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Severity of Blood Induced *Plasmodium cynomolgi* B and *Plasmodium cynomolgi cynomolgi* Infection in Pregnant Rhesus Monkeys *(Macaca mulatta)*

K.K. KAMBOJ[1] and G.P. DUTTA[1]

Course of blood induced Plasmodium cynomolgi B and *P. cynomolgi cynomolgi* infections in twenty two female rhesus monkeys in terminal stages of pregnancy and sixteen normal females, has been studied. It has been found that in pregnant monkeys there was a significantly higher level of peak parasitaemia and longer duration of patency compared to the normal (control) females. Pregnant monkeys also maintained 2.12 to 2.71 fold higher parasitaemia compared to normal females.

Major complications of untreated malaria during pregnancy were death of the mother with foetus *in utero* in 4 monkeys, premature abortion in 4, death of the monkeys after parturition in 2, and death of the new-born baby in 4 monkeys. Pregnant rhesus monkeys can serve as useful non-human primate model to study the complications of malaria in pregnancy and also for the evaluation of efficacy and toxicity of known and new antimalarials.

INTRODUCTION

Due to resurgence of malaria, the large populations belonging to South-East Asia, Africa and Southern America are exposed to a greater risk of malaria. Infact malaria and its complication remain a major infectious cause of morbidity and mortality in the world (WHO, 1971). The problem of malaria becomes more aggravated during pregnancy because of its reported adverse effects in pregnant women resulting in severe complications.

The incidence of malaria as well as the density of parasitaemia among pregnant women has been reported to be significantly higher compared to the non-pregnant women. Studies from areas with high incidence of malaria have shown that during pregnancy there is nearly 2-4 fold increase in the density of malaria infection (Madecki & Kretschmer, 1966; Gilles *et al.*, 1969; Pingoud, 1969; Otieno *et al.*, 1971; Kortman, 1972; Bray & Anderson, 1979).

Several studies in pregnant women show that malaria infection during this period can result in serious complications posing danger both to mother as well as the foetus. Wickramasuriya (1937) reported a high maternal loss among non-immune pregnant women during a malaria epidemic in Ceylon in 1937. Lee Van Hung (1951) reported 14% foetal death rate from mothers with positive placenta, many of whom aborted when they had severe attacks of malaria. Bruce-Chwatt (1952) and Jelliffe (1968) have reported reduction in mean birth-weights associated with placental parasitization. Tachakmov (1964) reported the oedamatous nature of placenta and its delayed retention leading to foetal anorexia among women who had malaria during terminal stage of pregnancy. Covell (1950) and McQuay *et al.* (1967) reported the rare occurrence of congenitally transmitted malaria. Galbraith *et al.* (1980) observed that maternal malaria may cause placental damage and thereby jeopardize the materno-foetal relationship among pregnant women.

Unfortunately there is no suitable experimental animal model reported so far in literature which can be reliably used for studies relating to mala-

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ria and its complications during pregnancy. The present communication reports the severity of malaria, in pregnant rhesus monkeys, as well as its adverse effects on the foetus and the mother experimentally infected with *P. cynomolgi* B and *P. cynomolgi cynomolgi* during the terminal stage of pregnancy.

MATERIAL AND METHODS

Twenty two female rhesus monkeys (weighing 7-8 kg) in terminal stage of pregnancy and sixteen normal females (weighing 5-6 kg.) which were free from tuberculosis as shown by negative tuberculin test and chest X-ray, were used in this study. The two strains of malaria parasite namely *P. cynomolgi* B and *P. cynomolgi cynomolgi* were obtained from Dr. W.E. Collins, CDC, U.S.A. and NICD, Delhi, respectively.

Infections with *P. cynomolgi* B were induced in both pregnant and normal monkeys by i/v inoculation of $1x10^5$ parasitized red cells and that of *P. cynomolgi cynomolgi* with $1x10^6$ parasitized red cells. The course of parasitaemia was monitored by routine examination of thick and thin blood smears stained with Giemsa. Most of the pregnant monkeys delivered their offsprings during the course of malaria infection. The data on still births, delayed retention of placenta, death occurring due to malaria in pregnancy and adhesion of placenta in the pregnant group were recorded and the possibility of transplacental transmission of malaria was studied by making regular blood smears of newborn and also by subinoculation of 2ml of blood into healthy baby monkeys (weighing 2 kg.).

RESULTS

The course of blood induced *P. cynomolgi* B and *P. cynomolgi cynomolgi* infections in pregnant and normal female rhesus monkeys was studied with a view to compare the severity of malaria in the two groups under identical conditions (Tables 1 & 2). The severity of malaria was judged by taking into account the height of peak parasitaemia as well as the duration of patency (level of parasitaemia >0.1%). The results show that *P. cynomolgi* B infection reached a peak of 8.36 ± 3.02% in pregnant females and 5.20 ± 1.35% in normal females. The difference in the peaks of the two groups was statistically significant ($p < 0.02$). There was also significant difference in the length of patency in the two groups ($p < 0.05$). Infection with *P. cynomolgi cynomolgi* also produced a significantly higher level of parasitaemia in the pregnant group compared to the normal females ($p < 0.001$). Further, the pregnant group showed a highly significant longer duration of patency compared to the control females ($p < 0.001$). The cumulative parasitaemia load was 2.12 fold higher in pregnant females infected with *P. cynomolgi* B and 2.71 fold higher in pregnant females infected with *P. cynomolgi cynomolgi* as compared to their corresponding control females.

The major complications of untreated malaria infection in 22 rhesus monkeys in terminal stage of pregnancy (with over 5 months of gestation) included the deaths of monkey before or after delivery in 4, premature abortion and foetal death in 6, delayed retention of placenta in 4 and death of monkey after delivery in 2 cases. In four cases the baby monkey died after birth because of poor health and neglect by mother. Parasties were found in the placenta but no transplacental transmission was encountered even when 2ml of the blood from four new born babies was transfused into healthy baby monkeys.

DISCUSSION

There is no suitable experimental model to study the problems of malaria during pregnancy particularly its adverse effects on the mother and foetus. It is extremely essential to have a non-human primate model to evaluate the efficacy and toxicity of known and new antimalarials during pregnancy.

The present study on malaria in female rhesus monkeys during their terminal stage of pregnancy using *P. cynomolgi* B and *P. cynomolgi cynomolgi* has shown that the parasite load dur-

Table 1. Prepatent period, peak parasitaemia* and patency of infection in pregnant and normal female *Macaca mulatta* monkeys infected with *Plasmodium cynomolgi* B.

Monkey No.	PREGNANT			Monkey No.	CONTROL		
	Prepatent period (Days)	Peak parasit-aemia	Patency >0.1% (Days)		Prepatent period (Days)	Peak Parasit-aemia	Patency >0.1% (Days)
1	6	560	28	9	6	540	22
2	6	820	28	10	6	625	28
3	6	824	24	11	5	756	18
4	6	780	24	12	7	484	20
5	6	984	24	13	7	280	21
6	6	360	24	14	6	546	25
7	5	1372	31	15	6	480	25
8	5	995	27	16	7	525	24
Mean	5.75	836.88	26.25		6.25	529.50	22.88
S.D	0.46	302.55	2.66		0.71	135.01	3.23
S.E	0.16	106.97	0.94		0.25	47.73	1.14
Significance	NS	<0.02	<0.05				

*Parasites/10^4 RBC's

ing pregnancy was double. The severity of malaria during pregnancy has been attributed to be due to a breakdown in the maternal immune system either under stress of pregnancy or withdrawal of proteins from the immune system by the foetus (McGregor & Smith, 1952), or lowering of IgG, IgM and globulins (Gilles *et al.*, 1969; Kortmann, 1972; McGregor *et al.*, 1970). Taufa (1978) asserted that reduced resistance to malaria during pregnancy might be due to immunological or hormonal changes.

Various materno-foetal complications like delayed retention of placenta observed during the course of present study are suggestive of some pathological changes in the placenta. The death of the mother before or after parturition might be due to some sort of toxaemia resulting from placental damages. The deaths of newborn were probably due to their poor birth weights (Jelliffe, 1968; Jilly, 1969). Premature abortion and foetal deaths that we observed are likely to result from placental damage and anorexia (Tachakmov, 1964). We could not come across any case of transplacental transmission of malaria parasites and this finding further supports the earlier view that this condition was extremely rare (Covell, 1950 & McQuay *et al.*, 1967).

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Table 2. Prepatent period, peak parasitaemia* and patency of infection in pregnant and normal female *Macaca mulatta* monkeys infected with *Plasmodium cynomolgi cynomolgi*

Monkey No.	PREGNANT			Monkey No.	CONTROL		
	Prepatent period (Days)	Peak parasit-aemia	Patency >0.1% (Days)		Prepatent period (Days)	Peak Parasit-aemia	Patency >0.1% (Days)
15	5	550	15	29	5	356	11
16	5	820	15	30	5	262	11
17	5	755	19	31	5	462	13
18	5	745	18	32	5	780	11
19	5	1150	20	33	6	628	39
20	5	935	20	34	6	790	11
21	5	1350	20	35	6	520	11
22	5	1465	16	36	6	330	12
23	5	1320	16				
24	6	1300	12				
25	5	660	14				
26	5	570	Died				
27	5	850	Died				
28	6	610	Died				
Mean	5.21	940.00	16.82		5.62	516.00	11.13
S.D	0.43	327.80	2.75		0.52	202.76	1.13
S.E	0.11	87.61	0.83		0.18	71.33	0.40
Significance	NS	<0.001	<0.001				

*Parasites/10^4RBC's

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Mosquitoes of Andaman Islands

B.N. NAGPAL[1] and V.P. SHARMA[1]

Twenty-four mosquito species belonging to five genera were collected from the south, middle and north islands of Andaman. Seven *Anopheles* species, *A. annularis, A. nigerrimus, A. nivipes, A. karwari, A. stephensi, A. subpictus* and *A. varuna* were recorded for the first time. The distribution, abundance and results of bait collections are recorded.

INTRODUCTION

For the first time, Christophers (1912) reported eight Anopheline species in his studies of the Mosquito fauna of Andaman islands. Later Covell (1927) recorded eight Anopheline species with one new record but *A. aitkenii* previously reported by Christophers was absent. Basu (1958) reported only *A. sundaicus* and *Culex* spp. which were collected from some human dwellings of these islands. Krishnan (1967) recorded nine Anopheline species, of which two were reported as new records, but *A. aitkenii* collected by Christophers and *A. umbrosus* by Covell were absent. This was the last published report of the mosquito fauna of these islands.

During a study tour in Jan.-Feb., 1982, intensive and extensive collections of mosquitoes were made from South, Middle and North Andaman. Results of this survey are reported in this paper.

MATERIAL AND METHODS

Indoor resting mosquitoes were collected from human dwellings and cattlesheds. Outdoor resting adult mosquitoes were collected from open sheds, bushes and vegetation, during day time. Evening collection were made in and around the cattlesheds and human dwellings. Mosquitoes were collected by suction tube method. In some rooms, mosquitoes were collected by space spraying using pyrethrum. All night (5.00 p.m. to 5.00 a.m.) man and cattle biting collections were made for one night each on two animal baits (outdoor) and two human baits (indoor) in Saitankhari, Wondoor, and Sipighat villages of South Andaman. Larvae were collected from the rice fields, ponds, creeks and tree crevices, and kept in the field laboratory until adult emergence. All newly emerged mosquitoes were killed with ether and packed in cellophene envelops. The packed mosquitoes were brought to the Malaria Research Centre, Delhi for identification and preservation. Identification of mosquitoes was done using the key of Christophers (1933) and Barraud (1934). The location of villages from where collections were made is given in Map I.

RESULTS AND DISCUSSION

Twenty-four species of mosquitoes belonging to 5 genera i.e., *Anopheles, Aedes, Armigeres,*

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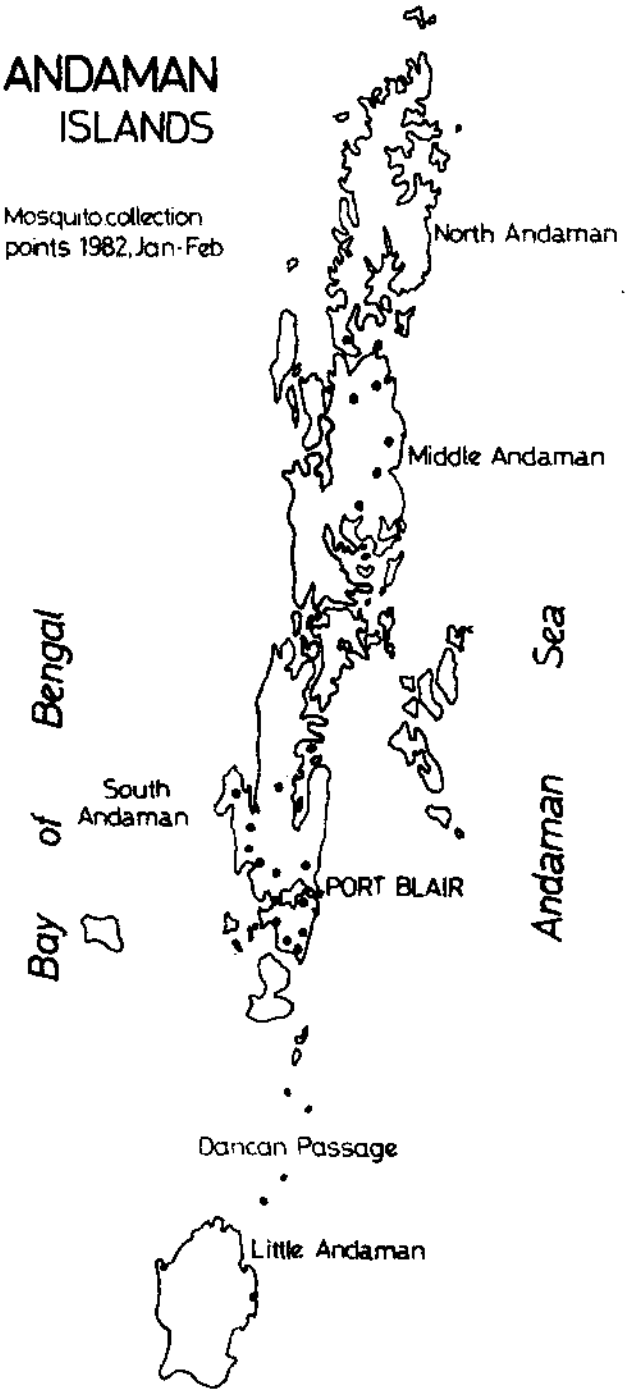

Map 1. Map of Andaman Islands. Solid circles on the map indicate sites from where the mosquitoes were collected.

Culex and *Mansonia* were collected in the present study. A total of 9,942 specimens of genus *Anopheles* comprising of 16 species, 1,023 specimens of genus *Culex* comprising of 4 species, 141 specimens of genus *Mansonia* comprising of 2 species and 20 specimens of *Aedes* and 23 specimens of *Armigeres* comprising of only one species were collected. The distribution of species in South, Middle and North Andaman is given in Table 1.

In the sixteen anopheline species collected from Andaman islands, the most prevalent species was *A. vagus* (80.79%) followed by *A. kochi* (8.11%) and *A. sundaicus* (3.26%). *A. aitkenii* and *A. umbrosus* were not found in this survey.

Among Culicines the most dominant genus was *Culex* and in this genus the most prevalent species was *Cx. quinquefasciatus* (44.96%) followed by *Cx. tritaeniorhynchus* (34.5%) and *Cx. vishnui* (19.06%). The populations of genus *Aedes*, *Armigeres* and *Mansonia* were found in very low numbers.

Results of all night bait collections made in Wondoor, Saitankhari and Sipighat villages of South Andaman are given in Table 2. In Wondoor, 28 *Culex* specimens were collected from one dwelling which had two human baits (indoor), but not a single Anopheline mosquito was collected. From a cattleshed which had two animal baits (Bovine, outdoor) 135 specimens belonging to genus *Anopheles, Culex* and *Mansonia* were collected. In Saitankhari, 392 mosquitoes were collected from two cattle baits (outdoor) belonging to genus *Anopheles, Culex, Mansonia,* and *Armigeres*. No mosquito was collected from the human baits (indoor). In Sipighat 416 mosquitoes were collected from two cattle baits (outdoor) belonging to genus *Anopheles, Aedes, Culex* and *Mansonia*, and only two specimens of *A. sundaicus* were collected from the human bait (indoor) at 5.45 p.m. The last specimen was collected at 1.45 a.m. from the animal bait of Sipighat and was identified to be *A. sundaicus*.

NOTES ON THE SPECIES COLLECTED

Anopheles aconitus Donitz: Eighty-nine adult specimens were collected from cattlesheds in the mornings and evenings from South and Middle Andaman. In South Andaman, *A. aconitus* was found in all collections while in Middle Andaman they were found only in village Billyground. Larvae were collected from ponds and pools of South Andaman.

A. annularis Van der Wulp, (New record): Seven adult females were collected from village Sipighat (South Andaman) and three from village Nimbudera (Middle Andaman) during evening time from the cattleshed. No larvae were found.

A. balabacensis Baisa: Four adults were collected during night time from animal baits (outdoor) at Wondoor (South Andaman). No larvae of this species were found.

A. barbirostris Van der Wulp: Eighty specimens were collected from South, Middle and North Andaman. Adults were collected from cattleshed in the evening and two adults were collected in the morning from a cattleshed in a village Collinpur (South Andaman). Larvae of this species were collected from streams, jungle pools, and rice fields in South and Middle Andaman.

A. karwari James, (New record): A total of 102 specimens were collected from villages Sholbay, Saitankhari, and Collinpur of South Andaman. Adults were collected from both open and closed cattlesheds in the evening. Larvae of this species were collected from streams and creeks of village Sholbay (South Andaman).

A. kochi Donitz: This was the second most prevalent species of South, Middle and North Andaman. Adult collections were made in the evenings from cattlesheds. The immatures of this species were collected from creeks, ponds, jungle pools, rice fields and breeding was found associated with green algae and weeds in Sholbay and Saitankhari villages of South Andaman. In seven adult specimens, four types of morphological variations in their palpi and hind leg were observed which are being published separately.

A. maculatus Theobald: Five adult female specimens of variety *willmori* were collected in the evening from open cattleshed in villages, Miletilak and Sholbay of South Andaman. No larvae were found.

Table 1. Results of mosquito collections in the Andaman Islands.

Sl. No.	Species collected	Total mosquitoes collected
	SOUTH ANDAMAN	
1.	*An. aconitus* Donitz, 1902	87
*2.	*An. annularis* Van der Wulp, 1884	7
3.	*An. balabacensis* Baisas, 1936	4
4.	*An. barbirostris* Van der Wulp, 1884	65
*5.	*An. karwari* James, 1902	102
6.	*An. kochi* Donitz, 1901	623
7.	*An. maculatus* Theobald, 1901	5
*8.	*An. nigerrimus* Giles, 1900	13
*9.	*An. nivipes* Theobald, 1903	2
10.	*An. philippinensis* Ludlow, 1902	134
*11.	*An. stephensi* Liston, 1901	6
*12.	*An. subpictus* Grassi, 1899	267
13.	*An. sundaicus* Rodenwaldt, 1925	359
14.	*An. tesselatus* Theobald, 1901	24
15.	*An. vagus* Donitz, 1902	6332
*16.	*An. varuna* Iyengar, 1924	4
17.	*Ae. aegypti* Linnaeus, 1762	15
18.	*Ar. kuchingensis* Edwards, 1915	14
19.	*Cx. fuscanus* Wiedemann, 1820	15
20.	*Cx. tritaeniorhynchus* Giles, 1901	331
21.	*Cx. quinquefasciatus* Say, 1823	388
22.	*Cx. vishnui* Theobald, 1901	189
23.	*M. annulifera* Theobald, 1901	108
24.	*M. uniformis* Theobald, 1901	10
	MIDDLE ANDAMAN	
1.	*An. aconitus* Donitz, 1902	2
*2.	*An. annularis* Van der Wulp, 1884	3
3.	*An. barbirostris* Van der Wulp, 1884	11
4.	*An. kochi* Donitz, 1901	173
5.	*An. philippinensis* Ludlow, 1902	1
6.	*An. subpictus* Grassi, 1899	2
7.	*An. sundaicus* Rodenwaldt, 1925	1
8.	*An. vagus* Donitz, 1902	1178
9.	*Ae. aegypti* Linnaeus, 1762	5
10.	*Ar. kuchingensis* Edwards, 1915	9
11.	*Cx. tritaeniorhynchus* Giles, 1901	14
12.	*Cx. quinquefasciatus* Say, 1823	47
13.	*Cx. vishnui* Theobald, 1901	4
14.	*M. annulifera* Theobald, 1901	23
	NORTH ANDAMAN	
1.	*An. barbirostris* Van der Wulp, 1884	4
2.	*An. kochi* Donitz, 1901	10
3.	*An. philippinensis* Ludlow, 1902	4
4.	*An. vagus* Donitz, 1902	519
5.	*Cx. tritaeniorhynchus* Giles, 1901	8
6.	*Cx. quinquefasciatus* Say, 1823	25
7.	*Cx. vishnui* Theobald, 1901	2

*Reported for the first time from these islands.

Table 2. Details of man and animal bait collections from South Andaman villages.

SPECIES COLLECTED*	ANIMAL BAIT (OUTDOOR)										HUMAN BAIT (INDOOR)				
	5.30 to 6.30 pm.	6.30 to 7.30 pm.	7.30 to 8.30 pm.	8.30 to 9.30 pm.	9.30 to 10.30 pm.	10.30 to 11.30 pm.	11.30 pm. to 12.30 am.	12.30 to 1.30 am.	1.30 to 2.30 am.	2.30 to 5.30 am.	5.30 to 6.30 pm.	6.30 to 7.30 pm.	7.30 to 8.30 pm.	8.30 to 9.30 pm.	9.30 pm. to 5.30 am.
An. aconitus	9	5	1	0	0	0	0	0	0	0	0	0	0	0	0
An. balabacensis	0	0	2	0	1	1	0	0	0	0	0	0	0	0	0
An. barbirostris	5	3	1	0	0	0	0	0	0	0	0	0	0	0	0
An. karwari	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
An. kochi	23	13	1	3	1	0	0	0	0	0	0	0	0	0	0
An. philippinensis	1	2	1	3	2	3	2	1	0	0	0	0	0	0	0
An. subpictus	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0
An. sundaicus	5	17	2	5	3	2	2	1	1	0	2	0	0	0	0
An. tessellatus	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
An. vagus	424	166	26	1	0	0	0	0	0	0	0	0	0	0	0
An. varuna	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Ae. aegypti	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ar. kuchingensis	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cx. tritaeniorhynchus	38	9	4	6	3	0	0	0	0	0	11	4	1	1	0
Cx. quinquefasciatus	44	11	3	6	4	1	0	0	0	0	7	3	1	0	0
Cx. vishnui	18	5	3	0	0	0	0	0	0	0	0	0	0	0	0
M. annulifera	24	6	1	0	0	0	0	0	0	0	0	0	0	0	0
M. uniformis	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0

*Collections were made from Wondoor, Saitankhari and Sipighat villages (One night in each village)

A. nigerrimus Giles, (New record): Ten adult female specimens were collected in the evening time from cattlesheds in village Sipighat (South Andaman) and three adult females emerged from the larvae collected from ponds and creeks of village Wondoor (South Andaman).

A. nivipes Theobald, (New record): Two adult female specimens were collected from cattlesheds in the evening in Collinpur (South Andaman). Identification of these specimens was based on the key given by Reid *et al.*, 1966. No larvae were found.

A. philippinensis Ludlow: Adult mosquitoes were collected from South, Middle and North Andaman. Only 139 specimens were collected from these islands. Larvae were collected from shady ponds, pools and streams which had green vegetation.

A. stephensi Liston, (New record): Two adult females were collected in the morning from cattlesheds in village Bimbalitan (South Andaman). Specimens were also collected in the evening from cattlesheds. No larvae were found.

A. subpictus Grassi, (New record): A total of 269 specimens were collected from the South and Middle Andaman. In South Andaman, specimens were found in all villages while in Middle Andaman they were found in the village Billyground, resting in the cattlesheds. Larvae were collected from rice fields, pools, creeks and drains of South and Middle Andaman.

