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CONTENTS

Volume 28
Number 2
June 1991

Repeat Regions of Malaria Parasite Proteins : A Review of Structure and Possible Role in the Biology of the Parasite 73
Ranjan Ramasamy

Observations on the Breeding of Anophelines in Rice Fields of Shahjahanpur District, Uttar Pradesh 83
S.N. Sharma and *R.N. Prasad*

Biting Rhythms of Malaria Vector *Anopheles culicifacies* in Kheda District, Gujarat 91
R.M. Bhatt, R.C. Sharma, V.K. Kohli, A.S. Gautam and *D.K. Gupta*

Vaccination of Rhesus Monkeys Against *Plasmodium knowlesi* with Aqueous Suspension of MDP as an Adjuvant 99
Raka Khanna, Sohail Ahmad, Haris Manzoor Khan, Hemant Kumar and *Abbas Ali Mahdi*

Prevalence of Malaria and Economic Loss in two Major Iron Ore Mines in Sundargarh District, Orissa 105
R.S. Yadav, S.K. Ghosh, S.K. Chand and *A Kumar*

The Haematology of *Plasmodium vivax* before and after Chloroquine and Primaquine Treatment in North Madras Area 115
P.R. Kumaresan and *R.Selvam*

Epidemiological Observations on Malaria in some parts of Tengakhat PHC, Dibrugarh District Assam 121
P. Dutta, D.R. Bhattacharyya and *L.P. Dutta*

Mosquito Survey in Tripura 129
S.C. Das, M. Bhuyan, I. Baruah and *P.K. Talukdar*

Short Note

Chloroquine Resistant *P. falciparum* Malaria in Arunachal Pradesh 137
S. Satyanarayana, S.K. Sharma, P.K. Chelleng, P. Dutta, L.P. Dutta and *R.N.S. Yadav*

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Repeat Regions of Malaria Parasite Proteins : A Review of Structure and Possible Role in the Biology of the Parasite

RANJAN RAMASAMY*

Tandemly repeated amino acid sequences are characteristic of many malaria parasite proteins that have been sequenced to date. Strong selective pressures must exist to maintain the repeat regions and also to diversify them in the case proteins containing strain-variant repeats. Repeats have been suggested to function in immune-evasion and in binding to host receptors. This article focusses on the structural characteristics of the repeats in relation to their postulated function.

INTRODUCTION

Many proteins of malaria parasites (*Plasmodium* species), that have been sequenced to date, contain regions composed of tandemly repeated sequences termed repeats. Repeats are also found in prokaryotes and in certain structural proteins of metazoan organisms e.g., collagen, *Drosophila* glue proteins[1] and freezing point depressing glycoproteins of Antarctic fish[2]. Repeats have been seen in the proteins of other parasitic protozoa, when the corresponding genes have been identified from DNA expression libraries by screening with immune sera[3]. However repeats have been most extensively documented in malaria parasite proteins, particularly in *Plasmodium falciparum* where the sequences of relatively large numbers of proteins have been determined. *P. falciparum* proteins will mainly be used as examples in this review which attempts to relate structural characteristics (particularly hydrophilicity and the predicted secondary structure) of the repeats to the possible functions of the repeats in parasite biology.

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Diverse *Plasmodium* proteins contain repeats

Because of the stability of epitopes in repeats and the presence of multimeric epitopes, it can be argued that screening expression DNA libraries with immune sera tends to select genes coding for antigens containing repeats. The ease of identification of repeat-containing antigens may also be a consequence of the biased representation of antibodies directed against repeat region epitopes in immune sera. The first few, and probably the more strongly reacting, antigens to be isolated from a λ gt 11 expression DNA library screened with immune human sera[4], were antigens containing extensive repeats e.g., the heat-stable S-antigen[5], the ring-infected erythrocyte surface antigen or RESA/

Pf155[6] and the falciparum interspersed repeat antigen or FIRA[7]. More recently however proteins lacking repeats e.g., the apical membrane antigen AMA-1[8] have been characterized from clones obtained by screening with immune human sera.

Repeats are seen in parasite antigens found in diverse locations and probably serving quite different functions. These include: (a) Membrane proteins e.g., the circumsporozoite or CS protein[9, 10], the 185-200 KDa merozoite surface glycoprotein termed PMMSA[11] and the glycosylated and myristilated smaller surface antigen or GYMSSA[12-14]; (b) Proteins in the parasitophorous vacuole that are released as soluble antigens into the exterior during schizogony e.g., the S-antigen[5]; (c) Proteins associated with the plasma membrane of the infected erythrocyte e.g., the knob associated histidine rich protein or KAHRP[15]; (d) Proteins present in the rhoptry-microneme complex e.g., RAP-1[16].

On the other hand several *P. falciparum* genes lack repetitive sequences. Proteins coded for by such genes include (a) Structural proteins e.g., actin[17] and tubulin[18]; (b) Rhoptry-microneme proteins e.g., AMA-1[8]; (c) Enzymes e.g., hypoxanthine-guanine phosphoribosyl transferase[19] and aldolase[20].

Structural features of repeats

The size of the repeats in *Plasmodium* antigens is quite variable. The clustered asparagine rich protein or CARP contains long tracts of asparagine[21], while the serine rich antigens or SERA is composed of 11% serine present in polyserine blocks[22]. Several histidine rich proteins have also been identified. Long repeats are seen for example in FC 27 strain GYMSSA which contains a 32 amino acid (a.a.) sequence repeated twice[13] and the glycophorin binding protein or GBP[23] which has multiple repeats of 50 a.a., the longest *Plasmodium* repeat known to date. The size of the repeat within a protein can vary considerably in different isolates of the parasite e.g., the 3D7 strain of GYMSSA has five tandem copies of the sequence GGSA that is unrelated to the 32 a.a FC 27 repeat[14]. More restricted diversity is seen within the repeats of S-antigens where there is an underlying homology in the DNA sequences that give rise to at least three distinct amino acid sequences [5, 24]. This indicates that the S-antigen repeats have evolved from a common ancestral gene sequence. In contrast, the two known nonapeptide repeats in the CS proteins of *P. vivax* show no homology at the DNA level and therefore could have evolved independently[25]. On the other hand, the repeats of RESA are conserved among all known isolates[6]. Immune pressure has been suggested to be the driving force for the generation of variant repeat sequences within a particular protein. Repeats within a particular region of a protein are often not exact, degeneracy of sequences being particularly evident towards the ends of the repeat regions. The genetic mechanisms for maintaining repeats and for generating new repeats in malaria parasites have not been elucidated, but known processes such as gene conversion and unequal crossing over probably play a part in this.

More detailed structural parameters in the repeat and non-repeat regions of *P. falciparum* proteins were examined in selected proteins[26]. Proteins were selected on the basis of the following criteria:

(a) The presence of clearly defined repeat regions composed of multiple amino acids;

(b) The availability of the complete sequence of the antigen and

(c) Only one sequence (chosen at random) for a given antigen when sequences from several isolates were available.

The selected proteins were (1) PMMSA-Wellcome[11], (2) GYMSSA-FC27[13], (3) GBP[23], (4) A heat shock protein HSP 70[27], (5) CS protein[9, 10], (6) KAHRP[15], (7) a heat stable S-antigen[5], (8) a small histidine and alanine rich protein located on infected erythrocyte membranes or SHARP[28], (9) RESA[6] and (10) an antigen located on the merozoite

surface and in the parasitophorous vacuole termed ABRA[29]. An analysis of amino acid composition of repeats containing fourteen or fewer amino acids (shorter repeats) and the non-repeat regions of these antigens showed significant variation (Table 1). Most marked is the paucity of cysteine and the absence or low proportion of the hydrophobic amino acids tyrosine, tryptophan, phenylalanine, methionine, leucine and isoleucine. With increasing size of the repeat regions, it was expected that differences between repeats and non-repeats would become less evident. This was borne out in an analysis of GBP and GYMSSA (FC27) which have 50 and 32 a.a. repeats, respectively (data not shown). This result shows that the larger repeats look more like normal protein segments present in parasite antigens.

Individual analysis of the ten antigens indicates that generally the repeat regions are more hydrophilic than the corresponding non-repeat regions (Table 2). This is particularly evident when the hydrophilicity values of Parker *et al.*[30] are used in the calculation. Seven out of ten proteins contained one or more amino acids with charged side chains while the other three contained an amino acid with a polar side chain in the repeats (Table 3).

Table 1. Amino acid composition in repeat and non-repeat regions of eight *P. falciparum* proteins with short ($\leq$14 a.a.) repeats

Amino acid	Repeat		Non-repeat		
	Number of residues	%	Number of residues	%	
A	164	16.21	226	4.75	**
R	11	1.09	114	2.40	*
N	146	14.43	463	9.74	**
D	74	7.31	275	5.78	N.S.
C	1	0.10	57	1.20	**
Q	1	0.10	186	3.91	**
E	214	21.15	409	8.60	**
G	43	4.25	245	5.15	N.S.
H	68	6.72	117	2.46	**
I	0	0.00	275	5.78	**
L	9	0.89	420	8.83	**
K	79	7.81	536	11.27	**
M	4	0.40	95	2.00	**
F	0	0.00	160	3.37	**
P	60	5.93	153	3.22	**
S	50	4.94	331	6.96	*
T	28	2.77	232	4.88	*
W	0	0.00	18	0.38	*
Y	1	0.10	202	4.25	**
V	59	5.83	240	5.05	n.s.
Total no. of residues	1012		4754		

Significance by Chi-square test ** $p \leq 0.001$; * $p \leq 0.05$; N.S. = Not significant.

Table 2. Hydrophilicity of repeat (R) and non-repeat (N) regions of parasite proteins

	Protein	PMMSA		HSP70		S-antigen		CS-angiten		KAHRP	
		R	N	R	N	R	N	R	N	R	N
Total a.a.		33	1607	16	665	193	116	184	258	120	537
Mean Hydrophilicity	(1)	–0.20	0.28	–0.33	0.23	1.35	0.33	0.00	0.38	1.42	0.32
	(2)	4.00	1.90	2.33	2.04	5.03	2.54	4.50	3.13	5.62	3.33
	Protein	RESA		ABRA		SHARP		GBP		GYMSSA	
		R	N	R	N	R	N	R	N	R	N
Total a.a		233	840	106	637	127	94	549	225	64	200
Mean Hydrophilicity	(1)	1.16	0.20	2.48	2.28	–0.13	–0.09	0.70	0.75	0.15	0.11
	(2)	4.67	1.91	7.38	1.96	3.39	1.86	3.13	3.37	5.04	2.78

Mean hydrophilicity was calculated from values for individual amino acids given by (1) Hopp and Woods[41] (2) Parker *et al.*[30].

Table 3. Repeat units on *P. falciparum* antigens

Antigen (Parasite strain)	Repeat	Predicted structure
PMMSA (Wellcome)	SVASGG	T
HSP70 (FCR3)	GGMP	T
CSP (Wellcome)	NANP	T
KAHRP (NF7)	SKKHKDNEDAESVK SKGATKEAST	T T/h H
S-antigen (NF7)	AR(L) KSDEAE	T/h
SHARP (FC27)	AHHAAN	T/h
RESA (FC27)	DDEHVEEPTVA, EENVEHDA EENV	H (Tin degenerate repeat) H H
ABRA (Camp)	V(T)NDE(D)ED, KEE	T H
GYMSSA (FC27)	32 a.a.	T
GYMSSA (3D7)	GGSA	T
GBP (FCR3)	50 a.a.	T/H/S

Predictions were performed according to the method of Chou and Fasman[31] using the Prosis software program (Pharmacia-LKB, Sweden) and the most strongly predicted structures are indicated. T indicates a significant tendency to form beta turns while H and h indicate strong and weak tendencies to form alpha helices respectively. S indicates a tendency from beta sheets.

The secondary structure assumed by the repeats in native proteins can be predicted with reasonable accuracy by the method of Chou and Fasman[31]. This procedure relies partly on assigning values for alpha helix, beta sheet and beta turn forming tendencies (P_α, P_β and P_t) for individual amino acids, based on their occurrence in these conformations in proteins of known three dimensional structure. The mean P_α, P_β and P_t values for segments of the protein sequence are then calculated, and with the application of certain other rules, the probable structure for segments of the protein determined. This is illustrated in the case of GYMSSA (3D7) with a graphical output from the analysis (Fig. 1). The results of such analysis of the ten proteins show that many repeats have a probable beta turn structure in a part if not in the whole of the repeat region (Table 3). Indeed circular dichroism studies indicate that the $(NANP)_n$, repeat of *P. falciparum* CS protein and the $(GQPQAQGDGANA)_n$, repeat of the *P. knowlesi* CS protein assume a reverse turn conformation in solution[32].

Functions of the repeats

The presence of extensive repeats in many malaria parasite antigens indicates a function for the repeats in the biology of the parasite. One possibility is that the repeats constitute ligands or receptors for host proteins, with high affinity of interaction being generated through multivalent binding. In support of this hypothesis are the observations on the binding of recombinant GBP to glycophorin. The binding affinity in this case is directly related to the number of repeats in the recombinant protein[23]. Since antibodies to the GBP repeat also inhibit merozoite invasion of erythrocytes, the results suggest that the GBP repeat is a functional ligand for glycophorin[23]. However, there is no

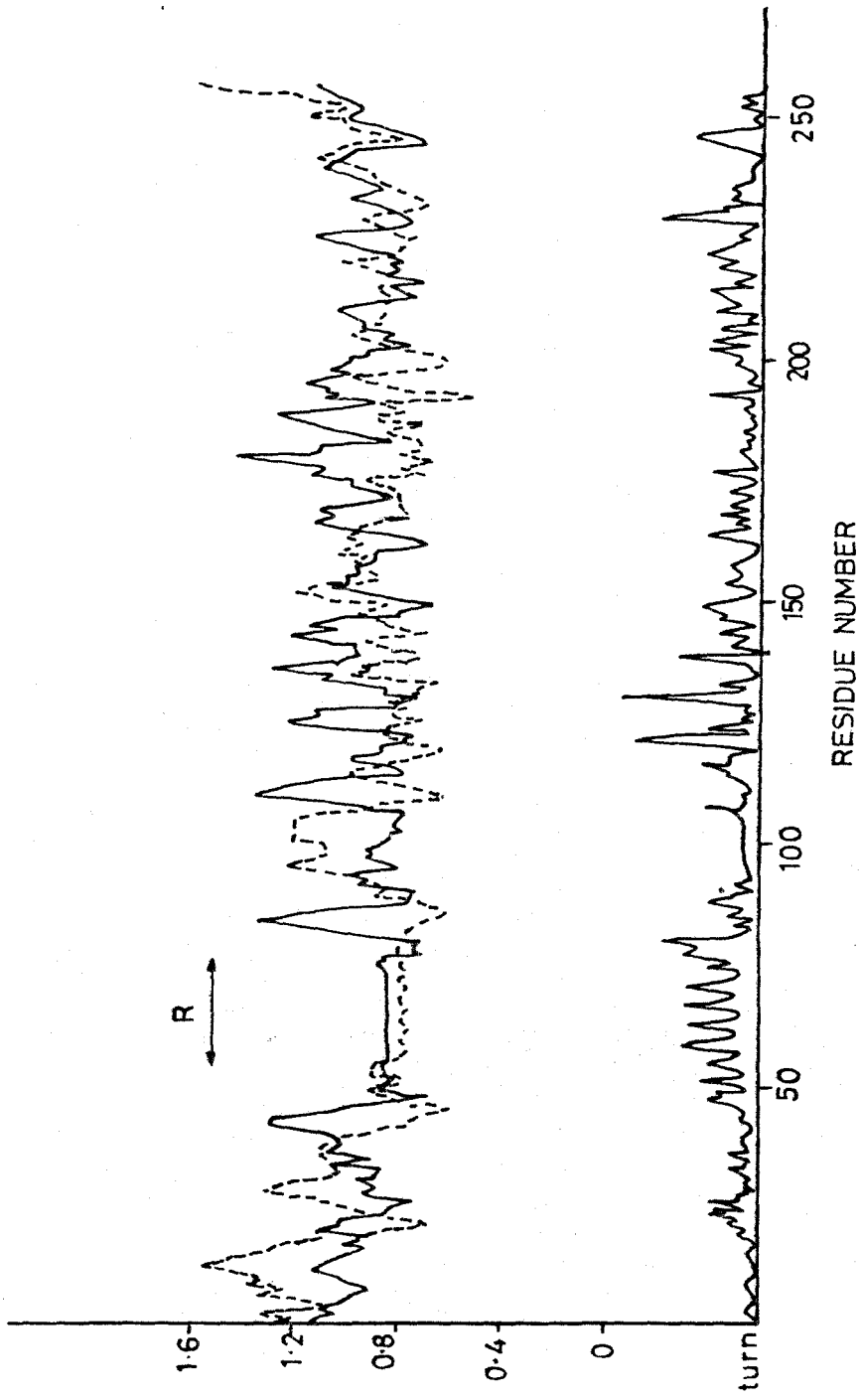

Fig. 1: Secondary structure prediction of GYMSSA (3D7 isolate) by the method of Chou and Fasman[31]. The tendencies to form α-helix and β-sheet are indicated by dotted and solid lines respectively in the middle of the figure. The tendency to form β-turn is shown by a solid line at the bottom of the figure. The region containing the repeat GGSA is from residue 57 to 76 and is marked R in the graph.

clear evidence for a similar function for repeats in other parasite proteins. Proteins with repeats that vary markedly between parasite strains are in any case unlikely to interact with the same receptor protein.

The possibility that the repeats play a role in evading or subverting a protective host immune response has been explored by a number of investigators. One suggestion has been that the highly immunogenic repeats may produce clonal exhaustion or general cell mediated immunosuppression[33]. These possibilities have not been directly tested experimentally. A second suggestion relies on the observation that there is extensive cross-reactivity between repeats in different antigens[34]. A consequence of this is an impairment of the affinity maturation of the host immune response to parasite antigens. This may be expressed as clonal exhaustion, production, of lower affinity antibodies, etc.

