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Evaluation of Certain Mosquito Repellents Marketed in India

M.A. ANSARI[1], V.P. SHARMA[1], R.K. RAZDAN[1] and P.K. MITTAL[1]

A study was carried out in certain villages of Distt. Ghaziabad (U.P.) to evaluate the efficacy of commercially available mosquito repellent preparations and devices. Results revealed that none of the repellents tested provided absolute protection against mosquito bites. The protection varied from 38 to 98% with different species of mosquitoes. In general, better protection was provided against *Anopheles* bite than against *Culex*. Of ten repellents evaluated, Mylol oil and Tortoise coils were marginally superior to Odomos cream and Rooster coils. Among electrical devices, Good Bye and Casper were marginally superior to Good Knight and Knight Queen.

INTRODUCTION

A variety of mosquito repellent preparations and electrical devices are marketed in the country. The market of mosquito repellents is increasing due to enormous build up of mosquito populations all over the country, in both rural and urban areas. Enquiries revealed that most products marketed in the country have not been tested scientifically or registered. New products and formulations are continually being added to meet the demand. The Centre often receives enquiries on the usefulness and relative value of the repellents available in the market, unfortunately there are no evaluation reports which could be referred. It was therefore decided to test the repellent action of the commonly marketed products in the country. Results of the study are reported in this paper.

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[1] Malaria Research Centre
22-Sham Nath Marg
Delhi-110 054, India.

MATERIAL AND METHODS

The study was carried out during August to October 1988 in Pipalhera and Ramgarh villages of Dhaulana and Dadri Primary Health Centres, District Ghaziabad (U.P.). These villages had high densities of *A. culicifacies* (80-130 MHD) during the transmission period, in spite of their being sprayed with HCH @ 200 mg/m^2.

The devices tested fall into three categories viz., (1) Oils and creams: Mylol oil (Boots Co. Ltd.), Odomos cream (Balsara Hygiene Products Ltd.); (2) Coils: Tortoise (Bombay Chemicals [P] Ltd.), Rooster coil (Sun-up Laboratories [P] Ltd.); (3) Mats: Knight Queen (Daffodil Chemicals [P] Ltd.; Good Knight (Trans Elektra Domestic Products [P] Ltd.), Good Bye (Priya Enterprises) and Casper (Chemicals and Plastics India Ltd.).

These repellents/devices were applied as per the instructions of the manufacturers. Human bait were used both indoors and outdoors for evalu-

Table 1. Field evaluation of oil and cream as mosquito repellents

Species	Control	No. of mosquitoes collected on human bait during the whole night (1800-0600 hrs)*			
		Experimental			
		Odomos	% protection	Mylol	% protection
Indoor					
A. culicifacies	21	2	89.0 ± 13.9	1	96.4 ± 6.2
A. subpictus	21	2	83.3 ± 11.8	0	100
Total anophelines	57	7	89.1 ± 9.0	3	96.8 ± 5.4
Total culicines	227	16	68 ± 39.8	2	98.5 ± 2.8
Total mosquitoes	284	23	82.5 ± 21.2	5	95.9 ± 4.4
Outdoor					
A. culicifacies	11	1	93.3 ± 11.5	1	93.3 ± 9.4
A. subpictus	27	10	69.5 ± 10.5	4	90.9 ± 9.1
Total anophelines	53	12	88.0 ± 11.5	5	95.4 ± 6.4
Total culicines	188	29	79.8 ± 20.7	16	89.6 ± 13.2
Total mosquitoes	241	41	83.9 ± 16.7	21	83.7 ± 13.4

* Total of six replicates.

Table 2. Field evaluation of repellent coils against mosquitoes

Species	No. of mosquitoes collected from 1900 to 0600 hrs.*					
	Tortoise coil			Rooster coil		
	Expt.	Cont.	% protection	Expt.	Cont.	% protection
A. culicifacies	29	83	65.3 ± 36.5	10	52	73.0 ± 36.9
A. subpictus	22	571	75.3 ± 35.3	16	717	96.0 ± 6.8
Total anophelines	84	860	77.6 ± 35	94	971	75.8 ± 19.5
Total culicines	59	149	84.0 ± 25.3	178	326	62.2 ± 25.8
Total mosquitoes	143	1009	68.6 ± 35.8	272	1297	67.7 ± 28.9

* Total of six replicates.

ation of cream and oil. Cream or oil was applied @ 1 gm or 1 ml per person on face, neck, fore arms and legs of human volunteers after washing thoroughly. These volunteers belonged to the staff of the Centre and are well trained in mosquito collection. Two human bait (protected) were used, one from 1800 hrs. to midnight and the second from midnight to 0600 hrs. Mosquitoes which landed on exposed body parts were collected by the volunteers themselves with the help of test tube and torch from dusk to dawn. Six replicates each were tested with oil and cream both outdoors and indoors.

Control bait (unprotected) were sited about 100 mts. from the test group. They did not apply oil or cream and collected mosquitoes by the same method as the protected volunteers. The experiment was supervised throughout night and mosquitoes were collected at hourly intervals.

Coils and electrical devices were evaluated in human dwellings. Coils were hung at central places in experimental dwellings throughout the night. Since the burning coils last only for 6 hours they were replaced with new coils at midnight. Six replicates were set up in human dwellings. Similarly, mats were placed inside electrical devices and switched on at 1900 hrs. No observations were made in cattlesheds. Mosquitoes were collected at an interval of 1 hour for 15 mins. in each experimental dwelling, identified and transported to the laboratory for reconfirmation in the morning. Similarly mosquitoes were collected and identified from control dwellings selected at a distance of 100 mts. in the same area. Per cent protection was calculated as described by Sharma *et al.* (1989).

All replicates were pooled together and protection was calculated based on control for whole night. Results were statistically analysed to compare the relative efficacy of different repellents.

RESULTS AND DISCUSSION

Altogether eight mosquito species were encountered on human bait and in human dwellings in the study area. These are *An. subpictus, An. annularis, An. culicifacies* and *Cx. quinquefasciatus*. Occasionally a few specimens of *An. stephensi, An. nigerrimus, An. pulcherrimus* and *Mansonia* species were also encountered.

Oil and Cream: The number of mosquitoes caught during six whole night collections on control and experimental human bait in indoor and outdoor conditions are given in Table 1. Odomos cream and Mylol oil showed varying degrees of protection. About 96 to 100% protection was observed in Mylol treated bait as against 68 to 89% in Odomos treated bait under indoor conditions. However, in outdoor bait collections the level of protection in Odomos and Mylol oil treated bait was more or less same but results were quite consistent with Mylol oil. This indicated that Mylol oil provided marginally better protection; but none of the repellents provided full protection (100%) against mosquito bites either indoors or outdoors. Comparison of indoor and outdoor collections revealed that repellent oil and cream gave marginally superior protection in indoor conditions.

Biting activity of total mosquitoes from 1800 to 0600 hrs. is shown in Fig. 1 and it appears from the graph that biting activity varies in indoor and outdoor conditions. Biting activity was found slightly higher during early hours of the night outdoors while indoors the activity of mosquitoes was quite low during the early hours but gradually increased after midnight. Results of biting activity also revealed that single application of cream and oil was found equally effective in providing protection for whole night. Protection was variable with different species of mosquitoes. These observations are in conformity with those of Curtis (1986) who reported that N, N-diethyl-m-toluamide (DEET) does not repel all mosquito species equally. The repellent effect was more pronounced with anophelines as compared to culicines.

Coils: Results of Tortoise and Rooster coils also

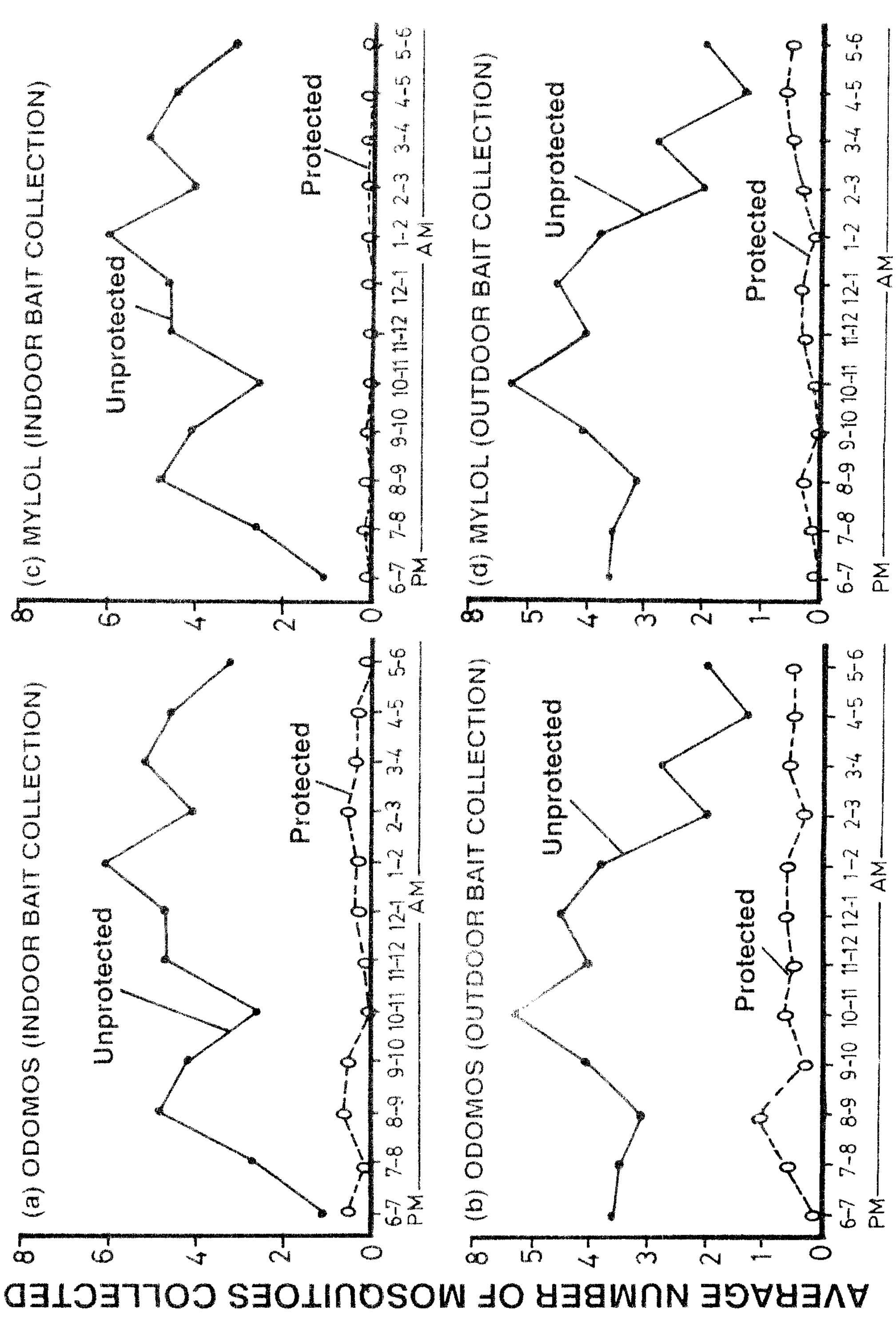

Fig. 1: Results of the effectiveness of Odomos (a,b) and Mylol (c,d) in preventing mosquito bites on human baits.

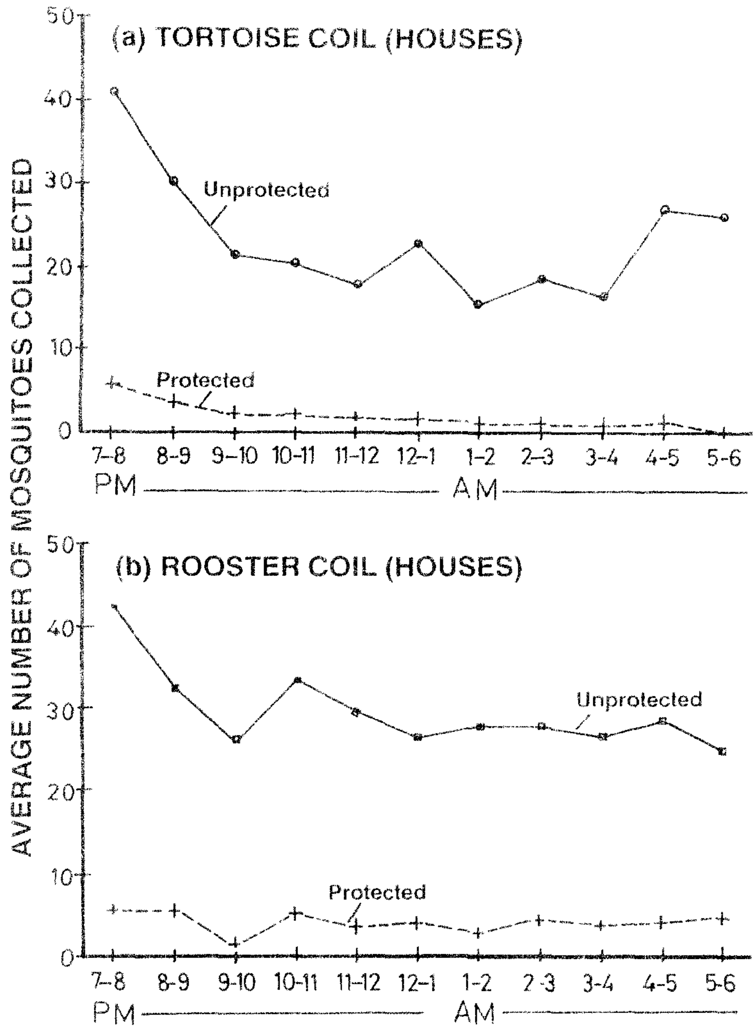

Fig. 2: Results of the effectiveness of Rooster coils and Tortoise coils inside houses.

showed varying degree of effectiveness in preventing the entry of mosquitoes in houses. Table 2 gives the results of six night collections of mosquitoes in control and experimental structures along with per cent protection. It is clear from the table that none of the coils provided adequate protection from mosquito bites. The protection varied from 65 to 84% and 62 to 96% with Tortoise and Rooster coil, respectively. It was interesting to note that Tortoise coil provided greater protection against culicines and Rooster coil was found marginally superior against anophelines. No difference was observed when per cent protection against total mosquitoes was compared between Tortoise and Rooster coils. Results of hourly entry of mosquitoes in houses revealed that mosquitoes do not enter houses at regular intervals either for feeding or resting and smoke from smouldering coils provided more or less equal protection throughout the night (Fig. 2). Studies on the safety of coils carried out by Saini *et al.* (1986) showed that smoke from coils is safe for humans.

Table 3. Field evaluation of repellent devices against mosquitoes

Species	Mosquitoes collected from 1900 to 0600 hrs.*											
	Casper			Good Knight			Knight Queen			Good Bye		
	Expt.	Cont.	%Protection	Expt.	Cont.	%Protection	Expt.	Cont.	%Protection	Expt.	Cont.	%Protection
A. culicifacies	1	9	91.6 ± 11.7	5	6	55.0 ± 45.8	4	8	50.0 ± 1.0	1	6	93.7 ± 10.8
A. subpictus	19	50	44.0 ± 44.4	17	67	82.3 ± 8.6	20	55	58.9 ± 41.5	15	68	81.8 ± 18.6
Total anophelines	48	127	61.5 ± 23.1	58	112	64.0 ± 16.5	69	132	59.7 ± 37.7	42	113	66.6 ± 29.8
Total culicines	253	639	58.3 ± 18.2	298	398	46.9 ± 18.6	334	687	48.8 ± 19.29	229	401	63.6 ± 29.1
Total mosquitoes	301	766	49.6 ± 13.3	356	510	51.7 ± 11.3	403	819	49.3 ± 22.9	269	514	63.9 ± 24.5

* Total of six replicates.

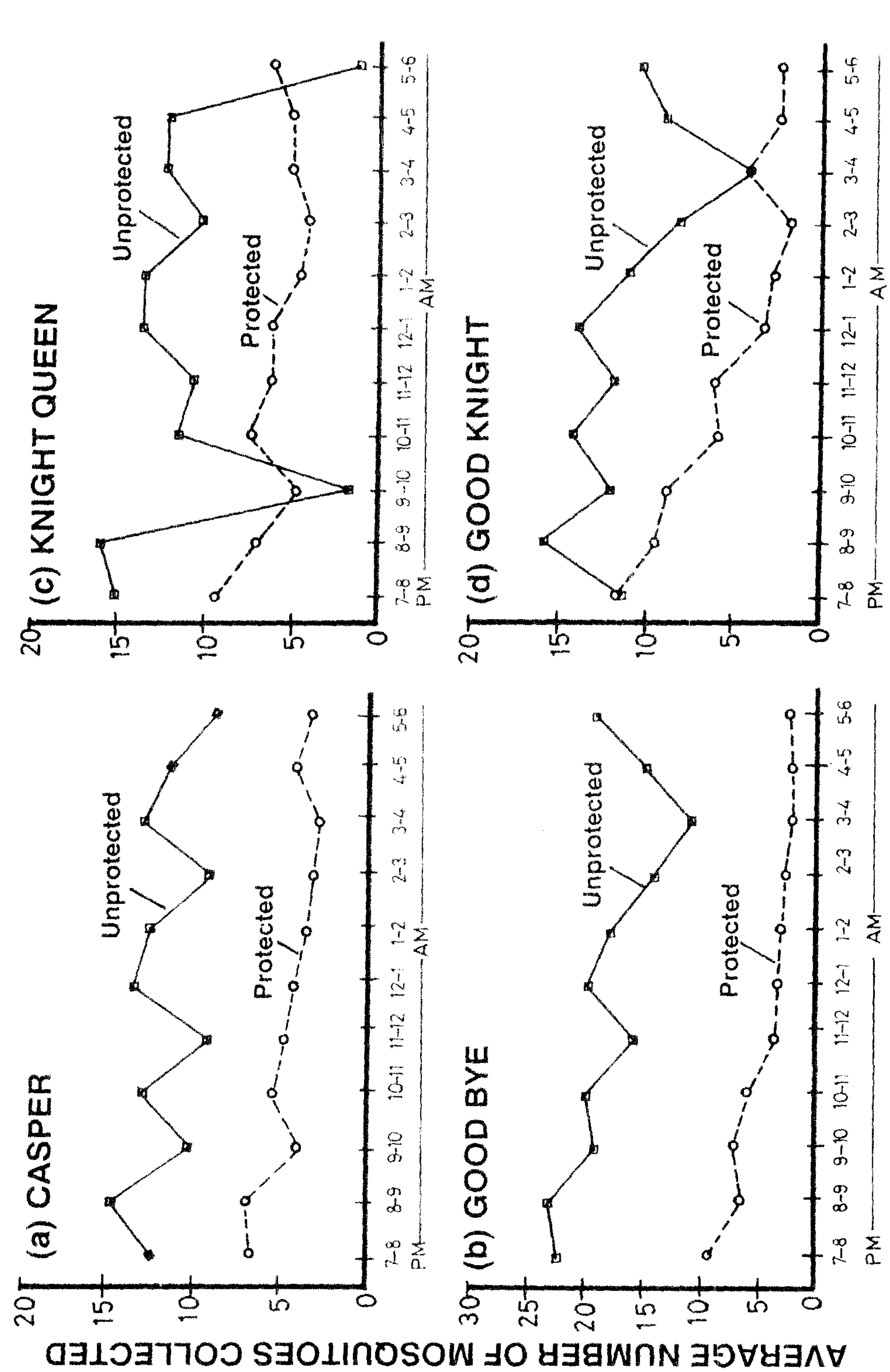

Fig. 3: Results of the effectiveness of four commercially available mats in preventing mosquitoes entering the houses.

Electrical devices: Results of electrical devices are given in Table 3. It was observed that these devices were not as good as oil/cream or coils in providing protection from mosquito bites. The degree of protection varied from one device to another. Of four types tested only "Casper" and "Good Bye" were able to provide more than 90% protection against *A. culicifacies*. However, no significant difference was observed when protection against total mosquitoes was compared. It was interesting to note that these devices provided better protection after midnight due to increased concentration of insecticide in the atmosphere (Fig. 3).

The study brought out that commercially available repellents do provide good protection from mosquito bites/nuisance but 100% protection was not obtainable against any species of mosquito prevalent in the study area. It may be noted that the composition of different repellents evaluated in this study is held as a trade secret. However manufacturers should print the composition for the benefit of the consumers and Indian Standards Institution (ISI) certification should be made mandatory.

The available repellents/devices are expensive and unsuitable for rural areas. A recent development is the burning of deltamethrin impregnated jute ropes from dusk to dawn in open rooms. Smoke from the smouldering rope gradually saturates the room and produces repellent and toxic effect on mosquitoes. This method has provided over 90% protection against the principal vector of malaria, *A. culicifacies* at 80 ppm dosage (Sharma *et al.*, 1989). Since jute and other ropes are produced locally in villages all over the country this method may prove most practical and useful in prevention of man mosquito contact during the peak transmission season instead of the use of expensive materials such as oil, cream and coils etc. Mats are also impractical as they require electricity.

ACKNOWLEDGEMENTS

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Fine Structure of the Erythrocytic Stages of *Plasmodium vivax* and the Host Cell Alterations

NUTAN NANDA[1]

Fine structural analysis of the trophic and sexual stages of *Plasmodium vivax* obtained from naturally infected humans revealed that in general, the structural features as well as certain specialized functions such as haemoglobin ingestion and utilization etc., are similar to those described for other malaria parasites. Young trophozoite is characterised by less condense cytoplasm with scattering of free ribosomes and minimum amount of endoplasmic reticulum. The trophozoite grows by feeding on the host cell cytoplasm with its cytostome. Older trophozoite gets considerably enlarged and its cytoplasm appears coarsely granular due to an increase in endoplasmic reticulum and ribosomal content. The two sexual forms, the macro- and microgametocyte, can be distinguished on the basis of their fine structure. The macrogametocyte has high ribosomal density and more osmiophilic bodies than microgametocyte and possesses a compact dense nucleus with nucleolus-like region whereas the microgametocyte has large diffuse nucleus without nucleolus. Caveola-vesicle complexes and cytoplasmic clefts are observed in all erythrocytes infected with *P. vivax* and the incidence of these host cell alterations increases as the intra-erythrocytic parasite matures.

INTRODUCTION

Erythrocytic phase of malaria parasites has been extensively studied by several workers. Numerous reports are available on the ultrastructure of erythrocytic (asexual and sexual) stages of various species of avian, reptilian and mammalian plasmodia. Among the less studied groups are the human malaria parasites. Out of four species of human plasmodia viz., *P. vivax, P. falciparum, P. malariae* and *P. ovale*, the former two account for more than 95% malaria cases in the world (WHO, 1980) and *P. vivax* predominates over *P. falciparum*. *In vitro* culturing of *P. falciparum* has made it possible to study various stages in the life cycle of this parasite in detail at ultrastructural level. However, similar information in case of *P. vivax* is lacking as it has not been possible to culture this parasite *in vitro*.

Plasmodium vivax, is the most common species of human malaria (about 70%) in India and its erythrocytic phase can only be studied in naturally infected cases. In the present study of erythrocytic stages of *P. vivax* obtained from naturally infected human volunteers, the fine structure of trophozoites, gametocytes and the changes induced by the parasite in its host erythrocytes have been described. Observations

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[1] Malaria Research Centre
22-Sham Nath Marg
Delhi-110 054, India.

made on the erythrocytic stages of *P. vivax* have been compared with those made by earlier workers in various other species of malarial parasites.

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 in J.S.B. stain (Singh *et al.*, 1953), and examined under light microscope for the malaria parasites. *P. vivax* infected individuals having trophozoites and gametocytes in their peripheral circulation were selected for this study. Blood was drawn by venepuncture in heparinized glass vials and processed immediately for ultrastructural studies. Patients were treated with 600 mg chloroquine on the day of examination and 600 mg 1 day after followed by 15 mg of primaquine for 5 days. The parasite was found sensitive to chloroquine treatment.

