}$
F/40	29700	4	$2 \times 10^{-8}$
F/25	1090	4	$2 \times 10^{-8}$
M/10	20400	2	$2 \times 10^{-8}$
M/42	18000	2	NT
M/40	550	2	NT
M/23	92000	16•	$2 \times 10^{-6}$
M/25	19600	32*	$2 \times 10^{-8}$
F/25	60000	5.7	$2 \times 10^{-6}$
F/24	2700	2	$^{8}$ -O1 $\times$ 5
F/30	17000	4	$2 \times 10^{-8}$
M/32	11000	4	NT
M/21	9500	2	NT
M/28	57500	4	NT
M/35	22800	4	NT
M/22	8800	5.7	NT
M/15	2600	2	NT
F/32	44000	4	NT
M/12	2000	2	NT

\*Indicates resistance to chloroquine NT = Not Tested

 $2 \times 10^{-9}$  to  $2 \times 10^{-8}$  M. Earlier, Thaithong and Beale (1985) reported chloroquine and sulfadoxine-pyrimethamine resistant P. falciparum isolates from Thailand, which were fully responsive to this drug in the in vitro, the MICs ranged from  $10^{-7}$  to  $10^{-8}$  M. Childs (personal communication) has also showed MIC values of  $1.43 \times 10^{-8}$  and  $1.48 \times 10^{-8}$  M for multidrug resitstant falciparum parasites from eastern Thailand. MIC values for qinghaosu obtained from the present study compare well with those reported earlier. There is also no evidence of cross resistance between chloroquine

and qinghaosu as indicated by Thaithong and Beale (1985) and Childs (personal communication).

The present study indicates a focus of chloroquine resistant strain in Calcutta. Further studies are indicated to confirm chloroquine resistance by standard WHO in vivo test as chloroquine is the first drug of choice for the treatment of falciparum malaria in Calcutta.

#### **ACKNOWLEDGEMENTS**

The authors are grateful to the Director, National Malaria Eradication Programme and Regional Director, Regional Office of Health and Family Welfare, Calcutta for extending facilities for carrying out this work. Thanks are due to Dr. D.K. Mukherjee, R.O., ICMR Monitoring Team, Calcutta and his staff for technical assistance. The authors are indebted to Dr. S.C. Pal. Director, National Institute of Cholera and other Enteric Diseases (ICMR) for extending excellent laboratory facilities. Qinghaosu was received through the courtesy of Dr. N.A. Roth.

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# In vitro antimalarial activity of Neem (Azadirachta indica A. Juss) leaf and seed extracts

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Components of ethanol extract of Azadirachta indica A. Juss (neem) leaves and seeds are reported as antimalarial for chloroquine sensitive and resistant strains of Plasmodium falciparum. Minimal efficacy doses of 25 to 50 fold concentration were noncytotoxic to Vero (African green monkey kidney) and Chang Liver (Human foetal liver) cultures.

Ethanol extracts of neem leaves and seeds were further fractionated on polarity basis. The noncytotoxic concentrations were added to cultures of *P. fulciparum* chloroquine sensitive strains FAN-5. FCK-3. FMN-17. FMN-33 and chloroquine resistant strain MP-11 and their antimalarial activities are reported in minimal efficacy doses showing complete clearance of parasites

#### INTRODUCTION

Azadirachta indica A. Juss (neem), a tropical tree with bitter root and shoot has been considered as a remedy for most afflictions, in ancient Indian medicine (Sushruta, 1931; Charaka, 1941; Bhav-Misra, 1984). Hot water extract of neem given to malaria patients cured them of this disease (Burkill, 1966; Sofovora, 1982). With a view to study the efficacy of the leaf and seed extracts of Neem against chloroquine sensitive

and resistant culture strains of *Plasmodium falci*parum, experiments were undertaken from May 1983 to August 1984 employing six culture strains

Maintenance of P. falciparum in culture

P. falciparum culture strains used are given in Table 1. These were maintained 'in vitro' employing candle jar method of Trager and Jensen (1976). Cultures were maintained in A Rh  $\pm$  verythrocytes using RPMI 1640 medium (Moore et al., 1967) supplemented with AB Rh  $\pm$  vehuman serum (10%), sodium bicarbonate (0.21%). HEPES Buffer (25 mM) and gentamycin (50  $\mu$ g/ml).

MATERIAL AND METHODS

Accepted for publication: 25 September 1987.

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Table 1. Plasmodium falciparum strains and their sources

S. No.	Strain	Chloroquine* sensitivity	Place of isolation	Name of the Institute that supplied the strain
1.	FAN-5	Sensitive	Alwar, Rajasthan (North India)	National Institute of Communicable Diseases, Delhi, India.
2.	FCK-2	Sensitive	Kolar, Karnataka (South India)	St. John's Medical College, Bangalore, India.
3.	FCK-3	Sensitive	Kolar, Karnataka (South India)	St. John's Medical College, Bangalore, India.
4.	FMN-17	Sensitive	Yavat. Maharashtra (Central India)	National Institute of Virology, Pune. India.
5.	FMN-33	Sensitive	Srigonda, Maharashtra (Central India)	National Institute of Virology, Pune, India.
6.	MP-11	Resistant	Thailand	Mahidol University. Bangkok. Thailand.

Sensitive: MED<sub>50</sub> < 0.05  $\mu$ g/ml at 72 hours. Resistant: MED<sub>50</sub> > 0.4  $\mu$ g/ml at 72 hours.

#### Evaluation of antimalarial activity

Cultures were set with an initial count of 0.5% parasitised erythrocytes (PPE) in tall glass vials  $(22 \times 45 \text{ mm})$  containing 100  $\mu$ l packed erythrocytes in 1.5 ml of complete medium. The PPE count of cultures at 24 hrs. was @ 1%. At this event cultures were treated in quadruplicate according to the method of Richards and Maples (1979) with selected concentrations of extracts. Medium was changed, smears drawn, stained with JSB stain (Singh, 1959) and PPE were recorded every 24 hours. Treatment was followed up to 72 hours. About 20,000 cells per slide were counted and PPE was calculated. Minimal efficacy dose 50 (MED<sub>so</sub>) meant a minimum dose at which 100% clearance of parasites was observed in at least 50% of cultures. Around 8 to 10 linear doses were selected within a trial range to determine MED so (Reed and Muench 1938).

#### Controls

- (a) Appropriate untreated parasitised cultures were maintained as controls.
- (b) Normal erythrocyte cultures treated with extracts were maintained as drug treated controls.

- (c) Drug pretreated controls: Normal erythrocyte cultures were supplemented with ethanol extracts of neem leaf doses ranging from 250-1000 μg/ml. The medium containing neem leaf extract was removed after 24 hrs. These erythrocytes were washed with RPMI 1640 medium and infected with parasitised erythrocytes to set a 0 hour PPE at 0.5. These cultures were followed upto 72 hours along with controls for the growth of parasites. This control was necessary to rule out the extraneous effect of neem extract on host cells.
- (d) Control for solvent: Cultures were set with 0.5 PPE at 0 hours. After 24 hours these were treated with doses ranging from 2.5 to 25 μl ethanol (Merck) per ml of culture. This treatment was continued upto 72 hours. This control was necessary to rule out the extraneous role of ethanol (used as solvent) in clearance of parasites.

#### Preparation of extracts of Neem

Leaf and seed extracts were prepared as follows:

#### Leaf extract

(a) One kilogram of leaves were extracted with ethanol (95%) for two days at room tempera-

ture. Yield of this extract was 25 gm. This was coded as  $L_1$ .

(b) Twenty gm of L<sub>1</sub> was washed with petroleum ether and acetone. The residue was 5 gm and coded as L<sub>2</sub>. It was soluble in water.

#### Seed extracts

- (a) One kilogram of dry neem seed powder was extracted with ethanol (95%) in a Soxhlet apparatus. It yielded 250 gm of extract and was coded as S<sub>1</sub>.
- (b) Hundred gm of S<sub>1</sub> was washed with petroleum ether. The ether extract weighed 65 gm. Fat free component (35 gm) was extracted with water. Yield of this extract was 10 gm. This was coded as S<sub>2</sub>.
- (c) Residue of S<sub>2</sub> (25 gm) was extracted with 50% ethanol which yielded 5 gm of extract and was coded as S<sub>3</sub>.
- (d) Residue left after taking out S<sub>3</sub> extract (20 gm) was extracted with 75% ethanol which yielded 10 gm of extract and was coded as S<sub>4</sub>.
- (e) Residue of S<sub>4</sub> yielded 10 gm and was coded as S<sub>5</sub>.
- (f) One gm of  $S_s$  was passed through a column (110 cm length and 3.5 cm diameter) of 400 gm silica gel (120 mesh) using acetone-ether (1:9) elution gradient with increasing polarity upto methanol. The elute was collected in 3 volumes. The extract from first volume of elute yielded 400 mg. This was coded as  $S_{S_1}$ .
- (g) Extract from the second volume of elute yielded 200 mg. This was coded as  $S_{5\mu}$ .
- (h) Extract from the third volume of  $S_5$  yielded 250 mg and was coded as  $S_{5_{\rm HI}}$ .

#### Dissolution and sterilisation of extracts

Extracts were dissolved in equal volume of ethanol as used in extraction, sterilised employing 0.22  $\mu$ m filter. The desired test doses were made by further diluting sterilised extracts in RPMI 1640 complete medium.

#### Cytotoxicity

Cytotoxic concentrations of extracts were determined employing Vero (African Green Monkey Kidney) and Chang Liver (Human foetal liver) cell lines. Cultures were set with  $2 \times 10^5$  cells/ml and after 24 hours these were treated with different concentrations of extracts in quadruplicate at doses ranging from 10 to 10,000  $\mu$ g/ml. Cytotoxic effect was assayed and recorded as tissue culture LD<sub>50</sub>/ml dose.

#### RESULTS

Supplementation of culture medium with ethanol to the extent of 25  $\mu$ l/ml (Fig. 1) and cultures of erythrocytes pretreated with neem leaf ethanol extract to the extent of  $1000 \, \mu$ g/ml of culture (Fig. 2) did not not show any inhibition of growth of parasites. The maximum ethanol content of drug dose was 1.25  $\mu$ l/ml. These observations clearly indicate specific antimalarial activity of different neem extract fractions and rule out any extraneous effect of solvents on parasites or neem extract fractions on erythrocytes.

Variation in the response amongst different strains of *P. falciparum* was negligible; even a chloroquine resistant strain MP-11 showed a pattern similar to that of average chloroquine sensitive strains (Table 2).

The MED<sub>50</sub> dose of ethanol extract of neem leaves ( $L_1$ ; 25-75  $\mu$ g/ml) was much lower than the cytotoxic dose (2000-2500  $\mu$ g/ml). 'Etheracetone extract' free component of  $L_1$  ( $L_2$ ) had

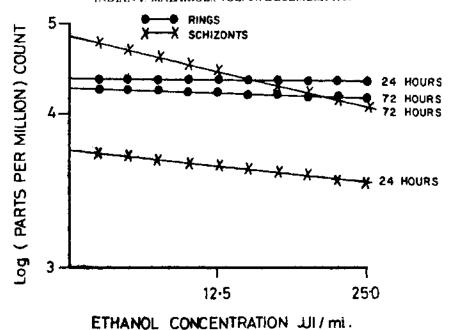


Fig. 1: Effect of ethanol on Plasmodium falciparum 'in vitro'.

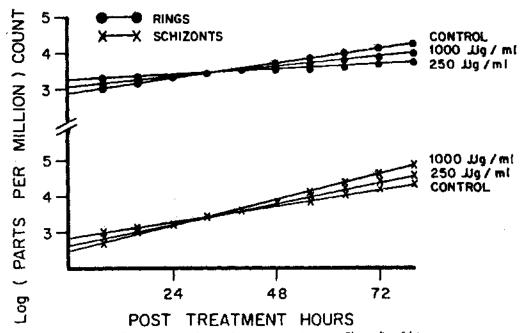


Fig. 2: Effect of neem leaf ethanol extract pretreatment on Plasmodium falciparum 'in vitro'.

Table 2. Effect of neem leaf and seed extracts on Plasmadium falciparum strains in vitro.

i	; ; ;	i   	 			1		1	1	
Code of	Concentra-		2:	$MED_{s0}(\mu g/ml)$		•	Average MFD of	MED <sub>50</sub>	Cytotoxi	c dove
fraction	tion in leaf / seed (%)	FAN-5	FCK-2	3 FCK-3	FMN-17	5 FMN-33	chloroquine sensitive strains (1 to 5)	MP-11	Vero Chang liver	Chang liver
1 1		9		9	05	9.	45	75	2500	2000
. آ	0.53	0001	750	1000	0001	0001	056	1000	8000	6500
, ,	60.95	00,	g, c	8	000	000	300	300	5000	4000
กัง	00.00	90	90,2	902	700	700	700	700	3000	2500
ດ້າ	96.7 96.4	300	300	300	902	300	300	300	3000	2500
ຄຳ	) ; <b>(</b>	95	150	001	[25]	100	125	100	3000	2500
ກັບ	95.5	3 15	£ 5	os Os	1 2	100	08	<u>6</u> 2	3000	2500
ก็ผ	0	2 5	କ	: €	9	S	50	70	3000	2500
กั <sup>-</sup> c	0.50	3 %	ř	35	5 t.	. <u>2</u> 2	25	95	2000	1500
e de	0.29	50	25	35	25	35	30	20	2000	1500
Chloroquine		0.03	70.0	0.01	0.04	0.05	0.03	0.40	ន	9
							! !!! ]   ! ! !	1		1 1 1 1 1 1 1

less antimalarial activity (MED<sub>50</sub>: 750-1000  $\mu$ g/ml). It meant most of the active principles were washed away by ether and acetone. Etheracetone extracts of neem leaves were too oily to be supplemented to the culture.

Ethanol extract of neem seeds (S<sub>3</sub>) also cleared the parasites (MED<sub>50</sub>: 200  $\mu$ g/ml), however, its effect was rather less than the L,: ethanol extract of leaves. Aqueous extract of fat free S<sub>1</sub> (S<sub>2</sub>) had less antimalarial activity (MED<sub>so</sub>: 700  $\mu$ g/ml). again confirming that most of the active principles are located in lipid portion. Extraction by 50% ethanol of S<sub>2</sub> residue (S<sub>3</sub>; MED<sub>50</sub>: 300  $\mu$ g/ml) had higher antimalarial activity than  $S_2$ . Repeating the trend, 75% ethanol extract of  $S_3$ residue (S<sub>4</sub>; MED<sub>50</sub>: 100-150  $\mu$ g/ml) showed higher antimalarial activity than S3 and residue of  $S_4$  ( $S_5$ ; MED<sub>50</sub>: 70-100  $\mu$ g/ml) had higher antimalarial activity than S4. Medium and low polar fractions of  $S_3$  ( $S_{5\mu} \pm S_{5\mu}$  ) had highest antimalarial activity (MED<sub>50</sub>: 25-50  $\mu$ g/ml) amongst all the extracts at doses which were non cytotoxic. These were comparable to ethanol extract of leaves.

The MED<sub>50</sub> doses for chloroquine sensitive strains (0.01 to 0.05  $\mu$ g/ml) were lower than that of chloroquine resistant MP-11 (0.4  $\mu$ g/ml).

#### DISCUSSION

Plant extracts have been reported to have antimalarial activity (Trager and Polonosky, 1981; Jiang et al., 1982; Guru et al., 1983). Rochanakij et al. (1985) have reported that ethanol extract of leaves A. indica var. siamensis is effective against resistant strain of P. falciparum. Bray et al. (1985) have also reported that bark and leaf extract of A. indica have antimalarial activity 'in vitro' against a multi-drug resistant strain of P. falciparum. It is evident from the present study that different fractions of neem (A. indica from India) leaf and seed exhibit efficacy against malarial parasites. The most potent being ethanol extract of leaf and medium polar fraction of seed extract. although all other fractions also suppressed the growth of parasites within 72 hours at slightly higher doses.

As against qinghaosu (Artemisinine) neem fractions are stable in ethanol.

Experiments conducted on the effect of neem leaf extract on erythrocytes clearly indicate that it is not toxic to erythrocytes. Ethanol extract of leaves and seeds of neem contain terpenoid lactone, nimbolide, triterpenes, nimbidin and sodium nimbidinate (ICMR, 1976). As nimbolide is the major constituent of these extracts, antimalarial activity could be due to it.

It is pertinent to mention here that practitioners of the Indian medicine system of "Ayurveda" prepare oral doses of neem for malarial patients by decocting about 500 gm of leaves or extracting them in alcohol or water. Antimalarial cum antipyretic activity of neem was reported by Charaka (2000 BC) and Sushruta (1500 BC) in their books on Ayurveda (Recent editions of these books are referred as Sushruta, 1931 and Charaka, 1941). Decoction contains lipids in the form of emulsion. Alcohol extract has better shelf life and decoctions are also preserved in alcohol.

Results of the present study 'in vitro' clearly indicate that neem from India has some active components in both leaf and seed extracts which are responsible for extirpating chloroquine sensitive and resistant malaria parasites from the cultures.

#### **ACKNOWLEDGEMENTS**

We are grateful to the Director, National Institute of Virology, Pune. Dr. (Miss) K.M., Pavri, for her constant encouragement. We are also thankful to Miss M.V. Apte and Mr. T.L.G. Rao for their skillful technical assistance. Cooperation and help rendered by Mr. V.B. Jadhav of Jehangir Nursing Home. Pune for prompt supply

of non citrated and citrated blood is gratefully acknowledged. Financial support of ICMR. New Delhi, for the project "Biological characterisation of merozoite and screening of drugs" is gratefully acknowledged.

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## Immunofluorescence Test in the Seroepidemiology of Malaria around Delhi

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Serological profile and parasitaemia was determined in 3 areas around Delhi in the peak transmission (I survey) and non-transmission (II survey) period of *Plasmodium falciparum*. Of the 6598 serum samples assayed for malarial antibodies by the IFA test, 4147 were assayed in the I survey, and 2451 in the II survey. A mathematical model is used for conversion of the serological data obtained to infection rates which provided a better estimate of the malarial problem in a community as compared to blood smear examination.

#### INTRODUCTION

The usual malariometric indices to determine malaria endemicity rates include splenomegaly and parasitaemia. Both being rather unsatisfactory, particularly at low levels of transmission, serological procedures have been used for epidemiological purposes in malaria in several parts of the world (McGregor et al., 1965; Draper et al., 1972b; Bruce-Chwatt et al., 1975; Lobel et al., 1976; Comille-Brogger et al., 1978) including the indirect fluorescent antibody test (Voller, 1971a; Draper et al., 1972a). We have employed IFA on serum samples collected from three different areas around Delhi and used the data to calculate malaria infection rates. It is shown that the data on age related

prevalence of malarial antibodies could be employed to determine infection rates in different communities, which correlate well with the results of smear examination for malarial parasites. We feel that seroepidemiology may provide a better tool for surveillance of malaria than the parasite index.

#### MATERIAL AND METHODS

The number of samples tested by IFA from three different locations around Delhi are shown in Table 1. The first survey was carried out during the peak transmission season of *P. falciparum* (November—December, 1985) and the second survey during the non-transmission season (February—March 1986). The three areas were chosen due to the known differences in the incidence of malarial infections, lowest being in Sonepat and highest in Ghaziabad. A number of villages were included in each of these three areas.

The details regarding the collection of samples, preparation of blood smears, their transpor-

Accepted for publication: 1 October 1987.