A third hypothesis of repeat region function related to immune-evasion has been advanced recently[35]. This is based on experiments in mice which show that the antibody response to the $(NANP)_n$, repeat of *P. falciparum* CS protein in intact sporozoites is a T-independent one[35]. This result is in contrast to synthetic $(NANP)_n$, peptides where T dependence has been extensively documented. It is postulated that the repeat regions act to induce a T-independent response by either cross-linking B-cell antigen receptors or by acting as potent B-cell epitopes to concentrate a polyclonal B-cell activating moiety on B cells specific for the repeats. Possible consequences of such a function are: (a) lower affinity antibody response; (b) a predominantly repeat region specific antibody response and (c) failure to present antigen to T-cells leading to lack of CS specific T helper cell sensitization[35].

However, not all repeat regions contain the extensive repeats seen in the CS protein. If 12-16 haptenic groups are necessary for cross-linking antigen receptors in order to activate B cells[36], this condition is not met in a large number of antigens containing repeats including GYMSSA (3D7, FC27) and PMMSA. Thus, it is possible that different repeats may generate different mechanisms of immune-evasion and that some repeats may have non-immunological functions such as binding to host receptors.

Structural characteristics of some repeats support : A possible role in immune-evasion

Many repeat region containing proteins are antigens that are externalised at some stage of the parasite life cycle and thereby exposed to the host immune system. In the eight proteins examined with short repeats (fourteen or fewer a.a.), the repeat regions are generally more hydrophilic than the rest of the proteins. This favours the location of the repeats on the exterior surface of the protein molecules. While beta turns are the most likely predicted structure for many repeats, the amino acid composition of the repeats are also consistent with the formation of the structurally related surface loops[37]. Loops and beta turns on the surface of proteins generally exhibit considerable atomic or segmental mobility[38]. Because mobile structures can assume slightly different conformations, they will contain epitopes that give rise to low affinity, cross-reactive antibodies[38, 39]. There is also a good correlation between beta turns and B-cell epitopes in many proteins[40].

It would, therefore, appear that the malaria parasite could have evolved repeats, in some antigens at least, as a means of producing surface-located, segmentally mobile structures with multimeric epitopes, that function in immune-evasion.

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Observations on the Breeding of Anophelines in Rice Fields of Shahjahanpur District, Uttar Pradesh

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Studies on ecological succession and association of anophelines in selected paddy fields of Dadraul PHC were carried out in 1988 during the paddy cultivation period from June to October. Breeding of five anophelines was observed in paddy fields. Out of the adults that emerged from larval collections the percentage of *An. subpictus* was the highest followed by *An. nigerrimus*, *An. culicifacies*, *An. annularis* and *An. barbirostris*. Further, *An. culicifacies* and *An. subpictus* breeding occurred in the early stage of rice cultivation and stopped before the breeding of *An. nigerrimus* started i.e., nearly 30 days after rice transplantation. *An. nigerrimus* breeding continued till harvesting. An inverse correlation between larval density of both *An. culicifacies* and *An. subpictus* and the height of the rice plants was observed.

INTRODUCTION

Establishment of a relationship between malaria and rice cultivation is of great importance because rice fields are a major source of anopheline breeding[1-3]. Observations of these investigators were confined either to Bengal or parts of south India, very few studies on anophelines in rice fields were made in Uttar Pradesh which is an important rice producing state of India.

Under the alternate strategy of malaria control by means of bioenvironmental improvement techniques, primary importance is given to antilarval operations[4]. Therefore, extensive search for mosquito breeding habitats and their proper management is of immense importance for mosquito control so as to achieve an effective impact on the transmission of the disease. Considering the importance of this aspect, investigations were carried out to find the pattern of anopheline breeding in relation to different stages of rice growth in paddy fields. Results of these investigations are presented in this communication.

MATERIAL AND METHODS

Paddy cultivation is mainly monsoon dependent and in Shahjahanpur district, paddy is the most important crop grown during June to October–November. Five villages viz., Bhedpur, Mauzampur, Rampur, Pipraula and Paintapur of Dadraul PHC were selected for study. In each village 3 paddy fields one each on the periphery, about 500 m and 1 km away from the village were selected. Villages were selected mainly on the basis of

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Table 1. Anopheline larval density in selected rice fields

S.No.	Location of field	Month of observation	No. of dips	Larval density					
				I & II		III & IV		Pupa	
				No.	D	No.	D	No.	D
1.	Periphery	Jun. 1988	5	42	8.4	2	0.4	0	0
		Jul.	100	402	4.02	75	0.75	1	0.01
		Aug.	90	165	1.83	25	0.27	1	0.01
		Sept.	50	81	1.62	13	0.26	0	0
		Oct.	Water level reduced leaving behind water patches						
2.	500 m away	Jun. 1988	Field under preparation						
		Jul.	90	247	2.74	66	0.73	0	0
		Aug.	80	118	1.47	18	0.22	0	0
		Sept.	45	99	2.2	20	0.44	0	0
		Oct.	Water level reduced leaving behind water patches						
3.	1 km away	Jun. 1988	10	16	1.6	0	0	0	0
		Jul.	110	319	2.9	110	1	10	0.09
		Aug.	95	147	1.54	35	0.36	1	0.01
		Sept.	35	62	1.77	6	0.17	0	0
		Oct.	Water level reduced leaving behind water patches						

extensive paddy cultivation. In each field, five paddy plants were chosen for weekly observations on their vertical and horizontal growth. Other information such as the mode of irrigation, type of insecticides or weedicides applied, fertilizer or manure used and rainfall were also recorded. Average depth of water in the fields was recorded at weekly intervals.

Seven varieties of rice are grown in the area viz., Jaya Indrasan, Seeta, Basmati type III, Pant IV and Gajraj. Lindane powder is used for pest control. Heptachlor 50% EC is being used as weedicide. Zinc sulphate is sprayed against chlorophyll specific deficiency. Paddy cultivation largely depends on rainfall except that some fields are irrigated by canal or tube-well.

In each rice field, weekly larval density was monitored using a standard dipper (500 ml capacity). Observations were made two weeks before the plantation of rice in June and were continued until 2 weeks after the harvest. Larvae were collected in containers and brought to the laboratory for adult emergence. Identification was carried out with the keys of Christophers[5] and Puri[6].

Adult mosquito density was also monitored from the villages selected for the study. Six adult catching stations, 2 human dwellings, 2 cattlesheds and 2 random stations were selected for collection of indoor resting mosquitoes from 0600 hrs to 0900 hrs. All hand collections were carried out at fortnightly intervals using suction tubes and torches. Per man hour density was calculated from these collections. One room was fixed in each village for monthly total catch using pyrethrum space spray technique.

RESULTS AND DISCUSSION

Mosquito breeding was observed irrespective of the variety of rice grown in the field. Results of larval density are given in Table 1. It may be noted that during June, rice fields situated at about 500 m from the villages were under preparation and rice transplantation was delayed. Anopheline breeding was encountered in all rice fields irrespective of their distance from the village.

From Fig. 1 it is evident that larval density per dip was highest when plant height was between 25 to 40 cms and the density varies from 39 to 43 per dip. Thereafter the larval density showed a gradual and steady decline. Densities ranged between 16 and 23 when the rice plants attained 55 to 95 cms height. The decline in larval density with the vigorous vegetative growth of paddy plants may be due to mechanical obstruction for oviposition. Similar results were obtained by Russell and Rao[2] in south India. Decrease in water level in rice fields, might have contributed to the decline in larval density of species breeding at later stages of rice growth.

Results of adult emergence and weekly records of rice growth are summarised in Table 2. It was seen that in addition to ubiquitous *Culex sp*. Five species of anophelines viz., *An. culicifacies*, *An. subpictus*, *An. annularis*, *An. nigerrimus* and *An. barbirostris* were collected in rice fields at different stages of paddy growth.

An. culicifacies breeding was observed in rice fields from the first to last week of July i.e., upto four weeks after transplantation. This observation that *An. culicifacies* larvae were found only in the early stages of paddy growth, agrees with the findings of Russell and Rao.[2] It is interesting to note that disappearance of *An. culicifacies* from the rice fields synchronized with the start of vigorous growth of the plants. *An. culicifacies* was found breeding in the stagnant water of irrigation channels and probably, these larvae were flushed into the fields especially during the first flow of irrigation water. *An. culicifacies* adults were found upto October but larvae were found only upto end of July. This suggests that rice fields are not the only source for the breeding of this species.

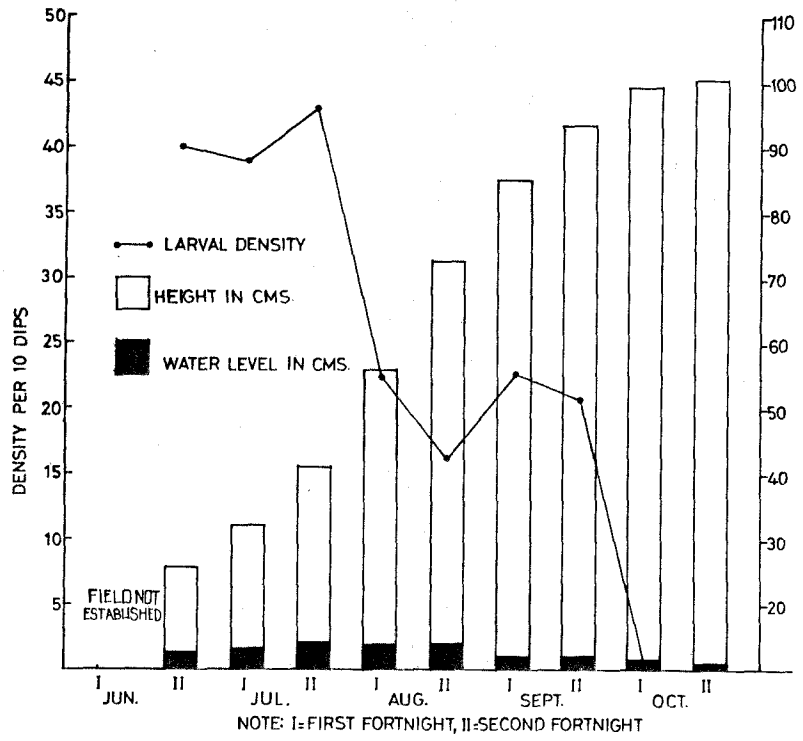

Fig. 1: Larval density and average growth of rice plants in cms.

Breeding of *An. subpictus* occurred during first week of July and continued till last week of August. The average height and distance from plant to plant during this period varied from 30.9 to 75.2 cms and 9.4 to 15.8 cms, respectively. Like many other water bodies, the water collection in rice fields also supported mixed breeding of both *An. culicifacies* and *An. subpictus*.

Breeding of *An. annularis* first appeared in the beginning of August and it was observed again in the last week of August. Its proportion varied from 3.3% to 5.9%. The average height and distance from plant to plant during the above period varied from 52.3 to 75.2 cms and 9.4 to 11.6 cms, respectively. During the present investigation, *An. annularis* prevalence was very low, but this species is considered to be a well-known rice field breeder and seeing its heavy breeding in rice fields in the submountainous area of U.P. (erstwhile United Provinces), Clyde[7] stated that all rice fields would become dangerous in the area if *An. annularis* was the vector.

An. nigerrimus first appeared in the second week of August and continued to breed till the end of October. The average height and the distance between the plants during this period varied from 60.3 to 100.3 cms and 6.2 to 10.2 cms, respectively. The proportion of *An. nigerrimus* larvae during this phase of rice growth varied from 4.3 to 100 per cent. *An. nigerrimus* was observed to be the sole breeder in the last phases of paddy growth (Table 2). The study revealed that *An. nigerrimus* breeding was never associated with that of *An. culicifacies*. Results on adult emergence from immatures collected from paddy fields further revealed that *An. barbirostris* was sparsely

Table 2. Results on anopheline emergence and growth of rice plants in selected paddy fields

Month	Week	Average growth of plants in cms.		*A. culicifacies*		*A. subpictus*		*A. annularis*		*A. nigerrimus*		*A. barbirostris*	
		Height	Distance	No.	%	No.	%	No.	%	No.	%	No.	%
Jun. 1988	IV	25.6	15.8										
	I	30.9	15.8	1	33.3	2	66.7						
	II	35.2	15.4	2	4.5	42	95.5						
	III	38.1	14.8	3	5.2	55	94.8						
	IV	44.2	12.6	2	6.9	27	93.1						
Aug.	I	52.3	11.6			21	70	1	3.3				
	II	60.3	10.2			22	95.7			1	4.3		
	III	68.2	9.6			27	69.2			12	30.8		
	IV	75.2	9.4			15	44.1	2	5.9	16	47.1	1	2.9
Sept.	I	83.9	7.9							11	100		
	II	85.8	7.7							21	100		
	III	91.6	7.3							4	100		
	IV	95.5	6.8							10	100		
Oct.	I	99	6.6							10	100		
	II	100.1	6.5							9	100		
	III	100.3	6.4							7	100		
	IV	100.3	6.2							11	100		
	Total			8	2.3	211	61.5	3	0.9	120	35	1	0.3

distributed in rice fields. It occurred only during August, the other anophelines found associated with this were *An. subpictus*, *An. annularis* and *An. nigerrimus*. Its breeding was found to be lowest as compared to either of the above mentioned species (Table 2). This indicated that rice fields may not be the preferred breeding sites of *An. barbirostris* in the area. Similar observations were made by Sen[1] in Bengal. Out of the total anopheline larvae during the study period the percentages of *An. culicifacies*, *An. subpictus*, *An. annularis*, *An. nigerrimus* and *An. barbirostris* were 2.3, 61.5, 0.9, 35 and 0.3, respectively.

It would be important to point out that breeding of *An. culicifacies* which is an important malaria vector of the area occurred in low densities in rice fields. This may be due to increased use of insecticides, fertilizers and other chemicals in agriculture. This statement is supported by the fact that extensive use of fertilizers and insecticides in paddy fields at Pattukkottai (Tamil Nadu) resulted in low adult density of *An. culicifacies* even during transmission season[3, 8].

Table 3 shows data on man hour densities of different species from June to October, collected from the fixed catching stations in the villages selected for the study. In addition to the 5 species of mosquitoes found breeding in rice fields, three additional species, viz., *An. aconitus*, *An. stephensi* and *An. pallidus* were obtained in hand collections. Breeding of *An. stephensi* in rice fields can be ruled out only during October when rice fields were almost dry and the crop was ready for harvest. Adult collection of *An. pallidus* and *An. aconitus* may indicate the probability of their breeding in paddy fields, although during the present study in spite of extensive search their breeding was not encountered in rice fields.

The MHD of *An. culicifacies* fluctuated from month to month. It was 23.5 in June and gradually rose to 34.7 in October. Density of *An. subp-*

Table 3. Per man hour density of anophelines collected from fixed catching stations of villages selected for rice field (Jun. to Oct. 1988)

S.No.	Species		Month of observation				
			Jun.	Jul.	Aug.	Sept	Oct.
1.	*A. culicifacies*	No.	212	320	347	194	132
		MHD	23.5	32	34.7	19.4	13.2
2.	*A. aconitus*	No.	0	1	1	0	32
		MHD	0	0.1	0.1	0	3.2
3.	*A. stephensi*	No.	0	0	0	0	6
		MHD	0	0	0	0	0.6
4.	*A. subpictus*	No.	462	1578	2189	727	534
		MHD	51.3	157.8	218.9	72.7	53.4
5.	*A. annularis*	No.	240	172	257	371	398
		MHD	26.6	17.2	25.7	37.1	39.8
6.	*A. pallidus*	No.	0	0	8	10	12
		MHD	0	0	0.8	1	1.2
7.	*A. barbirostris*	No.	0	0	0	2	7
		MHD	0	0	0	0.2	0.7
8.	*A. nigerrimus*	No.	0	0	0	0	1
		MHD	0	0	0	0	0.1

ictus and *An. annularis* during the above period varied from 51.3 to 218.9 and 17.2 to 39.8, respectively. Hand collection results revealed the prevalence of *An. culicifacies*, *An. subpictus* and *An. annularis* throughout the rice growing season (June to October). In hand collections *An. barbirostris* was encountered in very low numbers during September and October only. In the indoor catches *An. nigerrimus* had the lowest density, only one specimen of this species was found during October. Results of total catch showed complete absence of *An. nigerrimus*.

From these results it can be concluded that distribution and density of anopheline species in paddy fields depends on the stage of rice growth and the season of the year. These results further revealed that there existed a well defined species succession associated with different stages of rice growth.

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Biting Rhythms of Malaria Vector *Anopheles culicifacies* in Kheda District, Gujarat

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All night collections on bovine baits were made in 6 villages of Kheda district, Gujarat. During 70 all night bait collections from January to December 1989, a total of 2290 *An. culicifacies* were collected. *An. culicifacies* mostly exhibited bimodal rhythms with a seasonal shift in peak biting activities. During the cold season most biting occurred just after dusk and during the warm season late at night. *An. culicifacies* was more active during moonless hours and its activity showed no correlation with temperature and humidity during most part of the year.

INTRODUCTION

Information on the biting rhythms of mosquitoes, especially vectors is essential for determining the critical periods of potential disease transmission and maximim biting activity for scheduling the sampling efforts for various studies. Although in the past many studies have been made on the ecology and bionomics of the mosquitoes in India, few have described detailed biting rhythms. Vishwanathan *et al.*[1] made all night collections on bovine baits in specially constructed huts and studied the biting activities of *An. culicifacies* in a rural area near Pune. Reuben[2] presented observations on 18 mosquito species commonly collected in Madras (now Tamil Nadu) state. Kulkarni[3] studied the feeding behaviour of anophelines in Bastar district, Madhya Pradesh. Kumar *et al.*[4] studied the biting rhythm of mansonioides mosquitoes, the vectors of malayan filariasis in Alleppey district, Kerala. These authors provided valuable information on mosquito biting behaviour on baits but have not reported seasonal fluctuations in the biting rhythms. In Pakistan, Reisen and Aslamkhan[5] studied the biting rhythms of some mosquitoes using bovine baits and showed the seasonal shift in the feeding times of different species. The present investigation of all-night bovine bait collections was undertaken to study the night biting activity of primary malaria vector *An. culicifacies* in Kheda district, Gujarat.