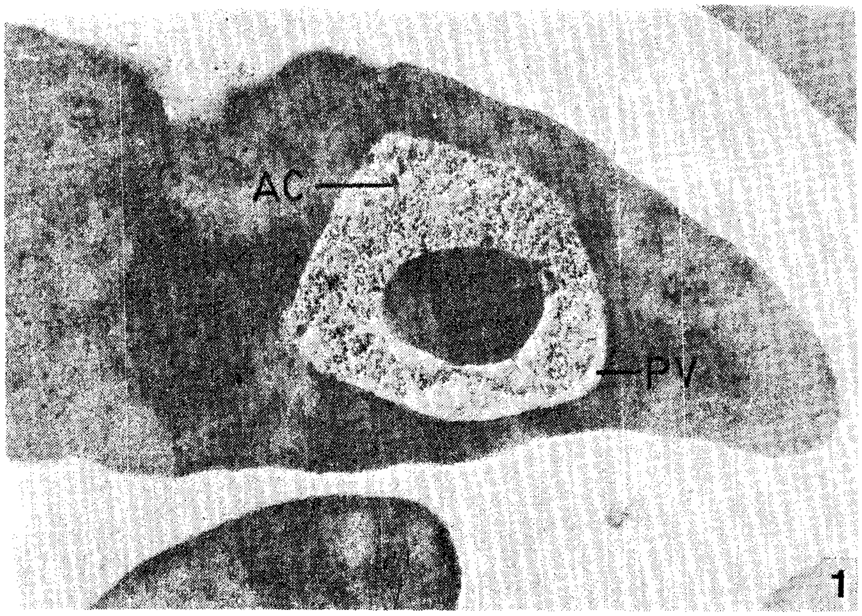
P. vivax infected blood samples were fixed according to the procedure described by Langreth *et al.* (1978). Infected blood was added slowly to a fixative consisting of 2% (v/v) glutaraldehyde, 0.12 M sucrose, 0.2 µml $CaCl_2$, 0.1 M sodium cacodylate—HCl buffer at pH 7.4. After one hour of fixation 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 hours 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

Trophozoites

A merozoite upon invading a red blood cell, changes its form and de-differentiates, losing a number of structures characteristic of this stage. Early trophozoite appears round or slightly oblong in shape (Fig. 1). Due to breakdown of inner pellicular membranes during de-differentiation process, the parasite at this stage is bounded by a thin membrane. An electron transparent parasitophorous space separating the parasite from the host cell is visible in some of the early forms. Though typical organelles of the merozoite are de-differentiated at this stage, some remnants of the apical complex of the merozoites can still be seen in the early trophozoites (Fig. 1). The cytoplasm is less dense and contains scattered ribosomes (Fig. 1), the endoplasmic reticulum is not well developed and is represented by few vesicles. As the trophozoite grows it becomes larger and amoeboid and the cytoplasm appears coarsely granular as it is filled with free and polymeric forms of ribosomes (Fig. 2). No typical mitochondria are observed and they appear as concentric membrane bound structures (Fig. 3). The trophozoite feeds on the cytoplasm of the host cell with its small cytostome which is composed of two concentric rings (Fig. 4). The membrane-bound food vacuoles thus formed have the same structure and density as the host cell cytoplasm (Fig. 5). Digestion of the host cell is indicated by the formation of electron dense malarial pigment particles within the food vacuole (Fig. 6).

Older trophozoite gets considerably enlarged. The amoeboid movement of trophozoite produces a tortuous extension and invagination of the cytoplasm and parasite becomes irregular in shape (Fig. 7). In addition to numerous ribo-



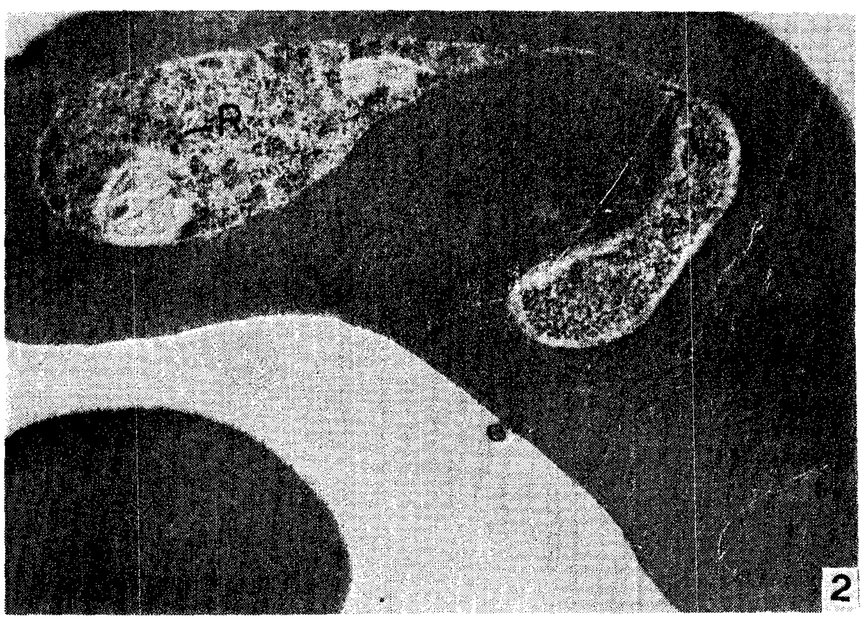


Fig. 1: Electron micrograph of early trophozoite stage of *P. vivax* showing loosely packed cytoplasm and remnants of apical complex (AC) of the merozoite. Parasitophorous vacuole (PV) formed by the entry of parasite is visible. x 17,000.

Fig. 2: Growing trophozoite of *P. vivax* showing increase in cytoplasmic ribosome (R) density. The parasite becomes irregular in shape. x 36,000.

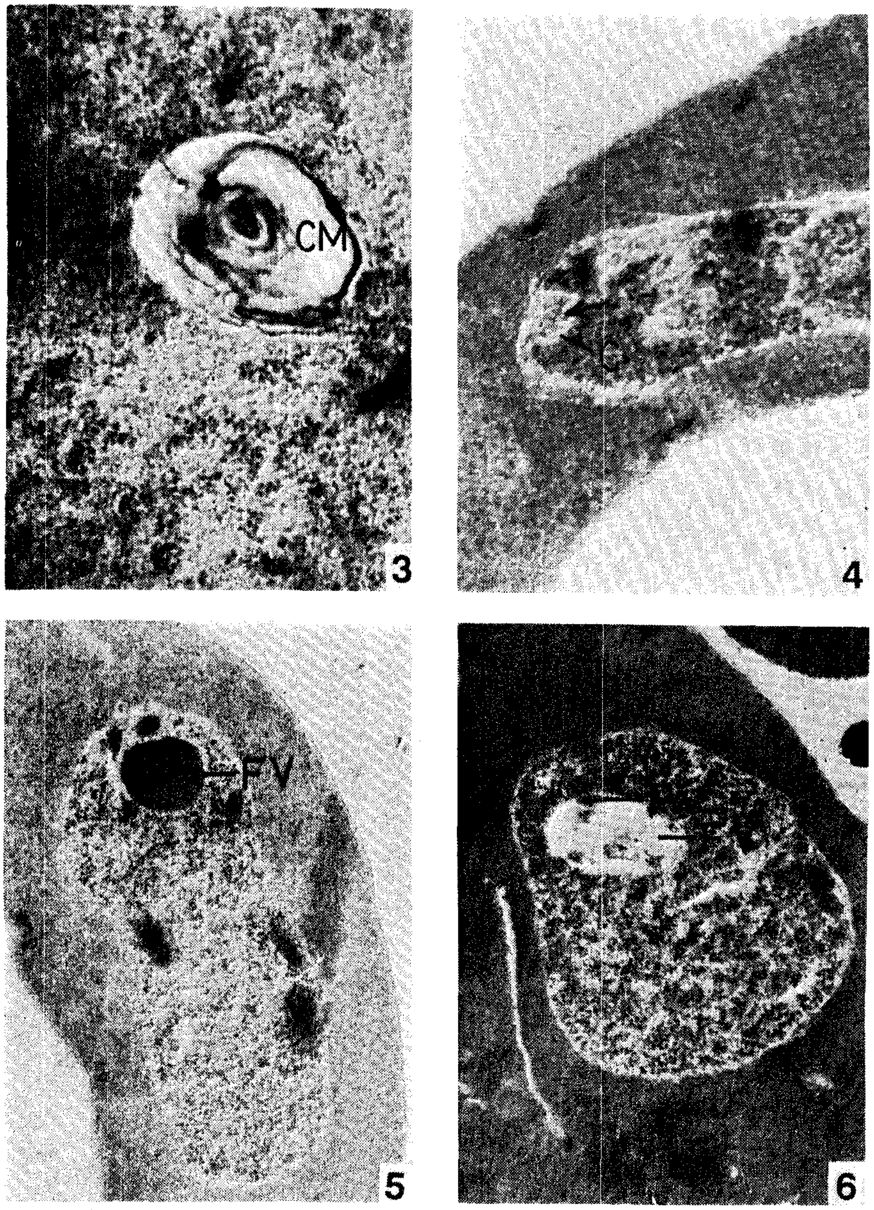


Fig. 3: High magnification electron micrograph showing concentric membrane whorls (CM) in trophozoite cytoplasm. x 60,000.

Fig. 4: Longitudinal section through a cytostome (C) of the trophozoite showing a developing food vacuole (arrow). x 47,000.

Fig. 5: Food vacuole (FV) in the trophozoite containing host cell cytoplasm. x 24,000.

Fig. 6: Trophozoite showing food vacuole (FV) with crystals of malarial pigment (P). x 27,000.

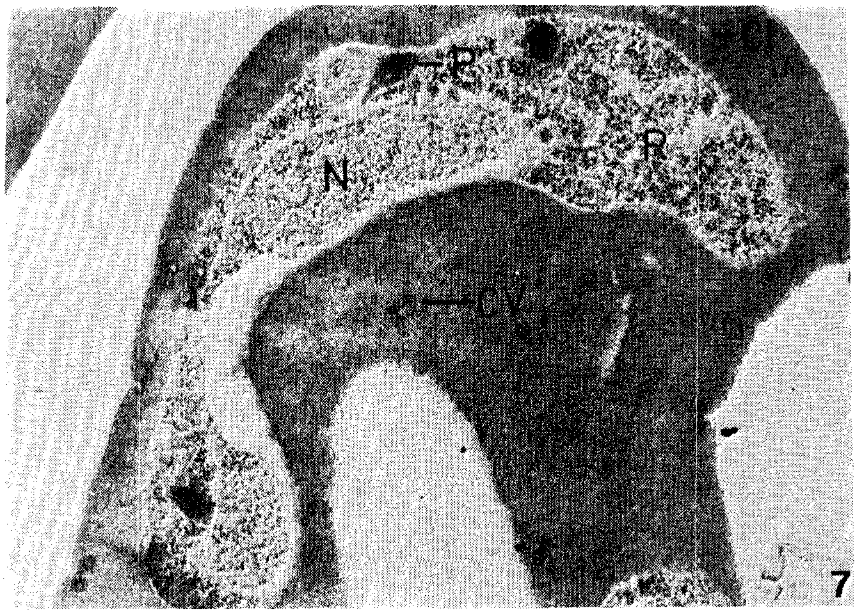


Fig. 7: Amoeboid, mature trophozoite of *P. vivax* showing large elongated nucleus (N), abundant ribosomes (R) and malarial pigment (P) in the cytoplasm. Note the caveola-vesicle complexes (CV) and cytoplasmic clefts (Cl) in the infected erythrocyte. x 25,000.

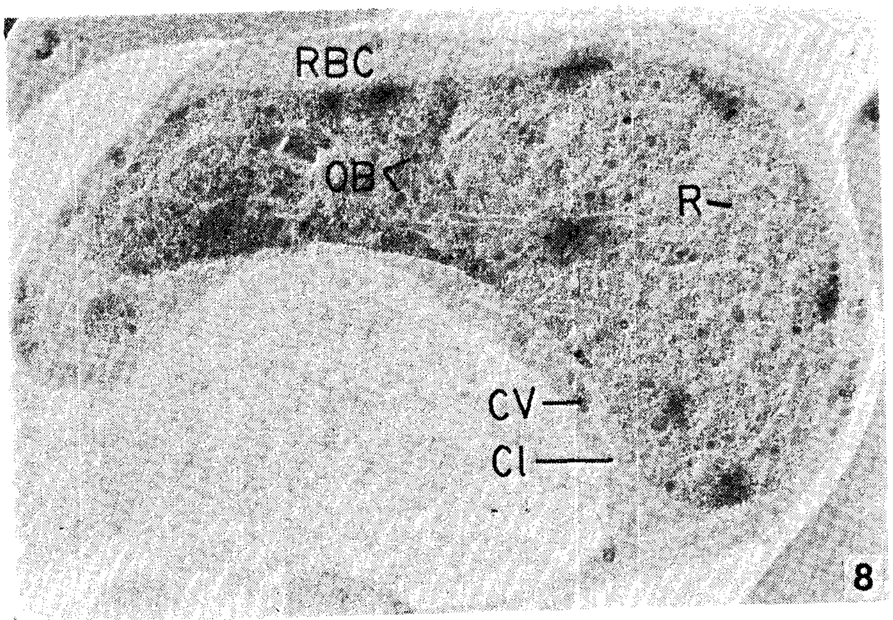
somes and canalicular endoplasmic reticulum, peripheral vacuoles containing malarial pigments are seen in the cytoplasm of the trophozoites. The trophozoite contains a single elongated nucleus often situated near the margin of the parasite cytoplasm. The nucleoplasm is composed of finely granular material and is devoid of any nucleolus-like dense area (Fig. 7).

Gametocytes

Gametocytes, the intra-erythrocytic sexual forms, develop usually after one or more generations of schizogony in the blood. Like trophozoites, the gametocytes are also uninucleate parasites and are characterized by the presence of electron dense osmiophilic bodies in their cytoplasm. Mature gametocytes are larger than the trophozoites and occupy most of the cytoplasmic area of the host cell (Fig. 8). The two gametocyte forms, macrogametocyte and microgametocyte can be differentiated on the basis of their fine structure.

The gametocytes are surrounded by two membranes but the pellicle is not very distinct as that in the gametocytes of avian and reptilian parasites (Fig. 9). No subpellicular microtubules are observed in any of the mature gametocytes. The cytoplasm of mature gametocytes is filled with ribosomes and most of them are in free form. They are more abundant in macrogametocyte whereas the cytoplasm of microgametocyte contains fewer ribosomes and is filled with fine granular material (Figs. 11 and 12). Endoplasmic reticulum of both macro- and microgametocyte is generally of rough type and is less apparent due to high ribosomal density. Cytostomal invaginations of the pellicle comprising of two concentric rings are occasionally seen in these sexual forms (Fig. 10). However, no food vacuoles are observed in the cytoplasm of the gametocytes.

The cytoplasm of both the gametocytes contains electron dense osmiophilic bodies which vary in shape. They are oval or elongated structures



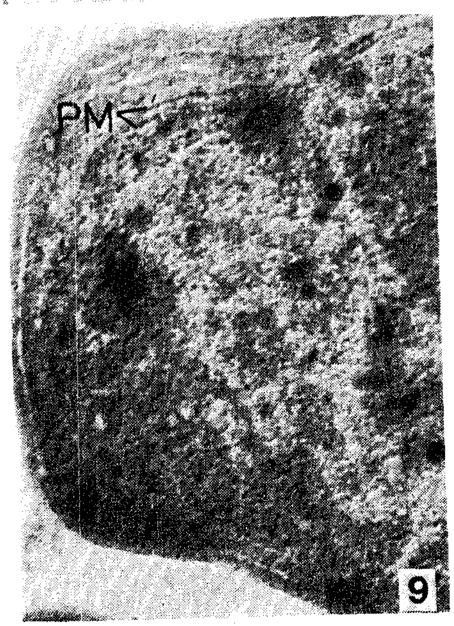


Fig. 8: Macrogametocyte of *P. vivax*. The cytoplasm contains many osmiophilic bodies (OB) and numerous ribosomes (R). The host cell (RBC) appears less electron dense and has a number of caveola-vesicle complexes (CV) and cytoplasmic clefts (Cl). x 23,000.

Fig. 9: Part of macrogametocyte showing outer and inner pellicular membranes (PM). x 37,000.

Fig.10: Part of gametocyte showing cytostome (C) comprising of two concentric rings. x 41,000.

measuring 0.2-0.3 μm in diameter. These osmiophilic bodies resemble the rhoptries and micronemes of the merozoites and are more frequently present in macrogametocyte than in microgametocyte (Figs. 11 and 12). Typical protozoan mitochondria are lacking and these are represented by double membrane-bound bodies without prominent cristae (Fig. 13).

Gametocytes have one large nucleus which often lies in peripheral position. The nuclear matrix contains uniformly scattered granules. The nucleus of microgametocyte is larger and shows moderately irregular protrusions and invaginations (Fig. 14). The macrogametocyte nucleus is comparatively small, more compact and contains a nucleolar like region but it is not so prominent as that in gametocytes of avian and reptilian parasites (Fig. 15).

Host cell alterations

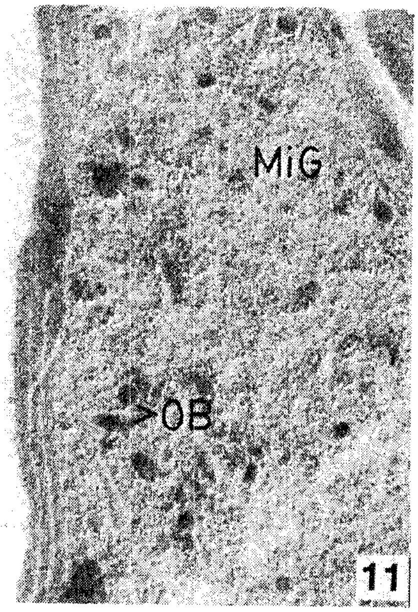
P. vivax causes great enlargement of the host erythrocyte. Parasitized red cells with larger developmental forms like late trophozoites, schizonts and mature gametocytes are 3 to 4 times larger in size than uninfected erythrocytes (Figs. 7, 8, 14, 15). In the erythrocytes having young parasites, the cytoplasm is dense but as the parasites grow and mature, the erythrocyte cytoplasm becomes less dense as if deprived of haemoglobin probably due to prolonged stay of these forms in the host cells. The parasitophorous vacuole membrane that originates from the host erythrocyte membrane during invasion of the parasite, expands with the development of parasite and is retained until the formation of next generation of merozoites.

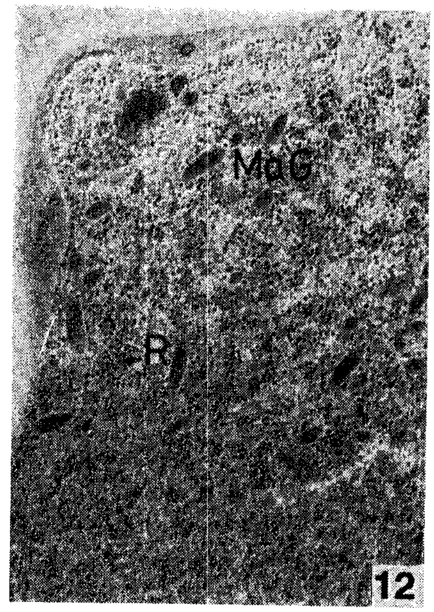
Schuffner's dots seen in erythrocytes infected with vivax-type (*P. vivax* [Vietnam strain], *P. cynomolgi* and *P. simium*) and ovale-type (*P. simiovale, P. fieldi* and *P. ovale*) malaria parasites have been demonstrated earlier by electron microscopy to be caveola-vesicle complexes along the erythrocyte plasma membrane (Aikawa *et al*., 1975; Sterling *et al*., 1975; Seed *et al*., 1976; Aikawa *et al*., 1977; Matsumoto *et al*., 1986b). In this study also, the caveola-vesicle complexes are frequently observed in infected erythrocytes. Small caveolae measuring approximately 120 nm in diameter are seen along the erythrocyte plasma membrane. These tiny infoldings are lined with unit membrane and have electron lucent lumen (Figs. 16 and 17). Each caveola is surrounded by small vesicles arranged in an alveolar fashion. These vesicles are round or slightly oblong in shape and measure approximately 80 nm in size. They are also delineated by a unit membrane and seem to be connected with caveola (Figs. 16 and 17). Most of these vesicles possess electron dense lumen while some are less electron dense. The incidence of caveola-vesicle complexes in the infected erythrocytes appears to be related to the developmental stage of the intracellular parasite. They are more frequently observed in the erythrocytes parasitized with late developmental forms.

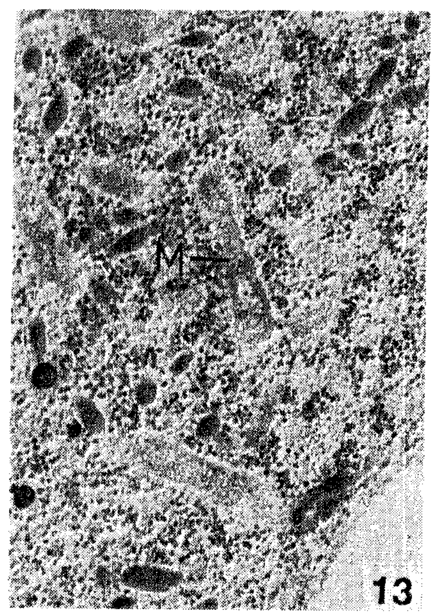
Apart from caveola-vesicle complexes, prominent tubular clefts are seen in the cytoplasm of infected erythrocytes. Their shape varies from straight, semicircular to irregular. The width of these clefts remains reasonably constant and measures approximately 40 μm and these are also lined by unit membrane on each side (Fig. 16). In some electron micrographs these clefts are seen in whorls in close proximity to the parasites. Multiple invasion of erythrocytes, characteristic of *P. falciparum* is uncommon in *P. vivax* infection. Erythrocytes harbouring two to three parasites are observed occasionally (Fig. 18).

DISCUSSION

Intraerythrocytic forms of various species of malaria parasites are extensively studied because much is known about their cultivation, *in vitro* and *in vivo*, their physiology, biochemistry and nutritional requirements. High levels of parasitaemia can be obtained using animal mode

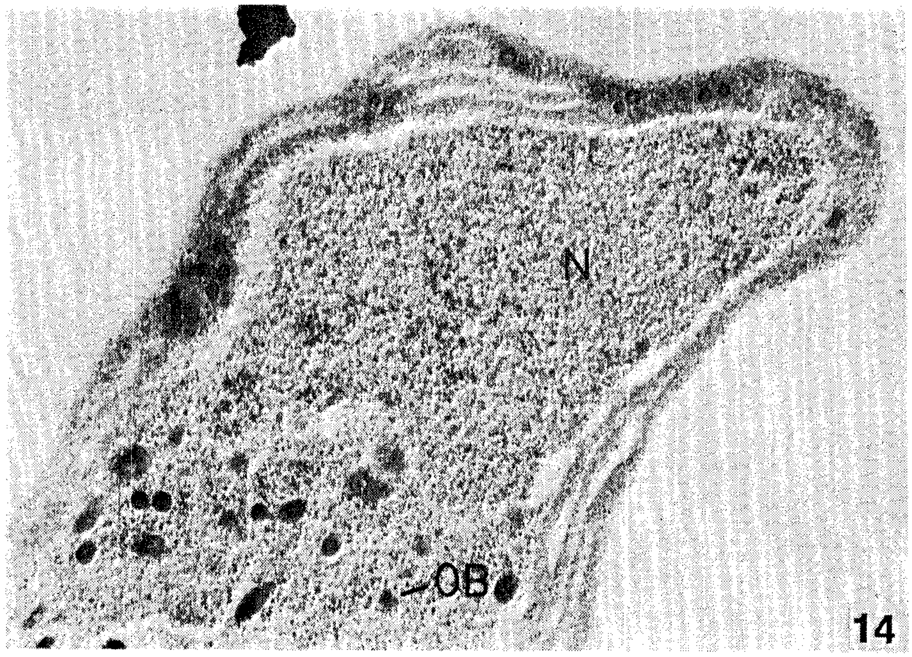






Figs. 11-12: Electron micrographs showing cytoplasm of micro- and macrogametocyte. The microgametocyte (MiG) has fewer ribosomes (R) and less number of osmiophilic bodies (OB) as compared to macrogametocyte (MaG). Fig. 11: x 48,000; Fig. 12: x 40,000.

