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Field Evaluation of Deltamethrin against Resistant *Anopheles culicifacies* in Distt. Ghaziabad (U.P.) India

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A field study to evaluate the impact of deltamethrin spraying on DDT and HCH resistant *A. culicifacies* population was carried out in Razapur primary health centre (PHC), Distt. Ghaziabad (U.P.) India. The PHC comprising of about 0.14 million population was divided into 3 sections of equal size and sprayed with (i) 3 rounds @ 12.5 mg/sq m at 6 weeks interval, and (ii) 2 rounds each @ 20mg/sq m and 25 mg/sq m at 8 weeks interval. One section in Dadri PHC located at a distance of about 22 kms was held as control. In this area 3 rounds of HCH were sprayed by the NMEP as was done to control malaria in this district. Deltamethrin spraying was carried out for 3 years. Results revealed that spraying @ 12.5, 20 and 25 mg/sq m resulted in drastic reduction of DDT and HCH resistant *A. culicifacies* population and in the interruption of malaria transmission. In control area high vector densities and malaria transmission was encountered throughout the period of study. Spraying of deltamethrin resulted in build-up of *Culex quinquefasciatus* population due to resistance.

INTRODUCTION

Anopheles culicifacies Giles is a principal vector of malaria and responsible for bulk of malaria transmission in rural and peri-urban areas of the country. The vector has become resistant to DDT and HCH in most parts of the country, while in Gujarat and Maharashtra it has also developed resistance to malathion (Sharma, 1984). There is therefore an urgent need in our armamentarium of a replacement insecticide to control the double or multiresistant *A. culicifacies*. In recent years several photostable pyrethroids have become available which show varying degree of toxicity to mosquitoes, chironomids and biting midges (Ali and Mulla, 1980). Of the several pyrethroids tested as larvicide and adulticide, deltamethrin (k-othrine) was found marginally superior for control of mosquitoes (Rajavel *et al.*, 1987; Amalraj *et al.*, 1987).

In 1985 a field trial was carried out in Bhanera village, Loni PHC, Distt. Ghaziabad to determine the impact of three rounds of residual spraying of deltamethrin @ 12.5 mg/sq m on DDT and HCH resistant *A. culicifacies* populations and malaria transmission. Results revealed that deltamethrin spraying controlled vector populations and interrupted malaria transmission (Ansari *et al.*, 1986). Therefore an extended field study was undertaken to demon-

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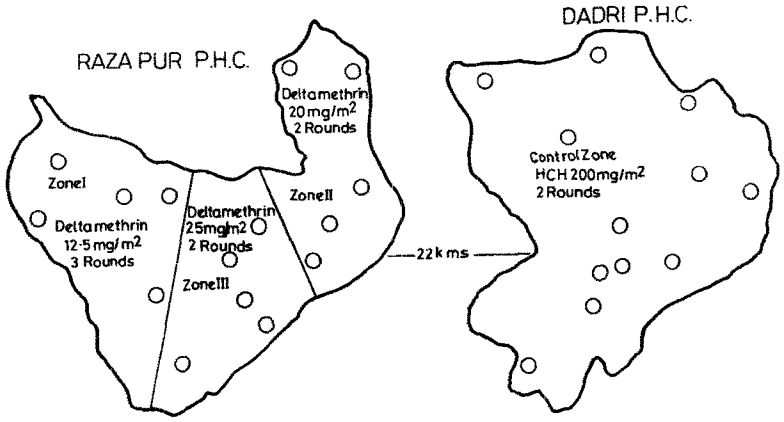
strate malaria control in Razapur PHC in *A. culicifacies* populations resistant to DDT and HCH. Observations were also made on the impact of spraying on non-target species. Results of a 3 year study are reported in this paper.

MATERIAL AND METHODS

Razapur PHC is situated in Distt. Ghaziabad (U.P.) about 35 kms away from Delhi. Adjoining PHCs are Loni, Murad Nagar, Bhojpur and Dhaulana. The area of the experimental PHC (Razapur) is about 185 sq kms with a population of about 140,000 living in 46 villages. There are about 18,000 human dwellings and majority of the inhabitants are engaged in agriculture. Major crops of the area are wheat, pulse, rice and maize and irrigation is through canals and tube wells. The ratio of man to cattle is about 4 to 1. The area of the control PHC (Dadri) is about 227 sq km with about 144,000 population (mainly agriculturists), living in 65 villages and irrigation is through canals and tube wells. The human and cattle ratio is about 6.5:1. In both PHCs HCH @ 200 mg/sq m was sprayed since 1980. In these PHCs about 25% houses were made of mud walls and thatched roofs and the remaining houses are cemented (Pukka).

Initially, villagewise API from 1983-85 of both Razapur and Dadri PHCs was collected from the National Malaria Eradication Programme (NMEP). Razapur PHC was selected because of high mosquito populations, DDT and HCH resistance in the vector species (*A. culicifacies*) and malaria transmission, although HCH was being sprayed. The PHC was divided into three zones on the basis of malaria situation and operational feasibility. The population of zone I,II and III was 49549, 45140 and 47668, res-



Dosages	No. vill.	Pop.	Monitored pop.	A.P.I.
12.5 mg	20	49549	18602	1.22
20 mg	17	45140	18649	2.18
25 mg	17	47668	19349	1.07
200 mg	65	160,000	19343	6.78

Fig. 1: Showing deltamethrin and HCH sprayed areas in Ghaziabad district U.P. Circles show the location of index villages used for monitoring the entomological and epidemiological parameters.

pectively. At the time of starting the experiment average API for the 3 year duration and mosquito breeding sites were comparable in the 3 experimental and control areas (Fig. 1). However, the density of *A. culicifacies* was higher in zone I as compared to zone II and III.

Deltamethrin 2.5% WP (K-othrine), a synthetic pyrethroid was supplied through the courtesy of M/s. Roussel Pharmaceuticals india Ltd. In zone I, three rounds of deltamethrin @ 12.5 mg/sq m were sprayed at an interval of six weeks, while two rounds each of deltamethrin @ 20 mg/sq m and 25 mg/sq m were sprayed at an interval of 8 weeks in the remaining 2 zones. Since it was not possible to undertake entomological and epidemiological evaluation in all villages of each zone, 5 villages located in different directions were selected to monitor the vector densities and incidence of malaria throughout the study period. The population of villages used for monitoring varied from 18602 to 19349 while API as reported by the NMEP ranged from 1.1 to 2.2. Similar observations were made in Dadri PHC held for comparison. In this PHC 3 rounds of HCH @ 200 mg/sq m were sprayed, and 12 villages with a population of 19343 were selected for monitoring. The average API (1983-1985) was 6.78 (see Fig. 1).

Indoor vector density was estimated by hand catch method during morning hours. Houses and cattlesheds were searched fortnightly for one hour each covering 16 structures in each village in a period of one month. During prespray period (May and June, 1986) only vector species viz., *A. culicifacies*, *A. stephensi* and *A. annularis* were collected. However, after 1st round of deltamethrin in Razapur PHC and from March 1987 in Dadri PHC the density of adult *Culex* mosquitoes was also monitored. Data of all villages in each zone was pooled together and per man hour density calculated. Door-to-door active surveillance was maintained on daily basis throughout the study period in both PHCs. Results of active parasitological surveys were subjected to 't' test. All fever cases were given presumptive treatment (600 mg chloroquine adult dose) and radical treatment (600 mg chloroquine with 45 mg primaquine in *Pf* cases or 15 mg primaquine for 5 days in *Pv* cases as adult dose) was given to all microscopically confirmed malaria cases. Children and pregnant women were not given primaquine. If radical treatment was delayed beyond one week the dosage of chloroquine was increased to 1200 mg and administered in 2 divided doses. The same drug schedule was followed for the 3 experimental and control areas.

Susceptibility tests were carried out in both PHCs by exposing fully fed field collected *A. culicifacies* for 1 hour on DDT (4%), dieldrin (4%), malathion (5%) and deltamethrin (0.025%) impregnated papers. A control was run concurrently and if the mortality exceeded 20% all tests were discarded. Mortality was recorded after 24 hours of post-exposure period. Spraying was carried out with the help of stirrup pumps. The discharge rate was maintained at 780 ml/40 strokes/minute during each round. All safety precautions were followed while spraying and efforts were made to spray all houses, cattlesheds and temporary structures in each round. A record of spray coverage was maintained throughout the study period.

RESULTS AND DISCUSSION

Results of susceptibility tests before starting the spray programme in 1986 revealed that *A. culicifacies* was resistant to DDT and dieldrin and fully susceptible to malathion and deltamethrin. The corrected mortality with discriminatory dosages of DDT, dieldrin and malathion was 17.8%, 16.07% and 100% respectively in the experimental PHC as against 21.9%, 21.9%, and 100% respectively in the control PHC. The susceptibility tests of vector species could not be performed during 1987 and 1988 because of very low mosquito populations in experimental areas. Results of susceptibility test against *Culex*

Table 1. Susceptibility of *Culex sp.* to deltamethrin and DDT

Areas sprayed with	Corrected mortality in subsequent years %			
	1987		1988	
	4% DDT	0.025% Deltamethrin	4% DDT	0.025% Deltamethrin
Deltamethrin 12.5 mg/m^2	0.0 (35)	17.3 (86)	0.0 (45)	10.0 (105)
Deltamethrin 20 mg/m^2	0.0 (45)	8.3 (130)	0.0 (30)	4.8 (135)
Deltamethrin 25 mg/m^2	0.0 (125)	18.05 (115)	0.0 (45)	4.6 (75)
HCH 200 mg/m^2	8.2 (105)	77.5 (136)	10.7 (30)	82.5 (90)

Figures in parentheses indicate number of mosquitoes tested.

Table 2. Spraying coverage (%) in different structures

Dosage	1986					1987					1988				
	HD	MD	CS	TS	T	HD	MD	CS	TS	T	HD	MD	CS	TS	T
Deltamethrin															
12.5 mg/m^2	97.7	92.1	100	100	92.8	97.4	92.6	100	100	93.3	98.2	96.6	100	100	96.7
20 mg/m^2	98.8	95.5	100	100	96.0	98.9	97.4	100	100	97.6	99.4	98.9	100	100	98.9
25 mg/m^2	98.3	97.0	99.8	100	97.3	99.4	98.7	100	100	98.8	98.1	98.0	100	100	98.0
Control															
HCH 200 mg/m^2	92.4	82.9	93.4	—	83.2	95.0	78.2	95.5	—	78.6	87.1	81.9	92.9	—	82.2

HD = Human Dwelling; MD = Mixed Dwelling; CS = Cattleshed; TS = Temporary structure; T = Total.

quinquefasciatus are given in Table 1. Studies revealed high degree of resistance to deltamethrin i.e., 8 to 17.3% mortality as against 77.5% obtained in control areas in 1987. The mortality was further reduced and ranged from 5 to 10% in 1988. Besides, resistance to DDT was also enhanced as indicated by no mortality of adults in 1987 and 1988 in the deltamethrin sprayed areas.

Results of spray coverage over the 3 years period are given in Table 2. The coverage in deltamethrin sprayed villages was always higher (> 90%) in comparison to control area and varied from 92% to 99.4% in human dwellings as against 100.0% in cattlesheds and other structures.

Entomological evaluation

Results of entomological evaluation revealed that average pre-spray density of *A. culicifacies* in 1986 in the experimental and control PHC villages was comparable. Residual spraying of 1st round of deltamethrin drastically reduced vector density in experimental PHCs. Subsequent spraying decimated *A. culicifacies*, *A. stephensi*, *A. annularis* and *A. subpictus* from the experimental villages. The impact of spraying was so pronounced on anophelines that the population never recovered during the 3 year period of spraying (Table 3). There was a marginal increase in the density of *A. culicifacies* during June-July 1987 and May 1988 in villages sprayed with 12.5 mg/sq m whereas in villages sprayed with higher dosages vector species remained negligible throughout the study period. In the control villages sprayed with HCH the density of *A. culicifacies* remained very high throughout the study period and attained a peak in the month of August (Table 3, Fig. 2). The gradual decline in mosquito population from October onwards was associated with climatic factors.

Similar reduction of other anophelines viz., *A. stephensi*, *A. annularis* and *A. subpictus* was observed but small numbers continued to be present in the experimental areas (Table 3). In contrast *Culex* sp. (mainly *Cx. quinquefasciatus*) densities increased after one year of deltamethrin spraying and continued to build up and reached enormous numbers in successive years of spraying (Table 3, Fig. 3). The impact of deltamethrin on other non-target species was not evaluated but inhabitants started complaining of houseflies and cockroaches which were eliminated in the first year of spraying. This had some adverse effect on the acceptability of the compound.

Epidemiological evaluation

Results of pre-spray data revealed that malaria picture was more or less comparable in the experimental and control villages. Average API for 3 years (1983-85) was slightly higher in the control villages as compared to the experimental villages. Similarly falciparum malaria during pre-spray period was more or less the same in both experimental and control villages. Table 4 gives the results of epidemiological assessment of the spraying. There was no immediate impact of deltamethrin spraying on the incidence of malaria. However, after second round of spraying, incidence of malaria particularly that of falciparum malaria started declining and remained very low in 1987 and 1988 in all experimental villages.

Similar trend was observed when SPR and SfR were compared with that in control area (Figs. 4 and 5). The malaria transmission during peak period in 1987 was very low in control PHC. This was mainly due to severe drought conditions in the country particularly in northern India. However, during the final year of trial i.e. 1988, intense malaria transmission occurred in the control villages of Dadri PHC although 3 rounds of HCH were sprayed whereas experimental villages were free from malaria except a few cases. There was no significant difference in reduction of malaria at the 3 dose levels of deltamethrin in experimental PHC (Table 4).

Table 3. Man hour

Month/ Year	*A. culcifacies* Deltamethrin mg/sq m				*A. stephensi* Deltamethrin mg/sq m				
	12.5	20	25	C	12.5	20	25	C	12.5
1986									
May	28.5	25.06	8.6	33.1	1.4	1.4	3.5	0.5	—
Jun	43.5	11.5	3.8	17.1	2.8	0.6	1.0	0.5	0.5
Jul	40.8	19.9	12.6	38.7	4.6	1.2	0.9	0.6	0.3
Aug	3.1	0.3	0.6	45.5	0.05	0.05	0.1	0.08	0
Sep	0.1	0.65	0.1	32.1	0.1	0.05	0.3	0.1	0
Oct	0.5	0.3	0	33.7	0.15	0	0	0.2	0
Nov	0	0	0	26.1	0	0.05	0	0.2	0
Dec	0	0	0	17.9	0	0	0	0.02	0
1987									
Jan	0	0	0	5.1	0	0	0	0	0
Feb	0	0	0	11.3	0	0	0	0.1	0
Mar	0	0.05	0	24.4	0.05	0	0	0.3	0
Apr	0.8	0.3	0	27.2	0.2	0	0	0.3	0.1
May	1.1	0.6	0	22.3	0.1	0	0	0.2	0.05
Jun	6.5	1.7	0.3	40.1	0.1	0	0	0.4	0.05
Jul	6.7	0	0.05	20.8	0.2	0	0.2	0.04	0
Aug	1.7	0.2	0	15.9	0.1	0	0	0.2	0.05
Sep	2.2	0.1	0	32.1	0.2	0	0	0.04	0
Oct	0	0	0	15.1	0	0	0	0.1	0
Nov	0	0	0	12.1	0	0	0	0.06	0
Dec	0	0	0	6.1	0	0	0	0	0
1988									
Jan	0.05	0.05	0	2.6	0	0	0	0.31	0
Feb	0.0	0	0	2.3	0	0	0	0.16	0
Mar	0.1	0	0	6.2	0	0	0	0.56	0
Apr	2.1	0	0.05	19.9	1.5	0.05	0.1	0.58	0.1
May	3.9	0.7	1.3	37.9	1.1	0.5	2.5	0.91	0.1
Jun	0	0.1	0.05	10.3	0.3	0.1	0	0.37	0
Jul	2.2	1.6	0.4	64.7	1.6	1.2	0.6	1.12	0.05
Aug	2.51	3.4	1.1	100.5	3.4	1.9	0.3	0.89	0.6
Sep	0.4	1.7	0.2	83.1	1.8	1.1	2.5	0.93	0.35
Oct	0.3	0.4	0.1	33.8	0.9	1.5	1.0	1.27	0.05
Nov	0	0.2	0	28.4	0.05	0	0.1	1.46	0
Dec	0	0	0	16.6	0	0	0	0.48	0

C = Control area sprayed with HCH 3 rounds @ 200 mg/sq m.