A. sundaicus Rodenwaldt: A total of 360 specimens were collected and of these, 359 specimens were collected from a single village Sipighat (South Andaman) and one adult specimen from Bakultala village (Middle Andaman). Adults of this species were collected from open cattlesheds during night time. The maximum numbers were found from 8.00 p.m. to 11.00 p.m. No specimen was found during day time either from the houses or cattlesheds. Larvae were collected from creeks and streams in village Sipighat of South Andaman. Larvae were found associated with green grasses and with other Anophelines such as *A. vagus, A. subpictus* and *A. aconitus*. Seven adult specimens were found from village Sipighat with morphological variations in their maxillary palpi. The description of these variations is being published separately.

A. tessellatus Theobald: 24 specimens were collected from villages Sipighat and Sholbay of South Andaman. Adults were collected from the open cattlesheds during the evening time, and no larvae were found. One adult specimen with variation in palpi and one in the wing was observed, and these are being published separately.

A. vagus Donitz: This was one of the most common species among Anophelines collected from South, Middle and North Andaman. Larvae were collected from a variety of breeding places such as small pools, creeks and ponds in both forest and plain areas, and could be found in almost every breeding habitat such as fresh, brackish and saline water and in association with other Anophelines and Culicines. A total of 366 specimens were found with morphological variations in their palpi, proboscis and wings (Nagpal and Sharma, 1983).

A. varuna Iyengar, (New record): Four adult females were collected from village Sipighat of South Andaman. Three specimens were collected during evening and one during night. No larvae of this species was found in the collections.

Aedes aegypti Linnaeus: Adult mosquitoes were collected from open cattlesheds and from the shrubs in the evening just after the sunset from South Andaman. Larvae were collected from stored water inside the houses and discarded tyres.

Armigeres kuchingensis Edwards: Specimens were collected from close and open cattlesheds of South Andaman and only from open cattlesheds of Middle Andaman. Maximum collections were made from Collinpur village in South Andaman. Larvae were collected from stored water in a house of village Collinpur.

Culex fuscanus Wiedemann: Specimens were collected only from South Andaman. Adults were collected in the evening from cattlesheds and in the morning from the houses. No larvae were found.

Cx. tritaeniorhynchus Giles: High populations of these mosquitoes were found in South, Middle and North Andaman. Larvae were found breeding in association with other *Culex* and *Anopheles* species in rice fields, pools, ponds and streams of South Andaman.

Cx. quinquefasciatus Say: This was the most prevalent species encountered in all the three islands. Adults were collected from cattlesheds and human dwellings in the evening and morning. Larvae were collected from creeks, rice fields and pools in South and Middle Andaman.

Cx. vishnui Theobald: This species was collected from all the three islands surveyed. Adults of this species were collected from cattlesheds during morning and evening hours. Larvae were collected from pools, ponds and rice fields in South and Middle Andaman.

Mansonia annulifera Theobald: Specimens were collected in the evening and morning from the houses and cattlesheds from the villages of South and Middle Andaman. A total of 131 specimens were collected from these islands. No larvae were found.

M. uniformis Theobald: Specimens were collected from villages of South Andaman. Ten specimens of this species were found in the evening from the cattlesheds in Wondoor and Sipighat villages. No larvae were found.

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Serum Proteins and Immunoglobulin Changes in Human Malaria

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Total serum proteins among 265 cases of slide positive malaria were found to be 7.79 ± 0.9302 gm. % and among 110 healthy subjects, 7.26 ± 1.135%. Among malaria cases, relative concentrations of serum protein fractions (%) were: Albumin, 48.95 ± 3.23; α_1-globulin, 5.45 ± 1.13; α_2-globulin, 8.96 ± 1.26; β-globulin 12.18 ± 2.18; and γ-globulin, 24.54 ± 3.82. The corresponding values obtained for normal healthy subjects were: Albumin, 61.85 ± 4.50; α_1-globulin, 3.14 ± 0.822; α_2-globulin, 10.98 ± 1.13; β-globulin, 9.16 ± 2.06 and γ-globulin, 14.51 2.60. A marked decrease in albumin and α_2-globulin fractions together with a significant increase in γ-globulin was observed in malaria infected cases. The albumin: globulin (A/G) ratio also showed a decrease from 1.73 ± 0.41 among healthy subjects to 0.972 0.133 in the malaria cases.

Sera from all the 265 cases of malaria and 36 normal human subjects, were analysed for quantitation of IgG, IgM and IgA by Mancini technique. Concentration of IgG in normal healthy subjects was 1185 ± 216.44 mg. %, 100.0 ± 31.31 mg. % and IgA, 188.3 ± 27.32 mg. %. The corresponding values among malaria cases were 1746 ± 154.94 mg.%, 163.11 ± 47.15 mg.% and 195.26 ± 22.45 mg. %, respectively. Locality-wise analysis of the data on malaria cases, showed that areas of early infection could be demarcated by higher levels of both IgM and IgG immunoglobulins. In areas of long standing infection, elevated levels of only IgG were obtained. Results of ELISA test for malarial antibody also showed a close correlation with the raised levels of IgG.

It may be concluded that the ELISA test complemented with quantitative determination of immunoglobulin classes can provide important information for delineating areas of early and long standing infection.

INTRODUCTION

Several workers have shown the association of increased gamma-globulin levels with malaria infection (Holmes *et al.*, 1955; Cohen and McGregor, 1963). Kuvin *et al.* (1962) demonstrated a positive correlation between the maximum malarial antibody response and maximum gamma-globulin formed. However, it is only a small proportion of the gamma-globulins which is specifically directed against the malarial parasite (Lunn *et al.*, 1966). It has now been shown that malarial parasite exerts a mitogenic effect on polyclonal B lymphocytes resulting in synthesis of large amounts of immuno-globulins without any specificity (Greenwood and Vick, 1975). Malaria is also known to cause decrease in albumin, α_2-globulin and albumin: globulin (A/G) ratio; increase in α_1-globulin and gamma-globulin and no consistent change in β-globulin fractions (Klainer *et al.*, 1968; Mousa *et al.*, 1972; Migasena *et al.*, 1978). Total protein values were found to be elevated in the *Plasmodium vivax* infected cases but slightly decreased in the *P. falciparum* infected cases. In the present study, levels of serum proteins and immunoglobulins have been studied among the slide positive malaria cases drawn from different localities and the normal healthy subjects. The results have been compared with the levels of specific antibody determined by the ELISA technique (Dutta *et al.*, 1982).

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MATERIAL AND METHODS

Serum samples from 265 slide positive malaria patients were obtained from district Shahjahan-

pur, Mathura, Mirzapur, Unnao (all in Uttar Pradesh), Ambala and Karnal (Haryana). Of these, 171 cases suffered from *P. falciparum* infection, 91 from *P. vivax* and the remaining 3 had mixed infection with both the parasites. Sera from 110 normal healthy subjects (medical students) were included as control samples in the study. None of the control subjects had any signs/symptoms of malaria. All sera were stored at −20°C.

Serum protein electrophoresis was carried out using cellulose-acetate membrane and Beckman Microzone (R) Electrophoresis system essentially according to the directions of the manufacturers. The stained cellulose-acetate membrane strips were scanned by means of Beckman scanning densitometer (model R-112) at 520 nm. The relative concentration of various serum protein fractions were determined as suggested in the Beckman Microzone Electrophoresis manual. Total proteins were determined according to the Lowry method.

Quantitative determination of immunoglobulin G(IgG), IgA and IgM was done by the single radial immunodiffusion technique, using Tripartigen (R) plates (Hoechst, India). The test was performed according to the instructions of the manufacturers. Briefly, well 1 of each plate was filled with the control serum (5 μ l) and wells 2 to 12, with 5 μ l of each of the respective test sera. For IgA and IgM, undiluted sera were used but for IgG, the sera were diluted 1:10 in normal saline. The charged plates were incubated at 4°C in a humid chamber. The incubation time for IgG and IgA was 50 hours and for IgM, 80 hours. At the end of incubation period, the diameter of the precipitin ring was measured accurately to 0.1 mm using a calibrated scale and the values in mg/dl obtained from the "Table of Reference Values" supplied by the manufacturers.

RESULTS

Average total serum protein in normal healthy subjects (Group I) was found to be 7.26 ± 1.13 gm.% while in slide positive malaria cases (Group II), it was 7.79 ± 0.9302 gm. % (Table I). Among 171 cases of *P. falciparum* infection, the average total serum protein was 7.18 ± 0.86 gm.% and among 90 cases of *P. vivax*, it was 8.14 ± 0.84 gm. %.

Relative concentration of various serum protein fractions among cases of Group I were (% mean ± SD); albumin, 61.85 ± 4.5; α_1-globulin 3.14 ± 0.822; α_2-globulin; 10.98 ± 1.13; β-globulin, 9.16 ± 2.06; and γ-globulin, 14.51 ± 2.60. The corresponding values obtained among cases of group II were: albumin, 48.95 ± 3.23, α_1-globulin, 5.45 ± 1.13; α_2-globulin, 8.96 ± 1.26; β-globulin, 12.18 ± and γ-globulin 24.54 ± 3.82.

The A/G ratio showed decrease from 1.73 ± 0.41 among group I to 0.97 ± 0.133 in Group II (Table 1). Among the cases of mixed infection the A/G ratio obtained was 1.1207 ± 0.17.

Results of quantitative determination of various immunoglobulin classes among cases of malaria (n=265) and healthy subjects (n=36) are presented in Table 2. Mean concentration of IgG in healthy subjects (group I) was 1185 ± 16.44 mg%; IgM, 100.0 ± 31.31 mg% and IgA, 188.3 ± 27.32 mg%. The corresponding values for malaria cases (group II) were 1746 ± 154.94 mg%; 163.11 ± 47.15 mg% and 195.26 ± 22.45 mg%, respectively.

Locality wise analysis of the levels of immunoglobulin classes has shown that in Shahjahanpur, Mathura (PHC, Ral and Mant), IgG was highly elevated as compared to Unnao (Table 3). On the other hand, serum samples collected from Unnao showed higher levels of IgM in comparison to other localities. Levels of IgA were not significantly altered.

DISCUSSION

It was observed in our study that malaria infection generally caused a slight increase in the total serum protein values. However, among *P. falciparum* cases there was a tendency towards slight

Table 1. Serum proteins in the control subjects and malaria patients

Group	Total serum protein (gm/dl.)	Albumin %	Globulin (%)				Albumin/ Globulin Ratio
			α_1	α_2	β	γ	
I Normal human subjects	7.2600	61.85475	3.1403	10.9782	9.1638	14.5121	1.72295
(n = 110)	±1.1333	±4.5023	±0.822	±1.125	±2.0633	±2.606	±0.4091
II Malaria patients	7.7879	48.9544	5.4484	8.9630	12.1838	24.5010	0.9720
(n = 265)	±0.9302	±3.2322	±1.1357	±1.2590	±2.1750	±3.8237	± .1335
(a) *P. falciparum* infected persons	7.1830	48.6691	5.1678	9.1008	11.5449	25.4555	0.9581
(n = 171)	±0.8667	±3.5704	±1.1680	±1.1006	±2.01922	±4.01784	±0.1361
(b) *P. vivax* infected persons	8.1407	49.0661	5.5621	8.9066	12.2888	24.1071	0.9757
(n = 91)	±0.8494	±3.0354	±1.0046	±1.3756	±2.0503	±3.5813	±0.1222
(c) Persons having both *P. falciparum* and *P. vivax* infections	8.04	52.6291	5.1812	9.3818	11.6679	21.1351	1.1207
(n = 3)	±1.0747	±3.8604	±0.61105	±0.3729	±0.8506	±4.666	+0.1701

Values are expressed as Mean ± SD

Table 2. Immunoglobulins in the control subjects and malaria patients

	Group	IgG (mg.%)	IgA (mg.%)	IgM (mg.%)
I.	Normal healthy subjects	1185.93	188.29	.100.0
	(n = 36)	±216.44	±27.32	±31.31
II.	Slide positive malaria cases	1746.71	195.26	163.11
	(n = 265)	±154.94	±22.45	±47.16

Values are Mean ± SD.

Table 3. Locality-wise break-up of Immunoglobulin levels in malaria positive cases.

	Group	IgG (mg.%)	IgA (mg.%)	IgM (mg.%)
1.	P.H.C. RAL	1734.15	194.92	142.85
	Distt. Mathura	±102.41	±13.53	±19.2306
	(n = 40)			
2.	P.H.C. MANT	1784.19	189.89	170.70
	Distt. Mathura	±266.12	±37.17	±55.98
	(n = 84)			
3.	P.H.C. KALAN	1706.35	193.91	160.61
	Shahjahanpur	±170.14	±24.37	±41.01
	(n = 54)			
4.	Shaktinagar,	1780.40	199.05	155.99
	Distt. Mirzapur	±166.82	±18.69	±43.76
	(n = 30)			
5.	P.H.C. PURWA	1538.33	196.32	185.83
	Distt. Unnao	±41.30	±16.83	±32.41
	(n = 18)			
6	Distt. Ambala	1907.37	189.61	170.15
	(n = 19)	±123.20	±18.34	±87.40
7.	Distt. Karnal	1776.20	203.17	153.66
	(n = 20)	±214.55	±28.22	±55.29

Values are Mean ± SD.

decline in these values, while in *P. vivax* there was an increase.

The rise in α_1-globulin and γ-globulin, and a decrease in albumin and α_2-globulin observed in our study are very characteristic of malaria infection as has been reported earlier also by Migasena *et al.* (1978), Lunn *et al.* (1966) and Mousa *et al.* (1972). In our study, a significant increase in β-globulin was recorded among the malaria cases. Similar observations were made by Abele *et al.* (1965) in human subjects and by Desowitz *et al.* (1968) among rhesus monkeys infected with *P. coatneyi*. Migasena *et al.* (1978), however, reported intermittent decrease in β-globulin fraction during malaria infection.

A significant increase in the levels of IgG and IgM was observed among malaria cases in the present study. This is in accordance with the results reported by McGregor *et al.* (1970) and Voller *et al.* (1971). Ganguly *et al.* (1980) who studied cases of early infection with *P. vivax* also reported elevated levels of IgG in 74% cases and IgM in 86% cases. It has been suggested that the levels of IgM initially rise and then decline. Other workers have also reported an extensive rise in IgG levels which continued to persist beyond the more transient IgM (McGregor *et al.*, 1970; Bruce-Chwatt, 1970).

Locality wise analysis of the levels of different immunoglobulin classes showed that there was an elevation in the IgM levels among cases from Unnao while in other localities an increase in IgG levels was observed. This finding suggests that Unnao represents a recent focus of malaria. This is further corroborated by the results obtained with the ELISA test for specific antibody (Dutta *et al.*, 1982). In the ELISA test, relatively lower extinction values (E_{492}) were obtained among cases from Unnao (0.115 ± 0.047) suggesting lower degree of exposure to infection than among cases from Shahjahanpur (0.168 ± 0.042) and Mathura (0.144 ± 0.046) which represented areas of long standing malaria infection.

It is felt that ELISA test complemented with quantitative determination of immunoglobulin classes will be a useful tool for delineating areas of early and long standing malaria infection.

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Studies on the True Incidence of Malaria in Kharkhoda (District Sonepat, Haryana) and Kichha (District Nainital, U.P.) Primary Health Centres

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Intensive surveillance revealed that malaria incidence was extremely high in Kichha and Kharkhoda PHCs. The study also revealed that NMEP's surveillance was recording a small number of malaria cases, and that the ongoing surveillance was missing the *vivax* and *falciparum* malaria peaks and was not sensitive enough to reveal the real effect of spraying. The study was useful in organizing malaria control operations in Kharkhoda PHC and pointed out that small focus of *falciparum* malaria should be stamped out from Kichha PHC. The importance of better surveillance is emphasized in the present day context of malaria control.

INTRODUCTION

The true epidemiological picture of malaria is required for planning and execution of malaria control operations, and to obtain feedback of the effect of intervention methods. Therefore, correct monitoring of the disease incidence is important for (i) the success of control operations, (ii) incorporating timely corrective measures, and (iii) tackling the epidemics. A few methods have been discussed that can be used to estimate the total incidence of malaria, and development of these methods would be useful in malaria control (Sharma, 1980).

In the National Malaria Eradication Programme (NMEP) malaria cases are detected through a surveillance system. At the peripheral level, a multipurpose worker (MPW) scheme was introduced under the modified plan of operations (MPO). The MPW was responsible for a variety of health activities in a population of 10,000 (now reduced to 7,000). *Inter alia* he is supposed to make house to house visits in each village at an interval of 2 weeks and collect blood smears from fever cases. Blood smears are also collected by the dispensaries, hospitals, fever treatment depots (FTDs) and occasionally mass blood surveys are also done. All these blood smears are examined under the microscope and slides found positive for malaria parasite represent the malaria positive cases. This information is also used in calculating various epidemiological indices viz., annual blood examination rate (ABER) which is the annual collection of blood smears from fever cases targetted to 10% of the population, slide positivity rate (SPR) represents the proportion of malaria parasite positive slides, slide *falciparum* rate (SfR) represents the proportion of slides found positive for *P. falciparum*, and annual parasite incidence (API) i.e., the number of malaria parasite positive cases per thousand population in one year. API reflects the total quantum of malaria parasite load in the community. This index is used to monitor malaria situation in the country, and therefore, API is the sheet anchor of decision making. It is understood that the effectiveness of various types of surveil-

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lance activities differs, being influenced by a number of factors such as the level of transmission, behaviour of population and motivation of the MPW. As a result, the ongoing surveillance does not provide true epidemiological picture, and the degree of error remains unknown. For this reason opinion regarding the total incidence of malaria varies considerably. Indian Drug Manufacturers Association (1981) estimated 2% malaria cases (12 million) in 1975, and 3% (20 million cases) in 1980, and the incidence would further rise to 5% level until 1990, whereas the recorded incidence of malaria in India was 5.16 million in 1975 and 2.89 million in 1980. In view of this situation, a study was initiated to obtain a realistic assessment of malaria incidence in 2 primary health centres (PHCs) and compare these results with the routine NMEP's surveillance. Results of one year study are reported in this paper.

MATERIAL AND METHODS

The study was carried out in 2 PHCs. These PHCs were selected randomly without any consideration of the previous history of malaria. For operational ease and adequate supervision, Kichha PHC was taken up near the field station in Haldwani and Kharkhoda PHC near the MRC, HQ's Office in Delhi. The population of each PHC was about 100,000. Since it was not possible to cover the entire PHC in one month, it was decided that a group of villages of about 8,000 population in each PHC would be taken up every month for the study. Each PHC was therefore, divided into 12 sections. Every month, one section was surveyed and the teams moved to the second section in the next month and this procedure continued until the entire PHC was covered in one year. In these villages, the following activities were carried out (i) listing of all houses, numbering and census of population age and sex wise (ii) blood smears were prepared from all fever cases and cases with the history of fever which have been referred to as febrile cases. All fever cases were given chloroquine presumptive treatment. (iii) one round of mass blood survey to cover 25% population (every fourth house) to ascertain the proportion of afebrile "inapparent" malaria cases. All blood smears were stained with JSB and examined in a central laboratory at the MRC. Work started in March 1981 and continued till February 1982.

RESULTS

1. *KICHHA PHC* : Sectionwise results of MRC and NMEP are given in Table 1. A total of 97,183 population was examined in 12 sections of the PHC. Monthly fever rate varied from 5% to 20%, highest being in September (19.8%). Similarly SPR varied from 3.5% to 38.0%, highest being in September. Most of the malaria was the result of *P. vivax* infection. Malaria cases per thousand population have been shown in Fig. 1. It was revealed that malaria peaks were observed in April, July and September followed by sudden drop in October and further drop during the winter months. The incidence of *falciparum* malaria was very low so that during one year period of febrile surveys, only 23 cases were found which included 3 mixed infections. Malaria cases detected through the mass blood surveys are given in Table 2, and cases per thousand population have been shown in Fig. 2. A total of 22,384 population was examined in 12 sections. There were 451 malaria parasite positive cases, of which 13 cases were of *falciparum* malaria. The proportion of afebrile or inapparent malaria cases were low throughout except during the winter months (see Fig. 2). A comparison of the actual malaria cases detected during active surveillance in each section by the MRC and NMEP revealed that NMEP was missing most of the cases (Fig. 3), including a small focus of *P. falciparum* (Table 1). It may be noted that the average SPR of 12 sections recorded by the MRC was 22.1 whereas NMEP recorded 4.7. NMEP was therefore, also missing a large number of positive slides during the microscopical examination. It is also noteworthy that during 1980-1981 and 1981-82 NMEP found 76 and 63 malaria cases respectively and no case of *falciparum* malaria was recorded, suggesting that NMEP's surveillance

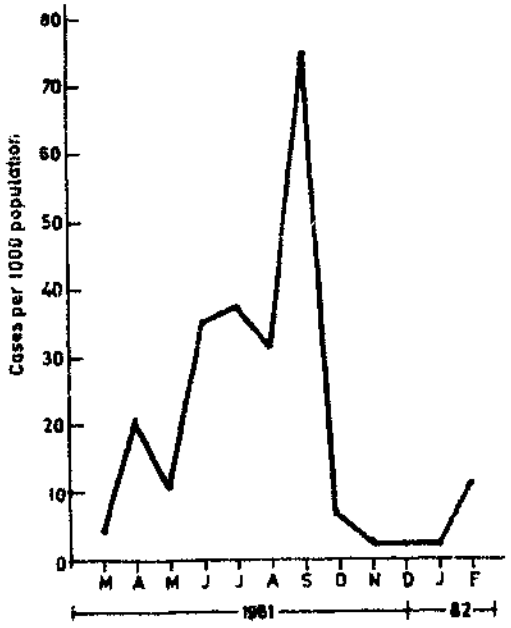

Fig. 1 Kichha PHC: Results of active surveillance showing section-wise monthly incidence of malaria cases per thousand population. Note the peaks of *vivax* cases in April, July and September.

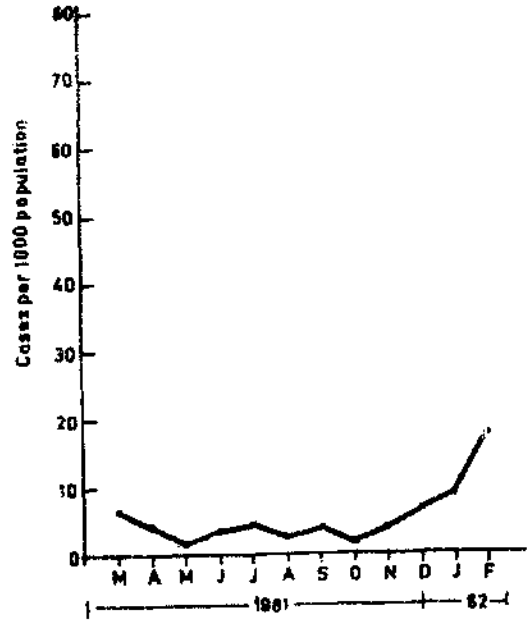

Fig. 2 Kichha PHC: Results of mass blood surveys showing section-wise monthly incidence of malaria cases per thousand population. Note the gradual increase of malaria parasite positive cases during the winter months.

in this area was collecting slides from a fraction of the febrile population. In the same population during 1981-82 MRC found 1784 malaria cases, of which 1761 were *P. vivax*, 20 *P. falciparum* and 3 mixed infections (Table 1).