MATERIAL AND METHODS

Study area

The study was carried out in six villages viz., Davda, Pansora, Sapla, Vanthvadi, Sanali and Kaniya of District Kheda, Gujarat (Fig. 1). Davda

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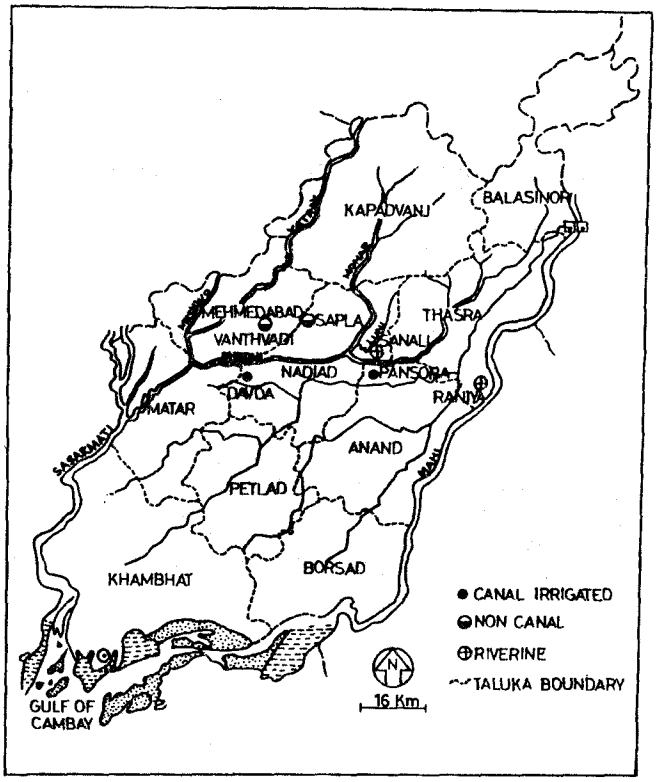

Fig. 1: Map of Kheda district showing location of the study villages.

and Pansora villages represented canal irrigated area with numerous breeding sources like canals, drains, seepage pools, wells, ponds and paddy fields. Sapla and Vanthvadi villages represented non-canal irrigated area with ponds and wells as the only perennial breeding sources. Sanali and Raniya villages situated on the banks of Shedhi and Mahi rivers, respectively, represented riverine area. In all the villages, cattlesheds are constructed very close to houses or attached to a verandah. Except during rainy days most of the population sleeps outdoors or on the verandah.

Major crops include paddy, wheat, millet and tobacco. In areas with irrigation facilities, two crops of paddy are cultivated every year during February-May and July-October.

The normal climate can be divided into three main seasons: the monsoon (mid June to mid October), the fair weather season (mid October to February) and the hot weather season (March to mid June). Monthly means of hourly observations on relative humidity and temperature recorded during the nights are presented in Fig. 2.

All-night bovine bait collections were performed once a month for one year in each village beginning January 1989, except in Vanthvadi village where collections could not be made during July and August. Outdoor bednet trap collections were made using a net of 450 x 450 x 240 cm size, hung about 15 cm above the ground with the help of supports like tree trunks, branches or bamboos. The net was provided with one entrance of over-

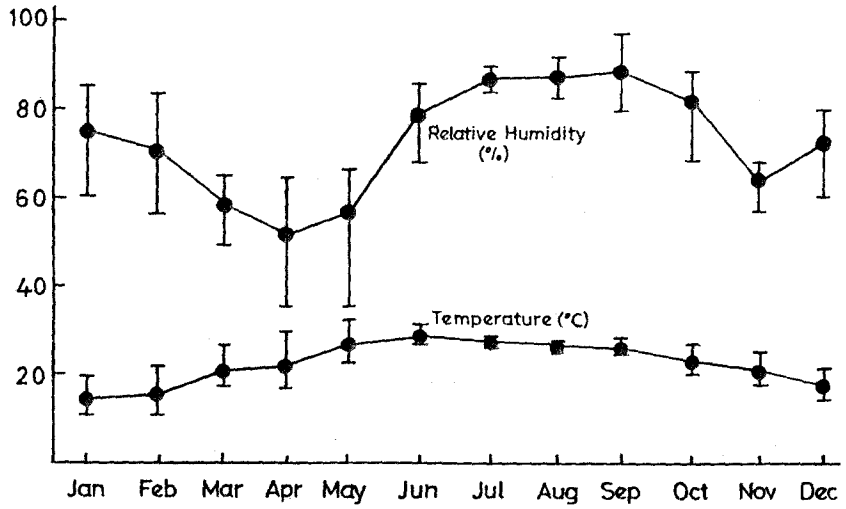

Fig. 2: Monthly mean temperature and relative humidity bracketed by minimum and maximum values recorded during the night collections in six villages.

lapping flaps and to minimise movement due to wind the lower ends were tied with weights. A bullock was tethered inside the net and collections were made in the last quarter of each hour from 1800 to 0600 hrs. To avoid any discrepancy in the collection of mosquitoes due to differential attraction to different type and size of the bait[6] only white coloured bullock was used as bait in all the collections. Keeping in view the environmental changes which have effect on the abundance and dispersal of different species within the month, collections were made preferably at a fixed site and in the first fortnight of every month. Observations on moonrise, moonset and moonphase were also recorded.

Fully-fed females resting on the inner surface of the net were carefully aspirated and transferred to 15 x 15 x 15 cm size mosquito cages after each hours collection and were brought to the field laboratory. Mosquitoes were anaesthetised with ether and only anophelines were identified up to species level using the key of Christophers[7].

As there was no significant difference in the biting rhythms among different physiographic areas, hourly collections of each village were pooled for each month for approximation of the biting curves to most favoured level. The biting rates were computed as logarithmic transforms known as Williams' mean (Mw) expressed in percentage[8].

RESULTS AND DISCUSSION

During a year long study involving 70 all-night bovine bait collections a total of 2290 *An. culicifacies* were collected. Maximum catch (1312) was made from the canal irrigated villages followed by 948 from riverine and only 30 from non-canal irrigated villages. Fig. 3 shows the biting rhythm of *An. culicifacies* during different months. From January to April there was a clear pattern of feeding in the early hours of the night with peak biting activity shifting by one hour from between 1800 to 1900 hrs in January to between 2100 to 2200 hrs in April. About 70 to 90 per cent of *An. culicifacies* population, caught during the whole-night, fed prior to midnight during these months. In May, the peak activity shifted to the second half of the night. During June and July two biting peaks were observed indicating a further shift towards second and third quarters of the night. Shift was pronounced during August and September when most biting took place in the later part of the night. Peak activity was spread between second and third

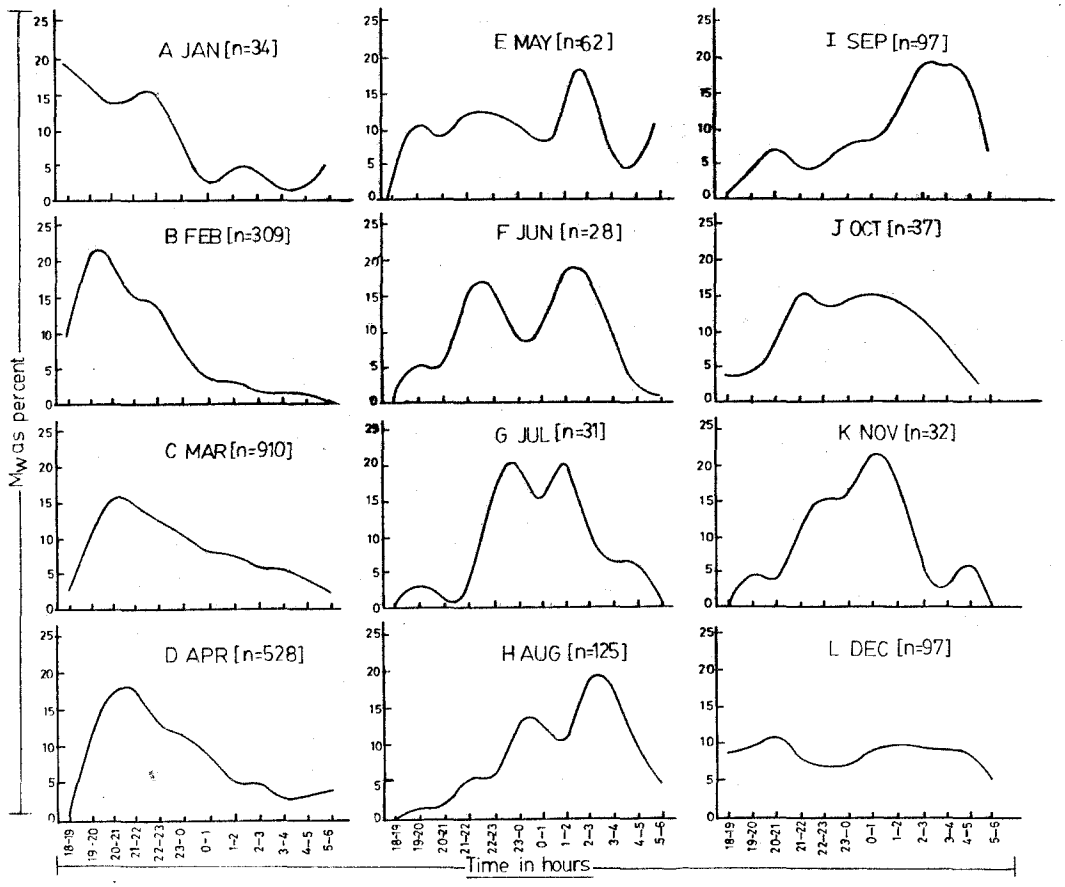

Fig. 3: Biting rhythms of *An. culicifacies* from January to December.

quarters of the night in October and November months. In December the biting activity was arhythmic and *An. culicifacies* was found equally throughout the night with statistically non-significant variations.

An. culicifacies exhibited marked seasonal shift in biting activity (Fig. 4). It was a more or less crepuscular feeder during the colder months although a few were collected late in the night. During the warm weather (May-September) it was a late-night feeder with maximum biting taking place during the third quarter of the night. It maintained the early and late night components of the biting curve in varying magnitude throughout the year.

From these observations it is apparent that biting activity varies from month to month and also between different segments of the night. There are conflicting reports in the literature about the seasonal variation in feeding times of *An. culicifacies*. Pal (1945)[9] and Reisen *et al.* (1976)[10] reported a gradual rise to a peak at 0100 hrs. Bhatia and Krishnan[11] in their review of the earlier works of Senior White in Jeypore Hill Tracts and Afridi and Puri at Delhi, suggested that maximum biting

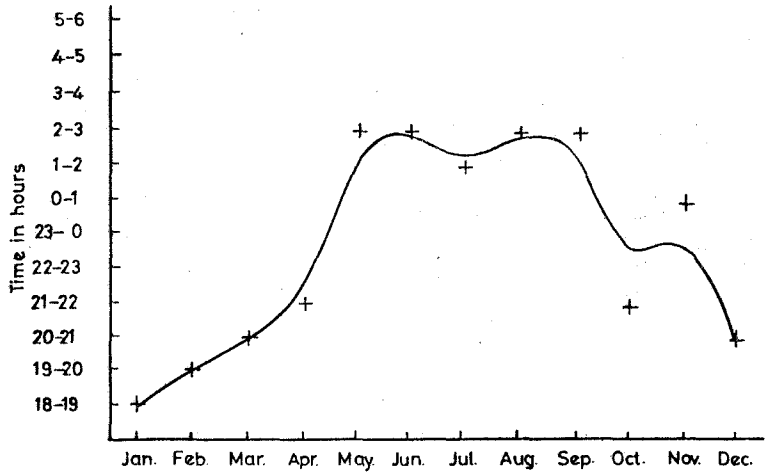

Fig. 4: Seasonal shift in peak biting activity of *An. culicifacies*.

occurs between 2230 and 2400 hrs. However, Senior White[12] found in Hazaribagh in central India that the night prevalence gradually decreased from 2300 up to 0500 hrs supporting the view that the period of highest activity was before 2300 hrs. Vishwanathan *et al.*[1] found that 67 per cent of the females entered the experimental huts to feed before midnight in a rural area near Pune. In the present investigation, 33 per cent of the females fed between 1800 to 2100 hrs, 36 per cent between 2100 to 2400 hrs, 20 per cent between 2400 to 0300 hrs and only 11 per cent between 0300 to 0600 hrs. Thus majority of the population fed prior to midnight. Present observations are in agreement to those made by Reisen and Aslamkhan[5] in Punjab Province, Pakistan. They found that *An. culicifacies* was extremely abundant throughout the year including the winter months except that marked changes occurred in its feeding times. They observed that most biting from January to April occurred prior to midnight and it shifted to midnight or early morning hours during May, June and July. The peak further shifted to the last quarter of the night during August and September months. In the present investigation all the essential components of biting *An. culicifacies* population were more or less retained.

An attempt was made to correlate the biting activity with two meteorological parameters, temperature and relative humidity recorded during different hours of the nights of each month. Biting activity was positively correlated with temperature during January ($r = 0.762$; $p < 0.001$) and February ($r = 0.888$; $p < 0.001$) months only. Strong negative correlation between biting activity and relative humidity was observed during these months ($r = -0.734$ and -0.895; $p < 0.001$). For the rest of the months the two parameters were negatively correlated with biting activity of *An. culicifacies*.

The data generated during the study were further analysed separately for moonlit and moonless hour based on the timings noted in the field and data published by Indian Express, Ahmedabad. From Fig. 5 it is apparent that *An. culicifacies* was more active during moonless hours than the moonlit hours ($t = 2.80$; $p < 0.005$). Similar observations on the effect of moonlight on vertical distribution of unfed females of *An. melas* were made by Snow[13] in west African Savanna.

From the foregoing it is clear that a single explanation for biting rhythm of *An. culicifacies* for all the

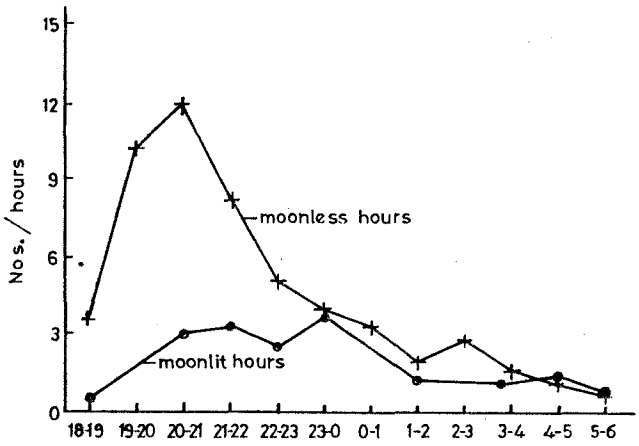

Fig. 5: Biting activity of *An. culicifacies* in relation to moonlight.

seasons would be improper. Little information is available on the physical and genetic factors influencing the biting behaviour or activity rhythms of mosquitoes. Haddow[14] suggested that a mosquito may be restrained from biting by unfavourable microclimatic conditions or unfavourable local weather and that on removal of the unfavourable factors it may be 'released' to bite. he further showed that various species of culicines bite (in nature) at a particular phase in the ovarian cycle. Reisen and Aslamkhan[5] postulated the possibility of genetic factors which could influence nocturnal behaviour on the basis of genetic causes associated with seasonal shift in the degree of anthropophilism, endophagy and endophily. Further research in the field of composition of differnt sub species, gonotrophic cycle and ovipositional behaviour may lead to more convincing explanation for the seasonal shift in the biting behaviour of *An. culicifacies*.

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Vaccination of Rhesus Monkeys Against *Plasmodium knowlesi* with Aqueous Suspension of MDP as an Adjuvant

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In these investigations two types of antigen preparations were used: (i) Whole Antigen (WAg), and (ii) Soluble Antigen (SAg). For immunization, Muramyl Dipeptide (MDP) was employed as an adjuvant. Highest reciprocal antibody titre values were recorded in ELISA and IHA tests from animals immunized by WAg-MDP. Skin reactions in animals sehsitized with WAg—MDP combination showed a well developed zone of erythema with induration after 24 hours of intradermal injection. Animals immunized with WAg-MDP showed a maximum leukocyte migration inhibition. All the animals in experimental and control groups showed patent infection following challenge with live parasites. Animals belonging to the control groups showed 100% mortality. On the basis of these experiments it could be concluded that MDP preparation afforded some protection to the test animals.

INTRODUCTION

The main task in vaccine development is generally the search of a safe and effective immunostimulant. Freund was the first person to demonstrate the importance of Freund's complete adjuvant (FCA) following its simultaneous inoculation with parasite antigen, for achieving protective immunity[1]. Although FCA as an adjuvant is very effective, its use in human subjects is considered unsafe due to various side effects[2]. In an earlier study MDP was shown to replace FCA for enhancing the immune response following its injection with mineral oil[3]. In yet another study, MDP in mineral or peanut oil was used as an adjuvant for vaccination of owl monkeys against *P. falciparum*[4,5]. Siddiqui *et al.*[6] demonstrated that stearoyl-MDP could replace FCA for effective immunization of owl monkeys against infection with *P. falciparum*[6].

In order to further evaluate the efficacy, or otherwise, of some of the above preparations, monkeys were immunized against *P. knowlesi* antigen using Muramyl dipeptide as an adjuvant. This communication describes the results of the study.