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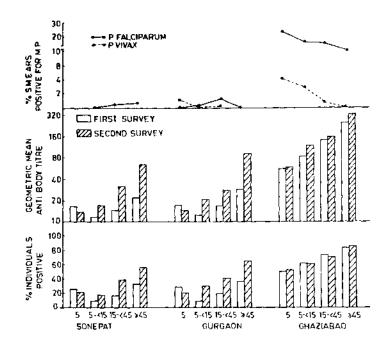


Fig. 1: Agewise distribution of IF malarial antibodies in different study groups. The smear positivity results of first survey are depicted. The second survey smear results are shown in Table 2.

Table 1. Number of samples assayed from different locations for the 1FA test for malarial antibodies

S. No. Area		1st Survey	2nd Survey	
1.	Sonepat	J211	901	
	(8 villages)			
2.	Gurgaon	1229	779	
	(15 villages)			
3.	Ghaziabad	f 707	771	
	(12 villages)			

tation to the laboratory etc., have been reported earlier (Kumar et al., 1986).

#### Serological procedure

The IFA test was carried out Voller, 1971b using an antigen prepared from in vitro cultivated

Plasmodium falciparum containing >5% mature schizonts (Trager and Jensen, 1976). Twelve smears were prepared on each microscopic slide. which after drying were wrapped and preserved at -70°C till tested. The filter paper eluates served as the material for antibody estimation (Kumar et al., 1986) and were diluted serially from 1/20 to 1/640. The antigen smeared slides were removed from-70°C and allowed to remain at room temperature for 30 mins., and then covered with diluted serum samples. Positive and negative control serum samples were included with each batch of tests. After incubation at 37°C for 30 mins, in a moist chamber the slides were extensively washed thrice with PBS pH 7.2 and covered with optimum dilution of antihuman IgG-FITC conjugate (Dakopatts). These were incubated at 37°C for 30 mins, and washed as before. The slides were mounted in 50% glycerol and examined under incident light fluorescent microscope (Carl-Zeiss). The results were expressed as reciprocal of the highest dilution of the test sample giving a clear positive reaction. The serum titres of 20 and above were taken as positive. End titres were not determined in samples giving a positive reaction in 1:640 dilution.

Statistical method: The mathematical model used for calculation of infection rate in the study population is described in detail by Draper et al., in 1972a. Briefly, the probability of a child never having been infected in n days =  $(1-\lambda)^n \sim e^{-n\lambda}$  where  $\lambda$  is the probability of a child getting infected in one day. According to the above method, the probability of being infected in one

year i.e., infection rate will be =  $1-e^{-uA}$  where u is 365 $\lambda$  and A is age measured in years.

#### RESULTS

The per cent individuals positive for antibodies, their mean IFA titres and the results of smear examination for malarial parasite are shown in Table 2. The antibody levels and the smear examination results show a fair correlation, particularly during the second survey. The age related distribution of antibodies is shown in Fig. 1. The IFA antibodies in different populations showed an increase with age, as expected. In areas of maximum transmission (Ghaziabad) the parasitaemia showed an inverse relationship with antibody titres. In Gurgaon on the other hand,

Table 2. Antihodies and parasitaemia in different locations investigated

	Anti	body			Blood sme	ars examined
Area	Per cent individuals positive		GM1		Per cent smear positive	
	Ist survey	2nd survey	1st survey	2nd survey	1st survey	2nd survey
Sonepat	14.86	23.64	13.92	21 66	0.25	0.00
Gurgaon	14.56	31.96	14.30	22.33	0.98	0.39
Ghaziabad	66.94	64.98	105.09	116 18	29.17	2.20

GMT = Geometric mean titre.

Table 3. Comparison of malarial antibodies during the transmission and non-transmission seasons in 45 years age group

Arca	Antibody titre			Neg.	χ²		
	640	320	160	80-20			
Ghaziabad							
Lsurvey	77	8	6	7	19	11.12	
II survey	69	ì	0	2	12		p < 0.02
Gurgaon							
1 survey	8	2	3	8	<b>3</b> 7	13.03	
II survey	21	1	3	6	19		p < 0.02
Sonepat							
1 survey	6	7	6	22	86	41.97	
II survey	40	2	4	6	82		p < 0.001

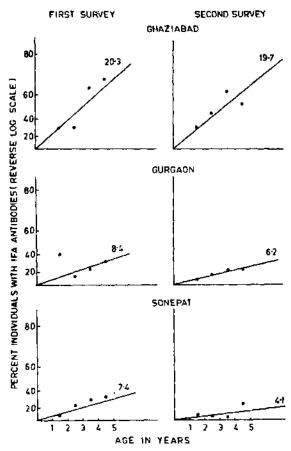


Fig. 2. Graphic representation of infection rates of malaria calculated from 1F antibody profiles in different study groups. For calculation of infection rates, the serological data in children belonging to the age group of 1-5 years only was taken into account.

initially the number of people with parasite in the peripheral blood increased with age but in later age groups they seemed to decrease. In Sonepat, with very few smears positive for malarial parasites, it is difficult to comment, but the general impression obtained is that smear positivity increased with age. Somewhat higher antibodies were found during the second survey in comparison to the first survey in all the areas in all age groups, with few exceptions.

In the age groups above 45 years a comparison between the first and second surveys (Table 3)

shows that the proportion of individuals with antibody as well as their titres are significantly higher in the non-transmission season in all the three areas. The infection rates calculated from the serological data obtained in the I-5 years age group for three localities are shown in Fig. 2. Since the calculated rates for the two surveys were not very different, the data was pooled and the final results are shown in Fig. 3. The infection rates in Ghaziabad, Gurgaon and Sonepat were 19.9, 6.9 and 5.6 respectively.

#### DISCUSSION

The immunologic profile of a population is the sum total of previous individual experiences. The

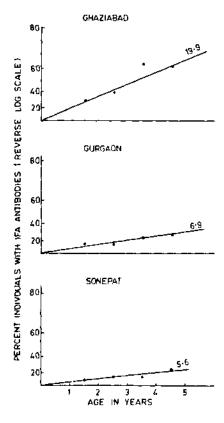


Fig. 3: Graphic representation of infection rates of malaria calculated by pooling the data from two surveys for 1F antibodies in the three populations studied.

response of the individual to these experiences is affected by age, immunological competence, cumulative exposure to malarial antigen and kind and amount of specific therapy used. In seroepidemiology, therefore, a sensitive test like 1FA provides period prevalence data which is more informative as compared to point prevalence data given by parasite rates (Collins et al., 1967; Mcfarlane et al., 1970; Meuwissen, 1974; Spencer et al., 1979). Our results of the surveys also show clearly that the seropositivity correlates well with parasitaemia, more so during the non-transmission season. The amount of antibodies were higher in the non-transmission season. As observed earlier (Kumar et al., 1986) lower antibody titres during the peak transmission could be due to absorption of antibodies by the parasites in the blood at a rate faster than they are produced. Therefore, non-transmission periods seem better for studying the antibody profiles for epidemiological purposes.

Repeated exposures in endemic areas should result in increasing antibody levels which would be reflected in an age related increase (Collins et al., 1967; Draper et al., 1972b). As seen in the results (Table 3) the IFA antibodies increased with age in all the three areas investigated. It is known that repeated exposures to malarial parasite in endemic areas result in induction of immunity (Mcfarlane et al., 1970). It is, therefore, reasonable to assume that with higher incidence of malaria, the immunity would manifest earlier than in areas with lower incidence of malaria. Our results on age related parasitaemia conform to this view. In Ghaziabad with highest incidence of malaria, the immunity was seen quite early, while in Sonepat there was no indication of immunity even in the >45 years age group.

Further analysis of the data is required to get a more definite mathematical expression of the serological profiles. For this, we have used a

mathematical model as advocated by Draper et al. (1972a). The congenital antibodies may be present in <1 year age group and the presence of antibodies in > 5 years age will not have any present epidemiological significance. Therefore, while calculating the infection rates using the above model, we have restricted ourselves to the serological data obtained in 1-5 years age group only. The infection rates as calculated are highest in Ghaziabad and lowest in Sonepat, and in between in Gurgaon. This correlates well with the results of blood smear examination for malarial parasites. However, the calculated infection rates for Gurgaon and Sonepat are much closer than would be expected from the results of smear examination. We believe that serological data provides a more realistic estimate of the infection in a community. Draper et al. (1972a) have claimed that seroepidemiology is likely to be most useful at lower levels of transmission, because of higher sensitivity and particularly in situations where transmission cycle has been disturbed by the use of insecticides or drugs.

In addition to their higher sensitivity, there are other reasons for serological methods to be more suited for malaria surveillance than smear examination because collection of samples is simpler, and their screening is quicker.

Serological methods do not permit a distinction to be made between *P. vivax* and *P. falciparum* infections, because of extensive cross reactions between the two parasites. Modern technology permits the identification of specific antigens of the two parasites, which should be attempted to further improve the value of seroepidemiology in malaria.

#### **ACKNOWLEDGEMENT**

The financial support provided by the Indian Council of Medical Research is gratefully acknowledged.

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## Specific IgM and IgG antimalarial Antibody responses in paired samples from Malaria patients

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Serial estimations of IgM and IgG antibodies are helpful in the diagnosis of different infectious diseases. However, in endemic countries the role of serial estimation of IgM titres in diagnosis of malaria is not known. IgG and IgM antibodies directed against *P. falciparum* have been studied by IFA-PF test in 21 slide positive malaria patients living in high endemic areas. It was observed that IgM antibody titres were raised in first samples and declined after 4 weeks time except in 2 patients. The IgG levels increased after 4 weeks interval. This suggests that serial IgM and IgG antibody estimations may prove useful in diagnosis of recent malaria infections.

#### INTRODUCTION

Antibodies to malarial parasite can be readily demonstrated by a variety of serological tests, namely indirect haemagglutination assay (IHA), indirect fluorescent antibody assay (IFA) and enzyme linked immunosorbent assay (ELISA) etc. (Collins et al., 1964; Voller et al., 1975; Agarwal et al., 1981; Dutta et al., 1982). These tests have been primarily used for epidemiological studies (Collins et al., 1968; Kagan, 1972; Voller et al., 1980; Agarwal et al., 1982) but there

too they suffer from the disadvantage of not being able to distinguish between recent and past infections (Collins et al., 1968; Voller and Bruce Chwatt. 1969; Das et al., 1985). This has generated interest in developing tests which can be used for detection of malarial antigen (Bidwell and Voller, 1981; Avraham et al., 1982; Mackey et al., 1980) or detection of the parasite by DNA probes (Barker et al., 1986; Pollack et al., 1985). Although these tests are successful but they are still in their infancy. An alternative approach could be to study the IgG and IgM type of antimalarial antibodies in paired samples. The results of such a study are presented in this paper.

Accepted for publication: 8 October 1987.

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#### MATERIAL AND METHODS

Paired serum samples were collected from 21 patients suffering from malaria who were attending Out Patients Department of Hindu Rao Hospital, Delhi. Of these, eight (38.095%) were

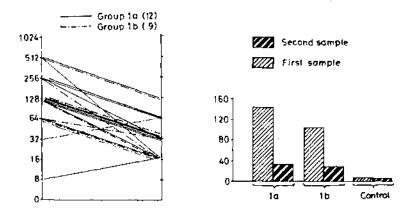


Fig. 1: (a) Distribution of IgM titres in paired samples of individual patient using P. falciparum antigen and IFA test.

(b) Comparison of GMRT's obtained in first and second samples of Group, la and lb.

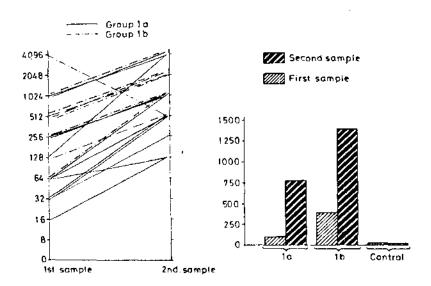


Fig. 2: (a) Distribution of IgG titres in paired samples of individual patient using P. falciparum antigen and IFA test.

(b) Comparison of GMRT's obtained in first and second samples of Group Ia and Ib.

positive for *P. falciparum*, eleven (52.38%) for *P. vivax* and two (9.52%) had both *P. falciparum* and *P. vivax* infections.

All patients gave characteristic history of high grade fever with chills and rigor. In 12 patients (54.65%) first blood sample was collected within 1 to 7 days of onset of fever. in 6 (27.27%) between 8 to 15 days, in 1 (4.44%) between 16 to 21 days, and in 2 (9.09%) between 21 to 28 days. All the patients were given chloroquine after collection of first blood sample and all responded to it. A second sample from the same subject was collected after four weeks of antimalarial treatment.

All sera were stored at 20°C with sodium azide (NaN<sub>3</sub>) as preservative and were inactivated at 56°C for 30 minutes prior to test.

IFA test: Smears of schizont stage of P. falciparum parasite from in vitro cultures were obtained from Dr. Krishna Ray, NICD, New Delhi. These slides were used as source of antigen for IFA test. All the serum samples were tested with the antigen (slides) of same batch. The IFA test was performed essentially as described by Voller and O'Neil (1971) except that the antigen slides were prefixed in chilled acetone for 30 minutes at 4°C. The results were recorded on Zeiss epifluorescence microscope, using exciter filter BP 546/12 and Barrier filter P 590.

#### RESULTS

IgM antimalarial antibody: The results of the IgM antimalarial antibody titre in paired serum samples from patients with malaria are shown in Fig. la. Both, in those where the first blood sample was collected within the first week of onset of fever (Group Ia) or later (Group Ib), the IgM antimalarial antibody titre showed a decline over a four week period (there were two exceptions, one in each group, where the IgM antibodies were higher in the second sample). Corresponding GMRT values are shown in Fig. Ib.

In Group Ia, GMRT for IgM antibodies in the 1st sample was 143.68. It fell down to 32 in the second sample. In group Ib, corresponding values for the first and second samples were 101.59 and 29.63, respectively. The degree of fall in individual subjects varied from 2 to 32 fold. These results are given in Table 1. The fall was comparatively more in Group Ia than Ib.

Table 1. Number of patients showing changes in IgM type of antimalarial antibody titre in paired samples

Change in titre		Group Ia*		Group 1b**	
		No.	(%)	No.	(%)
No change					
Decrease	2 fold			2	(22.2)
	4 fold	7	(58.3)	4	(44.4)
	8 fold	2	(16.7)	- 1	$(\Pi \Pi)$
	16 fold	J	(8.3)	J	(11.1)
	32 fold	1	(8.3)	_	
Increase		- 1	(8.3)	ž	(11.1)

<sup>\*</sup>la: where first blood sample was collected within seven days of onset of fever.

IgG antimalarial antibody: The results of IgG antimalarial antibody titre are shown in Fig. 2a. In all the subjects, except one, the IgG antimalarial antibody titre in the second sample was

Table 2. Change in titre of IgG antimalarial antibody in paired samples

Change in titre		Group la*		Group Ib**	
		No.	(%)	No.	(%)
No change					
Increase	2 fold	1	(8.3)	_	
	4 fold	4	(33.3)	6	66 6)
	8 fold	3	(25.0)	· ·	
	16 fold	3	(25.0)	2	(22.2)
	32 fold	1	(8.3)	_	
Decrease				1	(11.1)

 <sup>1</sup>a—where first bloods ample was collected within seven days of onset of fever.

<sup>\*\*</sup>Ib ...where first blood sample was collected after seven days of onset of fever.

<sup>•••1</sup>b—where first sample was collected after seven days of onset of fever.

higher than that of the first sample. The GMRT of IgG antibody for Group Ia in the 1st sample was 101.32. It rose to 767.13 four weeks later. Corresponding values for Group Ib were 449.14 and 1339.44 respectively (Fig. 2b). The rise in titre among individual subjects varied from 2-32 fold (Table 2).

# DISCUSSION

Results of the present study show that fall in IgM and rise in IgG antimalarial antibody titre in paired samples collected at an interval of four weeks can be used for confirmation of the diagnosis of recent attack of malaria. This approach appears to be useful even when the patient comes after first week of the onset of fever. Perhaps, a ratio of IgM/IgG antibody titre may be evolved which can distinguish between recent and past infections. A similar approach has been followed for serological diagnosis of rubella and toxoplasmosis (Alford et al., 1975).

For many infections it is known that first antibody formed during primary immune response is of the IgM type. It subsequently switches over to IgG type. In secondary immune response the antibodies are predominantly of the IgG type. Kuvin et al. (1962) and Collins et al. (1971) have observed that in both the simian and human malarial infections initial immune response is of the IgM type. However, the type of immune response to acute malarial infection in endemic areas, where past exposure to malaria can not be excluded, is not known. Our findings indicate that even in these circumstances the initial immune response consists of rise in IgM antibodies even though IgG antibodies are also elevated. One explanation for these findings could be the possibility of high degree of antigenic variability so that at each fresh infection the response is essentially of the primary immune type.

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# Comparative cost of mosquito control with Larvicidal Bacilli and Insecticides

K. BALARAMAN' and S.L. HOTI'

The production costs of the larvicidal bacilli. Bacillus thuringiensis H14 and Bacillus sphaericus were worked out on the basis of inputs used to produce them in a 13 lit working capacity laboratory fermentor. Whereas the production cost of one batch of B. thuringiensis H14 is US \$11.02, the production cost of B. sphaericus is US \$13.34. Also, the costs involved in controlling mosquito larvae in an area of one hectare with the bacilli and chemical larvicides were compared. The analysis showed that the cost of larval control with bacilli is more or less equivalent to that incurred if fenthion is used, but significantly lesser if mataria oil or paris green or abate is used.

#### INTRODUCTION

The use of biological agents for mosquito control, as alternative to chemical insecticides has been considered for several reasons. More energy and money has been spent in developing B. thuringiensis H14 and B. sphaericus for this purpose. The Vector Control Research Centre has been conducting in-depth studies in this area and has isolated, developed and tested several strains (Balaraman et al., 1981; Hoti and Balaraman, 1984; de Barjac et al., 1985; Balaraman and Hoti, 1987; Kuppusamy et al., 1987). On the basis of the data obtained from these studies an attempt was made to analyze the costeffectiveness of using the two larvicidal bacilli for mosquito control in comparison with insecticides. The details of the analysis and the conclusions drawn are presented in this paper.

Accepted for publication: 11 November 1987.

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#### MATERIAL AND METHODS

Two strains of *B. thuringiensis* H14. VCRC B17 (Balaraman et al., 1981) and VCRC MB24 (Balaraman and Hoti. 1987) and one strain of *B. sphaericus* H5 ab. VCRC B42 (de Barjac et al., 1985) were taken up for the study. These strains were cultured in a 13 lit working capacity laboratory fermentor using jaggery (country sugar) corn-steep liquor medium. After completing fermentation the biomass of the bacilli was harvested and prepared as briquette formulation using buoyancy and binding agents (Balakrishnan et al., 1986). The cost involved in producing the formulation was worked out taking into account the prevailing cost of inputs as detailed below:

The components of the jaggery-corn-steep liquor medium are. Jaggery: 1.5%; Corn-steep liquor: 2%; Potassium dihydrogen phosphate: 0.01%; Magnesium sulphate: 0.05%; Sodium chloride: 0.30%; Ferrous sulphate: 0.01%; and water: 1000

ml. Among the major components, jaggery is manufactured from sugarcane while corn-steep liquor is a by-product of starch industry. The biomass of the bacilli was harvested from the culture broth by sedimentation with alum (potassium aluminium sulphate) at the rate of 10 gm/lit and then decanting and freeze-drying.