2. *KHARKHODA PHC:* Sectionwise results of the active surveillance of MRC and NMEP are given in Table 3 and cases per thousand population have been shown in Fig. 4. A total of 91,806 population was examined in 12 sections of the PHC. Monthly fever rate varied from 6% to 42%, highest being in August (42.3%). Similarly SPR varied from 4% to 68%, the highest being in November. Malaria in this PHC was the result of *P. vivax* and *P. falciparum* infections. It was revealed that *vivax* malaria cases start increasing from March showing peaks in May and August followed by a gradual decline. Incidence of *falciparum* malaria was very low from March to May and slowly started increasing in the following months peaking in October followed by a declining trend. Even during extreme winters in January the incidence was quite high and declined during February and March. Results of mass blood surveys are given in Table 4 and cases per thousand population have been shown in Fig. 5. A total of 19,119 population was examined in 12 sections of the PHC in one year. There were 2,375 malaria cases (632 *P. vivax*, 1,772 *P. falciparum* and 22 mixed infection). It was revealed that *vivax* cases started increasing from March peaking in July followed by a gradual decline in the following months. Incidence of *falciparum* malaria increased from March onwards followed by a sudden spurt in September peaking in November. This was followed by a decline but the proportion of *falciparum* cases remained quite high even in February. *Vivax* and *falciparum* cases per thousand population have been shown in Table 5. The study revealed that the total parasite load in the community was extremely high even during the winter months. A comparison of the actual malaria cases detected during active surveillance in each section by the MRC and NMEP revealed that NMEP was missing most of the cases (Table 3 & Fig. 6). It

Table 1. Results of active surveillance (Febrile cases) in Kichha PHC

Section	Month	MRC DATA 1981-82 Popu-lation	Fever %	B.S. examined	*Pv*	*Pf*	Mixed *Pv*+*Pf*	TOTAL	SPR	NMEP DATA* 1981-82 Cases per 1000 popu-lation	B.S. examined	*Pv*	SPR	1980-81 *Pv*
1.	March	9912	5.1	504	45	0	0	45	8.9	4.5	93	11	11.8	5
2.	April	7002	8.9	624	147	0	0	147	23.6	20.9	181	7	3.9	5
3.	May	15634	5.1	799	169	0	0	169	21.2	10.8	373	14	3.8	7
4.	June	6583	10.2	673	230	2	1	233	34.6	35.4	119	5	4.2	17
5.	July	8210	10.5	858	301	3	2	306	35.7	37.3	122	9	7.4	11
6.	August	6759	12.8	868	215	1	0	216	24.9	31.9	129	4	3.1	4
7.	Sept.	6792	19.8	1342	506	4	0	510	38.0	75.1	171	8	4.7	21
8.	Oct.	8472	8.1	685	56	0	0	56	8.2	6.6	32	1	3.1	5
9.	Nov.	7029	5.0	354	12	2	0	14	3.9	2.0	33	4	12.1	1
10.	Dec.	9491	4.9	464	20	4	0	24	5.2	2.5	54	0	0	0
11.	Jan.	7218	6.7	482	15	2	0	17	3.5	2.4	21	0	0	0
12.	Feb.	4081	10.0	410	45	2	0	47	11.5	11.5	6	0	0	0
	--	97183	-	8063	1761	20	3	1784	22.1	--	1334	63	4.7	76

*No case of *P. falciparum* was detected.

Table 2. Results of mass blood survey in Kichha PHC

Section No.	Month 1981-82	Population checked in the section (%)	Malaria Parasite Positive cases					Malaria cases per 1000 population
			B.S. collected	*P.v.*	*P.f.*	Total cases	SPR	
1.	March	20.5	2035	13	0	13	0.64	6.4
2.	April	25.6	1792	72	2	74	4.1	41.0
3.	May	21.9	3437	51	0	51	1.5	15.0
4.	June	25.6	1683	54	1	55	3.3	33.0
5.	July	21.9	1800	74	0	74	4.1	41.0
6.	August	21.7	1467	35	1	36	2.5	25.0
7.	September	23.7	1607	61	1	62	3.9	39.0
8.	October	19.6	1657	28	0	28	1.7	17.0
9.	November	21 7	1527	5	1	6	0.4	4.0
10.	December	25 6	2431	14	1	15	0.6	6.0
11.	January	23.4	1690	13	2	15	0.9	9.0
12.	February	30.8	1258	18	4	22	1.7	17.0
	—	—	22384	438	13	451	—	—

Table 3. Results of active surveillance (Febrile Cases) in Kharkhoda PHC

SECTIONWISE INCIDENCE OF MALARIA

Sec. No.	Month 1981-82	Population surveyed	Fever rate	B.S. collected	MRC DATA						B.S. collected	NMEP DATA				
					Pv	*Pf*	*Pv*+*Pf*	Total	SPR	SfR		*Pv*	*Pf*	Total	SPR	SfR
1.	March	9273	6.2	571	17	4	1	22	3.9	0.87	162	5	2	7	4.3	1.2
2.	April	8122	10.9	882	111	10	0	121	13.7	1.13	226	10	0	10	4.4	0
3.	May	10835	13.9	1508	456	50	10	516	34.2	3.97	93	26	0	26	27.9	0
4.	June	9550	16.2	1546	401	16	8	525	33.9	1.55	223	21	2	23	10.3	0.9
5.	July	7305	18.0	1315	282	48	8	438	33.3	4.25	313	18	1	19	0.3	6.1
6.	August	4644	42.3	1965	306	38	1	745	37.9	1.98	74	3	0	3	4.1	0
7.	September	6381	33.6	2145	284	919	4	1207	56.3	43.03	126	8	15	23	18.3	11.9
8.	October	6692	30.9	2069	93	1178	4	1275	61.6	57.12	88	5	27	32	36.4	30.7
9.	November	7519	21.7	1630	31	1074	2	1107	67.9	66.01	16	0	7	7	43.8	43.8
10.	December	5448	22.0	1199	27	607	0	634	52.9	50.62	89	5	16	21	23.6	18.0
11.	January	6867	14.6	1006	63	320	4	387	38.5	32.20	33	1	4	5	15.2	12.1
12.	February	9170	6.8	621	33	107	0	140	22.5	17.23	9	1	6	7	77.8	66.7
		91806	--	16457	2104	4971	42	7117	43.2	30.46	1452	103	80	183	12.6	5.5

Table 4. Results of mass blood survey in Kharkhoda PHC

Section No.	Month 1981-82	Population checked (%)	B.S. Collected	Malaria Parasite Positive Cases				SPR	SfR
				Pv	*P.f.*	*Pv+Pf*	Total		
1.	March	17.9	1662	3	15	1	19	1.14	0.96
2.	April	30.6	2482	19	15	2	36	1.45	0.68
3.	May	19.7	2136	110	13	3	126	5.89	0.74
4.	June	20.4	1944	130	52	6	188	9.67	2.98
5.	July	20.3	1481	112	52	4	168	11.34	3.78
6.	August	28.7	1335	90	23	1	114	8.53	1.79
7.	September	18.9	1209	56	213	0	269	22.24	17.61
8.	October	20.5	1377	39	311	3	353	25.63	22.80
9.	November	13.9	1047	21	322	0	343	32.76	30.75
10.	December	20.2	1101	14	281	0	295	26.79	25.52
11.	January	19.9	1367	20	249	1	270	19.75	18.28
12.	February	21.6	1978	18	176	0	194	9.80	8.89
		—	19119	632	1722	22	2375	12.42	9.12

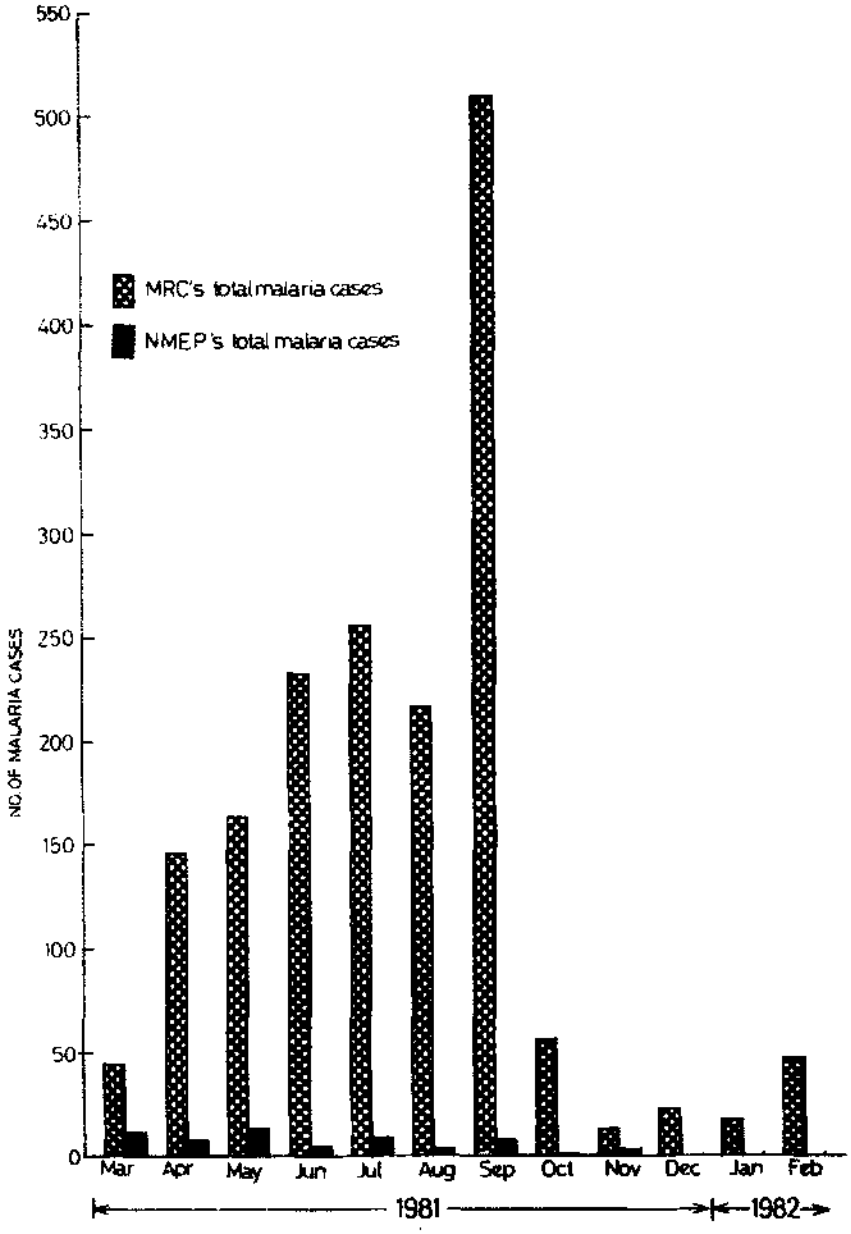

Fig. 3. Kichha PHC: Histogram showing sectionwise actual number of malaria cases in febrile population recorded by the MRC and NMEP independently. Note the discrepancy in data collected by the two agencies.

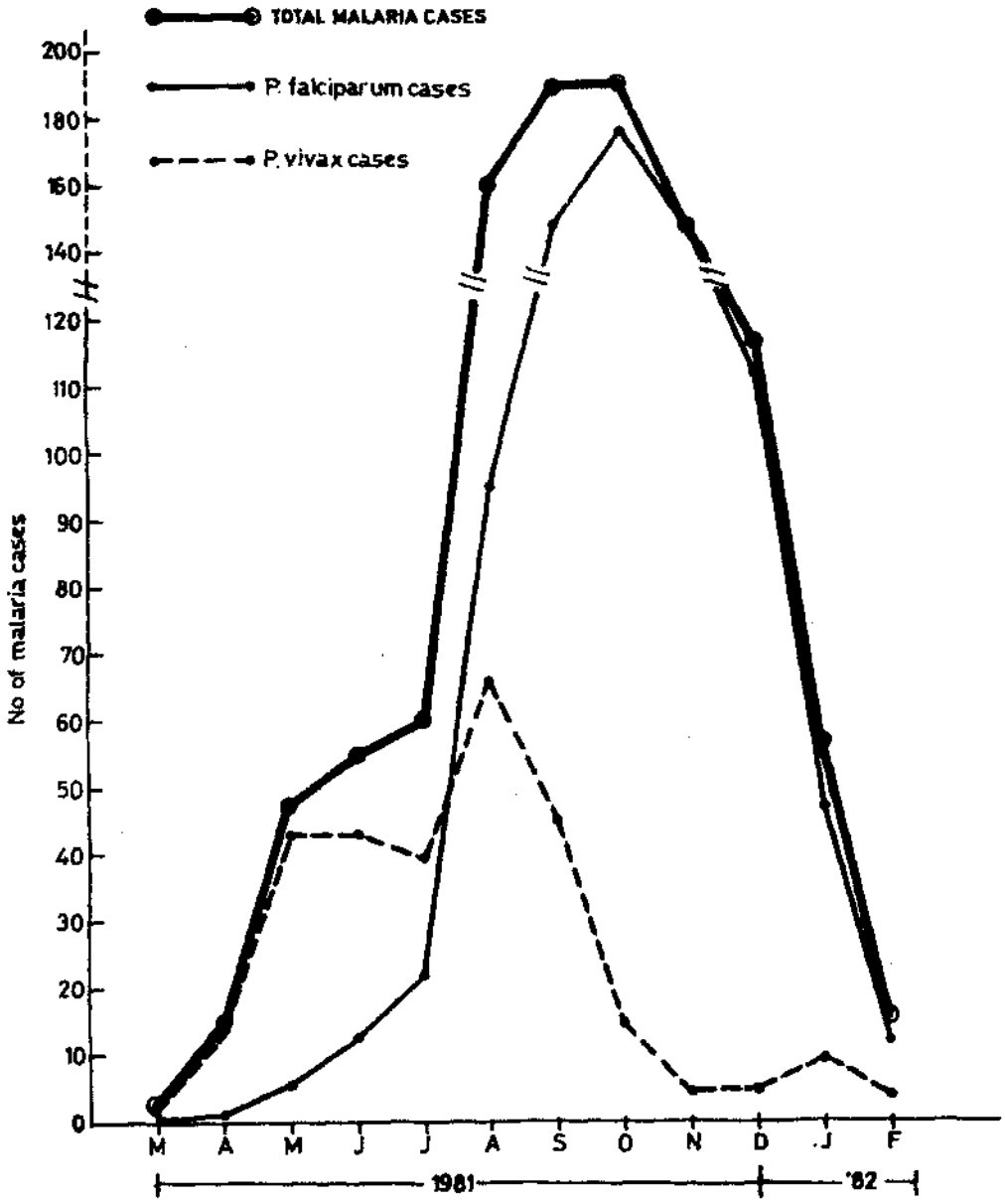

Fig. 4. Kharkhoda PHC: Results of active surveillance showing sectionwise monthly incidence of malaria (*P. vivax* and *P. falciparum* and total cases) per thousand population. Note the seasonal peaks of *vivax* and *falciparum* cases.

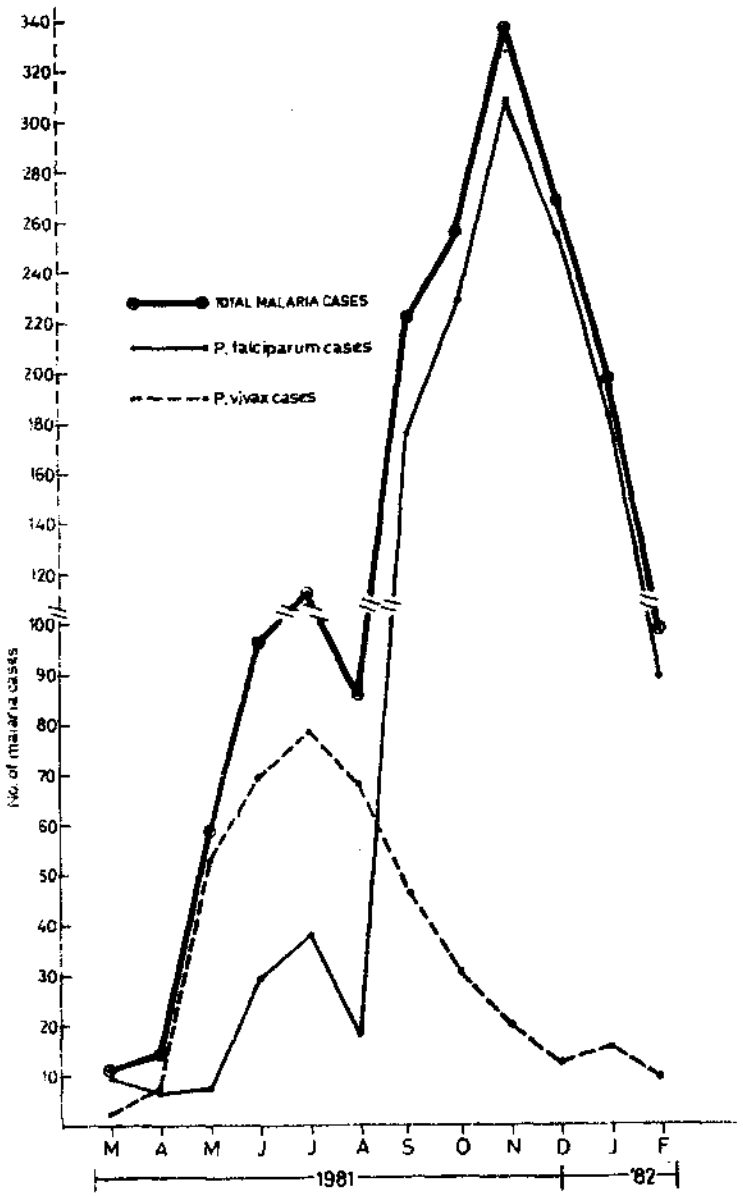

Fig. 5. Kharkhoda PHC: Results of mass blood surveys showing sectionwise monthly incidence of malaria (*P. vivax* and *P. falciparum* and total cases) per thousand population. Note the seasonal peaks of inapparent *vivax* and *falciparum* malaria cases which are similar to the peaks shown in Fig. 4. There was extremely high incidence of inapparent malaria cases in this PHC compared to Kichha PHC (see Fig. 2).

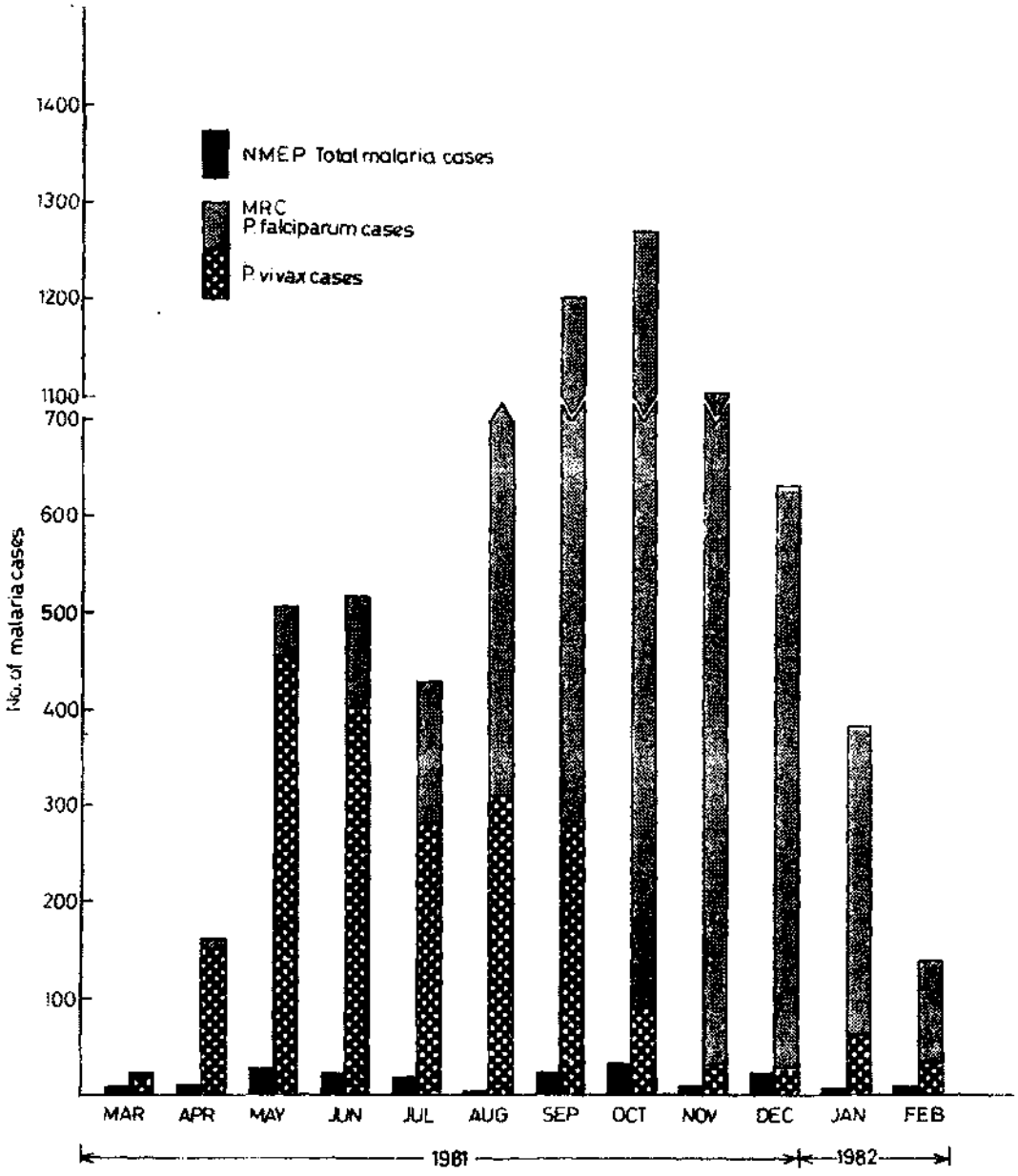

Fig. 6. Kharkhoda PHC: Histogram showing sectionwise actual number of malaria cases in febrile population recorded by the MRC and NMEP independently. Note the discrepancy in data collected by the two agencies.

may be noted that the SPR recorded by MRC in 12 sections was quite high compared to the SPR recorded by NMEP (Table 3). NMEP was therefore, missing a large number of *vivax* and *falciparum* positive slides during microscopical examination. It may also be pointed out that during 1981-82 NMEP recorded 183 malaria cases (103 Pv + 80 Pf) as against 7,117 (2,104 Pv+4,971 Pf+42 Pv+Pf) recorded by the MRC suggesting that NMEP's surveillance in this area was collecting slides, only from a fraction of the febrile population.

DISCUSSION

The spectacular success of malaria control/eradication under NMEP resulted in the near eradication of malaria from the country and there were about 100,000 cases in 1965. The eradication strategy was divided into the preparatory, attack, consolidation and maintenance phases. It was envisaged that as a result of insecticidal spraying during the attack phase transmission would be interrupted, and areas with 0.5 API would move into the consolidation phase. The monitoring of malaria therefore required a more sensitive index like the API through intensive surveillance. Resurgence of malaria started in late 1960s and continued unabated. In 1976, the highest incidence of 6.4 million cases were recorded. A revised strategy of malaria control known as the modified plan of operation (MPO) was implemented by the NMEP in 1977 (Pattanayak and Roy, 1980). As per the NMEP data, malaria incidence started declining every year and minimum number of cases (i.e. 2.1 million) were recorded in 1982. Under the MPO it was envisaged to spray areas with 2 or more API with residual insecticides, and distribute the antimalarials freely throughout the length and breadth of the country. Therefore, under the MPO, API continued to be the epidemiological index for the monitoring of malaria. In many areas of the country where adequate quantities of insecticides to cover all areas with 2 or more API were not available, spraying priorities were fixed on the basis of API. With this background, it was considered important to understand the reliability of API, although it may be noted that in many areas of the country, spleen enlargements are now commonly seen, and spleen surveys alone can easily reveal malaria situation at a much faster rate as was done in a recent study in Ghaziabad (Uprety *et al.*, 1982).

A comparison of the incidence of malaria in 2 PHCs, as recorded by the NMEP and MRC independently, at the same time in the same population, revealed that NMEP's surveillance was recording only a fraction of the total incidence of malaria, and parasite positive slides were being missed during the microscopical examination. NMEP's surveillance was therefore, not providing dependable information on the incidence of malaria, at least in the investigation areas (see Tables 1 & 3, Figs. 3 & 6). The study also revealed the continuity of uninterrupted transmission and that either the control measures were not applied or were not effective. It may however be pointed out that MRC's surveillance was very intensive and was carried out on daily basis to see that no case of malaria was missed: as against the NMEP, where slide collection from fever cases was carried out at an interval of 2 weeks by one person in a population of about 10,000. Also, mass-blood surveys under NMEP are done under special circumstances and not on routine basis. But the study revealed that the cases detected through mass blood surveys of the afebrile population alone constituted a heavy parasite load in the community. These cases of "inapparent malaria" are epidemiologically important. It may however be pointed out that the discrepancy recorded in this study may not be used in extrapolating malaria incidence in other areas, as the malaria situation and also the performance of surveillance workers would vary considerably from one region to another.

The importance of the study was underscored by the fact that NMEP's surveillance was not revealing the seasonal peaks of *vivax* and *falciparum* malaria in the community, as was obtained.

