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Anophelines of Siliguri-Naxalbari Block, Darjeeling, West Bengal

P. MALAKAR, S. DAS, G.K. SAHA, B. DASGUPTA and A.K. HATI[a]

A systematic survey has been carried out during 1993-94 to study the occurrence and distribution of anopheline mosquitoes in Siliguri-Naxalbari block of Darjeeling district. The anopheline fauna of this region consists of 13 species, namely, *An. vagus, An. culicifacies, An. annularis, An. barbirostris, An. subpictus, An. maculatus, An. tessellatus, An. jamesii, An. aconitus, An. "hyrcanus"* group, *An. karwari, An. fluviatilis* and *An. jeyporiensis* among which the first five species forms the major bulk (92%). All the species showed preference to cattlesheds than human-habitation for resting. The ratio between morning and evening collection was 1:0.87.

Keywords: Anopheline, Darjeeling, Habitat preference, Seasonal variation

INTRODUCTION

The malaria situation is extremely diverse in different parts of North Bengal. The foothills and its adjoining areas of Darjeeling district are considered worst affected with the problem. A gradual increase in the incidence of malaria has been noticed from areas of Siliguri-Naxalbari block in recent years. A high number of *P. falciparum* infections were encountered (data of Deputy Director of Health Services, Malaria, Government of West Bengal).

This unwelcome situation has called for an extensive entomological survey in this unique ecosystem for better un-

Post Graduate Department of Zoology, Darjeeling Government College, Darjeeling-734 101, India.
[a]Department of Medical Entomology, School of Tropical Medicine, Calcutta-700 073, India.

derstanding of the underlying causes behind transmission of malaria. Since Hati[1] carried out a survey in some areas of the district, no systematic and comprehensive survey has been made so far in recent years. Malakar *et al.*[2], however, published a brief note on the subject. The present study was carried out to supplement the information available on the subject.

MATERIALS AND METHODS

The block shares the slope of foothills in the north and is characterised by heavy rainfall, perennial slow-running streams and rivers, forest bustees, paddy-fields and abundant tea gardens. Mosquito collections were carried out at stations namely, Prodhan Nagar, Bhangapool and Rothkhola. Prodhan Nagar is an urban colony situated at the bank of Mahananda River, while Bhangapool bustee is a rural colony at the periphery of Siliguri near river Balasan. Rothkhola is also a small village near the semi-urban Naxalbari township and the area is a part of the sub-Himalayan terrain. A large number of families of all the three study areas of the block have a practice of rearing cattle in their house-complex.

Mosquito collections were made in each station at dawn (0600-0800 hrs) and dusk (1800-2000 hrs) from cattlesheds and human habitations in April 1993 to March 1994. In each study area, three selected cattlesheds and human habitations were searched thoroughly for adult collections. Adult collection was done twice a month spending two man-hours both in morning and evening. A total of 96 man-hours were spent in each study area during one year study period.

Mosquitoes were collected manually with the help of torches, test tubes and suction-tubes. All the specimens collected were identified following the key of Roy and Brown[3] in consultation with that of Das *et al.*[4] and kept place and habitat-wise in test tubes. The temperature and relative humidity were recorded during each collection.

RESULTS AND DISCUSSION

A total of 1371 anopheline mosquitoes were collected from April 1993 to March 1994, of which 1287 (94%) were females. The male and female ratio was 0.06:1. The anopheline fauna of this block consisted of 13 species namely, *An. aconitus*, *An. annularis*, *An. barbirostris*, *An. culicifacies*, *An. fluviatilis*, *An. "hyrcanus"* group, *An. jeyporiensis*, *An. jamesii*, *An. karwari*, *An. maculatus*, *An. subpictus*, *An. tessellatus* and *An. vagus* (Table 1). The most predominant species of this area was *An. vagus* (31.16%) followed by *An. culicifacies* (21.21%), *An. annularis* (16.24%) and *An. barbirostris* (14.99%). Among three collection spots surveyed the Rothkhola area harboured 12 anopheline species from October to December. A single specimen of each of *An. fluviatilis* (unfed) and *An. jey-*

Table 1. Relative abundance of different species of *Anopheles* in Siliguri-Naxalbari block (April 1993-March 1994)

Species	No. of specimen collected		
	Male	Female	Total
An. vagus	53	401 (31.16)	454
An. culicifacies	22	273 (21.21)	295
An. annularis	3	209 (16.24)	212
An. barbirostris	–	193 (14.99)	193
An. subpictus	6	105 (8.16)	111
An. maculatus	–	25 (1.94)	25
An. tessellatus	–	23 (1.79)	23
An. jamesii	–	22 (1.71)	22
An. aconitus	–	16 (1.24)	16
An. hyrcanus' group	–	15 (1.17)	15
An. karwari	–	3 (0.23)	3
An. fluviatilis	–	1 (0.08)	1
An. jeyporiensis	–	1 (0.08)	1
Total	84	1287	1371

Figures in parentheses are in per cent.

poriensis (fed) have been collected from cattlesheds of Rothkhola area during evening and morning hours respectively. Since *An. subpictus* was known to prefer wide range of habitations[5], they were present uniformly in different spots of the present study area.

Several anopheline species namely, *An. bengalensis, An. lindesayi, An. interruptus, An. varuna, An. theobaldi, An. ramsayi, An. culiciformis, An. balabacensis, An. annandalei, An. kochi, An. majidi, An. sintoni, An. aitkenii, An. gigas, An. leucosphyrus, An. nigerrimus, An. minimus* and *An. splendidus*, reported earlier from areas of Darjeeling district by several workers[2,6-11], were found absent during present survey. This may be attributed to the extensive spraying of DDT under National Malaria Eradication Programme[12-14] as a result of high susceptibility status of some species of anophelines. Besides, massive deforestation, extension of agricultural fields especially tea gardens, unplanned urbanization and large-scale use of insecticides and synthetic fertilizers might have altered the species balance and ultimately has led to an ecological succession of biological species in this

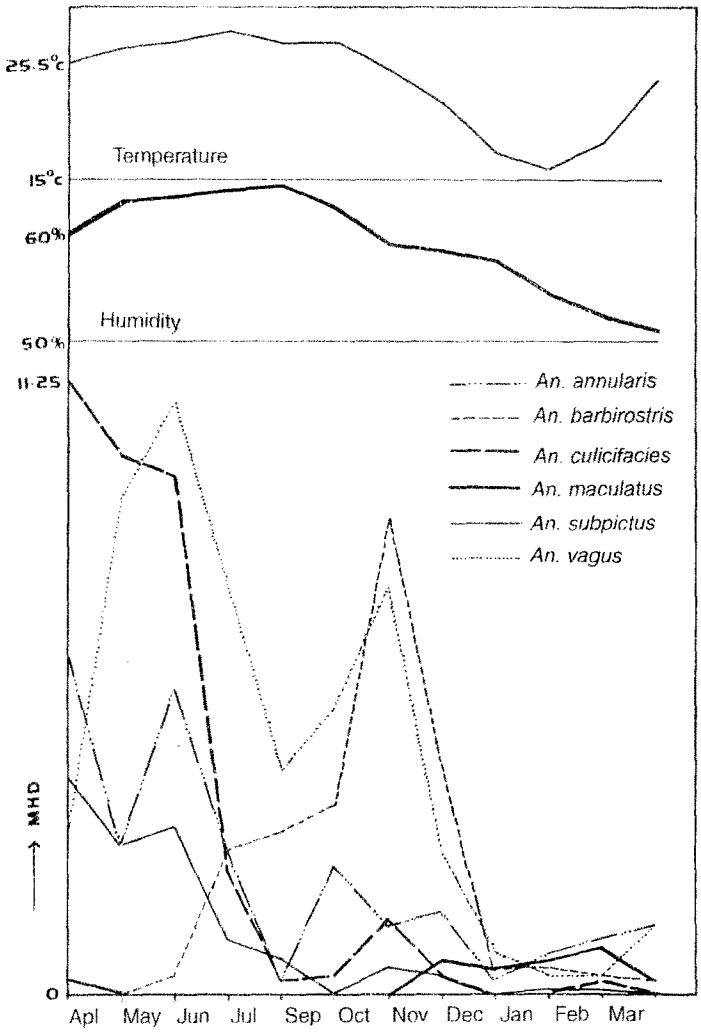

Fig. 1: Month-wise variation in the occurrence of common species of *Anopheles* in relation to relative humidity and temperature in the study area

area, as also suggested by Bansal and Singh[15] from Rajasthan.

Of the total females collected, the ratio between the morning and evening collection was found to be 1:0.87 and the ratio between the cattleshed and human habitation collection was 1:0.31. In morning collection, dominant species were *An. vagus* (36.68%), *An.*

annularis (19.5%), *An. culicifacies* (18%), *An. barbirostris* (10%) and *An. subpictus* (8.3%) and more than 75% specimens were collected from cattlesheds. In evening collections, 79% females were collected from cattlesheds and the notable species were *An. vagus* (25.17%), *An. barbirostris* (20.67%), *An. culicifacies* (20%), *An. annularis* (12.5%) and *An. subpictus* (8%). It was observed that all the 13 species of anophelines showed a marked preference to rest in cattlesheds except *An. vagus* that equally prefers to rest both in cattlesheds and human habitations. It is to be noted that in all collections both in cattlesheds and human habitations as well as in morning and evening, *An. vagus* remained the predominant species. The pattern of seasonality of *An. vagus* showed that the population built up with two distinct peaks (Fig.1), a high in June and another low in October, though the specimen were available throughout the year. Usually from November to March it had a comparatively lower prevalence. Likewise, bimodal peak was also found in *An. annularis* once in April and another in June.

The density of *An. culicifacies* remained higher (65%) in Bhangapool area reaching a peak at the end of April, while a relatively low density was attained as monsoon approached (Fig. 1). The fact may be attributed to the flushing effect in streams and rivers due to heavy rainfall during monsoon. The peak density of *An. barbirostris* attained in October and declined sharply in December, reaching a low figure in March to May, on the onset of monsoon. As the monsoon approached, the density again increased steadily. *An. maculatus* were found present from October to April and were absent during monsoon as a result of heavy rainfall in this area. In case of *An. subpictus* the density remained high during April to June and declined steadily, as monsoon approached, with an extremely low level in September. All the specimens of *An. 'hyrcanus'* group were collected from cattlesheds during August, while more than 90% specimens of *An. jamesii* were collected from Rothkhola during August and September. The density of *An. aconitus* remained high in winter with a peak in November. Only three specimens (two unfed and one fed) of *An. karwari* were found present in the evening collection caught from the cattlesheds of Rothkhola area. Though it is an uncommon species throughout the country, it was reported for the first time from this part of India.