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MATERIAL AND METHODS

Experimental host: Rhesus monkeys (*Macaca mulatta*) of both sexes weighing between 2-4 kg were purchased through a local animal supplier. All the animals were healthy and their tuberculin skin tests were negative. Tests showing negative antibody titres to *P. knowlesi* indicated no prior exposure to malaria.

Antigen: The procedure for antigen isolation has been described before[7]. Protein concentration was determined by the method of Lowry *et al.*[8]. The antigenicity of the antigen samples was checked against antisera obtained from clinically positive human subjects following an infection of *P. falciparum* malaria. Serologic testing was carried out by means of IHA[9].

Adjuvant: Muramyl dipeptide (MDP) used in these experiments was a commercial preparation (Choay Chimic Reactufs, France) containing 2 mg MDP per ml of sterile, pyrogen free water.

Immunization: Twenty four adult, healthy male rhesus monkeys, each weighing 2-4 kg were divided into six groups of four animals each. All the animals were given two intramuscular injections on each flank at an interval of 21 days. The immunization schedule is shown in Table 1.

Table 1. Immunization schedule

Animal groups		Immunization dose/ml	
		1st dose	2nd dose
1.	WAg + MDP	1 mg	2 mg
		1 mg	1 mg
2.	SAg + MDP	1 mg	2 mg
		1 mg	1 mg
3.	MDP	1 mg	1 mg
4.	WAg	1 mg	2 mg
5.	SAg	1 mg	2 mg
6.	Saline	1.5 ml	1.5 ml

Muramyl dipeptide was administered at the rate of 160 µg/kg body weight.

Total volume of each inoculum was made upto 1.5 ml with the addition of normal saline.

All injections were given intramuscularly.

Each group contained 4 animals.

WAg — Whole Antigen; SAg — Soluble Antigen;

MDP — Muramyl dipeptide.

Detection of humoral immune response: The Indirect Haemagglutination (IHA) test was performed according to the method of Mathews *et al.*[9] with few modifications.

Enzyme Linked Immunosorbent Assay (ELISA) was performed according to the method of Lin *et al.*[10] with slight modification.

Detection of cell mediated immune response

Skin test: Fifteen days after the completion of immunization, all the control and test monkeys were inoculated intradermally with 29 µg of *P. knowlesi* whole antigen. The animals were kept under observation for the appearance of positive skin reactions at 24 and 48 hrs intervals.

LMIT: Leucocyte Migration Inhibition test was performed according to the method of De Lucca *et al.*[11]. The total radial area of migration was outlined with Camera Lucida, and per cent migration inhibition was calculated as follows:

$$\text{Percent Migration Inhibition} = 100 - \frac{\text{Area of migration in antigen chambers}}{\text{Area of migration in control chambers}} \times 100$$

Challenge: Three weeks after the second immunization dose, experimental monkeys were challenged with lethal doses of live *P. knowlesi* parasite. The inoculum used for challenging each monkey contained 1.5×10^6 parasitized monkey erythrocytes. The route of all such inoculations was

intravenous. Daily blood smears were prepared from all the animals upto 25th day in the post challenge period and observed for the appearance of malaria parasites. The degree of protection was checked in terms of length of prepatent period, magnitude of peak parasitaemia, survival rate during post challenge period and the percentage of animals showing patent infection following challenge.

RESULTS

Effect of MDP on humoral immune response

Serum samples from the animals immunized with WAg-MDP and SAg-MDP showed highest IHA titres in the 6th week of immunization. The animal groups comprising antigen, adjuvant and saline control monkeys showed very low antibody titres. These results are shown in Table 2.

The results of ELISA test are shown as biweekly reciprocal titre values in Table 3. Animal groups immunized with WAg-MDP showed a maximum titre of 1024 in the 4th week of immunization. The animals immunized with SAg-MDP also showed a titre value of 1024 in the sixth week. Similarly, other control groups also showed slightly raised antibody titres.

Effect of MDP on CMI response

The erythema and skin indurations as measured after 24 and 48 hrs of intradermal antigen inoculation were used to determine the extent of such reactions. Skin reaction in animals sensitized with WAg-MDP combination showed a well developed zone of erythema and induration of 11.5 mm after 24 hrs. The above skin reactions receded to 9.7 mm after a period of 48 hrs. Hypersensitivity reactions in animals immunized with SAg-MDP showed induration values of 10.9 mm and 8.9 mm at 24 and 48 hrs, respectively. Saline control animal showed skin reaction of 8.0 mm after 24 hrs.

The results of LMIT are shown in Table 4. Animals immunized with WAg-MDP showed a maximum migration inhibition (45.5 per cent).

Table 2. Reciprocal IHA titres in biweekly sera samples from immunized monkeys

Animal groups	IHA titres* (Mean ± S.D.)		
	2nd week	4th week	6th week
WAg – MDP	64 ± 45.25 (32 – 128)	256 ± 181 (128 – 512)	512 ± 362 (256 – 1024)
SAg – MDP	32 ± 22.62 (16 – 64)	128 ± 90.5 (64 – 256)	256 ± 181 (128 – 512)
MDP	16 ± 11.3 (8 – 32)	32 ± 22.62 (16 – 64)	32 ± 22 (16 – 64)
WAg	32 ± 22.62 (16 – 64)	64 ± 45.25 (32 – 128)	64 ± 45 (31 – 128)
SAg	8 ± 5.65 (4 – 16)	32 ± 22.62 (16 – 64)	32 ± 22 (16 – 64)
Saline control	Negative	Negative	Negative

Each value is the arithmetic mean of 4 values; Values in parentheses indicate the range of these values; IHA titre values less than 32 were taken as negative; WAg = Whole antigen; SAg = Soluble antigen; MDP = Muramyl dipeptide.

Table 3. Reciprocal ELISA titres in biweekly sera samples from immunized monkeys

Animal groups	ELISA titres* (Mean ± S.D.)		
	2nd week	4th week	6th week
WAg – MDP	256 ± 181 (128 – 512)	1024 ± 724 (512 – 2048)	1204 ± 724 (512 – 2048)
SAg – MDP	256 ± 181 (128 – 512)	512 ± 362 (256 – 1024)	1024 ± 724 (512 – 2048)
MDP	32 ± 22.62 (16 – 64)	64 ± 45.25 (32 – 128)	64 ± 45.25 (32 – 128)
WAg	64 ± 45.25 (32 – 128)	128 ± 90.5 (64 – 256)	128 ± 90.5 (64 – 256)
SAg	64 ± 45.25 (32 – 128)	128 ± 90.5 (64 – 256)	64 ± 45.25 (32 – 128)
Saline control	Negative	Negative	Negative

Each value is the arithmetic mean of 4 values; Values given in parentheses indicate the range of values; WAg = Whole antigen; SAg = Soluble antigen; MDP = Muramyl dipeptide.

Table 4. Results of leukocyte migration inhibition test

Animal groups	Per cent migration	Per cent migration inhibition * ± S.D.	Statistical significance
WAg – MDP	54.5	45.5 ± 5.77	$P < 0.001$
SAg – MDP	60.75	39.25 ± 7.50	$P < 0.01$
MDP	90.00	10.00 ± 7.18	$P < 0.1$
WAg	90.75	9.25 ± 5.49	$P < 0.01$
SAg	95.50	4.50 ± 2.94	$P < 0.1$
Saline	99.50	0.50 ± 0.18	—

Each value is the arithmetic mean of 4 values ± S.D; Significance was calculated by student's 't' test; WAg = Whole antigen; SAg = Soluble antigen; MDP = Muramyl dipeptide; *$P < 0.05$ are significant.

Protection studies

The various parameters used to assess the degree of protection obtained against *P. knowlesi* infection following immunization with specific antigen, in combination with MDP adjuvant, are as follows:—

(1) Length of prepatent period.

(2) Percentage of animals showing patent infection following challenge.

(3) Magnitude of peak parasitaemia.

(4) Survival rate during post-challenge period.

A maximum prepatent period of 11 days was observed in animals immunized with WAg-MDP combination. The control groups showed a pre-

Table 5. Results of challenge experiment (Protection data)

Animal groups	Average prepatent period	Average peak parasitaemia* (%) ± S.D.	Per cent survival	Average day of death
WAg–MDP	11	10.5 ± 1.83 P < 0.001	50	16
SAg–MDP	9.5	25.5 ± 11.5 P < 0.01	25	13
MDP	6.0	75.0 ± 11.5 P < 0.05	0	10
WAg	6.0	70.0 ± 18.25 P < 0.05	0	10.5
SAg	5.0	72.6 ± 9.57 P < 0.05	0	9.2
Saline	5.0	80.1 ± 9.12	0	9.0

Each reading is the arithmetic mean of 4 values; WAg = Whole antigen; SAg = Soluble antigen; MDP = Muramyl dipeptide; *P values < 0.05 are significant. Significance was calculated as student's 't' test.

patent period of 5-6 days only (Table 5). All the animals in experimental and control groups showed a patent infection following challenge. Monkeys receiving WAg-MDP showed a survival rate of 50%. Animals in the control groups exhibited 100% mortality.

DISCUSSION

Numerous workers have attempted immunization of monkeys against malaria using blood stage parasites. Since blood stage parasites produce infections which are biologically similar to natural human malaria, the use of a similar model system in experimental animals appears to be slightly advantageous in determining the vaccine effectiveness through estimation of survival rate.

MDP has been shown to augment humoral antibody response to several antigens[12]. Certain lipophilic derivatives of MDP have also been shown to be active even when administered in saline[13].

The present study was aimed to determine the immunogenicity of the two antigen preparations, the whole antigen sample and the soluble antigen for vaccination of animals. The study further attempted to determine the efficacy of MDP as an adjuvant. An earlier study of this nature was carried out by Siddiqui *et al.* in 1979. In our study, monkeys receiving WAg-MDP and SAg-MDP showed a survival rate of 50% and 25%, respectively. In a parallel experiment Freund's complete adjuvant was also used to immunize the animals. These results were used to serve as reference data for comparison with MDP immunized animals[14]. In a few previous investigations, relatively unselected peripheral blood stages of *P. falciparum* were utilized as antigens. But a complete protection of vaccinated monkeys was however not obtained[4, 5, 15]. A synthetic derivative of MDP (Nor-MDP) given in mineral oil, has proved only partially effective as an adjuvant for merozoite vaccination of *Macaca mulatta* against *P. knowlesi*[16]. The MDP, whether used as such or emulsified in oil, was ineffective in the mouse system[17]. However, when MDP emulsified in oil was used in the rat model it was able to afford 100% protection to the immunized animals following the use of antigen—MDP mixture.

Our results further confirm that malaria antigen(s) serve as better immunogens in combination with a potent adjuvant for obtaining more durable protection. The serological tests employed for the detection of anti *P. knowlesi* antibodies indicated that ELISA is a more sensitive test. Also, whole antigen preparation in this study gave comparatively better results than soluble antigen. Further, the MDP preparation in saline solution did not appear to elicit good CMI response and as such afforded little protection in this study.

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Prevalence of Malaria and Economic Loss in two Major Iron Ore Mines in Sundargarh District, Orissa

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A cross-sectional study on malaria was undertaken in May 1989 in the settlements of Kalta and Barsuan iron ore mines situated in a hilly area of Sundargarh district. Fever surveys revealed slide positivity rates of 33.9% and 34.8% in Kalta and Barsuan, respectively. Malaria infection rates as recorded through mass blood surveys in the resident population were 23.5 and 13.8%, respectively. Children upto 9 years age suffered most from malaria and in the age group of 2-9 years 37.3%, children had enlarged spleens with an average enlarged spleen (AES) index of 1.7. *P. falciparum* was the most prevalent species followed by *P. vivax* and *P. malariae*. Malaria vectors viz., *An. fluviatilis* and *An. culicifacies* were present in high densities. The present paper also brings out the economic loss due to malaria to the mining industry.

INTRODUCTION

Orissa state is endowed with vast reserves of ferrous and nonferrous minerals. Although as many as twenty different types of minerals occur in Orrisa, only a dozen of them are being exploited commercially[1]. Sundargarh district, which lies in the northwestern part of the state has 81 operational mines of various types of ores like iron (22), limestone and dolomite (21), manganese (20), quartz (7), fire clay (8), bauxite (2) and lead and zinc (Galena) (1). The district has three revenue subdivisions as shown in Fig. 1. In Bonai subdivision which lies to the south the mines are concentrated in an elevated area on the Sundergarh-Keonjhar border and the region has a tropical semi-evergreen forest. It is mainly an iron ore belt. To the north of the district, in Panposh and Sundargarh subdivisions, the terrain is rocky and the mines are of limestone, dolomite and quartz. In the western part of Sundargarh subdivision which borders Madhya Pradesh fire clay mines are located. The district has about 800 million tonnes of iron ore and 600 million tonnes of limestone and dolomite reserves. As per estimates taken in 1981 the total value of minerals produced in the district is about Rs. 30 million[2]. Several thousand labourers are employed in mining activities. Therefore, there is constant labour movement as well as congregation of labour in the settlements at mining sites. The mines are open cast and situated in forested hilly terrain intersected by a network of perennial streams which maintain mosquitogenic conditions.

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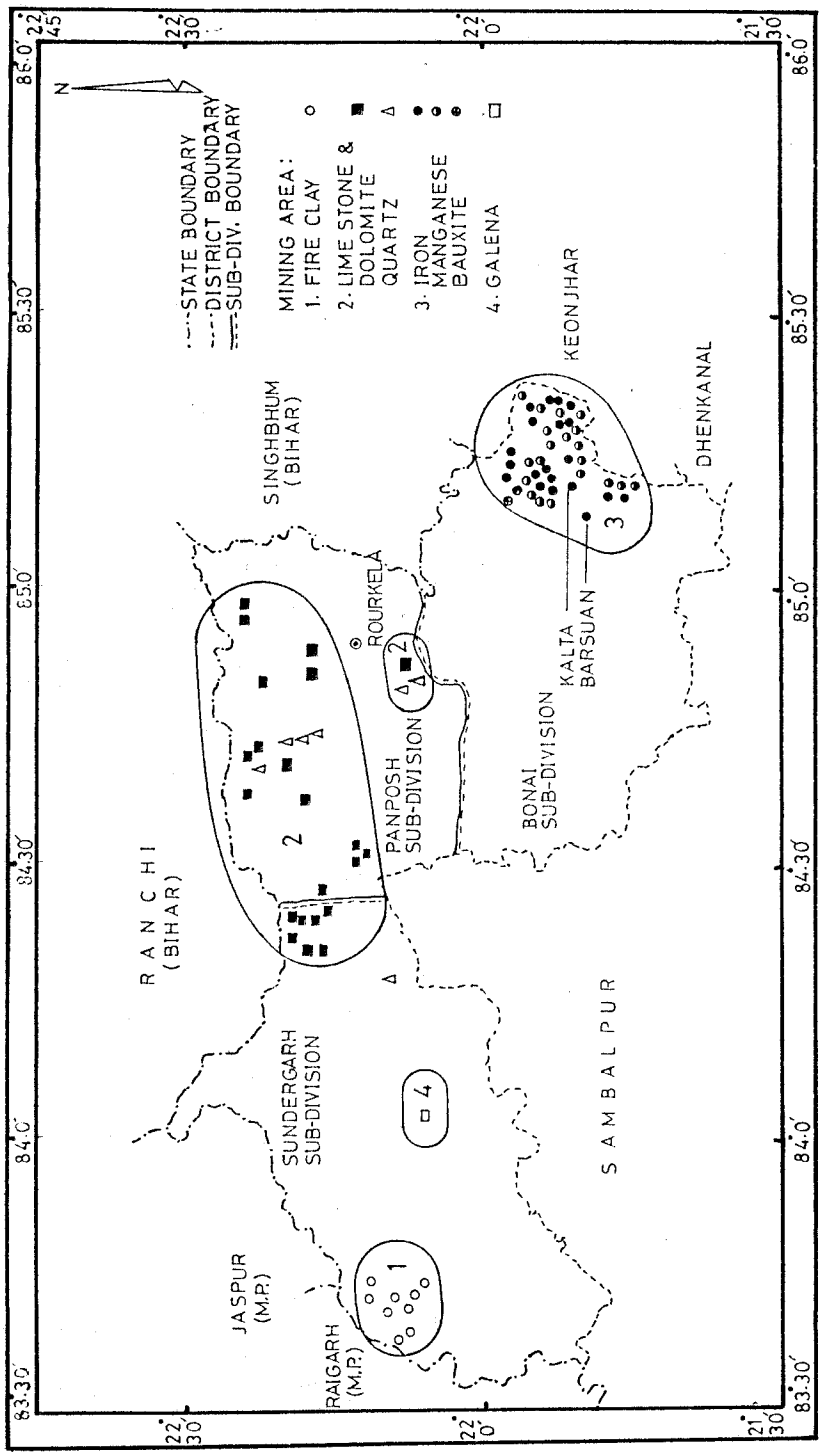

Fig. 1: Mining map of District Sundergarh showing Barsuan and Kalta iron ore mines.

Malaria in the mines is not a recent phenomenon. As reported by Senior White and Das[3] first investigations into damage caused by malaria to mining industry in Singhbhum hills were made by Christophers[4] and Watts[5]. Sinton[6] reported that malaria formed a great obstacle to the development of the mineral wealth of India in many localities. He reviewed the work of Christophers on the damage caused by malaria to the mining industry in Singhbhum areas of Orissa and observed that the high morbidity in the employees of iron and manganese mines was almost entirely due to malaria. He further quoted the report of Watts that "few diseases affect the working strength of labour gangs so much as malaria and immigration of non-immune people from healthier districts almost always gives rise to malarial epidemics of some magnitude, hence the necessity of suitable measures". Watson (1933) as described by Sinton[6] mentioned an occasion when the furnaces at the smelters were brought almost to standstill because no less than 7 trains, which had gone for ore, had been abandoned at the mines because drivers, firemen, guards and the staff at railway station and mine had gone down with malaria. Many more such reports have been given by Sinton for which original works need to be reviewed.