Fig. 13: Double membrane-bound mitochondria (M) without prominent cristae are present in the gametocyte of *P. vivax*. x 40,000.



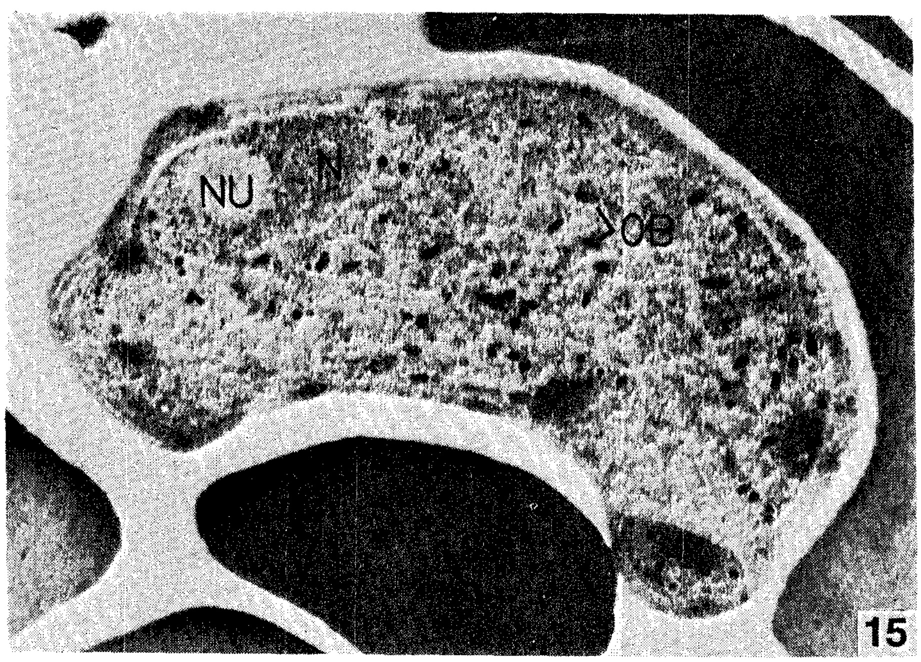
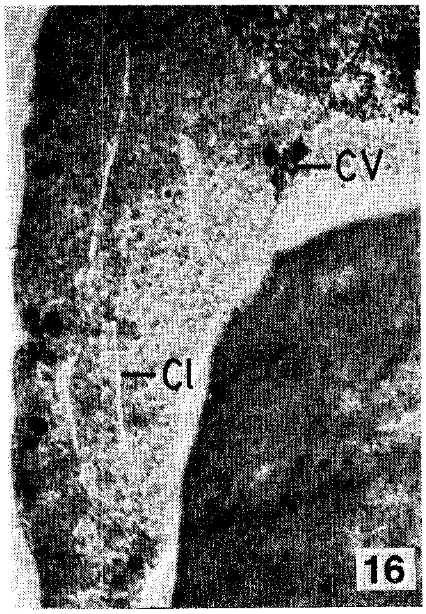


Fig. 14: Part of microgametocyte showing large diffused nucleus (N) and osmiophilic bodies (OB). *x* 39,000.

Fig. 15: Macrogametocyte showing small compact nucleus (N) with nucleolar zone (NU) and osmiophilic bodies (OB). x 19,000.



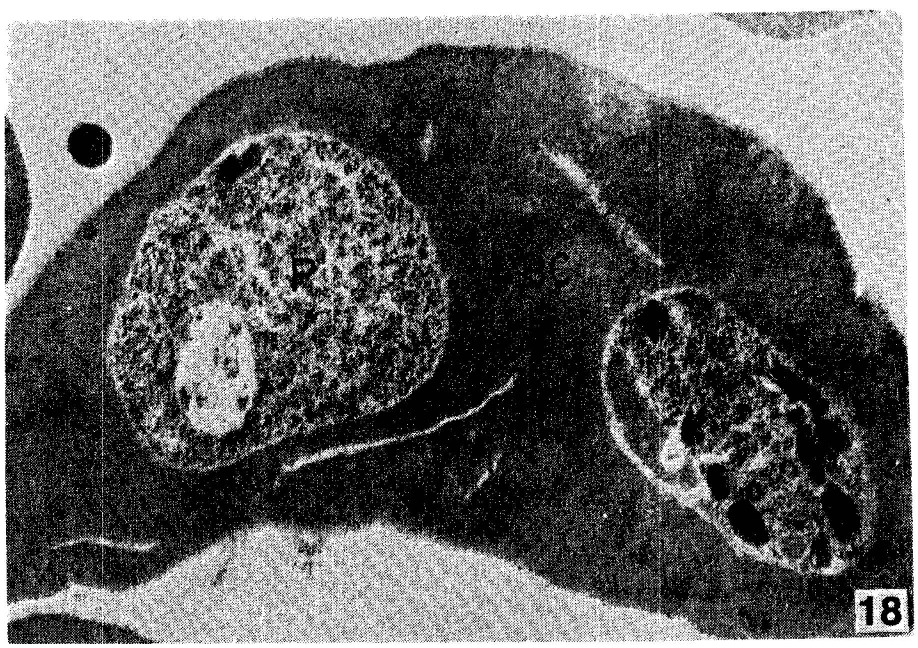


Fig. 16: *P. vivax* infected erythrocyte showing a number of caveola-vesicle complexes (CV) and narrow slit-like cytoplasmic clefts (Cl). x 32,000

Fig. 17: Cross section of a caveola-vesicle complex in *P. vivax* infected erythrocyte. Caveola (C) is surrounded by vesicles (V) in alveolar fashion. x 58,000.

Fig. 18: Multiple invasion of erythrocytes by *P. vivax*. Red blood cell (RBC) having two parasites (P). x 25,000.

which provide an opportunity to study these erythrocytic forms in detail. Numerous reports on the ultrastructure of erythrocytic stages of avian, reptilian and mammalian parasites (reviews by Rudzinska, 1969; Aikawa, 1971; 1977; Aikawa and Seed, 1980; Bannister and Sinden, 1982), indicate that the morphology of various species in their erythrocytic phase is basically the same with minor structural variations. Present study on the erythrocytic forms of *P. vivax* from natural infections had its limitations since not more than 1-2% parasitaemia was encountered in *P. vivax* infected individuals. Enrichment efforts using Plasmagel method were not very successful. Therefore, not enough number of parasites could be observed in the final preparations. Nevertheless, the ultrastructural features of the various erythrocytic forms of *P. vivax* studied are similar to those described for other malaria parasites. Young trophozoites are characterized by their simple ultrastructure because various organelles found in the merozoite are de-differentiated at this stage. The disappearance of these organelles after penetration of merozoites into red blood cell, suggests that they have specific functions that are not required for the stages concerned with growth and multiplication. Late trophozoite, a metabolically active stage, shows an increase in endoplasmic reticulum and ribosomal density which indicates increase in protein synthesis, a preparatory stage for future multiplication of the parasite. Though cytostomal ingestion of host cell cytoplasm by malaria parasites is well established (Aikawa, 1966; Aikawa *et al.*, 1967; Seed *et al.*, 1976; Langreth *et al.*, 1978; Matsumoto *et al.*, 1986a; Atkinson *et al.*, 1987), very few of them are observed in this study, therefore, possibility of food intake through pinocytotic surface vesicles cannot be ruled out.

Mature gametocytes of *P. vivax* show ultrastructural features similar to those of other mammalian parasites. The double layered pellicle of the gametocyte of *P. vivax* like in case of *P. knowlesi* and *P. cynomolgi* is less pronounced and is not so distinct as that of avian and reptilian parasites (Aikawa *et al.*, 1969). The gametocytes of avian and certain mammalian and human malaria parasites possess typical cristate mitochondria (Aikawa *et al.*, 1969; Langreth *et al.*, 1978; Matsumoto *et al.*, 1986) whereas the gametocytes of some mammalian parasites have double membrane bound structures which are considered to be mitochondrial equivalents (Rudzinska and Trager, 1968; Aikawa *et al.*, 1969; Aikawa, 1971). The gametocytes of *P. vivax* lack typical protozoan mitochondria and these are in the form of double membrane bound bodies without prominent cristae. The functional significance of cristate and acristate mitochondria in malaria parasites is still unknown, although it has been suggested that the presence of cristae is related to Krebs cycle (Aikawa, 1971).

Like in case of other mammalian malaria parasites the two gametocyte forms of *P. vivax*, when mature, can be differentiated on the basis of fine structure. The macrogametocyte of *P. vivax* has high ribosomal density and more number of osmiophilic bodies than microgametocyte. The macrogametocyte possesses a compact dense nucleus with a nucleolus-like region while a large diffuse nucleus without nucleolus is present in the microgametocyte. Studies on gametocytogenesis of *P. falciparum* have shown that significant RNA and ribosome synthesis occurs in the young gametocytes whereas in the mature male and female gametocytes there is apparently little, if any, RNA synthesis (Sinden and Smalley, 1979). This cessation of synthesis is accompanied in the macrogametocyte by the appearance of a nucleolus and maintenance of the cytoplasmic ribosome population. In marked contrast, the mature microgametocyte fails to form a nucleolus and suffers a sudden loss in cytoplasmic ribosome density. Therefore it has been suggested that the control of RNA synthesis in the two sexes is achieved by different mechanisms which coincidentally give rise to the characteristic differential sexual characters of the gametocytes (Sinden, 1982).

Small dense osmiophilic bodies present in the gametocyte resemble in structure and density with the micronemes present in merozoites and sporozoites. Aikawa *et al.* (1969), occasionally observed narrow ductules extending from osmiophilic bodies to the inner membrane of the pellicle in case of avian malaria parasites. These observations suggest that these osmiophilic bodies have a function similar to that of micronemes and rhoptries and are used in release of gametocytes from erythrocytes prior to fertilization.

It is still not clear how these sexual forms originate during the erythrocytic phase. Based on the structural similarity between the pellicle of merozoite and that of gametocyte in case of avian malaria parasites, Aikawa *et al.* (1969) proposed that gametocytes originate from the merozoites that have failed to undergo the process of de-differentiation i.e., breakdown of the inner membrane and microtubular layer of the pellicle, after penetrating the host cell. The structural and functional resemblance of the osmiophilic bodies with rhoptries and micronemes of merozoites provides further support to this hypothesis (Aikawa, 1971). On the other hand, Bannister and Sinden (1982), suggested that after red cell invasion and loss of distinctive merozoite features, some stressful stimulus like raised antibody levels, nutrient depletion or accumulation of metabolites from the intracellular microenvironment within the host cell induces a switch to the sexual pathway of development. The role of environmental factors influencing gametocytogenesis has also been suggested by several other workers (Carter and Miller, 1979; Sinden, 1983; Mons, 1985).

Regarding alterations induced by *P. vivax* in its host cell, the enlargement of parasitophorous vacuole membrane is quite apparent as the parasite grows particularly during schizogonic development. In fact the increase is even more than it is apparent since this membrane is continually being taken into the parasite with host cell cytoplasm during feeding process and is also involved in formation of cytoplasmic clefts in host erythrocytes. There are reports indicating changes in the molecular organization of parasitophorous vacuole membrane. In *P. knowlesi* freeze-fracture studies have shown that the frequency of intramembranous particles increases greatly indicating that the parasite is actively inserting new membrane components into the lining of the parasitophorous vacuole throughout the cycle (McLaren *et al.*, 1979). Cytochemical studies also indicate differences in surface charge, glycoprotein and enzyme distribution between erythrocyte membrane and vacuole membrane (Seed *et al.*, 1974; Langreth, 1977).

The caveola-vesicle complexes observed in *P. vivax* infected erythrocytes have been reported earlier in vivax-type, *P. vivax*, Vietnam strain, (Aikawa *et al.*, 1975), *P. cynomolgi* (Aikawa *et al.*, 1975), *P. simium* (Sterling *et al.*, 1975; Seed *et al.*, 1976) and ovale-type, *P. simiovale* and *P. fieldi* (Aikawa *et al.*, 1977), *P. ovale* (Matsumoto *et al.*, 1986b), malaria parasites. Caveolae without vesicles have been reported in *P. coatneyi, P. knowlesi* and *P. malariae* (Rudzinska and Trager, 1968; Wunderlich *et al.*, 1982; Atkinson *et al.*, 1987). In the present study, the caveola-vesicle complexes have been observed in all the erythrocytes infected with asexual and sexual forms though they are more frequently seen in erythrocytes having larger developmental forms like trophozoites schizonts and mature gametocytes. In addition to caveola-vesicle complexes, certain other alterations like surface blebs, excrescences and nodules have been reported in erythrocytes infected with *P. simium*, *P. simiovale*, *P. fieldi* and *P. ovale* (Seed *et al.*, 1976; Aikawa *et al.*, 1977; Matsumoto *et al.*, 1986b). However, these alterations are not seen in the erythrocytes infected with *P. vivax*. Regarding nature of these caveola-vesicle complexes, horseradish-peroxidase-labelled immunoglobulin from monkeys infected with *P. vivax* (Vietnam strain) has been shown to bind to the vesicle membrane, indicating the presence of malarial antigen in them. Incubating viable parasitized erythrocytes in case of *P. vivax*, *P. cynomolgi* and *P. simium* with ferritin has shown that the ferritin particles appear within the vesicles indicating that these vesicles are pinocytotic in origin (Aikawa *et al.*, 1975; Seed *et al.*, 1976).

Cytoplasmic clefts present in *P. vivax* infected erythrocytes have been demonstrated in erythrocytes infected by all species of malaria parasites studied so far. Regarding their origin, Rudzinska and Trager (1968) and Sterling *et al.* (1972) observed continuity between these clefts and the parasitophorous vacuole membrane in the erythrocytes infected with mammalian parasites and suggested that these structures might be derived from the membranes of the parasite. Similarly in case of *P. vivax, P. cynomolgi* and *P. falciparum* these clefts have been found extending from the parasite and surrounding vacuole membrane into the erythrocyte cytoplasm and occasionally seen fusing with the erythrocyte membrane (Aikawa *et al.*, 1975; Aikawa *et al.*, 1986). All these findings suggest that these structures are probably related to the membranes of the parasitophorous vacuole and the erythrocyte plasmalemma and could be extrusions of the parasitophorous vacuole membrane as suggested by Aikawa and Seed (1980).

Regarding functional significance of caveolae and vesicles observed in *P. simium* and *P. knowlesi* it has been suggested that the small vesicles may fuse with the host cell membrane to form caveolae or develop as micropinocytic vesicles from the host cell membrane (Sterling *et al.* 1975; Wunderlich *et al.*, 1982). These structures may provide a mechanism for the import or export of host or parasite derived proteins, independent of cytoplasmic clefts and may also play a role in the insertion of plasmodial antigens into the erythrocyte membrane. Similarly in case of *P. malariae* it is proposed that the caveolae may provide a mechanism for the intracellular transport of host and parasite derived material in the infected erythrocytes (Atkinson *et al.*, 1987). The cytoplasmic clefts present in the erythrocytes infected with *P. falciparum* and *P. malariae* are suggested to be involved in transportation of parasite derived material from the parasite plasmalemma to the erythrocyte membrane and in the formation of knobs (Aikawa *et al.*, 1986; Atkinson *et al.*, 1987). Recently, immunoelectron microscope studies in case of *P. vivax* have shown that the clefts and caveola-vesicle complexes make a contribution to the movement of some malaria proteins from the parasite to the erythrocyte surface (Matsumoto *et al.*, 1988). Further investigation is needed for understanding the precise role of these parasite-induced host cell alterations.

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Chloroquine Sensitivity of *Plasmodium falciparum* in Shankargarh Block of Allahabad District (U.P.)

P.K. TYAGI[1] and S.N. TIWARI[1]

Chloroquine sensitivity of *Plasmodium falciparum* was carried out in Shankargarh block of Allahabad district, U.P. to confirm the presence of resistant strain in the area. A total of 47 cases were subjected to *in vivo* extended test, of which resistance was detected in 11 cases at R-II/R-III level and in 9 cases at R-I level. One case out of 18 subjected to standard 7-day *in vivo* test showed resistance at R-III level. Micro *in vitro* test was done for 24 cases, of which growth was seen in 21 control samples. Out of these 7 cases were found to be resistant. The significance of the findings are discussed.

INTRODUCTION

High malaria incidence has been recorded in the rural areas of Shankargarh block for several past years. The local strain of *P. falciparum* does not respond to the usual doses of chloroquine. This might be because of development of resistance in the parasite against chloroquine. The existence of chloroquine resistant strains of *P. falciparum* has already been recorded from Shaktinagar (Mirzapur), Mathura and Terai areas of Uttar Pradesh (Sharma, 1984). The present study was undertaken to ascertain the presence of chloroquine resistant *P. falciparum* strain in Shankargarh block of Allahabad district (U.P.).

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[1] Malaria Research Centre (Field Station)
Primary Health Centre
Shankargarh-212 108, India.

MATERIAL AND METHODS

In vivo study

The study was conducted during the months of September, October, November and December, 1988 on patients from Pure Gangach and Beghel, Kasauta, Basehera, Karia Khurd, Bemera, Jilla, Joohi, Jigna and Karia Baj Bhadur villages of Shankargarh block. The test was carried out as per WHO (1973) method. Patients with acute attack of fever were selected for the test. After ascertaining that no chloroquine has been taken during the illness by taking history or examination of urine for chloroquine excretion, peripheral blood smears were made and stained with JSB (Singh, 1956). Patients showing adequate asexual parasitaemia were selected for the test. Chloroquine in total dose of 25 mg/kg body weight, equivalent to 10 tablets each of 150 mg base per adult was administered in three day. Four tablets each were given on two consecutive days followed by two tablets on 3rd day. The tablets were given after food in order to prevent vomiting. Blood smears were prepared prior to drug administration. However, on Day 2 after administering the drug in the morning blood smears were made in the evening. Eighteen patients were observed for 7 days and forty seven patients were followed for 28 days. However, in the extended study carried out during October to November 1988, only eight patients were followed up till Day 14. The parasitaemia was counted against 10,000 RBC.

In vitro study

In vitro test for drug resistance was carried out as per standard technique (WHO, 1981) in 24 strains picked up from patients attending the malaria clinic in Shankargarh and 3 residing in Kasauta and Pure Gangach and Beghel villages. 100 μl of blood drawn by finger prick from each patient was mixed with 0.9 ml growth medium and agitated gently to suspend the blood cells. 50 μl of the suspension was put in test wells containing different concentrations of chloroquine supplied by WHO alongwith the test kit and in-

Table 1. Results of *in vivo* test carried out at Shankargarh for determining chloroquine sensitivity with 1500 mg chloroquine dose

Period of test	7-Days study		
	Day 0	Day 2	Day 7
Sep. 1988	(a) 18	18	18
	(b) 18	9*	0
	(c) 100.0	50.0	0

	28-Days extended study				
	Day 0	Day 7	Day 14	Day 21	Day 28
Oct. and Nov. 1988	(a) 21	21	8	21	21
	(b) 21	7	0	5 (2)	1
	(c) 100.0	33.3	0	23.8	4.8
Nov. and Dec. 1988	(a) 26	26	26	26	26
	(b) 26	4	4 (3)	5 (3)	8 (1)
	(c) 100.0	15.4	15.4	19.2	30.8

(a) No. of cases examined; (b) No. of cases positive for asexual stage; (c) Per cent positivity.
*One case showed more than 25% asexual parasitaemia and eight cases showed less than 25% asexual parasitaemia on Day 2, complete clearance on Day 7.
Data given in parentheses indicate fresh cases.

Table 2. Results of *in vitro* test carried out in Shankargarh for determining chloroquine sensitivity during October 1988

No. of strains tested	No. showing growth in controls	MIC > 8 picomol	% Showing Resistance
24	21	7	33.3%

Remark: One showed inhibition at 64 picomol.

cubated at 37°C for 24 hours. After incubation slides were made from each well, stained with JSB and examined under oil immersion. Schizont maturation at 5.7 pmol or more was considered as an indication of resistance and the minimum inhibitory concentration (MIC) was ascertained.

RESULTS

Results of *in vivo* tests are given in Tables 1, 3, 4. Study carried out for one week revealed persistence of asexual parasites in 9 out of 18 cases on Day 2. Out of these nine cases, in one case percentage reduction in asexual parasitaemia was less than 75% i.e., R-III response whereas, remaining eight patients showed 81.2% to 99.8% reduction in parasite count, all these patients showed parasite clearance on Day7 i.e., sensitive or R-I response. Similarly extended study carried out during the months of October-November 1988 revealed lack of parasite clearance in 7 out of 21 cases on Day 7 i.e., R-II/R-III level resistance. Two patients showed reappearance of parasites on Day 21 i.e., showing R-I level of resistance. A third study carried out for 28 days during the months of Nov. to Dec., 1988 showed the persistence of asexual parasites in 4 out of 26 cases on Day7 i.e., R-III/R-II response. Asexual parasites were observed in 7 more cases on Day 14 to Day28 i.e., R-I response.

The data of micro *in vitro* test are shown in Table 2. Out of 24 strains growth was seen in 21 control samples, of these seven cases (33.3%) were found to be resistant at MIC >8 picomol.

DISCUSSION

Chloroquine is being used for malaria treatment since a long time. Its resistance in *P. falciparum* in the field was first reported from Venezuela in 1959 and later the problem rapidly spread to many other regions (Sharma, 1984). In India first report of *P. falciparum* resistant to chloroquine came from Diphu and Nowgong in Assam

Table 3. Parasite count of 7 day *in vivo* study cases

Case No.	Age (yrs)	Sex	Parasitaemia (Pf rings)/RBC x 10^6			Level of resistance
			Day 0	Day 2	Day 7	
1	13	M	2000	100	0	S/R-I
2	16	M	400	0	0	S
3	11	F	1500	100	0	S/R-I
4	25	F	2000	0	0	S
5	6	M	10200	0	0	S
6	6	F	1300	0	0	S
7	4	F	2500	0	0	S
8	20	F	9500	0	0	S
9	24	M	2600	200	0	S/R-I
10	4	F	48000	100	0	S/R-I
11	7	M	2800	0	0	S
12	20	F	200	0	0	S
13	25	F	1000	400	0	R-III
14	28	F	1200	0	0	S
15	22	M	800	100	0	S/R-I
16	38	M	1800	100	0	S/R-I
17	32	M	3200	600	0	S/R-I
18	35	M	1600	300	0	S/R-I

Table 4. Parasite count of 28 day extended *in vivo* study cases (Nov.-Dec. 1988)

Case No.	Age (yrs)	Sex	Parasitaemia (Pf rings)/RBC x 10^6					Level of resistance
			Day 0	Day 7	Day 14	Day 21	Day 28	
1	20	M	4200	0	0	0	0	S
2	36	M	3000	0	0	400	300	R-I
3	12	F	1200	300	0	0	300	R-II/R-III
4	26	M	500	0	0	0	0	S
5	17	M	2000	0	0	0	0	S
6	5	F	5000	200	0	0	0	R-II/R-III
7	10	F	2000	1000	800	*	300	R-II/R-III
8	13	F	13000	0	0	0	0	S
9	9	F	4500	0	0	3000	3000	R-I
10	13	M	1500	0	0	0	0	S
11	45	M	1800	0	0	0	0	S
12	26	M	4000	0	0	0	0	S
13	25	M	2500	0	0	0	0	S
14	22	M	3000	500	0	0	0	R-II/R-III
15	10	M	10000	0	0	0	0	S
16	30	F	2500	0	0	0	500	R-I
17	10	M	2000	0	0	0	0	S
18	16	M	3000	0	0	0	0	S
19	25	M	2400	0	0	0	200	R-I
20	10	M	2900	0	0	0	0	S
21	9	F	6800	0	0	0	0	S
22	5	M	5800	0	0	100	600	R-I
23	5	M	5300	0	0	0	0	S
24	25	F	2800	0	1000	0	0	R-I
25	3	F	8500	0	1000	3500	1000 (mixed)	R-I
26	35	M	2000	0	0	0	0	S

*Smear on Day 21 not available.

in 1973 (Sehgal *et al.*, 1973; 1974). Subsequently, resistant strains of *P. falciparum* were observed in different parts of India such as Arunachal Pradesh, Mizoram, Nagaland (Pattanayak *et al.*, 1979), Meghalaya (Chakravorty *et al.*, 1979), Orissa (Guha *et al.*, 1979), Andhra Pradesh, Madhya Pradesh (Ghosh *et al.*, 1981), Maharashtra (De *et al.*, 1979; Chowdhury *et al.*, 1981) and Haryana and Delhi (Choudhury *et al.*, 1983). In Uttar Pradesh, Dwivedi *et al.* (1981) first reported 33.3% resistance of R-II level in Shaktinagar area of Mirzapur district. Similarly Chowdhury *et al.* (1981) recorded the persistence of asexual stages of parasites on Day 6/7 in 248 cases out of 9191 cases studied in Terai area of U.P. Likewise Dwivedi *et al.* (1978) noticed one case either sensitive or resistant of R-I level in Mathura area of U.P. Two sets of extended *in vivo* studies carried out in present investigations revealed 23.4% R-III/R-II level resistance and 19.15% R-I level resistance. Similarly, *in vitro* test showed 33.3% resistance at MIC >8 picomol. The factors responsible for appearance of resistant strain of *P. falciparum* in Shankargarh area may be many but probably inadequate dosage of chloroquine is the most important cause.