For better understanding of the seasonal prevalence of individual species of anophelines and to find out the relation between the temperature, humidity and mosquito density, mean man-hour density of notable species, mean temperature and mean humidity during each collection is plotted in Fig. 1. The study indicates that the density of *An. vagus* has a significant positive correlation with both the temperature (r=0.806) and humidity (r=0.794), while *An. maculatus* showed

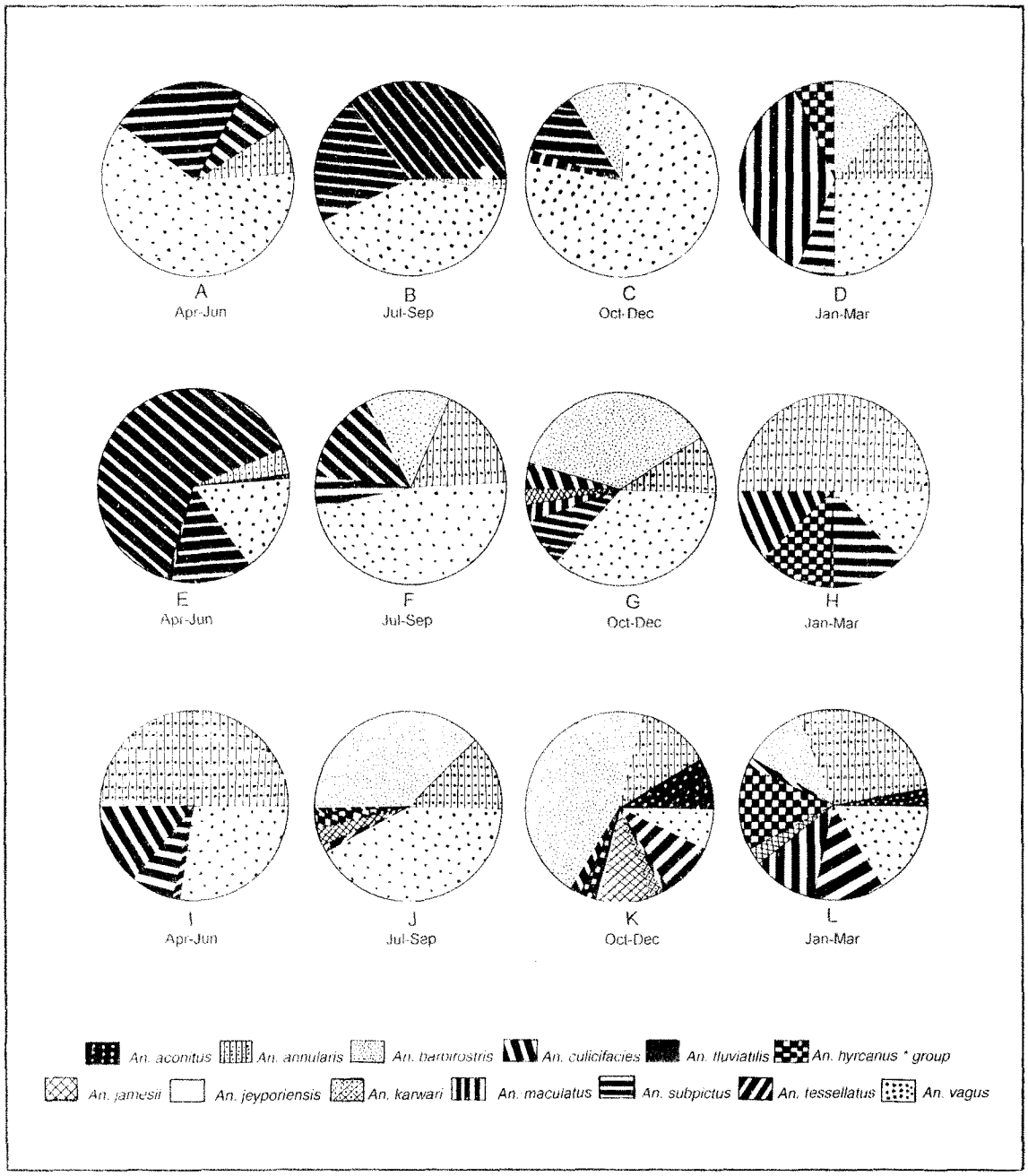

Fig. 2: Seasonal composition of different species of *Anopheles* in Prodhan Nagar (A,B,C and D), Bhangapool (E,F,G and H) and Rothkhola (I,J,K and L) areas during April 1993 to March 1994

a negative correlation with both of them (r= –0.698 and –0.683 respectively).

The species composition of the anopheline fauna in different seasons in three different collection spots were shown in pi-charts 'A' to 'L' (Fig. 2). Maximum number of species were found in October to December i.e. post-monsoon period, whereas less in the month of April to May.

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Epidemiological Observations on Malaria in Villages of Buhari PHC, Surat, Gujarat

H.C. SRIVASTAVA, RAJNI KANT, R.M. BHATT, S.K. SHARMA and V.P. SHARMA[a]

Investigations were carried out in the villages of Buhari PHC, Surat district, Gujarat to determine factors responsible for high prevalence of *P. falciparum*. The area is well-known for sugarcane cultivation, industrialization and frequent movement of labourers. The slide positivity rate (44.24%) and *P. falciparum* infections (95.37%) were significantly high. The children of 5-9 yrs of age group suffered maximum from malaria. The infection in children was found associated with splenomegaly also. High falciparum infection (82.35%) was found in labourers camping near villages out of which 90% afebrile cases were reported which were silent carriers and source of transmission. Chloroquine was effective in 75% cases despite of existence of resistant falciparum strain. Among seven anopheline species encountered, *An. culicifacies* was predominant and found breeding in most of the breeding sites. The status of the insecticide spray and its receptivity among the community were poor. The investigation led to the conclusion that adequate measures are required to deal with migratory labourers, improper insecticide coverage, poor surveillance and mass ignorance.

Keywords: Anophelines, Malaria, Resistance, Splenomegaly

INTRODUCTION

Several areas of Gujarat are endemic for malaria. The state contributes 19.6% *P. falciparum* (*Pf*) cases of the total cases in the country. Surat, one of the endemic district although accounting for only 8.2% of Gujarat's

Malaria Research Centre (Field Station), Civil Hospital, Nadiad-387 001, India.
[a]Malaria Research Centre, 20-Madhuvan, Delhi-110 092, India.

population contributes 20.94% of all malaria cases and 33.5% *P. falciparum* cases of the state. The district is well-known for sugarcane cultivation and rapid industrialization resulting in large-scale population migration. The present investigations were carried out during November-December 1993 to identify the epidemiological and entomological factors responsible for enhanced malaria incidence in general and high *P. falciparum* in particular in some villages of Buhari PHC of district Surat.

Study area

Studies were carried out in six villages of Buhari PHC namely, Valod, Moredevi, Kumbia, Kanjod, Bhimpol and Ranveri. The first four villages are located on the banks of river Jhakaria with canal irrigation while last two villages are away from the river (Fig. 1). Ranveri, Kanjod and Bhimpol villages contain 90 to 100% backward and tribal population followed by Kumbia (76.9%) and Moredevi (50%). The proportion of tribal and backward class is low (36.6%) in Valod compared to other villages. Sugarcane and paddy are the major cash crops. The area receives good rain during June to October and the average annual rainfall and rainy days are 1383.90 mm and 42.33 respectively. The meteorological data obtained from Gujarat Agricultural University, Anand and District Panchyat Office, Surat are shown in Fig. 2.

MATERIALS AND METHODS

Mass blood surveys were conducted to obtain the quantum of malaria in local people and in migratory labourers. All blood smears were brought to the field laboratory, stained with JSB and examined. Treatment was given according to NMEP drug policy. Screening of splenomegaly was performed in children of 2-9 yrs of age group[1]. Chloroquine sensitivity in *Pf* was investigated as per Rieckmann's method[2]. Mosquito density was estimated through hand collection from human dwellings and cattlesheds and parity rate was determined according to Detinova[3] to achieve the objectives. Larval sampling from all available breeding habitats in the villages was done using standard WHO method[4]. To assess the receptivity and quality of insecticide spray, a random survey of houses in the study villages was also carried out.

Observations on control measures by NMEP : The control activities included antiparasitic measures by instituting early detection and prompt treatment (EDPT) and anti-mostquito measures by intradomiciliary spray of conventional insecticide. Out of the seven sanctioned posts of MPHWs only three workers and one supervisor were engaged in surveillance activity in the study villages with a population of 19,900 and 39% scattered houses. Fortnightly, house-to-house visits by

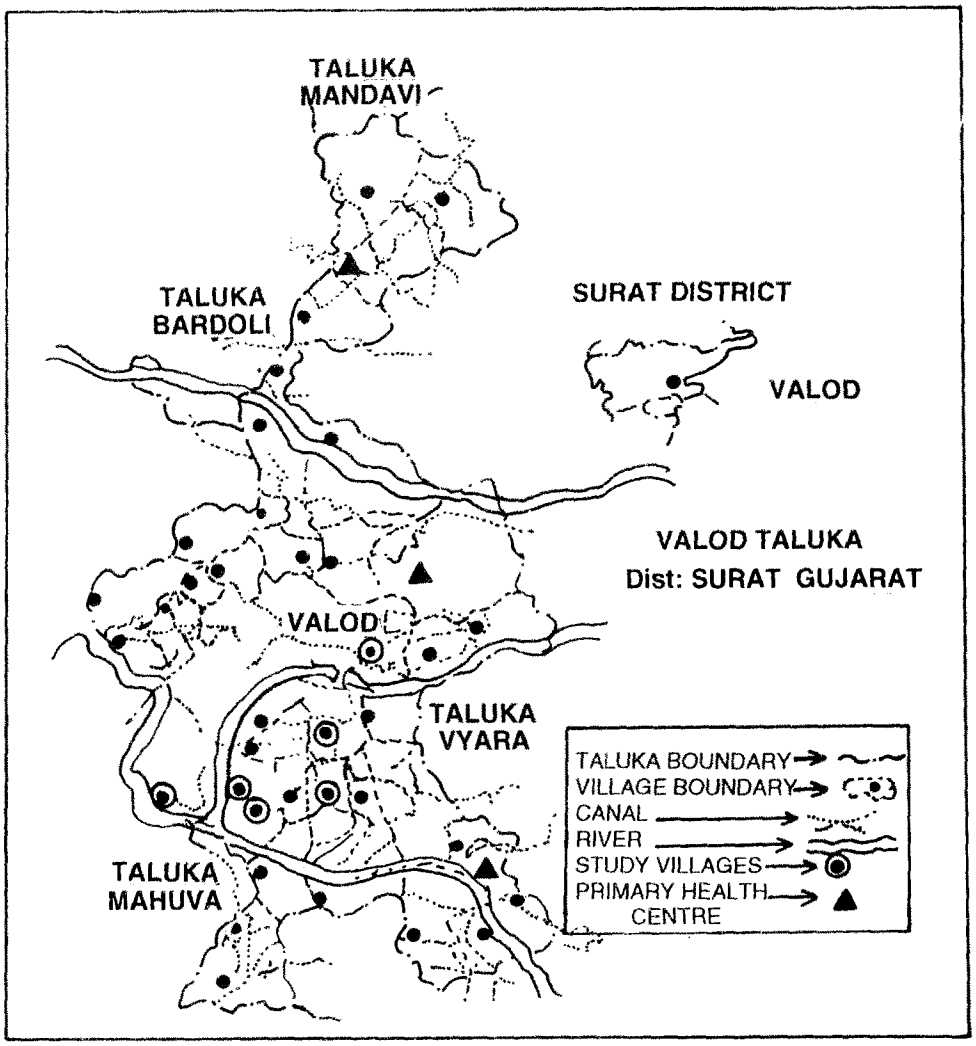

Fig. 1: Map of Valod taluka, Surat (village numbers are given against each village)

MPHWs was found irregular and some areas were untouched. However, annual blood examination rate (ABER) for the last five years has remained above 20%. Blood smears collected by ACD and PCD (1 CHC and 14 FTD) were not received by the PHC laboratory on the same day. However, no backlog of blood smear was shown, as excess load was distributed either to District malaria Office, Surat or to nearby PHC. Mean time lag between collection and examination was 5-7 days. Surveillance of labour settlements was neglected.