densities of mosquitoes

A. annularis Deltamethrin mg/sq m			*A. subpictus* Deltamethrin mg/s qm				*Culex* spp. Deltamethrin mg/sq m			
20	25	C	12.5	20	25	C	12.5	20	25	C
—	—	7.1	—	—	—	—	—	—	—	—
0.5	1.04	3.2	—	—	—	—	—	—	—	—
0.3	0.6	3.3	—	—	—	—	—	—	—	—
0.05	0.2	4.2	2.4	3.6	7.1	—	2.4	0.5	3.2	—
0.1	0.2	7.8	30.0	2.7	2.4	—	2.3	2.2	4.4	—
0.4	0	4.8	0.9	0.7	0.2	—	4.05	5.0	4.4	—
0.9	0.5	7.3	0	0.1	0	—	2.8	10.5	6.7	—
0	0	6.08	0	0	0	—	1.8	22.2	20.5	—
0	0	1.3	0	0	0	0	0.3	9.35	13.9	—
0	0	5.3	0	0	0	0	2.6	15.7	17.7	—
0.05	0	5.9	0	0	0	0	23.6	46.45	53.5	107
0.4	0.1	4.7	0	0	0	0	46.7	88.8	98.8	145
0.1	0	2.2	0	0	0	6.2	54.9	49.25	75.9	136
0.05	0.05	3.6	0	0	0	25.2	36.5	46.5	35.6	101.7
0	0	2.9	0	0.2	0.3	41.6	8.3	17.1	15.01	44.4
0	0	0.08	0.95	0.3	1.1	126.3	21.7	27.0	31.3	28.7
0	0	1.1	2.75	1.1	0.45	111.9	35.9	38.2	61.8	25.3
0	0	1.1	0.15	0.2	0.05	67.7	79.2	76.5	121.7	34.5
0	0	1.3	0	0	0	38.1	65.3	84.9	130.7	30.95
0	0	1.7	0	0	0	9.2	33.1	51.8	77.7	31.5
0	0	0.25	0	0	0	1.9	47.3	52.5	79.6	42.5
0	0	0.08	0	0	0	1.5	62.4	74.5	103.7	63.0
0	0.05	0.35	0	0	0	0.9	188.8	234.6	327.4	139.2
0	0.05	1.48	0	0	0	1.8	264.4	272.0	377.8	276.9
0	0.1	1.39	0	0	0	9.9	208.3	303.0	379.1	174.1
0	0	2.48	0.1	0.6	0	24.6	120.5	171.6	216.4	63.2
0	150.05	3.46	21.9	22.9	23.8	158.8	108.8	177.5	146.2	100.4
1.0	0.9	8.14	9.8	43.6	20.6	211.4	69.0	137.7	79.1	52.6
3.95	1.3	10.75	6.5	8.2	4.6	164.0	68.9	167.8	106.4	38.0
2.3	1.2	9.30	0.3	2.5	0.6	55.0	66.9	151.8	163.7	10.4
1.2	0.7	12.73	0	0.6	0.05	46.5	98.2	157.9	180.6	15.7
0	0	10.14	0	0	0	27.7	19.8	138.9	121.5	14.3

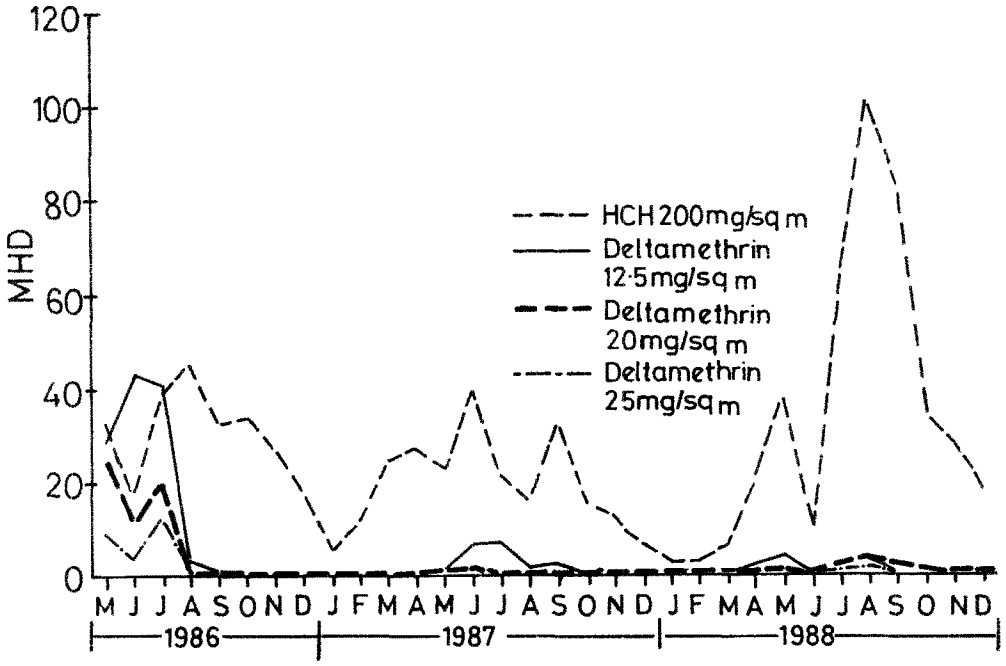


Fig. 2: Impact of deltamethrin spraying on man hour density of *A. culicifacies.*

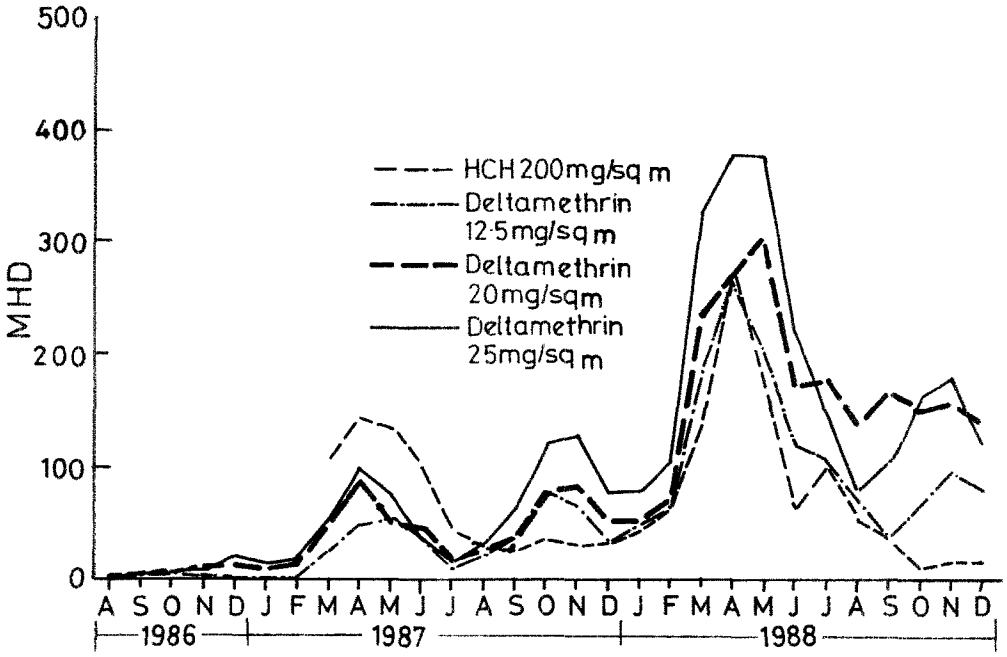


Fig. 3: Impact of deltamethrin spraying on man hour density of *Culex* sp.

The study revealed that residual spraying of deltamethrin successfully controlled DDT and HCH resistant *A. culicifacies*. Similar results were obtained by Rishikesh *et al.* (1979) in the control of *A. gambiae* and *A. funestus* with deltamethrin spraying @ 50 mg/sq m in Nigeria. It was interesting to note that the impact of residual spraying was quite evident when the density of vector species was compared with that in the control area. However, the impact of deltamethrin on *Culex quinquefasciatus* was not so pronounced though reduction of adult densities during post spraying period of 1986 was observed. This may be due to the fact that only 76% initial kill was obtained when pre-spray adults were exposed to discriminatory doses. Substantial increase in the density of *Culex* mosquitoes during 1987 and 1988 in experimental villages may be attributed to the development of resistance. There was also evidence of cross resistance to DDT as revealed by the fact that exposure to DDT on 4% papers did not produce any mortality during 1987 and 1988, whereas about 10% mortality was commonly encountered in areas not sprayed with deltamethrin. This observation was rather disturbing because DDT is still the cheapest and most useful insecticide in malaria control (Ansari *et al.*, 1988)

Halliday and Georghiou (1985) reported that resistance to pyrethroids is primarily due to Kdr gene, a recessive knock down resistance gene which also shows cross resistance to DDT in *Culex quinquefasciatus*. During the present study results of susceptibility tests have also given an indication of the presence of this gene in *Culex quinquefasciatus*. Similarly high level of permethrin resistance in houseflies (*Musca domestica*) was also reported by MacDonald *et al.* (1983) in Canada.

It was also revealed that of the 3 dose levels tried in the field 20 mg/sq m dosage is most suitable since 3 rounds of spraying with 12.5 mg is more expensive and operationally difficult and does not produce as good an impact as higher dosages. The results of 20 and 25 mg/sq m dosages are comparable with no additional advantage of enhanced dosage of 25 mg/sq m (see Table 4, Figs. 4 and 5).

Synthetic pyrethroids are an effective group of insecticides with residual toxicity making these chemicals suitable for use in public health. These insecticides are considered more acceptable from the environmental point of view because the yearly dosages used are very low as compared to other insecticides used in public health e.g., the dosages used for DDT, HCH and malathion in one year are 2 gm, 600 mg and 6 gm per sq m whereas deltamethrin dosage is 40 mg/sq m.

Results of field trial have clearly demonstrated that the spraying of deltamethrin resulted in

- control of DDT and HCH resistant *A. culicifacies*, and other anophelines.
- interruption of malaria transmission.
- rapid build up of deltamethrin and DDT resistance in *Cx. quinquefasciatus* and as a consequence high mosquito populations were encountered in all the sprayed villages.
- Spraying of two rounds of 20 mg/sq m dosage was found optimal for interruption of transmission.

Therefore deltamethrin spraying to control malaria can be resorted to in order to tackle epidemic situations for a short period of time, say for one year or so. Wherever possible this should be followed by the implementation of bioenvironmental control strategy as demonstrated in Nadiad to control rural malaria (Sharma and Sharma, 1989) BHEL, Hardwar to control industrial malaria (Dua *et al.*, 1988) and Mandla to control rural malaria in tribal area (Singh *et al.*, 1989).

Table 4. Epidemiological indices

Month/Year	BER				SPR				
	mg/sq m				mg/sq m				
	12.5	20	25	C	12.5	20	25	C	12.5
1986									
May	1.22	2.19	1.16	3.22	14.10	18.09	14.66	23.59	0.44
Jun	1.23	2.14	1.33	3.78	19.30	24.81	20.54	21.17	0
Jul	1.75	4.47	3.42	5.3	11.69	15.83	14.83	13.75	2.15
Aug	1.09	3.19	2.17	4.71	13.37	17.17	19.71	25.65	0.9
Sep	0.61	2.38	1.46	4.31	13.16	7.88	10.24	26.65	0.81
Oct	0.73	2.25	1.45	3.77	2.20	6.20	6.78	10.08	0.74
Nov	0.61	1.96	0.95	2.54	1.77	4.90	2.71	16.66	0.88
Dec	0.31	1.80	0.72	2.22	1.72	3.87	5.71	12.79	0
1987									
Jan	0.29	0.85	1.05	1.67	3.63	1.88	1.56	7.71	3.63
Feb	0.54	1.48	0.67	2.01	0	1.88	0	4.87	0
Mar	0.69	1.48	0.72	2.08	0.77	1.08	2.85	3.97	0
Apr	0.77	1.35	0.96	1.77	0	1.97	3.2	6.99	0
May	0.63	1.28	1.06	1.64	0.84	0.41	4.61	6.91	0
Jun	0.61	0.83	0.77	1.65	0	3.87	4.02	14.33	0
Jul	0.49	0.93	0.76	1.62	3	0.57	1.45	6.36	0
Aug	0.44	1.03	0.89	1.80	2.4	2.07	2.31	7.42	0
Sep	1.01	2.13	1.56	3.87	0	0.50	0.33	5.73	0
Oct	0.68	1.88	1.64	2.92	0.78	0	0.31	5.47	0.78
Nov	0.69	1.30	1.51	2.04	0	0	0	4.81	0
Dec	0.81	1.05	0.97	1.99	0	0.50	0	2.59	0
1988									
Jan	0.54	1.0	0.85	1.82	0	0	0	0.56	0
Feb	0.34	0.51	0.65	1.42	0	0	0.8	1.08	0
Mar	0.39	0.61	0.67	1.62	0	0	0	1.59	0
Apr	0.36	0.59	0.5	1.42	1.56	0	0	1.09	0
May	0.33	0.49	0.54	1.12	0	0	0	2.30	0
Jun	0.52	0.39	0.45	0.93	1.03	0	0	5.55	0
Jul	0.48	0.78	0.62	2.32	0	0	0	12.24	0
Aug	0.74	0.99	1.24	7.42	0	2.15	0.82	24.40	0
Sep	0.49	0.80	0.74	7.58	2.19	4.6	6.2	58.80	0
Oct	0.45	0.72	0.61	4.52	1.19	3.67	6.61	43.78	1.19
Nov	0.31	0.4	0.47	3.10	1.69	2.15	0.8	40.33	0
Dec	0.27	0.32	0.45	1.79	0	0	0	29.47	0

C = Control area sprayed with 200 mg/sq m HCH 3 rounds.

t - values with different doses of deltamethrin

Year	12.5 & 20 mg/sq m		12.5 & 25 mg/sq m		20 & 25 mg/sq m	
	Cases/1000	Pf/1000	Cases/1000	Pf/1000	Cases/1000	Pf/1000
1987	2.41	0.00	2.51	0.94	0.69	1.20
1988	1.56	2.59	1.32	1.59	0.01	0.32

t-values were not significant at 1% level of significance.

of the experimental villages

SFR			Cases/000				Pf/000			
mg/sq m			mg/sq m				mg/sq m			
20	25	C	12.5	20	25	C	12.5	20	25	C
0.49	0.88	0.64	1.72	3.97	1.71	7.59	0.05	0.11	0.1	0.2
0.5	1.16	0.54	2.37	5.31	2.74	8.01	0	0.11	0.15	0.2
2.16	1.21	1.36	2.04	7.08	4.96	7.29	0.38	0.97	0.41	0.7
7.07	8.55	10.31	1.45	5.47	2.29	12.09	0.11	2.25	1.86	4.86
1.88	1.50	17.88	0.81	1.88	1.50	11.47	0.16	0.70	0.78	7.7
3.58	3.21	14.65	0.16	1.39	0.98	6.82	0.50	0.80	0.47	5.5
2.18	0.54	14.84	0.1	0.96	0.21	4.24	0.05	0.43	0.05	3.77
2.68	1.43	11.39	0.05	0.69	0.41	2.84	0	0.48	0.1	2.53
0.63	0.62	0.17	0.1	0.16	0.26	1.29	0.1	0.05	0.1	1.03
0.36	0	1.79	0	0.16	0	0.98	0	0.05	0	0.36
0	0.71	0.24	0.05	0.16	0.21	0.83	0	0	0.05	0.05
0.39	0	0.58	0	0.26	0.31	1.24	0	0.05	0	0.1
0	0	0.31	0.05	0.05	0.46	1.13	0	0	0	0.05
0	0.67	0.31	0.05	0.32	0.31	2.37	0	0	0.05	0.05
0	0	1.27	0.16	0.05	0.10	1.03	0	0	0	0.2
0	0.57	1.42	0.1	0.21	0.21	1.34	0	0	0.05	0.25
0	0	0.93	0	0.10	0.05	2.22	0	0	0	0.36
0	0.31	2.47	0.05	0	0.05	1.60	0.05	0	0.05	0.72
0	0	3.54	0	0	0	0.98	0	0	0	0.72
0	0	0.51	0	0.05	0	0.51	0	0	0	0.1
0	0	0.28	0	0	0	0.1	0	0	0	0.05
0	0.08	0.72	0	0	0.05	0.15	0	0	0.05	0.1
0	0	0	0	0	0	0.26	0	0	0	0.1
0	0	0.36	0.05	0	0	0.15	0	0	0	0.05
0	0	0	0	0	0	0.31	0	0	0	0
0	0	0	0.05	0	0	0.53	0	0	0	0
0	0	4.07	0	0	0	2.84	0	0	0	1.08
1.07	0.41	12.92	0	0.21	0.1	18.19	0	0.1	0.05	9.61
1.98	4.1	40.08	0.1	0.37	0.46	44.61	0	0.16	0.31	30.39
0	3.30	33.06	0.05	0.27	0.41	19.85	0.05	0	0.2	14.99
2.15	0	34.66	0.05	0.21	0.05	12.51	0	0.21	0	10.75
0	0	26.30	0	0	0	5.27	0	0	0	4.70

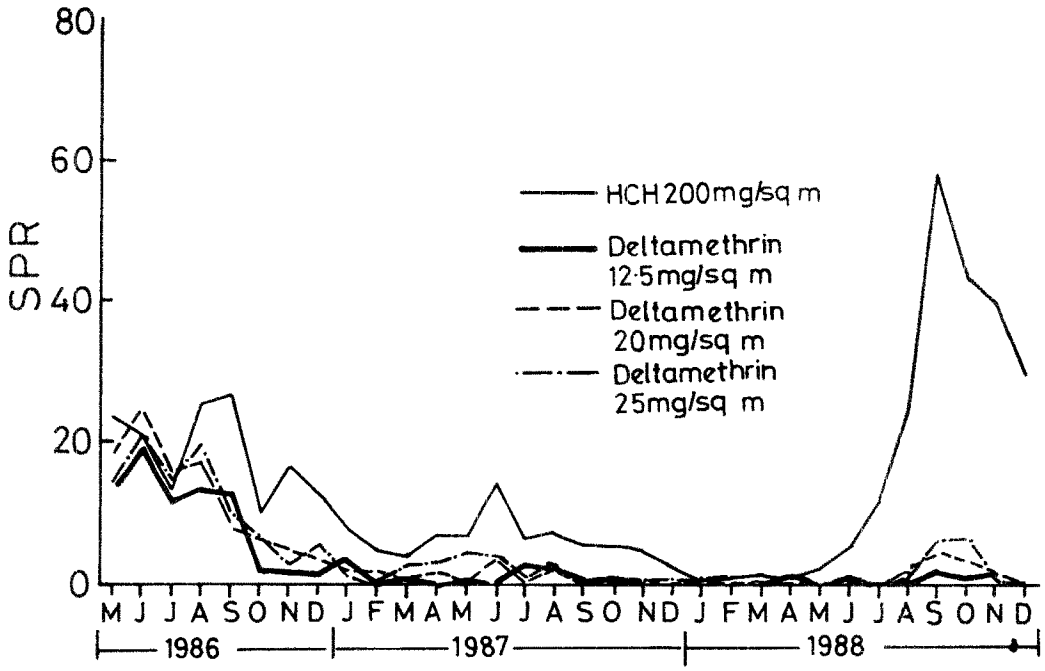


Fig. 4 : Impact of deltamethrin spraying on slide positivity rate.