The Indian strains of *P. falciparum* showed good response to chloroquine and a single dose of 600 mg was very effective. Later on this was followed by 1200 mg in two equally divided doses and presently total dose of chloroquine for treatment of *P. falciparum* infection is 1500 mg in two doses of 600 mg on Day 0 and 1 and 300 mg on Day2. It is possible that large scale inadequate use of chloroquine under NMEP might have resulted in selection of already existing mutant gene for resistance due to extensive drug pressure.

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Quartan Malaria—An Investigation on the Incidence of *Plasmodium malariae* in Bisra PHC, District Sundargarh, Orissa

R.S. YADAV[1], V.P. SHARMA[2], S.K. GHOSH[1] and A. KUMAR[1]

A longitudinal study on the incidence of *P. malariae* was taken up from September 1988 to December 1989 in Bisra block, District Sundargarh, Orissa covering 38,615 population, which is mainly tribal. The area is a known hard-core malarious region in the Garhjat hill range in eastern India. In this study, out of 22,217 blood smears examined through weekly active surveillance, 7362 (33.1%) were found malaria parasite positive. Out of the total positive cases, 82 (1.1%) were *P. malariae*. These occurred mostly (91.4%) in persons below 40 years of age and children below 9 years accounted for 36.6% of total quartan malaria cases. In this age group the disease was found to be associated with splenomegaly (average enlarged spleen 2.07; spleen rate 45.9%) and 9 out of 13 mixed infections of *P. malariae* with *P. falciparum* and/or *P. vivax* were detected from this age group. This is the first report of quartan malaria from this area.

INTRODUCTION

Quartan malaria is caused by *Plasmodium malariae*. The course of the disease is mild but, it is notorious for its long persistence in the blood, sometimes for the entire life time of the infected person (Garnham, 1980). It has been reported to cause nephrosis in most of the young children. Parasitaemia in *P. malariae* carriers is usually very low and at submicroscopic level it has been posing a constant threat of post-transfusion quartan malaria (Bruce-Chwatt, 1974; Loban and Polozok, 1985). The geographical range of quartan malaria extends over both tropical and subtropical areas, especially West and East Africa, Guiana and parts of India, but its presence in various zones tends to be patchy (Bruce-Chwatt, 1986). It exhibits a local and clustered pattern; its proportion is usually low and *P. malariae* is not as prevalent as *P. vivax* or *P. falciparum* (Loban and Polozok, 1985).

In India information on distribution of human malaria parasites, including *P. malariae*, was reported by Knowles *et al.* (1930). Covell and Singh (1942) reported the presence of *P. malariae* in the coastal belt of Orissa from Chilka lake to Gopalpur. Later Singh *et al.* (1952) compiled the information on distribution

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[1] Malaria Research Centre (Field Station)
N-8, Area 7 & 8, Civil Township
Rourkela-769 004, India.

[2] Malaria Research Centre
22-Sham Nath Marg
Delhi-110 054, India.

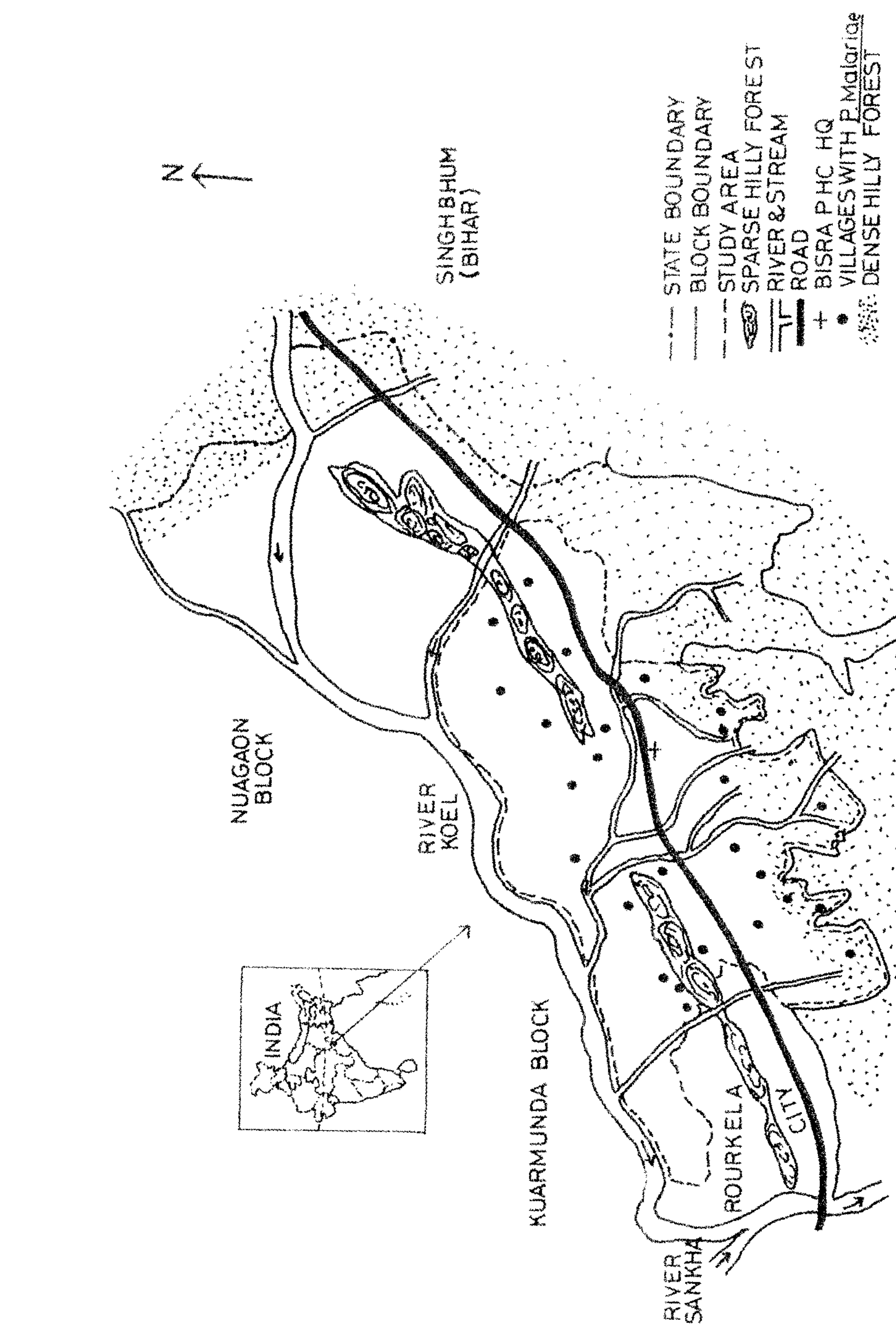

Fig. 1 : Distribution of *P. malariae* in Bisra PHC villages.

of human plasmodia in various states of India from 1931-1950. After a long gap Raichowdhuri *et al.* (1979) reported a solitary case of mixed infection of *P. falciparum* and *P. malariae* from Alwar in Rajasthan and Beljaev *et al.* (1985; 1987) reported the presence of *P. malariae* from passive blood smear collection from different Primary Health Centres in Phulbani, Sambalpur and Mayurbhanj districts of Orissa. Latter authors observed that *P. malariae* is practically not reported from Orissa nowadays but this species is by no means very rare. In the course of a longitudinal epidemiological study on malaria during the implementation phase of a project on Integrated Disease Vector Control in the tribal area of Bisra block in District Sundargarh, Orissa we came across several cases of *P. malariae*. As the detailed epidemiological information on the incidence of this species was lacking, the present study was conducted in Bisra PHC villages through a longitudinal survey.

MATERIAL AND METHODS

Geography and demography of the study area

The study was launched in September 1988 in 10 villages of Bisra block having 8000 population and was extended in January 1989 to cover a total of 39 villages having a population of 38,615. In the present report the results obtained upto December 1989 have been included. Bisra is one of the 17 Blocks of District Sundargarh which lies between 21^{O}35' and 22^{O}32' north latitude and between 83^{O}32' and 85^{O}22' east longitude. It is bounded on the north by Bihar, on the west by Madhya Pradesh, on the south by the district of Sambalpur, and on the east by Keonjhar district. It is one of the northern most districts of the highly malarious Orissa state (Fig. 1). The area presents ideal ecological conditions for malaria transmission resulting in meso- to hyperendemicity. It has a typical geographical picture of undulating upland intersected by forested hills, rocky streams, rivers, paddy fields and springs. The villages have mostly *kuccha* houses, either thatched or with tiled roofs and consist of clusters of hamlets which are often distantly located in the hilly terrain. The area has sub-humid to tropical savannah type climate with an annual rainfall between 160-200 cm (Sinha, 1981). More than 51% population is tribal, literacy rate is 36%, and although the economy is essentially agriculture and forest based, the Rourkela Steel Plant, its ancillary and other industries and mining operations do provide some supportive employment (Anonymous, 1980; 1981).

After conducting a baseline demographic survey in all the study villages of the project area, door-to-door surveillance of the fever cases was conducted on weekly basis throughout the study period from September 1988 to December 1989 by employing resident trained surveillance workers. Thick and thin blood smears of the fever cases were prepared using standard procedure. Slides were brought daily to the field station laboratory in Rourkela, stained with JSB stain and examined under oil immersion. Patients having *P. malariae* or *P. vivax* infections were treated promptly with a single dose of 600 mg chloroquine base (adult dose) and in the latter case primaquine at the dose of 15 mg daily for 5 days was also administered. Children received proportionate doses, whereas pregnant women and infants were not given primaquine. *P. falciparum* cases were treated with 600 mg chloroquine base plus 45 mg primaquine (adult dose) on Day 0 and 600 mg and 300 mg chloroquine based on Day 1 and 2, respectively.

A baseline spleen survey was also conducted in November 1988 among children (2-9 years) and the classification of spleen sizes in cases of splenomegaly was made according to Hackett's method as described by Bruce-Chwatt (1986). Blood smears of all the children were also examined for the presence of malaria parasites.

Parasite density of all the *P. malariae* cases was also recorded by counting the parasites against leucocytes in 200 fields of thick film (criterion: 8000 WBC per μl blood.)

RESULTS AND DISCUSSION

Results of the epidemiological investigations with special reference to *P. malariae* incidence have been presented in Table 1. A total of 22,217 blood smears were collected, out of which 7362 (33.1%) malaria parasite positive cases were recorded. Among the total malaria cases there were 5654 (76.8%) *P. falciparum* cases, 1387 (18.8%) *P. vivax* and 253 (3.4%) mixed infections. *P. malariae*, including the cases of its mixed infections with *P. vivax* and/or *P. falciparum*, accounted for 1.1% of the total cases; ranging between 0.2% of the total cases in October (wet season) and 3.2% in January (dry cool season). Nevertheless, the Annual Parasite Index for *P. malariae* was 2 in the year 1989. By

Table 1. *P. Malariae* **incidence in experimental villages of Bisra PHC**

Months	Blood smear examined*	Positive cases	*P. malariae* cases				Total	Slide malariac rate	Per cent Pm cases in total cases
			Pm	Pv+Pm	Pf+Pm	Pv+Pf+Pm			
Sep 1988	688	258	5	0	0	0	5	0.73	2.0
Oct	611	360	0	0	1	0	1	0.16	0.2
Nov	646	395	3	0	0	0	3	0.46	0.7
Dec	435	268	4	0	1	0	5	1.15	1.8
Jan 1989	1176	490	16	0	0	0	16	1.36	3.2
Feb	1153	288	5	0	0	1	6	0.52	2.0
Mar	1393	291	0	1	4	0	5	0.36	1.7
Apr	1138	267	7	0	0	1	8	0.7	2.9
May	1483	261	3	0	0	0	3	0.2	1.1
Jun	1430	212	2	1	0	2	5	0.35	2.3
Jul	2577	541	3	0	0	0	3	0.12	0.5
Aug	2774	739	6	0	0	0	6	0.21	0.8
Sep	2077	573	2	0	0	0	2	0.09	0.3
Oct	1675	786	5	0	1	0	6	0.36	0.7
Nov	1609	892	3	0	0	0	3	0.18	0.3
Dec	1365	741	5	0	0	0	5	0.37	0.7
Total	22,230	7362	69	2	7	4	82	0.36	1.11

* Population surveyed : Sep. to Dec.'88 = 8001; Jan. to Dec.'89 = 38615.

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

and large *P. falciparum* was the most prevalent species followed by *P. vivax* and *P. malariae*. During the entire study period a total of 82 *P. malariae* cases including 13 cases of mixed infections were recorded. Out of those 82 cases, 53 cases (64.6%) were found gametocyte positive. This type of situation of low proportion of *P. malariae* corresponds to the situation in Tanganyika (Africa) and among the imported malaria cases in USSR between 1963-73 (Loban and Polozok, 1985).

There was no apparent sex difference in the incidence of *P. malariae*, however, age did matter, hence, age-specific classification of the data has been presented in Table 2. Children below 9 years age who accounted for 25% of the total population under study, contributed 36.6% of the total cases and 37.7% of the total gametocyte positive cases. Similarly persons below 40 years age accounted for 91.4% (75 cases) and 96.2% (51 cases) of the total cases and gametocyte positive cases, respectively. Thus, in all probability quartan malaria was experienced most by children below 9 years and these findings are in agreement with the observations made by earlier workers in Africa. Since in the study area transmission is perennial, incidence above the normal among young children appears to be due to low immunity, whereas less malaria incidence beyond 40 years of age is most likely due to rise in immunity with age. Agewise details of 13 cases of mixed infections were interesting:

P. malariae + *P. falciparum* + *P. vivax*	(4 cases) : Aged—2, 2, 3, 6 years
P. malariae + *P. falciparum*	(7 cases) : Aged—2, 3, 5, 6, 8, 14,26 years
P. malariae + *P. vivax*	(2 cases) : Aged—25, 30 years

Table 2. Age-specific incidence of *P. malariae*

Age groups (years)	Population	No. of *P. malariae* cases	No. of *P. malariae* gametocyte positive cases	Cases (all stages) per 1000 population
≤ 1	983	1	1	1.01
2-4	3267	12	9	3.67
5-9	5735	17	10	2.96
10-14	4760	4	3	0.84
15-19	3996	11	6	2.75
20-29	7178	18	11	2.5
30-39	5355	12	11	2.24
40-49	3747	2	1	0.53
≥ 50	3594	5	1	1.39
Total	38,615	82	53	2.12

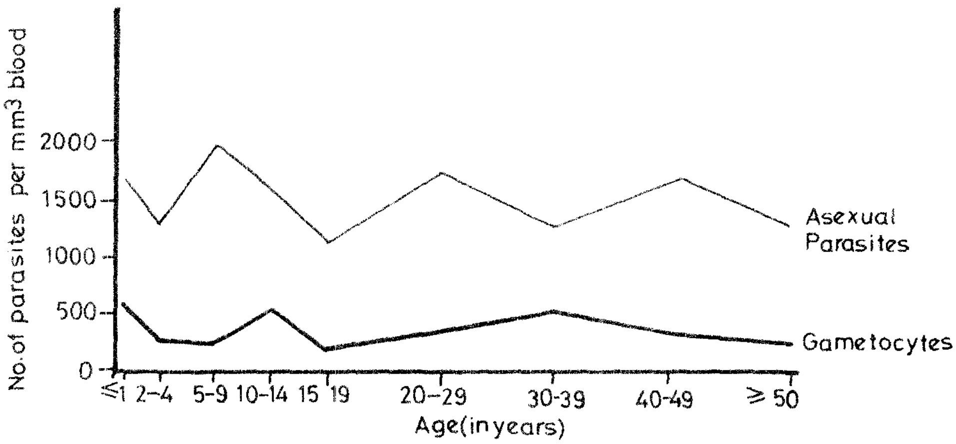

Fig. 2 : Age specific average parasite density of *P. malariae* cases in Bisra PHC villages.

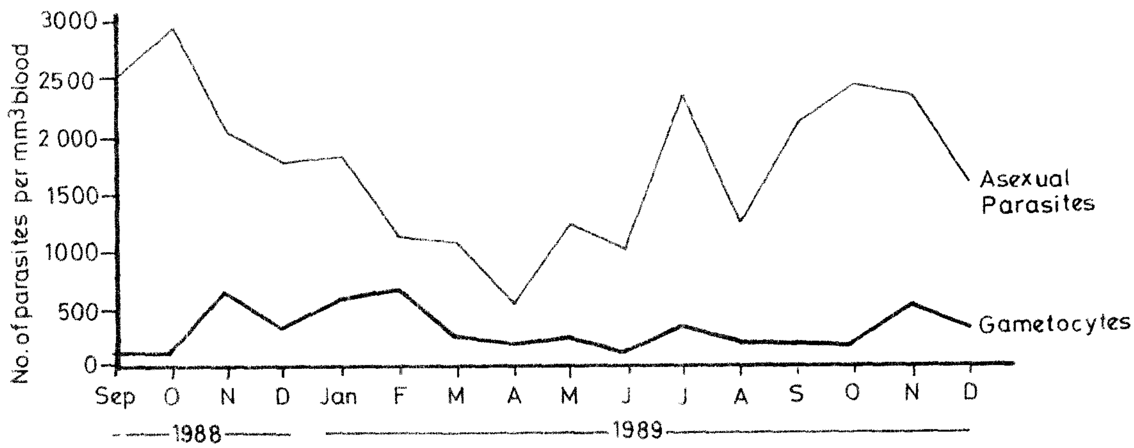

Fig. 3 : Monthly average parasite density of *P. malariae* cases in Bisra PHC villages.

It was, therefore, noteworthy that out of 13 cases, 9 cases were below 8 years age while the remaining 4 cases were 14, 15, 26 and 30 years old. Thus, mixed infections were mainly found in young children and were not observed in patients above the age of 30. Prevalence of mixed cases can be explained by the fact that the study area had a high *P. falciparum* incidence with moderate *P. vivax* incidence. Beljaev *et al.* (1987) also recorded one case each of *P. malariae* + *P. vivax* and *P. malariae* + *P. falciparum* + *P. vivax* infection from Orissa. No case of mixed infection of *P. malariae* and *P. falciparum* was observed by them as against 7 cases recorded in the present study, although in areas with *P. malariae* and *P. falciparum* incidence like west Africa mixed infections are not very rare (Molineaux and Gramiccia, 1980).

Age specific average asexual parasite/gametocyte densities of the positive cases have been projected in Fig. 2, which exhibit a typical pattern observed in meso- to hyperendemic areas. Highest parasite density was recorded in an infant aged 1 year. Asexual parasitaemia decreased in the age group of 2-4 years, increased in 5-9 years age group, again decreased upto 19 years of age, further showed a sharp increase in the age group of 20-29 years and thereafter decreased and stabilized in cases beyond 30 years. Gametocytaemia also behaved similarly but at long age intervals, possibly as a result of the equilibrium set off by acquired immunity in different exposure groups (Macdonald, 1957). In 13 cases (15.8%) out of 82 patients of quartan malaria, parasite density was below 500 per cu. mm. blood; in 14 cases (17.1%) it varied between 501 to 1000, in 20 cases (24.4%) it ranged between 1001 to 2000; in another 20 cases (24.4%) the range was between 2001 to 3500 parasites. Twelve cases (14.6%) showed parasite density between 3501 to 5000 and only in 3 cases (3.7%) it was between 5300 and 6400. As regards gametocyte density, out of 53 patients, 32(60.4%) had density below 500 per cu. mm; 14(26.41%) had between 501 and 1000 and remaining 7 cases between 1001 and 2050. Thus, parasitaemia was usually moderate or low but never exceeded 6400. The observation that parasite count in quartan malaria rarely exceeds 10,000 per µl blood (Bruce-Chwatt, 1986) has been found to hold good for this area as well.

Monthly average parasite densities have been shown in Fig. 3. Highest density of asexual parasites was recorded in the month of October and that of gametocytes in November. Asexual parasite density declined slightly in November but remained static at high level throughout the dry cool period from November to January. It further declined in February and was low in hot season (March-June). After the onset of monsoon in mid June there was an increasing trend which peaked in October. Though gametocyte density was very low in October it was quite high from November to February, thereby suggesting that this was the most suitable period for transmission of *P. malariae*. High slide malariae rate in December and January in the face of a long incubation period also suggest that November/December is the most conducive period for transmission when temperature and humidity are also quite favourable.

Results of the spleen survey have been given in Table 3, 45.94% children (range: 17.1 to 100% in different villages) were found to have enlarged spleens. Among the children with splenomegaly, parasite rate was 48.2%. Although a major proportion of the cases was accounted for by *P. falciparum* and *P. vivax*, four children were found to have *P. malariae* infection associated with grade 3 spleen extending upto umblicus. This indicates chronic infection caused by *P. malariae*. Although *P. malariae* is not a major cause of concern in this area, it has been reported to cause death in children due to nephrotic syndrome, as this species causes deposition of immunoglobulins (produced in response to *P. malariae* antigens) on the capillary walls of the renal glomeruli leading to glomerular sclerosis and tubular atrophy (Hendrickse *et al.*, 1972). In a study in west Africa, children with nephrosis were found to have quartan malaria as well, and *P. malariae* was rare in children without nephrosis. A detailed study on renal involvement in quartan malaria in this area is necessary and the same is under progress.

An analysis of the surveillance data of the last 35 years (collected by National Malaria Eradication Programme) revealed that the district which has 13.3 lakh population has been highly endemic for malaria. During the DDT era incidence was low, however, since 1973 the API has fluctuated between 11 and 29. Since 1965, 80 to 90% cases have been contributed by *P. falciparum*, the remaining being *P. vivax*, with no report of *P. malariae*. Knowles *et al.* (1930) reported that the proportion of *P. malariae* in total malaria cases in India was about 7% in 1930 which ranged between 0 and 39.1% in various states. After twenty years i.e., in 1950 there was no gross overall change in its distribution as well as in proportion which was at about 6% (Singh *et al.*,

Table 3. Baseline information on splenomegaly in children (2-9 years) in villages adjoining Bisra block

Month	No. of children examined	Spleen class	No. of children in each category	Spleen rate % (Range)	Average enlarged spleen (AES)	No. of blood smears examined	Positive cases					Child parasite rate (%)	Confidence interval at 95% probability level
							Pm	Pv	Pf	Mix	Total		
Nov 88	370	Enlarged (Grade: I to V)	170	45.94 (17.1-100)	2.07	170	4	7	68	3	82	48.2	43-57%
		Normal (Grade 0)	200	0	0	200	0	0	17	0	17	8.5	6-14%

1952). However, as per the annual reports of National Malaria Eradication Programme (NMEP) for the years 1965, 1976 and 1987 the only species recorded in India through regular surveillance were *P. falciparum* and *P. vivax* (Ray *et al.*, 1989).

Absence of *P. malariae* was not quite unexpected, as because of its low reproduction rate and long extrinsic developmental period in vectors it was the species which was expected to die out first under the extensive DDT spray coverage against mosquitoes since 1958 under NMEP.