Table 5. Results of the MRC Surveys of Malaria cases per thousand population in Kharkhoda PHC

Section No.	Month	Active case detection (febrile cases)			Mass Blood surveys		
		Pv	*Pf*	Total	*Pv*	*Pf*	Total
1.	March	1.9	0.54	2.4	2.4	9.6	11.4
2.	April	13.7	1.2	14.9	7.6	6.8	14.5
3.	May	43.0	5.5	47.6	52.9	7.5	58.9
4.	June	42.8	12.9	54.9	69.9	29.8	96.7
5.	July	39.7	21.4	59.9	78.3	37.8	113.4
6.	August	66.1	94.5	160.4	68.2	17.9	85.4
7.	September	45.1	144.6	189.1	46.3	176.2	223.5
8.	October	14.5	176.6	190.5	30.5	228.0	256.4
9.	November	4.4	143.1	147.2	20.0	307.5	327.6
10.	December	4.9	111.4	116.4	12.7	255.2	267.9
11.	January	9.2	47.2	56.4	15.4	182.8	197.5
12.	February	3.6	11.7	15.3	9.1	88.9	98.0

by the MRC (Figs. 1 & 4). Information on the timing of seasonal peaks was required for organizing spray operations and for adopting a correct drug treatment policy. The laboratory services also needed toning up. The study also showed that in Kichha PHC, the incidence of *falciparum* malaria was low, and that this was the most opportune time to eradicate this small focus or else the region may revert back to the hyperendemic conditions with high proportion of *falciparum* malaria. In fact this apprehension was confirmed in the following year (1982-83). In an adjoining area (Gadarpur PHC) investigations revealed an outbreak of *falciparum* malaria, and the transmission was so intense that it was comparable to the pre-eradication days of malaria endemicity (MRC Annual Report, 1982).

In Kharkhoda PHC, *P. falciparum* outbreak was quite serious and required immediate control measures. This information was used by NMEP, and the study villages were sprayed with malathion in 1982. There was drastic reduction in *Anopheles culicifacies* populations and the incidence of malaria.

The study also revealed that the NMEP's surveillance was recording a limited number of malaria cases, regardless of the total number of cases in the community. The surveillance was therefore, not sensitive enough to reveal the real effect of intervention methods or any fault/defect in spraying. Such a monitoring system may mislead the control strategy, and suggest a change of insecticide. In a recent study improved surveillance and coverage was utilized to demonstrate the epidemiological impact of DDT spraying in villages with DDT resistant *A. culicifacies* (Sharma *et al.*, 1982). It may be noted that decision to change the insecticide is expensive. Spraying cost to cover 1 million population is Rs. 14.01 lakhs for DDT, Rs. 28.02 lakhs for HCH and Rs. 177.02 lakhs for malathion and

spraying cost of some newer insecticides is many times more. Also the number of replacement insecticides are limited. Therefore for economic considerations, and the fact that there are limited number of replacement insecticides, the useful life of the DDT,'HCH and malathion should be extended, and to meet this objective surveillance should be tightened to provide correct picture of the disease.

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Morphological Variations in a Natural Population of *Anopheles vagus* Donitz (1902) Collected from Andaman Islands

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About 11,000 mosquitoes were collected from Andaman islands during a fauna survey in January-February, 1982. Of these 8029 were *Anopheles vagus*. This was also the dominant species during this period. A total of 366 *Anopheles vagus* showed variations in palpi, proboscis, wings and abdomen.

INTRODUCTION

There are some records of Indian Anophelines showing morphological variations from the type form. Few of these were first recorded by Christophers (1933). Ramakrishna (1954) and Rahman *et al.* (1960) observed an extra dark band on the palpi of female *A. fluviatilis*. Subramanian and Nagendra (1955) observed an extra dark band on the palpi of *A. subpictus* and *A. pallidus*. Bhatnagar *et al.* (1958) observed hypomelanic forms of *A. pallidus* and incomplete development of sixth wing vein in the colonized specimens of *A. stephensi stephensi*. Rajagopal and Chakraborty (1960) recorded variation in the palpi, hind tarsi and wings of *A. annularis*. Wattal *et al.* (1960) recorded a number of morphological variations in twenty species of Indian Anophelines from the reference collections of NICD. During a study tour, mosquitoes were collected from Andaman islands. Among these *A. vagus* collections had a large number of mosquitoes with morphological variations which have been described in this paper.

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MATERIAL AND METHODS

An extensive and intensive mosquito fauna surveys were conducted in 1982 (Jan-Feb) in South, Middle and North Andaman Islands. Adult collections were made from both indoors and outdoor using the suction tube method. In certain structures adult collection were made using the pryrethrum space spraying. Larval collections were made from creeks, ponds and pools in both forest and plain areas using a dipper. Larvae were held in field laboratory until adult emergence. Adults were killed with ether and packed in cellophene papers. About 11,000 mosquitoes were collected from 22 villages. They were brought to Malaria Research Centre, Delhi for identification and further analysis. Identification of the Anophelines was done according to Christophers (1933).

RESULTS AND DISCUSSION

About 11,000 mosquitoes collected, 8,029 were found to be *A. vagus*. A total of 366 (4.55%) adult specimens collected of *A. vagus* showing morphological abnormalities. Maximum variations were found in the palpi i.e., 18 variations

were observed in their banding pattern and in their sizes in 213 specimens. It was noted that colour of the palpi was blacker in the variant types. 124 specimens were observed with 5 types of variations in the proboscis and of these one specimen had two proboscis. Occurrence of two proboscis in one specimen is most unusual and this is the first record of its kind in any mosquito species examined. 18 specimens were observed with six types of variations in the wings. Eleven specimens had golden thorax and abdomen. Details of the site of collection, morphological variations and the number of specimens etc. are given in Table 1 and shown in Figures 1-31.

All the abnormal specimens have been preserved in the taxonomy section of Malaria Research Centre for future reference.

Variations were observed in mosquitoes collected from all the three islands i.e. from South, Middle and North Andaman and there was no specific trend of variations found due to geographical locations. Mostly variations were observed in female specimens, but two male specimens were also observed with variations in their wings and abdomen. Majority of the variants in *A. vagus* population were from cattle biting collections carried out in the evenings (5.30 p.m.—7.30 p.m.) and out door resting habitats from all the three islands. Studies on morphological variations are important for species identification and to understand the process of speciation. Occurrence of variations in about 4.5% *A. vagus* population in one survey indicated the need of more intensive and extensive mosquito fauna surveys to record such variations in other anophelines.

A. vagus is a secondary vector in Vietnam (Phan Vu thi, personal communication) but in India it is not a recognized vector, although Strickland *et al.* (1933) found two sporozoite positive mosquitoes from Terai and Russel *et al.* (1939) found one sporozoite positive mosquito from south east India. Studies on vector incrimination in different geographic regions of the country are indicated.

Table 1. Record of morphological variations in *Anopheles vagus*

Serial Number of variations	Date of Collection	Site (village) of Collection	Number of specimens collected from each village	Notes on variation from the type form*
1	2	3	4	5
			variations in palpi	
1	16.02.82	1. Sipighat, South Andaman	1	A continuous row of pale scales present on the basal and middle dark band of both the palpi, Fig. 2.
2	20.01.82	1. Wondoor, South Andaman	1	An extra dark band on the apical pale band of one of the palpi, total specimens 7, Fig. 3.
	28.01.82	2. Tugapur, North Andaman	2	
	31.01.82	3. Billyground, Middle Andaman	1	
	16.02.82	4. Sipighat, South Andaman	1	
	22.02.82	5. Chiriatapu, South Andaman	1	
	26.02.82	6. Sipighat, South Andaman	1	
3	17.01.82	1. Sholbay, South Andaman	1	Apical pale band is completely black in one of the palpi, total specimens 3, Fig. 4.
	05.02.82	2. Sipighat, South Andaman	1	
	26.02.82	3. Sipighat, South Andaman	1	

*Type Form palpus—size of the apical pale band 0.40mm and preapical dark band 0.12mm Fig. 1.

1	2	3	4	5
4	17.01.82	1. Sholbay, South Andaman	2	One of the palpi 1.3X as long as the other, total specimens 16, Fig. 5.
	19.01.82	2. Collinpur, South Andaman	1	
	20.01.82	3. Wondoor, South Andaman	3	
	21.01.82	4. Bimbalitan, South Andaman	1	
	31.01.82	5. Billyground, Middle Andaman	4	
	18.02.82	6. Saitankhari, South Andaman	2	
	20.02.82	7. Sholbay, South Andaman	3	
5	14.01.82	1. Sipighat, South Andaman	1	One of the palpi 1.6X as long as the other, total specimens 3, Fig. 6.
	18.01.82	2. Sipighat, South Andaman	1	
	20.01.82	3. Wondoor, South Andaman	1	
6	31.01.82	1. Billyground, Middle Andaman	1	Apical pale band of the palpi with a continuous row of black scales, total specimens 4, Fig. 7.
	01.02.82	2. Uttara, Middle Andaman	1	
	01.02.82	3. Kadamthala, Middle Andaman	1	
	23.02.82	4. Sipighat, South Andaman	1	
7	05.02.82	1. Sipighat, South Andaman	1	Apical and preapical pale bands of both the palpi dark in front, but with normal banding pattern below, Fig. 8.
8	06.02.82	1. Jangalighat, South Andaman	1	Preapical pale band increases, 0.46X as long as the apical pale band, Fig. 9.
9	14.01.82	1. Sipighat, South Andaman	1	Apical pale band reduced to half of the usual length, total specimens 14, Fig. 10.
	19.01.82	2. Collinpur, South Andaman	2	
	21.01.82	3. Bimbalitan, South Andaman	1	
	23.01.82	4. Miletilak, South Andaman	1	
	31.01.82	5. Billyground, Middle Andaman	2	
	06.02.82	6. Jangalighat, South Andaman	2	
	16.02.82	7. Sipighat, South Andaman	2	
	18.02.82	8. Saitankhari, South Andaman	2	
	22.02.82	9. Chiriatapu, South Andaman	1	
10	05.02.82	1. Sipighat, South Andaman	1	Basal dark band of one of the palpi bushy, preapical dark band absent on both the palpi, total specimens 2, Fig. 11.
	16.02.82	2. Sipighat, South Andaman		
11	01.02.82	1. Kadamthala, Middle Andaman	1	Basal dark band of both the palpi bushy, preapical dark band absent on both the palpi, total specimens 4, Fig. 12.
	05.02.82	2. Sipighat, South Andaman	1	
	06.02.82	3. Jangalighat, South Andaman	1	
	16.02.82	4. Sipighat, South Andaman	1	
12	31.01.82	1. Billyground, Middle Andaman	1	The tip of apical pale band of one of the palpi black, Fig. 13.
13	13.01.82	1. Chiriatapu, South Andaman	1	Palpi reduced, 0.76X as long as the proboscis, total specimens 7, Fig. 14.
	19.01.82	2. Collinpur, South Andaman	2	
	31.01.82	3. Billyground, Middle Andaman	2	
	16.02.82	4. Sipighat, South Andaman	1	
	18.02.82	5. Saitankhari, South Andaman	1	

1	2	3	4	5
14	18.01.82	1. Sipighat, South Andaman	1	Preapical dark band absent in one of
	19.01.82	2. Collinpur, South Andaman	1	the palpi, total specimens 8, Fig. 15.
	20.01.82	3. Wondoor, South Andaman	1	
	31.01.82	4. Billyground, Middle Andaman	1	
	18.02.82	5. Saitankhari, South Andaman	2	
	20.02.82	6. Sholbay, South Andaman	1	
	23.02.82	7. Sipighat, South Andaman	1	
15	05.02.82	1. Sipighat, South Andaman	1	Middle dark band with a continuous
	16.02.82	2. Sipighat, South Andaman	2	raw of pale scales, total specimens 6,
	18.02.82	3. Saitankhari, South Andaman	1	Fig. 16.
	23.02.82	4. Sipighat, South Andaman	2	
16	12.01.82	1. Bimbalitan, South Andaman	1	Preapical dark band reduced, apical
	13.01.82	2. Chiriatapu, South Andaman	1	pale band (6.0-10.0)X as long as the
	14.01.82	3. Sipighat, South Andaman	6	preapical dark band (also Wattal *et al.*,
	15.01.82	4. Saitankhari, South Andaman	4	1960), total specimens 72, Fig. 17.
	17.01.82	5. Sholbay, South Andaman	9	
	18.01.82	6. Sipighat, South Andaman	9	
	19.01.82	7. Collinpur, South Andaman	6	
	28.01.82	8. Tugapur, North Andaman	1	
	30.01.82	9. Bakultala, Middle Andaman	2	
	31.01.82	10. Nimbudera, Middle Andaman	5	
	31.01.82	11. Billyground, Middle Andaman	2	
	01.02.82	12. Kadamthala, Middle Andaman	1	
	16.02.82	13. Sipighat, South Andaman	7	
	18.02.82	14. Saitankhari, South Andaman	5	
	20.02.82	15. Sholbay, South Andaman	5	
	22.02.82	16. Chiriatapu, South Andaman	1	
	23.02.82	17. Sipighat, South Andaman	7	
17	13.01.82	1. Chiriatapu, South Andaman	1	Preapical dark band absent in both
	14.01.82	2. Sipighat, South Andaman	5	the palpi (also Wattal *et al.*, 1960),
	17.01.82	3. Sholbay, South Andaman	5	total specimens 57, Fig. 18.
	18.01.82	4. Sipighat, South Andaman	2	
	19.01.82	5. Collinpur, South Andaman	9	
	20.01.82	6. Wondoor, South Andaman	8	
	28.01.82	7. Tugapur, North Andaman	4	
	31.01.82	8. Billyground, Middle Andaman	5	
	31.01.82	9. Nimbudera, Middle Andaman	5	
	01.02.82	10. Kadamthala, Middle Andaman	2	
	16.02.82	11. Sipighat, South Andaman	3	
	18.02.82	12. Saitankhari, South Andaman	3	
	22.02.82	13. Chiriatapu, South Andaman	1	
	23.02.82	14. Sipighat, South Andaman	4	
18	14.01.82	1. Sipighat, South Andaman	1	Preapical dark band increases, apical
	15.01.82	2. Saitankhari, South Andaman	1	pale band 2.4X as long as the pre-
	28.01.82	3. Tugapur, North Andaman	1	apical dark band, total specimens 6,
	16.02.82	4. Sipighat, South Andaman	1	Fig. 19.
	23.02.82	5. Sipighat, South Andaman	1	
	26.02.82	6. Sipighat, South Andaman	1	

1	2	3	4	5
			variations in proboscis	
19	14.01.82	1. Sipighat, South Andaman	1	Proboscis longer, 1.15X as long as the palpi, Fig. 20.
20	14.01.82	1. Sipighat, South Andaman	1	With one extra proboscis, Fig. 21.
21	18.01.82	1. Sipighat, South Andaman	1	Apical half of the proboscis
	01.02.82	2. Kadamthala, Middle Andaman	1	flavescent, total specimens 3, Fig. 22.
	22.02.82	3. Chiriatapu, South Andaman	1	
22	12.01.82	1. Bimbalitan, South Andaman	7	Cup shaped proboscis, total specimens
	13.01.82	2. Chiriatapu, South Andaman	3	118, Fig. 23.
	14.01.82	3. Sipighat, South Andaman	28	
	17.01.82	4. Sholbay, South Andaman	19	
	18.01.82	5. Sipighat, South Andaman	23	
	19.01.82	6. Collinpur, South Andaman	5	
	20.01.82	7. Wondoor, South Andaman	4	
	21.01.82	8. Sipighat, South Andaman	25	
	28.01.82	9. Tugapur, North Andaman	2	
	31.01.82	10. Nimbudera, Middle Andaman	2	
23	23.01.82	1. Miletilak, South Andaman	1	Proboscis flavescent in front, Fig. 24.
			variations in wing	
24	15.01.82	1. Saitankhari, South Andaman	1	Fringe spot at wing vein 5.2 and 6 and
	23.01.82	2. Miletilak, South Andaman	1	between 5.2 and 6 absent in both the wings, total specimens 2, Fig. 26.
25	14.01.82	1. Sipighat, South Andaman	2	Fringe spot between wing vein 5.2 and 6
	17.01.82	2. Sholbay, South Andaman	1	absent in one wing, total specimens 4,
	21.01.82	3. Bimbalitan, South Andaman	1	Fig. 27.
26	17.01.82	1. Sholbay, South Andaman	1	Wings with a continuous fringe spot
	26.02.82	2. Sipighat, South Andaman	1	from wing vein 5.2 to 6, total specimens 2, Fig. 28.
27	14.01.82	1. Sipighat, South Andaman	1	Wings with an extra black spot on wing
	17.01.82	2. Sholbay, South Andaman	3	vein 1, total specimens 5, Fig. 29.
	20.01.82	3. Wondoor, South Andaman	1	
28	14.01.82	1. Sipighat, South Andaman	1	Dark spot at the base of wing vein
	17.01.82	2. Sholbay, South Andaman	1	5.1 absent, total specimens 2, Fig. 30.
29	12.01.82	1. Bimbalitan, South Andaman	1	Inner costa of both the wings completely
	18.01.82	2. Sipighat, South Andaman	1	dark, total specimens 3, Fig. 31.
	20.02.82	3. Sholbay, South Andaman	1	
30	17.01.82	1. Sholbay, South Andaman	6	Hairs and scale 6 of thorax
	18.01.82	2. Sipighat, South Andaman	1	and abdomen golden in colour,
	18.02.82	3. Saitankhari, South Andaman	1	Total specimens 11.
	20.02.82	4. Sholbay, South Andaman	3	

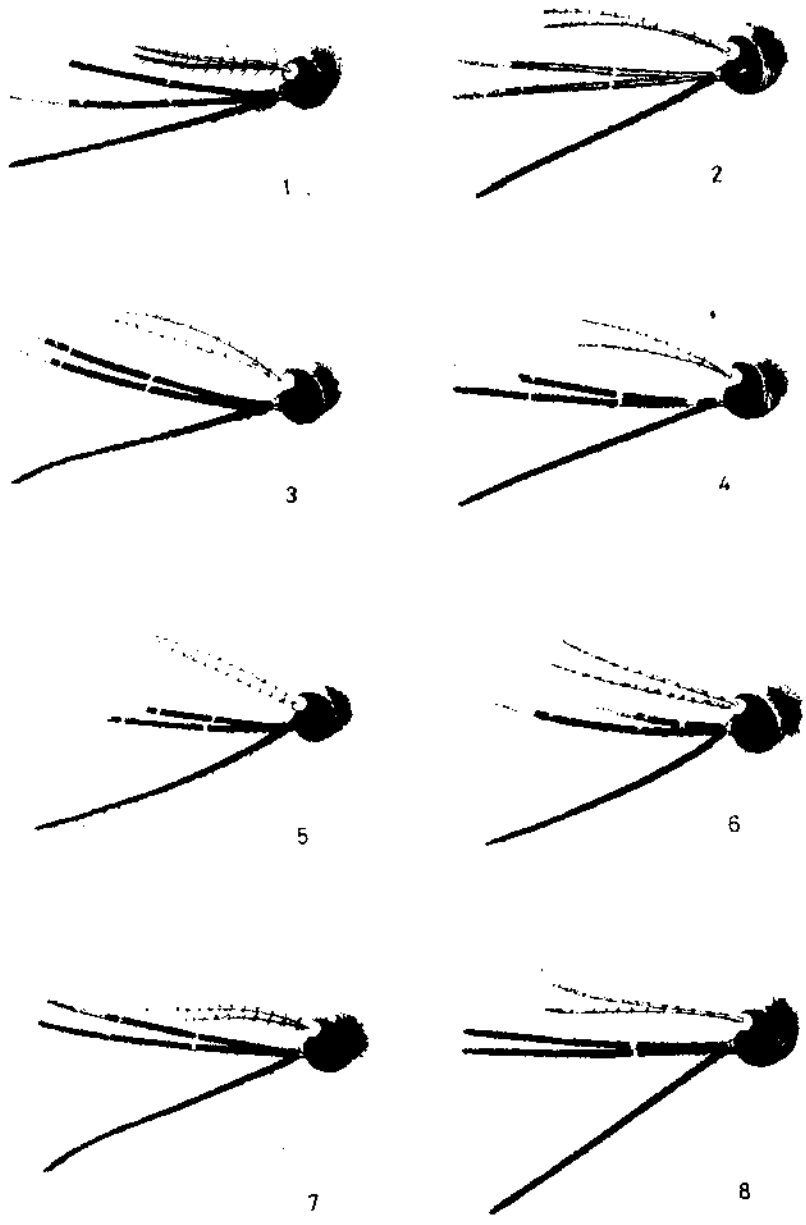

Fig. 1-8. Morphological variations in the palpi of *A. vagus*.

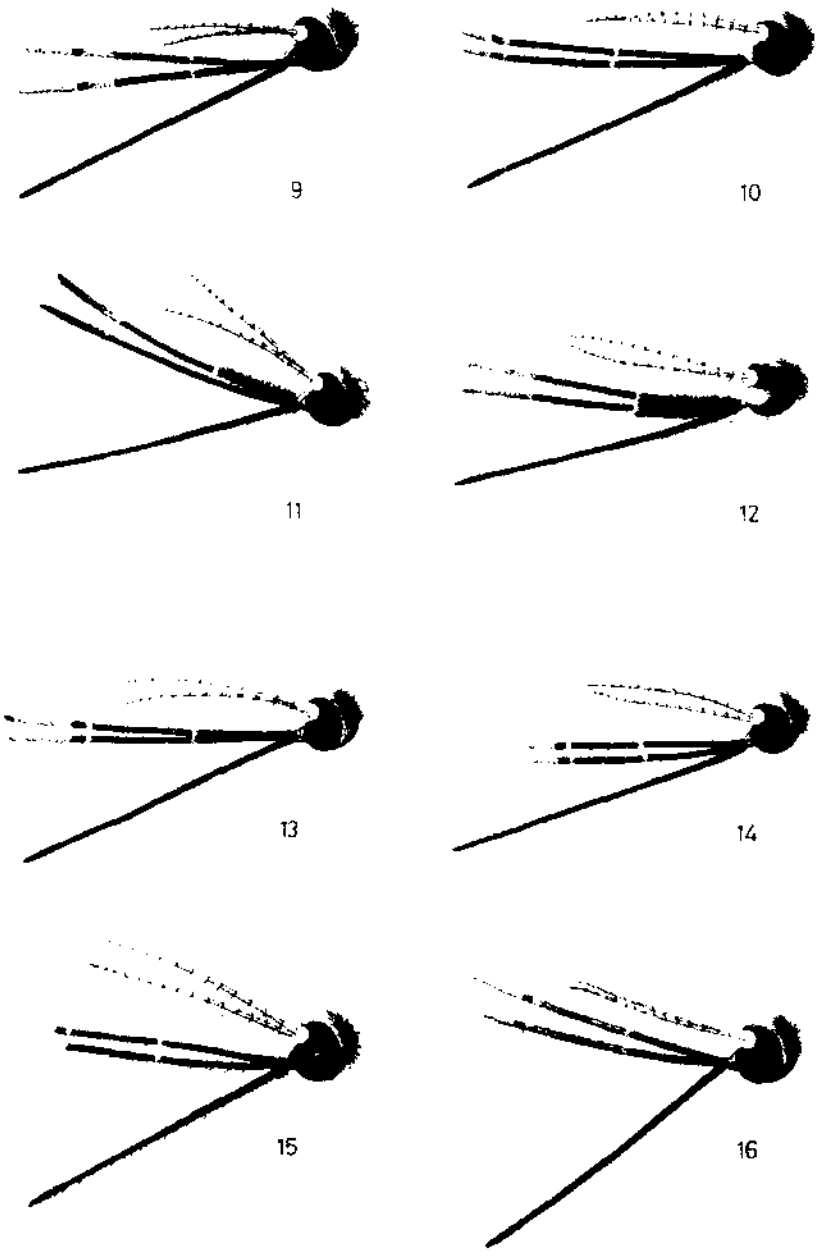

Fig. 9-16. Morphological variations in the palpi of *A. vagus*.