After the above mentioned reports, no study has been carried out on mining malaria and its economic impact, although almost six decades have passed. To ascertain the current status of malaria in the settlements of two major iron ore mines at Kalta and Barsuan, District Sundargarh and to assess the economic impact of malaria on the mining industry we conducted a cross-sectional study in May 1989. Barsuan and Kalta mines, which are located in the forest areas at an elevation (768-890 metres) about 100 km south of Rourkela city (Fig. 1), are functioning under the Steel Authority of India Limited (SAIL) since 1960 and 1966, respectively, and supply iron ore to Rourkela Steel Plant. Nearly 14,000 people including mine staff, labourers, personnel of Central Industrial Security Force and other people live in the settlements. Kalta mine has one main and two nearby labour settlements whereas Barsuan mine has two settlements viz., Tensa township and Barsuan valley.

MATERIAL AND METHODS

To begin with, hospital records on malaria morbidity at Kalta and Barsuan for the years 1986 to 1988 were reviewed. Subsequently, a point prevalence survey and a mass blood survey were carried out at both places. In the former, blood smears of all people with active fever were collected on glass slides by finger prick. In the latter case, certain representative sections of the settlements were selected and blood smears of nearly 20% of the residents were collected by taking smears of all the occupants of every fifth house. All the blood smears were stained and examined under oil immersion using Zeiss KF-2 binocular compound microscopes. Besides that, 75 children between 2 and 9 years age at Kalta settlement were also examined for presence of splenomegaly. Spleen examination was done as per Hackett's method as described by Bruce-Chwatt[7]. Blood smears of 30 children were also examined for presence of malarial parasite. Persons with *P. falciparum* infection were treated with chloroquine 600 mg base + primaquine 45 mg on Day 0, 600 mg chloroquine on Day 1 and 300 mg on Day 2 (adult doses). *P. vivax* and *P. malariae* cases were treated with chloroquine 600 mg base on Day 0 and in the former case primaquine 15 mg daily from Day 0 to Day 4 (adult doses) was also given. Children received proportionately low doses, whereas, primaquine, was not given to infants and pregnant women.

In Kalta, Barsuan and Tensa settlements mosquito collections were also made in the morning hours from 9 to 12 May 1989 using aspirator as well as space spray collection method. The collections were made for 15 minutes each in eight randomly selected houses and eight cattlesheds in each settlement. Mosquito species were identified and their man hour and room densities were recorded.

Besides this, meteorological data as recorded in Tensa in 1988 were analysed. Data on temperature

and rainfall were available, however, relative humidity was not being recorded in the observatory.

The expenditure incurred on account of treatment of malaria and mosquito control by mining establishments and labour contractors, as well as the loss that accrued to individual patients was estimated on the basis of direct and indirect information available. For this information the recurrent annual loss/expenditure was estimated under following heads:

(i) *Actual expenditure* met by mine hospitals on malaria treatment like drugs, diet and ambulance charges.

(ii) *Expenditure on anti-larval and residual spray* operations for mosquito control undertaken by mining establishments. As per 1988 estimate 1500 kg HCH and 840 litre MLO were used in mosquito control in Kalta and Tensa.

(iii) *Expenditure by the private contractors* on the treatment of malaria in labour and other staff through dispensaries.

(iv) *Man days lost* on account of sickness and subsequent debility due to malaria in mining settlements which was estimated as per the following criteria:

(a) Based on hospital records actual number of days spent by indoor malaria patients during hospitalization and subsequent rest taken on medical advice.

(b) A third (33%) of all the fever cases reporting annually to the OPDs of mine hospitals and dispensaries were excluded as they belonged to non-working age groups and in the remaining 43% were taken as malaria cases.

This was based on the fact that the annual slide positivity rate (SPR) in the area as recorded in Kalta hospital through actual blood smear examinations from 1986 to 1988 was 43%. For the total malaria cases estimated in that manner the loss of man days was calculated @ 5 days lost per malaria case. The average 5 day period per malaria case was taken after the analysis of sickness and sick-leave records of the OPD of Kalta hospital and also on confirmation during our survey.

For monetary estimation the man days lost were converted into equivalent wage loss on the basis of average prevailing wage rate i.e., Rs. 50 per day for every employee of Kalta and Tensa mines and Rs. 17 per day for every labourer or other person reporting to the contractor's dispensary in Kalta and in Barsuan valley dispensary.

For a more direct assessment of economic loss to the individual malaria patient, 240 persons, who had actually suffered from malaria during the period from May 1988 to April 1989, were contacted and interviewed by the survey team. These people included mining staff of the steel plant, labourers and other local residents in the settlements.

RESULTS AND DISCUSSION

Data on the malaria morbidity as gathered from the records of Tensa and Kalta mine hospitals for the years 1986 to 1988 and Barsuan dispensary for the years 1987 and 1988 have been given in Table 1. Out of over 4200 fever cases reporting to the OPD of Tensa hospital each year, between 68.2 and 73.1% cases were treated for malaria, mostly on clinical basis without blood smear examinations. Out of over 1900 indoor admissions each year, between 46.6 and 49% received treatment for malaria and in most cases treatment was given after blood smear examinations. In Barsuan dispensary laboratory facility did not exist and malaria was diagnosed on the basis of clinical symptoms.

In Kalta hospital, however, the cases were treated after blood smear examination. Out of 1752, 1902 and 442 fever cases reporting to OPD in 1986, 1987

and 1988, respectively, 741 (42.3%), 827 (43.5%) and 206 (46.6%) persons were treated for malaria. Out of over 500 indoor admissions each year between 53.5 and 62.2% cases were treated for malaria in different years. In both the hospitals uncomplicated malaria cases were treated orally which chloroquine and primaquine or sometimes even with Metakelfin, but the persons with moderate to severe complications were administered either chloroquine (i.m.) or quinine (i.v.) depending upon the general condition of the patient.

In Kalta a dispensary was also being run by a private company for the treatment of their labourers and other staff. During our observation for two hours in the dispensary we found that 35 out of 72 patients with fever reporting to the dispensary were diagnosed as malaria cases clinically in the absence of blood smear examination facility and administered chloroquine (i.m.) with follow-up doses of Lariago. In some cases Lariago was being prescribed alone or in combination with tetracycline. By and large malaria appeared to be the most common disease afflicting the residents of the mines.

For assessment of the true picture of malaria, parasitological data collected during the survey is presented in Table 2. It is apparent that in Barsuan and Kalta mine settlements 34.8 and 33.9% fever cases, respectively, were found malaria positive. Records of the Health Department showed that only *Plasmodium falciparum* and *P. vivax* species were known to exist, however, we recorded *P. malariae* also in that area. Out of 66 positive cases recorded from both the mining areas in prevalence survey there were 47 (71.2%) *P. falciparum*, 16 (24.2%) *P. vivax*, 1 (1.5%) *P. malariae* and 2 (3%) cases of mixed infections.

The results of mass blood survey revealed that 13.8% and 23.5% of all the slides collected in Barsuan and Kalta, respectively were found malaria positive, thereby suggesting a high infection rate among the community. From both the areas 6 cases of mixed infections (*P. vivax* + *P. falciparum* 4 cases; *P. malariae* + *P. falciparum* 1 case and *P. vivax* + *P. malariae* + *P. falciparum* 1 case) were also recorded.

Age-specific infection rate recorded in the prevalence survey has been presented in Fig. 2. It is evident that children upto 9 years age suffered most, with remaining groups having comparatively lesser prevalence, although considerably high.

Out of 75 children examined for splenomegaly 28 (37.3%) had palpable spleen (Class I to III) and the Average Enlarged Spleen (AES) index was 1.7, thereby indicating high malaria endemicity. Out of 12 blood smears taken from children with palpable spleens, 2 (16.6%) were positive for malaria. Out of the smears of 18 children having normal spleens, 10 (55.5%) had malaria which included 2 *P. vivax*, 1 *P. malariae* and 7 *P. falciparum* cases.

During entomological collections, five *Anopheles* and three *Culex* species were recorded from the settlements (Table 3). Among the well-known malaria vectors were *An. culicifacies* and *An. fluviatilis*. Man hour densities of *An. culicifacies* in Tensa and Barsuan valley and Kalta were 9.3 and 37.5, respectively, whereas, densities per room in above settlements were 1.2 and 2.5, respectively. *An. fluviatilis* was found only in Kalta with a man hour density of 6.5 and per room density of 0.5. Thus, the areas had high densities of malaria vectors. Of particular interest was the presence of *An. fluviatilis* which is a very efficient vector and has a highly anthrophilic nature. Among the culicine species *Cx. quinquefasciatus* (vector of bancroftian filariasis), *Cx. tritaeniorhynchus* and *Cx. bitaeniorhynchus* (both vectors of Japanese encephalitis) were recorded.

Analysis of the temperature and rainfall data suggested that the climate in the mining area was highly conducive to mosquito multiplication and longevity. In 1988 except for January and December the area received rainfall in all the remaining ten months with an annual precipitation of 1719 mm. However, nearly 85% of the rainfall occurred

Table 1. Malaria cases treated in iron mine hospitals

Hospital/ Dispensary	Years	OPD cases			Indoor cases		
		Total fever cases attended	No. of cases treated for malaria	Percentage of cases in all fever cases	Total cases admitted	No. of cases treated for malaria	Percentage of malaria cases in all fever cases
Tensa	1986	4260	2907	68.2	1925	933	48.5
	1987	4201	2870	68.3	2009	936	46.6
	1988	4404	3219	73.1	2003	982	49.0
Kalta	1986	1752	741	42.3	567	353	62.2
	1987	1902	827	43.5	584	322	55.1
	1988	442	206	46.6	534	286	53.5
Barsuan	1987	5357	2890	53.9	—	—	—
Dispensary	1988	4763	2570	53.9	—	—	—

— Indoor treatment facility did not exist.

Table 2. Results of the point prevalence and mass blood surveys in the mining settlements

Type of survey	Name of settlement	Total population	Population actually surveyed	Blood smears examined	Malaria							Slide positivity rate	Slide falciparum rate	Pf % (including mix cases of Pf)
					Pv	Pf	Pm	Pv + Pm	Pm + Pf	Pv+Pf +Pm	Total			
Point prevalence	1.Barsuan and Tensa	8090	8090	89	7	23	1	0	0	0	31	34.8	25.8	74.2
	2.Kalta	5658	5658	103	9	24	0	2	0	0	35	33.9	25.2	74.3
Mass blood survey	1.Barsuan and Tensa	8090	1675	340	13	32	0	1	1	0	47	13.8	10.0	72.3
	2.Kalta	5658	1100	234	12	39	0	30	0	1	55	23.5	18.4	78.2

Pv — *P. vivax*; Pm — *P. malariae*; Pf — *P. falciparum*.

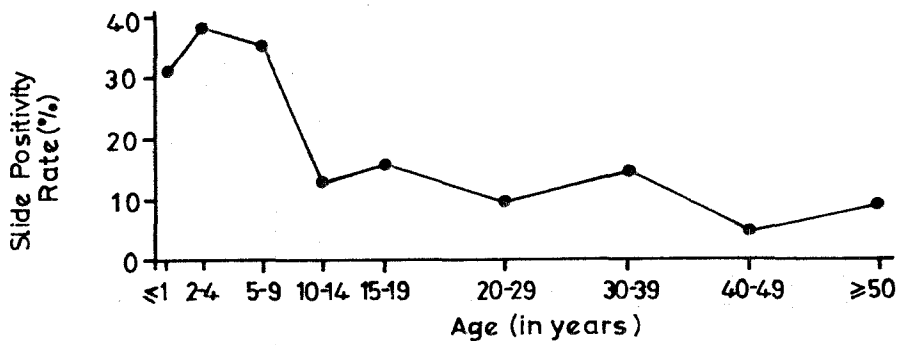

Fig. 2: Age-specific malaria infection rate in mining settlements.

from June to October. The average temperature ranged between 17°C in January and 31.2°C in May.

It is not possible to estimate the monetary loss caused by malaria in the community in absolute terms as the loss of human life or the chronic morbidity resulting into debility and poor health cannot be assessed in terms of money. Nevertheless, an estimated loss caused by malaria to people involved in mining industry has been made based on certain directly and indirectly quantifiable parameters (Table 4). For protecting a population of 13,748 residents in two mining settlements the annual expenditure/loss due to malaria stood at Rs. 1.1 million i.e., Rs. 80 per capita per year.

Survey among 240 malaria patients in the category of labourers, regular employees and others revealed that the per capita annual expenditure/loss per episode of malaria to the individuals was Rs. 178, Rs. 78 and Rs. 228, respectivley (Table 5). Highest individual loss was suffered by the people in the category of businessmen/others due to relatively higher expenditure per person on treatment, special diet and higher wage losses. This was followed by the labourers whose expenditure on treatment and loss in wages was higher than that of the regular mine staff. Labourers spent relatively less money on special diet. Individual loss to the mine staff was least among all owing mainly to no personal loss incurred on wages due to sickness. Expenditure on conveyance and spiritual care was

Table 3. Mosquito density in mining settlements

Species	Man hour density		Density per room	
	Tensa and Barsuan valley	Kalta	Tensa and Barsuan valley	Kalta
An. culicifacies	9.3	37.5	1.2	2.5
An. fluviatilis	0	6.5	0	0.5
An. annularis	0.7	8.0	0.2	1.0
An. subpictus	6.7	2.5	2.5	0.5
An. vagus	1.3	5.0	1.0	0
Cx. quinquefasciatus	38.2	5.0	17.7	2.5
Cx. tritaeniorhynchus	4.6	6.0	3.0	0
Cx. bitaeniorhynchus	0	3.5	0	3.0
Total	60.8	74.0	25.6	10.0

Table 4. Expenditure and loss due to malaria in mine (1988)*

I.	Expenditure by mining establishments		
	(i) Cost of treatment, diet and ambulance charges	Rs.	2,43,500.00
	(ii) Expenditure on anti-larval and residual spray operations for mosquito control	Rs.	22,446.00
II.	Expenditure by private contractors on treatment of their labourers and other staff	Rs.	20,000.00
III.	Loss due to sickness/debility		
	(i) 7166 man days lost at Tensa converted @ Rs. 50 per day	Rs.	3,58,300.00
	(ii) 3556 man days lost at Kalta converted @ Rs. 50 per day	Rs.	1,77,800.00
	(iii) 16,635 man days lost at Barsuan valley and Kalta labour settlements converted @ Rs. 17 per day	Rs.	2,82,795.00
	Total	Rs.	11,04,841.00

* Population : (i) Kalta : 5658, comprising of nearly 75% labourers; (ii) Tensa township : 5509, comprising of nearly 10% labourers and remaining SAIL employees and their dependents; and (iii) Barsuan valley : 2581, comprising of nearly 90% labourers.

Table 5. Annual economic loss due to malaria to the individuals of mining settlements (May 1988 to April 1989)

Category	Total no. of malaria patients interviewed	Average no. of man days lost per malaria episode	Expenditure loss (in Rs.)						
			Treatment	Special diet	Conveyance	Spiritual care	Loss to the nursing relatives	Income/ wage loss	Average annual loss/expenditure per patient/per episode
1. Labourers	121 (167)	7.6	36.49	22.52	0.30	0.15	9.21	109.71	178.38
2. Regular mine employees	105 (149)	5.4	16.40	55.57	0.80	0	5.16	0	77.93
3. Businessmen/ others	14 (42)	3.9	58.88	29.4	0.47	0	0	139.76	228.51

Figures in parentheses are total malaria cases occurred in the respective group due to multiple episodes.

almost negligible in all groups. From Table 5 it is further evident that average number of man days lost in each malaria episode in labourers was highest (7.6 days) followed by employees (5.4 days) and others (3.9 days). This is probably due to the reason that malaria afflicted the labourers in the low socio-economic group more seriously and longer than normal perhaps due to untimely or improper treatment. On the contrary other groups would have afforded better nourishment besides taking timely treatment.

In conclusion, malaria in mining areas of Sundergarh prevails in alarming proportions causing enormous economic loss. People employed in the mining industry are at a high risk of getting malaria and health authorities should heed this serious problem.

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The Haematology of *Plasmodium vivax* before and after Chloroquine and Primaquine Treatment in North Madras Area

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Changes in haematological parameters were studied in 35 *Plasmodium vivax* infected patients and compared with those in an equal number of normal subjects. Patients showed a high proportion of schizonts of *P. vivax* (1-2%). Hb, PCV and RBC values were significantly decreased ($p < 0.001$) with increasing parasitaemia. Osmotic fragility was slightly increased (15%) when compared to controls and ranged from 0.385-0.405 (50% hypo-osmotic haemolysis given at gm/dl of NaCl) with increasing parasitaemia in the patients. Decreased levels of lymphocyte and increased levels of eosinophils and monocytes were seen in *P. vivax* infected patients. However, after treatment with chloroquine and primaquine, all the haematological parameters were restored to near normal levels.

INTRODUCTION

Most malarial research has been conducted in endemic areas like Africa, infected with *P. falciparum*[1,2]. Very little study has been done on *P. vivax* malaria because of its racial insusceptibility[3], and because, unlike *P. falciparum* malaria, it is not a life threatening infection. However, it is important on account of the morbidity and debility it produces as a result of relapses. Man is the natural host of *P. vivax*[4], which is responsible for the highest incidence of malaria in the world[5]. This also has the widest distribution occurring through most of the temperate zone and the tropics as well[6]. Frequent relapses due to the presence of hypnozoites are observed in *P. vivax* and not in *P. falciparum* infection[7,8].

Malaria in India is predominantly *P. vivax* (70%), in Madras this proportion is 90%[9]. Therefore, studies were undertaken to elucidate the haematological changes before and after chloroquine and primaquine administration.

MATERIAL AND METHODS

The 55th division of Madras city was selected for this study. Most of the infected patients were economically and educationally backward. Further, this endemic area contained unapproachable open overhead tanks conducive for the growth of the *Anopheles* vector.