This probably did happen in most parts of the country except for the hard-core areas, like the present one, where malaria eradication programme could not be executed effectively. As against the proportion of *P. malariae* in all malaria cases in Orissa in 1930 and 1950, which was 1.2 and 4% respectively, in Bisra block it is now 1.1%, indicating no gross deviation. But the fact, that it has not been recorded under surveillance system, may be explained by the reason that either the species has been misdiagnosed as *P. vivax* or *P. falciparum* or missed completely either due to low parasitaemia or cursory examination of blood smears.

Based on passive slide collection from PHCs of Sambalpur and Mayurbhanj districts, Beljaev *et al.* (1987) observed that *P. malariae* was less common in Garhjat hills (which also covers Sundargarh district). However, we observed that it is quite common and in fact it was recorded from majority of the villages during our study in Bisra (Fig. 1), and was also recorded during a survey in iron ore mines located in hills about 100 kms south of Rourkela city.

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Relapse Pattern of *Plasmodium vivax* in Kheda District, Gujarat

R.C. SHARMA[1], A.S. GAUTAM[1], V. ORLOV[2] and V.P. SHARMA[3]

A study on relapse pattern of *P. vivax* in Kheda district of Gujarat revealed that the relapse rate in *P. vivax* within 8 months of primary attack was around 40% in untreated cases. It was 2.6% within one year in patients treated with 5-day course of primaquine. Relapses occurred more frequently from April to October and 82% relapses occurred within one year of the primary attack. Relapses occurred upto 4 years after primary attack but they were less frequent in 3rd and 4th year.

INTRODUCTION

Plasmodium vivax has inherent problems because of its mechanism of relapse. It has not developed resistance to 4-aminoquinolines but the parasite survives through the relapse mechanism.

Primaquine (8-aminoquinoline) is used against relapses in *Pv* cases but administering it daily for 5 days as required by National Malaria Eradication Programme (NMEP) poses operational difficulties. The drug is toxic and therefore not given to infants and pregnant women. Patients who recover from clinical attack invariably resent taking drug and are non-cooperative. Moreover, if the patient lives in an endemic area where transmission continues and reinfection is likely, treatment with primaquine has little value and is generally not recommended (Bruce-Chwatt, 1986). It has also been observed that primaquine may give a very high percentage of radical cure in vivax malaria in certain areas but not in others perhaps due to strain variations (Pampana, 1963).

Present study is an attempt to study the relapse pattern of *P. vivax* malaria in Kheda district where bioenvironmental disease vector control strategy has been successful in controlling malaria (Sharma, 1984; Sharma *et al.*, 1986; Sharma and Sharma, 1986).

MATERIAL AND METHODS

Weekly surveillance was done by door-to-door visits by a resident surveillance worker of each village. All fever cases and those having history

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[1] Malaria Research Centre (Field Station)
Civil Hospital
Nadiad-387 001, India.

[2] Martsinovsky Institute of Medical Parasitology and Tropical Medicine
Pirogovskaya 20
Moscow-119 435, USSR.

[3] Malaria Research Centre
22-Sham Nath Marg
Delhi-110 054, India.

of fever between the day of surveillance and last visit were given presumptive treatment of 600 mg chloroquine (adult dose) and a blood smear was prepared for examination in the laboratory. Blood smears were stained with JSB stain and examined within 24 to 48 hours.

All *Plasmodium vivax* cases occurring between January 1984 and June 1989 were given 600 mg chloroquine single dose and 15 mg primaquine daily for 5 days as adult dose, except those cases between April to October 1988, to whom only 1500 mg chloroquine (600 mg on Day 0, 600 mg on Day 1 and 300 mg on Day 2) was given. Same regimen was given in all relapse cases as well. Antimalarial drugs were administered by the worker in his presence. Patients not receiving primaquine were followed every fortnight from

Table 1. Relapse rate in *P. vivax* given 5-day anti-relapse treatment (chloroquine + primaquine)
(Results of follow-up upto June 89)

Year	Primary attack	Relapse within			
		I year	II year	III year	IV year
1984	77	0	0	0	1
1985	99	4 (4.0%)	0	0	0
1986	408	17 (4.2%)	3	1	
1987	894	17 (1.9%)	4		
1988	42*	2 (4.8%)			
Total	1520	40 (2.6%)	7	1	1

*Upto March 1988.

Table 2. Relapse rate in *P. vivax* treated with 1500 mg chloroquine only

No. of relapses										Per cent
Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	relapses
(29)	0	5	2	1	1	1	1	0	11	37.9
	(30)	1	6	1	2	2	0	0	12	40.0
		(35)	4	3	2	1	0	0	10	28.6
			(36)	1	5	1	0	0	7	19.4
				(28)	2	0	1	1	4	14.3
					(75)	1	2	2	5	6.7
						(31)	0	1	1	3.2
									50	18.9

Total cases = 264; Relapse cases = 50; % relapses = 18.9.
In parentheses are the number of cases of primary attack.

April to December 1988 and blood smears were prepared for examination.

RESULTS AND DISCUSSION

Table 1 summarises data collected between January 1984 and March 1988 from all *P. vivax* cases that were considered as cases of primary attack, who received 15 mg primaquine daily for 5 days alongwith 600 mg chloroquine as adult dose. From 1520 cases of primary attack a total of 40 (2.6%) cases relapsed within one year of primary attack.

Table 2 gives data of *P. vivax* cases given only 1500 mg chloroquine in divided doses. These cases were followed at fortnightly intervals for parasitaemia. From a total of 264 cases of primary attack between April and October 1988 there were 50 (18.9%) relapses. Relapse rate of 18.9% would have been higher had all the cases been followed for a period of one year.

Table 3 indicates that the highest number of relapse cases were recorded between April and October and further reveals that 7.8% of the patients contracting vivax infection in the months from January to April and 2.6% of those contracting infection in remaining months of the year, experience relapses. Sinha *et al.* (1989) in Hardwar observed that persons who had acquired infection during the month of September yielded maximum relapse. In the paper authors gave only the number of relapses and not the number of patients who contracted infection in September. Another reason of difference could be the presence of a different strain in Hardwar area.

To prevent relapses of vivax malaria in semi-immunes various regimens of primaquine ranging from 5 to 14 days have been administered. The success of these depends upon the strain of *P. vivax* as well as upon immune status of the patient. In India, for example, the 5-day course is probably followed by a 10% relapse rate (Wallace, 1980). In the present study with 5-day course of primaquine observed relapse rate was 2.6% (Table 1). This lower rate may be because of the fact that the patients were not followed actively as the relapse cases were detected during routine weekly surveillance. Because relapses can be clinical or simply parasitic (asymptomatic parasitaemia) the chances of missing some of the cases can not be ruled out. Other factors which influence detection of relapse cases are socio-economic conditions, immune status of the community, periodicity of surveillance and population migration.

Singh *et al.* (1953) reported relapse rate of 5% in 20 patients of *P. vivax* from Delhi who were treated with primaquine @ 10 mg base daily for seven days. In Tamil Nadu, of the 1889 vivax cases treated with 15 mg primaquine for five days, none was found positive up to one year on monthly follow-up in 7 divisions of the state. However, relapse rate of 26.7% (4 out of 15) in Pattukottai division and 17.1% (6 out of 35) in Thanjavur division were reported in a monthly follow-up for a period of one year (Roy *et al.*, 1979). These differences could be due to small sample size and differences in the methodology and periodicity of follow-up.

Garnham (1980) mentioned that relapses occur in infections in which the parasitised corpuscles are stippled with Schuffner's dots. In our own observation out of 345 blood smears positive for *P. vivax*, 291 showed stippling. Thus 84% of *P. vivax* infections should be of relapsing type. Therefore the parasite strain prevalent in Kheda district may have high relapsing characteristic and needs to be studied further.

During the period of study, slide positivity rate of *P. vivax* ranged between 0.40 and 1.22%, vector density was low, human blood index (HBI) did not exceed 2%, infant parasite rate was zero from January 1984 to March 1988 except for 1987 when it was 0.036% for the year, indigenous transmission was at a very low ebb as

Table 3. Relapse pattern of *P. vivax* treated with primaquine

Months	Primary attack	Month of relapse													Percentage
		J	F	M	A	M	J	J	A	S	O	N	D	Total	
Jan	32					1								1	3.12
Feb	33					1			1				1	3	9.09
Mar	52						1		1	1		1	1	5	9.61
Apr	75						2	2	1				1	6	8.00
May	115				1				1	1	1			4	3.48
Jun	133									2				2	1.50
Jul	142									1	1		1	3	2.11
Aug	154				1	1	2				2			6	3.90
Sep	264			1	1	2				1				5	1.89
Oct	289	1			1			1	1	1				5	1.73
Nov	199				1		2	1	1					5	2.51
Dec	32		1	1			1				1			4	12.5
Total	1520	1	1	2	5	5	8	4	6	7	5	1	4	49	
% relapse out of 1520 cases		0.07	0.07	0.13	0.33	0.33	0.53	0.26	0.39	0.46	0.33	0.07	0.26	3.2	

indicated by case study of *P. falciparum* cases, so the chances of reinfection can almost be excluded.

Relapse in strains from Kortweg (Holland) and Madagascar have been reported to be in 10 and 80% of cases respectively (Pampana, 1963). Manson-Bahr and Bell (1987) mentioned that in 60% of untreated cases clinical symptoms recur after a period of latency which varies with the strain of parasites showing striking differences in relapse pattern in different parts of the world. Therefore, radical treatment of malaria in patients living in an endemic area where reinfection is likely, needs to be reconsidered.

Primaquine, the only drug available for the radical cure of vivax malaria, is rapidly excreted (half life 4 hours) and must be given for several days. It is toxic, particularly in certain enzyme deficient individuals. A congenital deficiency of NADH methaemoglobin reductase leads to marked cyanosis. Individuals with a hereditary G-6-PD deficiency may develop an acute haemolytic episode leading to destruction of as many as 20% of older circulating red blood cells (RBC) (Wallace, 1980)

In view of the above it is suggested that more studies on relapse pattern in *P. vivax* should be undertaken in different geographical areas of the country as there may be more than one *P. vivax* strains with different relapse characteristics. Similarly the study should also be taken up to determine NADH methaemoglobin reductase and G-6-PD deficiencies in the populations living in different ecological areas. In endemic areas showing high incidence of *P. vivax*, first emphasis should be on vector control measures for reducing the transmission potential and to curtail reinfection.

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Status of *Plasmodium falciparum* Resistance to Chloroquine in Gujarat, Rajasthan and Maharashtra States of India

P. KARMAKAR[1], S.C. DUTT[1], M.V.V.L. NARASIMHAM[2] and R.C. SHARMA[3]

An extended study from 1978-88 revealed that in the western states of India i.e., Rajasthan, Gujarat and Maharashtra; *P. falciparum* resistance to chloroquine has a scattered pattern in its distribution. However in Gujarat, R-III level of resistance is established in southern districts showing four fold increase from 1.79% in 1985 to 8.2% in 1988. In this area use of some alternate drug is indicated.

INTRODUCTION

With the availability of DDT malaria control from 1953 and later malaria eradication programme in India from 1958 became a showpiece for the world until mid sixties. Subsequent resurgence of malaria became well-known through incoming reports and research publications quoting reasons for resurgence. Clyde and Beljaev (1984), Ray and Sharma (1984), Sharma (1984a) and Sharma and Mehrotra (1986) reviewed the factors which could possibly influence malaria situation in a community. Their studies revealed multiplicity of factors responsible for the resurgence of malaria.

Beside vector resistance to insecticides, among the technical reasons development of resistance in *Plasmodium falciparum* to chloroquine is important. Drug resistant *P. falciparum* in India was reviewed by Sharma(1984b).In India *P. falciparum* peak was observed in 1976 when 0.7 million cases were recorded. Implementation of Modified Plan of Operation in 1977 and introduction of Plasmodium falciparum Containment Programme (PfCP) in hard-core areas of Northeast region to begin with and later extension of the programme to more areas brought some respite but total number of reported *P. falciparum* cases in the country continued to stagnate at 0.5 million level every year. In Gujarat proportion of *P. falciparum* cases increased from less than 5% in 1980 to more than 34% of total malaria cases in 1988. Similar increasing trend in *P. falciparum* composition has been observed at national level. One of the important reasons of the resurgence of *P. falciparum* and problem in its control is the development of its resistance to chloroquine which is the mainstay of the malaria control programme as chemotherapeutic agent. Recently drug resistant *P. falciparum* has been

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[1] Regional Office for Health and Family Welfare
Ahmedabad-380 024, India.

[2] National Malaria Eradication Programme
22-Sham Nath Marg
Delhi-110 054, India.

[3] Malaria Research Centre (Field Station)
Civil Hospital
Nadiad-387 001, India.

reported from many parts of the country i.e., Raichowdhuri *et al.* (1984) from Assam, Barkakaty *et al.* (1984) from Assam and Meghalaya, Dutt *et al.* (1984) from Maharashtra, Gajanana *et al.* (1986) and Choudhury *et al.* (1987) from Delhi, Sinha *et al.* (1987) and Das *et al.* (1988) from Calcutta and Sharma and Sharma (1988) from Gujarat.

Monitoring of the drug resistance to delimit the foci for their subsequent elimination to prevent further build up is being done in the country through 12 *P. falciparum* monitoring teams. Results of the studies conducted in Gujarat, Rajasthan and Maharashtra are reported in this paper.

MATERIAL AND METHODS

Initially, mass blood surveys were conducted in selected villages of *P. falciparum* predominant PHCs. The PHCs were selected considering last 3 years epidemiological situation. The villages showing constantly high falciparum rate during the study period were chosen for mass blood survey.

The collected blood smears were stained with J.S.B. stain and were examined for presence of malaria parasite. Patients having asexual *P. falciparum* parasitaemia between 1000 to 80,000/µl of blood were subjected to urine test for presence of chloroquine. Those showing negative results for chloroquine in the urine were selected for study. Acutely ill patients, infants, pregnant women and old or debilitated persons were excluded.

Both *in vivo* and *in vitro* tests were conducted as per WHO methodology.

For *in vivo* assessment either WHO standard field test (7 days) or extended (28 days) follow-up test were performed (Table 4). Selected patients received chloroquine in the dose of 25 mg/kg of body weight in three divided doses and duplicate blood smears were collected daily for 7 days (standard test) and once/twice weekly for next 3 weeks (extended test). The blood smears were stained with standard Giemsa and were examined for presence of parasite and at the same time parasite count was made against 100 microscopic oil immersion field or against 1000 WBC. Levels of resistance were graded as per WHO schedule.

For *in vitro* (macro/micro) vials containing defibrinated parasitised blood (macro) was directly incubated or parasitised blood-media mixture (micro) charged in tissue culture plate was put in the desiccator having an atmosphere of CO_2 and water vapour and incubated in water bath/incubator at a temperature of 38.5°C for 30 hours. Validity of test was confirmed by the presence of 20% or more schizonts in control. Resistance and sensitivity was decided by the presence and absence of schizonts at or above and below the cut-off concentration of the drug respectively. The schizonts were counted against 200 asexual parasites in control as well as in different concentrations of the drug in Giemsa stained smears.

RESULTS AND DISCUSSION

Mass blood surveys were conducted in 90 PHCs/areas from 32 districts in the states of Rajasthan, Maharashtra and Gujarat. A total of 1,04,639 blood smears were collected from 1978 to 1988. Out of 12,737 malaria positive cases 8,571 (67.3%) were of *P. falciparum* having gametocyte rate of 24.2% (Table 1).

Results of *in vitro* test and *in vivo* test showing different levels of resistance of *P. falciparum* to chloroquine are given in Table 2. *In vivo* test in Rajasthan revealed drug-resistant *P. falciparum* of R-I level, whereas R-II level of resistance was seen in Maharashtra and R-III in Gujarat. *In vitro* test in Gujarat revealed highest level (56%) of drug resistant *P. falciparum* followed by Maharashtra (36%) and Rajasthan (23%). *In*

Table 1. Results of mass blood survey during the study period in three states

State	No. of Distt. covered	No. of PHCs/ areas covered	Total BSC Coll.	Total +ve	Pf cases		
					Pfr	Pfg	Mix
Rajasthan	9	15	17225	868	333	338	2
Maharashtra	9	22	25614	1646	819	343	18
Gujarat	14	53	61800	10223	5280	1396	42
Total	32	90	104639	12737	6432	2077	62

Pfr = *Plasmodium falciparum* rings; Pfg = *Plasmodium falciparum* gametocytes.

Table 2. Status of *P. falciparum* resistance

State	*In vivo* test						*In vitro* test		
	Total samples	S	S/R-I	R-I	R-II	R-III	Total samples	S	R
Rajasthan	65	64	—	1	—	—	13	10	3
Maharashtra	217	203	5	5	4	—	50	32	18
Gujarat	665	359	47	207	26	26	175	76	99
Total	947	626	52	213	30	26	238	118	120

S = Sensitive; S/R-I = Taken as S; R = Resistant.

vivo test showed 1.5, 6.5 and 46% drug resistant *P. falciparum* in Rajasthan, Maharashtra and Gujarat, respectively. Difference in the results of *in vivo* and *in vitro* tests may be due to differences in immune status of the test subjects.

Areawise status of drug-resistant *P. falciparum* in the states of Rajasthan, Maharashtra and Gujarat are given in Table 3. Data in Table 3 shows combined results of both *in vivo* and *in vitro* tests. Two PHCs/areas from 2 districts of Rajasthan, 7 PHCs/areas from 5 districts of Maharashtra and 26 PHCs/areas from 8 districts of Gujarat showed drug-resistant *P. falciparum*. Total districts and PHCs/areas where tests were conducted under the study were 2, 5, 8 and 2, 7, 26 in Rajasthan, Maharashtra and Gujarat, respectively. From the study it can be concluded that the phenomenon of drug resistance of *P. falciparum* to chloroquine is well established in the states of Rajasthan, Maharashtra and Gujarat. The differences in the degree of resistance may be due to variation in the strain in different areas and/or differential drug pressure which leads to the selection of drug-resistant parasite.

Status of *P. falciparum* resistance to chloroquine in the western states (Gujarat, Rajasthan, Maharashtra) from 1978 to 1988 is given in Table 4. *In*

Table 3. Areas showing *P. falciparum* resistance to chloroquine

State	District	PHC/area	Resistance	
			In vivo	*In vitro*
1. Rajasthan	1. Bharatpur	1. Rupbas	R-I	+
	2. Kota	2. Kota city		+
2. Maharashtra	1. Chandrapur	1. Gadchandur	R-I	
		2. Yetapalli	R-II	+
	2. Thane	3. Sakur	R-I	
	3. Bhandara	4. Deori		+
	4. Garhchiroli	5. Bhamragarh	R-I, R-II	+
		Bhamragarh	R-I	+
	5. Pune	6. Khatkala		+
3. Gujarat	1. Surat	1. Valod		+
		2. Utran	R-I	
		Utran	R-I	
		Utran	R-I, R-II, R-III	+
		3. Surat city		+
		4. Vanskui	R-I, R-II, R-III	+
		5. Earthan	R-I, R-II, R-III	+
		6. Kanav	R-I	
		7. Karanj	R-I, R-III	+
	2. Panchmahal	8. Shehera		+
	3. Baroda	9. Baroda city	R-I, R-II	+
		Baroda city	R-I, R-II, R-III	+
		Baroda city	R-I	
		Baroda city	R-I	
		Baroda city	R-I, R-II, R-III	+
		10. Chani	R-I, R-II	+
		11. Desar	R-I	+
	4. Banaskantha	12. Vav	R-I, R-II	+
	5. Junagadh	13. Talala		+
		14. Visavadar	R-I	+
	6. Bharuch	15. Lachhras	R-I, R-II	
	7. Valsad	16. Chanvai	R-I, R-II, R-III	+
		17. Nanapondha	R-I, R-III	
		18. Navsari city		+
		19. Alipore	R-I, R-II, R-III	
		20. Dungri	R-I, R-II, R-III	+
		Dungri	R-I	

Table 4. Status of *P. falciparum* resistance to chloroquine in western zone (States of Gujarat, Rajasthan and Maharashtra)

Year	*In vivo* test							*In vitro* test			
	No. of PHCs	No. of samples tested		Percentage of				No. of PHCs	No. of samples tested	Percentage of	
		ST	ET	S	R-I	R-II	R-III			S	R
A. *Gujarat*											
1978	2	0	72	100.00	0.00	0.00	0.00	—	—	—	—
1979	—	—	—	—	—	—	—	—	—	—	—
1980	4	0	59	100.00	0.00	0.00	0.00	3	12	83.33	16.67
1981	1	0	2	100.00	0.00	0.00	0.00	1	2	0.00	100.00
1982	4	0	6	100.00	0.00	0.00	0.00	4	11	90.90	9.10
1983	1	0	1	100.00	0.00	0.00	0.00	1	5	60.00	40.00
1984	2	0	81	88.89	9.88	1.23	0.00	1	8	12.50	87.50
1985	1	56	0	71.43	23.21	3.57	1.79	2	36	27.78	72.22
1986	7	3	130	45.11	44.36	6.02	4.51	6	41	56.10	43.90
1987	6	22	172	34.54	51.02	7.22	7.22	4	49	32.65	67.35
1988	4	0	61	44.26	45.90	1.64	8.20	2	11	27.27	72.73
B. *Maharashtra*											
1978	3	22	57	97.47	2.53	0.00	0.00	—	—	—	—
1979	2	21	22	93.02	0.00	6.98	0.00	1	13	30.77	69.23
1980	3	0	39	97.44	2.56	0.00	0.00	2	9	77.78	22.22
1981	1	0	7	100.00	0.00	0.00	0.00	1	4	100.00	0.00
1982	1	0	15	86.66	6.67	6.67	0.00	1	6	83.33	16.67
1983	2	9	15	95.83	4.17	0.00	0.00	2	18	66.67	33.33
1984	1	0	10	100.00	0.00	0.00	0.00	—	—	—	—
C. *Rajasthan*											
1980	1	0	30	100.00	0.00	0.00	0.00	1	6	66.67	33.33
1981	—	—	—	—	—	—	—	—	—	—	—
1982	1	0	2	100.00	0.00	0.00	0.00	—	—	—	—
1983	—	—	—	—	—	—	—	1	7	85.71	14.29
1984	1	0	33	96.97	3.03	0.00	0.00	—	—	—	—

(—) Denotes not done; S = Sensitive; ST = Standard test; S/R-I taken as S; R = Resistant; ET = Extended Test.

Table 5. Statewise break up of resistance status of *P. falciparum* in Gujarat, Rajasthan and Maharashtra (*in vivo* test)

Sl. No. District	PHC/Area	Year of occurrence	Percentage of			
			S	R-I	R-II	R-III
A. Areas where test could not be repeated						
Gujarat state						
1. Surat	Vanskui	1986	28.57	48.57	8.57	14.29
2. Surat	Kanav	1987	51.14	42.86	0.00	0.00
3. Surat	Earthan	1987	17.24	41.38	17.24	24.14
5. Surat	Karanj	1988	40.00	40.00	0.00	20.00
5. Valsad	Alipore	1986	15.79	63.16	15.79	5.26
6. Valsad	Chanvai	1987	29.17	54.17	8.33	8.33
7. Valsad	Nanapondha	1988	41.67	50.00	0.00	8.33
8. Banaskantha	Vav	1986	87.50	6.25	6.25	0.00
9. Junagadh	Visavadar	1986	37.50	62.50	0.00	0.00
10. Bharuch	Lachhras	1987	36.00	48.00	16.00	0.00
11. Baroda	Chani	1986	65.38	30.77	3.85	0.00
12. Baroda	Desar	1986	43.75	56.25	0.00	0.00
Rajasthan state						
1. Bharatpur	Rubas	1984	96.96	3.04	0.00	0.00
Maharashtra						
1. Chandrapur	Yetapalli	1979	86.96	0.00	13.04	0.00
2. Chandrapur	Gadchandur	1978	93.75	6.25	0.00	0.00
3. Thane	Sakur	1980	92.86	7.15	0.00	0.00
B. Areas where tests were repeated						
1. Surat (Gujarat)	Utran	1984	85.71	14.29	0.00	0.00
		1987	32.47	59.74	2.60	5.19
2. Valsad (Gujarat)	Dungri	1987	53.13	40.63	3.12	3.12
		1988	28.57	71.43	0.00	0.00
3. Baroda (Gujarat)	Baroda city	1985	91.11	6.66	2.23	0.00
		1985	71.43	23.10	3.57	1.79
		1986	46.15	53.85	0.00	0.00
5. Garhchiroli (Maharashtra)	Bhamragarh	1982	86.66	6.67	6.67	0.00
		1983	88.89	11.11	0.00	0.00

N.B.: S/R-I cases considered as sensitive.