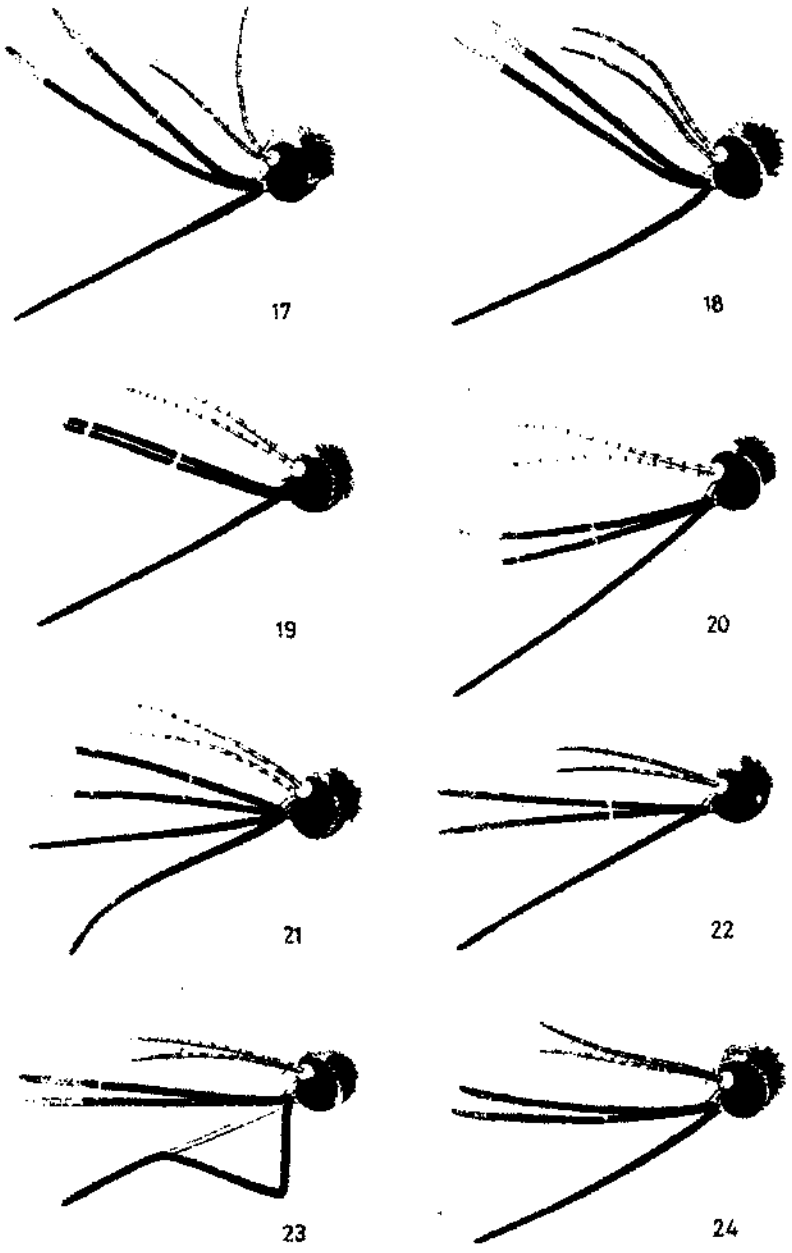

Fig. 17-24. Morphological variations in the palpi and proboscis of *A. vagus*.

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Fig. 25-31. Morphological variations in the wings of *A. vagus*.

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Chromosomal Translocations and Semisterility in the Malaria Vector *Anopheles fluviatilis* James

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Two male-linked and four autosomal aberrations were isolated after exposure to X-rays in the malaria vector *Anopheles fluviatilis*, James. 3000 rads was found to be the optimal dose for producing a larger number of translocations. A method is suggested for obtaining highly fertile females through selective inbreeding for 5-6 generations. The probable use of these translocations in a future genetic control programme of the vector is considered. In addition, it is presumed that these translocations would be the material of choice for studying linkage groups and chromosome correlations.

INTRODUCTION

Inherited semisterility due to translocations has long been suggested as possible means for control or eradication of insect pests (Serebrovskii, 1940; Laven, 1968; Curtis, 1968; Rai and Asman, 1968; Wagoner *et al.*, 1969). Searching for naturally occurring translocations in wild populations is time consuming since the incidence is rare. Alternatively, the other choice is to induce chromosomal translocations.

Among anophelines *Anopheles fluviatilis* James is an important malaria vector in India and in some parts of the eastern Oriental region. This paper describes X-ray induced reciprocal translocations in *A. fluviatilis*, isolation, nature and maintenance of translocations and also the implied application of such translocations in genetic control of the vector.

MATERIAL AND METHODS

A. fluviatilis stock used in the present study was obtained from the National Institute of Communicable Diseases, Mettupaliyam (Tamil Nadu), India. The mosquitoes were reared at 25°C with a photoperiod of 14 hours light and relative humidity of 70%. The colonies were maintained in 30 × 30 × 30 cm cages. Single pair matings were only partially successful in *A. fluviatilis*; therefore, mass matings were made in 30 × 15 × 30 cm cages. Females were blood-fed on mice. For digestion of blood 72-80 hours were allowed and the gravid females were isolated individually in 2 × 9.5 cm shell vials with water and filter paper lining for egg deposition. The larvae were reared in white enamel pans (15 × 30 cm) containing tap water and were fed on liver powder. Adults were provided with 10% sucrose solution.

In order to eliminate the recessive lethals or sterile genes, if any, in the laboratory populations, about 50 gravid females were randomly selected from the population cages and were isolated individually in separate vials for egg laying. Females giving the highest hatchable eggs were selected for further inbreeding. The process of selection and inbreeding was continued for 6 generations in order to increase the fertility rate. Stocks with high fertility rate were used for mass mating with males exposed to X-rays for studying the advent of reciprocal translocations.

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Males 2-3 days after emergence from the pupae of the parent generation (P) were irradiated with X-rays of 500 rads, 1000 rads, 2000 rads, 2500 rads, 3000 rads, 4000 rads, 5000 rads and 6000 rads of X-rays. In order to estimate the optimum dose of the mutagen and also to find out the incidence of translocations, the X-ray exposed males were immediately mass mated with virgin females of the same age. The total number of eggs laid and the number of hatched larvae were recorded and the percentage of induced sterility (dominant lethality) was calculated. Surviving offspring (F_1) from the crosses of both sexes were again outcrossed to normal individuals monitoring the fertility rate. Any reduction therefore, in the number of F_2 offspring from these crosses (semisterility) indicated the production of a translocation or of a pericentric inversion in the parental males. Reduced fertility was used as a marker for the recognition of translocations.

Maintenance of Y-autosomal translocation (TY) is easy. No selection is necessary in such a stock because the semisterility is inherited only through the males whereas all sister females were normal. In autosome-autosome translocations (Ta) the semisterility is inherited through both the sexes. Either a few males or a few females were selected from the families showing reduced hatchability (semisterility) and outcrossed to the wild type mosquitoes in each generation. X-autosome translocations were not observed in the investigation.

RESULTS

After obtaining some basic data on percentage fertility (induced sterility) at various doses of X-rays, a dosage of 3000 rads was chosen as an optimum dose for the production of translocations in *A. fluviatilis* of the 61 F_1 progeny tested 51 showed semisterility (Fig. 1). As semisterility is an indication of a possible chromosomal aberration, the per cent sterility was calculated which varied from 10-90%. Among these 6 lines which showed more than 40% sterility were selected for further study (Table 1) and the rest discarded. In order to verify genetically the nature of chromosomal aberration, such offspring were outcrossed for 2-3 more generations to confirm whether they were sex-linked or autosomal. It was found that in lines 5 and 11, the semisterility was inherited only through the males whereas all the sister females were normal and it is concluded that exchange had occurred between an autosome and the Y chromosome. All males in these lines showed constant levels of semisterility for seven generations. Because of this fact, these translocations are considered suitable for a field release study. In lines 19, 6, 13 and 35 the semisterility was inherited through a proportion of both sexes suggesting a translocation between two autosomes or a translocation between X-chromosome and an autosome or a pericentric inversion on X-or autosome. Experiments devised to test the viability and maintenance of translocations in these 6 selected lines, maintained in heterozygous condition, revealed that the degree of sterility and its variability was constant for seven generations. Table 1 lists the degree of variability with respect to semisterility for each of these lines. The fertility of translocation heterozygotes theoretically averaged around 50% which is just about the figure for most translocations in insect pests.

DISCUSSION

Based on the dose-response data, it is suggested that an X-ray dose of 3,000 rads can be used for generating maximum translocations leading to semisterility in *A. fluviatilis*.

It is mandatory that the females selected for mass mating with X-ray exposed males be highly fertile as indicated by egg hatchability. Such females are normally in very low frequency in wild populations of *A. fluviatilis*. The method described in this paper, to select such females and to restore fertility factor by repeated inbreeding for 5-6 generations, is of immense value in studies involving translocations and subsequent semisterility.

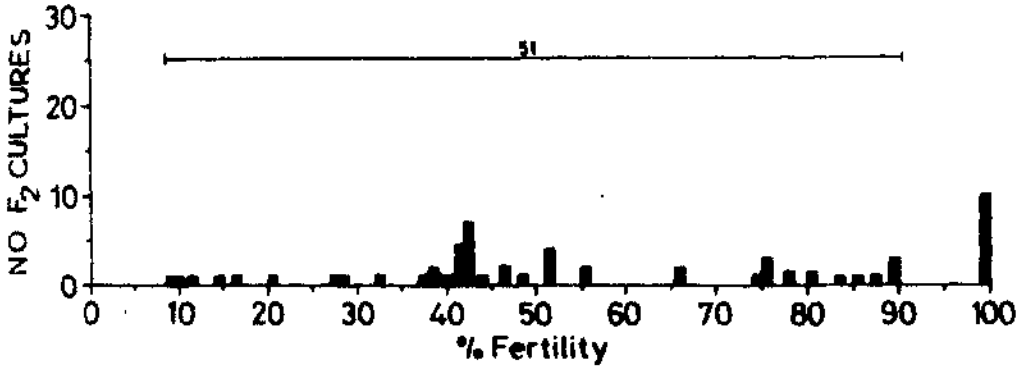

Fig. 1 Degree of fertility in 61 F_2 families of *A. fluviatilis* after 3000 rads irradiation of the Parental generation.

Table 1. Showing semisterility levels in 6 lines of *Anopheles fluviatilis*.

No. of line	Type of translocation tested	No. of families	No. of eggs	No. of larvae	Semisterility* %
T5	TY	10	1074	374	65.2 (56.8-70.8)
T11	TY	12	1957	925	52.7 (50.0-63.5)
T19	Not known	10	1013	390	61.5 (52.0-68.0)
T6	Not known	10	989	385	61.0 (52.0-70.0)
T13	Not known	13	1390	649	53.3 (41.0-64.0)
T35	Not known	15	1650	693	58.0 (53.0-63.0)

*Numbers in parenthesis indicate the range.

The Y-autosomal translocation heterozygotes may be helpful in genetic studies pertaining to sex separating systems in this species. In addition, such translocations could be used to assign linkage groups. The cytological studies, to ascertain if semisterility in 6, 13, 19 and 35 lines is due to translocations or pericentric inversions, are in progress. If the lines 13, 9, 6 and 35 turn out to be autosomal translocations, they would offer an excellent material for generating homozygous translocations in the species which in turn can generate double translocation heterozygotes which are often suggested as efficient mechanisms in the field studies involving genetic control programme (Curtis and Robinson, 1971).

The Y-autosomal translocations (lines 5 & 11), especially, are significant. Importance of such male-linked translocations (TM) in genetic control of other mosquitoes such as *Culex pipiens*, has been well established through field studies (Laven *et al.*, 1972). Hence the lines 5 and 11 established in the present study would be of value in the genetic control of *A. fluviatilis* in India, where the species is an important malaria vector.

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Resurgence of Malaria in Gadarpur PHC, District Nainital, Uttar Pradesh

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An outbreak of malaria was detected in Gadarpur PHC of Nainital district, Uttar Pradesh during 1982-83. Out of 19,318 blood slides collected from fever cases, 13,051 showed malaria parasites giving a slide positivity rate of 67.5%. Among the positives, 9,332 were *P. falciparum* (71.5%). *A. culicifacies* and *A. fluviatilis* were incriminated as vectors of malaria during the outbreak. In *A. culicifacies*, sporozoite rates of 0.79, 2.4 and 6.0% were found during September, October and November respectively. In *A. fluviatilis*, sporozoite rates of 1.4, 0.0 and 6.2% were found during the corresponding period.

INTRODUCTION

Nainital terai was hyperendemic for malaria (Clyde, 1931 and Hehir, 1927). The population in this region was scanty and remained so inspite of control measure taken up during the British rule. The Government of Uttar Pradesh, in 1947 initiated a pilot project for colonisation and large scale food production in this area. An anti-malaria organisation was also simultaneously started.

The initial survey carried out at that time showed hyperendemic malaria in 90% of the villages with spleen rate between 50 to 100% in both adults and children (Srivastava 1950). Srivastava and Chakrabarti (1952) found the incidence of *P. falciparum* to be three times that of *P. vivax*. Issaris *et al.* (1953) reported 84% infant parasite rate in eastern terai in February and the predominance of *P. falciparum* in this region. At one time people feared to visit these places even during day time (Chakrabarti, 1955).

Malaria control activity which was started in 1941 with the application of DDT and mass administration of paludrine showed spectacular success within a period of five years. Malaria came down from hyperendemic to nonendemic level i.e. spleen rate below 5% (Chakrabarti,loc cit.1955). About 16,000 acres were converted to a large agricultural farm in the middle region of the terai and this became the chief granary of Uttar Pradesh. Control of malaria ushered green revolution and thousands of displaced persons, both from West Punjab, Sind, North-West Frontier Province and East Bengal were rehabilitated by giving 10-15 acres of reclaimed land per family during the beginning of the colonisation scheme. A planned township and an agriculture university were also established. All these developments were possible mainly because of malaria control.

During epidemiological investigations in 1982-83, an outbreak of malaria was detected in Gadarpur PHC. Results of this investigation are reported in this paper.

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MATERIAL AND METHODS

The investigation was carried out in Gadarpur PHC which lies in Nainital terai adjoining Rudrapur township. The terai is situated in the foothills of the Himalayas bordering Kumaon division of Uttar Pradesh in the north. Gadarpur PHC is bounded by Kichha in the east, Bazpur on the west, Haldwani in the north and Rampur in the south (Fig. 1). It consists of 225 villages having a population of 77,000. The aboriginal tribes consist mostly of Bhuksas. For the purpose of the investigation, these villages were divided into 12 clusters. Survey of fever cases was started in April, 1982 and the teams moved from one cluster to another on monthly basis till March, 1983 so as to complete the entire PHC in one year. Thus there was a monthly follow-up of different groups of people inside the PHC and surveillance of fever cases was continued for one month in each cluster of villages. Blood slides were collected from fever cases and presumptive treatment of 600 mgm of chloroquine base per adult and proportionate dose to the children and infants was given. A total dose of 1.5 gm of chloroquine base per adult was also given to some cases where the presumptive treatment failed to bring down the fever and parasitaemia within a week. The thin smears were stained with J.S.B. and examined within a fortnight after collection. The slides were declared negative if 200 thin microscopic fields did not show any parasite.

Anopheline mosquitoes were collected with the help of sucking tubes in order to estimate the man hour density (MHD) and incriminate the vector. Collection was done in the morning between 6 to 8 a.m. from human dwellings, cattlesheds and mixed dwellings. These were brought to the laboratory, identified and dissected for sporozoites in their salivary glands. No attempt was made to detect infection in the mid gut as most of the mosquitoes collected had blood in them. The frequency of collection varied from weekly to fortnightly depending on the epidemiological conditions. The meteorological data for the year 1982 are given in Fig. 2.

RESULTS AND DISCUSSION

1. Parasitological:—The investigation covered 77,000 persons during one year. A total of 19,318 fever cases was detected and slides from these cases were taken for parasitological examination. Results of these slides are given in Table 1.

It can be seen that 13,051 slides showed malaria parasites giving slide positivity rate of 67.5. The slide positivity rate varied from 15.9 to a maximum of 94.3 which was found during the month of November. Among the positives, 3,652 (27.9%) were *P. vivax* and 9,332 (71.5%) were *P. falciparum*. The monthly incidence of *P. vivax* and *P. falciparum* is shown in Fig. 3. *P. vivax* showed a peak during August whereas a sharp peak was observed in *P. falciparum* during September and another higher peak in November with a steep fall in December. Higher incidence of *P. vivax* was observed during June to August with a sharp fall in September. From Table 1 and Fig. 3, it can be seen that there were two distinctly separate outbreaks of *P. vivax* and *P. falciparum*. The *vivax* outbreak during the spring was followed by the *falciparum* outbreak during the monsoon and post-monsoon months. Initially *P. vivax* cases were likely to be the relapse cases arising out of the cases of the previous year. The spring transmission of a short duration involving *P. vivax* possibly conferred a homologous immunity preventing super infection or subsequent reinfection. Ultimately a strong heterologous immunity was produced which explains the plateau seen in Fig. 3 due to *P. vivax*. An exactly similar pattern of prevalence of *P. vivax* and *P. falciparum* was found in an investigation carried out by this Centre in Haryana during 1980-81 (Sharma *et al.*, 1983). This was also observed in Garki district of Northern Nigeria where alternate outbreaks of *P. falciparum* and *P. malariae* occurred (Molineaux and Gramiccia, 1980). The number of mixed infection detected in this study was quite low i.e. 67 (0.5%) out of 13,051 positives found.

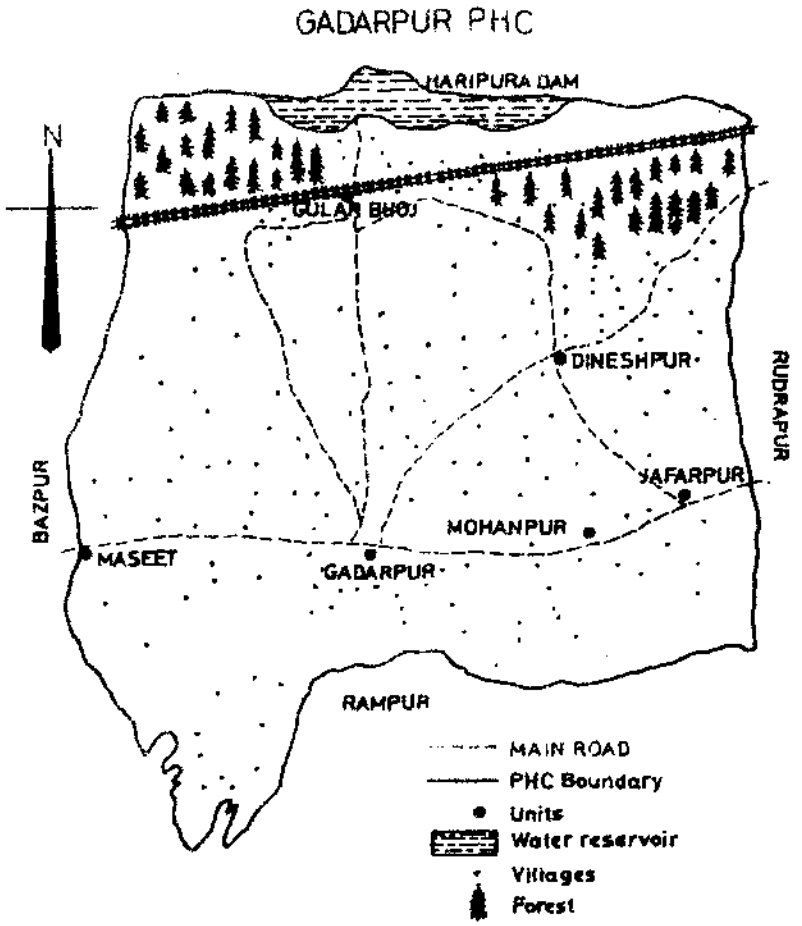

Fig. 1. Location of Gadarpur PHC in U.P. Terai region.

The age and sexwise distribution of the positive cases and the age specific attack rates are presented in Table 2 and Fig. 4. The same rates in the aboriginal Bhuksa population of this PHC are shown in Table 3. From the Tables 2 and 3, it can be seen that persons of all age groups and sex were affected in the outbreak. The attack rates show a progressive increase from infants to 16-25 age group when it reaches the maximum of 227.8. The following two factors i.e. increase in man vector contact and immunity may be responsible for this. To elucidate this further, a detailed study will be needed. The attack rates in Bhuksa and the same in Non-Bhuksa population by applying the age specific malaria risk of Bhuksa population is given in Table 5. From the table, it is seen that the estimated attack rate in Non-Bhuksa was 240.8 as against the observed value of 166.7. The Bhuksas were thus more prone to malaria than the rest of the population.

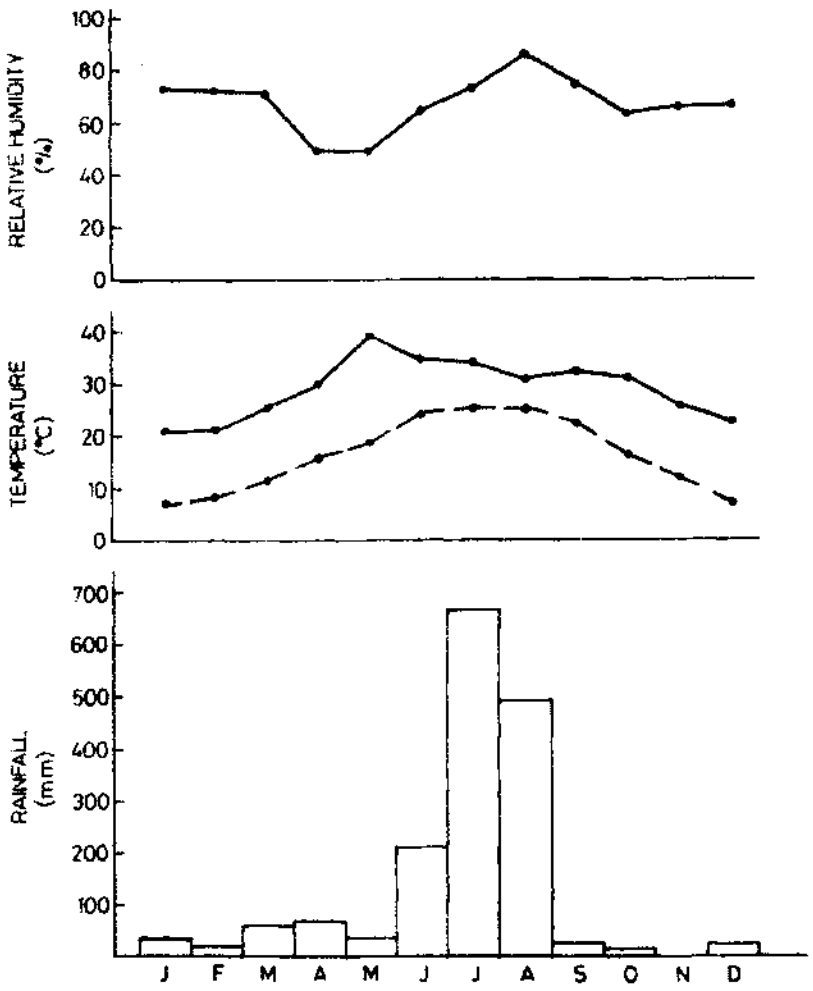

Fig. 2. Meteorological data recorded during 1982 at Pantnagar (Terai U.P.)

2. Entomological:— The following species of anopheline mosquitoes were collected during the investigation period:

1. *A. annularis*
2. *A. culicifacies*
3. *A. subpictus*
4. *A. hyrcanus*
5. *A. splendidus*
6. *A. vagus*
7. *A. fluviatilis*
8. *A. pallidus*
9. *A. stephensi*

Among these, *A. culicifacies, A. fluviatilis, A. stephensi* and *A. annularis* are known vectors of malaria in India. Dissection of these four species revealed sporozoite infection in the salivary gland of *A. culicifacies* and *A. fluviatilis*. The result of dissection of these two species are given in Table 4. Study revealed that the density of *A. fluviatilis* was high during May, but no infection

Table 1. Month-wise data of active surveillance in Gadarpur P.H.C.

Month 1982-83	No. of Villages	Population surveyed	No. of slides examined	*P. vivax*	*P. falciparum*	*P. vivax* + *P. falciparum*	Incidence (per 1000)	Slide Positivity rate
April	14	6894	788	126	—	—	18.2	15.9
May	15	7140	1396	596	1	—	83.4	42.7
June	14	7728	1421	773	1	—	100.0	54.4
July	15	6350	1325	699	10	1	111.8	53.5
August	15	6256	1597	705	237	13	152.6	59.7
September	18	5967	2832	156	1993	9	361.6	76.9
October	22	6799	2820	144	2062	3	324.6	78.3
November	33	7562	2826	38	2633	2	352.1	94.2
December	23	8498	1703	128	1143	12	150.9	75.3
January	16	4253	879	60	501	3	132.6	64.1
February	20	4676	1018	91	610	13	152.6	70.1
March	20	4883	713	146	141	11	61.0	41.7
Total	225	77006	19318	3652	9332	67		67.5

Estimated annual parasite incidence = 169.4.

Table 2. Age, sexwise distribution and age specific attack rates of malaria in Gadarpur P.H.C.

Age Group in years	Population	No. suffering from malaria			Attack rate	Attack rate in Bhuksa (For comparison)
		Male	Female	Total		
<1	1357	23	50	73	53.7	90.9
1–5	9347	580	593	1173	125.4	176.0
6–10	12430	1233	1143	2376	191.1	318.6
11–15	10259	1146	898	2044	199.2	255.1
16–25	13872	1875	1286	3161	227.8	343.2
26–50	21177	1877	1392	3269	154.3	207.8
>50	8562	548	407	955	111.5	118.0
Total	77004			13051		

Table 3. Age, sexwise distribution and age specific attack rates of malaria in Bhuksa villages, Gadarpur P.H.C.