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Table 1. Levels of Hb, PCV, RBC indices in whole blood of malaria patients before and after treatment

Measurement	Units	Control (n=35)	Pre-treatment (n=35)	After treatment (n=15)
Haemoglobin	g/dl	14.85 ± 1.00	13.31 ± 1.68†	14.04 ± 0.69
Packed cell volume	%	47.50 ± 3.00	42.50 ± 6.00†	44.00 ± 2.00
Red blood cell count	10^6cells/cu. mm	4.99 ± 0.44	4.55 ± 0.79*	4.84 ± 0.55
Mean corpuscular volume	per cu. μ	94.78 ± 9.13	93.40 ± 7.82‡	90.90 ± 7.50
Mean corpuscular haemoglobin	μ μg	29.98 ± 2.35	29.41 ± 2.14‡	28.70 ± 2.20
Mean corpuscular haemoglobin concentration	%	31.28 ± 1.59	31.31 ± 5.29‡	31.59 ± 1.04

For statistical evaluation of significant variation, the values of malarial patients were compared with that of the control group.
† $p < 0.001$; * $p < 0.01$; ‡ - Non-significant.

Table 2. Levels of Hb, PCV, RBC count and RBC indices with increasing parasitic density of malaria patients

Measurements	Units	Control (n = 35)	50-150 parasites/ cu. mm of blood (n = 18)	150-250 parasites/ cu. mm of blood (n = 8)	250-350 parasites/ cu. mm of blood (n = 4)	350-450 parasites/ cu. mm of blood (n = 4)
			(A)	(B)	(C)	(D)
Haemoglobin	g/dl	14.85 ± 1.00	14.32 ± 1.07†‡b,c	11.34 ± 3.30†d,e	11.05 ± 0.55†	10.35 ± 0.31†
Packed cell volume	%	47.50 ± 3.00	46.22 ± 3.52†‡b,c	39.33 ± 0.04†·e	33.50 ± 2.50†	31.50 ± 0.56†
RBC count	X 10^6 cells/cu. mm	4.99 ± 0.44	4.94 ± 0.49†‡b,c	4.37 ± 0.74†‡d,e	3.45 ± 0.05†	3.01 ± 0.31†
Mean cellular volume	per cu. μ	94.78 ± 9.13	93.51 ± 7.46‡	92.66 ± 9.12‡	90.75 ± 8.25‡	92.80 ± 1.00‡
Mean cellular haemoglobin	per μ μg	29.89 ± 2.35	32.29 ± 14.07*	29.51 ± 2.34‡	32.05 ± 2.05‡	29.62 ± 1.63*
Mean corpuscular haemoglobin concentration	%	31.28 ± 1.59	31.03 ± 1.95‡	31.10 ± 3.20‡	33.00 ± 0.80*	31.90 ± 1.40‡

For statistical evaluation of significant variation, the values with increasing parasitaemia (A,B,C,D) were compared with that of the control group.

† $p < 0.001$; * $p < 0.05$; ‡ - Non-significant.

b - Significantly different when compared with groups A and C; c - Significantly different when compared with groups A and D; d - Significantly different when compared with groups B and C; e - Significantly different when compared with groups B and D.

Blood samples were obtained from patients who reported to the Central Malaria Clinic, Elephant Gate, Madras. They were screened microscopically for the presence of the malarial parasite. Age and sex matched control samples were obtained from the same area.

Samples were taken from patients who tested positive for *P. vivax* with high proportion of schizonts on the first day and before any drug treatment (n = 35). Samples were also obtained from 15 infected patients on the 10th day after the commencement of drug treatment. The treatment consisted of an oral dose of 600 mg of chloroquine and 30 mg of primaquine on the first and second days followed by 15 mg of primaquine on the third day.

The haematological parameters studied were haemoglobin (Hb), packed cell volume (PCV), RBC, total, differential and platelet counts by routine laboratory methods as described by Wolf[10]. Thin blood smears were examined for red cell abnormalities. Osmotic fragility test was carried out by the method of Dacie and Lewis[11]. Parasitic density was determined by the method of Plinder[12].

Statistical significance was evaluated by student 't' test and the effect of parasitaemia by 'analysis of variance'.

RESULTS

Hb, PCV and RBC values were significantly decreased ($p < 0.001$), while insignificant changes were noticed in the levels of Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) in malaria patients, compared to controls (Table 1). Levels of the Hb, PCV and RBC count were significantly reduced ($p < 0.01$) with increasing parasitaemia, i.e., above 250 parasites/cu. mm (Table 2).

Red cell abnormalities observed in malarial patients were expressed as number of cells/100 RBC. The levels of reticulocytes, poikilocytes, hypochromic cells and parasite infected cells were 1.63, 2.66, 4.44 and 0.80, respectively before therapy. However, the values were decreased to 1.0, 1.8, 1.2 and 0 after therapy in all types of cells ($p < 0.01$).

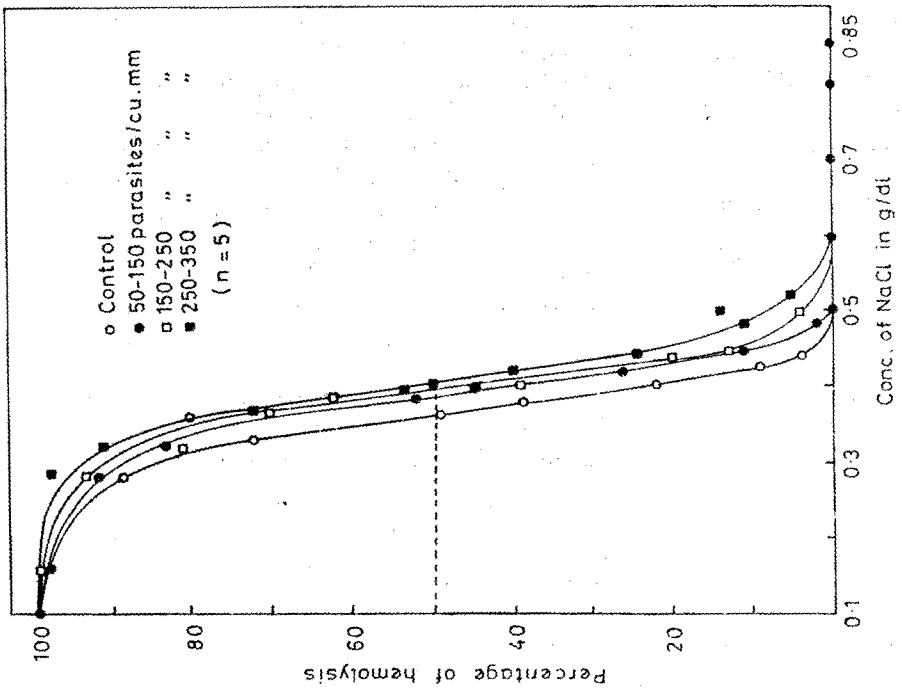
A marked decrease in the levels of lymphocytes ($p < 0.01$) and increase in eosinophils ($p < 0.1$) and monocytes ($p < 0.001$) without significant changes in leukocytes and platelet counts were observed in malarial patients when compared to controls (Table 3).

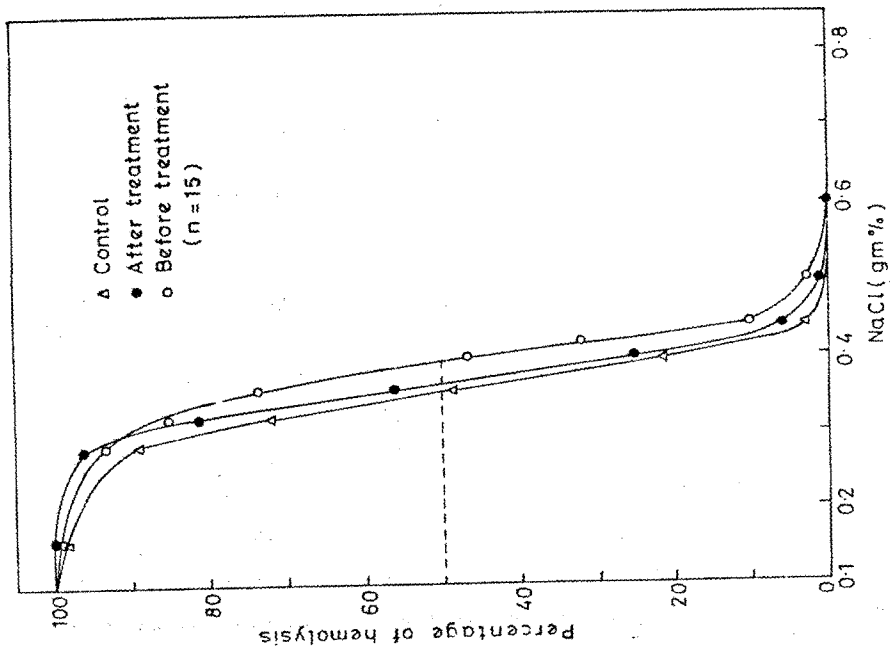
The osmotic fragility of RBC was studied before and after treatment (Fig. 1) and also at different levels of parasitaemia (Fig. 2). Malarial RBCs

Table 3. Levels of total count, differential count, platelet count observed in malaria patients before and after treatment with controls

Measurements	Units	Control (n = 35)	Pre-treatment (n = 35)	After treatment (n = 15)
Total leucocyte count	Cells/cu. mm of blood	7350 ±1650	6800 ±1550‡	6950 ±2850
Neutrophil	%	51.10 ± 8.10	58.30 ±11.00‡	50.50 ±10.30
Eosinophil	%	3.70 ± 2.20	5.30 ± 3.13*	3.10 ± 2.30
Lymphocytes	%	43.00 ± 7.00	37.00 ±11.00§	47.10 ± 9.20
Monocytes	%	1.50 ± 1.00	3.00 ± 2.10†	2.30 ± 1.80
Platelets	x 10^5 cells/cu. mm of blood	2.87 ± 0.36	2.70 ± 0.34	2.68 ± 0.32

For statistical evaluation of significant variation, the value of malaria patients were compared with that of the control groups. * $p < 0.1$; § $p < 0.01$; † $p < 0.001$; ‡ - Non-significant.





Figs. 1 & 2: Osmotic fragility test.

were found to be osmotically more fragile than that of controls. Treatment with drugs restored these levels to normal (Fig. 2). Fifty per cent haemolysis was observed at 0.4% saline solution (range 0.40-0.43) for malaria infected RBCs while normal RBCs showed 0.36 (range 0.312-0.395).

Further, osmotic fragility was found to increase with increasing parasitic density. The values were 0.36%, 0.39%, 0.39% and 0.40% at parasitic densities of 0, 50-150, 150-250 and 250-350, respectively (Fig. 2).

DISCUSSION

In this study, *P. vivax* infected patients have shown mild anaemia, increased osmotic fragility, increased monocytes and eosinophils. These effects correlate with increasing parasitaemia. All these changes are normalised after treatment. Similar observations of mild anaemia have been reported in *P. vivax* malaria with increasing parasitaemia[7]. However, the severity is not comparable to that reported in *P. falciparum*. This may be due to the fact that *P. vivax* prefers younger, lighter red cells and reticulocytes as reported by Kitchen[13]. Based on the values of MCV, MCH and MCHC it is assumed that the observed anaemia is not due to the deficiency of iron or folic acid but may be associated with *P. vivax* infection.

Red cell morphological abnormalities such as hypochromasia, polychromasia and poikilocytes are found less markedly in *P. vivax* infected patients when compared to that of *P. falciparum* infected patients[14,15]. Further P. vivax infected RBCs are found to be enlarged with subsequent hypochromasia. Similar observations of hypochromasia have been reported in *P. falciparum* infected RBCs[16].

Osmotic fragility is increased significantly in *P. vivax* infection and the fragility increases with parasitaemia. Similar observations have been reported in *P. berghei* infected RBCs[17]. This increased fragility may be associated with depletion of cholesterol from the RBC membrane as suggested by Sherman[18].

In *P. vivax* infection, decreased lymphocytes, increased monocytes and eosinophils have been observed without significant changes in total leucocyte, neutrophil and platelet counts. A similar observation of decreased lymphocytes, increased monocytes and eosinophils have been reported in *P. falciparum* infection[15].

All the above parameters have been normalized, following chloroquine and primaquine therapy.

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Epidemiological Observations on Malaria in some parts of Tengakhat PHC, Dibrugarh District, Assam

P. DUTTA*, D.R. BHATTACHARYYA* and L.P. DUTTA*

A three year study carried out in 44 villages (pop. 17,938) reveals seasonal transmission of malaria i.e., from June to November. The incidence of malaria was high in the year 1988 in comparison to that of 1987 and 1989. *P. falciparum* was the predominant species. The population living in forest fringe areas were more prone to malaria than the rest. *Anopheles nigerrimus*, *An. kochi*, *An. karwari* and *An. philippinensis* were most abundant among the 16 anopheline species collected. Known vectors of malaria such as *An. minimus*, *An. philippinensis*, *An. annularis* and *An. dirus* were detected. *An. dirus* was incriminated as vector during this study.

INTRODUCTION

Many parts of northeast India are highly afflicted with the problem of malaria. When *P. falciparum* infection in relation to resurgence of malaria is considered, there is a steady increase in the total number of cases as well as its ratio to total cases in the northeastern region[1]. Although *P. vivax* was the predominant species earlier in recent years, a greater prevalence of *P. falciparum* is observed. The resurgence of *P. falciparum* malaria is becoming a major problem in the foothill areas of the region. In Assam, malaria is still a great problem in the areas which neighbour hill states like Arunachal Pradesh, Nagaland, Meghalaya, Manipur, Mizoram etc.

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The present study was conducted in some parts of Tengakhat PHC of Dibrugarh district, Assam during 1987-89 to know the true incidence of malaria, the seasonal prevalence of parasite species, to survey the anopheline fauna and detect the vector/vectors responsible for the current transmission of malaria etc. A preliminary report on vector incrimination has already been published[2]. The details of those observations are presented in this paper.

MATERIAL AND METHODS

Some parts of Tengakhat PHC of Dibrugarh district, Assam which are not far away from the Arunachal Pradesh border (aerial distance is very short) were selected for this study. The area is about 75 km away from the Regional Medical Research Centre (ICMR), Dibrugarh and is very near to the oil town of Duliajan. It has a heterogeneous terrain with foothills, riverbeds, tea gardens, paddy fields and on one side there is a large

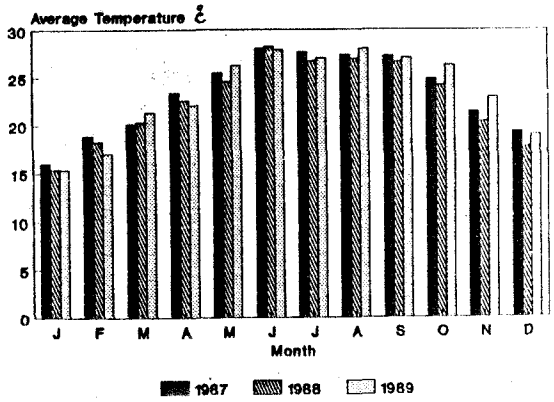

Fig. 1a: Temperature graph.

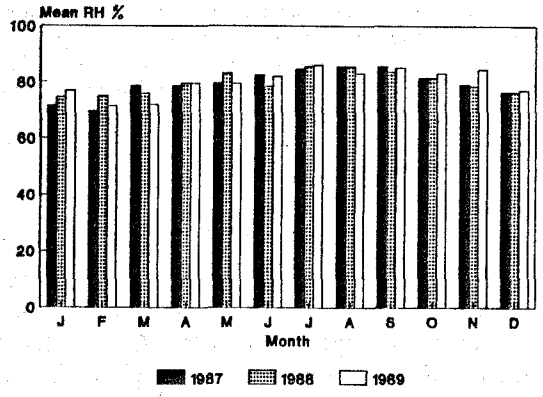

Fig. 1b: Relative humidity graph.

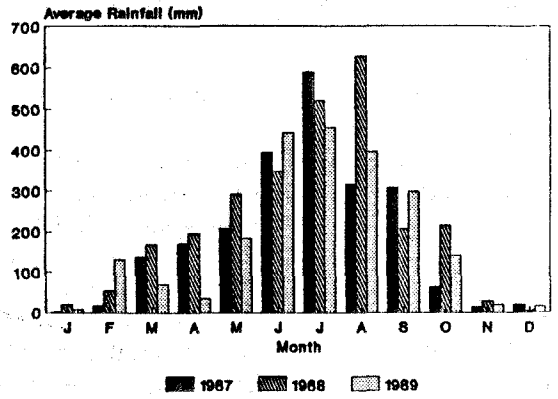

Fig. 1c: Rainfall graph.

forest range (Charaipung Forest Reserve). It consists of 44 villages with a population of 17,938. The population consists mostly of Ahom and Kachari tribes and tea garden labourers. The occupation of the majority of them is as tea garden workers and cultivators and a few are daily wage forest workers. For the purpose of the investigation, the area was divided into 4 sectors and in each sector, one surveillance worker was recruited. The surveillance workers enumerated the houses and collected information on a predesigned proforma. A weekly house to house survey was carried out by them to record fever cases in order to detect malaria patients. Fever cases were first registered and information on age, sex, name of the patient, name of the village, history of malaria, complaints of illness and drugs already taken etc. were noted. Blood smears were taken from the fever cases, stained with JSB and examined under the microscope for parasites. For presumptive treatment, all the fever cases were treated with 600mg of chloroquine (adult dose). After confirmation of parasites, radical treatment was given to the patients. Anopheline mosquitoes were collected weekly from different fixed catching stations of the study area by suction tubes between 1800 to 2400 hrs at night in cattlesheds and outdoors and also by operating CDC light traps from dusk to dawn i.e., from 1800 to 0500 hrs in cattlesheds and human dwellings. The collected mosquitoes were transported to the laboratory at Dibrugarh and were identified. The mosquitoes were dissected to detect the gland and gut infections. Meteorological data recorded during the study period have been shown in Figs. 1a, b, c.