The presumptive and radical treatment were given according to NMEP drug policy both by the workers and supervisors. Though radical treatment was administered to 92% positive cases during 1992, incidence of *Pf* has shown an increase. From September 1993 onwards FRT was administered. Delayed radical treatment (7-10 days), and through third person are the indications of improper radical treatment.

Out of 21 villages under Buhari PHC, only 12 villages received insecticide spray from 1991 to 1993. During 1991, focal spray with DDT was done in three study villages. Area received single round of spray during 1991 and 1992 in September and June respectively and two rounds in 1993 in August (1st round) and October (2nd round). Coverage of targetted population varied from 54 to 66% except in the year 1992, when it was 82%. However, spray coverage of room was less than 75% in every year. DDT (50%) in 1991 and 1993 and malathion (25%) in 1992 were used to cover targetted population.

Migrant labourer : Large-scale sugarcane cultivation throughout the year and eight sugar factories within 10 km range of study villages results in aggregation of labour. These labourers move into this area from the districts of Dang (22.10%), Panchmahal (16.14%) of Gujarat and Nasik (11.22%)

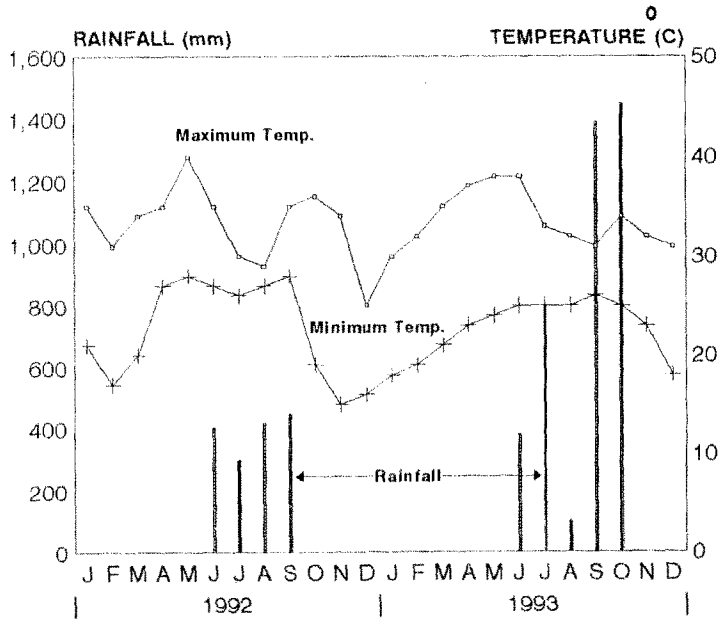

Fig. 2: Monthly means of maximum, minimum temperature and rainfall

Table 1. Epidemiological situation of Valod village, Buhari PHC and remaining PHCs (Rural)*

Year	BSE			(+) ve			API			%*Pf*		
	Valod	Buhari PHC	Remaining PHCs	Valod	Buhari PHC	Remaining PHCs	Valod	Buhari PHC	Remaining PHCs	Valod	Buhari PHC	Remaining PHCs
1989	2287	5979	2,27,279	1224 (53.51)	2375 (39.72)	37,565 (16.52)	107.40	26.35	19.30	35.45	34.77	35.70
1990	2762	7622	2,26,017	868 (31.42)	1555 (20.40)	24,341 (10.76)	76.19	36.16	12.51	23.04	24.82	36.14
1991	2773	6433	2,44,435	523 (18.86)	1084 (16.85)	25,032 (10.24)	45.90	25.21	12.86	27.15	27.58	47.05
1992	2655	5544	2,66,987	170 (6.40)	394 (7.10)	22,281 (8.34)	14.92	9.16	11.45	20.58	19.79	31.47
1993	4543	8609	2,75,247	624 (13.73)	1164 (13.52)	18,247 (6.63)	54.77	27.07	9.37	68.42	62.11	31.11

*Malaria cases reported from urban area have been excluded; Figures in parentheses are SPR; Population of Valod, Buhari PHC, and remaining PHCs are 11,392, 42,993 and 19,45,683 respectively.
Source: District Malaria Office, Surat.

and Dhuliya (50.52%) of Maharashtra states. These districts are known for malaria endemicity. Most of these labourers visit to their native places during monsoon season (June-September) due to scarcity of work, temporary nature of their settlement and cultivation practices at their native places.

RESULTS

Epidemiological observation: Malaria incidence during last five years (1989-93) revealed there was gradual decrease in SPR and API in rural areas of District Surat, while the proportion of *Pf* has not declined significantly. In Buhari PHC, particularly in Valod village all the three parameters have sharply increased in 1993 (Table 1). Maximum *Pf* cases were recorded during September to December in last two years which reflects the transmission season for *P. falciparum* (Fig. 3).

Results of survey in villages of Buhari PHC revealed high SPR and the contribution of *P. falciparum* infection was dominant over *P. vivax*. A low-level of mixed (*Pv*+*Pf*) infection (5.7%) was also observed. It is evident from Table 2, that former four villages situated near river were equally affected with malaria (*P. falciparum*), while Ranveri and Bhimpol villages had low malaria. Age and sexwise distribution of malaria given in Table 3 shows that persons of all age groups were affected. High slide positivity rate among the age group of 5-9 yrs indicate high attack rate which agree with the previous

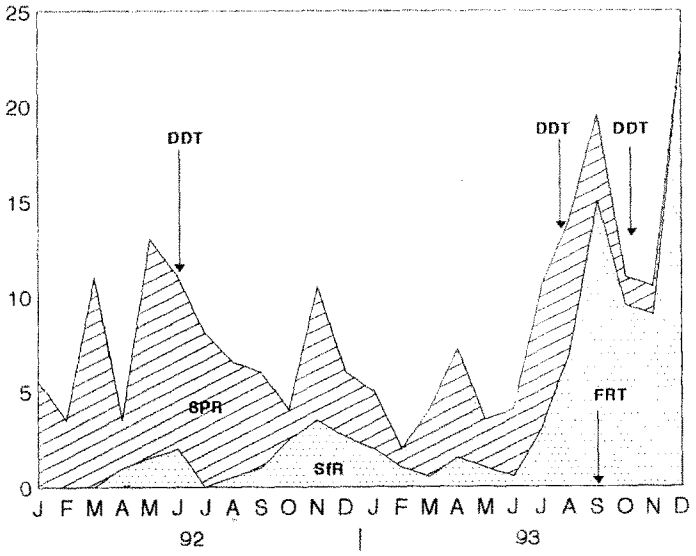

Fig. 3: Slide positivity rate and slide falciparum rate in Valod village of Buhari PHC

Table 2. Results of parasitological survey in villages of Buhari PHC, District Surat

Village	Population	BSE	(+)ve	*Pf*	Mix	SPR	SfR	%*Pf*
Valod	4252	283	117	102	9	41.34	39.22	94.87
Kanjod	800	19	7	6	0	36.84	31.58	85.71
Kumbia	675	29	14	14	0	48.28	48.28	100.00
Moredevi	900	50	31	29	1	62.00	60.00	96.77
Ranveri	450	4	0	0	0	0.00	0.00	0.00
Bhimpol	650	6	4	4	0	66.67	66.67	100.00
Total	7727	391	173	155	10	44.24	42.20	95.37

Table 3. Age and sexwise distribution of malaria cases in study villages

Age group (yrs)	Male						Female					
	BSE	(+)ve	*Pf*	Mix	SPR	SfR	BSE	(+)ve	*Pf*	Mix	SPR	SfR
< 1	–	–	–	–	–	–	2	–	–	–	0.00	0.00
1-4	5	4	2	1	80.00	60.00	14	7	5	2	50.00	50.00
5-9	18	12	10	2	66.70	66.70	30	22	18	2	73.33	66.70
10-14	29	19	18	1	65.50	65.50	30	20	18	1	66.70	63.30
15-29	51	26	24	–	50.98	47.06	51	21	20	1	41.18	41.18
< 30	73	16	15	–	21.92	20.55	88	26	25	–	29.55	28.41
Total	176	77	69	4	43.75	41.48	215	96	86	6	44.65	42.79

Table 4. Labour survey in Valod village of Surat district

	BSE	(+)ve	*Pf*	SPR	SfR	%*Pf*
Fever cases	59	7	5	11.86	8.47	71.43
Non-fever cases	258	10	9	3.86	3.49	90.00
Total	317	17	14	5.36	4.42	82.35

findings[5]. Persons above 30 yrs of age were found more safe with lowest malaria infection rate. Malaria positivity rate (57.8%) among 1-4 yrs of age group and high crescent rate (33-75%) were observed during the present survey.

Results of blood smears collected from labourers (mass blood survey), camping near the villages are shown in Table 4. Data shows that in spite of low SPR among afebrile cases, *Pf* per cent was higher compared to that among febrile cases. Spleen examination of 115 children in Valod village revealed splenomegaly in 11.3% subjects with average spleen enlargement of 1.8. Although grade 3 and 4 spleen were in low proportion (1.7%), the prevalence of falciparum and mixed infections among the children with enlarged spleen was 77.7% and 22.3% respectively.

To ascertain chloroquine sensitivity in *P. falciparum*, 48 cases with *Pf* rings were followed according to the Rieckman's method[2]. 75% cases responded to chloroquine and rest of the cases showed varying degree (RI 16.6%; RII 2.0%; and RIII 6.25% levels) of resistance. Chloroquine resistant *Pf* strains are existing in low proportion (Table 5) with maximum occurrence in 9-20 yrs of age group. Prevalence of resistant strain has also been reported from other parts of Surat and Kheda districts[6,7].

Entomological observation: It revealed the presence of seven anopheline species resting in houses. *An. culicifacies* was predominant constituting 88.7% of total anophelines collected followed by *An. subpictus* (9.6%). Other anopheline species namely, *An. aconitus*, *An. annularis*, *An. barbirostris*, *An. stephensi*, and *An. tessellatus* were found in small numbers (1.64%). Mean man hour density (MHD) of *An. culicifacies* was observed to be 59.58. Density of *An. culicifacies* was relatively higher in cattlesheds (MHD 136.8) than in mixed (MHD 54.0) and human dwellings (MHD 14.8) (Table 6). Results of mosquito dissections revealed that 52.63% *An. culicifacies* were parous, though only 7.89% were found with two dilatations.