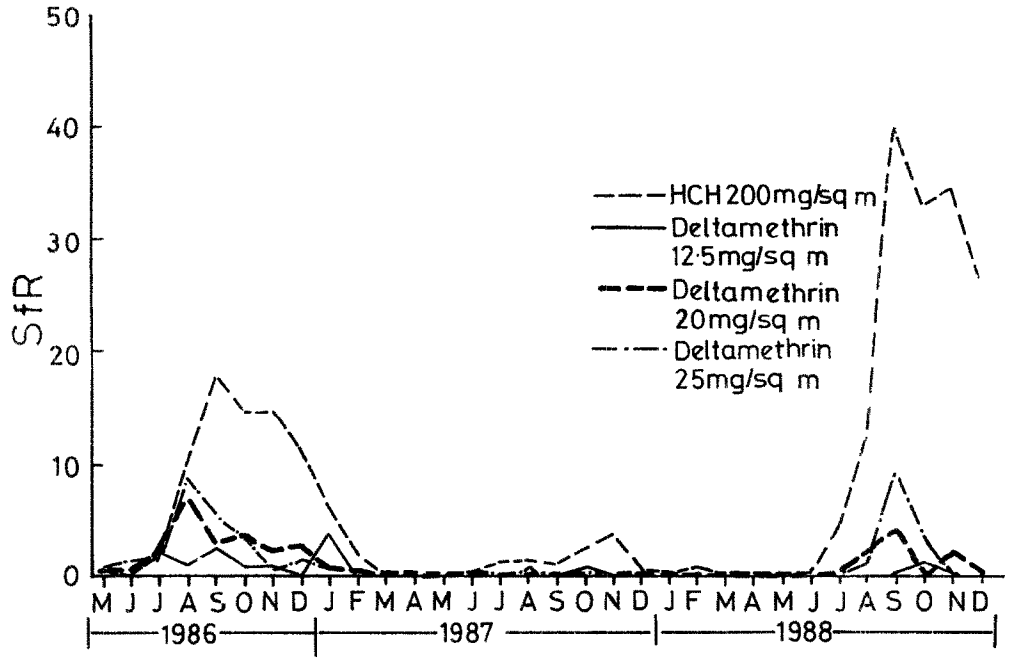


Fig. 5 : Impact of deltamethrin spraying on slide falciparum rate.

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Ultrastructural Study on the Erythrocytic Schizogony of *Plasmodium vivax*

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Erythrocytic schizogony is initiated by repeated division of the nucleus in rapid sequence. Another important feature of the developing schizont is the reappearance of segments of thick inner membrane and formation of lobes around these segments. These bulbous protuberances signal the start of budding process which ends in complete segmentation of the cell. The cytoplasmic organelles which de-differentiate at the beginning of trophozoite stage are formed again. Asynchrony during merozoite formation is observed in some schizonts where fully mature merozoites are seen lying in the parasitophorous vacuole while the mother cell is still in the process of giving rise to new merozoites.

INTRODUCTION

On their release from the liver cells, the merozoites invade erythrocytes and undergo repeated erythrocytic cycles. Each cycle includes a series of stages: the rapidly growing trophic forms (ring and trophozoite stages), the multinucleated proliferative schizonts, and the segmenter consisting of fully differentiated merozoites. The mature schizont bursts, liberating the individual merozoites which in mammalian species can only invade erythrocytes. Upon invading a new erythrocyte the merozoite can either initiate renewed blood schizogony or develop into a female or a male gametocyte.

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Ultrastructural organization of schizogonic stages during erythrocytic phase has been studied in several species of malaria parasites. These include, avian plasmodia, e.g., *Plasmodium cathemerium* (Aikawa, 1966), *P. elongatum* (Aikawa *et al.*, 1967), *P. fallax* (Aikawa, 1966), *P. lophurae* (Aikawa, 1966), reptilian plasmodia e.g., *P. floridense* (Aikawa and Jordan, 1968), and among mammalian plasmodia, *P. berghei* (Ladda, 1969), *P. chabaudi* (Scalzi and Bahr, 1968), *P. vinckei* (Scalzi and Bahr, 1968; Vickerman and Cox, 1967), *P. knowlesi* (Aikawa *et al.*, 1969) and *P. simium* (Seed *et al.*, 1976). Among the less studied groups are the human malaria parasites, e.g., *P. malariae* (Atkinson *et al.*, 1987), *P. ovale* (Matsumoto *et al.*, 1986) and *P. falciparum* (Langreth *et al.*, 1978).

The information on this aspect in the case of *P. vivax* is lacking mainly because of low levels of

parasitaemia (1-2%) encountered in *P. vivax* infected individuals and the long term *in vitro* culture of erythrocytic stages of this species has not been successful. In the present study schizogonic stages of *P. vivax* obtained from naturally infected human volunteers have been observed at the ultrastructural level and the subcellular organization of the successive stages involved in merozoite formation has been described.

MATERIAL AND METHODS

Thin blood smears were made from the patients attending malaria clinic of the National Malaria Eradication Programme (NMEP) situated in north zone of Delhi. Blood films were stained with JSB (Singh *et al.*, 1953) and examined under light microscope for the malaria parasite. *P. vivax* infected individuals having schizonts predominantly in their peripheral circulation were selected for this study. Blood was drawn by venepuncture in heparinized glass vials and processed immediately for ultrastructural studies.

The samples were added to a fixative consisting of 2% (v/v) glutaraldehyde, 0.12 M sucrose, 0.2u M $CaCl_2$, 0.1 M sodium cacodylate-HCL buffer at pH 7.4. After one hour of fixation the cells were washed several times in cold 0.1 M sodium cacodylate buffer containing 0.12 M sucrose. Samples were left overnight in the same buffer. Cells were then post-fixed in 1.5% (w/v) osmium tetroxide, 0.12 M sucrose, 0.1 M sodium cacodylate- HCl buffer pH 7.4 in cold for 1-2 hours. After rinses in buffer and distilled water, the cells were stained in 0.5% (w/v) aqueous uranyl acetate for 1-2 hrs and rinsed in distilled water. The material was dehydrated in ethanol-propylene oxide series and embedded in Epon-Araldite resin mixture (Mollenhauer, 1964). Sections were cut on Porter Blum MT-2 ultramicrotome and mounted on formvar-carbon covered 200 mesh copper grids. Sections were stained with saturated solution of uranyl acetate (BDH) in 50% ethanol and alkaline lead citrate (Reynolds, 1963) and examined at 80 KV in Siemens Elmiskop IA.

RESULTS

Repeated nuclear divisions proceeding in a rapid sequence in the late trophozoite initiate the process of schizogony (Fig. 1). The divided nuclei show distinct nuclear envelope and the chromatin which is in dispersed form during early stages of division becomes compact (Fig. 2). The daughter nuclei formed do not show nucleoli. The cytoplasm of the parasite at this stage shows an increase in endoplasmic reticulum which is mainly of tubular form and is studded with ribosomes (Figs. 1 and 2). The ribosomes are more numerous and occur as polysomes.

Another important feature of the developing schizont is the formation of lobes at various sites along its surface. Around these lobes, segments of thick inner membrane are seen beneath the parasite's limiting membrane (Fig. 3). The cytoplasmic organelles of the merozoite that are de-differentiated at the beginning of trophozoite stage are formed again. In close proximity to each lobe, the precursors of the rhoptries can be seen which finally give rise to oval electron dense rhoptries (Fig. 3). These bulbous protuberances with thick segments of inner membrane and rhoptries mark the anterior ends of the budding merozoites (Fig. 3). During this time there is an ingress of nucleus and other cytoplasmic organelles like ribosomes and endoplasmic reticulum into the developing merozoite from the mother schizont (Fig. 4). The cytoplasmic portion of the mother cell from which the merozoites are budded off is left with malarial pigment, some endoplasmic reticulum and ribosomes (Fig. 5). As the development proceeds the parasite plasma membrane becomes deeply indented marking off the differentiating merozoites and the residual body (Fig. 5). A fine coat of fibrous strands is observed on the surface of differentiating mero-

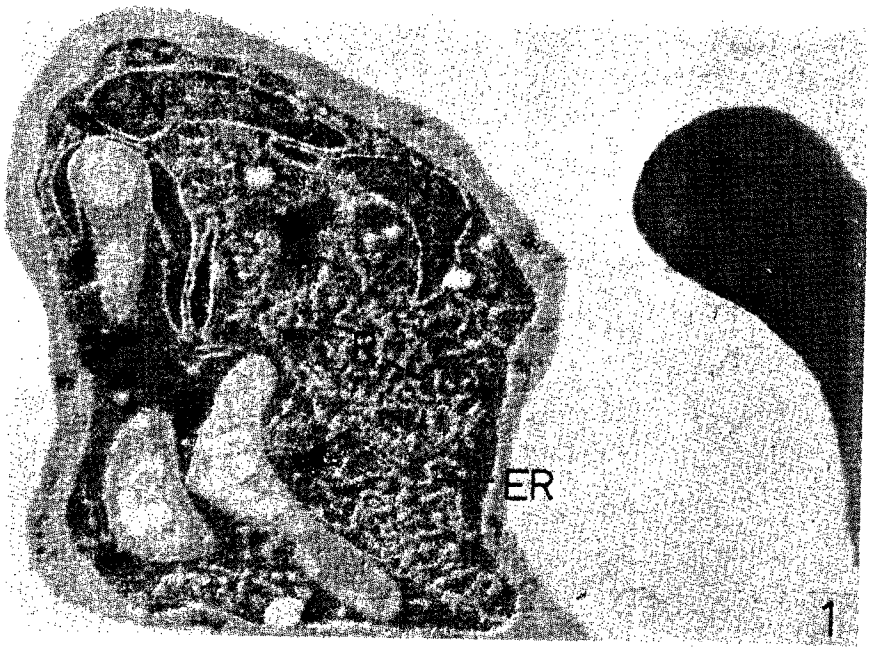

Fig. 1 : Presegmenting schizont showing divided nuclei (N), abundant ribosomes (R) and endoplasmic reticulum (ER) in the cytoplasm. x 19,000.

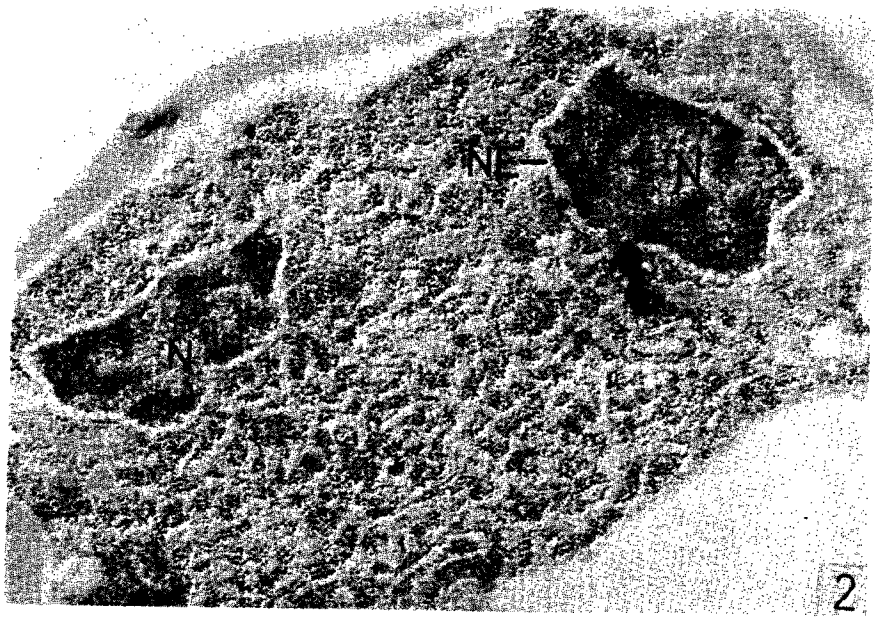

Fig. 2 : Presegmenting schizont with two daughter nuclei (N) showing compact chromatin and distinct nuclear envelope (NE). x 36,000.

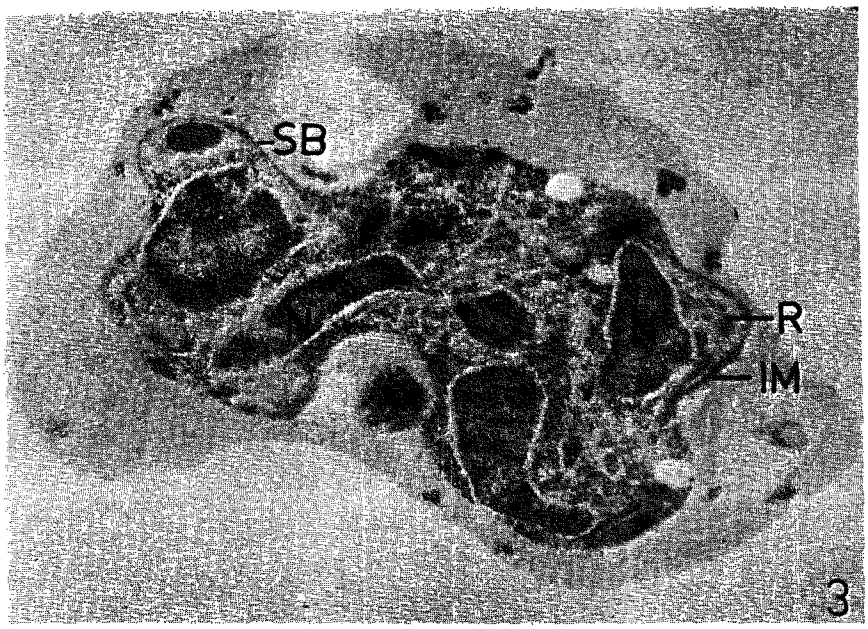

Fig. 3 : A schizont at an early stage of cytoplasmic segmentation. Note the localized surface buds (SB) with thick inner membrane (IM), rhoptry precursors (R) and a number of nuclei (N). x 20,500.

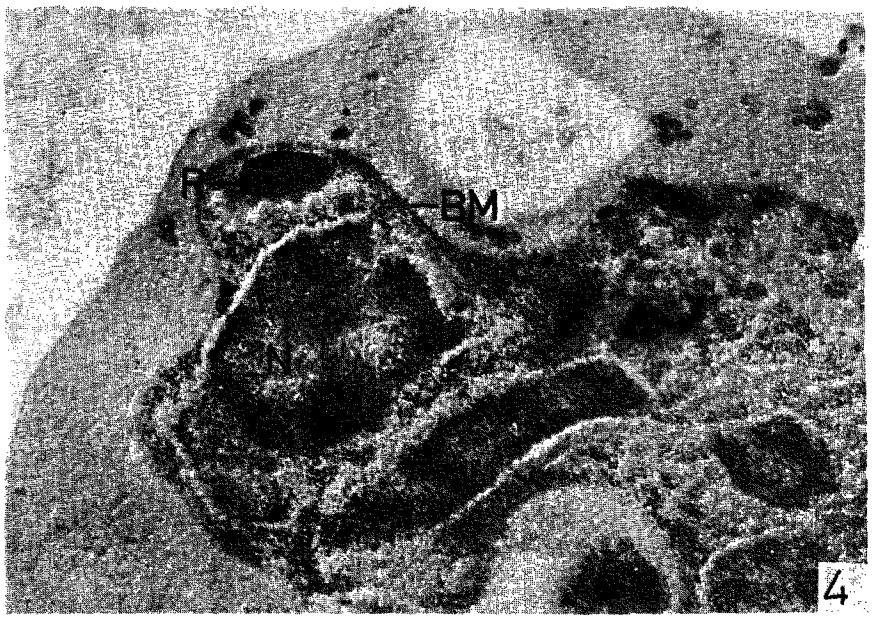

Fig. 4 : Enlarged view of the surface bud of segmenting parasite. Newly formed rhoptry (R) and ingress of nucleus (N) into the budding merozoite (BM) can be seen. x 33,000.