RESULTS AND DISCUSSION

Results of active surveillance conducted in the study area for three consecutive years (1987-89) are given in Table 1. It can be seen that malaria cases were maximum in the year 1988, SPR (Slide Positivity Rate) being 28.59 which is significantly higher in comparison with that of 1987 (16.05) and 1989 (12.90). The active surveillance reveals that the area is endemic for *P. falciparum* malaria as in three years of study, *P. falciparum* has been observed to be the dominant species over *P. vivax*. This has a similarity with the studies reported earlier in terai region of Uttar Pradesh where initially most of the malaria cases were due to *P. vivax* but during the last few years of observation, a considerable increase of *P. falciparum* cases has been observed[3-6]. The number of mixed infections detected in this study were quite low i.e., 1.22% out of total positives found. Maximum malaria cases were recorded in the months of September, October and November (Fig. 1) . *P. vivax* cases were found almost in association with *P. falciparum*. No separate outbreaks for these two species had been observed in this study area as observed in some other places of India[3,4].

The age and sexwise distribution and age specific attack rates are shown in Table 2. It can be seen that persons of all age groups and sex were affected in the outbreak. The attack rate is highest in the age group 2-9 years. It is interesting to note that the villages nearer to the forest reserve/forested foot hills are more prone to malaria than that of nonforested areas. A comparison of the age specific attack rates in the villages near forested areas and nonforested areas and observed and estimated attack rates in nonforest area villages by applying age specific malaria risk of the villages near forest reserve/forested foot hill are shown in Table 3. It is evident that the estimated attack rate in the villages of non-forested areas was 58.87 as against the observed value of 3.34. The villages near the forest fringes are thus more prone to malaria than the rest of the population.

The entomological survey reveals that a total of 16 anopheline species have been encountered. *Anopheles kochi*, *An. nigerrimus*, *An. karwari* were the most predominant species (Table 4). *An. dirus* (*An. balabacensis*) which constituted 2.6% of the total anopheline collection was incriminated as vector for current transmission of malaria in the area. The vector density was found to be high in the period from June to October. During this period high transmission of malaria took place as is evident from the high malaria positivity rate in this

Table 1. Results of active surveillance in the study area under Tengakhat PHC of Dibrugarh district during 1987-89

Population	Year	No. of blood slides examined	No. of total positive	Pf	Pv	Mix	SPR	SFR	API
	1987	529	85	60	22	3	16.06	11.34	6.45
17,938	1988	1435	409	359	46	4	28.50	25.01	31.06
	1989	612	79	66	13	0	12.90	10.78	6.00

SPR = Slide positivity rate; SFR = Slide falciparum rate; API = Annual parasite index.

Table 2. Age and sex specific attack rates of malaria in the study area

Age group	Population	No. suffering from malaria			Attack rate per 1000
		Male	Female	Total	
0 - 1 & >1	771	5	2	7	9.07
2 - 9	4483	128	114	242	53.98
10 - 20	4578	71	29	100	21.84
21 - 30	3580	69	57	126	35.19
31 - 50	3190	51	30	81	25.39
>50	1336	9	7	16	11.97

Table 3. Comparative age specific malaria attack rates in the forest and nonforest areas and observed and estimated malaria case rates in nonforest area villages

Age in years	Population		Cases of malaria		Probability of getting malaria in population		Attack rate per 1000 in population		Adjusted cases in Nonforest areas
	Forest areas	Non-forest areas	Forest areas	Non-forest areas	Forest areas	Non-forest areas	Forest areas	Non-forest areas	
	(P_F)	(P_N)	(C_F)	(C_N)	(P_b)	(P_N)	$AR_F = \frac{C_F}{P_F}$	$AR_N = \frac{C_N}{P_N}$	$P_b \times P_N$
0 - 1 & >1	381	390	7	0	0.0183	0.00	18.37	0.00	7
2 - 9	2282	2201	233	9	0.1021	0.0040	102.10	4.00	224
10 - 20	2431	2147	91	9	0.0374	0.0041	37.43	4.19	80
21 - 30	1831	1750	119	7	0.0650	0.0040	65.02	4.00	113
31 - 50	1631	1559	77	4	0.0472	0.0025	47.21	2.56	73
>50	703	633	16	0	0.0227	0.00	22.75	0.00	14

Estimated attack rate in nonforested areas $\frac{\Sigma (P_b \times P_N)}{\Sigma P_N} \times 1000 = 58.87$; Observed attack rate in nonforested areas $\frac{\Sigma C_N}{\Sigma P_N} \times 1000 = 3.34$.

Table 4. Anophelines collected by light-trap and suction tube from different sources during 1987-88

S. No.	Species	Light-trap collection*				Suction tube collection			
		Cattle shed		Human dwelling		Cattle shed		Out door resting	
		No.	%	No.	%	No.	MHD	No.	MHD
1.	*An. aconitus*	153	3.11	7	2.05	140	0.17	8	0.009
2.	*An. annularis*	87	1.77	0	0.00	95	0.11	7	0.008
3.	*An. barbirostris*	45	0.91	1	0.29	35	0.04	0	0.00
4.	*An. dirus* (*A. balabacensis*)	36	0.32	94	27.50	6	0.007	97	0.11
5.	*An. culicifacies*	0	0.00	1	0.29	0	0.00	0	0.00
6.	*An. nigerrimus*	941	19.14	11	3.22	955	1.05	20	0.02
7.	*An. jamesii*	1	0.02	0	0.00	2	0.002	0	0.00
8.	*An. karwari*	1413	31.65	9	2.34	615	0.75	45	0.05
9.	*An. kochi*	1556	28.74	8	2.63	220	0.27	14	0.01
10.	*An. maculatus*	31	0.63	1	0.29	105	0.12	0	0.00
11.	*An. philippinensis*	447	9.09	16	4.69	448	0.55	40	0.04
12.	*An. minimus*	68	1.38	3	0.87	20	0.02	0	0.00
13.	*An. splendidus*	1	0.02	1	0.29	9	0.01	0	0.00
14.	*An. tessellatus*	16	0.32	0	0.00	1	0.001	0	0.00
15.	*An. vagus*	135	2.74	189	55.42	643	0.79	14	0.01
16.	*An. gigas*	5	0.10	0	0.00	0	0.00	0	0.00

* Collected in 126 trap nights; MHD = Per man hour density.

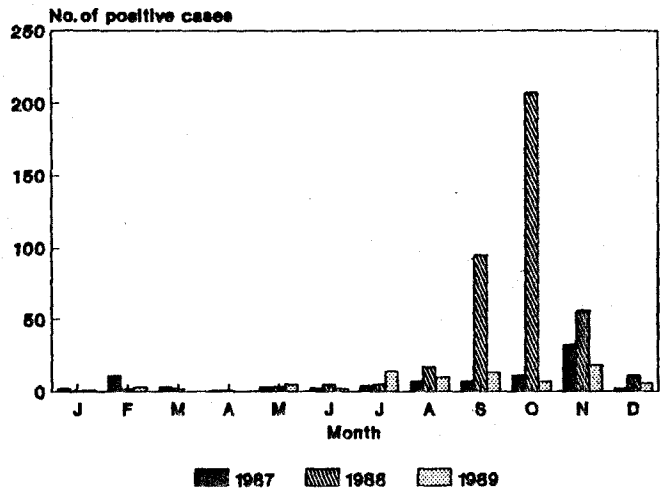

Fig. 2: Record of malaria cases in three consecutive years (1987-89).

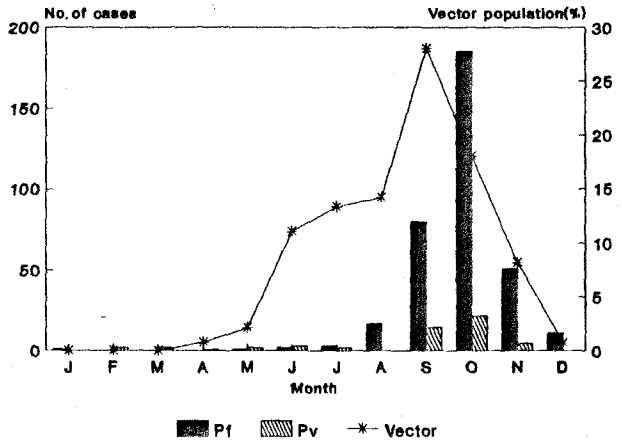

Fig. 3: Monthwise prevalence of malaria cases and vector (*An. dirus*) during 1988.

period (Fig. 2). The vector, *An. dirus* disappears on commencement of winter. It is also seen from the table that the vector *An. dirus* has been encountered more in human dwellings and also in outdoor resting sites (Fig. 3).

In the present study, it is observed that malaria is confined to the population near the forests. This can be attributed to the breeding habit of the vector *An. dirus* (*An. balabacensis*) in forest areas[7] and also to the short flight range of the species[8]. It was also reported that no *An. balabacensis* biting was observed in villages situated as little as 0.5 km clear of the forest[9,10]. In a study conducted in a tea estate of Sylhet, Bangladesh it was also observed that there was little prevalence of malaria at the factory line, which is situated in a clear area[11].

Our study on vectors in this area reports similarity with these reported earlier. Clark and Choudhury

(1941)[12] incriminated mosquitoes of *An. leucosphyrus* group as vector in Digboi area and Rajagopal (1979)[13] incriminated *An. balabacensis* from Nowgong district.

In the present study *An. minimus* was also encountered in low density and its distribution was patchy. Though *An. minimus* was thought to have disappeared from the northeastern region of India after DDT residual spray, it is still present and is a vector in this region specially in the areas of poor spray coverage[14-18].

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Mosquito Survey in Tripura

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Vector survey carried out in Tripura revealed the presence of 17 species of anophelines with a density of 92.39 per trap night. Major malaria vector encountered was *Anopheles philippinensis* which formed 30% of the total collection and is exophagic in nature. Another efficient malaria vector *An. minimus* which formed 5% of the total collection prevails equally both indoors and outdoors. The high parity rate of *An. philippinensis* (59%) and *An. mininus* (63.9%) gives a strong indication about their vectorial status in Tripura.

INTRODUCTION

The climate of northeast India provides an ideal environment for mosquito breeding due to high rainfall and prevailing hot and humid conditions.

In the last six decades several mosquito surveys have been carried out in northeast India. Due to resurgence of malaria, vector survey in these states was further emphasized by several workers. Malhotra *et al.*[1-3] carried out surveys in Nagaland, Mizoram and Arunachal Pradesh (Tirap district); Das *et al.*[4] in Meghalaya; Rajput and Singh[5] in Manipur and Nagpal and Sharma[6] in northeastern region as a whole. Simultaneously, incrimination of malaria vectors reported by Bhatnagar *et al.*[7] in Nagaland; Das and Baruah[8] in Mizoram and Dutta and Baruah[9] in Tirap district of Arunachal Pradesh. However, no vector survey was carried out in Tripura since 1955[10]. Due to nonavailability of data on vectors in last three decades, the effective malaria control programme in Tripura was greatly hampered. In view of the above, a detailed survey was carried out on vectors of malaria in Tripura during the wet season and the results are presented in this communication.

MATERIAL AND METHODS

Topographically, Tripura is a hilly and submountainous state with difficult inaccessible terrain. Hot and humid climate prevails almost throughout the year. Average annual rainfall is round 200 cm, which creates innumerable mosquito breeding habitats.

Adult mosquitoes were collected from human dwellings, cattlesheds, pig sties and goat cabins from 1800 hrs to 0600 hrs with the help of 6 volt battery operated CDC miniature light traps. Structures selected for trap collections were made of split bamboo plastered with mud. Like typical village huts, they had thatched roofs and mud floors. Traps were hung in the middle of the huts about 2 mtr above the ground. During trapping,

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the practice of burning hay and grass was prohibited in the huts. The collected mosquitoes were anaesthesized and identified in the laboratory with the help of standard keys[11-13].

Mosquitoes were also collected on cattle baits both indoors and outdoors between 1900 hrs and 2100 hrs with the aid of suction tubes. Fully-fed anophelines were tested for susceptibility against 4% DDT as per standard WHO procedure. Live mosquitoes were dissected to determine their physiological age by counting the number of dilatations in the ovarioles (Polovodova method). Salivary glands were also dissected for sporozoite infection.

Mosquito larvae (4th instar) and pupae were collected from natural and artificial breeding habitats such as ponds, paddy fields, ditches and roadside drains with the help of dippers, ladles and glass droppers. The larvae and pupae were reared till adult emergence in the field laboratory for confirmation of species.

RESULTS AND DISCUSSION

A total of 37,004 mosquitoes belonging to 32 species under 6 genera i.e., *Anopheles* (17), *Culex* (8), *Armigeres* (1), *Coquillettidia* (1), *Mansonia* (4) and *Aedes* (2) were collected in 146 trap nights (Table 1).

Highest mosquito density was recorded at Dharmanagar (458.8 per trap night) followed by Maharani (306.95 per trap night), Santir Bazar (185.51 per trap night) and Agartala (146.48 per trap night). Maximum number of species were encountered at Maharani (30) followed by Agartala (26), Santir Bazar (24) and Dharmanagar (19). Major species encountered were *Cx. gelidus* (6998), *Cx. tritaeniorhynchus* (6353), *An. philippinensis* (4014), *Cx. malayi* (3662), *An. karwari* (3477), *Cx. vishnui* (3235) and *An. crawfordi* (1350).

Vectors of Japanese Encephalitis viz., *Cx. tritaeniorhynchus*, *Cx. vishnui*, *Cx. bitaeniorhynchus* and *An. barbirostris* were found breeding in vast stretches of paddy fields, low-lying areas and roadside ditches. Adults were also encountered in large numbers both indoors and outdoors (Table 2). Thus the possibility of JE outbreaks cannot be ruled out if appropriate control measures are not adopted well in time.

In the present study malaria vectors; *An. maculatus*, *An. minimus*, *An. philippinensis* and *An. culicifacies* were also collected in good numbers. *An. minimus* was found to breed in clear streams, paddy fields and ditches with grassy margins. Breeding of *An. philippinensis* was observed in paddy fields, ditches and marshy land. *Cx. quinquefasciatus* the vector of filariasis was found breeding in the polluted waters of *kuccha* and cemented drains. Ponds and tanks with aquatic vegetation are the main source of *Mansonia* breeding. *Mansonia* adults were also collected in reasonable number (Table 1), however, there is not much chance of transmission of filariasis as the region is nonendemic for the disease.

Misra and Dhar[10] carried out a survey in Tripura and collected 1508 anophelines comprised of 10 species in 5 months (January–May 1954) in which the predominant species recorded were *An. vagus* (80%) and *An. minimus* (13%). In the present study, a total of 13,489 anophelines under 17 species were collected. Malaria vectors formed 35% of the total collection. The predominant species were *An. philippinensis* which formed 30% and *An. minimus* which formed 5% of the collection.

On animal baits, a total of 787 anophelines belonging to 9 species were collected indoors and outdoors (Table 2). Per man hour density was 10.45 indoors and 11.87 outdoors. Rajagopal[14] in Burnihat (Meghalaya) found 1417 *An. philippinensis* biting cattle and 187 biting man. Similar observations were made earlier by Reid[15] in Malaysia. Khin-Maung-Kyi[16] listed over 3000 *An. philippinensis* on cattle baits and only 62 adults on human baits both indoors and outdoors in Burma. In the

Table 1. Mosquito fauna in Tripura

S. No.	Mosquito species	Agartala			Maharani			Shantir Bazar			Dharmanagar			Total
		Human	Cowshed	Piggery	Human	Cowshed	Piggery	Human	Cowshed	Piggery	Human	Cowshed	Goat	
1.	*Anopheles aconitus*	8	75	13	18	400	–	2	56	–	–	–	–	572
2.	*An. barbirostris*	2	31	12	16	423	–	–	83	–	–	136	7	710
3.	*An. culicifacies*	–	5	–	–	–	–	–	–	–	–	3	–	8
4.	*An. crawfordi*	–	5	–	35	706	–	27	301	6	–	244	26	1350
5.	*An. jamesi*	–	64	3	–	537	–	10	300	1	–	21	–	936
6.	*An. karwari*	1	3	4	31	782	–	163	2406	5	–	62	20	3477
7.	*An. kochi*	–	1	3	4	248	–	20	355	8	–	47	10	696
8.	*An. dirus*	–	–	–	2	14	–	–	–	1	–	–	–	17
9.	*An. maculatus*	–	–	–	2	–	–	–	–	–	–	–	–	2
10.	*An. minimus*	1	66	–	5	222	–	12	286	–	–	103	4	699
11.	*An. nigerrimus*	–	–	5	–	14	–	–	–	–	–	–	–	19
12.	*An. majidi*	–	10	3	–	–	–	–	–	–	–	–	–	13
13.	*An. pallidus*	–	2	–	–	109	–	–	4	–	–	–	–	115
14.	*An. philippinensis*	3	52	13	53	2438	–	32	978	1	–	388	56	4014
15.	*An. subpictus*	1	9	–	1	18	–	2	3	–	–	–	–	34
16.	*An. tessellatus*	–	1	6	–	11	–	–	1	–	–	–	–	19

(contd.)