Survey of perennial as well as temporary mosquito breeding sources in the study villages revealed the positivity of 65.38%. Out of 26 larval samples collected 43.3% were positive for mix, 19.23% for anopheline and 3.8% for *Culex* mosquito breeding. Density of immature stages of anophelines was maximum in river (3.9/dip). Table 7 shows irrigation drain supported the breeding of maximum number of anopheline species (6). *An. culicifacies* was found to breed in most of the habitats available which agree with the earlier findings of its ubiquitous breeding habit[8].

Susceptibility test on *An. culicifacies* with different insecticides could not be conducted as the study villages were sprayed recently. However, tests carried out on *An. culicifacies* against

Table 5. Age and sexwise distribution of *P. falciparum* cases studied for chloroquine sensitivity

Degree of resistance	9-20 yrs		21-30 yrs		> 31 yrs		Total	
	Male	Female	Male	Female	Male	Female	Male	Female
S/RI	10 (66.7)	8 (38.1)	1 (6.7)	5 (23.8)	4 (26.7)	8 (38.1)	15 (41.7)	21 (58.3)
S/RI late	2 (66.7)	5 (100.0)	–	–	1 (33.3)	–	3 (37.5)	5 (62.5)
RI early/RII	–	1 (100.0)	–	–	–	–	0	1 (100.0)
RIII	–	2 (100.0)	1 (50.0)	–	–	–	1 (33.3)	2 (66.7)

Figures in parentheses are in per cent.

Table 6. Man hour density of mosquitoes in villages of Buhari PHC

Habitat	November			December			Total		
	Total mosquito	Total anophe-lines	*An. culici-facies*	Total mosquito	Total anophe-lines	*An. culici-facies*	Total mosquito	Total anophe-lines	*An. culici-facies*
Human dwelling	19.20	18.40	12.00	20.33	16.66	16.00	20.00	17.17	14.80
Mixed dwelling	70.00	64.00	54.00	–	–	–	70.00	64.00	54.00
Cattleshed	174.67	166.67	133.33	146.71	146.14	138.28	155.20	152.80	136.80
Mean	76.00	72.00	56.80	66.90	64.60	61.05	70.06	67.17	59.58

Table 7. Species specific mosquito breeding sources in villages of Buhari PHC

Species	River	Rocky pool	Sandy pool	Irrigation drain	Canal	Well
No. of samples	5	1	10	3	4	3
Larval density/dip						
Anophelines	3.94	2.6	2.3	2.4	0.09	–
Culicines	–	–	0.5	4.8	–	19.0
% frequency of species						
Anophelines						
An. aconitus	–	–	–	7.6	–	–
An. annularis	5.8	–	–	3.8	–	–
An. culicifacies	55.88	100.0	96.4	3.8	100.0	–
An. nigerrimus	–	–	–	15.4	–	–
An. subpictus	38.23	–	–	65.4	–	–
An. tessellatus	–	–	3.6	3.8	–	–
Culicines						
Cx. bitaeniorhynchus	–	–	–	93.7	–	100.0
Cx. quinquefasciatus	–	–	100.0	–	–	–
Cx. vishnui group	–	–	–	6.3	–	–

DDT, HCH and malathion by State Health Department during 1992-93 in Ukhalda, Champavadi and Buhari PHCs in Surat district revealed the mortality in the range of 8.8-22%, 8.3-20% and 11.11% respectively. These results confirm the presence of DDT, HCH and malathion resistant strain of *An. culicifacies* and support the previous observations[9].

Survey of 134 houses to assess the status of insecticide spray revealed low coverage 62.4% and the quality of spray was also poor (Fig. 4). The refusal rate by the villagers for not allowing their dwellings to be sprayed was 21.9%, the reasons for the same were irritating smell of the insecticide enhanced nuisance of bed-bugs and fleas and inadequate impact on mosquitoes. Similar reasons have also been reported from Orissa[10]. Among the houses sprayed, only 38.10% were found with insecticide deposits on the eaves, wall and roof that are the preferred mosquito resting surfaces. Important reasons behind low residual deposits on the surface were traditional house cleaning and mud plastering practices before Deepawali that falls during peak transmission season.

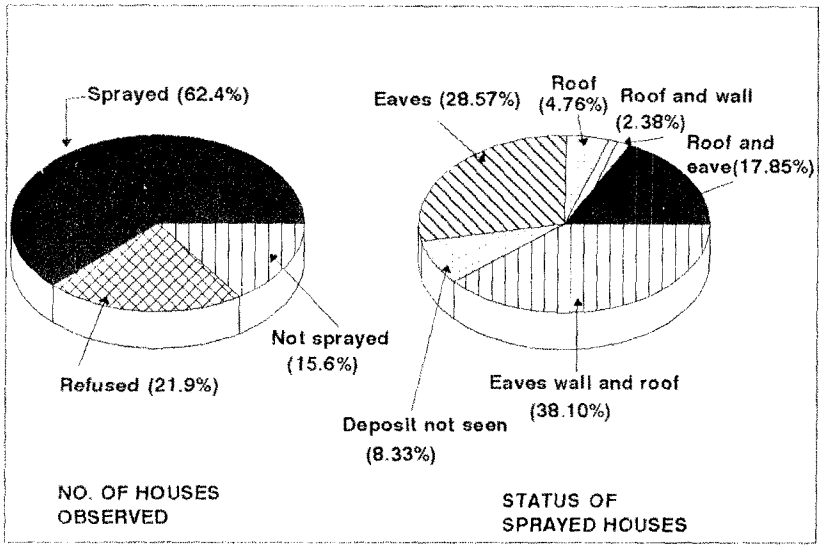

Fig. 4: Spray status of houses in Valod village, Surat

DISCUSSION

Undoubtedly, malaria has increased in Buhari PHC, especially prevalence of *P. falciparum* has enhanced in most of the villages surveyed. High proportion of SPR, SfR alongwith the high crescent rate observed during the present survey in this area indicate the inadequacy of surveillance and radical treatment of positive cases in containing the malaria transmission. Similar trend has been recorded from other places in past resulting in an outbreak[11-13]. High spleen rate and dominance of *P. falciparum* infections in children reflect continued malaria transmission. Labour inflow with afebrile *P. falciparum* infection during post-monsoon months confirms possibility of parasite load enhancement that was not included in surveillance. Sharma *et al.*[14] have reported continuous influx of labour for different work as an important source of malaria infection in Kheda.

Further the emergence of more RI chloroquine resistant *P. falciparum* strain is a great concern, which might have either been introduced by the migratory labourers camping near villages from September-October to May-June with incubating gametocytes or due to prolonged under dose treatment of malaria cases by private practitioners. High density of *An. culicifacies* was maintained due to favourable ecological and climatic factors as a result of unusual rainfall and vast stretch of river providing extensive breeding potential. Low level of susceptibility to insecticides and adequate longevity of *An. culicifacies* are suggestive of in-ef-

fectiveness of adulticide (DDT and malathion). Further, first round of DDT spray was carried out in second half of August in 1993 at the time when the transmission had already commenced. Under such situations use of some effective insecticide[15,16] at prescribed time schedule can only interrupt malaria transmission in this area.

The present study shows that if adequate precautionary measures are not taken to deal with migratory labourers, improper insecticide coverage, inadequate surveillance and mass ignorance, malaria situation may further worsen in future in this area. Above all, people's cooperation, knowledge of disease and communication between workers and community are pre-requisite to deal with the situation.

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Bionomics of Vector Anophelines in District Naini Tal, Uttar Pradesh

R.P. SHUKLA, A.C. PANDEY[a], V.K. KOHLI, V.P. OJHA and V.P. SHARMA[b]

Breeding behaviour and seasonal prevalence of vector anopheline in different habitats associated with rice land agroecosystem of terai and bhabar area of District Naini Tal was studied from July 1992 to June 1994. Adult prevalence in both the areas shows two distinct *An. fluviatilis* peaks in the months of March and October and the breeding places for the species were *pokhars* in bhabar and stream and irrigation drains in terai. Prevalence of adult *An. culicifacies* was observed during July to August in bhabar and April and August in terai. In bhabar high immature density of *An. culicifacies* was noted mainly in tanks, *pokhars* and paddy fields (early transplantation period). In terai high immature density of *An. culicifacies* was noted in stream, while low in paddy fields. Higher prevalence of immatures and adult anopheline vectors coincide with peak malaria incidence in both the areas. In bhabar in the months of August and September large number of malaria cases were contributed by *An. culicifacies* and in November by *An. fluviatilis*. In terai, malaria cases in April and from June to September were contributed by *An. culicifacies*, whereas *An. fluviatilis* might be responsible for transmission in October.

Keywords: Anophelines, Malaria, Vector bionomics

INTRODUCTION

Terai area of Naini Tal was hyper-endemic for malaria during the pre-DDT period and *An. minimus* was the prime vector[1-3], thereafter *An. fluviatilis* took over as the principal vector and *An. culicifacies* a secondary vector[4,5]. In

Malaria Research Centre (Field Station), Bhotia Parao, Inderjeet Garden, Haldwani-263 141, India.
[a]Malaria Research Centre (Field Station), BHEL Complex, Ranipur, Hardwar-249 403, India.
[b]Malaria Research Centre, 20-Madhuvan, Delhi-110 092, India.

1941, malaria control activity was started with application of DDT and mass administration of paludrine, resulting disappearance of *An. minimus* and malaria endemicity came down[6]. Reclamation of terai, clearing of forests, construction of seven water reservoirs for irrigation, colonization etc. in the DDT-era has entirely changed the ecosystem of the area. Since 1980's *An. culicifacies* has become a major vector followed by *An. fluviatilis* which was reported as primary vector in 1950's. Recently, *An. culicifacies* and *An. fluviatilis* both are incriminated as efficient vectors and evidence of their role in malaria transmission in terai is established[7-10].

District Naini Tal contains three longitudinal ecogeographical areas viz. terai, bhabar and Shiwalik ranges. Near the foothills of Shiwalik is a gentle slopping area called bhabar which constitutes 10-20 km wide belt with many forests. Bhabar is a piedmont area with dry upland, low water table, sandy loam and dispersed settlements. Terai is a low-lying leveled area with abundant ground water, clay loam soil and agglomerated settlements. In rice land agroecosystem to understand the vector bionomics in terai and bhabar area of District Naini Tal, studies on prevalence of immature anophelines in different breeding grounds, vector prevalence and malaria incidence were carried out in two villages of bhabar i.e. Bhawani Singh Newar and Himmatpur Chaumwal with a population of 288 and 392 and two villages of terai, i.e. Shantipuri No. 3 and CRC, Pant Nagar with a population of 709 and 529, respectively from July 1992 to June 1994. The results of the study are communicated in this paper.