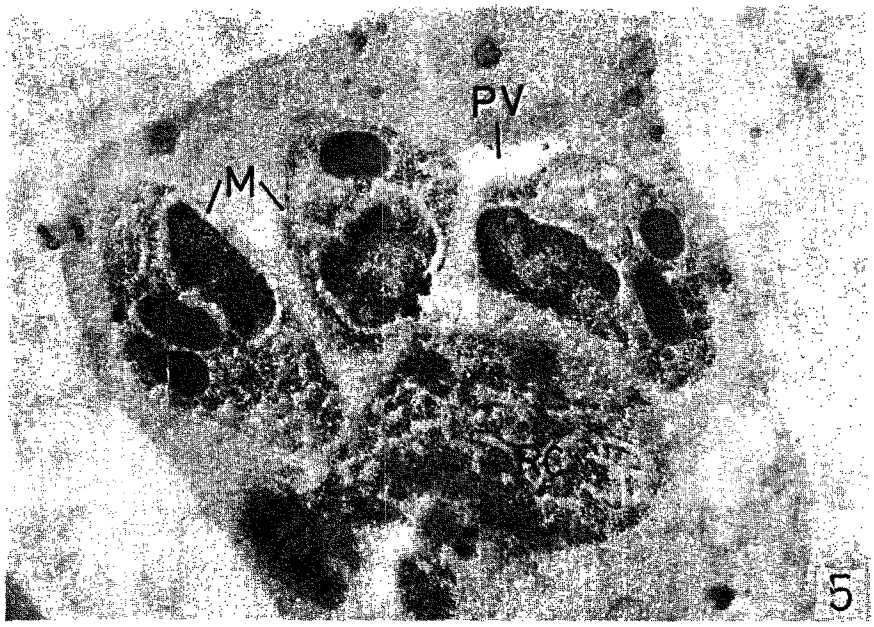

Fig. 5: A segmenter in the terminal stage of cell division showing newly formed merozoites (M), enlarged parasitophorous vacuole (PV) and residual cytoplasm (RC). x 34,000.

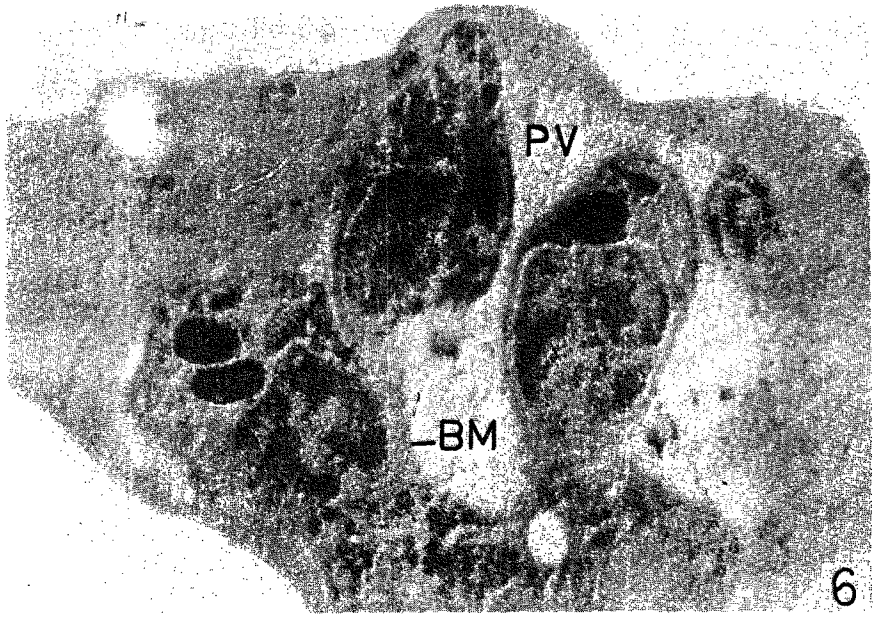

Fig. 6: Schizont of *P. vivax* showing budding merozoites (BM) in different phases of development. The enlarged parasitophorous vacuole (PV) is filled with coarse granular matrix. x 36,000.

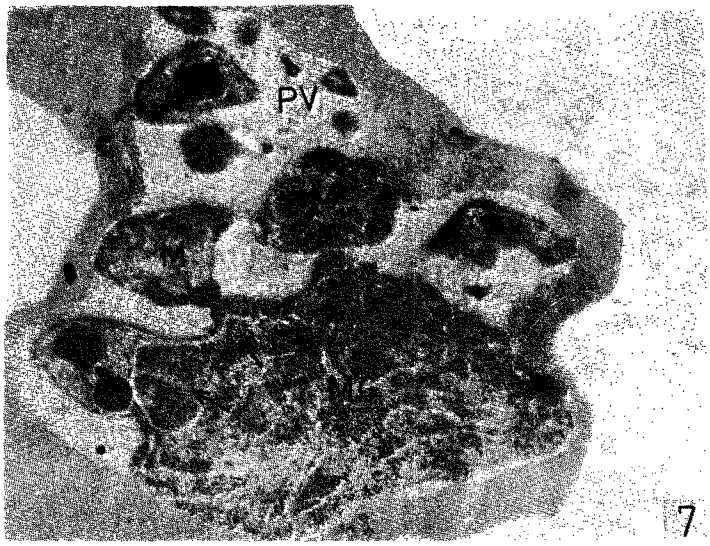

Fig. 7: Schizont of *P. vivax* showing asynchrony in cell division. Whereas many of the completely formed merozoites (M) are lying in the parasitophorous vacuole (PV), the mother cell (MC) is still in the process of forming new merozoite buds (arrows). x 24,000.

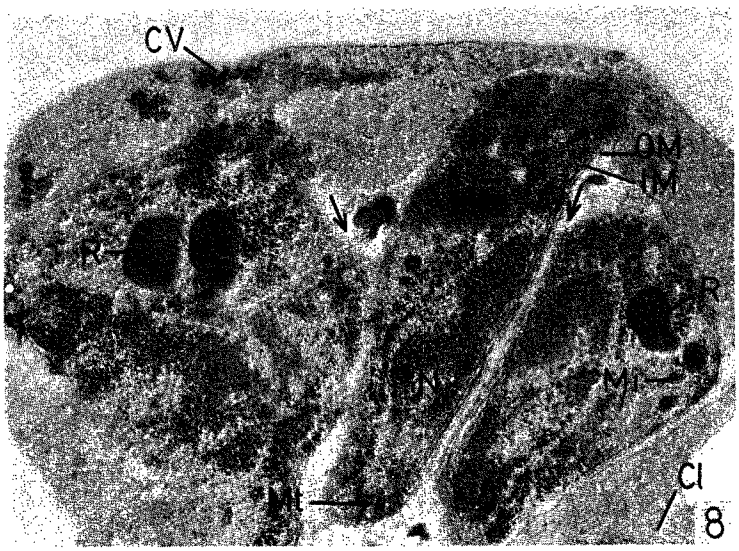

Fig. 8: Fully differentiated merozoites, prior to host cell lysis, showing well-developed pellicular membranes (OM, IM), microtubules (Mt), rhoptries (R), micronemes (Mi) and double membrane bound nucleus (N). Periodic surface strands appear to bridge the adjacent merozoites (arrows). Note the caveola-vesicle complexes (CV) and cytoplasmic clefts (Cl) in the host cell. x 37,000.

zoites and it appears to form bridges between the adjacent daughter cells (Figs. 5 and 8).

During schizogony the parasitophorous vacuole membrane enlarges considerably and appears to be discontinuous in the regions where the differentiating merozoites are in contact with it (Figs. 5-7). The parasitophorous vacuole becomes more and more apparent as the merozoite budding proceeds and appears to be filled with finely granular material (Figs. 5-7). Apart from this, the caveola-vesicle complexes and cytoplasmic clefts are invariably observed in the host cells harbouring schizogonic stages.

A wide variation in the rate of development of merozoites in a single schizont is observed. Merozoite buds at different developmental stages are seen in some schizonts (Fig. 6). At times fully mature merozoites are seen lying in the parasitophorous vacuole adjoining the mother cell which is still in the process of giving rise to another brood of merozoites (Fig. 7).

Fully differentiated merozoites of *P. vivax* like that of other species of malaria parasites, have well-defined anterior and posterior regions, specialized cytoplasmic organelles and complex pellicular structure. The pellicle of the merozoite is composed of a thin outer membrane and a thick inner membrane which shows discontinuity in some regions (Fig. 8). Microtubules beneath these pellicular membranes are not very apparent and are observed occasionally (Figs. 5 and 8).

The anterior region of the merozoite is recognized by its truncated appearance. Electron dense rhoptries and micronemes are located near this region (Fig. 8). A large oval nucleus is situated in the centre of the merozoite and at times it is seen extended up to the posterior region (Figs. 5 and 8). It is bounded by double membraned nuclear envelope and shows peripheral clumping of the chromatin material (Figs. 5 and 8). In addition to this, small dense particles and fine fibrils are seen scattered in the nucleoplasm. No nucleolus is observed in the nucleus of erythrocytic merozoite. The cytoplasm of the merozoites contains abundant ribosomes (both free and polysome forms) although little endoplamic reticulum is observed.

DISCUSSION

Merozoite formation during erythrocytic schizogony in *Plasmodium vivax* is similar to that observed in other plasmodial species. Prior to cytoplasmic segmentation the nucleus divides mitotically in a repeated sequence. The mitotic activity of the nucleus which begins in the trophozoite stage accelerates rapidly and the nucleus becomes large and multilobed finally giving rise to large number of nuclei. Since 8-24 merozoites are produced from each schizont then during S-phase there must be as many cycles of DNA synthesis. Though the dividing nuclei are not observed in any of the schizonts in this study, it is likely that their structural organization and mechanism of division would be the same as that described in case of *P. simium*, a vivax-type malaria parasite, by Seed *et al.* (1976). They found spindle fibres radiating from centriolar plaques located near the nuclear membrane of the dividing nucleus. Electron dense kinetochores which serve as attachment points to spindle fibres of individual chromosomes were seen occasionally. Similar observations have been made on the structural organization of the dividing nuclei during schizogony in case of avian malaria parasites (Aikawa, 1966) and human malaria parasites (Matsumoto *et al.*, 1986; Atkinson *et al.*, 1987).

The onset of merozoite budding is initiated by reappearance of segments of thick inner membrane. Soon after that, rhoptry formation takes place beneath these segments. It is observed that only those regions of the parasite bearing thick inner membrane and rhoptry precursors are extruded and form merozoite buds. This indicates that the thick inner membrane is

essential for the start of merozoite budding. It is also likely that the conoids of these buds govern the ingress of nucleus and other cytoplasmic organelles from the mother schizonts into the newly forming merozoites as suggested by Aikawa (1966) in case of avian malaria parasites.

Asynchrony during merozoite budding is observed in some schizonts of *P. vivax* as earlier described by Seed *et al.* (1976) in case of *P. simium*. Fully differentiated merozoites are seen lying in the parasitophorous vacuole while the mother cell is still in the process of giving rise to new merozoite buds. At the same time, single schizonts exhibiting budding merozoites in different developmental phases are also observed.

These observations suggest that as in simian species, the nuclear division in *P. vivax* also occurs in phases, not only before but also concurrently with cytoplasmic segmentation. Merozoites of *P. vivax*, like those of other species of *Plasmodium*, have well-defined pellicular membranes and specialized organelles which impart polarity to them. Aikawa (1966) suggested that inner pellicular membrane and microtubular structures function as a cytoskeleton providing rigidity to the merozoites. The truncated anterior end, bearing rhoptries and micronemes, of the merozoite is a characteristic feature of all the invasive stages of malaria parasites including ookinete and sporozoite. There are a number of reports suggesting that rhoptry-microneme complex plays a role during invasion of merozoites into the erythrocytes (Aikawa, 1966; Aikawa and Jordan, 1968; Aikawa *et al.*, 1978; Stewart *et al.*, 1986). The disappearance of these structures once the merozoite has invaded the host cell and their formation again during merogony suggests that these specialized organelles function during invasion of merozoites into the host cells. Histochemical studies in case of *P. falciparum* indicate that these structures contain proteins, (Kilejian, 1976; Kilejian and Jensen, 1977) and phospholipids (Stewart *et al.*, 1986). Surface active substances released from the rhoptries interact with host erythrocyte membrane causing invagination and parasitophorous vacuole formation (Ladda *et al.*, 1969; Bannister *et al.*, 1976; 1977; Holz, 1977).

Not much is known about the mechanism of release of merozoites from the host cells. In case of *P. simium*, Seed *et al.* (1976) observed local surface lesions in the regions where apical portions of the newly forming merozoites abutted against the plasma membrane of the host cell and suggested that these local membrane changes are mediated by release of chemical substances from the apical organelles. In case of *P. vivax* also, interruption in the parasitophorous vacuole membrane in the regions of contact between apical portion of the merozoite and host cell have been observed. Recently Stewart *et al.* (1986) observed membranous whorls (secretion of the rhoptries) associated with free merozoites from rupturing schizonts of *P. falciparum*. Thus it appears that cell penetration by merozoites and their release from the host cell involve similar mechanism. Differentiating merozoite buds have been shown to possess a cell coat of fine bristles in case of *P. knowlesi* (Bannister *et al.*, 1977; 1986), *P. simium* (Seed *et al.*, 1976) and *P. malariae* (Atkinson *et al.*, 1987). It has been suggested that these fibre strands on the surface of merozoites form bridges between the daughter cells and also connect merozoites with the vacuolar membrane thus providing a structural unity between the delicate buds, the mother cell and the enveloping parasitophorous vacuole (Seed *et al.*, 1976). In *P. vivax* it appears that this type of bridge is formed between adjacent merozoites and the vacuolar membrane but are not very distinct due to presence of fine granular material in parasitophorous vacuole.

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Morphological Variations in Natural Populations of *Anopheles stephensi* Liston 1901 collected from Kutch (Gujarat)

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A survey in February-March 1984 yielded a total of 9222 specimens of anopheline mosquitoes collected from 9 talukas of Kutch out of which 6729 were A. *stephensi*. This was thus the dominant species observed during this period. A total of 216 specimens of *A. stephensi* showed variations in palpi and wings.

INTRODUCTION

Anopheles (Cellia) stephensi is distributed in India, China, Thailand, Burma, Pakistan, Afganistan, Iraq, Iran, Bahrain, Trucial Oman, Oman, Saudi Arabia and Egypt (Knight and Stone, 1977). In India it is an important malaria vector in urban and some rural areas, and is distributed throughout the country except the high altitudes of the Himalayas and Andaman and Nicobar Islands. Sporozoite and oocyst positive specimens were recorded from Bombay city (Maharashtra), Hiriyur areas, Bellary, Bijapur (Karnataka), Calcutta city, Durgapur Steel plant (West Bengal), Hyderabad city, Visakhapatnam (Andhra Pradesh), Ahmedabad city, Bharuch town, Kutch (Gujarat), Madras city (Tamil Nadu) and Delhi (Rao, 1984).

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Morphological variations in the Indian anophelines are mostly restricted to ornamentation of palpal banding, legs and wings. These variations were recorded in *A. vagus* (Nagpal and Sharma, 1983a), *A. sundaicus* (Nagpal and Sharma, 1983b), *A. fluviatilis* (Ramakrishna, 1954; Rehman *et al.*, 1960), *A. subpictus* and *A. pallidus* (Subramanian and Nagendra, 1955), *A. annularis* (Rajagopal and Chakraborty, 1960; Sen, 1962), *A. philippinensis* (Azeez and Beig, 1959). Wattal *et al.* in 1960 recorded the morphological variations in 20 anophelines from the reference collections of the National Institute of Communicable Diseases (NICD) Delhi. There is no record of morphological variations in *A. stephensi*, except Bhatnagar *et al.* (1958) who reported incomplete development of 6th wing vein in colonized specimens. Recent work of Subbarao *et al.* (1987) has shown the occurrence of three forms of *A. stephensi* in nature viz., *Anopheles* (*stephensi*) *stephensi*, *Anopheles* (*stephensi*) *mysorensis* and intermediate form. These forms differ from each other in the number of ridges of the egg float which is a

maternally inherited character. During the present study morphological variations were observed in *A. stephensi* in the field collected specimens. Results of these observations are described in this paper.