The freeze-dried biomass of the two bacilli was used as active ingredient of the briquette formulation. The proportion of various components used to make 100 briquettes are. Cork powder: 166 gm; Inert matter: 400 gm; Plant resin: 100 gm: and Active ingredient: 100 gm.

Equipment: The depreciation value of the laboratory fermentor and other accessories after 10 years of use was assumed to be zero. The total cost of the laboratory fermentor. US \$ 36500 was thus divided by  $10 \times 365 \times 24$  in order to arrive at the cost per hour.

Personnel: To produce a batch (120 briquettes) of either B. thuringiensis H14 or B. sphaericus formulation the labour involved is 8 man hours each of a technician drawing a salary of US \$ 3.75 and an assistant drawing a salary of US \$ 2.92.

Energy: This was calculated on the basis of electricity consumed by the fermentor and other accessories used in the down stream processing.

Building: The initial value of the building space used for the process was assumed as US \$ 16670 and its depreciation value after 10 years was assumed to be zero.

Laboratory maintenance: The charges involved in maintaining a laboratory for quality control tests, inoculum preparation, etc., were worked out at the rate of 1% of the total production cost.

Servicing and maintenance of equipment: The servicing and maintenance charges of equipments were worked out at the rate of 20% of the equipment cost.

Chemical larvicides: The cost of larval control using chemical larvicides such as fenthion, paris green and abate was calculated on the basis of recommended doses (Mulla et al., 1974) and prevailing prices.

#### RESULTS AND DISCUSSION

The details on the production cost of B. sphaericus and B. thuringiensis H14 are given in Table 1. Out of the freeze-dried biomass obtained per run (of either of the bacilli) 120 briquettes were prepared. The production cost of one such batch of briquettes from the B. thuringiensis H14 strains, VCRC B17 and VCRC MB24 was US \$ 12.10 and US \$ 11.02, respectively. In case of B. sphaericus strain the cost was US \$ 13.34. The analysis thus shows that the production cost of B. thuringiensis H14 strains are lower than the production cost of the B. sphaericus strain by US \$ 1.24-2.32. This increase is due to more time (24-28 hr.) taken by the B. sphaericus strain to grow. sporulate and synthesize desired level of the larvicidal factor than B. thuringiensis H14 strains (Balaraman and Hoti, unpublished data) which

Table 1. Production cost of one batch\* of B. thuringiensis H14 and B. sphaericus strains (in laboratory fermentor, of 13 lit. working capacity)

Inputs		ringiensis strains	B. sphaericus H5ab strain		
	VCRC B17	VCRC MB24	VCRC B42		
	US \$	US \$	US \$		
Raw materials	1.25	1.17	1.25		
Equipment	0.42	0.42	0.42		
Personnel	6.67	6 67	6.67		
Energy	3.17	2.17	4.33		
Building	0.25	0.25	0.25		
Laboratory maintenance	0.17	0.17	0.25		
Servicing and maintenance of equipment	0.17	0.17	0.17		
Total	12.10	11.02	13.34		

One batch of product is equivalent to 120 briquette formulations.

consequently push up the cost of energy, laboratory maintenance and overhead charges. Among the *B. thuringiensis* H14 strains there is a difference of US \$ 1.08 between the production cost of VCRC B17 and VCRC MB24 due to less cost involved in harvesting (no need to add alum to sediment the biomass) of the latter and also due to consumption of less energy (VCRC MB24 can be harvested four hour earlier than VCRC B17) (Balaraman and Hoti, 1987).

In a laboratory study, the briquette formulation of B. sphaericus was found to release active ingredient and kill mosquito larvae continuously for 28 days (Kuppusamy et al., 1987). Similar type of briquettes made from B. thuringiensis H14 were found to control larval populations of Culex vishnui gr. breeding in disused wells for over 4 weeks at the dosage of 660 briquettes/ha. (Balakrishnan et al., 1986). Thus, based upon the production cost of the briquettes estimated in this study, the cost of larval control for 4 weeks in an area of one hectare works out to be US \$ 61.44-74.20 (Table 2). When the cost of larval control with malaria oil or fenthion or paris green or abate is compared with that of the two bacilli the following picture emerges (Table 2).

With fenthion, the cost of larval control works out to be US \$ 67.00 which is more or less equal to the cost incurred (US \$ 61.44-74.20) if B. thuringiensis H14 or B. sphaericus is used. However, if the other insecticides are considered for larval control the cost is pushed upto US \$83.68-159.00 which is much higher than the cost involved for either fenthion or the two bacilli. This is mainly due to the higher charges involved in the application of the chemical larvicides every week whereas the briquette formulation of the bacilli are to be applied once in four weeks.

The data leads us to conclude that the briquette formulation of both *B. thuringiensis* H14 and *B. sphaericus* are as economical as using fenthion for mosquito larval control and cheaper when compared with the cost involved in using other conventional larvicides, apart from the other advantages of using them. viz.; (i) safety to nontarget organisms; (ii) lack of environmental pollution and (iii) production is not dependent upon non-renewable sources of raw materials. Also, there is further scope to reduce the production cost of the two bacilli by scaling up the production to industrial level using improved strains and cheaper raw materials and by improv-

Table 2. Comparative cost of Jarval control (in unpolluted waters) per hectare for four weeks with chemical and microbial larvicides

			Cost of	larvicide	Cost of	labour	Tatal asst
Larvicides	Unit Price US \$	Dosage/ hectare	for one application US \$	for four applica- tions US \$	for one applica- tion US \$	for four applica- tions US \$	Total cost of larval control for four weeks US \$
Chemical				-			
Malaria oil	0.25/lit	142 lit	35,50	142.00	4.25	17.00	159.00
2. Fenthion (82%)	25.00/lit	500 ml	12.50	50.00	4.25	17.00	67.00
3. Paris Green	7.08/kg	4 kg	28.32	113.28	5.42	21.67	134.95
4. Abate (50%)	41.67/lit	400 ml	16.67	66.68	4.25	17.00	83.68
Microbial							
1. B. thuringiensis H 14 (B17)	12.10/120	660 brig	66.55	•	0.83	*	67.38
2. B. thuringiensis H 14 (MB24)	11.02/120	660 brig	60:61	•	0.83	•	61.44
3. B. sphaericus (B42)	13.34/120	660 brig	73.37	•	0.83	•	74.20

<sup>\*</sup>In the case of microbial larvicides single application is active for four weeks.

ing the residual activity of the formulation. Therefore, it is worthwhile to undertake further studies on these aspects to make better use of *B. thuringiensis* H14 and *B. sphaericus* as alternatives to chemical insecticides.

#### **ACKNOWLEDGEMENTS**

This investigation received financial support from the UNDP/World bank/WHO Special Programme for Research and Training in Tropical Diseases and the authors wish to thank them for the support. The authors wish to express their sincere gratitude to Dr. P.K. Rajagopalan. Director. Vector Control Research Centre. for critically reviewing the manuscript.

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# Studies on the Development of *Plasmodium vivax* in *Anopheles subpictus*

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Both A. subpictus, which is not established as a vector of human malaria in northern India, and A. stephensi, a known malaria vector in the region were artificially fed on the blood from patients infected with Plasmodium vivax. Development of the parasite in the non-conventional vector in terms of gut and gland infection was studied. The role of A. subpictus as a possible alternate vector of P. vivax has been discussed.

# INTRODUCTION

A. subpictus is a widely distributed species in the oriental region. In India it is most abundant in peninsular and northwest zones. This species has been incriminated as a vector of malaria in mid Java (Sundararaman et al., 1957) and Indonesia (Lien et al., 1975). In India, though it has been recorded positive in nature (Russell et al., 1939; Russell and Rao. 1940), its role in malaria transmission is not extensively studied. There are a few reports available where this species has been incriminated as vector in Maldive Island (Roy et al., 1978) and in coastal villages of Pondicherry and Tamil Nadu (Panicker et al., 1981).

Experimental feeding studies done in coastal areas of India have shown infection in A. sub-

pictus when fed on human volunteers (Covell. 1931; Russell and Mohan, 1939; Roy, 1943). However, similar studies carried out simultaneously with A. subpictus and A. stephensi have shown that none of A. subpictus when fed on a series of human volunteers became infected while a considerable number of A. stephensi were found positive for gut and gland infection (Das et al., 1979). There seems to be a variation in the susceptibility of A. subpictus in different areas. Moreover, recent studies (Suguna, 1982; Reuben and Suguna, 1983) have shown two cytologically and morphologically distinct species in A. subpictus of coastal areas. Thus, the 'importance of this anopheline in malaria transmission should also be ascertained.

Though A. subpictus is found in abundance. in northern parts of India no study has been done in relation to its ability to act as intermediate host to human malaria parasites. However, there is a report from Bastar district. Madhya Pradesh, where out of 12.107 females of A. subpictus dissected. 3 females showed the presence of sporozoites in the salivary glands (Kulkarni, 1983). Present work deals with the laboratory

Accepted for publication: 23 December 1987.

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infection of A. subpictus sensu lato collected from villages in Uttar Pradesh with Plasmodium vivax and a comparison is made with that of A. stephensi, a known malaria vector in the region.

# MATERIAL AND METHODS

A. subpictus sensu lato, collected from villages in U.P. were held in laboratory cages for egg laying. Adult females of A. subpictus from F<sub>1</sub> generation and laboratory colonized A. stephensi were fed through an artificial membrane on the blood of P. vivax infected human volunteers. For this purpose water-jacketed feeding chambers with sections of stretched parafilm membrane fastened to the base were used. Blood containing mature gametocytes from the patients with P. vivax malaria was drawn by venepuncture in heparinised glass vials and immediately added to the central well of the chamber which had the outer jacket connected to a constant temperature water circulator set to maintain the blood sample at 36 to 37°C. Two to four day old A. subpictus and A. stephensi females were held in 750 ml plastic containers with nylon screen top. The membrane base of the feeding chamber was placed against the nylon screen top of the mosquito container. The feeding apparatus was shaken at intervals to avoid settling of red cells and the mosquitoes were allowed to feed for one hour.

After the feeding period ended unfed females were removed and the fed mosquitoes were transferred to separate cages and were kept in the insectary at  $28 \pm 1^{\circ}\text{C}$  and 75 to 85% RH. The

mosquitoes were provided with 1% sugar solution and were dissected 7 to 10 days after the infective blood meal for gut and salivary gland infections. Average number of oocysts per gut was calculated by taking into account ten infected guts for each species.

For temporary mounts, mid-guts and salivary glands of A. subpictus and A. stephensi were dissected out in normal saline (0.85% NaCl). Mid-guts were stained with 0.5% aqueous solution of mercurochrome. Gut and glands were then mounted in normal saline on fresh glass slides. The preparations were observed under ordinary light and Nomarski interference phase contrast microscopes.

#### RESULTS AND DISCUSSION

Results of the membrane feeding experiments on A. subpictus and A. stephensi are summarised in Table 1. It is evident from the observations that the percentage of fed A. subpictus (23%) was comparatively much less than that of A. stephensi (66.5%). It was observed during the experiments that A. subpictus were sluggish and would rest at the bottom of the container while A. stephensi were quite active and they readily took the blood meal. Only 40% of the fed A. subpictus were able to survive beyond seven days after feeding whereas 85% of A. stephensi survived. When dissected between 7 and 10 days after the infected blood meal, 31% of A. subpictus and 80% of A. stephensi, were found positive for both gut and gland infections. Figs. 1 to 16 show gut and gland

Table ). Comparative account of development of P. vivax in two anopheline species

Anopheline species	No. of adult females used	No. took blood meal	% took blood mea!	% fed mosquitoes surviving on 7th day after feeding	% fed mosquitoes positive for gut and gland	Average no. of oocysts/gut
A. subpictus	195	45	23.0 + 3.29	40.0 + 3.29	31.0 <u>+</u> 3.10	39 ± 34.21
A. stephensi	335	223	65.5 ± 8.64	84.7 ± 5.38	$80.0 \pm 5.97$	$120.5 \pm 25.2$

Cumulative data from 5 replicates.

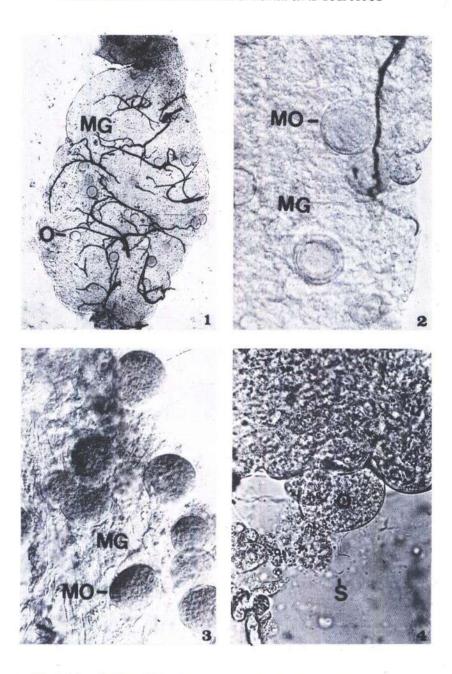
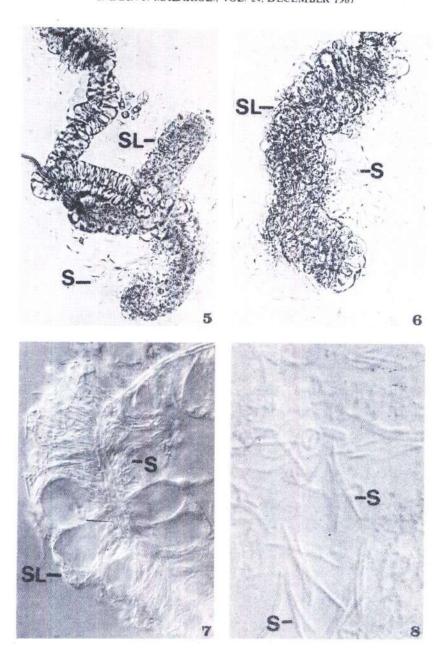


Fig. 1: Infected midgut (MG) of A. subpictus showing P. vivax oocysts (O) [×120].

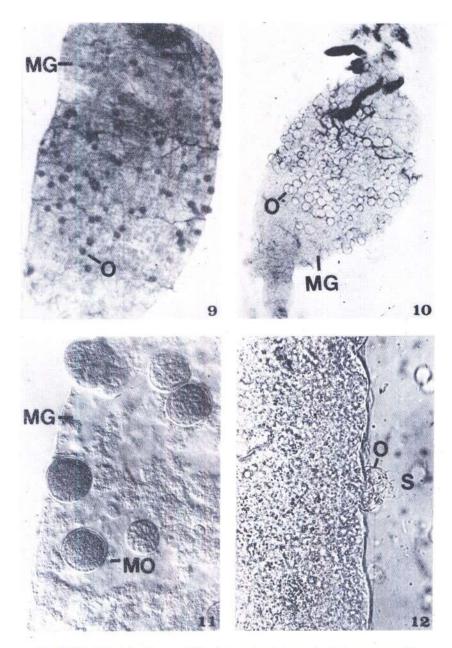
Figs. 2 & 3: Nomarski phase contrast micrographs showing mature oocysts (MO) of P. vivax on the midgut (MG) of A. subpictus [×1250].

Fig. 4: Midgut of A. subpictus showing dehisced oocyst (O) and liberated sporozoites (S) [ $\times$  450].



Figs. 5 &6: Infected salivary gland lobes (SL) of A. subpictus showing P. vivax sporozoites (S) [× 450].

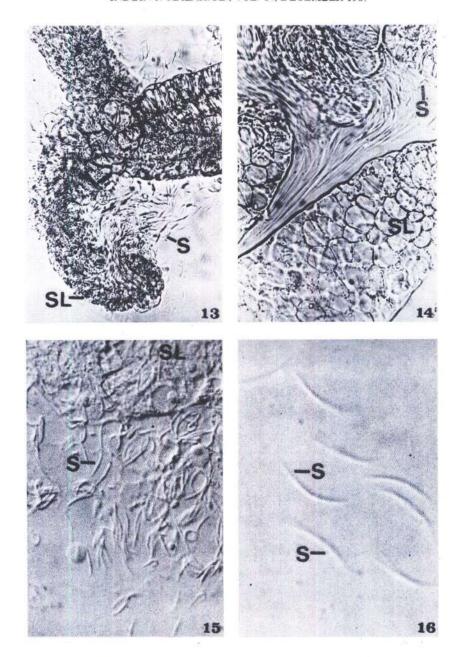
- Fig. 7: Nomarski phase contrast micrograph of infected salivary gland lobe (SL) of A. subpictus showing P. vivax sporozoites (S) [×1250].
- Fig. 8: Nomarski phase contrast micrograph of P. vivax sporozoites (S) liberated from salivary glands of A. subpictus [ × 1250 ].



Figs. 9 & 10: Infected midguts (MG) of A. stephensi showing P. vivax oocysts (O) [×120].

Fig. 11: Nomaeski phase contrast micrograph of mature oocysts (MO) of P. vivax on the midgut (MG) of A. stephensi [×1250].

Fig. 12: Photomicrograph showing dehisced oocyst (O) of P. vivax with liberated sporozoites (S) [ × 450].



Figs. 13 & 14: Infected salivary gland lobes (SL) of A. stephensi showing sporozoites (S) of P. vivax [× 450].

Fig. 15: Nomarski phase contrast micrograph showing P. vivax sporozoites (S) being released from the salivary gland lobe (SL) of A. stephensi [×1250].

Fig. 16: Nomarski phase contrast micrograph of P. vivax sporozoites (S) liberated from the salivary glands of A. stephensi [ x 1250].

infections in artificially fed A. subpictus and A. stephensi. The number of oocysts per gut was much less in A. subpictus as compared to that in A. stephensi (Table 1, Figs. 1,2 and 9) and no degenerating oocysts were observed in any of the two species.

Mahmood et al., 1984, observed higher gut infection rate in A. subpictus than that in A. culicifacies but no individual of the former species was found gland positive. In our experiments, no breakdown in the development of the parasite was observed and in all the infected A. subpictus, the parasite reached the salivary glands. The study clearly shows that although A. subpictus was less susceptible to human malaria infection as compared to A. stephensi, a certain proportion of the fed mosquitoes successfully completed the sporogony under laboratory conditions.

Cytotaxonomical studies by Suguna (1982) and Reuben and Suguna (1983) have shown two distinct siblings in A. subpictus populations of coastal areas. These are designated as species A and B. Species A which is less saline tolerant, breeds in fresh water and occurs in inland as well as in coastal localities, while the highly tolerant species B breeds in brackish water and has so far been recorded only on the coast. A wide variation in the susceptibility to malaria infection was observed during feeding experiments carried out on A. subpictus by several workers (Russell and Mohan, 1939; Roy, 1943; Das et al., 1979). This variation may be attributed to the probable differences in the susceptibility of these sibling species to malaria infection (Reuben and Suguna, 1983). Therefore, further studies are required to map the distribution of these sibling species and to evaluate their potential as a vector.