MATERIALS AND METHODS

Immature collections at fortnightly interval were carried out using standard dipper of 9.5 cm diam (300 ml capacity) from all the breeding grounds and densities of III and IV instar larvae were recorded. Regular adult collections were conducted on fortnightly interval using aspirator. Hand collections were carried out in four human dwellings and four cattlesheds of each villages and per man hour density of anophelines were recorded. Weekly active malaria surveillance from the above cited villages were made, blood smear of fever cases were prepared and presumptive treatment with 600 mg chloroquine base per adult was given. All malaria positive cases were given radical treatment.

RESULTS AND DISCUSSION

Larval density: Average anopheline immature density per dip during two years of study period in peridomestic sites was 2.3 and 1.2 in cemented tanks and *pokhars* (domestic ponds) in bhabar, and 1.0, 4.7 and 0.4 in shallow wells, stream and irrigation drains in terai areas, whereas in paddy field the immature density was 1.5 in bhabar from July to October and 1.4

in terai from March to October (two cropping season) (Table 1). During the study period a total of 4317 anopheline larvae were collected and identified from all the breeding grounds.

Species prevalence: Monthwise average density of immature anopheline vectors in bhabar is given in Table 2. Seasonal prevalence of *An. culicifacies* and *An. fluviatilis* at different breeding sites in bhabar area revealed that *An. culicifacies* breeds predominantly in cemented tanks, throughout the year and its density per dip was recorded highest in August (6.29), whereas, dur-

Table 1. Average immature density of mosquitoes (1992-1994)

	Bhabar			Terai			
	Pokhar	Tank	Paddy field	Shallow well	Stream	Irrigation drain	Paddy field
Anopheline	1.2	2.3	1.5	1.0	4.7	0.4	1.4
Culicine	0.5	0.8	0.9	9.2	0.03	0.4	2.5

Table 2. Average density/dip of immature anopheline vectors in bhabar area

Months	Tanks		Pokhars		Paddy field	
	An. culicifacies	*An. fluviatilis*	*An. culicifacies*	*An. fluviatilis*	*An. culicifacies*	*An. fluviatilis*
Jan	0	0	0	0.42	No cropping	
Feb	0.15	0	0.06	0.01	No cropping	
Mar	0.04	0	0.21	0.04	No cropping	
Apr	1.06	0.02	1.03	0.82	No cropping	
May	1.86	0	0.03	0.03	No cropping	
Jun	0.57	0	0.19	0	No cropping	
Jul	2.83	0	0	0	1.86	0
Aug	6.29	0	-	-	0.21	0.002
Sep	1.99	0	0.13	0	0	0
Oct	0.99	0	0	0.10	0	0
Nov	0.20	0.01	0.47	0.47	No cropping	
Dec	0.01	0	0.12	0	No cropping	

Table 3. Average density per dip of immature anopheline vectors in terai area

Months	Shallow wells		Irrigation drains		Stream		Paddy fields	
	An. culici-facies	*An. fluvia-tilis*	*An. culici-facies*	*An. fluvia-tilis*	*An. culici-facies*	*An. fluvia-tilis*	*An. culici-facies*	*An. fluvia-tilis*
Jan	0	0	0.11	0	0	0	No cropping	
Feb	0	0	0.03	0.27	0.39	1.12	No cropping	
Mar	0	0.02	0	0.30	0.08	0.78	0.6	0
Apr	0	0	0	0	2.58	0	1.81	0
May	0.98	0.16	0.63	0.11	15.92	0	0.05	0.05
Jun	0.49	0	0.02	0	7.23	0	0	0.01
Jul	0.13	0	0	0.2	1.0	0	0.37	0
Aug	0	0	0.07	0	3.25	0	0.04	0.01
Sep	0.13	0	0	0	0	0	0.02	0.01
Oct	0	0	0	0	2.25	0	0.02	0.01
Nov	0	0	0	0.2	0.80	0	No cropping	
Dec	0	0	0	0.2	–	–	No cropping	

ing the month of November to March the density was very low (0 to 0.2). A very low density of *An. fluviatilis* was observed in April (0.02) and November (0.01). In *pokhar, An. culicifacies* density was noted in April (1.03), whereas in remaining months the density varied from 0 to 0.47. Density of *An. fluviatilis* was observed from January to May and October and November which varied from 0.01 to 0.82. In paddy fields, *An. culicifacies* immature density was observed in July (1.86) and August (0.21) and during rest of the period density remained nil. Breeding of *An. fluviatilis* was observed only in August (0.002), which reflected that paddy fields were not preferred by *An. fluviatilis*[11] (Table 2).

The seasonal prevalence of immature anopheline vectors in terai area is shown in Table 3. Breeding of *An. culicifacies* was observed in stream for the greater part of the year and its highest density was recorded in May (15.92). Prevalence of *An. culicifacies* was observed from May to July and September which varied from 0.13 to 0.98 in shallow wells. In irrigation drains *An. culicifacies* prevalence ranged from 0.02 to 0.63 in January, Feburary, May, June and August, whereas during rest of the months density remained zero in both the breeding sites. It was further noted that in May, highest density of *An. culicifacies* was recorded in all breeding sites except paddy fields. During dry cropping season, highest *An. culicifacies* prevalence was recorded in April (1.81) and low (0.02) in wet cropping season, revealing maximum breeding during early paddy transplantation period.

An. fluviatilis density recorded was very low (0-0.05) in paddy fields, while in stream, density in February and March was 1.12 and 0.78, respectively and breeding was not observed in rest of the months. In irrigation drains breeding was recorded low in February and March, May, July, October and November ranging from 0.11 to 0.30. Comparative analysis of data on average density of immature anopheline vectors revealed that stream is much preferred site for breeding of both malaria vectors.

Adult density: Average per man hour density of indoor resting vectors in bhabar and terai area are presented in Table 4. In bhabar *An. culicifacies* density was very low (0.05-1.3) except during July to September, when it increased sharply and reached highest in August (48.3). This abrupt increase of *An. culicifacies* density might be due to vast area of paddy fields which contributed to enhance its density. Overall density of *An. fluviatilis* was observed low as compared to *An. culicifacies* throughout the study, but high density of *An. fluviatilis* was observed in the months of October (1.7), November (2.3), December (2.0) and March (1.0) and low for rest of the months (0.05 to 0.4). In terai *An. culicifacies* density was recorded high as

Table 4. Per man hour density of indoor resting anopheline vectors

Months	Bhabar		Terai	
	An. culicifacies	*An. fluviatilis*	*An. culicifacies*	*An. fluviatilis*
Jan	0.2	0.1	1.1	1.8
Feb	0.05	0.1	1.6	4.8
Mar	0.05	1.0	4.9	9.7
Apr	0.7	0.4	18.2	5.0
May	1.2	0.3	19.9	0.6
Jun	1.3	0.05	22.9	0.3
Jul	11.3	0	22.6	0.3
Aug	48.3	0.05	12.0	0.4
Sep	6.8	0.4	5.9	2.3
Oct	1.2	1.7	4.9	10.7
Nov	0.7	2.3	8.9	5.6
Dec	0.6	1.0	5.7	2.5

compared to bhabar throughout the year except in August. Its peak density was recorded during the month of June (22.9) and July (22.6), but low (1.6-8.9) from September to March. *An. fluviatilis* density was observed high in October (10.7) and March (9.7), whereas its density was observed low from late summer to mid rainy season which varied from 0.3 to 2.3. Incrimination study was not carried out during the course of investigations.

Malaria incidence: Results of malaria study conducted during the course of investigation in bhabar and terai are presented in Table 5. In terai a total of 277 blood smears from fever cases were collected and examined. Monthly fever rate varied from 1.01 to 25.5% and highest in August (25.5%). Average SPR and SfR were 7.2 and 0.7, respectively. Malaria cases per thousand population per year varied from 0 to 3.2. High incidence of malaria was observed in June (1.2), August (1.2), September (3.2) and sudden drop of cases was observed in October (0.8). Most of the malaria cases were due to *P. vivax*, whereas *P. falciparum* cases were recorded in August (SfR 1.4%) and October (SfR 5.3%).

In bhabar, a total of 283 blood smears were collected and examined. Monthly fever rate varied from 2.12 to 20.14%. Average slide positivity rate and slide falciparum rate was 5.3 and 0.3%, respectively. Malaria cases per thousand population per year varied from 0 to 4.4 during study period and more malaria cases were recorded in May (2.9), Au-

Table 5. Malaria cases in bhabar and terai area

Months	Terai (Popu. 1238)						Bhabar (Popu. 680)					
	BSE	*Pv*	*Pf*	Total (+) ve	SPR	SfR	BSE	*Pv*	*Pf*	Total	SPR (+) ve	SfR
Jan	3	0	0	0	0	0	6	0	0	0	0	0
Feb	11	1	0	1	9.1	0	9	0	0	0	0	0
Mar	10	0	0	0	0	0	0	7	0	0	0	0
Apr	16	2	0	2	12.5	0	10	0	0	0	0	0
May	7	0	0	0	0	0	20	4	0	4	20.0	0
Jun	19	3	0	3	15.8	0	9	0	0	0	0	0
Jul	55	1	0	1	1.8	0	45	1	0	1	2.2	0
Aug	70	2	1	3	4.3	1.4	44	3	0	3	6.8	0
Sep	47	8	0	8	17.0	0	57	6	0	6	10.5	0
Oct	19	1	1	2	10.5	5.3	45	0	0	0	0	0
Nov	7	0	0	0	0	0	15	0	1	1	6.7	6.7
Dec	13	0	0	0	0	0	16	0	0	0	0	0
Total	277	18	2	20	7.2	0.7	283	14	1	15	5.3	0.35

Table 6. Meteorological data of Pant Nagar (terai)

Months	1992			1993			1994		
	Av. temperature (0°C)	Av. relative humidity (%)	Total rainfall (mm)	Av. temperature (0°C)	Av. relative humidity (%)	Total rainfall (mm)	Av. temperature (0°C)	Av. relative humidity (%)	Total rainfall (mm)
Jan	13.85	72.0	9.2	10.9	72.3	5.2	14.20	71.9	29.45
Feb	14.45	69.65	16.8	17.55	66.25	11.0	15.64	71.48	37.52
Mar	20.5	56.2	0.0	18.55	60.55	66.6	21.3	64.20	0.0
Apr	26.4	39.6	0.0	25.55	48.5	0.6	25.18	45.16	24.9
May	29.25	38.65	26.6	31.65	47.7	37.2	31.04	47.64	27.9
Jun	30.75	50.85	75.6	31.05	58.55	110.4	31.55	60.54	66.3
Jul	28.85	74.15	302.6	29.05	76.4	299.6	29.52	78.49	334.8
Aug	28.65	79.25	199.8	29.05	83.2	308.4	28.28	85.16	411.68
Sep	27.5	76.15	183.0	26.7	84.35	690.3	27.71	75.78	51.6
Oct	23.55	26.8	11.6	23.85	65.0	0.0	23.96	63.95	0.0
Nov	19.3	64.4	6.0	19.45	65.5	0.0	19.22	61.54	0.0
Dec	14.8	6.35	0.0	15.35	65.1	0.0	15.15	67.10	0.0
Total	23.15	62.7	831.2	23.2	66.2	1529.0	23.56	66.08	984.07

gust (2.2) and September (4.4). A sudden drop of malaria cases were recorded in October, whereas one case of *P. falciparum* (SfR 6.7%) was reported in November, thereafter no malaria case was observed till April (Table 5). The meteorological data for the year 1992 to 1994 collected from G.B. Pant University of Agriculture and Technology, Pant Nagar is given in Table 6. The relative humidity and temperature during July to September was favourable for transmission of malaria and the rainfall from June to September further supported the reproduction of vectors.