MATERIAL AND METHODS

An extensive mosquito fauna survey was carried out in 41 villages of 9 talukas of Kutch (Gujarat) during February and March 1984. Adult collections were made both indoors and outdoors using suction tubes and torch lights. Total catch collections were made by space spray method from cattlesheds and human dwellings using "Finit" a product of Hindustan Petroleum which is a mixture of pyrethrum (0.05%) and malathion (100%) in kerosene base.

The villages were also searched for larval breeding sites. Larvae were collected from ponds, pools, ditches, canal and from intradomestic pots and reared in the field laboratory until eclosion. Different types of collections were made in an effort to obtain maximum number of species and specimens of a representative cross-section of the mosquito population, so that specimens are not missed. All the field collected adults and the adults that emerged from larvae were packed in cellophane paper and brought to the MRC HQs laboratory in Delhi for identification and preservation. Identifications were made using the keys of Christophers (1933) and Barraud (1934). During the identification 34 types of morphological variations from the type form were found in 216 *A. stephensi* specimens. These variations were observed in maxillary palpi and wings. India ink diagrams were drawn of all the variations. The specimens have been preserved at the Centre as reference material.

RESULTS AND DISCUSSION

During the survey, a total of 9222 specimens of anopheline mosquitoes were collected from 9 talukas of Kutch. Out of these 6729 were *A. stephensi* (Singh *et al.*, 1985). Of these 216 (3.20%) specimens were found with morphological variations in their palpi and wings. Variations were observed oftener in palpi (25 variations) than in wings (9 variations). These variations were observed in the banding patterns, speckling and size. Details of collection sites, morphological variations and the number of specimens collected from each village are given in Table 1 and Figs. 1-36.

Table 1. Morphological variations in *Anopheles stephensi*

S.No.	Date of collection	Site (village) of collection	No. of specimens collected from each village	Description of variations from type form
1	2	3	4	5
			*Variations in Palpi**	
1.	24.2.84	Karagora	3	Apical pale band almost half of the normal size and equal to intervening dark band, **Fig. 2.**
	24.2.84	Baraya	5	
	25.2.84	Anjar	2	
	28.2.84	Deshalpur	5	
2.	25.2.84	Anjar	1	Apical pale band with a row of black scales, Fig. 3.

Contd...

Contd...

1	2	3	4	5
3.	25.2.84	Anjar	1	An extra dark band on apical pale band of both the palpi,
	6.3.84	Madanpur	1	**Fig. 4.**
4.	23.2.84	Karagora	2	Intervening dark band absent in both the palpi, **Fig. 5.**
	24.2.84	Karagora	2	
	24.2.84	Mundra	9	
	25.2.84	Anjar	1	
	28.2.84	Deshalpur	24	
	6.3.84	Rajda	5	
5.	25.2.84	Anjar	2	Intervening dark band absent in one palpus, **Fig. 6.**
	28.2.84	Deshalpur	3	
	5.3.84	Trambo	2	
	6.3.84	Rajda	2	
	9.3.84	Lilpur	1	
6.	23.2.84	Khavada	1	Intervening dark band short and apical pale band 3.9 x
	25.2.84	Anjar	2	compared to intervening dark band, **Fig. 7.**
7.	20.2.84	Parapur	1	Intervening dark band increases; apical pale band 1.3 x
	22.2.84	Bari Amia	1	compared to intervening dark band, **Fig. 8.**
	24.2.84	Karagora	4	
	24.2.84	Mundra	5	
	25.2.84	Anjar	3	
	2.3.84	Dayapur	1	
	6.3.84	Rajda	2	
8.	24.2.84	Karagora	3	Intervening dark band increases and nearly equal to the
	24.2.84	Baraya	3	apical pale band **Fig. 9.**
	25.2.84	Anjar	2	
	28.2.84	Deshalpur	5	

* Type form: Length of apical pale band 0.27mm, subapical pale band 0.225 mm and intervening dark band 0.19 mm, Fig. 1.

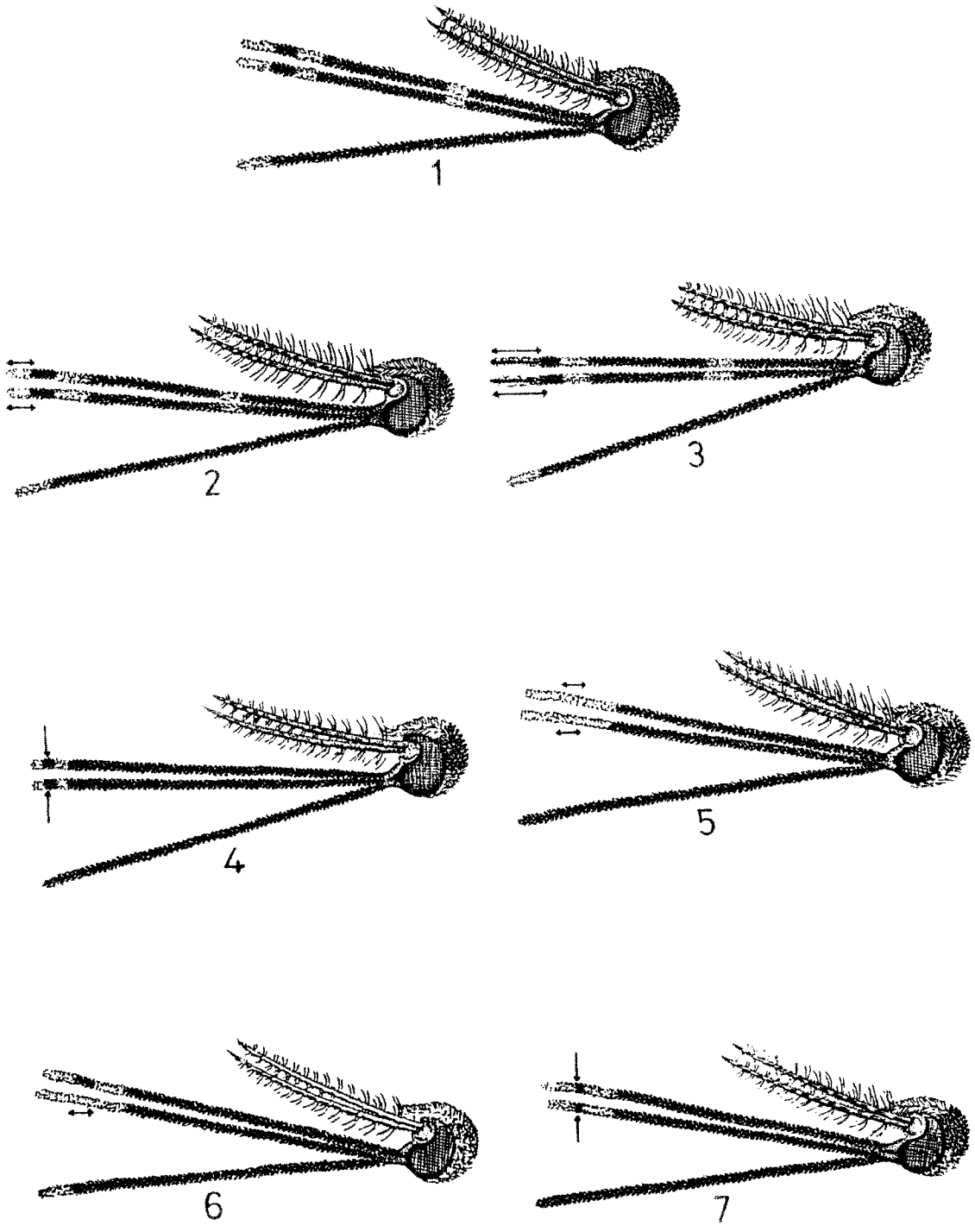
9.	28.2.84	Deshalpur	1	Intervening dark band increases apical pale band 0.9 x compared to intervening dark band, **Fig. 10.**
10.	24.2.84	Mundra	1	Intervening dark band with few pale scales, **Fig. 11.**
11.	24.2.84	Karagora	2	Middle dark band with a row of white scales, **Fig. 12.**
	6.3.84	Madanpur	4	
	6.3.84	Rajda	1	
12.	24.3.84	Baraya	9	Five banded palpi; two extra pale bands present on the middle dark band, **Fig. 13.**
13.	25.2.84	Anjar	1	Seven banded palpi; four extra pale bands on middle dark
	6.3.84	Rajda	4	band, **Fig. 14.**
14.	24.2.84	Karagora	1	Tip of both the palpi dark, **Fig. 15.**
	25.2.84	Anjar	2	
	6.3.84	Rajda	1	

Contd...

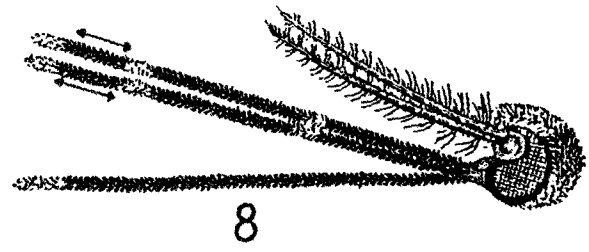
Contd...

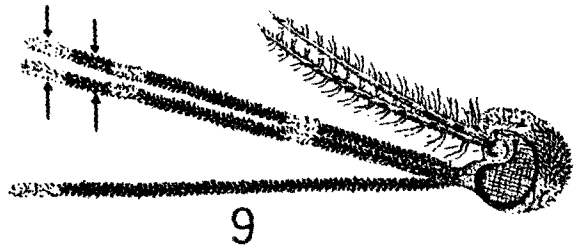
1	2	3	4	5
15.	23.2.84	Khavada	1	Only basal dark bands with speckling, **Fig. 16.**
	24.2.84	Karagora	3	
	25.2.84	Anjar	1	
	28.2.84	Deshalpur		
16.	6.3.84	Rajda	1	Basal dark band with speckling in one of the palpi, **Fig. 17.**
17.	6.3.84	Rajda	1	Speckling absent in both the palpi, **Fig. 18.**
18.	25.2.84	Anjar	1	Subapical and basal pale bands absent, **Fig. 19.**
19.	6.3.84	Rajda	1	Intervening and middle dark bands absent, **Fig. 20.**
20.	24.2.84	Mundra	1	One of the palpi 1.15 x as long as the other, **Fig. 21.**
	25.2.84	Anjar	3	
	28.2.84	Deshalpur	1	
21.	28.2.84	Deshalpur	1	One of the palpi 1.14 x as long as the other, intervening dark band absent in one of the palpi and the tip of one of the palpi black, **Fig. 22.**
22.	25.2.84	Anjar	3	Palpi short 0.68 x as long as the proboscis, **Fig. 23.**
	6.3.84	Rajda	1	
23.	2.3.84	Dayapur	1	Palpi short 0.75 x as long as the proboscis and an extra dark band present on the apical pale band, **Fig. 24.**
24.	28.2.84	Deshalpur	1	Palpi size increases; palpi 1.16 x as long as the proboscis, **Fig. 25.**
25.	8.3.84	Vondh	1	One of the palpi 1.16 x as long as the other and apical, subapical and basal pale bands absent, **Fig. 26.**
			*Variations in Wings**	
26.	24.2.84	Mundra	2	Fringe spot on vein VI absent, **Fig. 28.**
	28.2.84	Deshalpur	2	
	6.3.84	Madanpur	1	
	6.3.84	Rajda	2	
27.	23.2.84	Khavada	1	Vein V pale at bifurcation, **Fig. 29.**
	23.2.84	Karagora	34	
	28.2.84	Deshalpur	4	
	6.3.84	Rajda	9	
28.	25.2.84	Anjar	1	Wing vein IV dark at bifurcation, **Fig. 30.**
29.	6.3.84	Rajda	1	Wing vein V completely pale, **Fig. 31.**
30.	6.3.84	Rajda	1	Wing vein V completely black and fringe spot absent on vein VI, **Fig. 32.**
31.	26.2.84	Deshalpur	1	Wing vein III with two dark spots, **Fig. 33.**
32.	26.2.84	Karagora	1	Wing vein I with an extra dark area, **Fig. 34.**
33.	24.2.84	Mundra	1	Inner costa completely pale, **Fig. 35.**
	6.3.84	Rajda	1	
34.	6.3.84	Rajda	1	Fringe spot on vein 5.1 and 6 absent, **Fig. 36.**

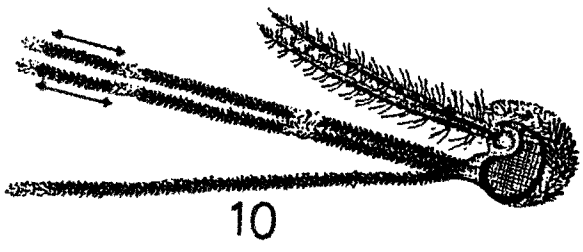
* Type form—Fig. 27.

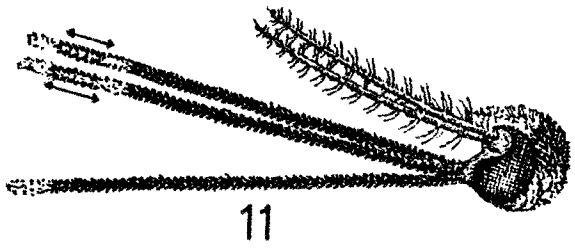


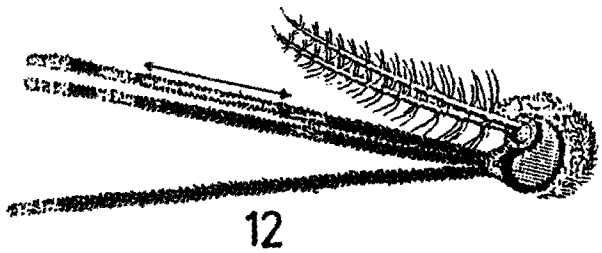
Figs. 1-7: Morphological variations in the palpi of *A. stephensi*.