Longevity comparable to that of A. culicifacies and A. stephensi has been observed for A. subpictus (Reisen et al., 1982; Kulkarni, 1983) and there are reports indicating high anthro-

pophilic index for this species (Roy, 1943; Bruce-Chwatt et al., 1966; Kulkarni, 1983). Laboratory feeding experiments have also revealed that A. subpictus can be a potential vector but the epidemiological evidences do not confirm the role of A. subpictus as a vector of malaría in India. The population of A. subpictus is extremely high in most parts of the country but the incidence of malaria does not seem to have any correlation with the densities observed. It might be a marginal or accidental vector under certain situations. In order to delimit the role of A. subpictus in malaria transmission screening of A. subpictus populations for sporozoites using IRMA or ELISA technique and simultaneous identification for sibling species should be undertaken in different parts of the country particularly in those areas where the prevalence of established vector species is low. It may then be possible to define the role of A. subpictus, if any, in malaria transmission.

### **ACKNOWLEDGEMENTS**

We gratefully acknowledge the technical assistance of Mr. K.B. Masiwal, Mr. Y.P. Chawla and Mr. H.D. Joshi. Thanks are due to Mr. E.A Daniels for help in photography.

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# Survey of Mosquito Fauna of Northeastern Region of India

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### INTRODUCTION

The first anopheline survey was done by Challam (1923) from Kamrup district of Assam. Later the surveys were carried out by Christophers (1925). Strickland (1929). Ramsay (1930). Macdonald and Chowdhury (1931). Gupta and Mazumdar (1932). Rice and Savage (1932), Rice and Mohan (1936). Viswanathan et al. (1941). Sarkar et al. (1981) in Assam. In other states the surveys were carried out by Mortimer (1946) in Manipur; Shortt (1924) and Rajagopal (1976) in Meghalaya; Sarkar et al. (1980) in Nagaland; Misra and Dhar (1955) in Tripura; Misra (1956) and Sen et al. (1973) in Arunachal Pradesh. In recent years surveys have been carried out by Malhotra et al. in Nagaland (1982), Manipur (1983) and Mizoram (1982; 1984). The number of anopheline species collected by these authors ranged from 6 to 19 in Assam; 7 to 13 in Manipur; 8 to 13 in Meghalaya; 8 to 10 in Nagaland; 11 in Tripura state; 6 to 13 in Arunachal Pradesh and 14 in Mizoram. The well-known vector species which were collected from this region were A. minimus, A. balabacensis, A. philippinensis, A. culicifacies, A. varuna, A. annularis and A. fluviatilis.

Accepted for publication: 4 January 1988.

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None of the above authors reported tree hole breeding of anophelines from this region except Barraud in his monograph "The fauna of British India" (1934) who reported tree hole breeding of culicine mosquitoes.

Since the last surveys were done, major ecological changes have occurred in this region particularly deforestation, industrialization, agriculture, urban development, migration of populations, new settlements etc. These changes may have brought about ecological succession of biological species which may have direct bearing on disease transmission. In view of this, mosquito fauna surveys were undertaken in Assam, Meghalaya, Arunachal Pradesh and Mizoram. Results of this study are reported in this paper.

# MATERIAL AND METHODS

Mosquito collections were made from 22 localities of Assam, Meghalaya, Arunachal Pradesh and Mizoram during Sept. 1986 (Fig. 1). Adult mosquitoes were collected with the help of suction tube from different resting places viz., cattlesheds, human dwellings, mixed dwellings, forests, tree holes and other man-made structures. Immatures were collected from intradomestic containers, discarded tyres, coconut shells and other breeding habitats. Mosquito larvae were also collected from 329 tree holes of Bamboo.

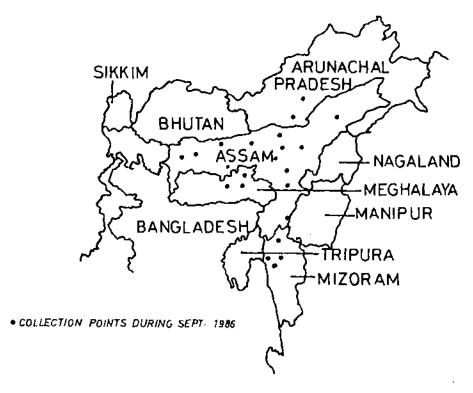


Fig. 1: Map showing northeastern region of India.

Neem. Pipal. Kathal, Seasum, Mango, Chikoo, Litchi and Gulmohar with the help of dipper or pasteur pipette. The immatures collected from the field were held in a cage in the field laboratory until adult emergence. All field collected emerged mosquitoes were killed with ether, packed in cellophane paper and transported to MRC for further studies. Mosquitoes were identified using the key of Christophers (1933). Barraud (1934) and catalog of Knight and Stone (1977).

# RESULTS AND DISCUSSION

A total of 10,224 mosquitoes collected from 22 villages of northeastern region were identified into 61 species of 8 genera viz., Anopheles, Aedes, Armigeres, Coquillettidia, Culex, Malaya, Mansonia and Toxorhynchites. The number of specimens and species collected in each genus are

given in Table 1. The most dominant genus was Anopheles followed by Culex, Aedes and Mansonia. The species collected and their numbers are given in Table 2. The most prevalent species in the genus Anopheles was A. vagus (3152 specimens), followed by A. nigerrimus (1914 specimens) and A. nivipes (1574 specimens). In genus Culex the most dominant species was Cx. quinquefasciatus (1110 specimens) followed by Cx. tritaeniorhynchus (743 specimens) and Cx. vishnui (235 specimens). Aedes albopictus (253 specimens) was the most prevalent species in the genus Aedes and it was followed by Ae. chrysolineatus (95 specimens) and Ae. aegypti (65 specimens). In genus Mansonia the most common species was Ma. annulifera (210 specimens) followed by Ma. uniformis (127 specimens) and Ma. indiana (14 specimens). The other 4 genera viz. Armigeres (202 specimens of 3 species).

Table 1. Occurrence of mosquito genera and their species in northeastern regions

Gener	a	Species found	Total specimens
Аѕѕал	n		
i.	Aedes	9	343
2.	Anopheles	27	4832
3.	Armigeres	3	175
4.	Coquillettidia	1	1
5.	Culex	13	1742
6	Malaya	1	1
7.	Mansonia	3	274
8.	Toxorhynchites	1	39
Arun	achal Pradesh		
1.	Acdes	6	53
2.	Anopheles	19	482
3.	Armigeres	1	2
4.	Culex	10	216
5.	Mansonia	3	34
6.	Toxorhynchites	1	11
Megi	halaya		
1.	Aedes	5	90
2.	Anopheles	20	973
3.	Armigeres	2	6
4.	Culex	11	255
5.	Mansonia	2	29
6.	Toxorhynchites	)	15
Mizo	ram		
1	Aedes	5	26
2.	Anopheles	14	374
3.	Armigeres	3	19
4.	Culex	10	213
5.	Malaya	1	5
6	Munsonia	2	14
7.	Toxorhynchites	]	4
	Total	60	10224

Coquillettidia (1 specimen of a species), Malaya (2 specimens of 1 species) and Toxorhynchites (69 specimens of 1 species) were also encountered.

Out of 329 tree holes of bamboo (Bambusa sp.), Neem (Azadirachia indica), Pipal (Ficus religiosa), Seasum (Dalbergia sisso), Mango (Mangifera indica), Gulmohar (Delonix regia). Chikoo (Sapota spp) and Litchi (Litchi chinensis) 237 were found positive for mosquito breeding. Out of 102 negative tree holes 94 were found dry. Maximum breeding was observed in bamboo holes. The details of species emerged from the immatures collected from different tree holes are given in Table 3. Although culicine breeding in tree holes was reported from the northeastern region by Barraud in 1934, this is the first report of the tree hole breeding of A. philippinensis, A. nivipes and A. splendidus. Recently the breeding and resting of A. culicifacies in tree holes was reported from two hill districts viz., Koraput and Phulbani of Orissa (Nagpal and Sharma, 1985).

A total of 1655 specimens of A. pallidus, A. philippinensis and A. nivipes were collected and identified. Of these 19 were A. pallidus, distinguished very easily by the lack of any apical pale band on the first hind tarsal segment and the presence of scattered pale scales over most of the venter. In 1574 specimens, the presector dark mark of vein I extending basally beyond the distal end of the humeral dark mark on the costa suggested the specimen to be A. nivipes. In 62 specimens the

Table 2. List of mosquitoes collected from northeastern region

Species	Assam	Arunachal Pradesh	Meghalaya	Mizoram	Total
Aedes (Aedimorphus) vexans (Meigen). 1830	 []	1		3	15
Aedes (Finlaya) alholateralis (Theobald), 1908	7	2	3	2	14
Aedes (Finlaya) chrysolineatus (Theobald), 1907	62	15	14	4	95
Aedes (Finlaya) dissimilis (Leicester), 1908	9	4	2	-	15
Aedes (Finlaya) novoníveus Barraud, 1934	13	_		_	13
Aedes (Finlaya) pseudotaeniatus Giles, 1901	23	1	1	_	25
Aedes (Neomelaniconion) lineatopennis, (Ludlow), 1905	-		-	2	2
Aedes (Stegomyia) aegypti (Linnaeus), 1762	65	_		_	65
Aedes (Stegomyia) albopictus (Skuse), 1894	138	30	70	15	253

contd...

Table 2. (contd.)

Table 2. (	contd.)				
Species	Assam	Arunachal Pradesh	Meghalaya	Mizoram	Total
Aedes (Stegomyia) annandalei (Theobald), 1910	15				15
Anopheles (Anopheles) ahomi Chowdhury, 1929	7	2	-		9
Anopheles (Anopheles) barbirostris Van der Wulp, 1884	76	7	3	6	92
Anopheles (Anopheles) gigas Giles, 1901	1			_	1
Anopheles (Anopheles) nigerrimus Giles, 1900	8.33	24	4.3	14	914
Anopheles (Anopheles) sinensis Wiedemann, 1828	14				14
Anopheles (Cellia) aconitus Doenitz, 1902	14		1	I	16
Anopheles (Cellia) annularis Van der Wulp, 1884	29	2	12	l l	44
Anopheles (Cellia) balabacensis Baisas, 1936	2	_			2
Anopheles (Celha) culicifacies Giles, 1901	3	_	14	_	17
Anopheles (Cellia) fluviatilis James, 1902	37	16	11		64
Anopheles (Cellia) jamesii Theobald 1901	21	•••	2		23
Anopheles (Cellia) jeyporiensis James, 1902	7	J			8
Anopheles (Cellia) kurwari James, 1902	4			1	5
Anopheles (Cellia) kochi Doenitz. 1901	221	2	26	12	261
Anopheles (Cellia) maculatus Theobald, 1901	98	41	2	14	155
Anopheles (Cellia) majidi Yong and Majidi, 1928	10	8	3	2	23
Anopheles (Cellia) minimus Theobald, 1901	24	3	4	_	31
Anopheles (Cellio) nivipes Theobald, 1903	1041	171	227	135	1574
Anopheles (Cellia) pallidus Theobald, 1901	17	2		_	19
Anopheles (Cellia) philippinensis Lucilow, 1902	40	4	14	4	62
Anopheles (Cellia) samsayi Covell, 1927	1.5	3	8	1	27
Anopheles (Cellia) splendidus Koidzumi. 1920	12		ž		14
Anopheles (Cellia) subpictus Grassi, 1899	8	_	4		12
Anopheles (Cellia) tessellatus Theobald, 1901	4	***			4
Anopheles (Cellia) theobaldi Giles, 1901	24	13		2	39
Anopheles (Celha) vagus Doenitz, 1902	2221	158	595	178	3152
Anopheles (Cellia) maculatus var. willmorei (James), 1903	35	23	1	3	62
Anopheles (Cellia) varuna lyenger. 1924	16	2	i		19
Armigeres (Armigeres) kuchingensis Edwards, 1915	60		3	2	65
Armigeres (Armigeres) subalabatus (Coquillett), 1898	110	2	3	15	130
Armigeres (Leicesteria) magnus (Theobald), 1908	5	4	3	2	7
Coquillettidia (Coquillettidia) crassipes(Van der Wulp), 1881	ĭ	_		4	í
Culex (Culex) bitaeniorhynchus Giles, 1901	15	3	2	7	27
Culex (Culex) cornutus Edwards, 1922	13	. <u> </u>	2	2	
Culex (Culex) gelidus Theobald. 1901	75	15	7	8	17
Culex (Culex) mimulus Edwards, 1915	13	13		0	105
Culex (Culex) quinquefasciatus Say, 1823	877	115	6	73	6
Culex ( Culex) yinnyuejustianis 3ay, 1823 Culex ( Culex) sinensis Theobald, 1903	1		45		1110
			2	16	19
Culex (Culex) tritaeniorhynchus Giles, 1901	504	37	131	71	743
Culex (Culex) vishmui Theobald, 1901	142	31	45	17	235
Culex (Culex) whitmorej Giles, 1904	70	4	11	15	100
Culex (Culiciomyia) pallidothorax Theobald, 1905	7	2	2	_	11
Culex (Eumelanomyia) brevipalpis (Giles), 1902	81	2	_	2	22
Culex (Eumelanomyia) malayi (Leicester) 1908	3	2		2	7
Culex (Lophoceraomyia) minor (Leicester), 1908	5		2	_	7
Culex (Lutzia) fuscanus Wiedemann, 1820	12	5	_	<del></del>	17
Malaya (Maorigoeldia) genurostris Leicester, 1908	. 1	_		1	2
Mansonia (Mansonioides) annulifera (Theobald). 1901	168	15	25	2	510
Mansonia (Mansonioides) indiana Edwards, 1930	12	2	<del>-</del> .		14
Mansonia (Mansoniaides) uniformis (Theobald), 1901	94	17	4	12	127
Toxorhynchites (Toxorhynchites) splendens (Wiedemann), 1819	39	11	i5	4	69
Total	7407	798	1368	651	10224
· <del></del>					

Table 3. Details of tree hole breeding in northeastern region

Species	Ξ	(3)	$\widehat{\mathfrak{S}}$	€	(5)	9	9	(8)	6)	(10)
Aedes (Stegomyia) albopictus (Skuse), 1894	991	2	m	-	2	   ~	5	4	   ~	77
Aedes (Finlaya) albolateralis (Theobald), 1908	==	C)	I	-		- 1	ļ	!	. 1	<u> </u>
Aedes (Finlaya) chrysolineatus (Theobald), 1907	**	i	ì	7	C.I	rr,		C	!	95
Aedes (Finlaya) dissimilis (Leicester), 1908		ļ	;	7	_	ļ	}	' I	!	: :2
Aedes (Finlaya) novoniveus Barraud. 1934	<u>;</u>	ŗ	!	!			I	I	ı	-
Aedes (Finlaya) pseudotacniatus Giles, 1901	17	1	I	L)	r4	I	1		64	5.
sedes (Sregomyia) aegypti (Linnacus), 1762	1~	I	7	4	}	I	4.	٠,	۱ ا	4
stedes (Stegamyia) annandalei (Theobald), 1910	15	!	!	ļ	1	i		!	I	~
Inopheles (Cellia) nivipes (Theobald), 1903	δ.	į	1	ł	١	ļ	I	I	1	٠,٠
Anopheles (Cellia) philippinensis Ludlow, 1902	E4	!	ļ	I	I	1	!	ı	İ	. •
Inopheles (Cellia) splendidus Koidzumi, 1920	۲.	1	;	!	1	1	ı	i	ı	' -
Culex (Eumelanomyla: brevipalpis (Giles), 1902	7.	i		;	er.	!	1		i	۶
Culex (Lophoceraomyia) minor (Leicester), 1908	7	1	;	;	١	I	!			,
Toxorhynchires (Toxorhynchites) splendens (Wicdemann) 1819	7	C	Ş	\$	v	7	СI	Ci	cı	69
Total	308	192	12	)¥	   =	=	; ; æ; 	- =	2	565

(1) Bamboo; (2) Neem; (3) Pipal; (4) Kathal; (5) Gulmohar; (6) Scasum; (7) Mango; (8) Litchi: (9) Chikoo; (10) Total.

presector dark mark of vein one extended basally beyond the end of the corresponding mark on the costa, but did not reach up to the distal end of the humeral dark mark. In these 62 specimens, pale scales were also present on the last few apical segments of the venter. This identification confirms the specimens to be A. philippinensis as based on the observations of Reid. 1967. The study of mosquito fauna of northeastern India showed that the prevalent species understood to be resembling the identification of A. philippinesis was in fact A. nivipes.

No specimens of previously recorded A. aitkenii, A. bengalensis, A. insulaeflorum, A. lindesayi and A. umbrosus were found during this survey. It is likely that more extensive surveys or searches during other months may reveal the presence of these anophelines in certain habitats. Some species found during this survey viz., A. majidi, A. theobaldi, A. nivipes and A. maculatus variety willmorei were not reported from this region in earlier surveys.

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# Mosquito Survey in Tirap and Subansiri districts of Arunachal Pradesh

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A total of 48 species of mosquitoes belonging to nine genera i.e., Anopheles, Aedes, Armigeres, Culex, Coquillettidia, Malaya, Mansonia, Toxorhynchites and Tripteroides were collected during a survey carried out in Tirap and Subansiri districts of Arunachal Pradesh in 1979 and 1982 respectively. Culex gelidus and Culex tritaeniorhynchus were found in large numbers in Tirap. Culex quinquefasciatus was prevalent in Subansiri forming 65.47 per cent of the total larval collection while Anopheles maculatus was the next dominant species (40.75 per cent of the total adults collected). Six new species i.e., A. culicifacies, A. gigas, A. jamesii, A. karwari, A. maculatus var. willmorei and A. tessellatus are reported for the first time from Arunachal Pradesh. A. minimus, earlier reported malaria vector, was altogether absent.

# INTRODUCTION

Defence Research Laboratory. Tezpur has taken up mosquito survey in various States/Union Territories of northeastern region to study the occurrence, distribution and species composition of various mosquitoes with a view to identify vectors. The survey programme comprises of districtwise studies divided into different phases and carried out through various seasons of the year in order to present a representative cross-section of information.

Mishra (1956) studied malaria transmission in North East Frontier Agency (now Arunachal Pradesh) and reported the occurrence of six Anopheles species. Subsequently, Sen et al. (1973) recorded 14 Anopheles species from Tirap district with preponderance of Anopheles balabacensis balahacensis which formed nearly 60 per cent of the total catch. However, there is no published record dealing with mosquito fauna of Arunachal Pradesh.

The present paper relates to the results of the mosquito survey carried out at Miao. Deomali and Jairampur of Tirap district; Kimin. Raga. Hapoli and Ziro of Lower Subansiri district and Sippi. Daporijo and Taliha of Upper Subansiri district of Arunachal Pradesh.

# MATERIAL AND METHODS

# Topography and climate

Tirap district covers an area of 7.024 sq km between 26°39′ and 27°44′N latitude and 95°12′ and 97°12′E longitude, with a population of 1.28.050 (as per 1981 census). Population density per sq. km. is highest in Tirap district which forms the southeastern part of Arunachal Pra-

Accepted for publication: 22 February 1988.