Prevalence trend of immature and adult anopheline vector and malaria in bhabar and terai area is shown in Figs. 1 and 2. In bhabar, an increase in malaria cases in the months of August and September indicated that cemented tanks and rice fields contribute for an abrupt increase in larval and adult *An. culicifacies* density. Malaria cases reported in the month of November might be due to increase in *An. fluviatilis* adult density which was contributed by *pokhars*. It was further observed that in bhabar high *P. vivax* malaria in August and September coincides with increase in *An. culicifacies* density, whereas *P. falciparum* malaria coincided with *An. fluviatilis* density in November (Fig. 1).

In terai, malaria cases observed in April, June to September is because of *An. culicifacies* density which was contributed by paddy fields and stream.

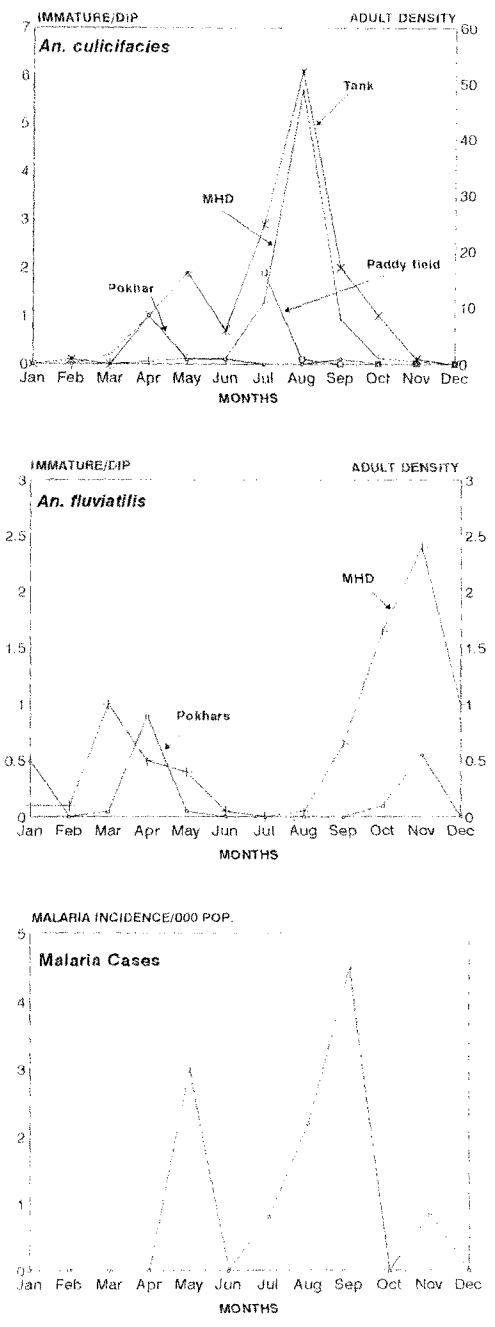

Fig. 1: Prevalence trend of immature and adult anopheline vectors and malaria in bhabar area

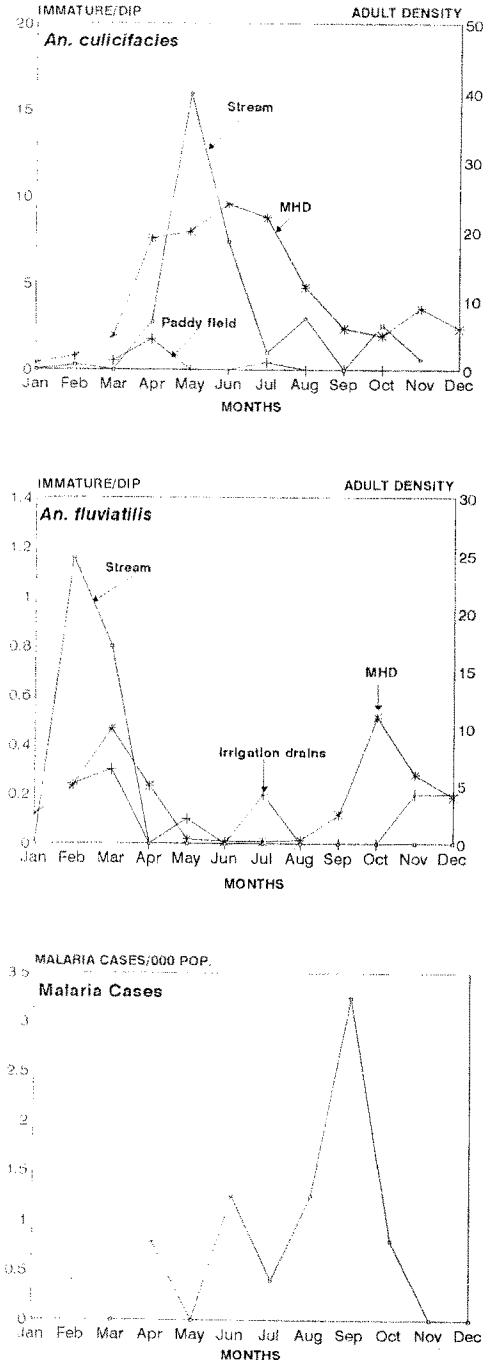

Fig. 2: Prevalence trend of immature and adult anopheline vectors and malaria in terai area

Density of *An. culicifacies* remained high for longer duration because of presence of stream and cultivation of crops during dry and wet cropping season and density recorded in paddy fields was low as compared to stream. Malaria cases in October might be due to high *An. fluviatilis* density which was contributed by stream (Fig. 2). During the study period *P. vivax* malaria was recorded highest in September and low from April to July followed by lowest in October. In above cited months, except in October *An. culicifacies* adult density was recorded high and was responsible for transmission of malaria[11,12]. Peak density of *An. culicifacies* being the main vector of the area coincides with rice cultivation period i.e. early cropping period and more than 95% *P. vivax* malaria was reported during rainy season. Regarding the contribution of *An. fluviatilis* in transmission of malaria it was reported earlier that this species is zoophilic. Now it was found responsible in transmission of malaria from June to October suggesting prevalence of both anthropophilic and zoophilic races[5]. Presently, *An. fluviatilis* is considered a secondary vector due to its low anthropophilic index, resting habitats and host preference, thus its role in malaria transmission needs further investigation[13]. Though, due to ecological change in the area and its flexible behaviour, possibility of malaria transmission was considered during post-monsoon period, i.e. October and November. In rice growing foothills area a high API is due to *An. fluviatilis* which

breeds in stream[14] and *pokhars* and *An. culicifacies* in tanks, paddy fields and stream which agrees with our present findings. Presently, low malaria cases recorded from both the areas during the study period, might be due to less reservoirs and parasitic load in the community resulting in low transmission of malaria. Thus, cemented tanks, paddy fields and *pokhars* in bhabar and stream, paddy fields and irrigation drains in terai contributes for vectors population in malaria transmission.

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Application of Simple Peptide ELISA for Stratification of Malaria Endemicity

ARATI ROY, SUKLA BISWAS, LALITHA KABILAN and V.P. SHARMA[a]

A serological investigation was conducted in north India to determine malaria endemicity based on the antibody levels against a nonapeptide RI (EENVEHDA–Cys) from the *P. falciparum* antigen *Pf* 155/RESA. *P. falciparum* sonicated crude antigen was also used in the study. Subjects of all age groups from various strata of malaria endemicity were included in this study. A total of 4273 finger prick blood samples from 49 villages of five districts were collected during January to March 1991 which is a non-transmission season and the antibody levels were estimated by ELISA. Although a good correlation was found between the antibody titre to the RI peptide and that to the crude antigen, the most consistent results were obtained with the RI peptide. When compared with the annual parasite index (API) values, an established method for defining malaria endemicity, mean anti-RI antibody titres obtained from several villages within a single zone correlated (r=0.94, p=0.023) with mean API value of the area. Thus, our results suggest that by using the RI peptide as antigen in seroepidemiology, it is possible to stratify malaria endemicity. We didn't distinguish between endemicity of *Pv* and *Pf* since each area experiences cycle of transmission of *P. vivax* followed by *P. falciparum* and our sera were from individuals having no fever.

Keywords: Malaria, *P. falciparum*, Seroepidemiology

INTRODUCTION

To determine the degree of malaria transmission in an area no simple method is available. For efficient monitoring and surveillance[1], the existing entomological and parasitological methods are either tedious or inadequate[2,3].

Malaria Research Centre, 22-Sham Nath Marg, Delhi-110 054, India.
[a]Malaria Research Centre, 20-Madhuvan, Delhi-110 092, India.

Importance of seroepidemiology in disappearing malaria is well-recognized[4]. Crude *Pf* antigen for ELISA has been widely used[5,6] in such studies. However, this presents a major limitation of serology, that is the lack of standardized reagents, particularly defined antigens[7].

To overcome this limitation a standardized peptide antigen for ELISA[8] was developed using 3' repeat sequence (EENVEHDA-Cys) of *Pf* 155/RESA antigen[9]. Its seroreactivity has been assessed in individuals from different malaria endemic situations.

Study area

The study was conducted in Srinagar, Haldwani, Shankargarh, Allahabad, Jabalpur and Mandla regions of northern India. In the study areas, an arbitrary classification was assigned, namely, low, moderate, high and non-endemic according to the annual parasite incidence (Table 1). All areas were under malaria control programme of the Malaria Research Centre, except Srinagar (Kashmir, non-endemic) and one malaria endemic (Jabalpur) area. Active malaria surveillance (weekly collection of blood smears from fever cases by door-to-door survey)[10] in the study areas was well established by Malaria Research Centre. As a result of intervention, malaria incidence declined in some places that had high transmission[11], such as villages in Haldwani, as ascertained by a weekly active surveillance (Table 1). Whereas, in Shankargarh, the incidence of malaria remained virtually stable probably because of new cases due to constant influx of migratory labour. In addition to the existing malariometric indices, a serological study of estimation of antimalarial antibodies against *Pf* and RI was carried out in Jabalpur (endemic/no malaria intervention measures) and Mandla (endemic/malaria control measures). These two areas were presented as control and experimental villages, respectively.

MATERIALS AND METHODS

Sample collection: Finger prick blood (50 µl) samples were collected from individuals having no fever as described earlier[8] during January to March 1991, known as the non-transmission season.

Peptide antigen R1: The synthetic nonapeptide EENVEHDA-Cys, an immunodominant epitope of ring infected erythrocyte surface antigen (RESA) was custom made (Cambridge Research Biochemicals, England) with 80 per cent purity as assessed by HPLC.