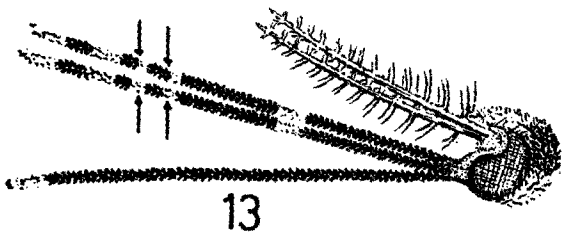






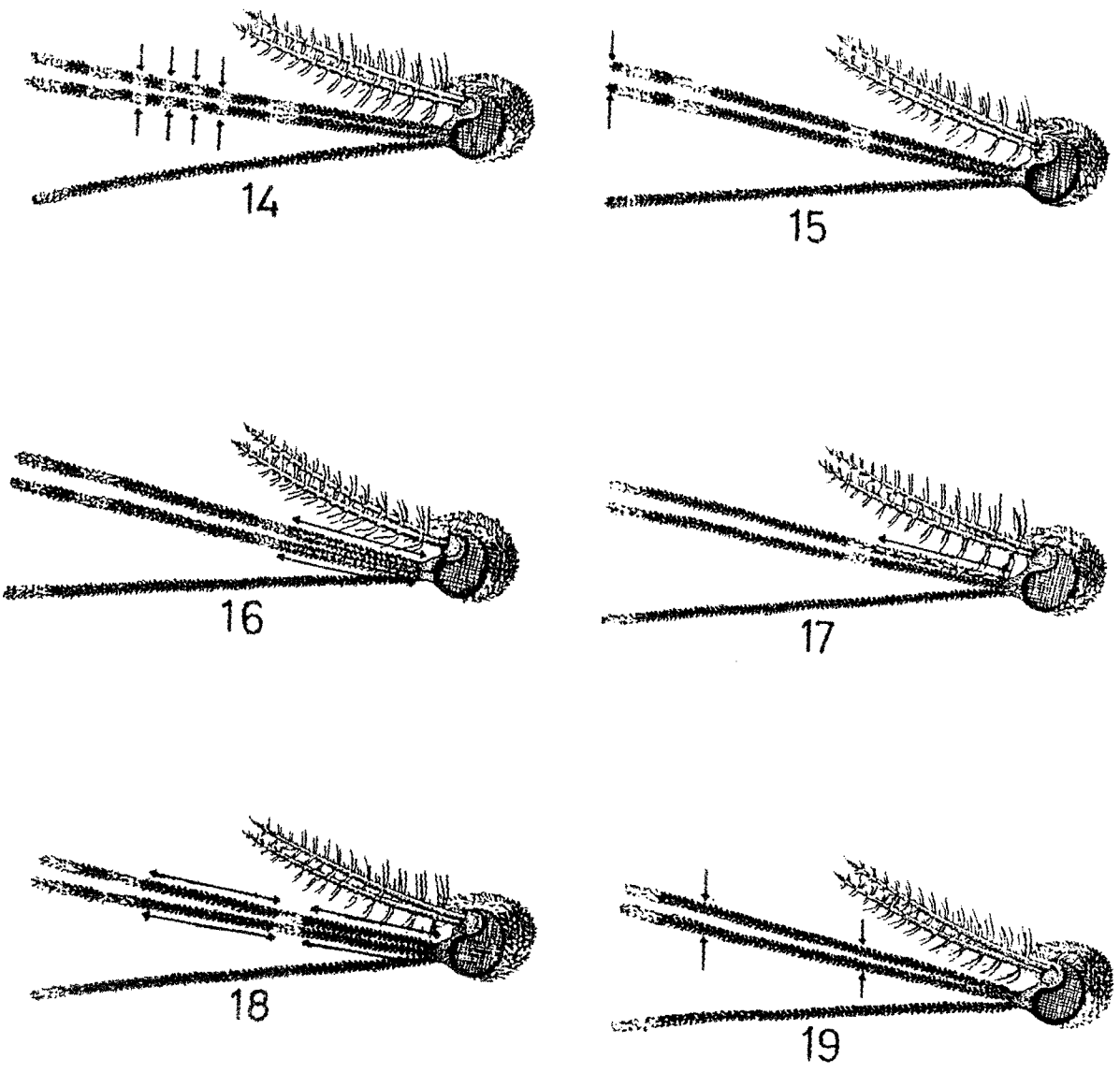






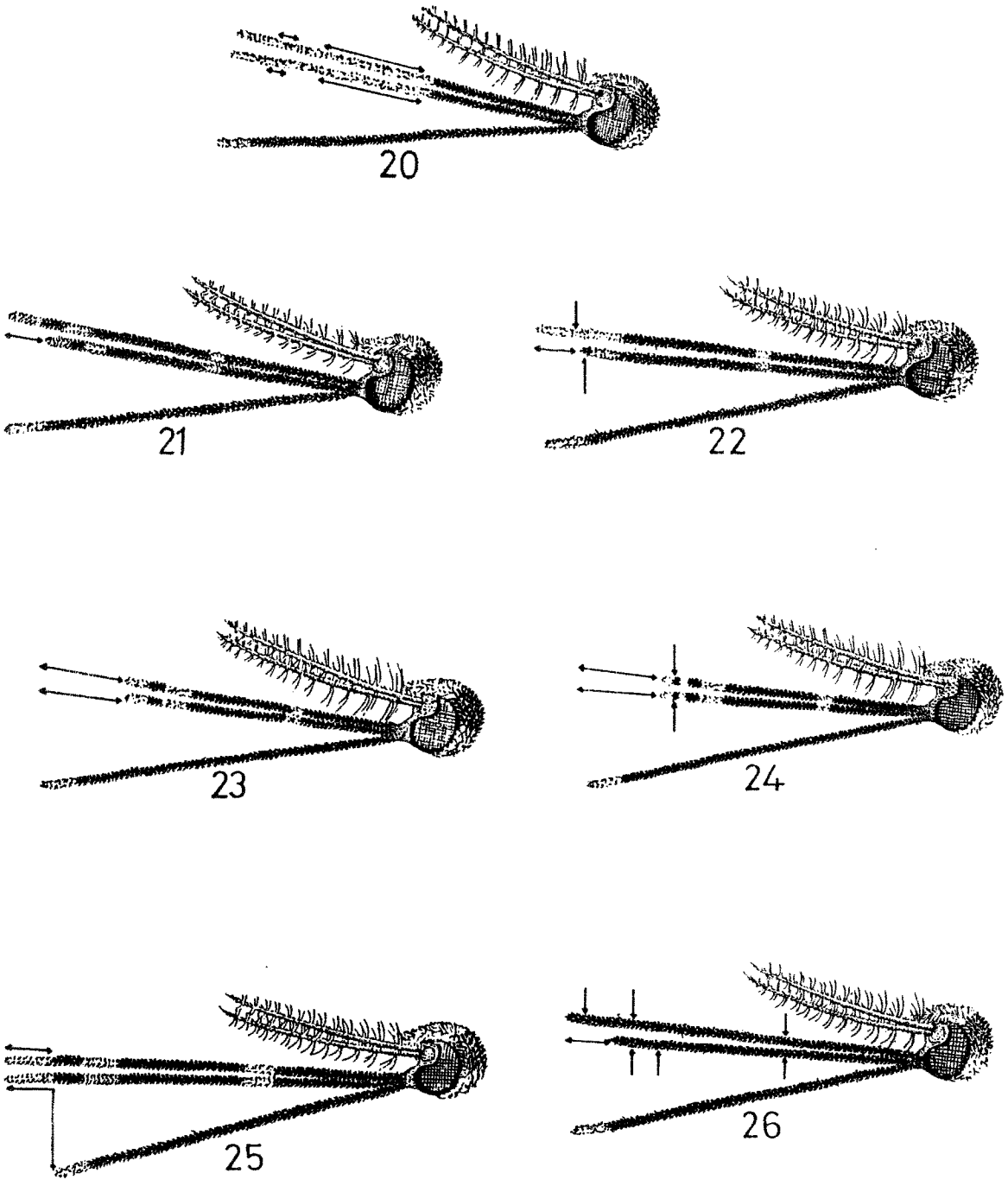
Figs. 8-13: Morphological variations in the palpi of *A. stephensi.*

It was not possible to distinguish between *A. stephensi (stephensi), A. (stephensi) mysorensis* and the intermediate form in the field collected specimens based on morphological characters. Aslamkhan and Hafeez (1972) from Pakistan recorded 50 morphoplogical variations in larvae, pupae and adults in reared specimens of *A. stephensi*. This was the first report of morpho-



Figs. 14-19: Morphological variations in the palpi of *A. stephensi*.

logical variations in A. *stephensi* from field collected specimens. However from the morphological variations recorded in the present study, five variations in palpi and two in wings resembled those reported in laboratory reared specimens by Aslam Khan and Hafeez (*loc. cit.*). No specific trend of variations could be found as a result of topography or any other factor related



Figs. 20-26: Morphological variations in the palpi of *A. stephensi*.

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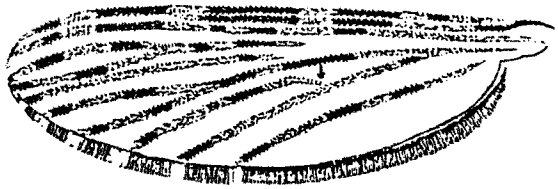

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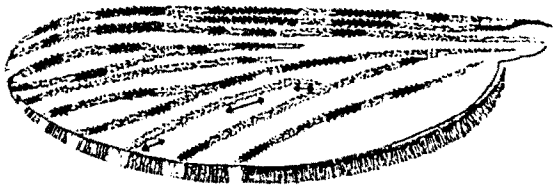

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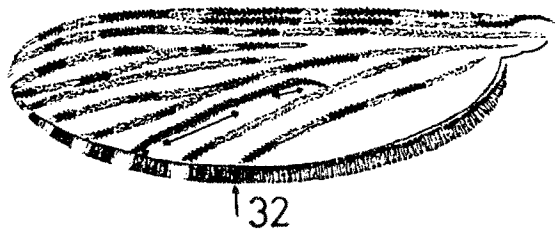
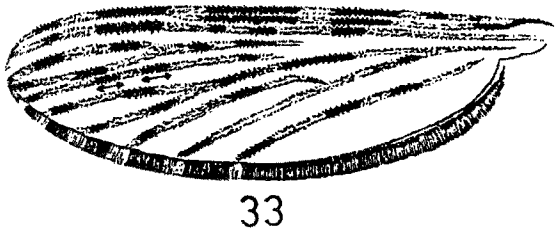
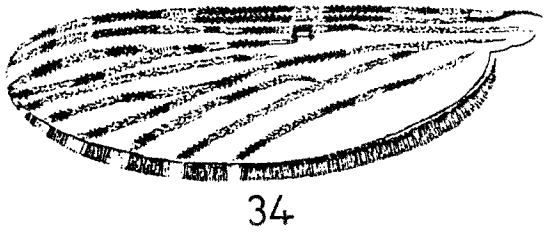
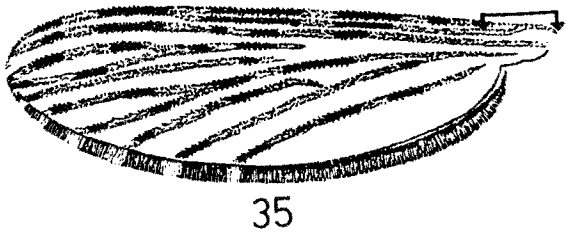
Figs. 27-31: Morphological variations in the wings of *A. stephensi*.

to Kutch district. It may be noted that Kutch district can be divided into three well-defined regions. Bhuj taluka, the headquarters of Kutch represents the central mountainous region, Mandavi and Mundra the southern coastal alluvial tract, whilst Lakhpat taluka has barren low-lying terrain, and *A. stephensi* is found in all the regions of the district. Since palpi, wings and









Figs. 32-36: Morphological variations in the wings of *A. stephensi*.

legs are important for identification, these variations may cause confusion in the correct identification of *A. stephensi*. It would therefore be advisable that correct identification should be based on more than one character on the appendages. The variations may be due to aberrations during embryonic and post-embryonic development or at times mutations. It is also possible that some specimens may be representing a speciation process. Further studies are indicated, particularly because of the importance of *A. stephensi* in malaria transmission in Kutch (Afridi *et al.*, 1938) and other urban areas of the country (Sharma and Mehrotra, 1986).

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Micro *In vitro* Assessment of *Plasmodium falciparum* Sensitivity to Chloroquine and Mefloquine in Gujarat

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Micro *in vitro* tests conducted in 1987 in Surat district of Gujarat on sensitivity status of *P. falciparum* to chloroquine and mefloquine revealed that the parasite has developed resistance to chloroquine upto 32 pmol. The ED 99 in Hazira, Gothan and Umra areas of the district was found to be 17.3, 18.5 and 8.7 pmol/well for chloroquine and for mefloquine it was 14.5, 4.8 and 6.8 pmol/well respectively. Monitoring of *P. falciparum* resistance is indicated under National Malaria Eradication Programme.

INTRODUCTION

Resistance of *Plasmodium falciparum* to schizontocidal drugs particularly chloroquine is a serious problem for malaria control programme in India. Delimitation of drug resistant *P. falciparum* foci for their subsequent elimination was undertaken by six *Pf* monitoring teams in 1978. Since 1986, 12 *Pf* monitoring teams are working in the country. Drug resistant *P. falciparum* malaria in India has been reported by several authors from 55 different places in the country upto 1983 and has been reviewed by Sharma (1984). Later Raichowdhuri *et al.* (1984) from Assam, Barkakaty *et al.* (1984) from Assam and Meghalaya, Gajanana *et al.* (1986) and Choudhury *et al.* (1987) from Delhi, and Sinha *et al.* (1987) and Das *et al.* (1988) from Calcutta reported drug resistant *P. falciparum*. Sharma and Sharma (1988) reported drug resistant *P. falciparum* from Kheda district, Gujarat and stressed the need for careful monitoring and proper treatment. In search of a suitable alternative of chloroquine, mefloquine, a new antimalarial is being tested for its efficacy against *P. falciparum* throughout the country since 1982 following micro *in vitro* technique (WHO, 1982). *In vitro* test minimizes the variations in apparent drug response due to immunity and obviates the operational difficulties of following up test subjects (Bruce-Chwatt, 1986). Using micro *in vitro* technique Dutt *et al.* (1984) reported mefloquine resistant *P. falciparum* from Gadchiroli district of Maharashtra. To study sensitivity of *P. falciparum* to chloroquine and mefloquine micro *in vitro* tests were conducted in Surat district covering Utran and Earthan

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PHCs. Results of the studies are reported in this paper.

MATERIAL AND METHODS

Initially a mass blood survey was conducted in June in Hazira complex and in August 1987 in Gothan and Umra villages for the selection of suitable cases. Persons found having asexual parasitaemia were subjected to urine test by Dill and Glazko method (Lelijveld and Kortman, 1970). Patients showing negative chloroquine excretion in urine were selected for the study. Tests were conducted following WHO (1982) methodology using micro test kit having tissue culture plate of mefloquine upto 16 picomol and of chloroquine upto 32 picomol.

RESULTS AND DISCUSSION

Table 1 summarises the results of mass blood survey for selection of suitable cases for the study. In Gothan and Umra villages of Earthan PHC of Surat district very high SPR values of 80.0 and 86.4%, respectively were observed. Most of it was due to *P. falciparum* as is evident from SfR values of 70.5 and 79.5%, respectively. Against this, in Hazira complex SPR (15.2%) and SfR (9.7%) were indicative of low malaria incidence in the area which could be due to the fact that mass survey was conducted in the month of June. June is normally the month of low malaria incidence, specially of *P. falciparum*. Epidemiological investigation was carried out of all study cases and all were found to be indigenous.

Table 2 shows the results of micro *in vitro* tests of *P. falciparum* to chloroquine and mefloquine. Out of 34 cases tested for sensitivity of *P. falciparum* to chloroquine and mefloquine first 15 cases were from Hazira complex of Utran PHC, 9 from Gothan and 10 from Umra village of Earthan PHC. No schizont maturation took place in 5 cases i.e., 2 cases at serial numbers 20 and 30 with chloroquine and 3 cases at serial numbers 11,19 and 27 with mefloquine.

Average schizont count in control (Mean K) in Hazira complex, Gothan and Umra areas was 33.8, 76.55 and 31.05, respectively. Following the WHO protocol (1982) wherein cut-off points for chloroquine and mefloquine were given as 5.7 and 4.0 pmol, respectively the percentage of schizont count in above mentioned three areas at cut-off point of chloroquine (5.7 pmol) was observed to be 7.2, 17.70 and 5.15, respectively. Drug resistance of parasite at cut off point of

Table 1. Results of mass blood survey (Surat district)

Area	Population covered	Blood smears collected	Malaria parasites							
			Pv	Pfr	Pfrg	Pfg	Mix	Total	SPR	SFR
1. Hazira Complex (Utran PHC)	5500	2184	120	121	56	32	2	331	15.2	9.7
2. Gothan (Earthan PHC)	2650	180	17	102	20	5	0	144	80.0	70.5
3. Umra (Earthan PHC)	2094	44	3	28	4	1	2	38	86.4	79.5
Total	10244	2408	140	251	80	38	4	513	21.3	15.5

Pv = *Plasmodium vivax*; Pfr = *P. falciparum* rings; Pfrg = *P. falciparum* rings and gametocytes; Pfg = *P. falciparum* gametocytes.

Table 2. Results of micro *in vitro* test of *P. falciparum* to chloroquine/mefloquine

Case No.	No. of asexual parasites/ 8000 WBC	Mean K	Schizont count per 200 asexual parasites in concentration (pmol) of chloroquine/mefloquine						
			1.0/0.5	2.0/1.0	4.0/2.0	5.7/4.0	8.0/5.7	16/8.0	32/16
A. Hazira Complex									
1	5547	22	34/28	17/16	2/7	0/2	0/2	0/1	0/2
2	1067	20	3/5	2/4	1/2	2/2	1/2	0/0	0/0
3	17653	41.5	8/6	3/4	2/2	2/1	3/1	2/1	0/0
4	15520	54	46/67	-/42	11/-	4/2	1/1	2/2	0/1
5	960	31	28/41	28/27	18/10	-/2	0/1	0/1	0/0
6	1227	35	3/4	2/2	2/1	0/0	0/1	0/1	0/0
7	4240	44.5	14/68	8/36	4/21	0/3	0/0	0/0	0/0
8	693	23	18/14	14/11	20/9	14/2	2/2	2/3	0/2
9	3600	25	8/8	3/6	3/3	2/1	2/0	1/0	0/0
10	10853	24	9/2	2/1	1/0	0/0	0/0	0/0	0/0
11	1067	24	6/0	0/0	0/0	0/0	0/0	0/0	0/0
12	5733	59	-/59	3/29	2/2	1/1	0/0	0/0	0/0
13	13680	26	16/25	11/18	8/4	1/1	1/1	0/1	0/0
14	3707	52	45/46	18/24	6/18	4/4	0/2	0/1	0/0
15	2267	25.5	4/4	2/4	0/3	0/1	0/2	0/0	0/0
		33.8	c (51.21)	(23.90)	(15.79)	(7.20)	(1.98)	(1.39)	(0.00)
			m (74.43)	(44.22)	(17.36)	(4.35)	(2.96)	(2.16)	(0.98)
B. Gothan									
16	5600	74.5	35/18	6/5	6/1	2/0	0/0	0/0	0/0
17	38133	200	200/200	200/200	200/200	77/13	25/0	8/0	0/0
18	13600	73.5	58/70	29/25	4/0	0/0	0/0	0/0	0/0
19	10933	43	10/0	0/0	0/0	0/0	0/0	0/0	0/0
20	43200	25	0/6	0/4	0/3	0/0	0/0	0/0	0/0
21	6880	105.5	80/48	35/8	7/6	0/1	0/0	0/0	0/0
22	15680	37	8/20	0/12	0/3	2/0	0/0	0/0	0/0
23	4533	110.5	96/0	93/40	84/7	38/0	37/0	4/0	0/0
24	65920	20	2/2	2/4	2/1	3/0	2/0	2/4	1/1
		76.55	c (70.97)	(52.97)	(43.98)	(17.70)	(9.23)	(2.03)	(0.14)
			m (52.83)	(43.25)	(32.07)	(2.03)	(0.00)	(0.57)	(0.14)
C. Umra									
25	17600	26	18/6	15/0	14/0	8/0	0/0	0/0	0/0
26	8950	25.5	4/2	0/0	0/0	0/0	0/0	0/0	0/0
27	1867	24	18/0	15/0	8/0	5/0	4/0	0/0	0/0
28	19200	22.5	1/2	0/2	0/1	0/0	0/0	0/0	0/0
29	15520	43	31/26	14/15	3/1	2/0	0/0	0/0	0/0
30	9067	62	0/7	0/2	0/1	0/0	0/0	0/0	0/0
31	22987	26.5	2/5	1/4	0/2	0/0	0/0	0/0	0/0
32	16480	22.5	16/19	5/21	2/18	0/18	0/11	0/0	0/0
33	27680	30.5	2/1	1/1	1/1	0/0	0/0	0/0	0/0
34	59787	28	4/15	2/12	1/14	1/10	1/3	0/0	0/0
		31.05	c (30.91)	(17.07)	(9.43)	(5.15)	(1.61)	(0.00)	(0.00)
			m (26.73)	(18.36)	(12.24)	(9.02)	(4.50)	(0.00)	(0.00)

c = Chloroquine; m = Mefloquine; Figures in parentheses are percentages.