Table 1. Mosquito fauna in Tripura *(contd.)*

S. No.	Mosquito	Agartala			Maharani			Shantir Bazar			Dharmanagar			Total
		Human	Cowshed	Piggery	Human	Cowshed	Piggery	Human	Cowshed	Piggery	Human	Cowshed	Goat	
17.	*An. vagus*	19	90	17	26	283	–	21	270	4	–	75	3	808
18.	*Aedes albopictus*	–	1	–	1	9	–	–	20	–	–	–	–	31
19.	*Ae. pseudotaeniatus*	–	1	–	–	–	–	–	–	–	–	–	–	1
20.	*Armigeres subalbatus*	2	40	19	27	218	–	2	355	6	–	292	37	998
21.	*Culex bitaeniorhynchus*	11	97	69	72	98	–	6	426	13	–	159	–	951
22.	*Cx. (lutzia) fuscanus*	–	–	–	4	12	–	–	–	–	–	–	–	16
23.	*Cx. gelidus*	43	504	947	90	4500	–	9	303	17	–	579	6	6998
24.	*Cx. malayi*	15	164	225	54	1744	–	26	453	6	–	930	45	3662
25.	*Cx. quinquefasciatus*	1	6	3	–	138	–	–	51	–	–	–	–	199
26.	*Cx. sinensis*	–	–	–	2	8	–	4	11	–	–	–	–	25
27.	*Cx. tritaeniorhynchus*	16	225	113	303	3834	–	57	963	19	–	778	45	6553
28.	*Cx. vishnui*	6	114	130	160	2005	–	29	413	10	–	349	19	3235
29.	*Mansonia annulifera*	–	20	54	20	142	–	–	1	–	–	43	–	280
30.	*Mn. indiana*	–	2	3	–	7	–	–	–	–	–	4	–	16
31.	*Mn. longipalpis*	–	–	–	2	2	–	–	–	–	–	–	–	4
32.	*Mn. uniformis*	2	7	–	66	334	–	12	82	3	–	121	21	648
33.	*Coquillettidia crassipes*	–	–	–	2	7	–	–	64	–	–	25	–	98
	Total collection	131	1596	1642	996	19263	–	434	8185	100	–	4359	229	37004
	Total trap nights	7	14	2	16	50		5	39	3		9	1	146
	Per trap night	18.71	113.93	821.0	62.25	385.26		86.80	209.87	33.33		484.33	299.0	253.45

present study *An. philippinensis* showed a marked preference for biting outdoors (53) then indoors (29), probably indicating their exophagic biting habit in Tripura. However, *An. minimus* did not show any specific preference either for indoor or outdoor biting.

Adults emerged from field collected larvae/pupae were *An. barbirostris* (5), *An. crawfordi* (6), *An. minimus* (8), *An. vagus* (1), *Cx. tritaeniorhynchus* (9) and *Cx. vishnui* (3).

To determine the epidemiological importance of anopheline vectors, *An. philippinensis* (1155) and *An. minimus* (300) were dissected (Table 3). Neither of the species was found positive for sporozoite infection in salivary gland, however, high parity rate was observed for both *An. philippinensis* (59%) and *An. minimus* (63.9%). This gives a strong indication about their vectorial status. *An. philippinensis* is a local vector in the Burma-Bangladesh border area. In Bangladesh, Quraishi *et al.*[17] found three *An. philippinensis* specimens positive for sporozoites. Rajagopal[14] found one specimen gland positive out of 195 *An. philippinensis* dissected in Burnihat.

The results of the susceptibility tests revealed that *An. philippinensis* was susceptible to 4% DDT at one hour exposure (Table 4) though verification is

Table 2. Indoor/Outdoor collection of mosquitoes (Anophelines) on animal baits in South Tripura

S.No.	Mosquito species	Collection hour (1900-2100 hrs)		Total
		Indoor 48 men hour	Outdoor 24 men hour	
1.	*Anopheles barbirostris*	19	12	31
2.	*An. crawfordi*	17	7	24
3.	*An. jamesi*	2	4	6
4.	*An. karwari*	3	—	3
5.	*An. kochi*	—	2	2
6.	*An. minimus*	48	30	78
7.	*An. philippinensis*	29	53	82
8.	*An. subpictus*	5	—	5
9.	*An. vagus*	379	177	556
Total collection		502	285	787
Per man hour collection		10.45	11.87	10.93

Table 3. Results of mosquito dissection in Tripura

Species	No. collected	No. dissected*	Parity %	Gland(+)ve
An. philippinensis	4096	1155	59.0	Nil
An. minimus	777	300	63.9	Nil

*Only alive mosquitoes were dissected.

Table 4. Susceptibility of adult *Anopheles* to 4% DDT in Tripura

Species	No. treated	No. dead	% morality	Remark
An. philippinensis	58	55	94.82	Verification required
An. vagus	528	85	16.1	Resistant

required as 95% mortality (less than 98%) was recorded. Well-known indoor resting species *An. vagus* yielded 16% mortality and is thus recorded resistant to DDT.

It can be stated from the present study that *An. philippinensis* and *An. minimus* might be playing an important role in active transmission of malaria in Tripura. Methodical spray of residual insecticides with maximum coverage, in addition to the reduction of parasitic load in the community by adequate chemotherapy can reduce malaria incidence in Tripura.

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SHORT NOTE

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Chloroquine Resistant *P. falciparum* Malaria in Arunachal Pradesh

S. SATYANARAYANA*, S.K. SHARMA*, P.K. CHELLENG*, P. DUTTA*, L.P. DUTTA* and R.N.S. YADAV**

The detection of chloroquine (CQ) resistance in *P. falciparum* (Pf) has become essential as it is still the first line of treatment recommended by the Government. Resistance to chloroquine in *P. falciparum* is believed to have arisen on the Thai-Kampuchean border around 1957[1,2] and it spread to many other countries[3]. In India, the first report came from Diphu, Karbi Anglong district, Assam in 1973[4] and many areas in the northeastern region of India have subsequently reported resistance[3].

The present study was conducted in the Nampong circle of Changlang district of Arunachal Pradesh during the years 1987–89. The area is situated at an altitude of 305 to 455 mts above sea level with a population of about 10,000 inhabited by Nocte and Tangsa tribes. As this place is famous for its forest resources, a large number of migrants visit the area in search of jobs from time to time, especially during monsoon season.

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A clinic was organised at the primary health unit, Jairampur. Patients with fever complaints were screened for malarial infections. *P. falciparum* cases with a minimum of 1,000 asexual parasites per cubic mm of blood were selected for the WHO *in vivo* test[5]. Cases with history of chloroquine treatment were excluded by performing the urine test with Dill and Glazko reagent. Choloroquine sulphate 25 mg base/kg body wt in divided doses over three consecutive days were given to the test subjects. The absorption of the drug was confirmed by the Dill and Glazko urine test[6].

Blood smears were collected on Day 2,7,10,14,21 and 28 and stained with 10% Giemsa in phosphate buffer. The asexual forms were counted against 300 WBC on thick film, multiplied accordingly and recorded.

From Table 1, it is evident that *P. falciparum* is the predominant species in the study area, the slide positivity rates going up to epidemic proportions during monsoon season. Out of 37 cases studied for the *in vivo* test (Table 2), 3(8.1%) cases were detected to have RIII type resistance, 10 (27.02%) had RII type, 16(43.24%) had RI, 2(5.4%) were S/RI type and 6(16.21%) cases were found sensitive. All the cases found resistant to chloroquine were

Table 1. Record of malaria cases during the years 1987-89 at different periods

Total slides	Total (+) ves	Pf	Pv	Mix	SPR	SFR	Year	Month
429	19	17	2	0	4.4	3.96	1987	Aug.
543	177	131	34	12	32.59	24.15	1987	Sep.—Oct.
254	8	6	2	0	3.15	2.36	1988	Feb.
2614	480	341	126	13	18.36	13.05	1988	May—Sep.
2252	290	149	122	10	12.28	6.61	1989	May—Nov.

treated with pyrimethamine plus sulphamethoxypyrazine. As it is a field study and chances of reinfection could not be ruled out some difficulty was experienced in deciphering the results.

A similar study conducted by the Directorate of Regional Health and Family Welfare, Shillong during 1982 (personal communication) (Fig. 1) revealed that there are only 3.3% sensitive cases, but in the present study it has increased five times. It is seen that RIII and S/RI type of resistance remained same, 10.55% increase in the RII type of resistance is noticed. The RI type was reduced to 43.24% from 60%.

In a separate study 27 cases were followed up till 28 days after treatment with pyrimethamine plus sulphamethoxypyrazine combination. All the cases were found sensitive to the drug, i.e., slides remained negative till Day 28.

Twelve CQ resistant cases were also treated with pyrimethamine-sulfalene combination followed-up to 10 days. In this case also all the slides remained negative till Day 10.

Cases with acute attack of *Pf* malaria were selected for quinine study. Out of 13 cases, 12 were found to be sensitive. In one case S/RI type of resistance

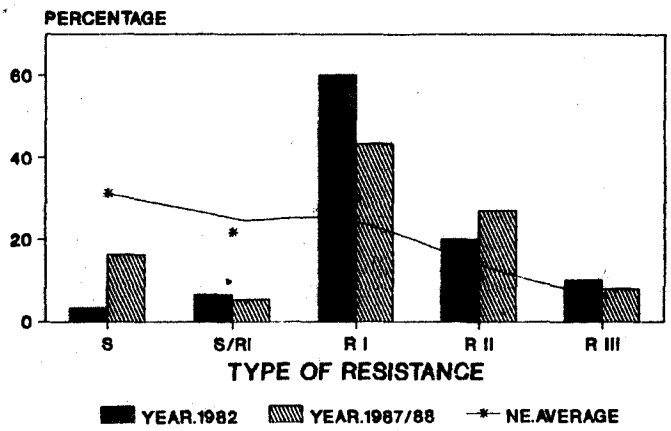

Fig. 1 : Comparative chloroquine resistance chart of Nampong circle (Arunachal Pradesh).

Table 2. Results of WHO *in vivo* 28 Day extended field test

S.No.	Age	Wt.(Kgs)	Asexual parasite count/mm^3 blood in different days intervals							Degree of resis-tance
			D0	D2	D7	D14	D17	D21	D28	
1.	30	50	25800	NEG	NEG	875				RI
2.	25	50	3125	625	NEG	NEG		100		RI
3.	28	45	11400	3900	NEG	N.D.	450			RI
4.	26	54	8550	275	NEG	900(D10)				RI
5.	15	28	38250	27750	NEG	N.D.	250			RI
6.	30	58	2700	NEG	NEG	350(D10)				RI
7.	26	54	3000	500	NEG	800				RI
8.	16	37	13000	1525	NEG	7750(D10)				RI
9.	45	48	4050	900	NEG	3000				RI
10.	25	60	10000	8000	NEG	5400				RI
11.	52	45	2075	150	NEG	NEG		NEG	225	RI
12.	12	26	8300	175	NEG(D4)	825(D10)				RI
13.	13	30	5250	N.D.	NEG	3000				RI
14.	25	50	4650	2750	125	NEG		NEG	100	RI
15.	28	45	16875	250	NEG	100				RI
16.	40	60	44750	16250	NEG	NEG	250			RI
17.	13	37	1000	175	50					RII
18.	23	43	1275	11500	775					RII
19.	35	47	1575	34975	425					RII
20.	35	42	109925	9375	8550					RII
21.	50	45	10275	750	3575					RII
22.	20	45	3150	250	750					RII
23.	6	12	76500	4200	17400					RII
24.	35	49	900	175	150	900				RII
25.	6	15	98550	475(D5)	800					RII
26.	21	42	925	100	600					RII
27.	50	55	11875	3500	6000					RIII
28.	26	55	6450	10257	7000(D4)					RIII
29.	15	45	10275	9375	19575					RIII
30.	31	45	5500	1900	NEG	NEG		NEG	NEG	S
31.	11	22	9000	125	NEG	N.D.		NEG	NEG	S
32.	35	60	5475	200	NEG	NEG		NEG	NEG	S
33.	25	39	2500	500	NEG	NEG		NEG	NEG	S
34.	32	45	3125	1000	NEG	NEG		NEG	NEG	S
35.	11	22	9000	125	NEG	NEG		NEG	NEG	S
36.	32	45	3725	1900	NEG	N.D.		NEG	200	S/RI
37.	25	50	7775	950	NEG	N.D.		2825		S/RI

N.D.—Not done; NEG—Slide negative; Figures in parentheses are the actual follow-up days.

was registered by showing the parasitaemia on Day 17.

From this study it is observed that resistance to pyrimethamine combination and quinine is not a problem in the study area presently. Judicious use of these drugs and the gametocytocidal agent primaquine may bring about control over the chloroquine resistant *Pf* malaria in the study area.

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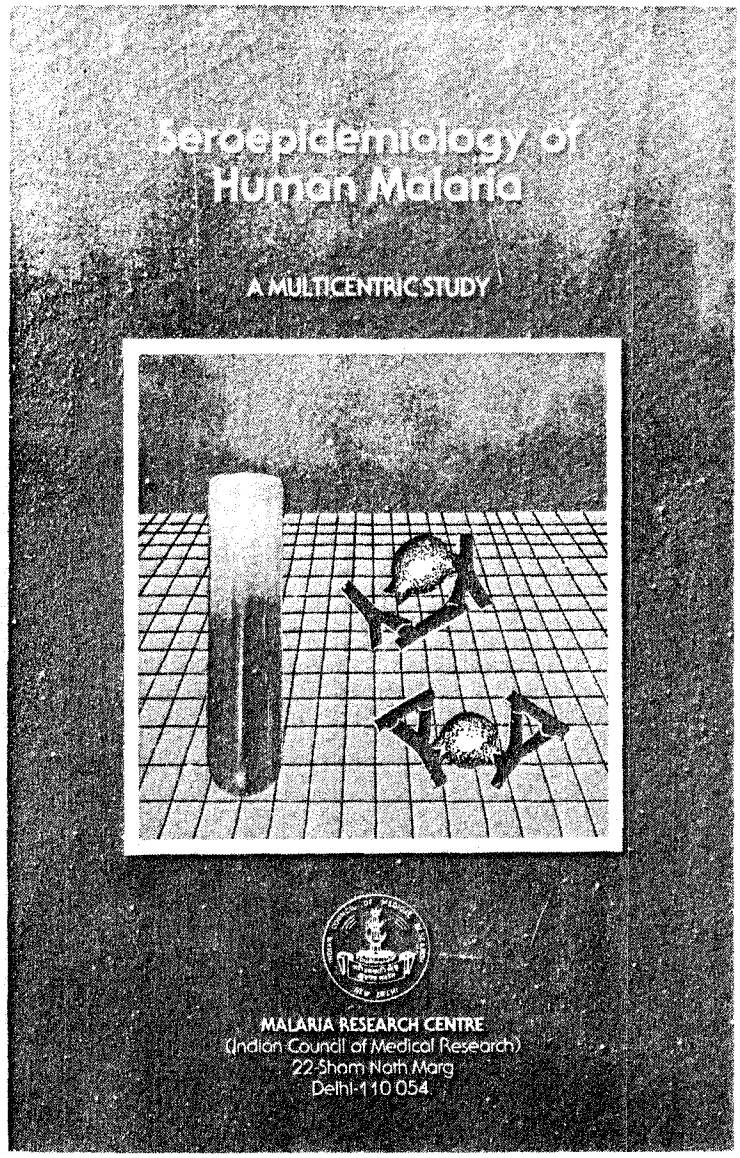

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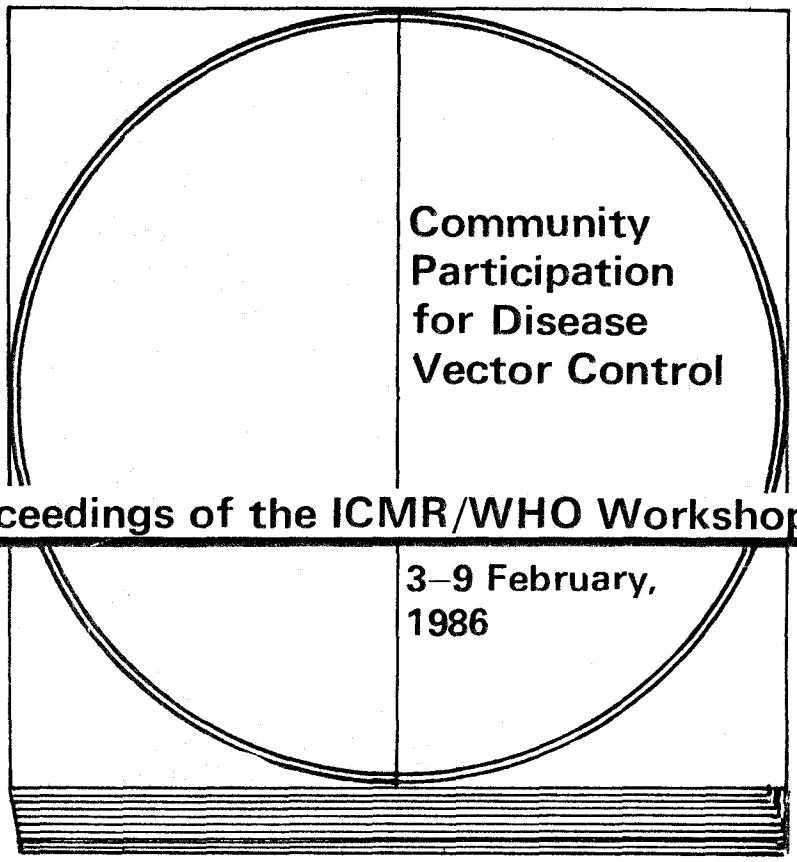

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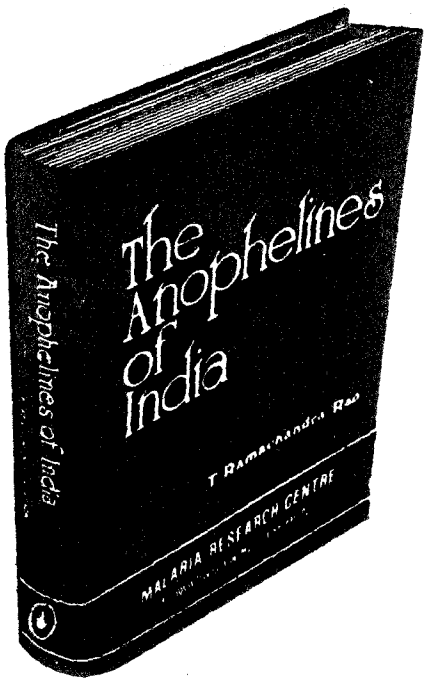

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