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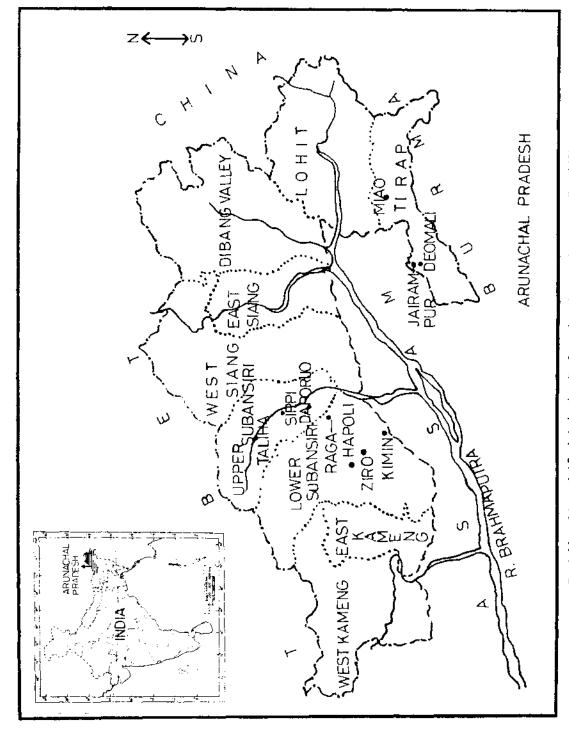


Fig. 1: Map of Arunachal Pradesh showing sites from where the mosquitoes were collected ( )

desh, bordering Dibrugarh in the west. Burma on its east and southeast, Nagaland in the south and Lohit district in the north (Fig. 1). The main river flowing through the district is the Tirap. The altitude of the hills ranges between 125 and 3760 mtrs. Climate is hot and humid. Temperature varies from 11 to 30.5°C and annual rainfall ranges between 300-350 cms.

Subansiri covers an area of 16,939 sq. km. between 26°55' and 28°24'N latitude and 92°41 and 94°37'E longitude. Lower Subansiri has a population of 1,12,650 and Upper Subansiri only 39,410, as per 1981 census. The density of population per sq. km. is about 7. The population is entirely rural. The district is bounded on the north by Tibet (China) and partly by Siang district of Arunachal Pradesh, on the east by Siang, on the south by Lakhimpur and Darrang districts of Assam and on the west by Kameng district of Arunachal Pradesh (Fig. 1). The entire Subansiri district is mountainous forming part of the Eastern Himalayas. The climate of the district is largely influenced by nature of its terrain.

Rainfall in the foothill region is extremely heavy and the humidity is high throughout the year. Temperature varies a lot, January and February being the coldest months.

# Methodology

Mosquito larvae and pupae were collected from cemented tanks, ponds, pools, lakes, ditches, riverbeds, roadside drains, tree holes and crevices, plant axils, cut bamboos, tyre dumps and earthen pots etc., with the help of dippers (14 cm. dia, and 375 ml. capacity), glass droppers and pipettes. The collected larvae and pupae were reared in the field laboratories until adults emerged.

Adultmosquitoes were collected from cattlesheds and human d gellings with battery operated CDC Miniature and Monk Wood light traps from 1800 to 0600 hours. Besides trapping, adult mosquitoes were aspirated with suction tubes from human dwellings (indoor resting) in the morning hours and from cattle bait during evening hours. Adult mosquitoes were collected from breeding and outdoor resting places during the daytime.

All the adult mosquitoes including those emerged from larval and pupal collections, were killed with ether. Mosquitoes were identified with the help of standard keys of Christophers (1933). Barraud (1934). Puri(1955). Wattal and Kalra (1961) and catalog of Knight and Stone (1977). The identified specimens are preserved in the departmental collection.

# Period of study

The present study was undertaken in July-August 1979 in Tirap district and March-April 1982 in Upper and Lower Subansiri districts.

# RESULTS AND DISCUSSION

A total of 927 larvae and 6474 adult mosquitoes were collected from Tirap district and identified into 40 species of 9 genera (Table 1). The genus Anopheles comprised of 13 species (268 larvae

Table 1. Occurrence of mosquito genera and their species

S.	Managha	No. of encou	T.,	
No.	Mosquito genera	Tirap	Suban- siri	Total
1.	Anopheles	13	17	18
2.	Acdes	5	4	7
3.	Armigeres	2	2	3
4.	Culex	12	9	12
5.	Coquillettidia	1	-	3
6.	Malaya	£	_	j
7.	Mansonia	4	2	4
8.	Toxorhynchites	1		1
9.	Tripteroides	1	ī	1
	Total	40	35	48

Table 2. Species distribution in Arunachal Pradesh

	Ţ	irap	Sul	oansiri	
Mosquito species	Adult	Larvae	Adult	Larvae	Tota
Aedes (Finlaya) albolateralis (Theobald), 1908	7			178	185
Ae. (Fin.) chrysolineatus (Theobald). 1907				52	52
Ae. (Fin.) shortii (Barraud). 1923		5			5
Ae. (Neomelaniconion) lineatopennis, (Ludlow). 1905	4				4
Ae. (Stegomyia) albopictus (Skuse). 1894	7	172	4	1044	1227
Ae. (Steg.) annandalei (Theobald), 1910				58	58
Ae. (Steg.) vittatus (Bigot). 1861		30			30
Anopheles (Anopheles) barbirostris Van der Wulp. 1884	62		110		172
An. (Ano.) gigas Giles. 1901	52			25	77
An. (Ano.) nigerrimus Giles. 1900	41		10	12	63
An. (Cellia) aconitus Doenitz, 1902	2		8	_	10
An. (Cel.) annularis Van der Wulp. 1884	17		11		28
An. (Cel.) balabacensis Baisas, 1936	18				18
An. (Cel.) culicifacies Giles, 1901	90		12		102
An. (Cel.) jamesji Theobald. 1901	<b>3</b>		5		5
An. (Cel.) karwari James, 1902	14		103		117
An. (Cel.) kochi Doenitz. 1901	94		37		131
An. (Cel.) maculatus Theobald. 1901	613	80	1009	146	1848
An. (Cel.) maculatus var. Willmorei (James), 1903	*****		39	12	51
An. (Cel.) pullidus Theobald, 1901			.,,	1.2	5
An. (Cel.) philippinensis Ludlow. 1902	62	85	30	5	182
An. (Cel.) splendidus Koidzumi. 1920	·-	(7)	10		10
An. (Cel.) subpictus Grassi, 1899		35	90		125
An. (Cel.) tessellatus Theobald, 1901		2.2	, · · ·	28	28
An. (Cel.) vagus Docnitz, 1902	38	68	19	20	) 25
Armigeres (Armigeres) kuchingensis Edwards, 1915	J.11	On-	17	3	3
Ar. (Ar.) subalbatus (Coquillett). 1898	257		60	. 25	342
Ar. (Leicesteria) magnus (Theobald), 1908	12		00	. 23	12
Coquillettidia (Coqui.) crassipes (Van der Wulp), 1881	7				7
Culex (Culex) bitaeniorhynchus Giles, 1901	66	10	1	81	95
	39	10	•	10	39
Cx. (Cx.) cornutus Edwards. 1922	2033	0	10		
Cx. (Cx.) gelidus Theobald. 1901	4	9 20	10	190	2052 215
Cx. (Cx.) mimeticus Noc. 1899 Cx. (Cx.) quinquefasciatus Say. 1823	223	103	178	3690	4194
	34	105	170	3090	35
Cx. (Cx.) sinensis Theobald. 1903	1934	50	663	9.3	
Cx. (Cx.) tritaeniorhynchus Giles. 1901		50		83	2730
Cx. (Cx.) vishnui Theobald. 1901	165	216	22		403 30
Cx. (Eumelanomyia) brevipalpis (Giles). 1902	106	30 2	31		
Cx. (Eu.) malayi (Leicester). 1908 Cu. (Lygie) (isoonya Windomann. 1820	485		31	o	518 40
Cx. (Lutzia) fuscanus Wiedemann, 1820	30	2		8	40
Cx. (Lut.) halifaxii Theobald, 1903	5	7			5
Malaya (Maorigoeldia) genurostris Leicester, 1908	6	1			13
Mansonia (mansonioides) annulifera (Theobald), 1901	1		,		1
Ma. (Ma.) indiana Edwards. 1930	25		]		26
Ma. (Ma.) longipalpis Van der Wulp, 1881	12		,		12
Ma. (Ma.) uniformis (Theobald). 1901	13	,	5		18
Toxorhynchites (Tox.) splendens (Wiedernann), 1819	•	3	,		3
Tripteroides (Rachionotomyia) aranoides (Theobald). 1901	2		I	59	62

and 1103 adult specimens), genus Aedes 5 species (207 larvae and 18 adults), genus Armigeres 2 species (269 adults), genus Culex 12 species (442 larvae and 5018 adults), genus Coquillettidia 1 species (7 adults), genus Malaya 1 species (7 larvae and 6 adults), genus Mansonia 4 species (51 adults), genus Toxorhynchites 1 species (3 larvae) and genus Tripteroides 1 species (2 adults).

In all 5636 larvae and 2476 adult mosquitoes, collected from Subansiri district, were identified into 35 species of 6 genera (Table 1). The genus Anopheles comprised of 17 species (228 larvae and 1498 adult specimens), genus Aedes 4 species (1332 larvae and 4 adults), genus Armigeres 2 species (28 larvae and 60 adults), genus Culex 9 species (3989 larvae and 907 adults), genus Mansonia 2 species (6 adults) and genus Tripteroides 1 species (59 larvae and 1 adult).

# Larval distribution

In all, 927 larvae belonging to 5 genera and 18 species were collected from Tirap district during the present survey (Table 2). These comprised of 9 species of Culex, 4 of Anopheles, 3 of Aedes and 1 each of Malaya and Toxorhynchites. The maximum number of species (11) were recorded from Jairampur while Miao and Deomali yielded 9 species each. Among anophelines. A. vagus larvae were found in all the places surveyed. while A. maculatus, A. philippinensis and A. subpictus only from Jairampur. Among Culex larvae (442). C. vishnui was found in large numbers (216) followed by C. quinquefasciatus (103). Larvae of Ae. alhopictus were collected in good number and recorded from all the three places of survey.

A total of 5636 larvae belonging to 5 genera and 18 species were collected from Subansiri district (Table 2). Species composition comprised of 6 species of *Anopheles*, 4 of *Aedes*, 5 of *Culex*, 2 of *Armigeres* and only one species of *Tripteroides*. Maximum number (2138) were collected at Ki-

min. followed by Daporijo (1702) and Raga (1093). Culex quinquefasciatus was found in very large numbers. It formed 65.47 per cent of the total larval collection. These larvae were collected from all the places of survey and from different breeding habitats e.g., G.1, drums, cut bamboos, cemented tanks, water pits, ditches, ponds, drains, tyres, etc. Their predominance may be due to wide range of larval adaptability to physico-chemical characteristics of the water sources (Sarkar et al., 1978). Larvae of Aedes albopictus occupied the second position (1044 out of 5636 larvae). These larvae were found in tyres and cut bamboos. At Kimin, 290 tyres were checked, out of which 78 (26.89% had water in them and 24 were positive (30.76%) for the presence of larvae and pupae of Ae. albopictus. Among anophelines, larvae of A. maculatus were encountered in good number from the riverbed.

The incidence of associated breeding of immature stages of different mosquito species are presented in Tables 3 and 4. It was observed that A. maculatus, A. philippinensis and A. subpictus shared breeding habitats with C. vishnui while A. vagus was found breeding with C. tritaeniorhynchus, C. vishnui, C. gelidus, C. quinquefasciatus and A. subpictus in Tirap district. A. philippinensis shared its breeding habitats with C. tritaeniorhynchus in Subansiri district. Associated breeding of A. maculatus with C. mimeticus and C. mimulus have earlier been reported (Bhat. 1975; Sarkar et al.. 1980).

During the present survey, it was seen that Ae. albapictus, C. tritaeniorhynchus and C. vishnui were found in association with a number of species in the same breeding habitats. Similarly, A. gigas and Ar. kuchingensis shared their breeding with C. quinquefasciatus individually. Other mosquito species were found in association with many species on a number of occasions. Mosquito species which can share with others, are always found in plenty. In hilly terrain, there is always a great scarcity of breeding habitats. Therefore, associated breeding is very common.

Table 3. Incidence of associated breeding of different species of mosquitoes in Tirap district (Arunachal Pradesh)

Mosquito species	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
C. quinquefasciatus								_	_	_a	1	
Ae. albopictus	-		_		_			1	1	1	2	
C. tritaeniorhynchus	_	1		1	1	_			_	_	1	
C. gelidus	£				J	1	ſ					
A. vagus	1	1	1	1	_	_	ļ					
Tx. splendens			_	]	_	_	t					
C. malayi			_	_	- **	_	1					
A. maculatus	1											
A. philippinensis	1											
A. subpictus	. ]											

<sup>(1)</sup> C. vishnui; (2) C. gelidus; (3) A. subpictus; (4) C. quinquefasciatus; (5) A. vagus; (6) C. brevipalpis; (7) C. tritaeniorhynchus; (8) Ac. shortii; (9) Ae. vittatus; (10) C. mimeticus; (11) Tx. splendens; (12) C. (L) fuscanus

Table 4. Incidence of associated breeding of different species of mosquitoes in upper and lower Subausiri districts (Arunachul Pradesh)

Mosquito species	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
Ae. albopictus			_		_		1	3	2	6	1
Ac. albolateralis		_	_	_	_		1	1	1		
Ar. kuchingensis					_		ı				
A. gigas	_	_			_		ı				
A. tessellatus	1	2	~-	2	1	1					
A. maculatus	3	3	2	_	ı	1					
C. mimeticus	1		1	1		1					
A. barbirostris	1	1	;	1	J						
A. nigerrimus	1	1	1	1							
A. philippinensis	į										

<sup>(1)</sup> C. tritaeniorhynchus; (2) C. mimeticus; (3) A. tessellatus; (4) A. maculatus; (5) A. nigerrimus; (6) C. (L) fuscanus; (7) C. quinquefasciatus; (8) Tr. aranoides; (9) Ae. annandalei; (10) Ae. albolateralis; (11) Ar. kuchingensis

### Adult distribution

A total of 6474 mosquitoes were collected on 20 trap nights from human habitations and cattlesheds in Tirap district giving an average of 323.7 mosquitoes/trap night (Table 2). Mosquito collection was much more in cattlesheds (426.7 per trap night) as compared to human habitation (83.3). The catch comprised of 35 species belonging to 8 genera. Specimens of all the species were trapped in cattlesheds, while human habitations yielded only 24 species. Even the highly anthropophilic species, viz.. A.b. balabacensis and C. quinquefasciatus—were trapped in cattlesheds collections. Of the places surveyed, Miao showed

the highest density (394.33 mosquitoes/trap night), followed by catches at Deomali (318.22) and Jairampur (248). The maximum species (29) were encountered at Deomali. Next in order was Jairampur (28) and Miao (22). The largest collection of a single species in the total adult catch was C. gelidus (31.40%) followed by C. tritaeniorhynchus (29.87%). Among the areas surveyed. Deomali had the highest density of C. tritaeniorhynchus (1333) while C. gelidus was the most common at Miao (and Jairampur. Among anophelines, A. maculatus (55.57%) was predominant and recorded from all the places of survey. Its collection was highest at Miao.

A total of 2476 adult mosquitoes were collected during the survey of Subansiri district (Table 2). The catch comprised of 28 mosquito species belonging to 6 genera i.e.. Anopheles (1498 specimens of 15 species). Culex (907 specimens of 8 species). Aedes (4 specimens of 1 species). Armigeres (60 specimens of 1 species). Mansonia (6 specimens of 2 species) and Tripteroides (1 specimen of 1 species). A. maculatus was found in large numbers. It formed 40.75 per cent of the total catch. C. tritaeniorhynchus was the next (663 specimens). Specimens of C. quinquefasciatus, A. barbirostris and A. karwari were collected in fairly good number.

Misra (1956) had reported only 6 Anopheles species from Arunachal Pradesh. As his study had lasted for about six weeks only (November 28. 1955 to January 14. 1956) and that too in winter months, the collection of less species may be justified. Sen et al. (1973) confirmed the existence of all the species reported by Misra (loc.) cit.) except A. minimus, which was incriminated as malaria vector. The complete absence of A. minimus was attributed to large-scale use of DDT and other insecticides under NMEP as well as by agriculturists. Sen et al. (loc. cit.) made intensive entomological studies in Tirap district commencing from February 1969 to November 1969 and thus covering all the seasons. These workers reported 14 Anopheles species from Tirap,

We have found 18 anopheline species in all. Of these, 12 were in conformity with that of Sen et al. (loc. cit.). Only 6 A. jeyporiensis and 1 A. varuna were collected in 914 man nights by these workers. It indicates that the densities of these species were very low in Arunachal Pradesh. Six species i.e., A. culicifacies, A. gigas, A. jamesii, A. karwari, A. maculatus var. willmorei and A. tessellatus are reported for the first time from Arunachal Pradesh. As we used light traps also for adult mosquitoes, our collection in terms of density/species composition is much better than earlier workers.

Rao (1984) is of the view that no true A. hyrcanus exists in India. In fact, "A. hyrcanus" is a group consisting of more than ten species of which A. argyropus, A. nigerrimus, A. nitidus, A. sinensis and A. crawfordi are recorded in India. A.b. balabacensis collected in the present survey may have a few specimens of A. dirus also, based on the description of Peyton and Harrison (1979).

No A. minimus has been encountered by Sen et al. (loc. cit.) nor in the present survey. However, A. minimus has recently been incriminated as malaria vector in Nagaland and Mizoram (Bhatnagar et al., 1982; Das and Baruah, 1985). The species was reported from Assam also in recent years (Kareem et al., 1985). As the present distribution of A. minimus in northeastern region is very patchy, the presence of A. minimus in Arunachal Pradesh cannot be ruled out. More studies on its occurrence, distribution and role in malaria transmission are warranted.

Thus the mosquito fauna of Arunachal Pradesh (based on the recent findings) swells to 48 species of 9 genera, viz., Anopheles (18 species). Aedes (7 species). Armigeres (3 species). Culex (12 species). Mansonia (4 species). Malaya, Tripteroides, Coquillettidia and Toxorhynchites (1 species each).

#### **ACKNOWLEDGEMENTS**

The authors are grateful to Dr. B. Singh. Director, Defence Research Laboratory, Tezpur who took pains to go through the manuscript and extended valuable comments. We acknowledge our sincere gratitude to Dr. M.C. Joshi, Consultant (formerly Director of Agriculture, Arunachal Pradesh) for providing valuable suggestions. Thanks are also due to Shri A.K. Sen, Retd. Entomologist, Arunachal Pradesh and Dr. C. Nagaraj, Epidemiologist (Tirap) PfCP for their kind help.

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# Incrimination of Anopheles minimus Theobald as a Vector of Malaria in Arunachal Pradesh

P. DUTTA1 and B.D. BARUAH1

Anopheles minimus, A. balabacensis, A. philippinensis, A. annularis and A. culicifacies have been detected in Tirap District of Arunachal Pradesh during the course of entomological studies carried out during April and May, 1986. Sporozoites were detected in the salivary glands in one specimen of A. minimus.

#### INTRODUCTION

Anopheles minimus was considered to be the chief vector of malaria in the foothills of Himalayas extending from Uttar Pradesh to Assam. because of its predominantly domestic habits and preference for human blood (Ramsay et al., 1936; Iyengar, 1940; Viswanathan et al., 1941; Misra and Dhar, 1955; Misra, 1956). However, subsequent studies after application of DDT as residual insecticide showed the absence of this species from the Terai region of Uttar Pradesh (Chakraborty and Singh, 1957) and Burnihat areas of Meghalaya (Rajagopal, 1976). The possible role of other species e.g., A. philippinensis and A. balabacensis in transmission of malaria in Meghalaya and Arunachal Pradesh had been considered by Rajagopal (1976) and Sen et al. (1973).