Age Group in years	Population	No. suffering from malaria			Attack rate per 1000
		Male	Female	Total	
<1	66	2	4	6	90.9
1–5	426	33	42	75	176.0
6–10	499	87	72	159	318.6
11–15	392	59	41	100	255.1
16–25	472	106	56	162	343.2
26–50	794	97	68	165	207.8
>50	305	21	15	36	118.0
Total	2954			703	

was found in this species. *A. culicifacies* density was low during this month. No entomological data could be collected during June to August. During September, higher density in *A. culicifacies* was seen as was expected after the rains. Infected mosquitoes were found during this month and the sporozoite rate in collections from different locations varied from 0.6 to 2.7 with an overall rate of 0.79. Though there was a comparative fall in the density during October, the sporozoite rate increased and varied from 1.2 to 10.2 in different samples. The overall rate

Table 4. Vector density and sporozoite rate in *A. culicifacies* and *A. fluviatilis*

Month 1982	*A. culicifacies*				*A. fluviatilis*			
	No. collected and dissected locality wise	MHD	No. positive gland	Sp. rate	No. collected and dissected locality wise	MHD	No. positive gland	Sp. rate
May	41	6.8	Nil	0.0	123	20.5	Nil	0.0
	22	3.1	Nil	0.0	117	16.7	Nil	0.0
	37	4.6	Nil	0.0	73	9.1	Nil	0.0
	117	14.5	Nil	0.0	18	2.2	Nil	0.0
Total	217	7.48	Nil	0.0	331	11.34	Nil	0.0
	105	52.5	1	0.95	16	1.1	Nil	0.0
	74	18.5	2	2.7	40	8.0	1	2.5
September	132	22.0	2	1.5	14	4.6	Nil	0.0
	317	63.4	2	0.6				
	254	84.6	Nil	0.0				
Total	882	44.1	7	0.79	70	3.1	1	1.43
	23	5.7	1	4.3	38	19.0	Nil	0.0
	104	43.6	Nil	0.0	29	14.5	Nil	0.0
	49	12.2	5	10.2	32	8.0	Nil	0.0
	35	19.5	Nil	0.0	26	1.2	Nil	0.0
	12	3.0	1	8.3				
October	15	7.5	1	6.6				
	18	9.0	1	5.5				
	43	21.5	Nil	0.0				
	83	27.5	1	1.2				
	19	9.5	1	5.2				
	40	20.0	Nil	0.0				
Total	441	14.8	11	2.4	126	4.25	Nil	0.0
	11	3.6	1	9.0	4	0.6	Nil	0.0
	13	4.3	2	15.3	2	0.5	1	50.0*
November	14	3.5	Nil	0.0	10	1.6	Nil	0.0
	12	2.0	Nil	0.0				
Total	50	3.12	3	6.0	16	0.95	1	6.2

*The rate is inflated as only two mosquitoes could be collected.
MHD = Man hour density.
Sp. = Sporozoite.

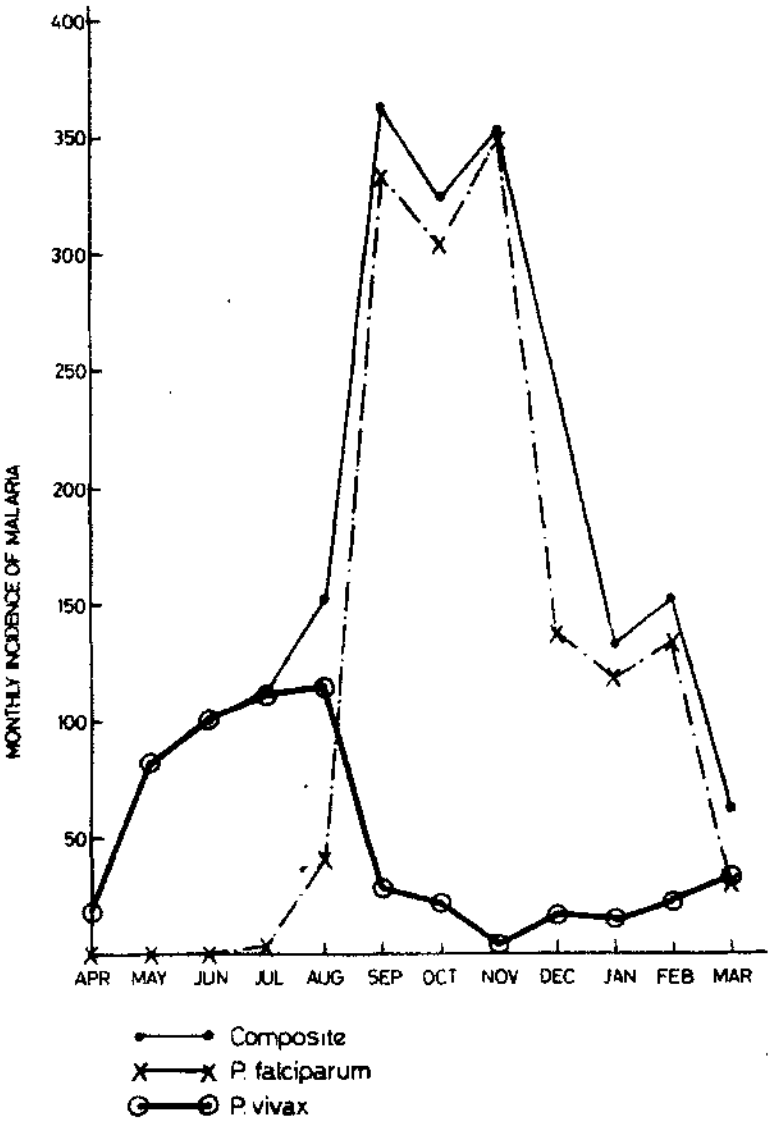

Fig. 3. Monthly incidence of malaria in Gadarpur PHC, Nainital District 1982-83.

Table 5. Estimation of comparative malaria case rates in Non-Bhuksa population by applying age specific malaria risk of Bhuksa population

Age in years	Population (P)		Cases of malaria		Probability of getting malaria		Attack Rate (1000)		Adjusted cases in Non-Bhuksa $= p_B \times P_N$
	Bhuksa (P_B)	Non-Bhuksa (P_N)	Bhuksa (C_B)	Non-Bhuksa (C_N)	Bhuksa (p_B)	Non-Bhuksa (p_N)	Bhuksa $AR_B = \frac{C_B}{P_B}$	Non-Bhuksa $AR_N = \frac{C_N}{P_N}$	
<1	66	1291	6	67	0.0909	0.052	90.9	51.9	117
1--5	426	8921	75	1098	0.1760	0.123	176.0	123.1	1570
6--10	499	11931	159	2217	0.3186	0.186	318.6	185.8	3801
11--15	392	9867	100	1944	0.2551	0.197	255.1	197.0	2517
16--25	472	13400	162	2999	0.3432	0.224	343.2	223.8	4599
26--50	794	20383	165	3104	0.2078	0.152	207.8	152.3	4235
<50	305	8257	36	919	0.1180	0.111	118.0	111.3	974
Total	2954	74050	703	12348			237.98	166.75	17813

Estimated attack rate in Non-Bhuksa $\frac{\Sigma(p_B \times P_N)}{\Sigma P_N} \times 1000 = 240.5$

Observed attack rate in Non-Bhuksa $\frac{\Sigma C_N}{\Sigma P_N} \times 1000 = 166.75$

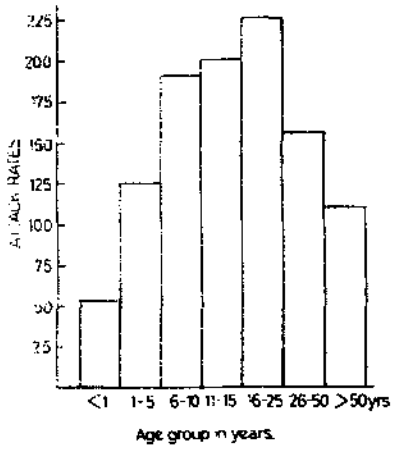

Fig. 4. Age specific attack rates-- all ethnic groups (Gadarpur PHC, Nainital dist. 1982-83).

during this period was 2.4. During November, the density of the species had fallen further but rate as high as 15.3 was found with an overall rate of 6.0. Gland infection was found in *A. fluviatilis* during September with a sporozoite rate of 2.5 in one sample. Another infection in this species was seen during November inspite of low density. The overall sporozoite rate was 6.2. This study re-established *A. fluviatilis* as a vector in this region. The infectivity in these two species of mosquitoes was found to run parallel to the rise in *P. falciparum* cases during September to November. The meteorological data in Fig. 2 show that relative humidity and temperature during July to November was favourable for transmission. The rainfall in June to August further helped the reproduction and survival of the vectors. Conditions for a short period during May were favourable for spring transmission.

Resurgence of malaria in India started in mid sixties. NMEP data show an increase in *P. falciparum* cases to the extent of 7.5 folds during the period 1970-76 before the modified plan of operation was started in 1977. The increase was noted in North Eastern Region, Orissa and some parts of Madhya Pradesh and Bihar. In Punjab, Haryana and Uttar Pradesh, there was a significant increase of *P. falciparum* cases during 1976-77 as compared with 1974 figures and in fact a warning signal was sounded by Ray (1979). The eradication programme was converted in 1977 to a control programme with emphasis on chemotherapy and less stress on surveillance and insecticides. As a result of these changes, a small focus of *P. falciparum* in terai was not timely detected and contained. The small focus resulted in an outbreak and intense transmission in a short time. The slide positivity rate in fever cases reached an astounding figure of 94.4% in the month of November. The monthly incidence of cases per thousand population was 361.1, 324.6 and 352.1 during September, October and November respectively. The annual parasite incidence in this PHC was estimated to be 169.4. The aboriginal tribes, the Bhuksas had long been known to have a relative tolerance to malaria (Srivastava and Diwan Chand, 1951). As a result of the control/eradication of malaria their tolerance decreased and they became susceptible to malaria again as revealed by this investigation.

A. minimus was the chief vector in this region in the past (Clyde loc. cit. 1931, Das, 1930). Subsequently, *A. fluviatilis* was considered to be the principal vector (Srivastava and Chakrabarti loc. cit., 1952). *A. culicifacies* was considered to play a secondary role. They reported a sporozoite rate of 11.1 in 1948 and 1.6 in 1952 in *A. fluviatilis* in terai. Issaris *et. al.*, loc. cit. (1953) working in this region found both *A. fluviatilis* and *A. culicifacies* as vectors and also considered the former to be more important as they found the predominance of *A. fluviatilis* during pre and post monsoon period. They reported a lower sporozoite rate of 0.13 in 1951 and 0.2 in 1952 which might have been due to the active malaria control operations taken up in 1947. It is interesting to note the reappearance of *A. fluviatilis* in large numbers in some of the villages. The association of this species with the spring transmission remains to be established as recorded in the past. It may be pointed out that people were found to be repeatedly suffering from attacks of malaria and some unusual deaths had occurred in this region at the peak of the outbreak. The presumptive treatment of 600 mgm of chloroquine base per adult was at one time successful in containing an acute attack of *P. falciparum* malaria. In this study, it has been repeatedly observed that this dosage was not effective in bringing down the fever and parasitaemia in many cases (Choudhury *et al.*, 1983). It is essential that adequate dose of chloroquine is administered to *P. falciparum* cases to provide relief to the people. This would reduce the chance of the appearance of resistant strains which might occur as a result of administration of repeated small ineffective doses of the drug. The incidence of malaria supported by high sporozoite rates in two vector is a danger signal and emphasises the urgency of effective control measure to retain the achievements gained due to green revolution.

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Inheritance Pattern of *Vermilion-eye* in *Anopheles culicifacies* Species A

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An eye colour mutant *vermilion-eye* was isolated from *Anopheles culicifacies* species A, from a laboratory colony. Genetic crosses have shown that it is an autosomal recessive mutant with complete penetrance. Mosquitoes of this strain express vermilion colour in eyes at the larval, pupal and adult stages.

INTRODUCTION

Anopheles culicifacies is an important vector of malaria in the Indian subcontinent. Recent chromosomal studies in this species revealed the existence of three sibling species, species A, species B (Green and Miles, 1980) and species C (Subbarao *et al.*, 1983). Species A has been incriminated as a vector in villages around Delhi (Subbarao *et al.*, 1980). To date only six phenotypic markers have been isolated and studied in *A. culicifacies* species A (Sakai *et al.*, 1977, 1979, 1981, Sakai and Baker, 1980, Dubash *et al.*, 1982 a & b) and three phenotypic markers in *A. culicifacies* species B (Subbarao *et al.*, 1982). This paper presents the genetics of one more autosomal eye colour mutant from species A.

MATERIAL & METHODS

The following strains were used in the crosses:
1. ***vermilion eye*** **(*vr*)**: Pupae with vermilion eye were observed in the rearing pans of a laboratory strain from Basantpur (Haryana). These pupae were isolated and inbred to establish a true breeding *vermilion eye* colony. Basantpur laboratory strain from which *vermilion eye* strain was isolated was identified as species A (Subbarao *et al.*, 1980). This mutant expresses its phenotype *vermilion eye* colour from the instar II through pupae to adult stages. Penetrance and expressivity of this mutant is constant. Aged mutant adults can easily be distinguished from the wild type although vermilion colour darkens considerably after the eclosion from pupae to adult.

2. Mandora: This strain was established as species A after the cytological identification of the progeny from single cultures of females collected from Mandora (Haryana) (Subbarao *et al.*, 1980) and was used as wild type in the genetic crosses.

Mosquitoes were reared in the insectary maintained at 27-28°C ± 1°C and 70-80% R.H. and fitted with an automatic machine which simulated the artificial dawn and dusk condition. Crossing experiments were carried out in 30 x 30 x 30 cm cloth cages and mosquitoes were offered fresh water soaked raisins

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and 1% glucose on cotton pads. Females were offered rabbits as a source of blood meal.
Eggs were collected in a pool and reared. Since these phenotypes could easily be distinguished at instar II, larvae were separated at instar II and were rechecked at pupal stage. This procedure provided accurate numbers in each category and helped in avoiding errors due to occasional mortality in the late instars.

RESULTS & DISCUSSION

Results of crosses showing mode of inheritance of *vermilion eye* are given in Table 1. Vermilion and wild type strains were reciprocally crossed and F_1 progeny were inbred and backcrossed to obtain F_2 progeny. Absence of mutant phenotypes in F_1 progeny (crosses 1 and 2) indicated that *vr* is recessive to wild type. This was further supported by 3:1 ratio of wild to vermilion observed in the progeny of crosses 3 and 4 (non-significant X^2-value). Absence of males with mutant phenotype in cross 2 indicated that *vr* is not sex linked, since *A. culicifacies* has the X-Y sex determination mechanism (Sakai *et al.*, 1977). This is further supported by the non-significant chi-square value obtained for linkage tests between sex and *vr* in crosses 7 and 10. Backcrossing of the F_1 progeny with *vermilion eye* strain resulted in wild and mutant types in a ratio of 1:1 (crosses 5,7,9 and 10) while in the backcrosses with wild type all the progeny were of wild type (crosses 6,8,11 and 12).

The results from inbreeding and backcrosses of F_1 progeny confirmed that *vermilion eye* is an autosomally inherited recessive mutant. This is the first phenotypic marker isolated in *A. culicifacies* species A in our laboratory.

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We thank Shri H.D. Joshi and Shri K.B. Masiwal for isolating the mutant and for their technical assistance.

Table 1. Inheritance pattern of *vermilion-eye* in *Anopheles culicifacies* A

Sl. No.	Parental genotypes		Progeny phenotypes: Vermilion		Wild		Total	testing for X^2		
	♀	♂	♀	♂	♀	♂		*vr*:+ 1:1	*vr*+ 1:3	Linkage sex-*vr*
1.	+/+	*vr*/*vr*	—	—	151	196	347	—	—	—
2.	*vr*/*vr*	+/+	—	—	65	62	127	—	—	—
3.	+/*vr*	+/*vr*	207	182	638	555	1582	—	0.15	—
4.	*vr*/+	*vr*/+	74	49	168	182	473	—	0.25	—
5.	+/*vr*	*vr*/*vr*	207	177	241	210	835	5.4	—	—
6.	+/*vr*	+/+	—	—	261	235	496	—	—	—
7.	*vr*/*vr*	+/*vr*	160	133	156	144	593	0.1	—	0.4
8.	+/+	+/*vr*	—	—	89	62	151	—	—	—
9.	*vr*/+	*vr*/*vr*	126	119	140	128	513	1.0	—	—
10.	*vr*/*vr*	*vr*/+	207	194	235	214	850	2.7	—	0.04
11.	+/+	*vr*/+	—	—	409	379	788	—	—	—
12.	*vr*/+	+/+	—	—	439	427	866	—	—	—

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Response of *Plasmodium falciparum* to Chloroquine in Delhi, Sonepat District of Haryana and Terai Region of Uttar Pradesh

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Out of eleven cases of chloroquine resistance (R1 type) detected in *P. falciparum* during 1980 and 1981 by using the standard WHO *in-vivo* test, eight belong to Sonepat district of Haryana and three to Delhi city. Using the WHO macro *in-vitro* test, another four cases from Delhi city did not show inhibition at 1.5 nmol concentration of chloroquine during 1982 and 1983. Decreased sensitivity to chloroquine was also found in terai region of Uttar Pradesh where a single dose of 600 mgm chloroquine (base) failed to control 247 cases of acute infection due to *P. falciparum* out of 9191 tested (2.68%). The recrudescence of parasites and fever in these cases occurred within 7-9 days.

INTRODUCTION

Monitoring of sensitivity of *P. falciparum* to chloroquine was taken up by National Malaria Eradication Programme in 1977 under the auspices of Indian Council of Medical Research in different parts of the country. The chloroquine resistance against *P. falciparum* was first detected in Assam (Sehgal *et al.*, 1973). Subsequently chloroquine resistance was detected in Arunachal Pradesh, Mizoram, Meghalaya and Nagaland (Pattanayak *et al.*, 1979, Chakraborty *et al.*, 1979 and Das *et al.*, 1979). The resistance was also found in Maharashtra, Orissa, Uttar Pradesh and Madhya Pradesh (De *et al.*, 1979, Guha *et al.*, 1979 and Dwivedi *et al.*, 1981).

The resurgence of malaria after the failure of the eradication programme in India brought *P. falciparum* again in the forefront. In 1970, there were 100,115 cases of *P. falciparum* in this country which rose upto 753,713 by 1976 before the introduction of modified plan of operation in 1977. Two major outbreaks due to *P. falciparum*, one in Haryana and another in U.P. terai were investigated by Malaria Research Centre during 1981-1983. Patients from different parts of Delhi also visited the Centre for advice and treatment during the period. Sensitivity to chloroquine was tested in *P. falciparum* cases of these places as part of the epidemiological investigations.

This paper records chloroquine resistance in *P. falciparum* in Delhi city, Sonepat district of Haryana and decreased sensitivity in Uttar Pradesh terai region.

MATERIAL AND METHODS

Both *in-vivo* and *in-vitro* tests were carried out according to the procedure laid down by WHO (1973). The patients selected for the study were having fever and showing rings and early trophozoites in the peripheral blood. For macro *in-vitro* test, the kit supplied by WHO was used and the tests were carried out according to the procedure laid down by WHO. For *in-vivo* test the excretion of chloroquine in the urine was

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monitored to ensure the absorption of chloroquine in the blood (Lelijveld and Kortmann, 1970). The test dose consisting of 1.5 gm of chloroquine base was administered to the adult patients and proportionate doses were given to the children as indicated by WHO for the *in-vivo* test.

The patients belong to different villages of Sonepat district of Haryana and Delhi city. They were included in the investigation when inspite of repeated treatment with chloroquine in adequate doses, the patients failed to respond as judged by failure of parasite clearance and presence of pyrexia. In U.P. terai, the patients belong to different villages of Gadarpur PHC of Nainital District. They were given a single dose of 600 mgm of chloroquine (base) as a preliminary screening procedure for chloroquine resistance.

RESULTS

The results of *in-vivo* test are given in Table 1 and *in-vitro* macro test in Table 2 and Fig. 1. The results of test with single dose of 600 mgm chloroquine (base) in U.P. terai villages are shown in Table 3.

From Table 1, it can be seen that all the 11 cases showed R_1 level of resistance. Five of them showed early recrudescence and the rest delayed between two to three weeks. All these cases were indigenous. Nine of them had previously been given one to two courses of chloroquine, each of 1.5 gm, one to eight weeks before the test dose. All the nine cases responded to Metakelfin* with termination of fever within 48 to 72 hours. The slides taken on D_5 to D_7 did not show any asexual stages. Crescents were however seen occasionally during the course of one month of post treatment follow-up. Of the two cases receiving 1.5 gm of chloroquine, one had recrudescence with fever and parasitaemia on the 20th day after treatment and had to be treated with Metakelfin. The other cases responded to chloroquine and did not complain of fever during the follow-up of one month.

*Metakelfin-Sulfamethopyrazine + Pyrimethamine

From Table 2, it can be seen that the parasites from all the four patients did not show inhibition even in 1.5 nmol concentration of chloroquine. Complete inhibition was only seen in one case when exposed to 3.0 nmol. All the four cases had also previously been given courses of 1.5 gm doses of chloroquine in recent past and failed to respond properly indicating the possibility of resistance. They were treated with Metakelfin successfully without any recrudescence.

It can be seen from Table 3, that 624 out of 9191 cases from U.P. terai showed breakthrough in the form of fever and parasitaemia after administration of a single dose of 600 mgm of chloroquine (base). Among the breakthrough cases, 248 (2. 68%) had recrudescence of parasites and fever within 7-9 days after the adminsitration of treatment indicating the possibility of appearance of decreased sensitivity to chloroquine in this community. Majority of the cases had repeated attacks of malaria during 1982 as a result of an epidemic outbreak in this area and received chloroquine treatment (Choudhury *et al.*, 1983). Regular monitoring of chloroquine resistance by *in-vivo* and *in-vitro* test is expected to be carried out in near future.

DISCUSSION AND CONCLUSION

During the National Malaria Eradication Programme, the main emphasis was given on insecticides. Chemotherapy had only a supporting role. On the contrary, in the modified plan of operation, the emphasis has been shifted to chemotherapy with insecticides playing a secondary role. This is due to the fact that most of the vector mosquitoes have become resistant to commonly used insecticides and the cost of other potent insecticides has gone up considerably due to their production being oil based. Chloroquine is being used extensively and in many cases inadequately by the Drug Distribution Centres, the Fever Treatment Depots and the patients themselves. The presumptive treatment with chloroquine in a single dose of 600 mgm (base) per adult was very effective in the past (Roy *et al.*, 1966) against *P.*

Table 1. Results of *in vivo* test carried out in Sonepat District of Haryana and Delhi during 1980-81

Sr No.	Period of test	Locality	Age/ Sex	D-0	D-2	D-4	D-6	D-7	D-9	D-11	D-13	D-15	D-17	D-19	Remarks
1.	1980	Sonepat	16/F	+	+	—	+	+	+*						One course 15 days before test dose.
2.	1980	Sonepat	35/F	+	+	+	—	—	—	—	—	+	+*		Two courses during 5 weeks before test dose.
3.	1980	Delhi	26/M	+	+		—	—		—	—	—	+*		Two courses during 4 weeks before test dose.
4.	1981	Sonepat	50/M	+	+	—	—		—	—	—	+**			
5.	1981	Sonepat	38/M	+	+	—	—	—	—	—	—	—		+*	Two courses during 4 weeks before test dose.
6.	1981	Sonepat	32/M	+	+	—	—	—	+**						
7.	1981	Sonepat	35/M	+	+	—		—		—	+*				One course 14 days before test dose.
8.	1981	Sonepat	25/F	+	+	—	—	—	+*						One course 7 days before test dose.
9.	1981	Sonepat	20/M	+	+	—	—	—							One course 10 days before test dose.
10	1981	Delhi	26/M	+	+	—		—	—	—	+*				One course 18 days before test dose.
11.	1981	Delhi	24/M	+	+	—	—	—	—	—	—	+*			Two courses during 42 days before test dose.

Course: 1.5 gm of Chloroquine.

\+ *P. falciparum* rings with fever

* Metakelfin—Sulfamethopyrazine 1 gm and Pyrimethamine 50 mgm. All the patients responded this dose.

** Chloroquine 1.5 gm.

Table 2. Results of *in-vitro* macro tests for chloroquine resistance in Delhi: 1982-83.