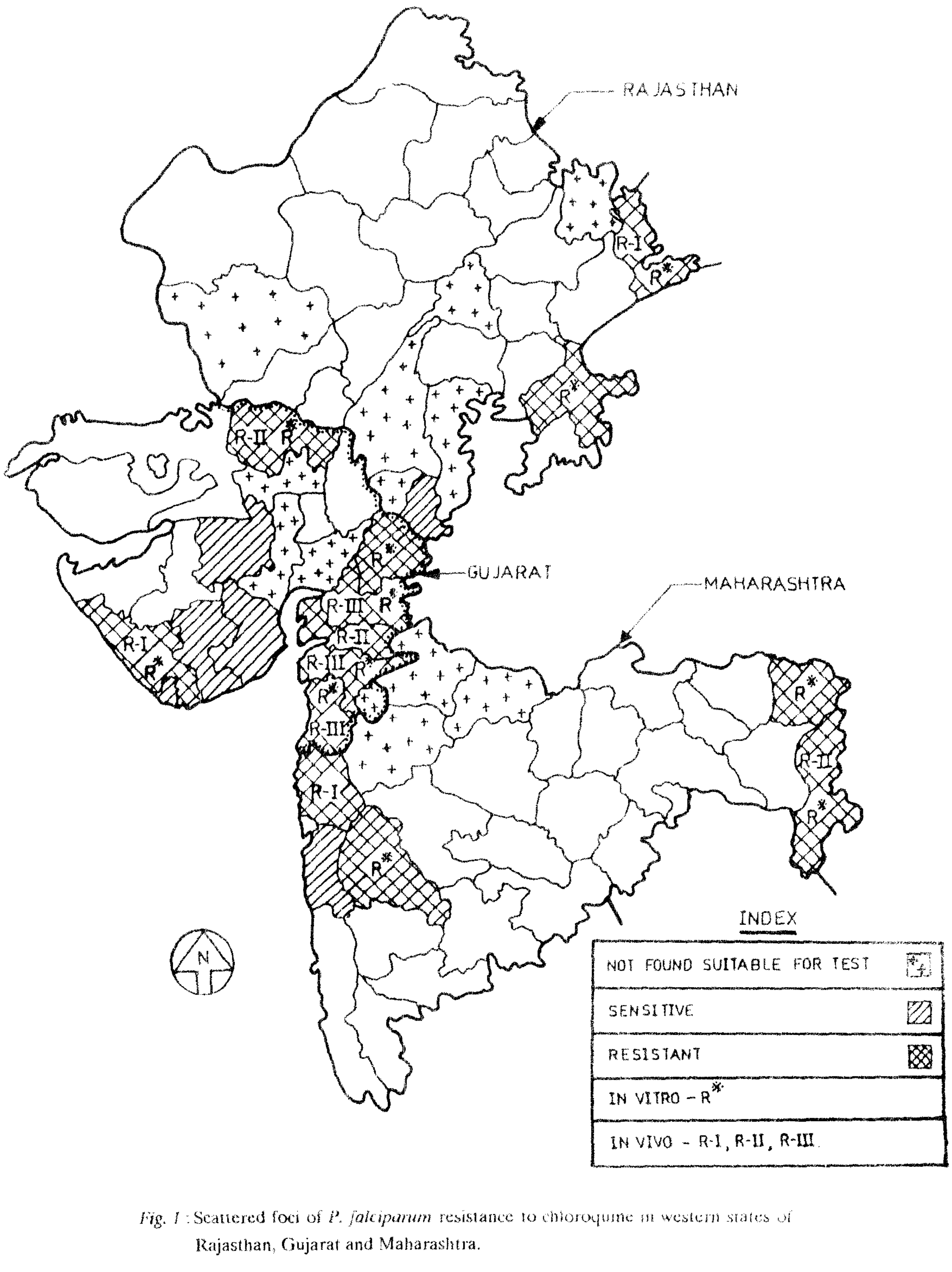

Fig. 1 : Scattered foci of *P. falciparum* resistance to chloroquine in western states of Rajasthan, Gujarat and Maharashtra.

vitro test results indicate that drug-resistant *P. falciparum* was present in all the three states showing an increasing trend in Gujarat whereas in Maharashtra and Rajasthan the results indicate a declining trend. Similar observations were made with *in vivo* test also. In Maharashtra at least the declining trend could be possibly due to the use of amodiaquine instead of chloroquine for the treatment of malaria cases beginning 1981. In case of Rajasthan the reasons are not clear. Results seen may be due to inadequate sample size over a period of time. It is important to note that in Gujarat increasing trend of *P. falciparum* resistance and increase in its level to R-III is a matter of concern specially because proportion of R-III level has increased about 4-fold over a period of 4 years from 1985.

Statewise and areawise break up of *P. falciparum* resistance is given in Table 5 showing different level of resistance in areas where tests could not be repeated and where tests were repeated. The areas where tests were repeated are Utran, Dungri, Baroda city areas/ PHCs of Surat, Valsad, Baroda district, respectively of Gujarat and Bhamragarh PHC in Garhchiroli district of Maharashtra. In Gujarat as indicated by the yearwise pooled data given in Table 4 repeat tests also confirm the observation that both the level and proportion of *P. falciparum* resistance show an increasing trend over a period of time.

Scatter of foci of *P. falciparum* resistance in the states of Rajasthan, Gujarat and Maharashtra has been given in Fig. 1. Districts of South Gujarat show a focus of R-III level of resistance whereas R-I and R-II level of resistance appears to be scattered in different districts of all the three states.

The study clearly indicates that in Gujarat *P. falciparum* resistance to chloroquine is showing an increasing trend both in its level and proportion since 1984 whereas *in vitro* resistance was observed as early as 1980. It is suggested that in the areas of R-III foci some alternate drug needs to be tried in order to eliminate the foci in combination with some effective vector control measures. The study also clearly brings the point home that the monitoring of *P. falciparum* as currently being done under National Malaria Control Programme should continue to delimit the foci with the ultimate objective to find a suitable alternate drug to control malaria, particularly *P. falciparum* which is hard to control.

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Active Malaria Transmission in South Mizoram

S.C. DAS [1], M. BHUYAN[1] and I. BARUAH[1]

Anopheles dirus was incriminated as malaria vector with 1.12% sporozoite rate in Tlabong (Demagiri) subdivision, Mizoram. High parity rate of *A. dirus* (33.69%) and *A. minimus* (52.87%) further confirmed their vectorial status. Both the vectors were found highly susceptible to 4% DDT.

INTRODUCTION

Mizoram a hill state of the N.E. region is situated between 20°20' N and 24°27' N latitude and 92°20' E and 93°29' E longitude. This state is of great strategic importance as three-fourths of its border is exposed to Burma and Bangladesh. Due to difficult and inaccessible terrain, very little information is available on mosquitoes and their role in transmission of malaria in Mizoram. Malhotra *et al.* (1982; 1984) carried out a preliminary mosquito survey in certain places of Mizoram, which was further supplemented by the first incrimination report of *A.b. balabacensis* (*A. dirus*) and *A. minimus* as malaria vectors in the state (Das and Baruah, 1985). Resurgence of malaria in N.E. region has posed a serious health hazard which warranted detailed studies on bioecology of malaria vectors (Sharma, 1987). The present study was conducted to identify the vectors responsible for active transmission of malaria in Tlabong subdivision (Demagiri) during wet season (August-September 1984).

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[1] Defence Research Laboratory
Post Bag No. 2
Tezpur - 784 001, Assam.

MATERIAL AND METHODS

Study area: Tlabong is a border town of south Mizoram situated at an altitude of 900 metres. The sylvan habitat of the place is mainly dominated by bamboo bushes, tall trees and other vegetations. It is often frequented with small hill streams and rivers. The place is sparsely populated, mostly inhabited by Mizo and Chakma tribes. Village huts and labourer camps were scattered, located by the side of road and stream. Low-lying areas of the locality are mainly used as paddy fields. Hot and humid climate prevailing in the area is highly congenial for the breeding of mosquitoes and other haematophagous insects like midges and culicoides.

Epidemiology: Malaria is the major vector-borne disease which is rampant since last few years in this subdivision.

Disease profile is given in Table 1.

The malaria situation was further aggravated by induction of casual labourers (susceptible popu-

Table 1. Malaria profile of study area*

Mizoram state

Year	Positive cases	Pf cases	SFR	Death
1977	5500	2200	40	1
1981	17600	9200	52.27	4
1985	16300	8300	50.92	—
1986	19000	10100	53.16	34

Tlabong subdivision

Year	Slide examined	Positive cases	SPR	Pf cases	SFR
1982	4612	692	15	486	70.23
1983	3099	485	15.65	212	43.71
1984	853	106	12.42	45	42.45

Upto June.
*Received from Health Deptt., Mizoram.

lation) for maintenance and construction of roads by Border Roads Organisation (BRO).

Of the blood samples collected from suspected malaria cases of the casual labourers, 25% were found positive for *P. falciparum* parasite.

For further investigations vectors were collected from labour camps, village huts (human), cattle-sheds and goat cabins. CDC miniature light traps operated by 6 volt motor cycle battery were used for collection of mosquitoes. Traps were hung indoors, in the middle of the structure at about 2 mts. above the ground level. Collections were made dusk to dawn (1800 hrs.-0600 hrs.) without hampering the normal activities of the inhabitants. However, smoking and illumination were prevented during the collection. Hourly incidence of vectors was also determined with the help of CDC light traps to find out the hour of peak intensity inside human dwellings (huts). Adult anopheline mosquitoes were also collected by aspirator tube for susceptibility test as per standard WHO technique (WHO, 1963). Trap collections were sorted out and identified in the camp laboratory with the help of standard keys (Christophers, 1933; Puri, 1960; Wattal and Kalra, 1961). Physiological age of anopheline mosquitoes was determined by parity status. For this ovarioles of adult mosquitoes were dissected to check the number of dilatations as per Detinova (1962). Gland infections were also determined by dissection of live *Anopheles* mosquitoes. Larvae were collected by standard dipper and density was recorded (number of larvae per 100 dips).

RESULTS AND DISCUSSION

A total of 2533 mosquitoes which comprised of 26 species under 7 genera viz., *Anopheles* (9), *Culex* (9), *Aedes* (4) and one each of *Armigeres*, *Mansonia*, *Coquillettidia* and *Ficalbia* were collected in 107 trap nights (Table 2). *A. dirus*

Table 2. Collection record of mosquitoes at Demagiri (Tlabong) subdivision

Sl. No.	Mosquito species	Human habitation	Cowshed	Goat cabin	Total	% of total collection
1.	*Anopheles aconitus*	11	—	—	11	
2.	*A. dirus*	1005	1	1	1007	39.75
3.	*A. jamesi*	1	—	2	3	
4.	*A. kochi*	4	3	—	7	
5.	*A. maculatus*	40	17	12	69	2.72
6.	*A. maculatus* var. *willmorei*	3	—	2	5	
7.	*A. minimus*	788	—	3	791	31.22
8.	*A. nigerrimus*	5	1	—	6	
9.	*A. vagus*	6	—	4	10	
10.	*Aedes albolateralis*	70	—	1	71	2.80
11.	*Ae. albopictus*	9	—	—	9	
12.	*Ae. chrysolineatus*	1	1	—	2	
13.	*Ae. poicilius*	34	2	1	37	1.46
14.	*Culex bitaeniorhynchus*	5	—	1	6	
15.	*Cx. gelidus*	5	—	1	6	
16.	*Cx. malayi*	70	6	2	78	3.07
17.	*Cx. mimeticus*	44	—	2	46	1.81
18.	*Cx. quinquefasciatus*	102	5	4	111	4.38
19.	*Cx. sinensis*	1	1	—	2	
20.	*Cx. (L) fuscanus*	2	—	—	2	
21.	*Cx. tritaeniorhynchus*	21	—	10	31	1.22
22.	*Cx. vishnui*	6	—	1	7	
23.	*Armigeres subalbatus*	111	22	25	158	6.23
24.	*Mansonia uniformis*	53	—	—	53	2.09
25.	*Coquillettidia crassipes*	2	—	—	2	
26.	*Ficalbia chamberlaini*	3	—	—	3	
	Total collection	2402	59	72	2533	96.75

Total 107 trap nights collection.

Table 3. Comparative collection from different habitats

Mosquito species	Human habitation	Cowshed	Goat cabin	Total
Anopheles dirus	23	1	1	25
A. jamesi	—	—	2	2
A. kochi	—	3	—	3
A. maculatus	2	17	7	26
A. minimus	36	—	1	37
A. nigerrimus	1	1	—	2
A. vagus	—	—	4	4
Aedes albolateralis	9	—	1	10
Ae. chrysolineatus	—	1	—	1
Ae. poicilius	4	2	1	7
Armigeres subalbatus	6	22	24	52
Culex bitaeniorhynchus	—	—	1	1
Cx. gelidus	1	—	1	2
Cx. malayi	1	6	1	8
Cx. mimeticus	3	—	—	3
Cx. quinquefasciatus	1	5	3	9
Cx. sinensis	—	1	—	1
Cx. tritaeniorhynchus	—	—	10	10
Cx. vishnui	2	—	—	2
Mansonia uniformis	3	—	—	3
Coquillettidia crassipes	1	—	—	1
Total collection	93	59	57	209
Total trap nights	4	4	4	12
Density per trap night	23.25	14.75	14.25	17.41

which was the dominant vector species, formed 39.75% of total catch and 52.75% of anopheline collection. Another malaria vector, *A. minimus* was the second predominant species. It constituted 31.22% of the total and 41.43% of the captured anophelines. Altogether 624 culicine mosquitoes belonging to 17 species under 6 genera were collected, of which *Armigeres subalbatus* was the predominant species followed by *Cx. quinquefasciatus* and *Cx. malayi*.

In south Mizoram, unlike other places of N.E. region, mosquito density was highest in human habitations, while it is comparatively low in cattlesheds and goat cabins (Table 3). In all 26 species were collected from human habitations, of which malaria vectors *A. dirus* and *A. minimus* were the most dominant species. Statistical analysis of data also revealed that the *Anopheles* density in human habitation was significantly high compared with that in goat cabins and cattlesheds ('F' value = 19.965 at 5% level). Hourly collections (two hourly blocks) were made from BRO labour camps and were analysed as per Fisher's 'F' test (Table 4). The collection of early evening hours (1800-2000 hrs.) and early morning hours (0400-0600 hrs.) were found significantly low in case of *A. dirus* (F = 6.869). High density of *A. dirus* was observed between 2000 hrs. and 0200 hrs. with a peak at 2200–2400 hrs. However, there was no significant difference among three blocks of collection. In case of *A. minimus*, density increased since beginning of the collection. Significantly high density was observed in two blocks of collection between 2000 and 2400 hrs. (F = 5.520). Collections of early evening and early morning hours had significantly low densities of *A. dirus*.

It is evident from the present study that *A. dirus* is predominantly a midnight biter though it was observed throughout the night. This once again confirms the earlier observations made by Rajagopal (1979) in the tea gardens of Nowgong district of Assam. Similar incidence of *A. dirus* was recorded at Burnihat of Assam (now Meghalaya) and in adjacent countries, Burma and Thailand (Rao, 1984). Earlier authors have also recorded the midnight incidence of *A. dirus* during hourly collection of vectors in Mizoram (Das and Baruah, 1985).

Table 4. Hourly collection of *A. dirus* and *A. minimus* in 16 trap nights at Demagiri

Hours	*A. dirus*	*A. minimus*
1800--2000	2*	18*
2000--2200	33	71
2200--2400	44	53
2400--0200	33	44
0200--0400	16	20
0400--0600	6*	1*

* Level of significance 0.05.

High incidence of *A. minimus* in first part of the night with gradual fall till morning hours is well known (Ismail *et al.*, 1974 a;b; 1978) In foothills of Nagaland hourly incidence of *A. minimus* was recorded by Bhatnagar *et al.* (1982) during dry (April) and wet (June) season. Peak incidence was observed in the first part of night during dry season and during late part of night in the wet season. Earlier authors had also observed the incidence of *A. minimus* almost throughout the night in the same area (Das and Baruah, 1985).

A total of 178 *A. dirus* and 159 *A. minimus* were dissected to determine their parity rate and gut/gland infection for malarial parasite (Table 5). Two *A. dirus* were found gland positive for sporozoites, indicating 1.12% infection rate in the species. No *A. minimus* was found positive for sporozoites. This once again substantiates the earlier findings of Das and Baruah (1985) in the same locality in which two specimens of *A. minimus* and one specimen of *A. dirus* were found with sporozoite infection. *A. minimus* was incriminated in N.E. region by Kareem *et al.* (1983) in Assam, Bhatnagar *et al.* (1982) in Nagaland and Dutta and Baruah (1987) in Arunachal Pradesh. Earlier Sen *et al.* (1973) found three specimens of *A.b. balabacensis* positive for sporozoites, out of 1,811 specimens dissected in Arunachal Pradesh.

On updating the earlier observation of July 1984 (Das and Baruah, 1985) altogether three specimens of *A. dirus* and two specimens of *A. minimus* were found to carry sporozoite infection out

Table 5. Records of dissection of vector species

Mosquito species	No. dissected	No. of glands found positive	Sporozoite rate %	Parity%
Anopheles dirus	178	2	1.12	33.69
A. minimus	159	Nil	Nil	52.87

of 312 and 217 mosquitoes dissected respectively between July and September 1984. The infection rate was 0.96% for *A. dirus* and 0.92% for *A. minimus*. High parity rate (31.22% for *A. dirus* and 58.48% for *A. minimus*) were maintained throughout the malaria season (July to September 1984.)

In the present study, parity was recorded at 33.69% for *A. dirus* and 52.87% for *A. minimus* which indicates high survival rate of the species in nature. This may be due to conducive climatic conditions in Mizoram. In addition to the parasitic load in the community, long survival of vectors may be a major cause of high infection rate in *A. dirus* and *A. minimus*. Out of the blood slides collected from 104 fever cases in the study area, 22 slides were found positive for *Plasmodium falciparum*. This is an indication of active transmission of malaria among the local populace. Presence of chloroquine resistant strains of *P. falciparum* parasite has further aggravated the situation. Dutta and Baruah (1987) also observed high parity status in *A. minimus* (56.41%) in Arunachal Pradesh. This once again confirms the high survival rate of vector species in northeastern region.

A. dirus and *A. minimus* were found highly susceptible to 4% DDT as cent per cent mortality was achieved within two hours of holding period after 30 mins. exposure as per standard WHO technique (Table 6). The first round of spraying was going on in the villages during the period of study. Efforts were made to give full coverage although the spraying was late as per schedule. In addition the Border Roads Organization is also spraying their newly constructed huts before occupation. It has been observed during biting collection inside the huts and barracks (doors and windows completely open) that mosquitoes come inside freely to take blood meal and fully engorged mosquitoes go out from the hut through the open doors, windows and split bamboo walls. Thus the mosquitoes never come in contact with the sprayed surface which could have caused mortality. Moreover, the occupants of the huts rest outside in the evening hours as a habit after the day's hard work. This is mainly due to congested and stuffy condition of the huts caused by sultry climatic conditions of Mizoram prevailing during the wet season.

Profuse breeding of *A. minimus* was observed along the grassy margins of clear flowing streams, seepages and perennial rivers. *A. dirus* was found to breed in typical forest ditches under shady trees. Similar breeding habitats of *A. minimus* and *A. dirus* were reported earlier by various authors (Rao, 1984).

Table 6. Adult susceptibility test of *A. dirus* and *A. minimus* against 4% DDT

Mosquito species	Exposure period	% mortality
A. minimus	1 hr	100
	30 mins	100
	15 mins	100
A. dirus	1 hr	100
	30 mins	100
	15 mins	92.43

It appears from the present study that the parasitic load in the community, particularly of resistant *P. falciparum* parasite is aggravating the malaria problem in Mizoram. In addition, the exophilic nature of the vectors is further worsening the situation. It may be concluded from the present study that malaria situation in South Mizoram is a serious health problem which requires utmost devotion, care and a systematic approach to undertake an effective control programme. In view of the susceptible nature and high indoor incidence of vectors, it can be stated that reasonable coverage and methodical spray of residual insecticides, in addition to the reduction of parasitic load in the community can substantially reduce malaria incidence in Mizoram.

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SHORT NOTES

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Seasonal Incidence of Water Mites (*Arrenurus* sp.) Parasitising Mosquito Vectors at Tezpur, Assam, India

P.K. SARKAR[1], D.R. NATH[1], P.K. TALUKDAR[1] and P.R. MALHOTRA[2]

Parasitic water mites have the potentiality of limiting the reproduction and survival of natural populations of host mosquitoes (Lanciani and Boyt, 1977). Presence of mites is also a possible indicator of nulliparity in female mosquitoes (Corbet, 1970; Mullen, 1975). Water mites (*Arrenurus* species) parasitising mosquitoes were first reported as early as 1900 A.D. in India which is still being updated with additional information (Biswas *et al.*, 1980; Malhotra *et al.*, 1983). The present communication is related with the seasonal incidence of water mites (*Arrenurus* sp.) parasitising malaria, filariasis and Japanese encephalitis vectors.

Collection sites were selected in various habitats of Tezpur (Assam) after initial survey. CDC miniature light traps were used for dusk to dawn collection of mosquitoes fortnightly for a year from cattlesheds. Mite infested mosquitoes were sorted and identified as per standard key. Mites attached to different parts of host were recorded and preserved for identification as per the key of Mullen (1974). Abdominal condition and parity status of the females were also recorded to determine the age composition of host species.

In the present study 8750 mosquitoes belonging to 27 species were collected in 48 trap nights. Of which only 182 mosquitoes under 15 species were found parasitised with water mites (Table 1). This is the first report of mite infestation in *Anopheles ramsayi* and *Ficalbia chamberlaini* in India. Among vector species, mite infestation was highest in *Mansonia uniformis* (15.9%) followed by *Mansonia indiana* (9.9%), *Mansonia annulifera* (7.5%), *Anopheles philippinensis* (6.6%), *Culex tritaeniorhynchus* (1.0%) and *Culex vishnui* (1.0%) as shown in Table 1.

Positive correlation (r) was observed between rainfall and mite infestation in *An. philippinensis* (r = 0.263), *Ma. annulifera* (r = 0.016), *Ma. indiana* (r = 0.522), *Ma. uniformis* (r = 0.756), *Cx. tritaeniorhynchus* (r = 0.571) and *Cx. vishnui* (r = 0.471).

Incidence of mite infestation was observed uniformly for nine months i.e., from April to December. The highest number of vectors (40) infested with mites was recorded in September (comprising of 5 species). Mullen (1977) observed mite incidence only for five months (May to September) in tropical Africa.

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[1] Defence Research Laboratory
Tezpur-784 001, India.
[2] Defence Research and Development Estt.
Tansen Marg
Gwalior-474 002, India.