Antigen *Pf*: Parasites from the continuous *in vitro* cultures were enriched for schizonts (95%) by centrifugation on a percoll gradient. Parasite pellet was saponin lysed, sonicated and soluble extract was used as an antigen[12].

Table 1. Study of antibody response under varying endemicity

District code no.	District	Sample size	Mean API	API range	RI	Pf
I	Srinagar (Kashmir)	66	0	0	0.15	0.25
II	Gangapar/Duaba (Allahabad)	1251	3.11	3-8.6	0.197	–
III	Yamunapar (Allahabad)	430	10.6	1-17	0.193	–
IV	Haldwani (Naini Tal)	161	22	3-64	0.321	0.24
V	Mandla (Bizadandi PHC, MP)	358	165	80-291	0.37	0.52
VI	Shankargarh (Allahabad)	1002	218	99-431	0.834	0.83
VII	Jabalpur (MP)	1005	272	182-454	0.796	0.90

Control sera: Ten pooled serum from an endemic and non-endemic areas were taken as positive and negative sera respectively.

Enzyme linked immunosorbent assay (ELISA): Enzyme immunoassay was performed in Costar (USA) fast binding ELISA plate as described before[8]. Peptide (RI) and *Pf* antigen plates were coated with 50 ng/well and 10 µg/well respectively in carbonate bicarbonate buffer (0.1 M, pH 9.6) and incubated overnight at 4^0C and then 1 h at 37^0C. The plates were then washed three times with PBS T (0.05% Tween 20) and blocked with 200 µl/well of 1% BSA in PBS for 1 h at 37^0C. Later the plates were washed again as described above. 50 µl of various sample eluant in 1 ml PBS was added to the wells at 1:40 and 1:1024 dilution as developed by checkerboard titration for RI and *Pf* respectively. The plates were incubated for 1 h at 37^0C, washed thrice with PBST and then 50 µl of goat anti human-HRPO conjugate (Dakopatt, Denmark) at 1:3000 dilution was added to each well. After 1 h incubation at 37^0C, the plates were washed with PBST thrice, 50 µl orthophenylene diamine/H_2O_2 diluted in enzyme substrate buffer (0.1 M phosphate citrate buffer pH 5.0) was added to each well. Finally, the reaction was stopped by adding 25 µl of H_2SO_4 (8N). The OD of reaction product was recorded at 490 nm in Titretek ELISA reader. In each assay one set of negative and positive sera were used as con-

trols to determine day-to-day variation in the tests. Seropositivity was determined using an OD value equivalent to the mean + 2 SD obtained in the control population (non-endemic) as the cut-off value.

RESULTS

Antimalarial antibodies in the finger prick blood samples to the *P. falciparum* crude antigen and to a synthetic nonapeptide derived from the blood stage antigen of *P. falciparum*, *Pf* 155/RESA, were estimated by ELISA in subjects of various age groups living in different regions. Study areas have different levels of endemicity for malaria.

Fig. 1 shows the heterogeneous distribution of antibody reactivities of donors from the different regions to the synthetic peptide RI and the *Pf* crude antigen. The presence of IgG type antibodies were tested in serum. In general, antibody level to the *Pf* crude antigen were elevated (Fig. 1). Results of individual antibody titre from non-endemic population against the *Pf* crude antigen showed a wider scattering in comparison to the responses directed against the RI antigen (Fig.1). There was a statistically significant positive linear correlation (r=0.86; p=0.085) between the results obtained for the two antigens from a total population of 26 villages (Fig. 2). Usually endemicity of malaria is classified based on the spleen rate and API. We also investigated whether estimation of anti-RI antibody levels can serve as an additional parameter in determining malaria endemicity. To establish how the different test-systems are related to

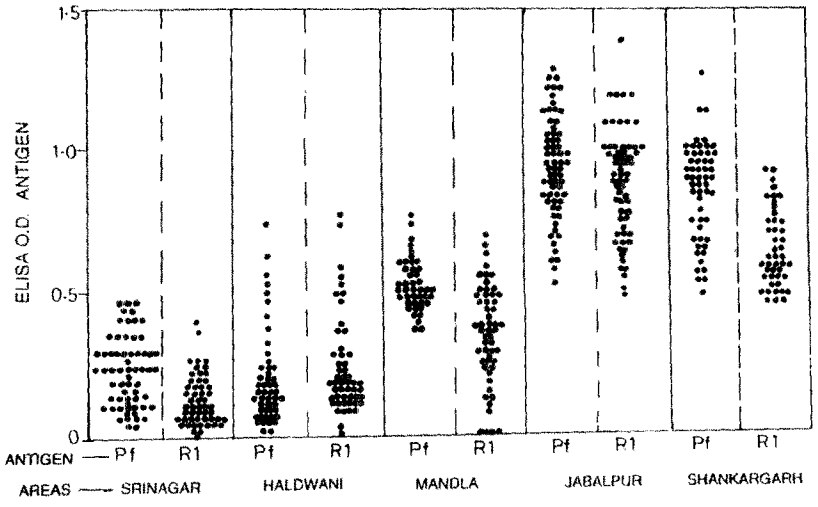

Fig. 1: Each dot presents a single individual, RI and *Pf* used as antigens under different endemicity

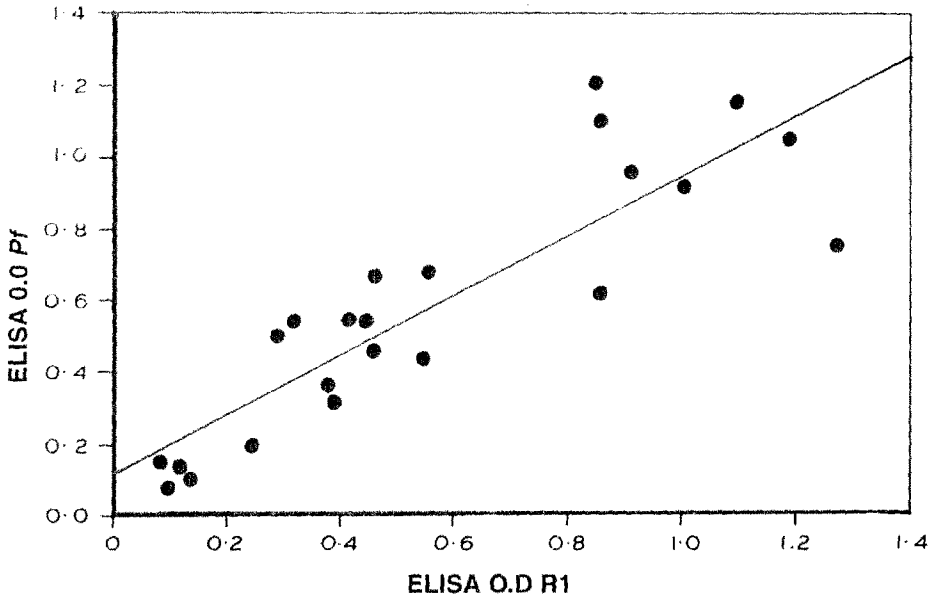

Fig. 2: Each dot presents RI, *Pf* values of individual village under variable malariogenic conditions

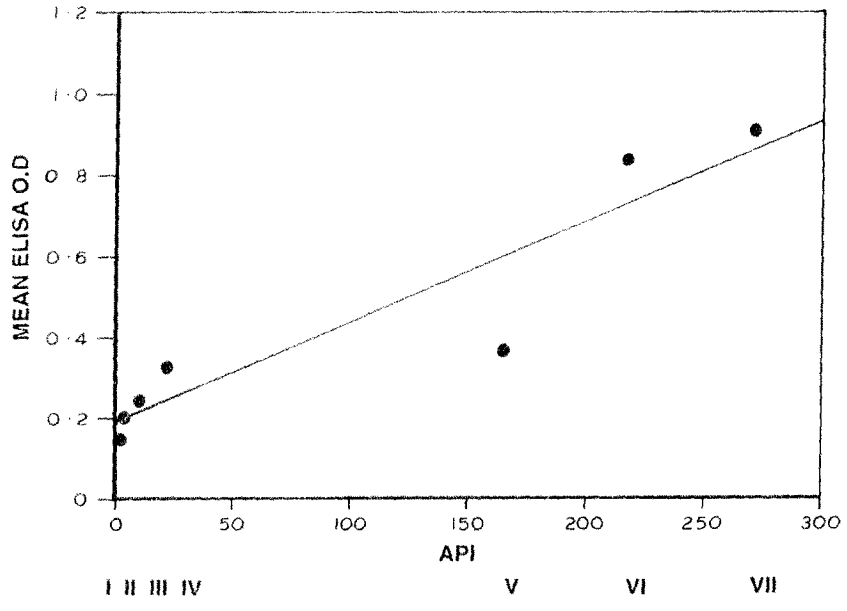

Fig. 3: Each dot presents one group comprising several villages

each other in classification of malaria endemicity, correlation analysis between API and antibody levels (mean ELISA OD values) from the study areas was done. The results showed a significant correlation (r=0.94; p=0.023) (Fig. 3).

To assess the significance of antibody responses in classification of the malaria endemicity, the study areas were arbitrarily classified into low, moderate, high and non-endemic depending on the mean ELISA OD values (Table 2). The classification was compared

Table 2. Village wise mean ELISA OD values between RI and *Pf* antibody with API

Area	Villages	API	RI	*Pf*	% population examined
Haldwani (Low)	Baira Pokhra	19	0.125±0.06	0.124±0.06	26
	Badi Mukhani	5.8	0.242±0.15	0.194±0.10	8
	Ratanpuri	35	0.099±0.04	0.071±0.04	45
	Brijwalpur	64	0.380±0.18	0.347±0.13	–
	Kishanpur Gurdwar	15	0.455±0.16	0.458±0.17	35
	Haripur Motia	13	0.547±0.17	0.433±0.12	38
	Deval Chaur	4	0.385±0.18	0.321±0.13	10
	Manpur	3	0.138±0.08	0.127±0.07	37
	Jeetpur Negi	9	0.136±0.07	0.108±0.04	14
	Anandpur	22	0.134±0.07	–	–
	Himmatpur Baijnath	42	0.16±0.04	–	–
	Gusaipur	31	0.134±0.06	–	–
Mandla (Moderate)	Chargoan	100	0.42±0.17	0.54±0.08	10
	Ghota	189	0.44±0.22	0.53±0.13	25
	Somnopur	291	0.29±0.10	0.49±0.10	29
	Vijaypur	81	0.32±0.16	0.54±0.11	18
Jabalpur (High)	Tarwani	235	0.85±0.11	1.2 ±0.10	13.5
	Dnduwa	210	0.86±0.19	1.09±0.20	13.9
	Magardha	195	1.00±0.13	0.927±0.09	15.3
	Chargaonkala	293	1.19±0.05	1.045±0.23	–
	Majhgaon	381	0.913±0.10	0.95±0.17	–
	Tikariya	231	1.27±0.08	0.75±0.11	–
	Khapa	327	0.86±0.20	0.61±0.17	32.8
	Dungariya	454	0.56±0.12	0.67±0.18	20.9
	Bilnagari	210	1.10±0.16	1.15±0.19	25.5
	Banjartola	182	0.46±0.16	0.66±0.19	36.7

with malaria endemicity based on the API values (Table 2.). It is apparent from the results that there was a distinct positive correlative pattern between the anti-malarial antibody titres and API values (Fig. 3). Mean OD value of RI and *Pf* antigen showed a rising trend with increased endemicity.