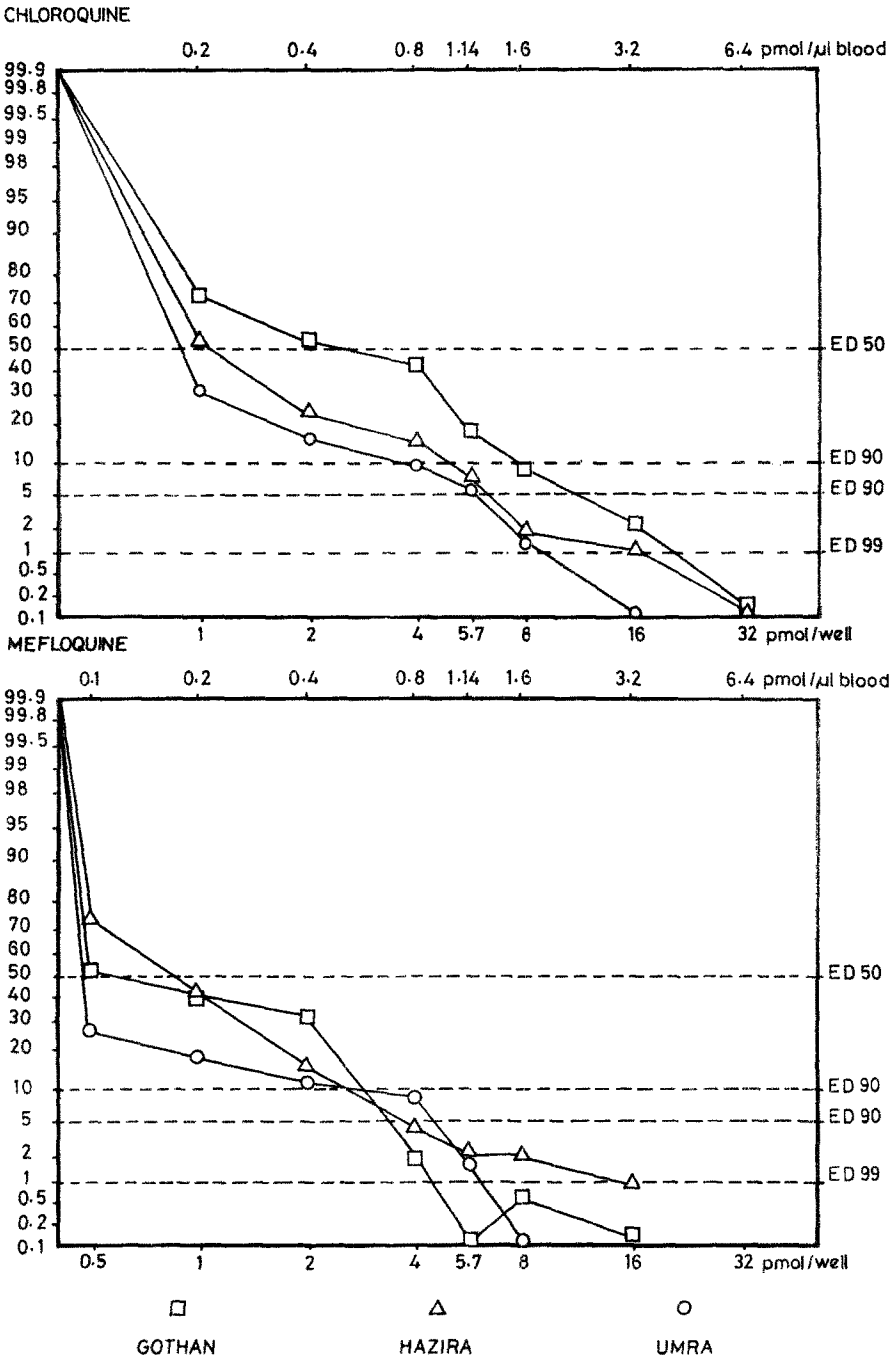

Fig. 1: *In vitro* response of *P. falciparum* to chloroquine and mefloquine.

mefloquine (4.0 pmol) was 4.35, 2.03 and 9.02% in the said areas, respectively. This indicates presence of the parasite, *P. falciparum*, resistant to both chloroquine and mefloquine in all the three areas surveyed.

Data given in Table 2 further indicate that in Gothan area, *P. falciparum* showed resistance to chloroquine concentration of 32 pmol and mefloquine concentration of 16 pmol. Similar observations were made from Hazira complex except that the parasite showed susceptibility to 32 pmol concentration of chloroquine. Both Hazira complex and Gothan were found to have parasite showing higher degree of resistance both to chloroquine and mefloquine than the parasite prevalent in Umra area where it was found to be resistant to chloroquine and mefloquine upto the concentration of 8.0 and 5.7 pmol of chloroquine and mefloquine, respectively.

Fig. 1 shows *in vitro* response of *P. falciparum* to chloroquine and mefloquine. Effective doses (ED99) for *P. falciparum* in Hazira, Gothan and Umra were found to be 17.3, 18.5 and 8.7 pmol/well, respectively for chloroquine. In case of mefloquine ED 99 was 14.5, 4.8 and 6.8 pmol/well, respectively. Thus chloroquine resistance status of *P. falciparum* in Hazira and Gothan was similar and approximately twice of that observed in Umra. As regards *P. falciparum* resistance to mefloquine, the parasite showed approximately half or less resistance level than that observed in Hazira. Therefore, *P. falciparum* showing high degree of resistance to both chloroquine and mefloquine in Hazira and to chloroquine in Gothan should be viewed seriously. The observed differences in the resistance levels of *P. falciparum* to chloroquine may be due to variation in strains which require further investigations and detailed study.

Micro *in vitro* tests with mefloquine were conducted in 15 PHCs/areas of Gujarat state. The parasites except those in Utran and Earthan PHCs were found to be sensitive to the drug *in vitro*.

However, as per the changed criteria of WHO (1987) where cut-off points for chloroquine (8 pmol) and mefloquine (64 pmol) or more are indicators for resistance, all the three study areas showed *P. falciparum* resistance to chloroquine. The study indicates that resistance could develop very quickly to mefloquine as well, when it is used extensively in the programme.

It is interesting to note that in spite of the fact that mefloquine has not been used in the study area, the parasite showed some low level resistance to it. This is likely to be a case of cross resistance which should be a matter of great concern. Therefore, any alternative antimalarial proposed to be used in Surat district or elsewhere needs to be screened first for cross resistance. It is further indicated that monitoring of drug resistance as in vogue at present should remain a continuous feature in our national malaria eradication/containment programme with the objective to eliminate the foci before they spread to other areas.

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SHORT NOTES

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Role of Splenomegaly in Diagnosis and Epidemiology of Malaria

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It is an age old practice to use splenomegaly as an important parameter for diagnosis and epidemiological assessment of malaria. Even before the discovery of malaria parasite by Laveran in 1880, Dempster (Russel *et al.*, 1963) pointed out the significance of splenomegaly in malaria in 1848. In recent years, Mashaal (1986) has regarded splenomegaly as one of the cardinal features of malaria, the other two being fever and anaemia. A study was designed during June 1988 to January 1989 to determine the correlation between malaria and splenic enlargement.

Suspected malaria cases (on the basis of clinical features such as fever, chill, rigor, headache and bodyache etc.) were taken from the outpatients of Calcutta School of Tropical Medicine. Their peripheral blood smears (both thick and thin) were examined for malaria parasite. Of these, 53 parasitologically positive malaria cases of different age and sex groups were selected for the present study. All the selected patients were from central Calcutta which is endemic for malaria. They were examined thoroughly for detection of splenomegaly in particular.

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Out of the 53 patients, 13 were of good nutritional status, 12 of them were suffering from *P. vivax* malaria and one from mixed infection of *P. vivax* and *P. falciparum*, with fever for 7 to 10 days. Out of those 13 patients, 4 (30.7%) had enlarged spleen. A total of 40 patients were of poor nutritional status and of these, the cases of *P. vivax* and *P. falciparum* were 35 and 5, respectively, with fever for 7 to 10 days.

Splenomegaly was detected in only 3 (7.5%) patients of poor nutritional group. Irrespective of nutritional status 13.2% (7 out of 53) had enlarged spleen. Splenomegaly ranged from 'just palpable' to 3 cm. In 3 cases the spleen was just palpable. In three other cases splenomegaly was 2 cm and only in one case splenomegaly was 3 cm. Nutritional status of the patients was determined by measuring the height and weight and by estimation of Serum Total Protein and Albumin/Globulin ratio.

Clinical history revealed that 40 patients *(P. vivax)* suffered from previous malarial infection. Of them, 18 had one, 15 had two and 7 had frequent malarial attacks. However, all these patients took antimalarial drugs (chloroquine) during their previous attacks. All seven cases of splenomegaly were found in relapse or reinfection cases. A control survey of splenomegaly in non-malarious patients attending the OPD of

Calcutta School of Tropical Medicine was undertaken. The rate of splenomegaly was 6% (6 out of 100 patients) in control cases.

Maegraith (1985), Manson-Bahr (1987) and Woodruff (1974), showed a positive correlation between malaria and splenomegaly. However, the observation of the present study was quite different. Splenomegaly was detected only in 13.2% malaria patients which does not signify a positive correlation between splenomegaly and malaria. Vanderkay (1976) and Mamtani *et al.* (1979) opined that enlargement of slpeen is insignificant in diagnosis and epidemiological survey of malaria, where widespread use of antimalarial drugs is prevalent. Use of antimalarials, specially chloroquine, was widespread in the present study areas, and may be one of the causes of low splenomegaly among the patients. According to Suntharasamai and Marsden (1972), splenomegaly in malarious mice fed on a low protein diet was less pronounced than in those on higher protein diet.

Similarly in our study area, though total splenomegaly rate in malaria patients was low, it was higher in the good nutritional status group (30.7%) than in the poor nutritional status group (7.5%). In our study area poor nutritional status may be another cause for lower percentage of splenomegaly. This particular non textbook phenomenon should be kept in mind during diagnosis and epidemiological studies of malaria in such areas.

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Outbreak of Malaria in Banda PHC of District Shahjahanpur (U.P.)

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The initial success in control of malaria under National Malaria Eradication Programme (NMEP) was followed by resurgence of the disease in the late 60s. Focal outbreaks of malaria were recorded in some areas of consolidation as well as maintenance phases of the NMEP (Sharma and Mehrotra, 1986). Modified plan of operation (MPO) was introduced by NMEP in 1977 to control the situation (Pattanayak and Roy, 1980). In spite of the implementation of the revised strategy of malaria control under MPO focal outbreaks of malaria occurred in different parts of the country resulting in high morbidity and reports of mortality due to malaria.

In 1983, in Shahjahanpur district, Nigohi and Tilhar PHC villages experienced an epidemic of malaria. In this epidemic approximately 349 persons died due to *P. falciparum* infection (Chandrahas and Sharma, 1983; Sharma *et al.* 1985). Malaria situation has not changed in this area except in Dadrol PHC where the bio-environmental control strategy is being used. This is currently under expansion to cover the entire district. During periodical surveys in 1988 some Banda PHC villages of Shahjahanpur district reported high morbidity and mortality due to fever. An investigation was taken up to study the cause of this epidemic. Results of this study are reported below.

Banda is one of the fourteen PHCs of the district. It is surrounded by Pilibhit district in northwest and Powayan and Khutar PHCs of Shahjahanpur district in southeast, respectively. The four villages of Banda PHC namely, Marena, Habibnagar, Balemau and Anantapur were included in the present investigation.

The study villages are situated about 50 kms north of Shahjahanpur. A branch of Sharda canal flows through Banda PHC. The affected villages are situated in canal irrigated zone and have innumerable seepage and rain water collection sites. There are 303 houses, in the four villages with a population of 2003 (1142 males and 861 females). The number of cattlesheds was 271 containing 1395 animals (969 cattle, 309 goats and 27 pigs). Thus the human to animal population ratio in the area was 59:41.

As a result of heavy rainfall during 1988 the villages were marooned and became inaccessible. The rainfall was 428.9 mm in 1988 as against 150.1 mm in 1987 during July to September

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alone. The number of rainy days during 1988 were 45 as against 26 in 1987.

Parasitological data for 1987 and 1988 collected from the District Malaria Officer for the 4 villages showed that during 1987 a total of 259 blood smears were collected of which 34 were positive (28 *Pv* + 6 *Pf*). The SPR and SfR were 13.1 and 2.3, respectively. The collection of blood smears was 15 to 40 per month and in September only 6 blood smears were collected.

During 1988 a total of 703 blood smears were collected. Bulk of the collection i.e., 433 were in September and October due to reports of epidemic. There were 85 positive (63 *Pv* + 22 *Pf*) cases. The SPR and SfR were 12.0 and 3.1, respectively. During 1988 fever rate in the villages was very high and DDCs, FTDs and VHGs treated 4079 fever cases with 13235 chloroquine tablets. Two rounds of HCH (50% WDP) were sprayed. First round was completed in June and July and the second during September 1988. Third round was not carried out but focal spray was done in all villages from 6.10.1988 to 15.10.1988. Spray coverage in the first 2 rounds varied from 93-100%. Besides active and passive case detection mass radical treatment (MRT) was given to all fever cases from 25-27 September 1988. Out of a total population of 2311 in 4 villages 2046 were given MRT and the percentage coverage was 88.1.

Project staff carried out fever surveys from 6 to 10 October 1988 in the 4 affected villages. Blood smears were collected from the fever cases and stained with JSB. Morning hand collections of indoor resting mosquitoes were carried out from 6.10.88 to 9.10.88. The collected mosquitoes were identified and dissected in normal saline solution for gut and gland infections.

Results of parasitological surveys are given in Table 1. A total of 88 blood smears were collected from the four villages. Out of these, 42 blood smears were positive for malaria parasite giving a slide positivity rate (SPR) of 47.7. Among the positives, 41 were *P. falciparum* and only one was *P. vivax*. Slide falciparum rate and *P. falciparum* percentage were 46.59 and 97.6, respectively.

A major branch of Sharda canal flows nearly 3 kms away from the area. Many irrigation channels originating from this canal result in heavy seepage in the area. Other mosquito breeding sites include ponds, wells and water collections in rice fields. *A. culicifacies* was

Table 1. Results of parasitological survey in the four affected villages of Banda PHC, District Shahjahanpur, U.P.

S. No.	Villages surveyed	Population	No. of blood smears collected	Results			
				Pv	Pf	SPR	SFR
1.	Marena	1415	19	0	9	47.4	47.4
2.	Habibnagar	211	31	0	15	48.4	48.4
3.	Balemau	214	23	0	14	60.9	60.9
4.	Anantapur	163	15	1	3	26.7	20.00
	Total	2003	88	1	41	47.7	46.59

Dates of Survey : 6 to 10 October 1988.

found breeding in seepage water of the irrigation channels and *A. stephensi* in the wells. Breeding of *A. annularis* and *A. subpictus* was found mainly in waste water collections in the streets. Mixed breeding of anophelines and *Culex* was found in many wells of the area.

A total of 17 man hours were spent in mosquito collections which resulted in the collection of 433 specimens of anopheline mosquitoes. The following 7 anopheline species were present in the area viz., *A. culicifacies, A. aconitus, A. stephensi, A. subpictus, A. annularis, A. barbirostris* and *A. nigerrimus*.