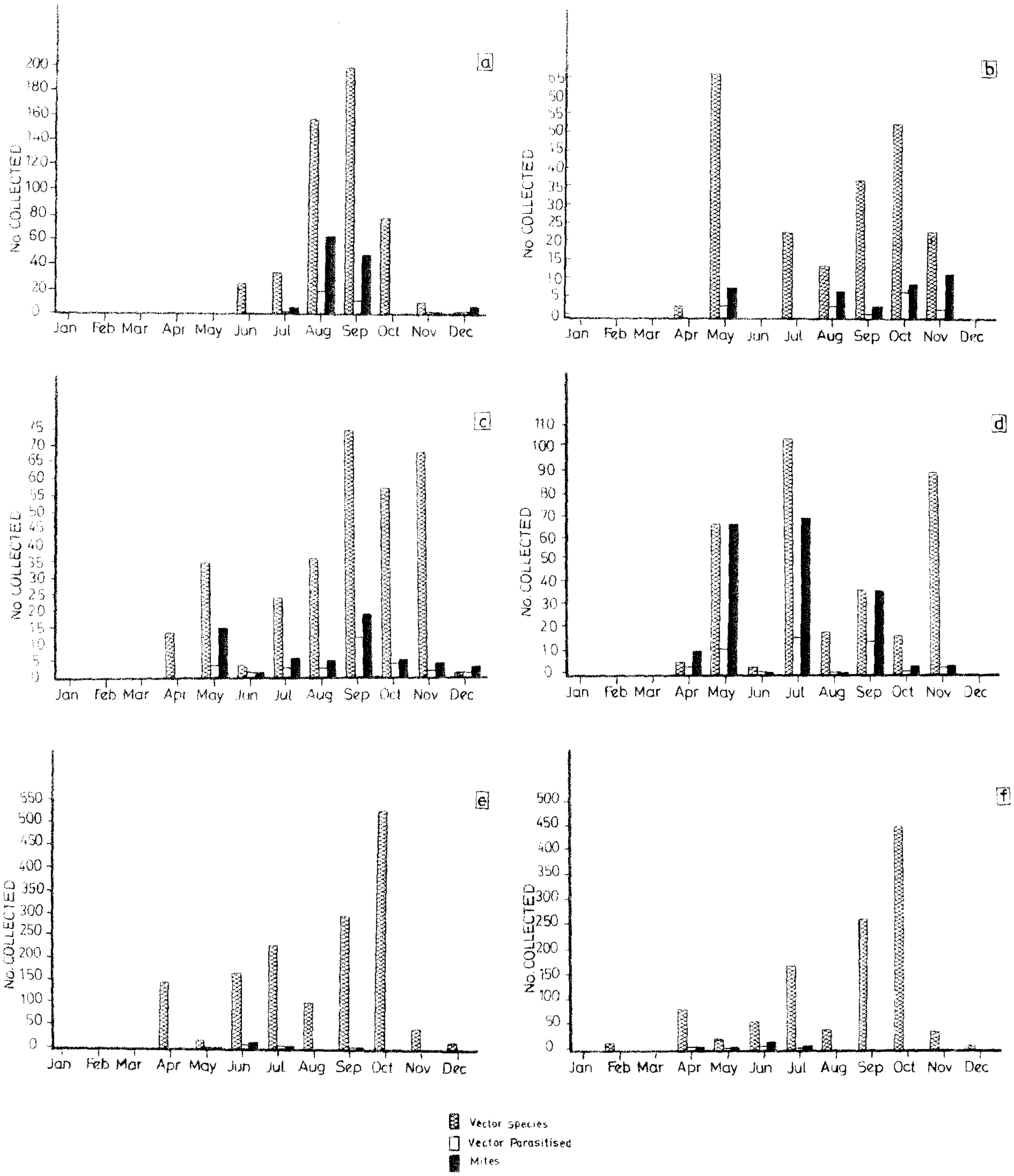

Fig. 1: Monthwise parasitised vectors: (a) *An. philippinensis;* (b) *Ma. annulifera;* (c) *Ma. indiana;* (d) *Ma. uniformis;* (e) *Cx. tritaeniorhynchus;* and (f) *Cx. vishnui.*

Table 1. Mite infestation on mosquito species

Mosquito species	Total catch	No. parasitised	Mite infestation %
Anopheles annularis	60	1	1.7
A. barbirostris	375	4	1.1
A. nigerrimus	444	1	0.2
*A. philippinensis**	502	33	6.6
A. ramsayi	26	1	3.8
A. vagus	16	3	18.8
Coquillettidia crassipes	93	1	1.1
*Mansonia annulifera***	214	16	7.5
*Ma. indiana***	314	31	9.9
*Ma. uniformis***	346	55	15.9
Culex bitaeniorhynchus	107	8	7.5
Cx. sinensis	7	1	14.3
*Cx. tritaeniorhynchus****	1558	15	1.0
*Cx. vishnui****	1152	11	1.0
Ficalbia chamberlaini	7	1	14.3
Noninfested species	3529		
Total	8750	182	
Major Vectors:			

*Malaria; **Filariasis; ***JE.

Seasonal incidence of mite on six vector species are shown in Fig. 1. Peak infestation of mites on J.E. vectors was observed in June and on malaria vectors in August. Filariasis vectors i.e., *Mansonia* species have shown fluctuations in mite infestation between May and October. Peak infestation in case of *Ma. annulifera* was observed in October and for *Ma. indiana, Ma. uniformis* in September. In south India, Daniel *et al.* (1986) reported maximum mite infestation during July in *Ma. uniformis*. Mite infestation was observed in *Cx. vishnui* and *Ma. uniformis* since the appearance of the species (April), while in others infestation was recorded after one month of their appearance.

Mite infestation was highest (56%) in *Mansonia* species. This conforms with the earlier findings of Malhotra *et al.* (1983) in Assam. The high infestation on *Mansonia* species is probably due to static immature stages (that remain attached to hydrophytes) which provides ample scope to the mites to locate the host and subsequently to the emerging adults. Around 100% nulliparity among the parasitised mosquitoes (Table 2) further strengthen this view.

Table 2. Results of physiological conditions and distribution of water mites on mosquito vectors

Host species	No. parasit-ised	% of		No. dissec-ted	% of		No. of mites in			Total no. of mites	Average mites/ host species	Range
		Unfed	Fed		Nulli-parous	Bipa-rous	Head	Thorax	Abdo-men			
Anopheles philippinensis	33	72.7	27.3	25	100	—	—	19	102	121	3.7	1-12
Mansonia annulifera	16	37.5	62.5	11	100	—	—	2	37	39	2.4	1-10
Ma. indiana	31	27.3	72.7	17	100	—	—	24	35	59	1.9	1-9
Ma. uniformis	55	40.6	59.4	29	96.6	3.4	7	66	122	195	3.5	1-23
Culex tritaeniorhynchus	15	53.3	46.7	8	100	—	1	10	11	22	1.5	1-2
Cx. vishnui	11	18.2	81.8	7	100	—	1	13	14	28	2.5	1-7
Total	161			97			9	134	321	464		

Smith and McIver (1984) also found 87.5% newly emerged *Coquillettidia perturbans* parasitised with *Arrenurus danbyensis*. Maximum 23 mites recorded in a single specimen of *Ma. uniformis* followed by *An. philippinensis* (12). However, average density was only 2.9. Mites were mostly attached to head, thorax and abdomen. Attachment of mites on abdomen was significantly high in *Ma. annulifera* (mean = 3.083) and *Ma. indiana* (mean = 2.917) while in others there was no significant difference in body part preference.

Mite infestation was more in unfed population of *An. philippinensis* and *Cx. tritaeniorhynchus* while it was more in fed specimens in case of *Ma. annulifera, Ma. indiana, Ma. uniformis* and *Cx. vishnui* (Table 2). However, significantly high infestation was observed only in fed population of *Ma. indiana* ($t = 1.974$, $P = 0.05$ level).

To be an effective natural enemy as a biological control agent, an efficient method of dispersal is necessary. As these mites are parasitic on mosquitoes, they have access to any potential breeding habitat used by their mosquito associates. Moreover research has shown that parasitism by larval *Arrenurus* mites can have a substantial effect on both individuals and populations of *Mansonia* and *Anopheles* mosquitoes. How and to what extent this effect can be augmented should be investigated (Smith, 1983).

Mites are known to have selective breeding habitats e.g., springs, seepage areas, pools, ponds, lakes etc. (Smith and Oliver, 1986). Thus detection of mites on vector and age grading may help in locating the breeding sites in epidemiological studies and control programmes.

ACKNOWLEDGEMENTS

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Morphological Variations in Some Indian Anophelines from Koraput District, Orissa, India

K. GUNASEKARAN[1], S.S. SAHU[1], C. SADANANDANE[1], S.K. PARIDA[1], K.P. PATRA[1] and P. JAMBULINGAM[1]

The anopheline fauna in India has been classified into 51 species and 7 sub-species or varieties (Rao, 1981). Recently one more species, *A. sergenti* was recorded in Orissa state (Gunasekaran, *et al.*, 1989). Many of these species are known to show some variations in morphological characters and these variations are usually found in the ornamentation of palpi, tarsi of hind legs and the wings. Since the variability in ornamentation of wing, palpi and tarsi of hind leg can cause confusion in taxonomy, morphological variations observed in 16 anopheline species from Koraput district of Orissa state, India, is reported. Some of the variations have already been documented by Christophers (1933), Ramakrishna (1954) and Wattal *et al.* (1960) from other parts of India. A total of more than 45,000 anophelines were collected from different areas of the district and the variations were recorded in 163 mosquitoes. Of these many of them are not reported so far.

The species, with the variations from the type form, the month and places of collection are furnished in Table 1. The illustrations include both type and variant forms.

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[1] Vector Control Research Centre
Medical Complex, Indira Nagar
Pondicherry-605 006, India.

The variations were observed in 163 anophelines belonging to 16 species. Some of these variations occur seasonally. For example, *A. annularis* is known for its melanic forms. The forms with an extra dark band in the apical segment of the palpi is considered to be the so called variety *nagpori* and the forms with an extra dark band on the hind tarsi is *adiei* (Christophers, 1933). While the forms with palpal variation were observed in Punjab and U.P. during winter months (Wattal *et al.*, 1960) both the melanic forms were noticed recently in Orissa state (Dash *et al.*, 1988). Because of their prevalence in winter months these forms are generally considered as winter forms. In the present study, both of these forms have been recorded and with a similar seasonal preference. But interestingly in one specimen these two variations were found together (Figs. 1h and k). This could also be a melanic variant like *adiei* and *nagpori*.

Some variations reported in this study, have been considered as usual characters by earlier workers. In *A. culicifacies*, the presence of a second interruption at inner end of inner dark costal spot was reported to be usual character by Christophers, 1933, but it is not so. Out of over 10,800 specimens examined during the present investigation, only 9 specimens had such second pale interruption (Fig. 7c). Hence, this second pale interruption can not be considered as a

Table 1. Morphological variations in some Indian Anophelines from Koraput, Orissa

Sl. No.	Month of collection	Place of collection	No. of specimens collected	Variation from the type form
1	2	3	4	5
				A. annularis **Van der Wulp, 1884**
Variations in palpi				
1.	Dec'88	Deulaguda	1	A patch of black scales present in the apical pale band (Fig. 1e, also reported by Wattal *et al.,* 1960).
2.	Dec'88 Jan'89 May'89	Deulaguda Benasur Deulaguda	14 2 } 17 1	Apical pale band with an extra narrow black band in the middle (Fig. 1f, also reported by Christophers, 1933; Wattal *et al.*, 1960; Dash *et al.*, 1988).
3.	Nov'88 Dec'88	Deulaguda Bonda Hills	7 } 8 1	Subapical black band, middle black band and the proximal black band, all having pale areas towards the inner margin (Fig. 1g, also reported by Rajagopal and Chakraborty, 1960).
Variations in wing and palpi				
4.	Feb'89	Bonda Hills	1	(a) No fringe spot in 6th vein. (b) Other fringe spots are all very narrow. (c) Tip of palpi black, the length of the palpi shorter than proboscis (Figs. 1b and c).
Variation in hind leg				
5	Jan'88 Feb'88 Dec'88 Feb'89	Deulaguda Benasur Deulaguda Benasur Deulaguda Deulaguda	5 13 2 } 28 1 1 6	An extra black band present basally in tarsal segment 3rd (Fig. 1j, also reported by Christophers, 1933; Puri, 1938 and Dash *et al.*, 1988).
Variations in palpi and hind leg				
6.	Feb'88	Bonda Hills	1	(a) Apical pale band with an extra narrow black band in the middle. (b) Hind legs with an extra black band basally in tarsal segment 3 (Figs. 1h and k)
				A. majidi **McCombie Young & Majid, 1928**
Variations in hind leg				
7.	Feb'88	Bonda Hills	1	(a) T-2 completely dark not involved in banding. (b) T-3 with more pale area basally and less dark area apically and (c) T-4 with basal dark band, the apical white band equal in length to the basal black band (Fig. 2b).

Contd...

Table 1. Morphological variations in some Indian Anophelines from Koraput, Orissa (*contd.*)

1 2	3	4	5
		A. fluviatilis **James, 1902**	
Variations in palpi			
8. Jan'89	Ambaguda	1	Apical white band with a narrow extra black band in both palpi. (Fig. 3e, also reported by Ramakrishna, 1954 and Wattal *et al.*, 1960).
9. Aug'88	Masipodar	2	Palpi have two white bands apically, the sub-apical one as broad as the apical thus the intervening black band reduced in length (*A. minimus* like) (Fig. 3f).
Dec'88	Malkangiri	1	
Dec'88	B. Singpur	1	
	Deulaguda	2 } 16	
	Bonda Hills	1	
Jul'89 Aug'89	Champapodar	9	
10. Dec'88	Ambaguda	1	In the apical white band an extra narrow black band present in only one palpi (Fig. 3g).
11. Sep'89	Champapodar	1	One of the palpi (right side) shorter and tip reaching about sub-apical white band of the other one (Fig. 3h).
Variations in wing			
12. Mar'89	B. Singpur	1	3rd vein completely black (Fig. 3b, also reported by Puri, 1938).
13. Feb'89	B. Singpur	2	In the apex of the wing the apical dark spot absent (Fig. 3c).
		A. pallidus **Theobald, 1901**	
Variation in palpi			
14. Jul'89	Seamalaguda	1	The apical pale band with an extra narrow black band in the middle (Fig. 4b, also reported by Wattal *et al.*, 1960).
Variations in hind leg			
15. Nov'88	Deulaguda	1	2-3/4 segment of hind tarsi continuously white (Fig. 4d).
16. Sep'89	Deulaguda	1	In hind leg 3-1/8 of tarsal segments continuously white (Fig. 4e).
		A. jeyporiensis **James, 1902**	
Variations in wing			
17. Jan'88	Bonda Hills	1 } 2	No fringe spot in vein 6 (Fig. 5b).
Jan'89	Champapodar	1	
18. Feb'88	Masipodar	2 } 3	3rd vein completely black (Fig. 5c, also reported by Christophers, 1933).
Jan'89	Benasur	1	
19. Nov'88	Champapodar	5	Usual costal pale interruptions absent, and only a pale spot present near inner costal dark spot towards inner margin (Fig. 5d).
Dec'88	Deulaguda		

Contd...

Table 1. Morphological variations in some Indian Anophelines from Koraput, Orissa (*contd.*)

1	2	3	4	5
20.	Nov'88 Dec'88 Jan'89	Deulaguda Bonda Hills B. Singpur	6	Base of costa uninterrupted (Fig. 5e).
21.	Dec'88 Aug'89	Deulaguda Champapodar	1 2 } 3	Costa with one pale interruption (Fig. 5f)
22.	Jan'89	Champapodar	1	Sub-costal pale area absent (Fig. 5g).
Variation in palpi				
23.	Jan'88 Dec'88	Bonda Hills Bandhuguda	1 1 } 2	One of the palpi (left side) shorter in length-1/2 the size of the other (Fig. 5h).
				A. karwari **James, 1903**
Variation in hind leg				
24.	Aug'88 Sep'88 Aug'89 Aug'89	Benasur Bonda Hills Bhalumahul Champapodar	5	The basal portion in 3rd tarsal segment is black. (Fig. 6b)
				A culicifacies **Giles, 1901**
Variations in wing				
25.	Sep'87 Nov'88	Doraguda Malkangiri	2 1 } 3	3rd vein with more pale area in the middle (Fig. 7b, also reported by Christophers, 1933).
26.	Sep'88	B. Singpur	1	Costa with two pale interruptions (Fig. 7c, also reported by Christophers, 1933).
27.	Dec'87	Deulaguda	4	Absence of fringe spot at 4-2, 5-1 (Fig. 7d, also reported by Christophers, 1933).
28.	Jan'89	B. Singpur	1	Fringe spots present also in 4-1, 5-2, and 6th veins (Fig. 7e, also reported by Puri, 1938).
29.	Dec'88	Deulaguda	1	(a) Costa with two pale interruptions. (b) Apical black spot is equal to pre-apical costal spot. (c) In apex of the wing the pale area extended upto 2-1 only. (d) Vein 1 dark spot opposite to basal costal interruption extended upto the wing root (Fig. 7f).
30.	Sep'88	Bonda Hills Deulaguda	1 5 } 6	(a) Two pale interruptions at base of costa. (b) Fringe spots present also in 3, 4-1, and 5-2. (c) A pale spot in the centre of vein 3 (Fig. 7g).
31.	Jul'89	Naranaguda	1	An inconspicuous fringe spot at 5-2 only in left wing. Costa with two pale interruptions (Fig. 7h).

Contd...

Table 1. Morphological variations in some Indian Anophelines from Koraput, Orissa (*contd.*)

1 2	3	4	5
32. Jun'89 Jul'89	Masipodar Malkangiri	1} 2 1}	All veins ending with a pale spot at the base. The apical dark spot is equal to pre-apical costal spot. Fringe spot present in 5-2 also (Fig. 7i).
33. Jun'89	Naranaguda	1	4-2 fringe spot absent, but fringe spot present at 5-2. Two unequal pale interruptions at base of costa (Fig. 7j).
34. Mar'89	B. singpur	1	(a) Uninterrupted costa. (b) In the apex of the wing the pale area extended upto 2-1 only. (c) Vein 1 dark spot opposite to basal costal interruption is twice that of type form (Fig. 7k).
		A. splendidus **Koidzumi, 1920**	
Variation in palpi			
35. Aug'88	B. Singpur	1	Palpi unspecked (Fig. 8b, also reported by Wattal *et al.*, 1960).
		A jamesi **Theobald, 1901**	
Variation in palpi			
36. Apr'89	Malkangiri	1	The apical pale band with an extra narrow black band in the middle (Fig. 9b, also reported by Wattal *et al.*, 1960).
		A. subpictus **Grassi, 1899**	
Variations in palpi			
37. Aug'88	Bonda Hills	1	The subapical black band about twice the length of the apical pale band (Fig. 10b, also reported by Wattal *et al.*, 1960).
38. Jan'88	Bonda Hills	2	Subapical pale band with a narrow black band in the middle (Fig. 10c).
		A. vagus **Donitz, 1902**	
Variations in palpi			
39. Aug'89	B. Singpur	1	(a) Subapical black band is incomplete, only some dark scales present in lateral side of both palpi. (b) Middle black band with a row of pale scales towards inner margin (Fig. 11b).
		A philippinensis **Ludlow, 1902**	
Variation in palpi			
40. Nov'88	Deulaguda	1	An extra narrow black band in the middle of the apical pale band (Fig. 12b, also reported by Wattal *et al.*, 1960).

Contd...

Table 1. Morphological variations in some Indian Anophelines from Koraput, Orissa (*contd.*)

1 2	3	4	5
Variation in hind leg			
41. Aug'89	Masipodar	1	2/3 area of 2nd tarsal segment is white (Fig. 12d).
			A. sergenti **Theobald, 1907**
Variations in wing			
42. Apr'88	B. Singpur	1	6th vein completely dark (Fig. 13b).
43. Dec'88	Deulaguda	1	(a) 6th vein completely dark. (b) No fringe spot in all vein (Fig. 13c).
			A. varuna **Iyengar, 1924**
Variations in wing			
44. Apr'88	B. Singpur	3	(a) All veins extensively dark. (b) 6th vein completely dark (Fig. 14b).
45. Dec'88	B. Singpur	1	(a) Sector pale area absent. (b) Both subcostal and pre-apical pale areas very narrow (Fig. 14c).
			A. maculatus **Theobald, 1901**
Variations in palpi			
46. Oct'88 Dec'88 Jan'89	Bonda Hills B. Singpur	1 5 } 6	White scattered scales in the middle black band giving speckled appearance (Fig. 15e, also reported by Wattal *et al.*, 1960).
47. Dec'88	B. Singpur	4	(a) The intervening apical dark band absent. (b) Middle black band very narrow, with white band pattern in one palpi and in another with two black spots (Fig. 15f).
Variations in palpi and wing			
48. Sep'88 Nov'88	Bonda Hills Deulaguda	1 1 } 2	(a) White scattered scales in middle black band of palpi. (b) Base of costa with two interruptions, and (c) Sixth vein with three black spots (Figs. 15b and c).
Variation in hind leg			
49. Dec'88	B. Singpur	4	The black band in 4th tarsal segment of hind leg very narrow (Fig. 15h).
			A. theobaldi **Giles, 1901**
Variations in palpi			
50. Aug'89	Champapodar	1	The subapical dark band is absent (Fig. 16b, also reported by Wattal *et al.*, 1960).
51. Sep'89	Deulaguda	1	The apical intervening black band not prominent. Only some brownish scales present (Fig. 16c).
52. Aug'89	Bhalumahul	1	The black band in the hind tarsal segment 3 is smaller in length (Fig. 16a).

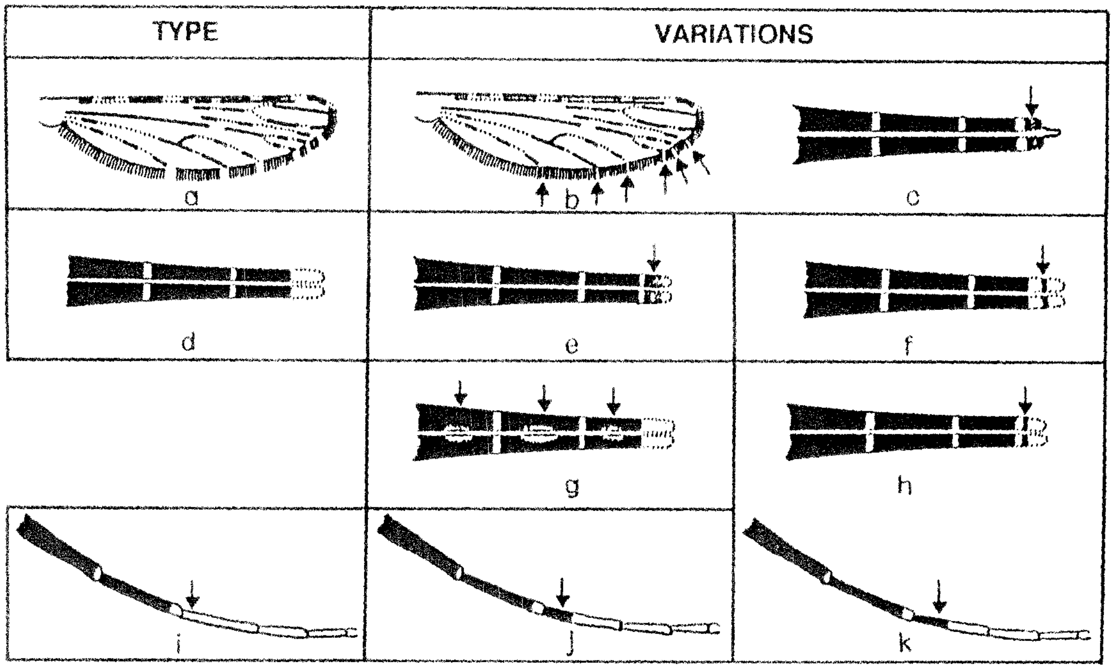

Fig. 1: Anopheles annularis, Van der Wulp, 1884.

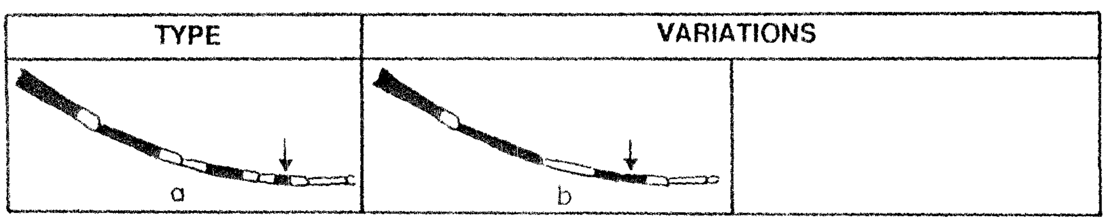

Fig. 2: Anopheles majidi, McCombie Young & Majid, 1928.