Sl. No.	Age/ Sex	Date	Control per 300 Leucocytes			No. of schizonts per 300 Leucocytes and % of schizonts relative to control in parenthesis. (Control—100%)							
						Chloroquine concentration in nmol.							
			I	II	Mean	0.25	0.5	0.75	1.0	1.25	1.5	2.0	3.0
1*	38/M	16.10.82	168	176	172	--	135 (78.4)	128 (74.4)	--	42 (24.4)	27 (15.6)	13 (7.5)	9 (5.3)
2**	45/M	17.01.83	352	384	368	367 (99.9)	328 (89.1)	311 (84.5)	288 (78.2)	219 (59.5)	176 (47.8)	93 (25.2)	17 (4.6)
3*	45/M	17.01.83	415	384	399.5	386 (96.7)	314 (78.6)	275 (68.9)	216 (54.0)	204 (51.1)	182 (47.1)	94 (23.5)	28 (7.0)
4*	20/M	18.02.83	112	92	102	--	73 (71.5)	64 (62.7)	56 (54.9)	48 (47.0)	26 (25.4)	17 (16.6)	0 (0.0)

* Had 3 courses of 1.5gm of chloroquine in 8-12 weeks before test.

** Had 2 courses of 1.5gm of chloroquine in 11 weeks before test.

All the patients responded to a single dose of Metakelfin.

Table 3. Results of treatment with 600 mgm chloroquine (base) in patients of Gudarpur PHC, suffering from *P. falciparum* malaria, 1982-83.

Number treated	No. showing *P. falciparum* rings with fever and breakthrough* after treatment (%)							
	7-9 days	%	10-12 days	%	13-14 days	%	Total	%
9191	248	2.68	236	2.54	140	1.5	624	6.78

*Breakthrough—recrudescence of parasites with fever.

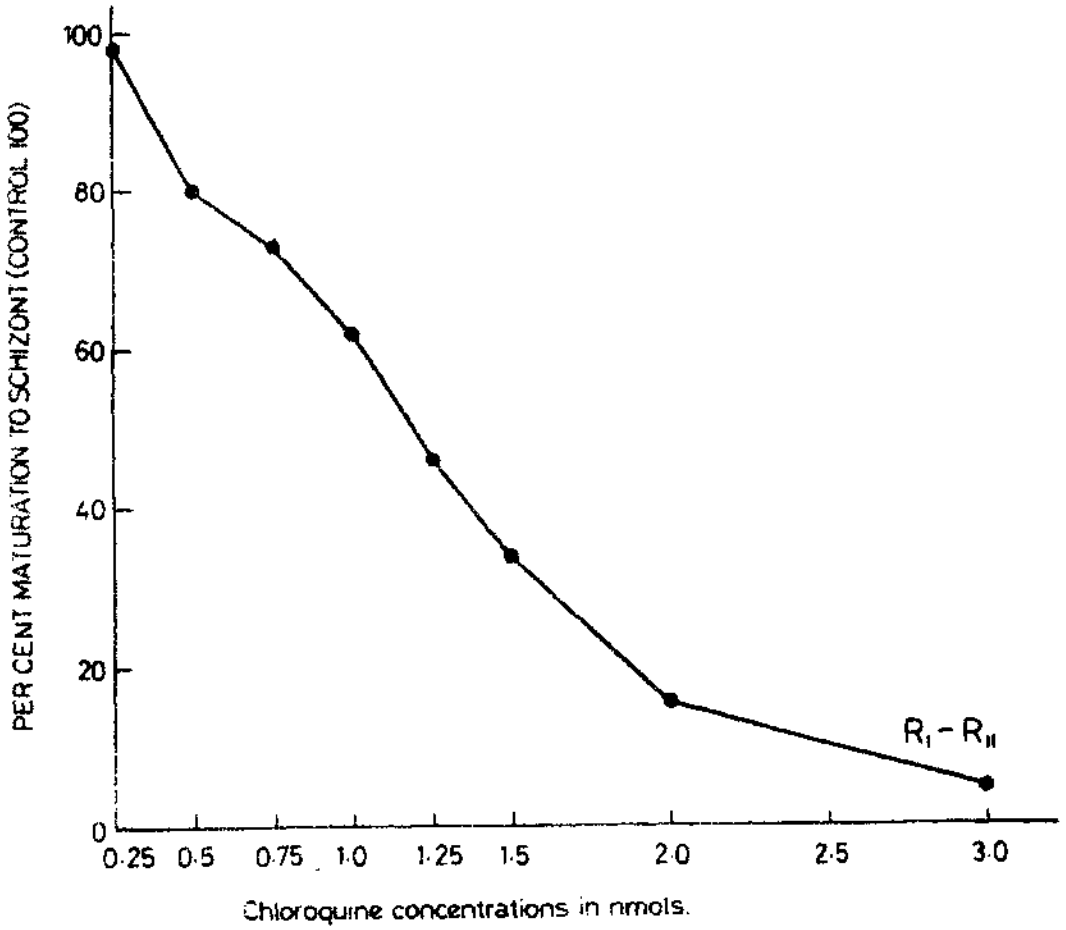
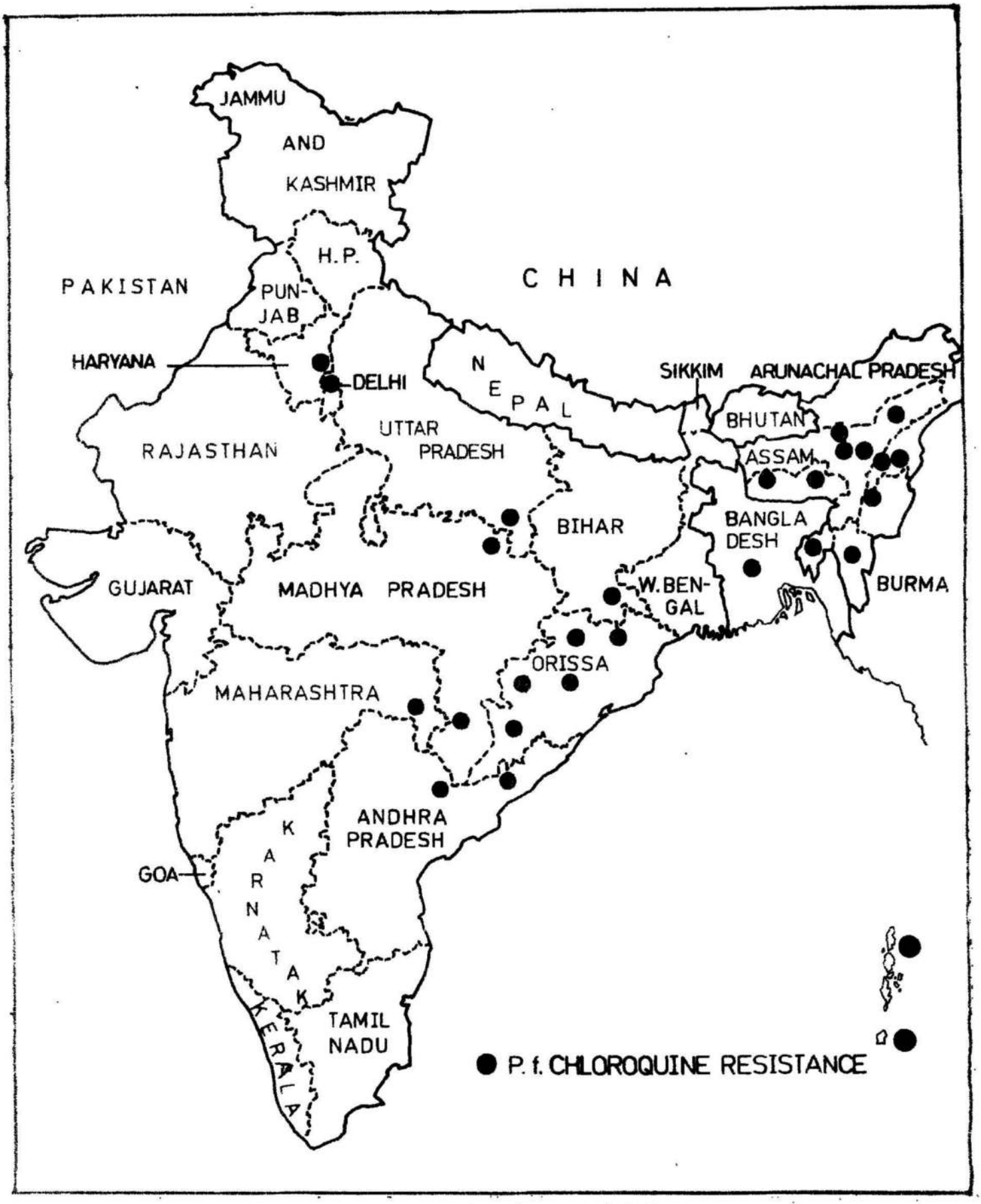


Fig. 1. *In vitro* chloroquine sensitivity of *P. falciparum* Delhi 1982-83.

falciparum cases. This is no more that effective in controlling an acute attack of *P. falciparum* malaria as has been seen in the present study. This is in contrast to the findings of Jaswant Singh *et al.* (1953) working on the same area and the adjoining Rampur district of U.P. They observed 100% clearance of *P. falciparum* parasites within 72 hours in 122 cases with a single dose of 600 mgm of chloroquine (base). Three cases showed recrudescence of parasites in 6 to 8 weeks after treatment (2.4%).

The drug must be exerting a constant selection pressure as attempts are being made to control *P. falciparum* outbreaks with drug alone or ineffective insecticides in some parts of the country.

Chloroquine resistant *P. falciparum* is being detected in widespread areas in this country. Delhi and Haryana are new additions to the already increasing list of chloroquine resistant *P. falciparum* foci in this country (Map I). *P. falciparum* containment programme (PfCP) was taken up in 1977 with WHO/SIDA assistance to contain *P. falciparum* outbreaks in this country. Intensive work with proper insecticides and chemotherapy should be taken up to liquidate these foci immediately on detection, on a priority basis.



Map 1. Chloroquine resistant areas in India.

Efforts are also urgently needed to delineate newer areas where *P. falciparum* outbreaks have started occurring, and include them under *P. falciparum* containment programme. Unless this is done and these areas are left under the existing modified plan of operation, where unfortunately much laxity has crept in, catastrophic consequences may happen.

For the treatment of chloroquine resistant *P. falciparum*, quinine or sulfonamide with pyrimethamine can be used. The latter is quite effective in controlling the chloroquine resistant *P. falciparum* at present in this country. In fact all the resistant cases found during this investigation responded to a single dose of sulfamethopyrazine and pyrimethamine combination (Table 1 and Table 3). This drug is being used now-a-days in a large scale even for the treatment of *P. vivax* cases because of the simplicity of administration and the number of tablets to be taken in each course. Caution is to be exercised in the use of this drug extensively as resistance is likely to come up against this combination. In future, if chloroquine resistance in *P. falciparum* come up in a big way in this country, this drug may be useless to be effective when we will be requiring it very badly. In fact there is already an indication of drug resistance coming up against this combination in different parts of South East Asian countries (Black *et al.*, 1981). Nguyen-Dinh *et al.* (1982) feel that the use of this combination may be reserved for well defined occasions such as allergy to other antimalarials or clearly defined cases of chloroquine resistance.

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Studies on Malate Dehydrogenase of *Plasmodium knowlesi*

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Different developmental stages of *Plasmodium knowlesi* (rings, trophozoites and schizonts) showed significantly high levels of malate dehydrogenase (MDH). Sequential studies on MDH showed that level of the enzyme of infected monkey RBC's generally increased with rise in parasitaemia. The cell-free parasites possessed highest level of MDH activity. MDH of the host and of the parasite were found to vary markedly in respect to their properties such as Km, pH, heat stability and electrophoretic mobility etc. The parasite MDH was found to be more heat labile as compared to the host enzyme. Polyacrylamide gel electrophoresis for MDH isoenzymes showed 5 bands in the normal RBC's and two bands in the parasite preparation.

INTRODUCTION

Malate dehydrogenase (MDH) of *Plasmodium* species had been studied by many workers. Sherman (1966) reported the presence of MDH in *P. lophurae* and *P. berghei*, and later Nagarajan (1968a) demonstrated this enzyme in cell-free preparations of *P. berghei*. Momen (1975) and Carter (1970 and 1973) carried out the electrophoretic study of *P. berghei* enzyme and demonstrated distinct bands for the host and the parasite. According to Carter and Voller (1973), who studied human and simian malarial parasites, the presence of distinct MDH enzyme in the parasite could not be established.

In the present report, MDH of *P. knowlesi* and that of host RBC along with some of their kinetic properties have been studied.

MATERIAL AND METHODS

Collection of blood: Adult rhesus monkeys *Macaca mulatta*, of either sex, weighing about 4-6 kg were kept under 12 hrs. photoperiodicity with fluorescent lights on from 7.00 hr to 19.00 hr. A strain of *P. knowlesi* (w_1), kindly donated by Professor P.C.C. Garnham was used in this study to infect healthy rhesus monkeys which were certified to be free from tuberculosis as shown by negative tuberculin test and chest X-ray.

Blood from *P. knowlesi* infected monkeys was collected in acid citrate dextrose (ACD) solution (Kessel *et al.*, 1965) by cardiac puncture. It was centrifuged at 800×g for 10 min. Plasma and buffy coat were removed and the schizont infected cells, which form a brownish layer, and the underlying erythrocytes were washed three times with chilled phosphate buffer saline (PBS). The erythrocytes of normal monkey were also taken in ACD, centrifuged at 800×g for 10 min. and the plasma and buffy coat were removed as described above. The pellet of normal erythrocytes was washed three times with chilled PBS.

Normal as well as infected erythrocytes were lysed according to the method of Cook *et al.* (1969) with 0.2% saponin in 0.85% saline with

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continuous stirring in cold for 30 min and centrifuged at 7000g×10 min. The parasites were collected from the sediment and washed thrice with chilled PBS.

Preparation of Homogenate:— The packed erythrocytes or the parasites were homogenised in Potter-Elvehjem homogeniser at 4°C in chilled PBS and centrifuged at 2000g for 10 min and the supernatant was used as enzyme source.

Subcellular fractionation: Homogenate was prepared in 0.32M sucrose and centrifuged at 2000g for 15 min for nuclear fraction. The 2000g supernatant was further centrifuged at 9,200g for 30 min for mitochondrial fraction. The microsomal and soluble fractions were separated by centrifuging the above supernatant at 1,04.000g for 1 hr.

Assay of malate dehydrogenase: (L-malate: NAD^+ oxidoreductase, E.C. No. 1.1.1.37) Enzyme activity was measured spectrophotometrically according to the method of Ochoa (1955). The reaction mixture contained 2.7 ml phosphate buffer (0.1M of required pH), 0.1ml oxaloacetate (0.0078M, freshly prepared), 0.1 ml NADH (0.0015M) and suitably diluted enzyme preparation. Decrease in O.D. at 340nm was recorded at 30 sec. intervals for a period of 3 min.

One MDH unit is defined as the amount of protein required to catalyze the oxidation of one μ mol. of NADH per min, under the specified experimental conditions.

Protein estimation:— Protein was estimated according to the method of Lowry *et al.* (1951), using bovine serum albumin as standard.

Electrophoresis: MDH was separated electrophoretically according to the technique of Davis (1964) using 7% polyacrylamide and the activity was located on the gels by the method of Fine and Costello (1963).

RESULTS

Comparison of the MDH activities of normal and different developmental stages of *P. knowlesi* infected monkey erythrocytes as well as in cell-free parasites showed that the enzyme level in infected cells was considerably high as compared to normal erythrocytes whereas different stages of cell-free parasite showed highest enzyme activity as compared to that of normal and infected red cells. The aqueous extract of trophozoites, among three stages of parasite revealed highest activity which was nearly five folds higher as compared to the activity of normal red cells. However, schizonts and ring stages also possessed significantly high levels of MDH (Table 1). Studies on infected RBC's showed progressive increase in MDH activity with the increase in level of parasitaemia. Estimations with infected samples ranging from 0.2-60% infection, showed an increase in specific activity from 86.00×10^{-3} (at 0.2% parasitaemia) to 151.00×10^{-3} (at 60.0% parasitaemia). Cell-free parasites (schizonts) showed considerably higher levels of MDH (283.93×10^{-3}) (Table 2). Sub cellular fractionation of parasite (schizonts) indicated that more than 50% of the enzyme was localized in the soluble fraction (Table 3).

Polyacrylamide gel electrophoresis of normal erythrocytes and cell-free parasites (schizonts) showed that the normal erythrocytes possessed five isoenzymes of MDH whereas the cell-free parasites contained only two. The parasite enzymes were different in mobility as compared to the host enzymes (Fig. 1).

Table 1. MDH activity of different stages of *P. knowlesi* and infected erythrocytes and host RBC.

S No.	Stages of Parasite host RBC	Cell-free parasite	Infected RBCs (100% infection)	Normal RBC
		μ mg Protein $\times 10^{-3}$		
	Parasite			
1.	Rings	150.373	140.42	
2.	Trophozoites	414.34	139.99	
3.	Schizonts	285.93	85.78	
	Host RBC (Normal)			82.24

Table 2. MDH activity of normal and *P. knowlesi* infected erythrocytes at different levels of parasitaemia

S. No.	Parasitaemia	μ/mg/protein $\times 10^{-3}$	S. No.	Parasitaemia	μ/mg protein $\times 10^{-3}$
1.	Nil (Normal)	82.24	11.	15.0	116.80
2.	0.2	86.00	12.	16.0	117.20
3.	0.8	90.00	13.	20.0	124.20
4	2.0	99.40	14.	35.0	136.80
5	2.5	109.40	15.	40.0	142.00
6.	2.8	109.00	16.	45.0	139.00
7	3.0	92.00	17.	50.0	145.20
8.	5.0	108.50	18.	60.0	151.00
9.	7.0	116.00	19.	Parasite (Cell-free)	285.94
10.	10.0	118.20			

Kinetic properties of parasite MDH were markedly different from the host MDH. The MDH of normal monkey red cells was found to be most active at pH 7.5 whereas the pH optima of parasite MDH was 6.8 (Fig. 2). The Km values of host and parasite MDH, at pH 7.5 for oxaloacetate, were nearly the same (i.e. 4.5×10^{-5} M and 5.0×10^{-5} M for normal RBC's MDH and parasite MDH respectively), but at pH 6.8, Km values of MDH from both the sources, differed markedly (5.5×10^{-5} M for erythrocytes and 2.5×10^{-5} M for *P. knowlesi* enzyme) (Table 4). Both the enzymes were inhibited by the high concentrations of oxaloacetate although the inhibition was not much (Fig. 3). Heat resistance of the host and parasite MDH were not alike. The host enzyme showed better stability towards temperature as compared to the corresponding enzyme of the parasite (Table 5).

DISCUSSION

The present study showed that the cell-free preparations of different developmental stages of

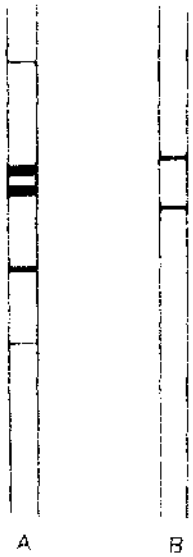

Fig. 1. MDH isoenzymes, A=normal red blood cells, B=host cell-free parasites.

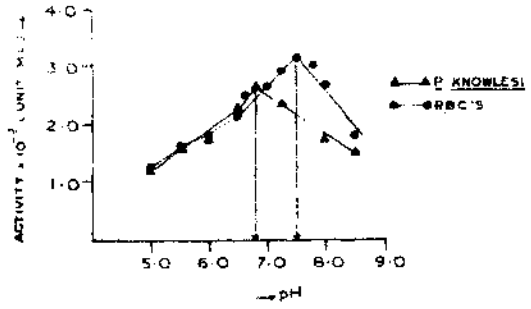

Fig. 2. Optimum pH of RBC's and *P. knowlesi* MDH

P. knowlesi, namely rings, trophozoites and schizonts possessed significantly high activity of MDH indicating that the parasite possessed this enzyme at every stage of its development, which is different from that of the host erythrocytes' MDH. It has been reported that the mammalian malarial parasites are able to catabolise glucose by glycolysis but they lack the TCA cycle (Homewood, 1977).

Table 3. MDH activity in various subcellular fractions of *P. knowlesi*

S. No.	Fraction	% of MDH activity
1.	Crude	100%
2.	Nuclear	4.42%
3.	Mitochondrial	13.64%
4.	Microsomal	4.54%
5.	Soluble	53.54%

Table 4. Km of normal RBC's and *P. knowlesi* MDH at different pH

S. No.	Sample	pH 6.8	pH 7.5
1.	Normal RBC's	5.5×10^{-5} M	4.5×10^{-5} M
2.	*P. knowlesi*	2.5×10^{-5} M	5.0×10^{-5} M

Table 5. Stability of normal RBC's and *P. knowlesi* MDH at different temperatures.

S No.	Temperature (°C)*	% Activity after heat treatment	
		RBC's	*P. knowlesi*
1.	0	100	100
2.	37	98	73
3.	40	93	62
4.	50	84	58
5.	60	54	12
6.	70	8	8
7.	80	0	0

*The enzyme preparations were exposed to various temperatures for 30 min. prior to assay.

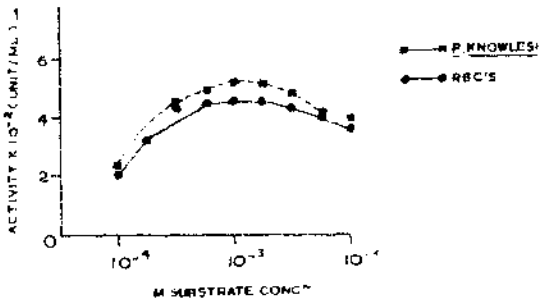

Figure-3. Substrate inhibition of RBC's and *P. knowlesi* MDH.

The existence of two forms of NAD^+ dependent MDH, a soluble cytoplasmic form and a particulate form associated with mitochondria has been reported in mammalian tissues (Oelshlegel and Brewer, 1975). The present findings suggest that probably the soluble form of MDH is predominant in all stages of *P. knowlesi* since the well-defined mitochondria are not found in this parasite. Further, subcellular fractionation of cell-free parasites (schizonts) confirmed that more than 50% of the MDH was present in the soluble cytoplasmic fraction whereas the association of fairly good amount of MDH activity with mitochondrial fraction may be indicative of presence of unorganised mitochondrial organelle in the parasite.

The activity of this enzyme increased gradually with the rise in parasitaemia although there was no concomittant increase in enzyme activity along with the rise in parasitaemia. Every stage of parasite showed higher activity of the enzyme with respect to corresponding infected erythrocytes suggesting that the parasite enzyme contributes to observed activity of the infected RBC. We had also reported similar observations while studying lactate dehydrogenase of *P. knowlesi* and *P. knowlesi* infected erythrocytes at different levels of parasitaemia (Saxena *et al.*, 1982).

On the basis of kinetic properties and substrate analogue techniques, Sherman (1966) had dem-

onstrated that *P. berghei* as well as *P. lophurae* contain a NAD^+ dependent MDH which can be distinguished from the corresponding host enzyme. The present study has shown that MDH of parasite is markedly different from that of the erythrocytic enzyme in electrophoretic mobility. The parasite MDH exists in two isoenzymic forms which have different electrophoretic mobilities as compared to the five isoenzyme forms of the host enzyme. Carter (1970 and 1973), Momen *et al.*, (1975) and Momen (1979) had also detected in addition to the MDH of erythrocytes, other isoenzymes in the red cells of mice infected with *P. berghei*. Shernan (1966) demonstrated an anodal band of MDH in normal duck erythrocytes whereas the *P. lophurae* MDH moved towards cathode on starch gel

Soluble malic dehydrogenase is an enzyme of considerable importance as it plays an important role in reoxidation of reduced NAD^+ and thus facilitates glycolysis since higher $NADH/NAD^+$ ratio is rate limiting in glycolysis. In this way the function of MDH in malarial parasite is similar to that of the parasite LDH. At present it is difficult to conclude about other functions of plasmodial MDH. However, it may have other functions related to amino acid metabolism.

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SHORT NOTES

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Mosquito Breeding Survey in Urban Delhi

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A high fever epidemic raged Delhi from September to November, 1982. The fever lasted for 7 to 10 days and affected all age groups. During routine malaria surveillance it was revealed that the fever was not due to malaria. Later the dengue virus was isolated from *Aedes aegypti* collected from affected colonies in Delhi (Dhanda, personal communication). The outbreak of dengue prompted us to locate the mosquito breeding sites in Delhi.