Per man hour density of *A. culicifacies* and *A. stephensi* in the 4 study villages varied from 0.2 to 12.5 and 1.2 to 6, respectively, whereas *A. annularis* densities varied rom 3.3 to 13. *A. subpictus* was the most dominant anopheline and accounted for 56.8 per cent of the total collections (MHD 25 to 30). Other anophelines such as *A. aconitus, A. barbirostris* and *A. nigerrimus* were also found but in very low numbers i.e., only few specimens of each species.

To detect natural infection, salivary glands of 69 *A. culicifacies*, 20 *A. stephensi*, 4 *A. aconitus* and 86 *A. annularis* were dissected. In village Habibnagar, out of 8 *A. culicifacies* 1 specimen was found with sporozoites and in village Balemau out of 25 *A. culicifacies* one more specimen was found with gland infection. The overall sporozoite rate of *A. culicifacies* was 2.89. No infection was found in any other anopheline species dissected during this period.

Studies revealed that although HCH was being sprayed in Shahjahanpur district to interrupt transmission, the spraying was not producing the desired impact on transmission. The transmission was so high that mass distribution of drugs through the DDCs, FTDs and VHGs was not helpful in suppressing the epidemic. The surveillance in the area was totally inadequate as could be seen by the number of blood smears collected. The laboratory services required to be strengthened as the slide positivity rate in the blood smears examined was very low compared to the reports of surveys by the project staff.

A. culicifacies is the primary vector of malaria and sporozoite positive specimens were found in 2 villages in a few days. There was evidence of transmission under the pressure of insecticides and drugs. Although no insecticide susceptibility tests were done, earlier studies by the project and NMEP staff showed that *A. culicifacies* was resistant to HCH. Spraying of HCH was therefore unlikely to suppress the epidemic. Besides this, instead of 3 rounds only 2 rounds were given during 1988. In this area *A. culicifacies* comprises of sibling species A and B, of which species A is the vector. This species is more susceptible to DDT than species B, whereas there is no difference in susceptibility to HCH in species A and B. It would therefore be advantageous to revert to DDT spraying which was likely to produce better impact than HCH (Subbarao *et al.*, 1988a;b). In case of poor impact of DDT the choice should be in favour of malathion and not HCH. As soon as the epidemic is suppressed the areas should be brought under the bioenvironmental control strategy (Sharma, 1987;1988) and insecticides should be used only during epidemic situations.

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Susceptibility Status of *Anopheles fluviatilis, A. annularis* and *A. culicifacies* to Insecticides in Koraput District, Orissa

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Koraput district is hyperendemic for malaria since many decades (Perry, 1914; Rajagopalan *et al.,* 1990), and the funestus group of anophelines was identified as the main vector (Senior White 1937; 1938). Recently *Anopheles fluviatilis, A. annularis,* and *A. culicifacies* have been incriminated as vectors in this area (Gunasekaran *et al.,* 1989; VCRC, unpublished data). Since 1958, this area received two rounds of DDT indoor residual spray (May-June; I round and Dec-Jan; II round) and in some PHCs in 1972, DDT was replaced by HCH mainly due to availability and not due to susceptibility status of the vector. At present out of 42 Primary Health Centres (PHCs) of the district, 24 PHC areas are covered with DDT spray (two rounds) and 18 PHC areas are covered with HCH (three rounds) spray every year. Since the susceptibility status of these malaria vectors to the insecticide being used is not known, a study was carried out in April 1989 (prior to I round spray) and October 1989 (prior to II round spray) to determine the susceptibility of these vectors to DDT, HCH, malathion and deltamethrin and the results are presented here.

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The insecticide papers used for this test were prepared at VCRC following standard method. Susceptibility tests were conducted as per standard procedure (WHO, 1975). Fully fed, wild caught females of *A. fluviatilis, A. annularis* and *A. culicifacies* were used for the test. Since the density of the vector was low, only 10 mosquitoes were exposed to the insecticide in each replicate. The data obtained on mortality in different replicates were corrected using Abbott's formula.

The susceptibility test results for *A. fluviatilis, A. annularis* and *A. culicifacies* to different insecticides are summarised in Table 1. The results indicate that *A. fluviatilis* is susceptible to all the insecticides tested viz., DDT, HCH, malathion and deltamethrin. Except some unconfirmed reports on the resistance of *A. fluviatilis* to DDT, this species has not developed resistance yet to either DDT or HCH in other parts of the country (Rao, 1984; Bhatia *et al.,* 1958; Azeez,1964) probably due to its exophilic behaviour. Moreover in this area there is no pesticide pressure in agriculture especially in kharif season when extensive, rain fed, paddy cultivation is done. During rabi season, paddy is cultivated in limited areas and a few pesticides like democran, miraculan (monocil), etc. are occasionally used.

Table 1. Per cent mortality in three vector species exposed to diagnostic dosages of DDT, HCH, Malathion and Deltamethrin

Insecticide	Species	Exposure period					
		1 hr		2 hr		3 hr	
		No. exposed	Corrected % mortality	No. exposed	Corrected % mortality	No. exposed	Corrected % mortality
DDT (4%)	*A. fluviatilis*	40	95.8	—	—	—	—
	A. annularis	20	10.3	20	10.0	20	25.0
	A. culicifacies	50	35.7	30	53.4	30	78.6
HCH (0.4%)	*A. fluviatilis*	20	93.3	0	—	—	—
	A. annularis	30	19.3	20	47.1	20	60.0
	A. culicifacies	20	10.0	20	40.0	20	45.0
Malathion (5%)	*A. fluviatilis*	20	100.0	—	—	—	—
	A. annularis	20	100.0	—	—	—	—
	A. culicifacies	20	83.6	—	—	—	—
Deltamethrin (0.025%)	*A. fluviatilis*	20	100.0	—	—	—	—
	A. annularis	20	100.0	—	—	—	—
	A. culicifacies	30	96.6	—	—	—	—

Hence selection pressure has been minimal in this species.

A. annularis and *A. culicifacies* are found to be resistant to both DDT and HCH. The resistance of these two species to DDT is well established in many parts of the country (Rao, 1984). However, in some parts of Orissa state, *A. annularis* was found to be susceptible to these chlorinated hydrocarbon insecticides (Das, 1976). It is noteworthy that *A. annularis* and *A. culicifacies* collected from DDT sprayed area, have developed resistance not only to DDT, but also to HCH, which shows the presence of cross resistance. Earlier, bioassay tests carried out on sprayed surfaces also showed negligible mortality in *A. culicifacies* (VCRC, unpublished data).

A. culicifacies and *A. annularis* are found to be susceptible to malathion (5%) and deltamethrin (0.025%) which are not in use in this area. The development of resistance in *A. culicifacies* and *A. annularis* to DDT and HCH is due to prolonged selection presssure maintained by the control programme and the predominant indoor resting and biting habits of these species.

Though *A. annularis* has been incriminated with sporozoites; *A. fluviatilis,* is the major vector of this area (Gunasekaran *et al.,* 1989) which is found biting indoors, though it predominantly rests outdoors (VCRC, unpublished data). In other parts of the country, indoor residual spray with DDT was successsful against exophilic *A. fluviatilis* (Rao, 1984). However, in this area malaria continues to persist (Rajagopalan *et al.,* 1990) in spite of repeated application of DDT against indoor biting and susceptible *A. fluviatilis.* The failure of DDT spray could be attributed to the inadequacy of the spray operation as well as to the frequent mud plastering of sprayed surfaces by the villagers. This nonco-

operative attitude of the villagers to residual spray and resistance in *A. culicifacies* and *A. annularis* to DDT and HCH necessitates the development of an alternative control strategy.

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Radical Treatment of Vivax Malaria in Madhya Pradesh, India

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In India about 2 million parasite positive cases are detected every year by active and passive case detection. *P. vivax* is the dominant infection (70% cases) and large parts of the country have very high proportion of vivax malaria. Falciparum malaria is concentrated in Orissa, North Eastern states and certain hard core and tribal areas. Therefore, a study on the relapse pattern of vivax malaria would be of importance to the control of malaria in India. The anti-malaria drug policy of the National Malaria Eradication Programme for the cure of *P. vivax* infection provides 600 mg chloroquine presumptive treatment (PT) followed by radical treatment (RT) comprising of 600 mg chloroquine and 15 mg primaquine daily for 5 days (75 mg total dose for adults).

We report the results of a 2-year study of the treatment of *P. vivax* cases with 1500 mg chloroquine i.e., 600 mg PT followed by RT within 2-3 days comprising of chloroquine 600 mg on day 1 and 300 mg on day 2; starting on the same day 15 mg primaquine was given daily for 5 days or RT was done without primaquine administration. Children were given proportionate lower doses. All cases were checked for malaria parasite at 15 day interval during the 8 month follow-up period. Those found positive after RT were given second and third RT of 15 mg daily for 5 days. The study was carried out in Bizadandi Primary Health Centre (PHC) in Mandla district of Madhya Pradesh. The PHC is under bioenvironmental control of malaria since 1986, and as a result of intervention measures the incidence of malaria in the experimental areas was relatively less i.e., in 1988 SPR, SfR and API were 111.9, 17.8 and 7.4, respectively as compared to neighbouring areas with SPR 48.1, SfR 42.6, API 316.4 and Pf% 80 (Singh *et al.*, 1989). Indigenous population is mainly Gond tribe (80%) with 10-15% G6PD deficiency genetic disorder.

During 1987 a total of 3403 malaria (2028 *Pv* + 1364 *Pf*) cases were detected (SPR 16.53). Of the total *P. vivax* cases 995 were given RT and followed for an 8 month period. At each relapse radical treatment was administered. First relapse occurred in 104 (10.34%) cases i.e., 27, 30, 18, 10, 32, 10 and 4 cases, respectively relapsed at monthly intervals for 8 months. Second relapse occurred in 14 (13.46%) cases

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i.e., 7, 4, and 3 cases relapsed at monthly intervals. Third relapse occurred in 2 cases in the first month itself. There was no other relapse.

Studies continued in the same area in 1988 without 5-day primaquine radical treatment. During the year 4290 positive (3009 *Pv* + 2259 *Pf*) cases were detected (SPR 17.28). A total of 2500 *P. vivax* malaria cases were followed. Relapses were recorded every month upto 8 months i.e., 82, 54, 44, 27, 5, 7, 1, and 2 cases relapsed, and the relapse rate was 8.92% in 222 cases. Second relapse occurred in 36 (16.13%) cases and the number of cases relapsed were 19, 13, 2, 1, 0, 1 each month. Third relapse occurred in 7 cases (19.44%) and 3 cases relapsed in first month followed by 1 case each for 5 months.

From the above study it is clear that relapse rate in *P. vivax* strain of this region was low (10%). A 5-day radical treatment was inadequate to prevent relapses. Maximum relapses occurred in the first few months of chloroquine administration. The study brought out that for the radical treatment of vivax malaria a 14-day course of 15 mg primaquine daily recommended by WHO may be adopted. It may be pointed out that efficacy of 14-day course has not been tested against the Indian *P. vivax* strain. In this area 5-day RT may be discontinued as it does not provide radical cure, there are operational problems encountered in the administration of primaquine in different field areas, besides there is some risk of primaquine induced haemolysis in G6PD deficient cases. A similar observation has been reported recently by Sinha *et al.* (1989) from Hardwar, U.P.

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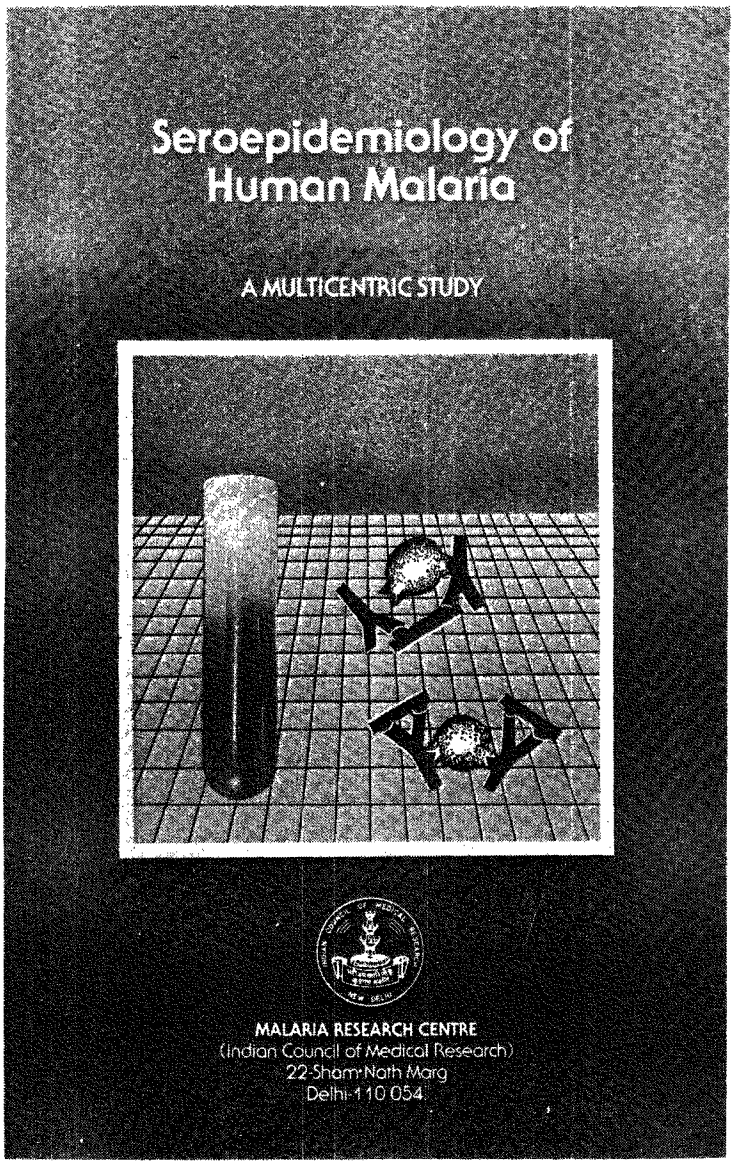

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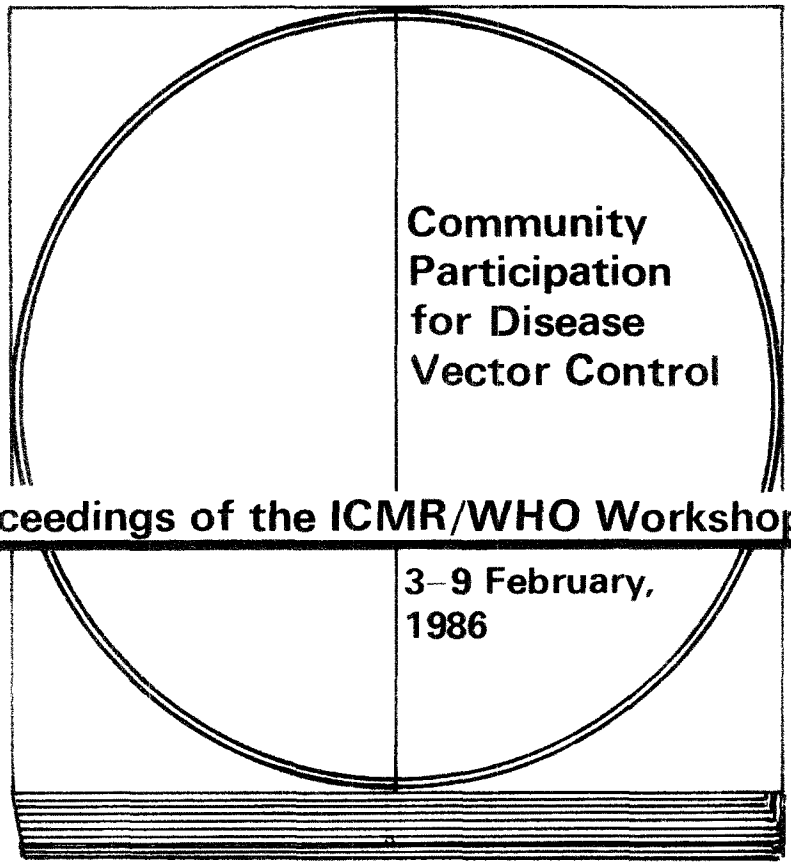

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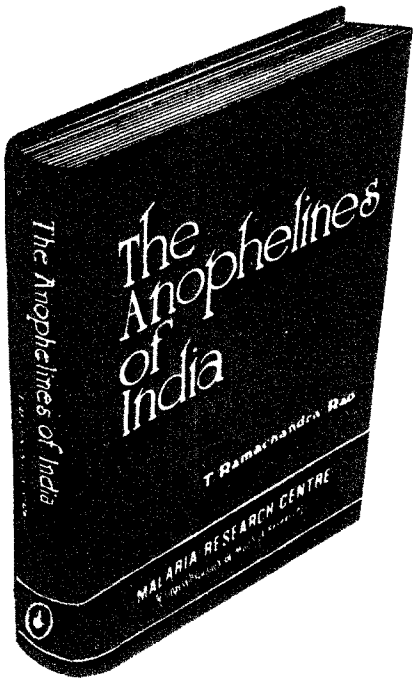

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