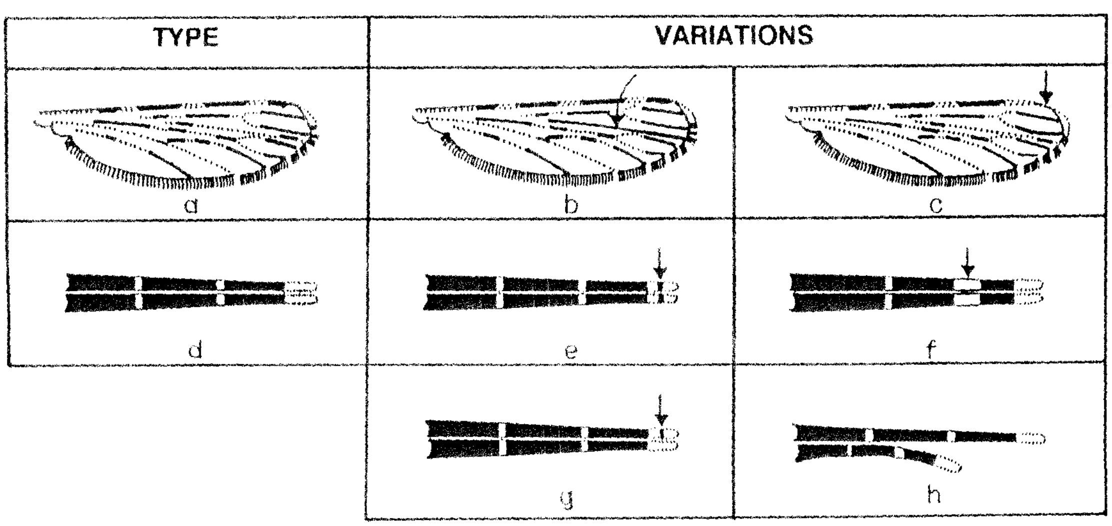

Fig. 3: Anopheles fluviatilis, James, 1902.

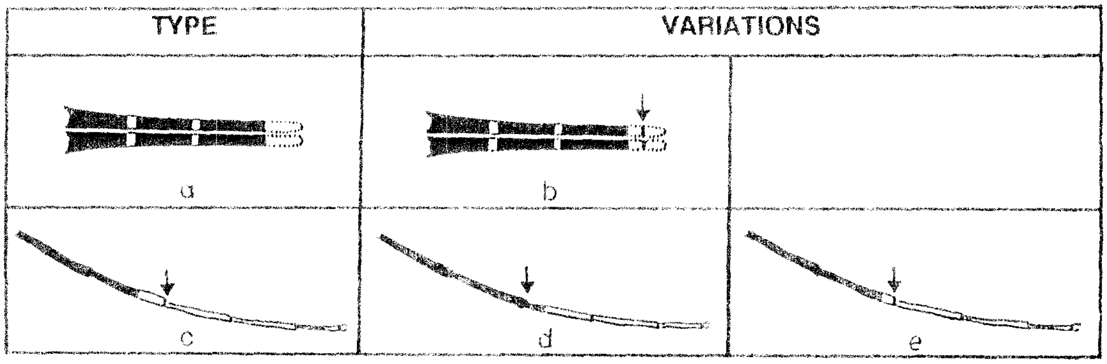

Fig. 4: Anopheles pallidus, Theobald, 1901.

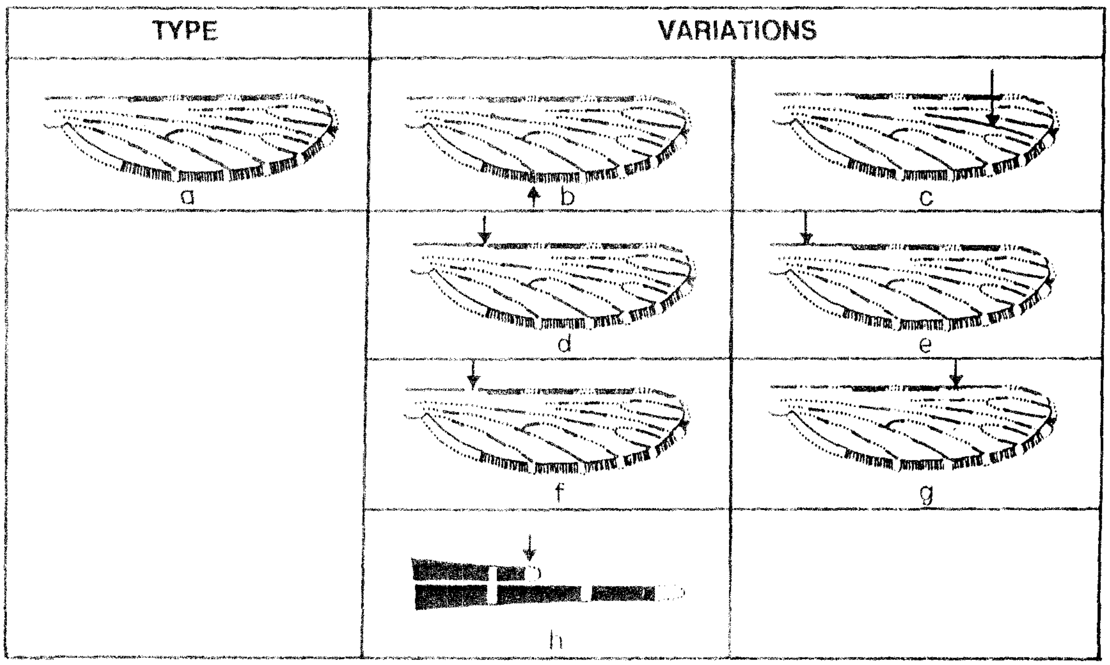

Fig. 5: Anopheles jeyporiensis, James, 1902.

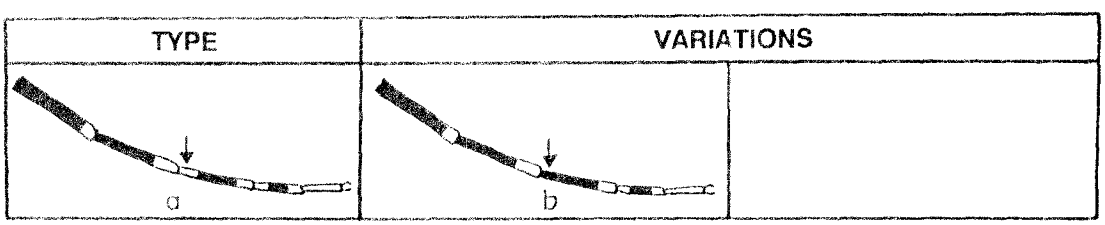

Fig. 6: Anopheles karwari, James, 1903.

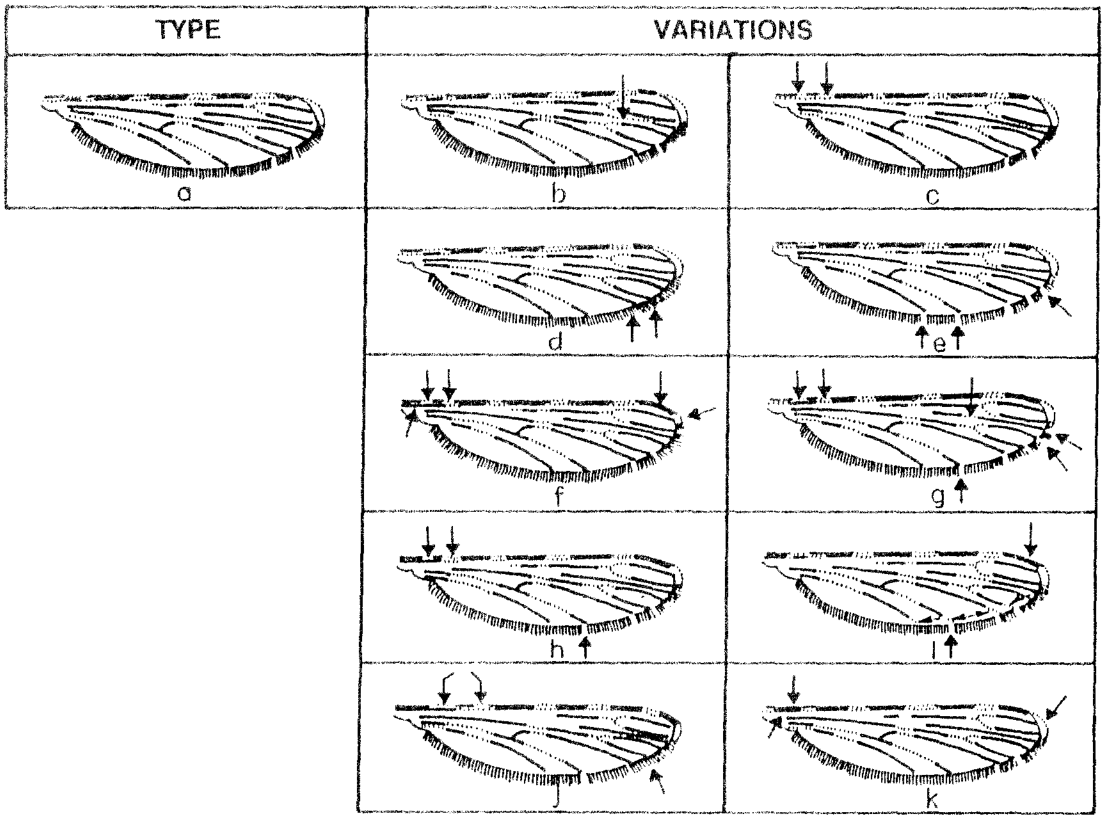

Fig. 7: Anopheles culicifacies, Giles, 1901.

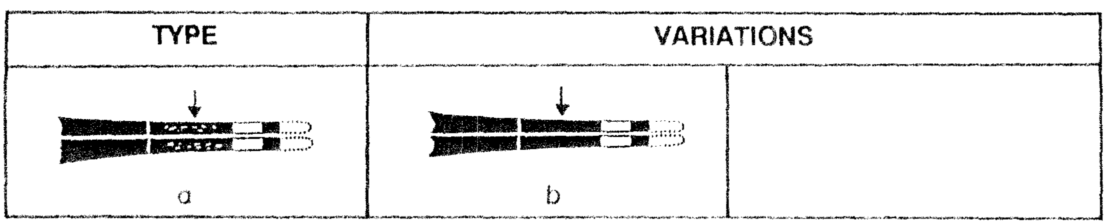

Fig. 8: Anopheles splendidus, Koidzumi, 1920.

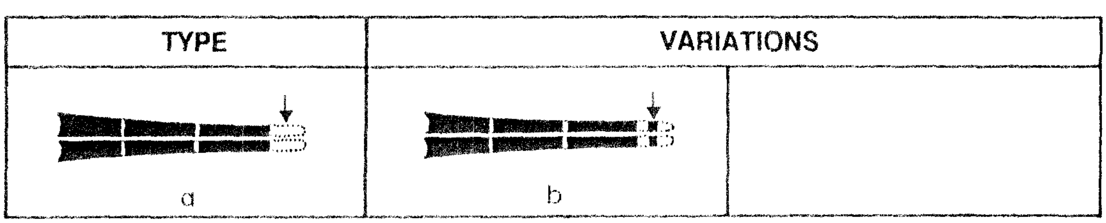

Fig. 9: Anopheles jamesi, Theo, 1901.

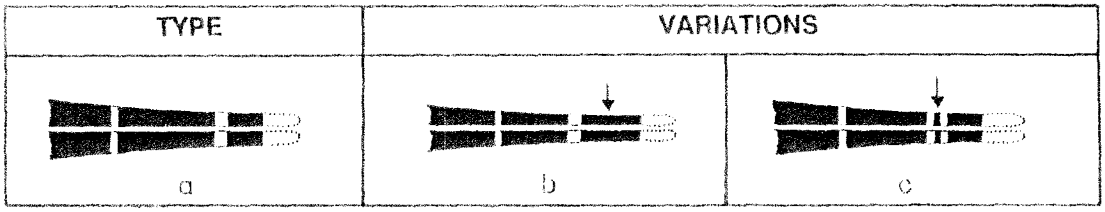

Fig. 10: Anopheles subpictus, Grassi, 1899.

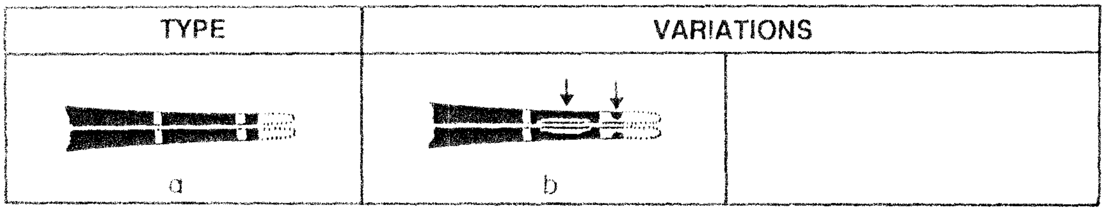

Fig. 11: Anopheles vagus, Donitz, 1902.

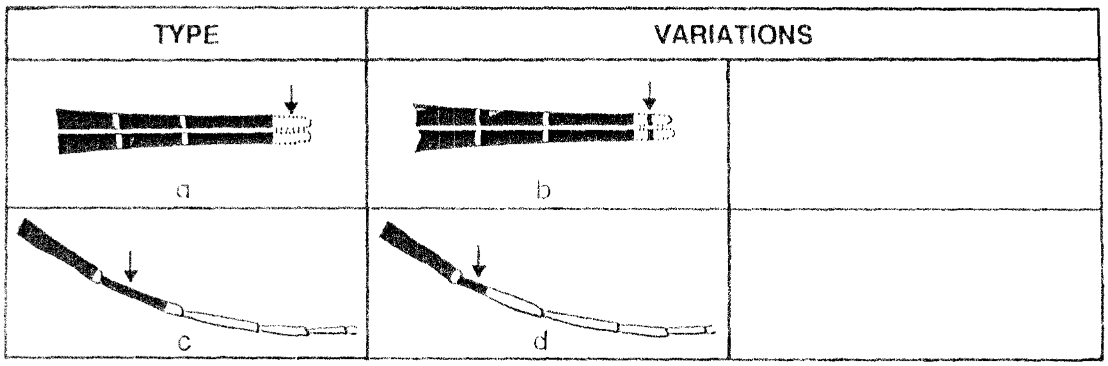

Fig. 12: Anopheles philippinensis, Ludlow, 1902.

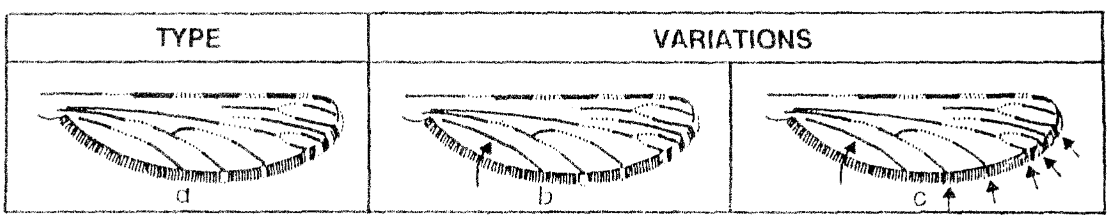

Fig. 13: Anopheles sergenti, Theobald, 1907.

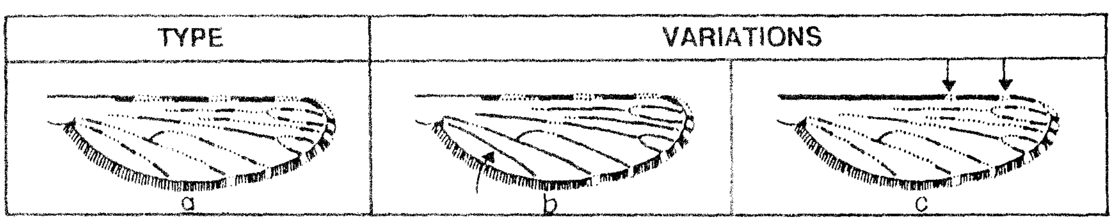

Fig. 14: Anopheles varuna, Iyengar, 1924.

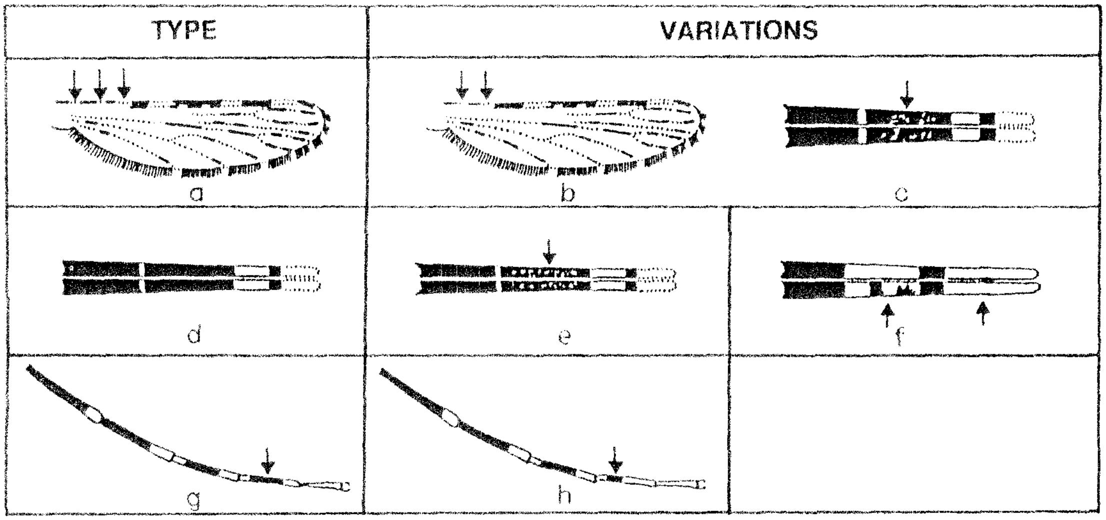

Fig. 15: Anopheles maculatus, Theobald, 1901.

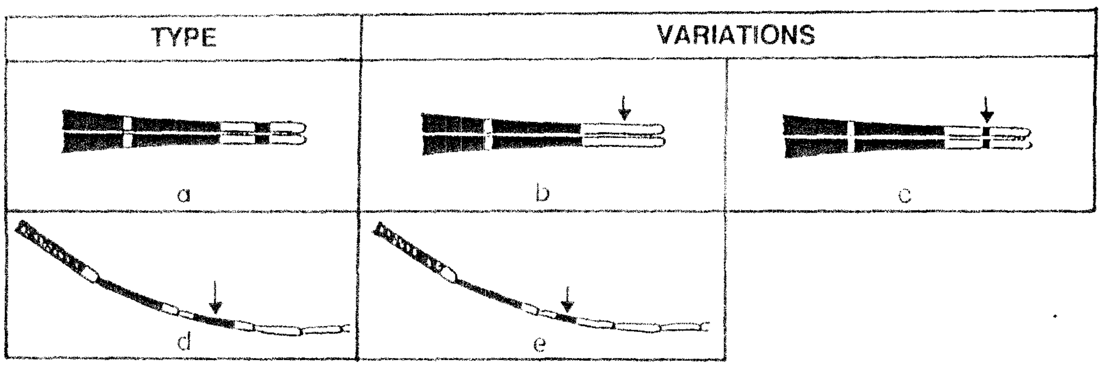

Fig. 16: Anopheles theobaldi, Giles, 1901.

usual character and the same, if present, is variation only.

Some of the characters, which are usually present in the variety form of a species, are also sometimes found in the type form. This needs careful examination of other key characters to confirm whether it is the type form or the variety. For example, in two specimens of *A. maculatus* (type form) two pale interruptions at the base of costa, and three black spots in sixth vein (Fig. 15b) were observed. These are the characters of variety *willmorei*. But there was a preapical dark spot in the wing which resembled type form (Fig. 15a). Further, the usual characters of variety *willmorei* like profuse and broad scales on the whole area of tergites III-VII, a well developed patch of broad scales on segment II, fore tarsi frequently apically banded only etc. were not found in these specimens. Hence these specimens could not be called as variety *willmorei* and the two characters, two pale interruptions and 6th vein with three black spots were considered to be the variations in type form.

In another six specimens while scattered scales were found in the middle black band of palpi, giving speckled appearance (Figs. 15c and e). The presence of speckling in palpi is considered to be the conspicuous feature of the variety *willmorei*. However in these specimens except palpal speckling no other characters of variety *willmorei* were found. Hence the palpal speckling was also included as a variation in the type form of *A. maculatus*.

As a rare phenomenon in some anophelines one of the palpi was found to be shorter in length (Figs. 3h and 5h). This may probably be due to the developmental abnormality.

All these phenotypic variations in anophelines were detected mostly in forested hilly areas and might probably be due to some micro-evolutionary process underway in this area to overcome environmental stress brought about by deforestation and prolonged use of insecticide.

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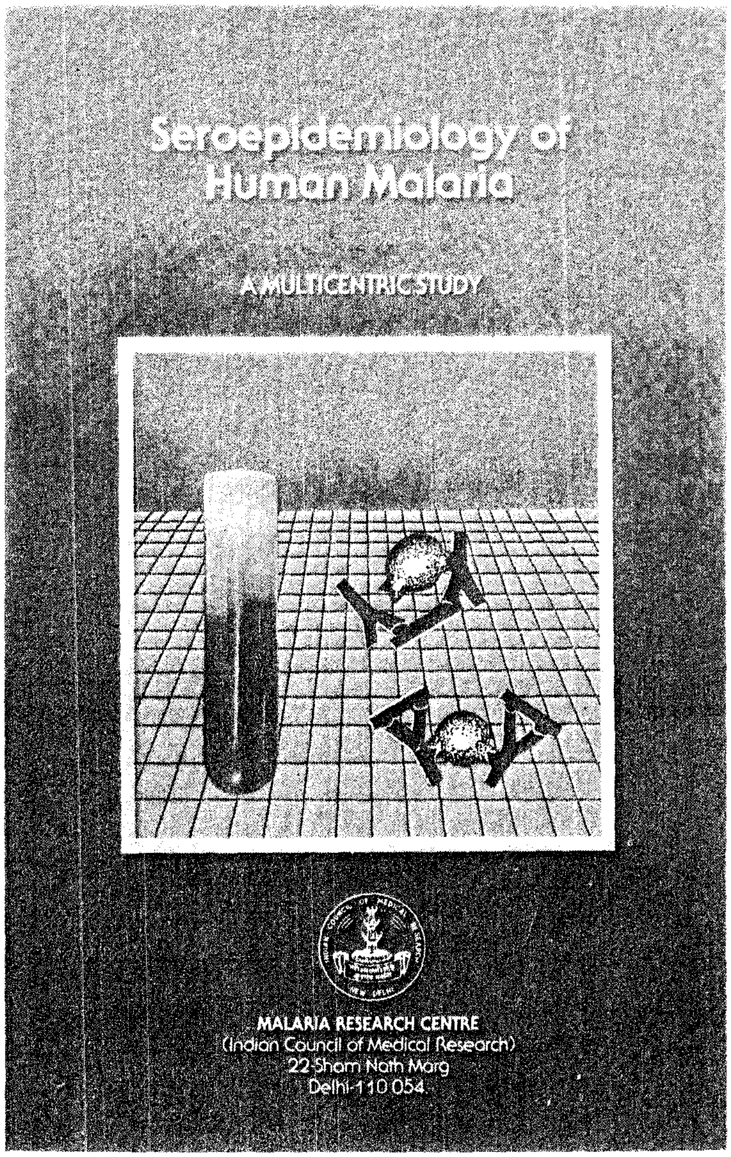

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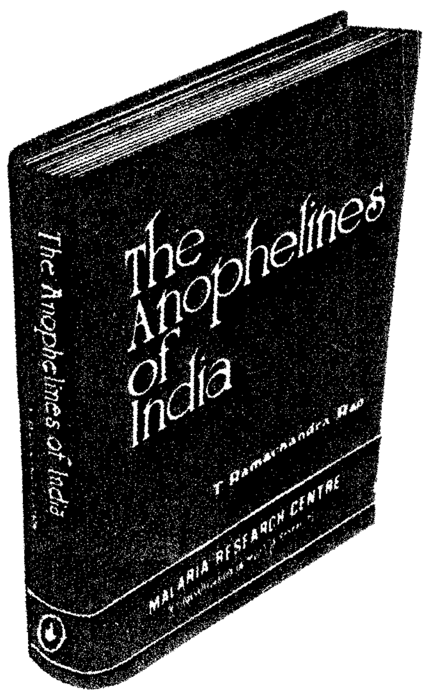

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