A survey was undertaken in selected areas of Tirap District of Arunachal Pradesh for determining the prevalence of known vector mosquitoes and their possible role in the current transmission of malaria. The preliminary observations are presented in this paper.

# MATERIAL AND METHODS

Certain localities under Namsang Circle of Tirap District of Arunachal Pradesh were selected for the study. These are mostly foothill areas ranging from 1000-1400 ft. above sea level. The region is covered by a large forest traversed by hill streams and rivers. The people of these localities are mostly Arunachalis (Nokte tribe) with a few Nepali settlers. The area has been under DDT spray since 1960.

These places were visited twice a week during the observation period. The meteorological information recorded in the area during this period was as follows—average temperature Max. 24.18°C and Min. 21.54°C, average relative humidity 67.36% and average rainfall 4.51mm.

The mosquitoes were collected with the help of suction tubes between 1800 hrs to 2400 hrs mostly from cattlesheds adjacent to human dwellings

Accepted for publication: 6 January 1988, 'Regional Medical Research Centre N.E. Region (ICMR) Dibrugarh-786003, India.

Table 1. Suction tube night collection from cattlesheds in April and May 1986 and results of dissection

, v		Average	•	Abdominal condition	condition		Dissection.	tion.	Positive		Sporo-	Parity
		collection	UF	   <u>   </u>  - 	SG	0	Gland	B	Gland	Gat J	zoife rate (%)**.	%
	A. annularis	$0.083 \pm 0.28$ $(33.739)$	0	   ~- 	   0 	0	-    -	_	;   c 	0	0.00	1 
7	A. balabacensis	0.83 + 1.52	¢1	7	-	0	σ.	6	0	0	0.00	4
rá	A. barbirostris	0.16 + 0.38	0	2	0	0	0	0	0	0	0.00	00'0
4.	A. nigerrimus	1.83 - 1.48	7	8	0	0	0	0	œ	0	00.00	0.00
	A. kochi	0.16 + 0.57	-	0	-	0	0	0	0	0	0.00	0.00
<b>y</b> i	A. minimus	$3.66 \pm 5.56$	œ	13	23	I	39	39	-	0	1.408	56.41
r <del>.</del>	A. maculatus	38.66 ± 27.91	217	110	136	_	163	163	0	0	00:00	43,55
જાં	A. philippinensis	0.33 + 0.88	2	0	7	0	2	7	0	0	0.00	100
6	A. vagus	$3.33 \pm 5.17$ (155.25)	2	<del>-</del>	9	۲-	0	O	С	0	000	0.00

Figures in parentheses are % coefficient of variation.

\* Only fresh mosquitoes dissected.

\*\* Calculated from the nos. of dissected mosquitoes that were collected both by suction tube and light-trap.

and also by CDC light-traps from dusk to dawn i.e., from 1800 hrs. to 0500 hrs. The collected mosquitoes were transported to the laboratory and were identified. They were dissected to detect the gland and gut infection.

# RESULTS

Data of collection by suction tubes is given in Table 1 and of collection by light-traps in Table 2.

From Tables 1 and 2, it can be seen that A. maculatus is the predominant species in these areas. Among the known vector species, A. minimus, A. balabacensis, A. philippinensis and A. annularis were encountered in the study areas. Both A. minimus and A. balabacensis were collected in the later part of the night from 2000 hrs. onwards. During the morning and day only few mosquitoes could be collected.

The mosquitoes were dissected for gland or gut infection. Out of 71 A. minimus dissected, one captured from cattleshed was found positive with

sporozoites in the salivary gland, giving a sporozoite rate of 1.4%.

#### DISCUSSION

Our findings are in conformity with those of earlier investigators (Bhatnagar et al., 1982; Kareem et al., 1983; Das and Baruah, 1985). A. minimus which seemed to have disappeared from the North Eastern Region has reappeared and has re-established itself in the region as a vector of malaria. Bhatnagar et al. (1982) reincriminated it in Nagaland and Das and Baruah (1985) in Mizoram. Kareem et al. (1983) reported its prevalence in Boko area of Kamrup District, Assam.

In a study carried out about three decades ago, A. minimus was considered to be the most important malaria vector in Arunachal Pradesh (Misra, 1956). Sen et al. (1973) incriminated A. balabacensis and established its role in malaria transmission in Tirap District of Arunachal Pradesh. The present study, has re-established

Table 2. CDC Light-traps collection in April and May 1986 and results of dissection

S.	Species	Numbers collected on 12 trap nights	Collection per trap night	<b>Abd</b> o	minal o	conditi	០ឮ	Numbers dissected*	Gland posi- tive	Parity (%)
No.				UF	FF	SG	G			
1.	A. balabacensis	4	0.33 (0.46)	2	2	0	0	4	0	0.00
2.	A. barbirostris	4	0.33 (0.46)	I	3	0	0	0	0	0.00
3.	A. culicifacies	4	0.33 (0.96)	0	3	1	0	3	0	66.66
4.	A. nigerrimus	28	2.33 (6.74)	5	22	1	0	0	0	0.00
<b>5</b> .	A. maculatus	320	26.66 (77.10)	52	235	33	0	58	0	15.51
6.	A. minimus	35	2.91 (8.43)	3	22	10	0	32	0	40.62
7.	A. philippinensis	8	0.66 (1.92)	0	7	1	0	5	0	40.00
8.	A. vagus	12	1.00 (2.69)	0	2	8	2	0	0	0.00

Figures in parentheses are percentage values.

<sup>\*</sup> Only fresh mosquitoes dissected.

the role of A. minimus in the transmission of malaria in foothill areas of Tirap District.

Known vector mosquitoes like A. minimus, A. balabacensis, A. philippinensis and A. culicifacies were captured by operating CDC light-traps. It is evident that CDC light-traps can be used effectively in place of human baits to determine the relative density of malaria vectors, particularly where the baits are liable to come in contact with chloroquine resistant strains of P. falciparum (Ismail et al., 1982; Das and Baruah, 1985).

# **ACKNOWLEDGEMENTS**

Authors are thankful to Dr. D.S. Choudhury. Deputy Director, Malaria Research Centre, Delhi for his kind advice and suggestions to take up this study in this area. The help rendered by the Circle Officer of Namsang, Tirap District (Arunachal Pradesh) in carrying out the investigation is gratefully acknowledged. Thanks are due to Dr. B.N. Nagpal of Malaria Research Centre, for confirming the identification of the specimens. Technical assistance received from Mr. C.K. Sharma, Mr. B.K. Goswami, Mr. A.C. Rabha, Mr. J.N. Gogoi and Mr. D. Dutta in conducting mosquito surveys is also gratefully acknowledged.

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# Feeding Behaviour of Anopheline mosquitoes in an area endemic for Malaria in Bastar district, Madhya Pradesh

S.M. KULKARNI<sup>1</sup>

Feeding behaviour of anopheline mosquitoes was studied in an area endemic for malaria in south Bastar district. Madhya Pradesh, between February 1980 and January 1981. Anopheles subpictus was found active throughout the night with a peak just after midnight. An hyrcanus and An karwari showed bimodal activity with first peak after dusk and second after midnight. An culicifacies showed a bimodal activity on cattle but fed intermittently on man. An fluviatilis, An jeyporiensis and An maculatus were found throughout the night with higher activities till midnight and a peak around dusk. Host feeding behaviour indicated that An jeyporiensis was the most common species found on man followed by An karwari, An maculatus, An hyrcanus and An fluviatilis. An culicifacies was rarely encountered on man. On bovid bait, An hyrcanus and An subpictus were the most abundant species followed by An jeyporiensis, An karwari, An jamesii and An maculatus. Biting rates (per bait/per night) and seasonal prevalence for all the 19 species of anophelines collected are given. An culicifacies and An fluviatilis are the important vector species for malaria in the region. The biting rates of these along with other potential vector species, their seasonal prevalence and oocyst/sporozoite detection in different species during different months of the year are considered responsible for maintaining a perennial transmission of malaria in the region.

# INTRODUCTION

Bastar district of Madhya Pradesh is highly endemic for malaria. Anopheles culicifacies Giles. 1901 and Anopheles fluviatilis James, 1902 are the primary vectors of malaria in the region (Kalra, 1978; Vaid and Nagendra, 1964). Natural infection in these mosquitoes in addition to Ansubpictus has been reported earlier (Kulkarni and Wattal. 1982; Kulkarni. 1983). High Annual Parasite Indices (API) of 31.3 for 1979, 23.3 for 1980 and 25.8 for 1981 have been reported (PfCP Report of Madhya Pradesh for 1980 and Malaria

Unit, Jagdalpur). Even the total number of malaria cases increased from 35,465 in 1965 to 1,72,736 in 1981 in south Bastar, for a population of over nine lakhs. These factors are compounded by the development of insecticidal resistance in mosquito vectors. Therefore, adequate knowledge on the feeding behaviour of anopheline mosquitoes was necessary for delineating periods of active malaria transmission, in order to find a suitable control strategy. This communication presents the data on the feeding behaviour of 19 species of anopheline mosquitoes in Bastar district.

Study area: Bastar district has an area of 39,086 sq km. and a population of 15,15,956 (1971 census). It lies in the southeast of Madhy?

Accepted for publication: 2 March 1988.

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Pradesh. Climate is moderate to hot throughout the year. The area is drained by many perennial rivers and streams and receives about 150 cm rain from both southwest and northwest monsoons.

Seasons are roughly marked as monsoon (July to September), winter (October to December), spring (January to March) and summer (April to June). The vegetation is largely tropical with moist deciduous forests covering about 70 per cent of the area. The human population consists mostly of tribals. Two study areas viz., Darbha and Kurandi comprising of 4 localities were selected. These were surrounded by thick forests, hilly streams and had mosaic paddy cultivation. Other topographical and ecological features have been described earlier (Agarwal, 1968).

#### MATERIAL AND METHODS

All night (1800 hrs. to 0600 hrs.) biting collections of anopheline mosquitoes were made outdoors on human and bovid baits at four localities once a month from February 1980 to January 1981.

Human bait collection: Batches of two men each were made to sit opposite each other, one exposing his legs upto thigh region and arms upto armpits and the other with a torch light and a suction tube to catch the mosquitoes biting the man sitting opposite to him.

Bovid bait collection: A bovid animal (generally a moderate size calf) was tied to a wooden peg about 100 m. away from the human bait. A mosquito net (size  $2 \times 1.2$  m.) was hung by four equal bamboo sticks keeping the net about 30 cm. above the ground. Mosquitoes entered the net to feed on the animal bait and remained inside the net after the blood meal. The insect collector entered the net for 10 mins, after every hour to collect all the fed mosquitoes.

Each hourly collection was kept separately and was transferred either into test tubes or when the

number was large into Barraud cages. All the mosquitoes thus collected were brought to the laboratory, anaesthetised and identified upto species level with the help of keys (Christophers, 1933; Puri. 1937). They were then dissected in normal saline under binocular microscopes. Gut and salivary glands were examined for oocysts and sporozoite infection.

#### RESULTS AND DISCUSSION

A total of 3745 adult female anophelines (738 on humans and 3007 on bovid baits) comprising of 19 species were caught during the 12 month period of study. The data of the hourly collections is presented in Table 1, and that of the total collections in four different seasons of the year in Table 2. The feeding behaviour and seasonal prevalence for each species are discussed below.

Anopheles hyrcanus 'group': Highly zoophilic species found throughout the night, showing bimodal activity with first peak just after dusk and second after midnight. Per night per bait density was nearly 1 on man and 20 on cattle. Prevalence was highest during October-December but also occurred from July to March. It is reported that this species has been collected biting man as well as cattle, both inside and outside houses and it does not have any relationship to human malaria (Rao, 1984).

An. barbirostris: Mostly zoophilic and feeds during dusk hours. Per night per bait density was 0.09 on man and 2.5 on cattle. Prevalent during October to March.

An. tessellatus: Zoophilic, feeds any time of the night. Per night density was very low. Generally found during October to December.

An aconitus: Feeds on both man and animal upto midnight with a peak just after dusk. Density per night per bait was less than one. Prevalent during October to December. Husainy

Table 1. Number of female mosquitoes collected on human (H) and bovid (B) batts for the period from February, 1980 to January, 1981

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98 89	27	8	[9	39	۶.	33	22	۲.,	13	ç	တ	392	10.89
An subpictus H 5	m	۲.	I	ş	'n	er,	۲	CI	Ļì	-	۴.	349	0.45
8 19	œ	5	15	<b>æ</b>	13	115	133	88	5	7.	۲۰	527	14.64

(Contd.)

Table 1. (Contd.)

-	cı i	3	<del>-</del>	Š	9	۲-	œ	6	10	= ;	15	13	2	15	16
An. vagus	Ħ	_	r-I	_		ı	i		1	:				4	0.0
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An annularis	Ξ	-	S	ĊI	-		۴.	-			!	ļ	!	15	0.17
	B	91	S	Ξ	7		-1	C)	!	-	I	-	-	67	186
An kurwari	I	2	6.	Z	7	=	16	→	6	•	۲.	9	7	971	1.46
	<b>6</b> 0	61	7	32	77	17	70	27	40	9	Ξ	1	o	272	7.55
.4n. moghulensis	Ξ	ì	ı		I	I	ł	ļ	Į	ŀ	I	I	1	ļ	ì
1	20	i	•	I	ļ	I			1	1	1	1		•	0.25
An pallidus	Ξ	~	7	~	I	۲.	1	1	1	7	1	Ì	١	=	0.13
•	æ	2	7	13	56	77	35	<u>\$</u>	4	5	1	-	-	185	5.14
An philippinensis	Ξ	ſ	!	l	I	-	ı	1	~	ļ	1	1	1	7	0.02
:	æ	I	7	-	9		-	1	-	į	l	ı	ı	=	0.25
An jamesii	I	8	m	7	I	7	-	í	-	ł	1	-	I	32	0.37
1	æ	55	48	27	43	22	••	9	9	1	-	4	1	230	6.38
An maculatus	=	9:	6	15	~	7	7	S	<b>~</b> 1	7	7	4		83	1.01
	В	21	53	21	61	∞	15	15	15	<u>-</u>	S	9	7	173	4.80
An splendidus	Ξ	23	7	I	I	7	7	4	7	_	*			45	0.52
-	æ	28	œ	15	17	17	9	-	7	4	e	7	-	<b>6</b> 0	3.03
An theobaldi	I	ſ	ļ	4	-	7	1		4	ø	*	-	1	53	0.32
	æ	1	-	4	6	<b>∞</b> !	9	9	7	4	۲,	-	-	65	1.36
Total	=	293	93	75	18	8	53	32	39	90	91	26	٠	738	8.58
	œ	765	318	251	206	231	183	747	264	52	7	85	~	3007	83.53

Total no. of nights on Human  $\approx 43$  for two baits. Total no. of nights on Bovid  $\approx 36$  for one bait.

Table 2. Seasonal prevalence of anophelines collected on human (H) and bovid (B) baits February, 1980 to January, 1981

			Seasons and mo	nths of the year		
Species	Bait	Spring (Jan to Mar)	Summer (Apr to Jun)	Monsoon (Jul to Sept)	Winter (Oct to Dec)	Totai
An. hyrcanus 'group'	н	6		5	70	81
	В	8	_	112	597	717
An. barbirostris	55	3		_	5	8
	В	1	1	2	88	92
An tessellatus	H		<del></del>	1	_	!
	В	-	ŀ	1	6	8
An aconitus	н	-	_		15	15
4 0 1 11	В	_	~-	1	23	24
An. fluviatilis	H	14	22 9	20	11	67
4	В	17 I	,	8 1	19	53
An. varuna	H B	2		3	14 23	16
An. culicifacies	H	ī		2	23	28 3
An cuncijacies	B		- 1	26		28
An jeyporiensis	H	80	_	36	47	163
AR JEJJOHENSIS	В	146	_	106	[40	392
An. subpictus	н	7	2	29	1	39
An surprises	В	8	7	504	8	527
An vagus	н	2	<u>,</u>	1	Ĭ	4
	В	_	1	21	11	33
An annularis	H	5		7	3	15
	В	4	-1	28	34	67
An. karwari	H	_	_	122	4	126
	В	1		259	12	272
An moghulensis	Ħ		_	-		0
	·B	1	<b>–</b> .	_	8	9
An. pallidus	H	6	1	2	2	- 11
	В	2	4	130	49	185
An. philippinensis	H	<del></del>	_	2	_	2
	В		<del></del>	10	i	11
An. jamesii	H	5	-	6	21	32
	В	45		76	109	230
An. maculatus	H	16	<del>-</del>	51	20	87
	В	42	1	93	37	173
An. splendidus	H	11	_	15	19 16	45
An theobaldi	B H	41 3	3	29	39	109
AR ineogua	В	4		17 45	. ~	23 49
Total	Н	160	28	317	233	738
	В	322	26	1454	1205	3007

(1978) reported this species to be zoophilic and exophilic in Bastar district. In Indonesia, Indochina, Java and Sumatra, it is reported to be a malaria vector of some importance. In India, it is

considered a secondary vector, next to An annularis especially in central India (Rao, 1984). However, its role in malaria transmission in Bastar district can not be ruled out.

An fluviatilis: It feeds throughout the night on man as well as on cattle with a higher activity till midnight and a peak around dusk. Per night density on man was <1.0 and on cattle it was 1.47. The proportion of biting man was more than cattle (1.2:1) and was higher during transmission seasons viz., summer and monsoon. Prevalent throughout the year. It is one of the important vectors of malaria and was found positive for both oocyst and sporozoite infections during July and August and their rate of infection were 2.08 for gut and 1.2 for gland (Kulkarni and Wattal, 1982). In earlier studies, the sporozoite rate varied from 0.1 to 37.0 per cent (Rao, 1984). Hence this species could be responsible for maintaining transmission and endemicity of malaria in the region. In India, much work has been done on this species from Assam in the North upto Tamil Nadu in the South. Feeding behaviour and seasonal patterns were same as in the present study.

An. varuna: Feeds on man as well as on cattle. Feeding was observed mostly during early hours of the night as reported in Burma and not in the second half of the night as reported earlier for central India (Rao, 1984). Per night per bait density was 0.18 on man and 0.78 on cattle. Husainy (1978) found this species to be zoophilic and exophilic. It was prevalent during October to December. This species was found positive for oocyst infection in the month of December in the present study. This species is being considered as a secondary vector of local importance in east central India or northern peninsular India (Kalra, 1978).

An. culicifacies: Mainly zoophilic but occasionally found on man the ratio being 9.3:1. Feeding was throughout the night but showed a bimodal activity with a peak after dusk and second after midnight on cattle. Per night density was very low on man but it was 0.77 on cattle. Most prevalent from July to September. It is one of the most important species of malaria vectors. Some of the specimens collected resting indoors were found positive for oocyst and sporozoite infection during March and September (Kulkarni, 1983). Feeding habits and seasonal prevalence vary greatly in different parts of India (Rao, 1984).

An jeyporiensis: Feeds on man as well as on cattle throughout the night with higher activity till midnight and a peak just after dusk. It is the most common species found on man. Density per night per bait was nearly 1.9 on man and 11 on cattle. It is found from July to March. Total absence was seen during April to June. No infection was found in any mosquito during this study. However, this species is considered to be a secondary vector of local importance in India, especially in Orissa state (Wattal, 1961).