Sample size is 20-25% of the total that have been covered. Children of 0-5 yrs age group and adult >20 yrs have been compared in the study. Age and sexwise status did not show any impact on antibody profile. Studies of antibody response in the pre- and post-monsoon season and their relation with API values are currently in progress.

The data from our studies suggest that the RI peptide can be useful in seroepidemiological investigation in determining endemicity. This nonapeptide differs as quoted previously[8], from that of octapeptide as reported elsewhere[9]. Whether the terminal cystine in nonamer which is absent in the octamer may enable a better binding to the plate[8] or even undergo oligomerization during storage remains to be elucidated. However, the reproducibility, the simplicity and the specificity of the assay using RI antigen indicate the applicability of this system in seroepidemiological surveys. Moreover, the correlation seen between the levels of anti-RI antibodies and the intensity of transmission (API) also suggest that RI peptide ELISA may be useful as an additional parameter in malariometric survey. However, further work is needed to explore the potential of this antigen in malaria surveillance.

DISCUSSION

Usual malariometric surveys include parameters of host origin like spleen rate and API. Now in India, availability of anti-malarial drugs is very good. When the parasites are cleared, the spleen size reduces to normal so it is difficult to locate palpable spleen. Moreover, spleen examination requires trained personnel. This calls for inclusion of additional parameters like serology in malaria survey. In malaria surveillance, the need of a simple test that will be complementary to parasitological surveillance cannot be over emphasized[13]. Serological approach to achieve this end has been tried by many workers using crude malaria antigens[14]. Results obtained in different studies however, are not comparable and at times are inconsistent[15]. Scattering of *Pf* OD values of non-endemic area, not seen with RI antigen, may suggest that the RI antigen is relatively pure and thus may be more specific for malaria.

There are reports of cross reacting protozoal antigens[16]. However, it will be difficult to know whether the antibodies seen in the non-endemic population to the *Pf* crude antigen are due to multiple cross-reactivity. A good correlation was observed between the results of RI and *Pf* antigen which may

indicate that humoral responses against the RI antigen reflects the anti-*Pf* responses. The main limitation of these studies was the batch-to-batch variation of the crude plasmodial antigen which was used in the assays. Establishment of *in vitro* culture system of the parasite is necessary to procure crude antigenic material. Thus the standardization of the assay system was not feasible. Serological investigations using peptides as capturing antigens could overcome such difficulties, since results obtained with the peptides are consistent, reproducible and specific. The simplicity of the assay facilitates screening of many samples in epidemiological investigation.

In this study, we have tried to investigate whether estimation of antibody levels against a nonapeptide (RI) from *Pf* 155/RESA can be a complementary index in determining levels of endemicity of malaria in a particular area. Our serological data with the RI antigen revealed the distinct differences in the antibody levels in individuals living in endemic and non-endemic areas in India. Increased levels of anti-RI antibodies in endemic areas seen here reflects the continuous boosting of individuals under perennial transmission. It was shown elsewhere that antibody responses remained constant over the years in such individuals[17]. It was also shown that antioctapeptide antibodies are short lived. These results showed that the categorization of endemicity correlated significantly with the rate of malaria transmission of *P. falciparum* malaria except in moderate endemic area.

In Mandla, where the anti-RI antibody level was low, a pilot malaria control programme was in practice during the period of our study and thus may attribute to the low levels of anti-RI antibodies. However, it is difficult to state now, whether the antibodies against the RI peptide are due to cross-reactivity to other malarial antigens, since *P. vivax* is also prevalent in the study areas. Cross-reactivities to plasmodial antigens in serum have been described by others[18]. Further studies correlating antinonamer antibody with incidence of *Pv* and *Pf* are in progress to investigate this question.

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SHORT NOTE

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Drug Resistant *Plasmodium falciparum* in Mandla District, Madhya Pradesh

NEERU SINGH, A.K. TYAGI and V.P. SHARMA[a]

Keywords: Drug resistance, Malaria, *Plasmodium falciparum*

The hilly and forested tribal belt of Madhya Pradesh (M.P.) is highly malarious. Transmission is generally perennial with preponderance of *Plasmodium falciparum* in the presence of two vector species, i.e. *Anopheles culicifacies* and *An. fluviatilis*[1]. Chloroquine has been a highly effective antimalarial drug in M.P., and no chloroquine resistant cases were reported till 1983[2]. Resistant strains were first detected *in vitro*[3] and also *in vivo* in Mandla[4]. Subsequently, resistance to the drug was reported from other areas of M.P.[5] Therefore, a follow-up study was taken up to assess changes in *P. falciparum* prevalence and its response to chloroquine after several years of drug pressure to obtain information for determining appropriate malaria treatment policy in these hard-core malarious areas.

For the purpose of this study a malaria clinic was established in PHC hospital of Bizadandi block, District Mandla during September to October 1994. After obtaining patient's consent visiting malaria clinic a simplified *in vivo* test system[6,7] was applied to de-

Malaria Research Centre (Field Station), Medical College Building, Jabalpur-482 003, India.
[a]Malaria Research Centre, 20-Madhuvan, Delhi-110 092, India.

tect chloroquine resistance. All patients were given 600 mg chloroquine phosphate orally and those below 14 years were given a dosage of 10 mg/kg body weight. Blood smears were taken before treatment and these were re-examined to determine asexual parasite density per cu mm of blood. The day of blood smear collection and drug administration was taken as Day 0. On the next day, the same dose of chloroquine was given as on Day 0. On Day 2, the patients were given 300 mg chloroquine or 5 mg/kg body weight. Follow-up blood smears were taken on Day 2 and the asexual parasite density was determined. If the level of asexual parasitaemia detected on Day 2 was more than 25% of that recorded on Day 0, the resistance to malaria parasite was considered to be at RIII level. These patients were administered a single dose of metakelfin (sulfalene 1500 mg, pyrimethamine 75 mg). The patients whose asexual parasite density on Day 2 was less than 25% of that of original smear on Day 0, follow-up blood smear were taken on Day 7, if the later was negative for malaria parasite, the degree of resistance was S/RI late. If parasites were still present, RI early/RII level of resistance was presumed and later treated with metakelfin. If the patient's blood smear were found negative for asexual parasite on Day 2, they were considered as S/RI.

A total of 1231 fever cases were screened, out of which 496 were positive for malaria (375 *Pf* and 121 *Pv*). Only 78 patients came for follow-up of which four were pregnant women. In all 44 cases showed asexual parasites after 48 h of which three were pregnant. The individual responses observed in these patients are presented in Table 1. RIII level resistance was recorded in 35% cases and overall failure rate of chloroquine to clear *P. falciparum* was 55% as against 15-20% in 1989-90 from this area[3]. Perusal of malaria records of PHC hospital from the last five years revealed that prevalence of *Pf* was on increase, i.e. from 56% in 1989 to 68% in 1993.

P. falciparum had been a major problem in foot hills of M.P. and other regions of high transmission. The tribals inhabiting these regions are one of the weakest sections of society and are responsible for a sizeable number of falciparum malaria cases[8] and deaths due to malaria. State record (NMEP)[9] showed that average API of Mandla for the last five years is on increase i.e. 8,7,10,12 and 16 respectively from 1989 onwards and is third among 44 districts in M.P. This indicates that control measures carried out by NMEP in the state are not effective to contain the disease.

The spread of resistant strain to other receptive areas within the district and outside is of great concern considering the fact that there is substantial degree of human movement for various purposes. As majority of *P. falciparum* cases were found to be resistant by *in vivo* method, an immediate change in

Table 1. Chloroquine sensitivity* of *P. falciparum* by simplified *in vivo* test in Bizadandi PHC, District Mandla

Sl. No.	Age/Sex		Parasitaemia/mm^3			
			Day 0	Day 2		Day 7
1.	30	M	3494.74	1300	(37.2)	Nil
2.	35	F	6846	2800	(41)	Nil
3.	22	F	33654.32	10387.30	(31)	Nil
4.	30	F	9859	3368	(34.2)	Nil
5.	26	M	3181.74	1310.58	(41.2)	Nil
6.	8	F	12686	2952	(23.3)	Nil
7.	9	M	12969	12027	(93)	Nil
8.	5	M	22807	13013	(57)	Nil
9.	8	M	31107	7242	(23.3)	Nil
10.	30	M	42377	11864	(28)	Nil
11.	7	F	1927	3259	(169)	Nil
12.	13	M	2957	1098	(37)	Nil
13.	28	F**	1994	11284	(566)	10146***
14.	25	F**	2443	1743	(71.4)	205
15.	23	F**	2404	205	(8.5)	Nil
16.	24	F**	1994	1308	(65.6)	405

*Only few cases are given here for brevity; **Pregnant women were given repeated dose of chloroquine; ***Referred to hospital for quinine. Figures in parentheses indicate the percentage parasitaemia, 100% being the level on Day 0.

drug policy is warranted. Since migration of labourers from other states to several irrigation project areas in Madhya Pradesh (29 major, 135 medium and 3000 minor irrigation projects) will continue for many years to come for development of the state, it is necessary to establish several check posts at each project site to prevent spread of drug resistant strains and deaths due to malaria.

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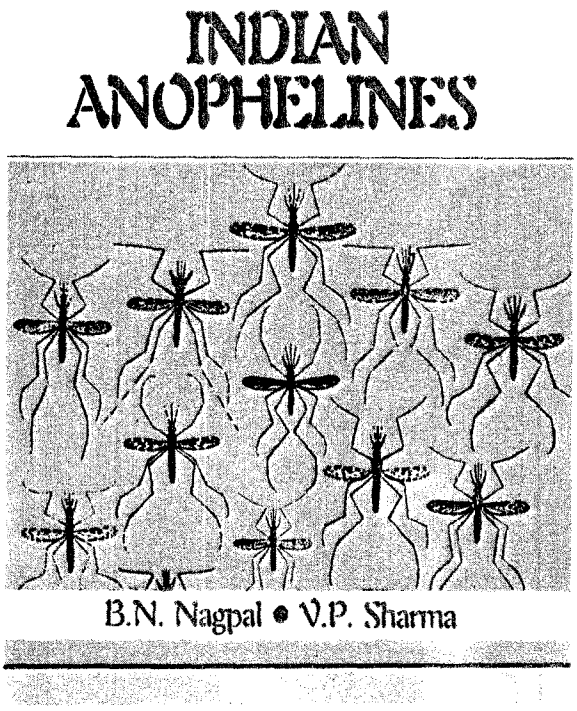

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