Mosquito breeding surveys were carried out from November 4 to 27, 1982. Initially surveys revealed that breeding was confined to the overhead tanks (Fig. 1). Most of these tanks were poorly maintained. They were not cleaned for a long time, the lids were broken or left open, some of the tanks were not in use and had rain water (Fig. 2). The tanks were made of cement or steel and placed on the house tops. In some localities they were installed high on the roof tops making them inaccessible. The house owners of these tanks were ignorant or disinterested, and some of the owners were taken aback at the quality of stored water they were using. The survey of mosquito breeding was done in localities shown in Fig. 3. It was revealed that mosquito breeding was found throughout urban Delhi including trans-Jumna colonies.

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Results of survey given in Table I revealed that in November most of the overhead tanks were breeding for *Ae. aegypti*. In some tanks larvae of *Ae. aegypti* and *Ae. stephensi* were found. No other mosquito species was found breeding in the overhead tanks. The intensity of breeding per tank varied considerably i.e., from 10-20 larvae to a few thousand or more. The study revealed that overhead tanks were a permanent source of mosquito breeding. Earlier Balaya *et al.* (1969) investigated an outbreak of dengue in Delhi in the month of September-October, 1967. The main areas involved in the epidemic were thickly populated central parts of Delhi. They observed that epidemic started in the third week of September, prevailing throughout October and declining towards the end of October and in the first week of November, 1967. The 1967 epidemic did not appear to have reached the outskirts of Delhi, where housing was more open or in localities of central part which were not continuous with the affected areas. In September-October, 1970 Delhi was again under the grip of another outbreak of dengue fever (Diesh *et al.*, 1972). The investigations showed that the epidemic was in the central part as well as in the peripheral part of Delhi. It may be due to the fact that *Ae. aegypti*, the vector of dengue, had spread to new areas in Delhi due to rapid urbanization of suburban areas, thereby spreading the disease to the peripheral parts of the city. The periodical fogging operations under the Urban Malaria Control

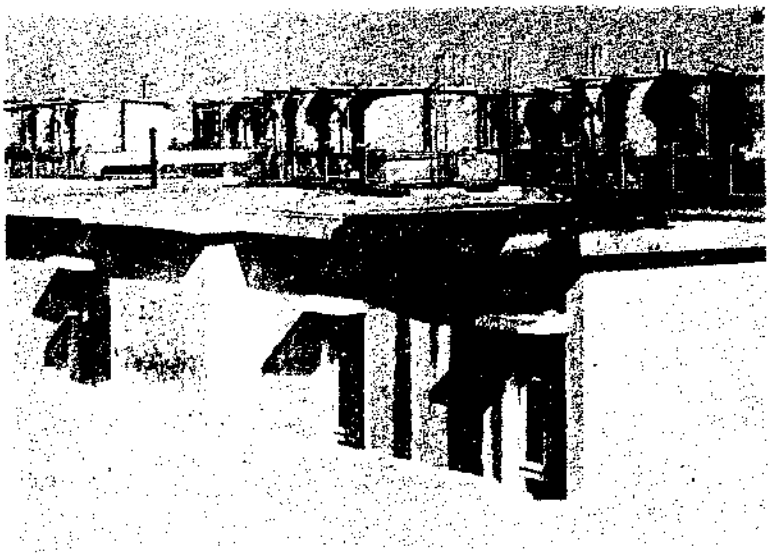

Fig. 1. A view of the overhead tanks found breeding for mosquitoes.

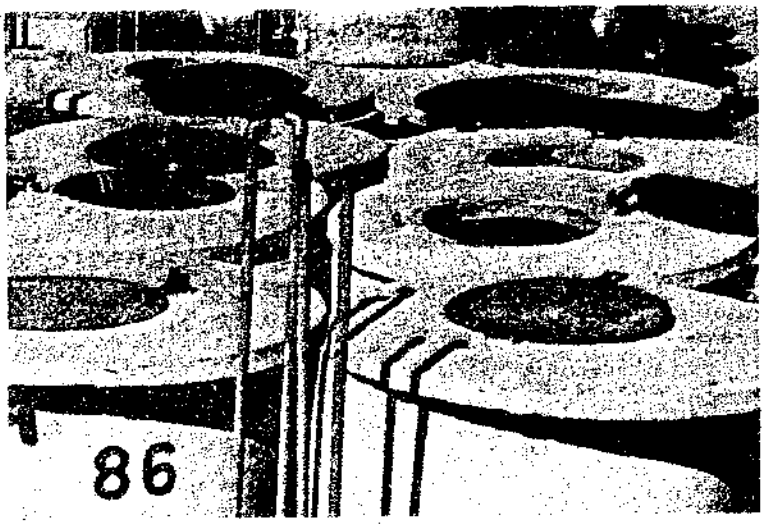

Fig. 2. Overhead tanks with open lids found breeding for mosquitoes.

Table 1: Breeding of mosquitoes in overhead tanks in Delhi.

Date of survey	Locality	Total Tanks Examined			Tanks Breeding for *Aedes*			Tanks Breeding for *Anopheles*	Tanks with Mixed Breeding	Per cent Tanks Breeding
		Cement	Steel	Total	Cement	Steel	Total			
4-11-82	Shahpur Jat Colony	79	8	87	15	0	15	1	5	25.3
5-11-82	Hauzkhas Enclave	18	4	22	4	0	4	0	1	22.7
6-11-82	Ayurvijnan Nagar	156	0	156	32	0	32		0	20.5
8-11-82	Shahpur Jat Colony (DDA)	48	0	48	9	0	9	0	0	18.8
9-11-82	Sadiq Nagar	136	0	136	28	0	28	3	8	28.7
10-11-82	Seva Nagar	110	0	110	22	0	22	0	0	20.0
11-11-82	Lodi Colony	44	100	144	12	63	75	0	0	52.0
13-11-82	Lawrence Road	142	0	142	30	0	30	0	0	21.1
18-11-82	Dilshad Garden Colony	122	0	122	19	0	19	0	0	15.5
22-11-82	Karkar Dooma	146	0	146	61	0	61	0	0	41.8
23-11-82	Mayapuri, D.D.A. Colony	114	0	114	14	0	14	0	0	12.3
27-11-82	Reserve Bank Quarters	108	0	108	8	8	8	0	0	7.4
27-11-82	Masjid Moth, D.D.A.	180	0	180	8	0	8	0	9	9.5
27-11-82	R.K. Puram, Sec. 7	12	117	129	4	10	14	0	0	10.9
	Total	1,415	229	1,644	266	73	339	4	23	22.3

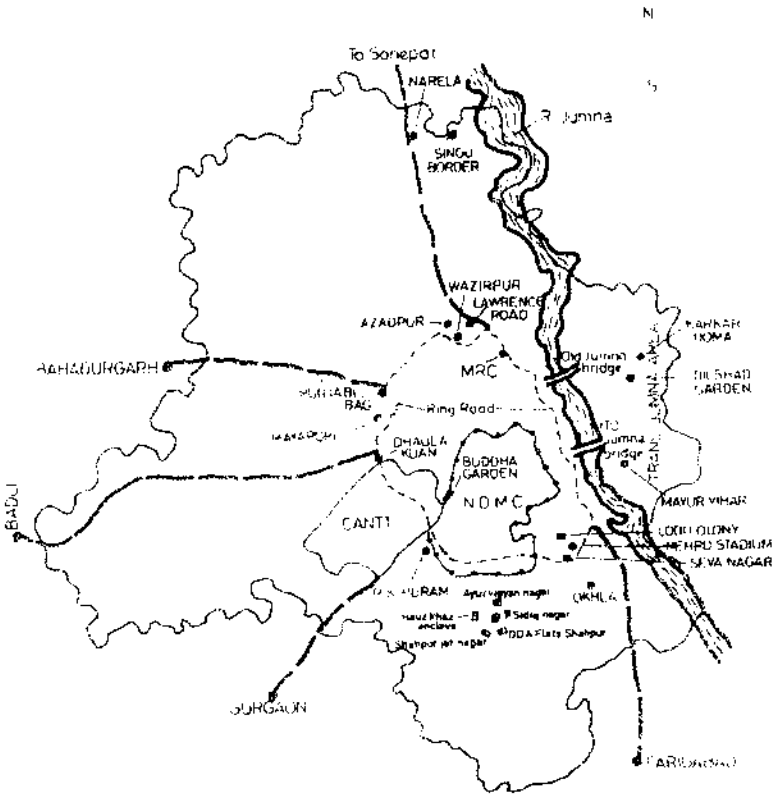

Fig. 3. Larval survey sites in Delhi.

Programme were not very helpful in the containment of the disease. *Ae. aegypti* is a day biting mosquito and daily new population was emerging from the overhead tanks. Also, in urban Delhi, animal population was very limited and most of the mosquito feeding was confined to man, thus enhancing disease transmission.

In order to control breeding, the overhead tanks should be periodically cleaned and lid placed tightly so that there were no openings left for the entry and exit of mosquitoes. It would also be advisable to have community overhead tanks which can be more easily managed. The control of breeding in the overhead tanks would greatly reduce the density of vector populations and thus eliminate/reduce the vector borne diseases.

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Vital Staining of the Malaria Parasites

V.P. SHARMA

Malaria case is confirmed by the demonstration of plasmodial parasite in blood smears. Development of this method was the result of the discovery of malaria parasite by Laveran in 1880, staining by polychromed methylene blue by Romanowsky in 1891; and thick smear preparation by Ross in 1903 (Russell *et al.*, 1963). The method is used in all laboratories throughout the world. Blood smear preparation and examination requires experienced technicians, and negligence may result in the misclassification of slides or parasite misidentification. In a study in Garki project it was shown that the percentage of parasite positive slides increased both by doubling the time of slide examination, and also by re-examination of slides by their supervisors (Molineaux and Gramiccia, 1980). The method is tiring and time consuming. In endemic areas it is commonplace to find backlog of slides, especially during the transmission season. To overcome these problems, vital staining of the malaria parasites was developed. Details of this technique are given below.

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Stain is prepared by diluting 1.0 ml Giemsa stock solution in 7.5 ml distilled water and 2.5 ml acid-citrate-dextrose (ACD) solution, filtered and used. A drop of finger prick blood is mixed with the stain on a microslide, and cells were allowed to settle for 5-10 minutes under the coverslip. The slide is then examined at 400× magnification. The microscope is first focussed on the floating cells followed by the examination of other cells. It would be seen that nuclear material of some leucocytes takes blue stain. The malaria parasites (*P. vivax*, *P. falciparum* and *P. falciparum* in culture) are stained in shades of blue, greenish blue and purple with brown to deep brown pigments. Platelets and red blood cells are not stained. The stained parasites with their familiar morphology are easily identified within the unstained red blood cells (Fig.1). Detection of parasites is easy since the slide is examined at a magnification of 400× and also the stained parasitized cells stand out against the translucent background. In fact all stages of *P.vivax* and crescents of *P. falciparum* are easily identifiable at 250× magnification while the *P. falciparum* rings which are small could generally be seen best at 400× magnification. Examination of parasitized cells at 1000× may be required to study the structural details of the parasite.

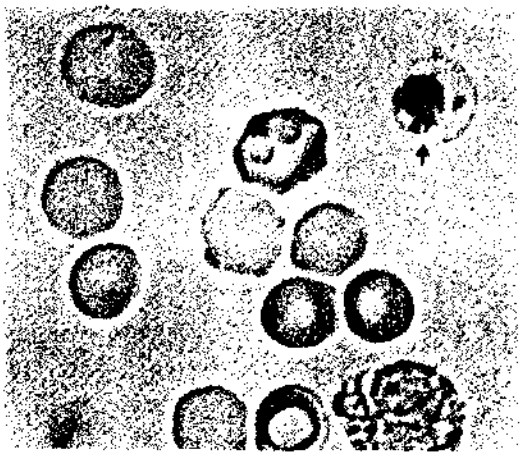

Fig. 1. Arrow showing stained parasite amongst the unstained RBCs

We tested this technique in the field in Delhi and nearby villages. A total of 752 blood samples were examined. One drop of blood from finger prick was taken on a microslide and mixed with a drop of stain. At times blood was also mixed with the stain in small vials in the field and transported to the laboratory for examination. Concurrently thin blood smears were prepared and stained with JSB. Examination of slides stained with vital dye revealed 217 *P.vivax* and 72 *P. falciparum* cases. Smear examination revealed 210 *P. vivax* and 70 *P. falciparum* cases. Re-examination of these smears revealed 9 parasite positive slides which were missed in the first blood smear examination. It was also estimated that the new technique was about 5 times faster than the existing method of blood smear examination. The technique was found simple, rapid, accurate, and economical in the detection of malaria parasite positive cases. The application of this technique in malaria clinics and routine surveillance would provide an opportunity of an early administration of the correct antimalarials to the parasite positive cases. The procedure would also obviate the need of presumptive treatment, and in turn help in extending the useful life of the antimalarials.

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Variations in Ornamentation of Palpi of *Anopheles sundaicus* Rodenwaldt (1925) Collected from Andaman Islands, India

B.N. NAGPAL and V.P. SHARMA

Anopheles (Cellia) *sundaicus* is distributed in India, Bangladesh, Burma, Indo-China, Thailand, Malaysia, Singapore, Indonesia, Sunda Island, South Sulawai and China. In India it was reported from West Bengal, Orissa, Coastal regions of Andhra Pradesh and Andaman (Rao, 1981). It is one of the most important malaria carrying species in India (Christophers, 1933). Sporozoites and oocysts positive specimens were recorded from Bengal (Iyengar, 1931 and Sen, 1938), Orissa (Senior White *et al.*, 1939, Panigrahi, 1942 and Covell *et al.*, 1942) and Andamans (Covell, 1927).

Morphological variations in Indian Anophelines including some vectors of malaria have been described by Ramakrishna (1954) Subramanian and Nagendra (1955), Bhatnagar *et al.*, (1958), Rajagopal and Chakraborty (1960) and Wattal *et al.*, (1960). During January-February 1982, a total of 360 specimens of *A. sundaicus* were collected from South and Middle Andaman. Adult specimens of *A. sundaicus* were collected from the cattle sheds and human dwellings, using the suction tube and pyrethrum space spray methods. Larval collections were made from the creeks and streams of village Sipighat with the help of a dipper. Out of 360 specimens, 262 adults were collected from a single village Sipighat (South Andaman) and one adult was collected in the evening from cattle sheds from village Bakuntala (Middle Andaman). In all night baited collections, 38 specimens of *A. sundaicus* were collected from two cattle baits (5.30 p.m. to 2.30 a.m.) and 2 specimens were collected from two human baits (5.30 p.m. to 6.30 p.m.) from village Sipighat. From the larval collections of village Sipighat 47 *A. sundaicus* emerged. These larvae were found breeding with other Anophelines species such as *A. vagus*, *A. subpictus* and *A. aconitus*.

Eight adult female specimens of *A. sundaicus*, collected from Sipighat village showed variations in the palpal banding. The palpi had four types of banding patterns. In the typical specimens the length of apical pale and preapical dark bands has been found to be 0.31 mm and 0.3 mm respectively. In comparison to the typical palpi of *A. sundaicus* the length of apical pale and preapical dark bands in variants has been found to be, (i) 0.16 mm apical pale band and 0.30 mm

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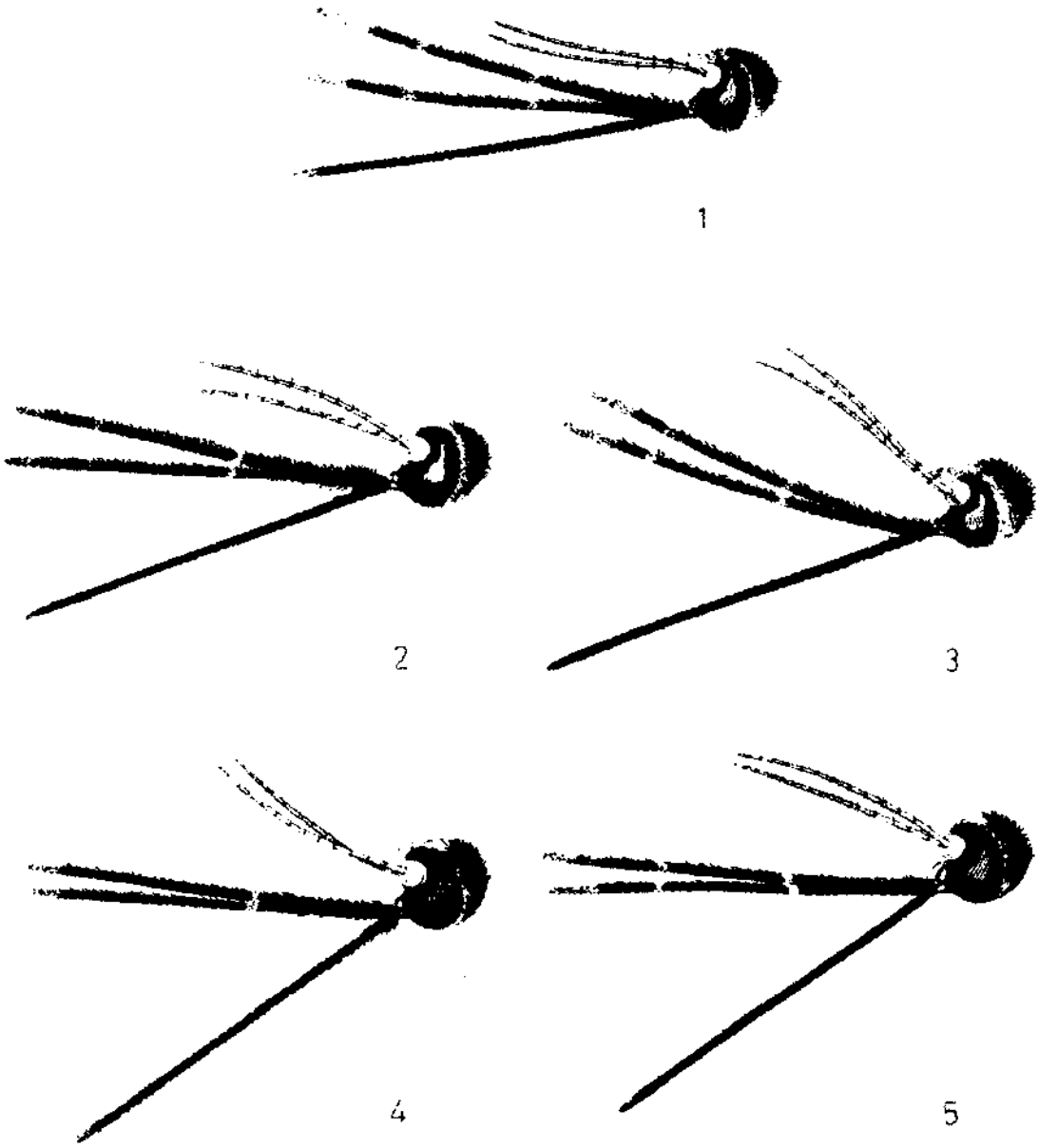

Plate I. Variation in the ornamentation of palpi of *A. sundaicus*.

Table 1. Morphological variation in *A. sundaicus* collected from village Sipighat (South Andaman).

Serial number of variations	Date of collection	Number of specimens collected	Notes on variations of palpi from the type form
1	14.1.82 21.1.82	1 2	Apical pale band short (0.53x) as compared to the preapical dark band in both palpi, Fig. 2.
2	5.2.82 16.2.82	1 1	Apical pale band long (2.1 3.1 x) as compared to the preapical dark band in both palpi. Fig. 3.
3	18.1 82 23.2.82	1 1	Preapical pale band absent in both palpi, Fig. 4.
4	28.2.82	1	An extra dark band on the apical pale band of one palpus. Fig. 5.

*Type Form palpi - Length of the apical pale band 0.31 mm and preapical dark band 0.30 mm, Fig. 1

preapical dark band, and (ii) 0.31 mm 0.46 mm apical pale band and 0.15 mm preapical dark band. Drawings of these variations in relative sizes were made and have been presented in Plate I. Details of palpi variations, site, and date of collection are given in Table 1. All the eight specimens have been preserved in the taxonomy section of Malaria Research Centre. Such variations in the palpal banding of *A. sundaicus*, have not been reported so far.

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BOOK REVIEW

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Anopheline Names, Their Derivations and Histories by James B. Kitzmiller

Thomas H.G. Aitken[1]

From time to time one chances on a book which refuses to be put down until it has been read from cover to cover. Kitzmiller's "Anopheline Names" falls in such a category, but I hasten to add it was not non-stop reading on my part. The book is much too long (and I am a slow reader). However, like a butterfly flitting from flower to flower, I found myself leafing here and there for "nectar delights", searching for entomological heroes of yesteryear who had been blessed with assignments to some far away romantic spot.

Perhaps I am biased in my thoughts since all of my life I have yearned for a life abroad, and, indeed, I began such a career at the age of seven. It wasn't until college days, however, when I had ample opportunity to glean among the tropical medicine, travel and natural history books of my college library that my imagination was really fired to the point where tropical service had to be my goal. I avidly read accounts of 18th and 19th century naturalists and medical officers who were unravelling the mysterious life cycles of parasites, pathogens, mosquitoes and what not in such distant places as the Straits Settlements, the Western Ghats, the upper reaches of the Congo, etc. What I'm trying to say is that I wanted to experience the (to me) exciting lives of such heroes as Ronald Ross (1857), Wilhelm Schuffner (1867), Oswaldo Cruz (1872), Malcolm Watson (1873), Rickard Christophers (1873), Jacques Schwetz (1876), Emile Brumpt (1877), Carlos Chagas (1879), Emile Roubaud (1882), John Sinton (1884), Raymond Shannon (1894), and a host of others.

Many of these tropical medicine specialists (medical men, entomologists, parasitologists, etc.) bear names well known in the field, but there are others more obscure and how much do we really know about any of them? What were their origins, what was their schooling and what influenced their lives? How did it happen they were posted to Sarawak or Timbuktu? How did a medical doctor become involved in mosquito taxonomy or mosquito control? Obituaries and biographies abound but they are widely scattered. The stories are all here (or most of them); some read like western thrillers or John Masters' "The Lotus and the Wind", *vide baileyi*. Jim Kitzmiller has put together a fascinating account

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of one aspect of tropical medicine history through his biographical accounts of a multitude of individuals associated with anopheline mosquitoes. These sketches may be very detailed (witness over 5 pages devoted to Clara Ludlow) and provide an enlightening insight into the development of knowledge on a variety of diseases but with emphasis on malaria. One poignant historical reminder are the name of those workers whose lives were cut short in the World War II conflict in the Pacific.

Not only are all patronymics beautifully and painstakingly researched but other specific (and generic) epithets of a descriptive or geographic nature are analyzed in a most scholarly fashion. It has always unsettled me that so many young students fail to understand the naming of the insects they are working with. A part of the fun of studying a group of animals (or plants) is in knowing their historical background. This book is a most useful and entertaining aid in this respect, especially to young culicidologists but also to the broader group of biologists as well. I note with pleasure the book's dedication to John Alexander Reid, outstanding student of southeast Asian anopheline biology and classification.

Time has not permitted reading all of the 767 entries, but rest assured they will be read by me and many times over. Very few errors mar the book. Aside from some printer's errors, the following are noted: p. 173 (*edwardsi*) spelling of Blephariceridae (par.4); p. 177 (*emilianus*) spelling of Emilio; p. 187 (*Feltinella*) spelling of Ephraim; p. 481 (*sawyeri*) spelling of Hudson (par. 3); p. 491 (*shannoni*) discovery and isolation of yellow fever virus from *Haemagogus*, with due respect to Ray Shannon, is a controversial subject involving several investigators and is perhaps best stated in a less definitive manner. Shannon's posting to Trinidad was to a Rockefeller Foundation malaria laboratory; the Trinidad Regional Virus Laboratory was established after the war in 1954. A frequently cited biographical reference in the text is that of Pamela Gilbert which is "lost" on p. 589; it would have been helpful to list it again among the "G's" on p. 596.

The book is printed by the photo offset process, vi+640 pp, with a Preface written by Leonard Jan Bruce-Chwatt. It represents Volume VIII 1982 of the Thomas Say Foundation published by the Entomological Society of America.

INDIAN JOURNAL OF MALARIOLOGY

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

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

Books/Monographs

Rao, T.R. (1981). *Anophelines of India* (W.Q. Judge Press, Bangalore).

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

Paper presented to Symposium/Conference

Subbarao, S.K. *Cytoplasmic incompatibility in mosquitoes*. Paper presented to the International symposium on recent developments in the genetics of insect diseases vectors, Bellagio, Italy, 20-24 April, 1981.

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