An subpictus: Predominantly zoophilic but to some extent feeds on man. Feeding was active throughout the night with a peak just after midnight. Per night density on man was 0.45, while on cattle it was as high as 15. Highly prevalent during July to September. Earlier. sporozoite detection has been reported in this species in the month of February and July from the study area and its anthropophilic index was 35.6% in the month of June and per man hour density was 66 to 84 during June to August (Kulkarni, 1983). It has also been reported as malaria vector in other regions (Panicker et al., 1981). Because of its high man biting rate and density, the probable role of this species as a secondary vector under certain ecological conditions cannot be ruled out.

An vagus: Mainly zoophilic but occasionally found on man. Biting activity was higher till midnight. Mostly found during July to December. Per night density was very low on man while it was 0.9 on cattle.

An annularis: Predominantly zoophilic but feeds on man also. Most of the feeding occurred till midnight and a peak around dusk. Per night density was 0.17 on man while it was 1.86 on

cattle. Mostly prevalent during July to December. Venkat Rao (1961) and Dash et al. (1984) found high biting rate during August and February on cattle. However, the present observations agree with the observations made in Pakistan (Reisen and Aslamkhan, 1978).

An. karwari: This species is zoophilic as well as anthropophilic. Feeding occurs throughout the night but showed a bimodal activity with first peak after dusk and a second after midnight. Per night density was nearly 1.5 on man but on cattle it was 7.5. Highly prevalent during July to September. Although a large number were collected on man, this species does not carry any importance in the transmission of malaria (Rao, 1984).

An moghulensis: It is a rare species found in the eastern part of central India. A few specimens were collected on cattle during dusk in October to December.

An pallidus: Predominantly zoophilic. Feeds throughout night with a peak around midnight. Maximum feeding occurred before midnight. Per night density was 0.13 on man but on cattle, it was as high as 5.14. Prevalent from July to December.

An philippinensis: Although a few specimens (2 on man and 11 on cattle) were collected, this species appears to be zoophilic. It was mainly collected in the first half of the night during July to September.

An jamesii: Mostly zoophilic, feeds throughout night with higher activity till midnight and a peak around dusk. Per night density on cattle was 6.38 and 0.37 on man. More prevalent during October to December. Total absence was observed during April to June.

An maculatus: Zoophilic as well as anthropophilic. Feeding was observed throughout the night with a peak around dusk. Per night density was 1.01 on man while it was 4.8 on cattle. Predominantly found during July to September but period from October to March was also quite favourable for this species. Almost disappears during summer. One specimen collected outdoors was found positive for sporozoite infection in the month of April. This species has been reported to be a primary vector of Indo-Chinese region and secondary vector of Malaysian region (Bruce-Chwatt, 1985).

An. splendidus: Found on man as well as on cattle but more on animal. Feeding was throughout the night with a peak at dusk. Density per night was 0.5 on man and 3 on cattle. Prevalent from July to March. It was absent during summer.

An theobaldi: Zoophilic as well as anthropophilic. It feeds throughout the night with a higher activity during second and third quarter of the night. Per night density was 0.32 on man and 1.3 on cattle. Prevalent during July to September.

The results have shown that An hyrcanus 'group' and An subpictus were the most abundant species found in the collection. However, the latter was found active throughout the night with a peak just after midnight. An hyrcanus along with An karwari showed a bimodal feeding activity with a first peak after dusk and the second after midnight. An culicifacies showed a bimodal activity on cattle but fed intermittently on man. Three species viz. An fluviatilis, An jeyporiensis and An maculatus were found feeding throughout the night and they had a peak feeding activity just after dusk. The remaining species had their peak feeding activities after dusk.

Host feeding behaviour has indicated that An jeyporiensis was the most common species found on man with a highest per night density of 1.9, followed by An karwari, An maculatus, An hyrcanus and An fluviatilis and others had <0.5 density. An culicifacies was rarely encountered

on man. On bovid bait. An. hyrcanus and An. subpictus were the most abundant species with per bait per night density of >14 followed by An. jcyporiensis, An. karwari, An. jamesii, An. maculatus etc.

Regarding seasonal prevalence, only 3 species viz.. An. fluviatilis, An. subpictus and An. pallidus were found throughout the year. However, majority of the species were predominant in either July to September or October to December. Except three species viz.. An. fluviatilis, An. pallidus and An. subpictus, all other sixteen species were either totally absent or remained dormant during April to June.

During an earlier study and the present study, the data on both oocyst and sporozoite infections were obtained for different species in different months of the year as shown in Table 3.

Table 3. Monthwise infection of anophelines\*

S. No.	Species	Oocyst + ve in months	Sporozoite + ve in months
1.	An culicifacies	September	March and September
2.	An. fluviatilis	July	August
3.	An subpictus	_	February and July
4.	An maculatus	_	April
5.	An. varuna	December	<del>-</del>

<sup>\*</sup>Source: Kulkarni and Wattal, 1982; Kulkarni, 1983 and present study.

Vaid et al. (1974) and Kalra (1978) have reported that Bastar district has a perennial type of malaria and active transmission occurs between July and October. However, the data for 1978 obtained from the NMEP unit, Jagdalpur of south Bastar showed that the period of active transmission extends upto December. In the present study, it was observed that An fluviatilis was found throughout the year with higher prevalence during April to December, covering

summer, monsoon and winter seasons. It showed high predilection to man was observed to feed throughout the night. Sporozoite and oocyst infections have been recorded in July and August. Therefore, this species plays an important role in the transmission of malaria during April to December in this area.

The data in Table 3 shows that sprozoite infections in An. culicifacies were detected in the months of March (spring season) and September (monsoon season). Though, it showed a high predilection to cattle, it fed intermittently on man. Earlier, this species was reported to be responsible for spring transmission (January to March) and for monsoon transmission (July to October) in the northwestern parts of Madhya Pradesh (Vaid et al. loc. cit.). Therefore, An. culicifacies could be considered as the primary vector in the area.

Earlier, sporozoite infections in An. subpictus were found in the months of February and July and its anthropophilic index was high (35.6%) in the month of June (Kulkarni, 1983). The per man hour density of this species ranged from 66.0 in June to 84.0 in August. Therefore, this species could support malaria transmission during the summer season. Other species An. maculatus and An. varuna may act as potential vectors in the area. Hence, malaria transmission in this area remains perennial and mosquito control measures should be carried out during all the seasons of the year in Bastar district.

#### **ACKNOWLEDGEMENTS**

Thanks are due to the Director, National Institute of Communicable Diseases, Delhi, for his technical guidance and to all staff members of the Field Operational Research Station, Jagdalpur Distt. Bastar, Madhya Pradesh for their help in various ways. Thanks are also due to Dr. H.N. Kaul, Deputy Director, National Institute of Virology, Pune for his helpful suggestions.

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# Small-scale Field Trials with Polystyrene beads for the Control of Mosquito Breeding

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Wells and overhead tanks are the principal sources of mosquito breeding in urban and semiurban situations. In villages around Delhi, wells are the major sites for mosquito breeding. Most of the wells are unused and abandoned. Culex quinquefasciatus is the predominant species associated with well breeding. Other species found breeding in wells were the members of C. vishnui group. Anopheles stephensi and A. culicifacies. In urban situations, overhead tanks are of considerable importance as far as mosquito breeding is concerned. In Delhi city almost every locality has an innumerable number of overhead tanks and the mosquitoes found in association with overhead tank breeding are the vectors of urban malaria. A. stephensi and dengue haemorrhagic fever. Aedes aegypti.

In an effort to find a convenient approach for the control of mosquito breeding in unused wells and overhead tanks, the effectiveness of Expanded Polystyrene beads (EPS) was evaluated in Delhi and its neighbourhood. The results of the obser-

Accepted for publication: 28 August 1987.

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vations made between February 1985 and February 1986 are summarised in this communication.

Well surveys were undertaken in South Delhi (Basantpur area) and West Delhi (Gommanahera and Pindiwala Khurd areas) in January 1985. All wells found with mosquito breeding were numbered. The population of immatures in the wells was sampled using a galvanized bucket (5 litre capacity). A total of 5 dips were taken from each well. The anophelines and culicines were then separated and counted instarwise in enamel trays. The number of pupae collected were also recorded. Polystyrene beads were then introduced with the help of a bucket (16 litre capacity) and allowed to form a thick layer. Generally two buckets of beads (about 650 to 700 gms) were introduced into each well of about 1.5 m diameter and in bigger wells 2.5 to 3 buckets of beads (about 800 to 900 gms) were introduced.

The overhead tanks located in Ayurvigyan Nagar, R.K. Puram (Sector 9), INA Colony and Pushpa Vihar areas were surveyed during June/July 1985 and numbered. Thereafter, the entrance of the ingoing pipe was covered by a small piece of steel mesh to prevent the entry of beads into the pipe line. About 200 gms of polystyrene beads were introduced into each overhead tank.

Post-treatment observations in wells were carried out at weekly intervals. After taking one sample, the beads drawn along with the bucket were removed with the help of a metal strainer and transferred to an enamel tray and the larvae (instarwise), and pupae were counted. The larvae (instarwise) and pupae which were found dead were also counted and recorded separately. The beads collected from all the five samples were returned to the well.

The post-treatment observations in overhead tanks were also carried out at weekly intervals. The breeding was checked with the help of an aluminium spoon (60 ml capacity) or an enamel bowl (150 ml capacity). Generally three dips were taken. The contents were transferred to an enamel tray, beads separated carefully and then checked for the presence of immatures.

The EPS beads were introduced into 42 wells of which 25 were found breeding exclusively culicines and the remaining 17 wells had mixed breeding of anophelines and culicines at the time of application. The EPS beads were introduced into eight wells each during February 1985 first and second fortnights respectively, six and three wells in March first and second fortnights respectively, nine wells in July second fortnight and five and three wells during the first and second fortnights of August respectively. A total of six wells served as control. The post-treatment observations were continued for a period of 46 weeks in wells treated during the first and second fortnights of February 1985; for 42 weeks in wells treated during the first and second fortnights of March; for 26 weeks in wells treated in July (second fortnight) and for 22 weeks in wells treated during the first and second fortnights of August 1985. However, in the presentation of post-treatment data, the results of weekly collections have been computed and the monthly average number of immatures per dip sample with range for the entire observation period from February 1985 to February 1986 have been given

in Table 1 with density of larvae and pupae recorded in the control group of wells.

EPS beads were introduced into a total of 879 overhead tanks in Ayurvigyan Nagar, R.K. Puram and INA Colony. Pushpa Vihar with 476 overhead tanks was retained as control. The results of overhead tank survey are furnished in Table 2.

There are inherent difficulties associated in the effective control of mosquito breeding in wells. While a larvicide requires weekly application, larviciding is a difficult operation in wells because of long distances between the wells and difficulties in reaching them during the rainy season. The present study on the application of EPS beads has shown that there was a drastic decline in the density of culicine larvae and pupae in the successive weeks after treatment. Further, the EPS beads suppressed adult emergence as seen by considerable mortality of pupae and emerged adults. In case of anophelines, breeding was detected in 6 of the 8 wells treated in February 1985 and one of the 3 wells treated in March 1985. Following application of beads, the anopheline breeding was found to continue for a period of one to three weeks except in two wells where the breeding was recorded for a period of 19 to 22 weeks after application. In all these wells only the early instars were encountered. Fourth instar larvae could be detected during 7 out of 17 weekly observations. However, no pupae could be detected during the entire period of observation. Based on laboratory studies (Sharma, 1984) it was observed that application of EPS beads at the rate of 1.0 gm/m<sup>2</sup> produced high larval and pupal mortality in Cx. quinquefasciatus, A. culicifacies and A. stephensi. In a field study, Sharma et al. (1985) found that EPS beads were effective in the control of mosquito breeding in biogas plants and wells in Nadiad taluka, Kheda district, Gujarat. The results of the present study support the observations of the above authors. The method has several advantages, a) it

Table 1. Effect of polystyrene beads on mosquito control in wells

					ואוסשוווא— אספר ווכמיוווכיוו	* * * * * * * * * * * * * * * * * * *	Catherin						
	Feb 1985	Mar	Apr	May	Jun	Jul	Aug	Sept	Ö	Nov	Dec	Jan 1986	Feb
February 1985 study	Pre- treat-	Post- treat-								   			
Number of collections Average No. of	ment 16	ment 65	99	8	苯	51	19	Ģ	\$	17	4	40	92
immatures  -      Pange	458.0		28.0	17.6	0.025	<b>C</b> =	2.5	0.02	0	2.6	2.67	20.6	9.05
range 1V	38.0		0.445	0.44	, 0		0.06	0.006	0	0.095	0.143	0.73	0
Range	18-58:		0-71	0-523	0		0-11	10	,	01~0	01-0	68-0	
Range	1-267	0-10	- 0	0	0	٥	<b>-</b>	5	5	<b>-</b>	<b>=</b>	<b>c</b>	<del>c</del>
March 1985 trentment		Pre- treat- ment						j	}	   			
Number of		,		!									
collections Average number of immatures		<b>&gt;</b>	વ	<b>4</b>	\$	£	9	37	20	<u>s</u>	12	%	<del></del>
1-111	I	524.2	32.2	1.7	0.07	90.0	0	0.04	Φ	90	6.6	127.1	, 4
Range	}	306-7632	0-1801	0-158	0-3	0-15		0-7		0-148	0-453	0-6-0	0-343
10	I	6.5	6.0	0.03	0	0	0	¢	0	9.7	0.00	3.0	0
Range	}	28-1441	0 - 149	0-3						0 - 128	1	0-285	
Pupac	I	5.2	0.001	Ç	0	0	0	0	0	0.001	0	0	0
Range	}	0-127	0-1							7-0			

contd.

Table 1. (contd.)

Feb Mar 1985  Treatment No. of collections Average number of immatures 1-111 Range Range Range Range Range Range		 	Months	Months—post treatment	Batment .				;   	!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!	
Feb 1985 sent ons promber satures				-	***************************************						
July 1985 Treatment No. of collections Average number of immatures 1-111 Range IV Range Range Range Range	Apr	May	Jun	] [2]	Aug	Sept	) Ost	Nov	Dec	Jan 1986	Feb
Treatment No. of collections Average number of immatures 1-III Range IV Range Pupar			İ	- - -	Post.	[ [				   	
No. of collections Average number of immatures 1-III Range IV Range Range				treut-	treat-						
collections of immatures of immatures Range Pupae Range CONTROL				ment	ment						
Average number of immatures 1-ill Range IV Range Range Range CONTROL				:	;	į					
of immatures of immatures Range Rupae Range Range				Ď	 	9.	55	91	=	<u>.</u> .	~
I-ill Range IV Range Pupae Range											
Range IV Range Pupae Range				64.6		-	~	0.0	,	r 14	,
Range Pupac Range						;	1	= !	,	5.0	*
Range Pupac Range				0-1-0			77-0	0.874	00	0-120	0.87
Kange Pupac Range CONTROL				9.15		0	0.008	7.0	0	0.03	С
Pupac Range CONTROL				0.215			-0	0.55		6-3	
Range				1.6	_	0	0	0.04	c	0	C
CONTROL				0 - 35	0-1			6-3			;
	       	     	   	; 	   	!   		 			j
No. of collections 21	25	27	26	24	7.7	~	82	a	0	ç	÷
Average number			•	í	ì	;	2	n.	o	?	ю.
matures											
I-III 461.5	664.8			37.2		6.7	89	77	0.52	067	3 771
Range 40-667		941 0-3140	19-0	0-2346	0-4064	0-384	0-358	0-915	0-7454	0-5815	7,000
				7.6		0.83	0.0		15.0	119.0	
				0-723		0-43	<del>-</del> 0	;	0-473	0-477	0 104
			_	3.0		0.3	0.03	c		235	2 -
Range 0-269				0 - 303	_	81-0	[	:	5-0	;	# 'V

Table 2. Efficacy of EPS beads in overhead tanks

Area		Ayurvigyan Nagar	R.K. Puram	INA Colony	Pushpa Viha (Control)
Number of tanks treated		317	172	276	476
July 1985 Number of tanks sampled/ positive					
Week	I	0			
	][	0			
	111	72/1			48/7
	11	72/0	140/3		88/12
August			<del></del>		
Week	1	60/3			
	11				96/4
	ĺΠ	161/2	52/3		80/6
_	ìv	_	143/0		80/0
September					
Week	I	86/0		80/4	331/25
	17	60/0		247/10	96/12
	111	116/0	116/1	125/8	65/19
	1 <b>V</b>	141/0	96/2	_	_
October		•			
Week	1	157/2	172/0	144/1	249/30
	11	68/1	167/2	251/1	414/14
	111	157/0	201/1	280/3	463/7
	IV	223/0	199/ l	249/4	465/7
November					
Week	1	286/3	92/0		64/1
	11	186/0		146/4	318/3
	111	185/0	37/0	270/2	472/7
	IV	_	172/0	276/0	_
December					
Week	1	281/0	_	80/0	97/1
	11	68/0	172/0	_	379/1
	111		_	90/0	
	IV.	317/0	172/0	199/0	227/0
lanuary 1986		***			
Week	1	317/0		80/0	265/0
	11	_	172/0	_	211/0
	111	317/0	_	90/0	476/01
	١٧		172/0	199/0	201/0

Note: In a part of Pushpa Vihar, the beads were constantly being removed as the residents were in the habit of drawing water directly from the overhead tanks. In view of this the entire Pushpa Vibar area was taken as control although the area was treated in the beginning.

forms a thick blanket on the water surface thus preventing oviposition on the water surface; b) the beads remain on the water surface for a long time; c) they are environmentally safe and d) they prevent adult emergence in case oviposition does take place.

In case of overhead tanks, no breeding could be detected wherever the beads were not disturbed. In all the tanks which were found to be positive for mosquito breeding either the beads were removed or disturbed so as to form patches.

During the field study the following observations were made.

- (a) If the terrace is easily approachable, the EPS treated overhead tanks were subject to disturbance.
- (b) If the water regulators are not maintained properly, the beads were apt to be washed away along with the overflowing water.

However, constant contact with the residents and health education efforts served to improve the situation. Based on this trial it could be concluded that EPS beads are useful on a long term basis for the control of mosquito breeding in wells and overhead tanks. Community contact will help in eliminating drawbacks in the latter habitat.

#### **ACKNOWLEDGEMENTS**

The authors are grateful to Shriyuths K. Sachdeva, O.P. Pal, Sri Bhagavan, S.M. Dash, Narinder Kumar, Ravi Kant and Dinesh Sharma for their assistance in the studies.

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# Importation of Malaria Cases from Sri Lanka to Rameswaram Island (Tamil Nadu)

A. RAMALINGESWARA RAO!, A.V. SADANAND! and N. JALEEL!

There was large-scale influx of refugees from Sri Lanka to Rameswaram Island (Tamil Nadu) during 1985 and 1986. In view of this, arrangements were made to screen the refugees for malaria infection on their arrival at Rameswaram Port and subsequently during weekly surveillance at the transit camp established at Mandapam. A total of 28760 refugees arrived during 1985 and 1986 (23734 in 1985 and 3026 refugees in 1986). Of 32291 blood smears collected (26569 blood smears in 1985 and 5722 in 1986), 214 were found positive for malaria parasite: 159 P. vivax, 45 P. falciparum and 10 mixed infections (Table 1).

Table 1. Monthwise record of mataria cases among refugees from Sri Lanka during 1985 and 1986

Months .			Epidemiological	data		
,wiomas	Refugees arrived	Blood smears collected	No positive	Pv	Pſ	Mix
1985		· · · · · · · · · · · · · · · · · · ·				
Feb	10139	7914	1.5	*	. 4	0
Маг	6590	6868	49	32	14	3
Apı	1167	1560	29	16	2	3
May	812	1136	15	1.3	2	0
Jun	1123	1410	25	14	10	0
Ju	730	1073	15	15	0	0
Αug	1376	1380	3	3	0	0
Sep	1907	1979	2	2	0	0
Oci	1442	1939	8	2	4	2
Nov	282	732	10	5	4	J
Dec	166	573	5	4	1	0
Total	25734	26564	165	118	38	9

contd

Accepted for publication: 18 September 1987.

Directorate of Public Health and
Preventive Medicine
259. Anna Salai
Madras-600006. India.

Since the blood smears were collected from the refugees on the day of their arrival either at Rameswaram Port or at the Mandapam Camp within a week, all positive cases have been classified as imported from Sri Lanka. The

Table 1. (Contd.)

			Epidemiological	data		
Months	Refugees arrived	Blood smears collected	No. positive	Pv	Pf	Mix
1986						
Jan	454	166	6	5	J	0
Feb	319	135	2	2	0	0
Mar	292	138	9	8	I	0
Apr	348	209	4	4	0	0
May	461	286	5	5	0	0
Jun	764	588	4	4	0	0
Jul	758	<b>56</b> 6	5	5	O	0
Aug	481	341	4	2	. 2	0
Sep	487	319	5	1	3	ı
Oct	39 <b>9</b>	236	2	2	0	0
Nov	409	15	2	2	0	0
Dec	550	27	1	1	0	0
Total	5722	3026	49	41	7	

Table 2. Agewise classification of malaria cases recorded among Sri Lanka refugees (1985-1986)

S			Age group (in years	s)		_
Species	0-11+	1 - 4	5-15	16-50	50 +	Total
P. vivax	3	18	55	76	7	159
P. falciparum	1	6	12	24	5	48
Mixed	0	2	3	2	0	7
Total	4	26	70	102	12	214
Percentage	1.8	12.1	32.7	47.6	5.6	

<sup>\* 0-11</sup> months

agewise classification of the positive cases revealed that the age group 5-15 and 16-50 accounted for about 80% of the total positives (Table 2).

The occupational classification of the refugees showed that 50% were fishermen, 40% labourers

in tea gardens and 10% engaged in trade. The source of importation of malaria cases revealed that 60% of the positive cases originated from Besalai, Mannar, Vavuniya, Marugan, Chettikulam and Thlaimannar. Chettikulam has contributed to a maximum number of *P. falciparum* cases (33).

# Incrimination of *Anopheles stephensi* Liston as Malaria Vector in Calcutta

S.K. CHOUDHURI! and A.K. SEN1

There has been resurgence of malaria in Calcutta beginning from 1981. A progressive increase in the number of *P. vivax* and *P. falciparum* cases occurred during 1981 onwards. Chloroquine resistance in *P. falciparum* has also been detected in parts of Calcutta during 1986.

The city of Calcutta is under the NMEP Modified Plan of Operation. Antilarval operations are being carried out by the Health Department of the Calcutta Municipal Corporation. Besides this, pyrethrum solution of 0.1% strength is being sprayed focally where malaria cases are detected through passive agencies of Government of India, Government of West Bengal and Calcutta Municipal Corporation. Labourers engaged in Metro rail construction and other developmental projects in Calcutta suffer from malaria and usually receive inadequate treatment thus creating a reservoir of infection.

There is an urgent need to control this resurgence of malaria as the problem is getting exacerbated and complicated. A. stephensi was incriminated as vector of malaria in Calcutta about four decades ago. Nothing definite is known as to the current status of the vector of malaria in Calcutta. Hence an intensive study was carried out to incriminate the species of mosquitoes responsible for the present resurgence of malaria. After preliminary investigations, the areas of persistent transmission of malaria in the city were demarcated.

Adult anopheline mosquitoes were collected from fixed dwellings (mixed) during 1800 hours to 0400 hours in Chetla area of the city. Outdoor collections of resting anophelines were also made in the same area during the day. The collected mosquitoes were brought to the laboratory in the morning. They were dissected for gut and gland infections after identification. Glands found positive were stained and preserved.

Regular larval collections were also made from clear water collections in Metro rail sites in the heart of the city. Larvae were also collected from water collections around buildings under construction and other manmade peridomestic and domestic water collections. The larvae and pupae were brought to the laboratory for identification, both in the larval stages as well as after adult emergence.

The incidence of malaria in Calcutta since 1981 to 1986 is shown in Table 1. This indicates that

Calcutta, India.

Accepted for publication: 11 December 1987.

Calcutta Municipal Corporation

Table I. Malaria incidence in Calcutta

Year	Blood smears examined	No. Positive	Pf cases	Slide positi- vity rate	
1981	39000	6600	21	16.9	
1982	47000	6785	15	14.4	
1983	67000	19389	1330	28.9	
1984	71000	26056	1184	36.7	
1985	75000	18001	1667	24.01	
1986	103830	15723	898	15.2	

Table 2. Monthly man hour density of different anopholines in Calcutta\*

Species	A. stephensi		A. subp	A. vagus		
	No. Coll.	MHD	No. Coll.	MHD	No. Coll.	MHD
Months						
Jan	30	0.07	20	0.05	5	0.01
Feb	20	0.05	30	0.07	2	0.005
Mar	15	0.03	43	0.10	J	0.002
Apr	40	0.10	39	0.09	2	0.005
May	30	0.07	<b>2</b> 5	0.06	3	0.007
Jun	50	0.12	44	0.11	3	0.007
Jul	86	0.21	37	0.09	2	0.005
Aug	128	0.32	84	0.21	4	0.01
Sep	183	0.45	97	0.24	5	0,012
Oct	112	0.28	83	0.20	5	0.02
Total	694	0.17	502	0.12	32	0.008

<sup>\*</sup> Jan to Oct 1986. MHD = Man Hour Density

the incidence of malaria had been rising from year to year. It was only in 1985 that the increasing trend of malaria could be checked, but in this year there was a significant rise in *P. falciparum* cases.

A total of 1228 anophelines represented by 3 species. A. stephensi, A. subpictus and A. vagus were collected between 1800 hours to 0400 hours during the period January to October 1986.

A. stephensi was the most predominant species forming nearly 56.52% of the total catch. The second predominant species was A. subpictus (40.86%) and the third was A. vagus (2.60%). The

monthly man hour density of the different anopheline species is shown in Table 2.

In the outdoor collections during the period January to October 1986, 4 A. subpictus and 2 A. stephensi were found from empty drums and fencing.

A total of 982 anophelines were dissected and of these, gland infection in one specimen of A. stephensi was found during the month of September.

Spot checks were made both for the detection of untreated and newly established breeding places.

Breeding of larvae was found in masonry tanks, cisterns. low-lying land, water collections in buildings, tyres, borrow pits, ditches, ponds, empty artificial containers, depressions, shallow wells, pits, drains, nullahs, canals etc. Of a total of 59762 sites checked 10592 were found positive for breeding.

The earlier work on incrimination of vectors of malaria in Calcutta was done by Siddons (1946). He incriminated A. stephensi as the vector of malaria in Calcutta. Out of 1052 A. stephensi dissected and examined, 6 glands were found positive.

Neogy and Sen (1962) also incriminated A. stephensi as the vector of malaria in Durgapur. West Bengal when there was an epidemic of malaria during construction of Durgapur Thermal Plant (D.V.C.). Recent studies by Hati and Mukhopadhyay (1980) and Hati et al. (1987) also implicate A. stephensi as the vector of malaria in Calcutta.

#### ACKNOWLEDGEMENTS

The authors thank Dr. D.S. Choudhury, Malaria Research Centre for confirmation of sporozoite slide and also acknowledge the cooperation and help of Mr. B.K. Roy, Institute of Urban Management and Lt. Col. S.C. Chandra, S.H.O. Calcutta. The assistance of Shri Sandip Dutta, Shri Kamal Ghosh and Shri Gora Chand Das of vector control section is also acknowledged.

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## EDITORIAL ACKNOWLEDGEMENT

The Editor gratefully acknowledges the help of the following scientists who have kindly reviewed the papers for the 1987 issues of the Indian Journal of Malariology.

Dr. C. Achuthan, Vijayanagar Extension, Bangalore; Prof. T.N. Ananthakrishnan, Entomology Research Institute, Loyola College, Madras; Dr. H.M. Bhatia, Institute of Immunohaematology, Seth Medical College. Bombay; Dr. D.S. Choudhury, Malaria Research Centre. Delhi; Dr. C.F. Curtis. School of Hygiene & Tropical Medicine. University of London, London; Dr. D.M. Denapurkar. Haffkine Institute for Training, Research and Testing, Bombay; Dr. G.P. Dutta, Central Drug Research Institute, Lucknow; Dr. S.I. Farooqui, Indian Agricultural Research Institute, New Delhi; Dr. N.K. Ganguly, Postgraduate Institute of Medical Education and Research. Chandigarh; Dr. S.C. Lahiri, Rashbehari Avenue, Calcutta; Dr. Ram Lakhan, Banaras Hindu University. Varanasi; Dr. R.C. Mahajan, Postgraduate Institute of Medical Education and Research, Chandigarh, Dr. K.N. Mehrotra, Indian Agricultural Research Institute, New Delhi; Dr. E.B. Myhre, PfCP, National Malaria Eradication Programme, Delhi; Dr. Ramesh Kumar, All India Institute of Medical Sciences. New Delhi; Dr. A.P. Ray, PfCP, National Malaria Eradication Programme. Delhi; Dr. R. Reuben. Centre for Research in Medical Entomology, Madurai; Dr. R.G. Roy. Bidhan Nagar. Calcutta; Dr. Santokh Singh, School of Entomology, Agra; Dr. Q.B. Saxena, Malaria Research Centre, Delhi; Dr. Shobha Sehgal, Postgraduate Institute of Medical Education and Research, Chandigarh; Dr. G.K. Sharma, National Malaria Eradication Programme. Delhi; Prof. B.S. Srivastava. Jawaharlal Nehru University, New Delhi; Dr. D. Subrahmanyam, Hindustan CIBA-GEIGY Research Centre, Bombay, Dr. G.P. Talwar, National Institute of Immunology, New Delhi, Dr. Sarala K. Subbarao, Malaria Research Centre, Delhi, Dr. S.C. Tewari, Delhi University. Delhi; Dr. V.K. Vinayak, Postgraduate Institute of Medical Education and Research, Chandigarh.

## INDIAN JOURNAL OF MALARIOLOGY

### Author Index

Subbarao, Sarala K. 9, 29, 33, 135

Sinha, S. 95, 107

Tewari, S.C. 21

Vasantha, K. 9

Waghs U.V. 111

Yajnik, Vijay 65

Srivastava, I.K. 125

Subhash Chandra 17, 59

Usha Devi. C. 95, 107

Abuzar, Syed 17 Adak, T. 9, 135 Agarwal, S.S. 79, 125 Ansari, M.A. 119 Arif, A.J. 59 Badam, Lalita III Balaraman, K. 131 Baruah, B.D. 159 Beljaev, A.E. 97 Bharla, S.C. 79 Bharadwaj, Y. 119 Biswas, D. 85 Brohult, J.A. 97  Kulkarni, S.M. 163 Lahiri, S.M. 163 Lahiri, S.M. 163 Lahiri, S.C. 45 Malhotra, P.R. 151 Mathur, P.D. 59 Nagpal, B.N. 143 Nagsampgi, B.A. 111 Nanda, Nutan 135 Nihal Singh 89 Pal, N.L. 39 Passey, M.N. 125 Raghavendra, K. 29, 33 Ramasamy, Ranjan I Ramesh Kumar 119 Rao, A. Ramalingeswara 181
Agarwal, S.S. 79, 125       Malhotra, P.R. 151         Ansari, M.A. 119       Mathur, P.D. 59         Arif, A.J. 59       Nagpal, B.N. 143         Badam, Lalita III       Nagsampgi, B.A. 111         Balaraman, K. 131       Nanda, Nutan 135         Baruah, B.D. 159       Nihal Singh 89         Beljaev, A.E. 97       Pal, N.L. 39         Bhalla, S.C. 79       Passey, M.N. 125         Bharadwaj, Y. 119       Raghavendra, K. 29, 33         Bhattacharya, U. 45       Ramasamy, Ranjan I         Biswas, D. 85       Ramesh Kumar 119         Brohult, J.A. 97       Rao, A. Ramalingeswara 181
Ansari, M.A. 119 Arif, A.J. 59 Badam, Lalita HI Balaraman, K. 131 Baruah, B.D. 159 Beljaev, A.E. 97 Bhalla, S.C. 79 Bharadwaj, Y. 119 Bhartacharya, U. 45 Biswas, D. 85 Brohult, J.A. 97  Mathur, P.D. 59 Nagpal, B.N. 143 Nagsampgi, B.A. 111 Nanda, Nutan 135 Nihal Singh 89 Pal, N.L. 39 Passey, M.N. 125 Raghavendra, K. 29, 33 Ramasamy, Ranjan I Ramesh Kumar 119 Rao, A. Ramalingeswara 181
Arif. A.J. 59  Badam, Lalita III  Balaraman, K. 131  Baruah, B.D. 159  Beljaev, A.E. 97  Bhalla, S.C. 79  Bharadwaj, Y. 119  Bhattacharya, U. 45  Biswas, D. 85  Brohult, J.A. 97  Nagpal, B.N. 143  Nagsampgi, B.A. 111  Nanda, Nutan 135  Nihal Singh 89  Pal, N.L. 39  Pal, N.L. 39  Passey, M.N. 125  Raghavendra, K. 29, 33  Ramasamy, Ranjan I  Ramesh Kumar 119  Rao, A. Ramalingeswara 181
Badam, Lalita III  Balaraman, K. I3I  Baruah, B.D. 159  Beljaev, A.E. 97  Bhalla, S.C. 79  Bharadwaj, Y. I19  Bhattacharya, U. 45  Biswas, D. 85  Brohult, J.A. 97  Nagsampgi, B.A. III  Nanda, Nutan 135  Nihal Singh 89  Pal, N.L. 39  Pal, N.L. 39  Passey, M.N. 125  Raghavendra, K. 29, 33  Ramasamy, Ranjan I  Ramesh Kumar 119  Rao, A. Ramalingeswara 181
Balaraman, K. 131 Baruah, B.D. 159 Nihal Singh 89 Beljaev, A.E. 97 Pal, N.L. 39 Bhalla, S.C. 79 Passey, M.N. 125 Bharadwaj, Y. 119 Raghavendra, K. 29, 33 Bhattacharya, U. 45 Riswas, D. 85 Ramesh Kumar 119 Brohult, J.A. 97 Rao, A. Ramalingeswara 181
Baruah, B.D. 159  Beljaev, A.E. 97  Bhalla, S.C. 79  Bharadwaj, Y. 119  Bhattacharya, U. 45  Biswas, D. 85  Brohult, J.A. 97  Nihal Singh 89  Pal, N.L. 39  Passey, M.N. 125  Raghavendra, K. 29, 33  Ramasamy, Ranjan I  Ramesh Kumar 119  Rao, A. Ramalingeswara 181
Beljaev, A.E. 97 Bhalla, S.C. 79 Passey, M.N. 125 Bharadwaj, Y. 119 Raghavendra, K. 29, 33 Bhattacharya, U. 45 Riswas, D. 85 Ramesh Kumar 119 Brohult, J.A. 97 Rao, A. Ramalingeswara 181
Bhalla, S.C. 79 Bharadwaj, Y. 119 Bhattacharya, U. 45 Biswas, D. 85 Brohult, J.A. 97  Passey, M.N. 125 Raghavendra, K. 29, 33 Ramasamy, Ranjan I Ramesh Kumar 119 Rao, A. Ramalingeswara 181
Bharadwaj, Y. 119 Raghavendra, K. 29, 33 Bhattacharya, U. 45 Riswas, D. 85 Ramesh Kumar 119 Brohult, J.A. 97 Rao, A. Ramalingeswara 181
Bhattacharya, U. 45  Biswas, D. 85  Brohult, J.A. 97  Ramasamy, Ranjan I  Ramesh Kumar 119  Rao, A. Ramalingeswara 181
Biswas, D. 85  Brohult, J.A. 97  Ramesh Kumar 119  Rao, A. Ramalingeswara 181
Brohuft, J.A. 97 Rao, A. Ramalingeswara 181
Chanan Singh 59 Rastogi, M. 39
Chandrahas, R.K. 175 Razdan, R.K. 119
Chatterjee, K.K. 85 Reuben, R. 21
Choudhuri, S.K. 183 Rooney, W. 89
Choudhury, D.S. 79, 95, 107 Roy, Arati 51, 65
Das, N.G. 151 Roy, Kunal B. 65
Das, P.K. 17 Roy, S. 45
Das, S.K. 79, 125 Sadanand, A.V. 181
Dass, C.M.S. 135 Samantaray, K.C. 97
Deolankar, R.P. 111 Sarkar, P.K. 151
Dutta, G.D.P. 17 Sen, A.B. 39, 59
Dutta, G.P. 17, 125 Sen, A.K. 183
Dutta, P. 159 Sharma, G.K. 97
Ghosh, S.K. 95, 107 Sharma, R.C. 73
Gopal, Krishan 125 Sharma, Satyavan 17
Gupta, D.K. 73 Sharma, V.P. 9, 29, 33, 51, 65, 73, 79, 95,
Hati, A.K. 85 107, 119, 135, 143, 175
Hazarika, S. 151 Sinha, Ragini 125

Kar, P.K. 45 Kondrashin, A.V. 89 Kudesia, Madhur 125 Kulkarni, M.M. 111

Joshi, Hema 29, 33

Hiriyan, J. 21

Hoti, S.L. 131

Jaleel, N. 181 John, V.M. 151

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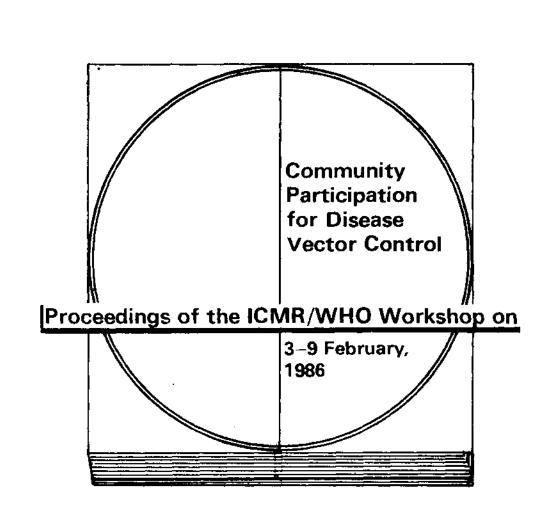
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2. Periodicity of its publication

Half yearly

3. Printer's Name Nationality Address V.P. Sharma Indian

Maiaria Research Centre (ICMR)

22-Sham Nath Marg Delhi—110054

4. Publisher's Name Nationality Address Dr. V.P. Sharma

Indian

Malaria Research Centre (ICMR)

22-Sham Nath Marg Delhi—110054

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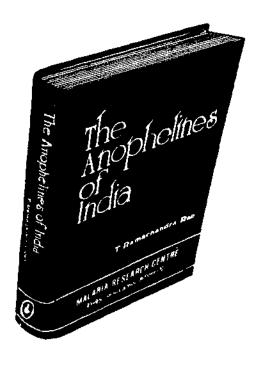
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