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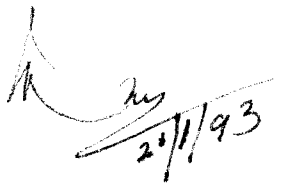

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Esbiothrin-Impregnated Ropes as Mosquito Repellent

M.A. ANSARI*, V.P. SHARMA* and R.K. RAZDAN*

Esbiothrin [(±)-3-allyl-2-methyl-4-oxocylopent-2-enyl-(+)-*trans*-chrysanthemate] is an improved isomeric composition of allethrin series and consists essentially of esters of chrysanthemic acid and allethrolone. Jute rope was impregnated with esbiothrin and the smoke from smouldering ropes was evaluated as mosquito repellent in human dwellings and cattlesheds with open doors and windows at different dosages. Esbiothrin-impregranted (500 ppm) ropes prevented the entry of more than 95% *An. culicifacies* and other anophelines, 90.9-88.8% *Culex quinquefasciatus* and 96-95.1% total mosquitoes in open rooms of houses and cattlesheds respectively. The impact of ropes was more pronounced on the biting rate of mosquitoes. Indoors and outdoors human baits seated at a distance of about 3 m from smouldering esbiothrin ropes experienced no bite at all from *An. culicifacies*. An iron mesh around the rope prevents fire hazards.

INTRODUCTION

Prior to the discovery of synthetic chemicals, personal protection measures were considered most practical to prevent mosquito bites. The use of natural products such as plant barks, leaves, flowers and oils was quite common during ancient times and these are still being used in several countries[1]. In the middle of this century, synthetic non-toxic products were developed to repel mosquitoes. In 1954, Deet (N, N-diethyl-m-toluamide) was discovered which has a good repellent action against mosquitoes and even against blood-sucking land leeches[2,3]. There are a variety of chemical devices marketed in the country but mosquito coils and mats are quite popular due to their good repellent action, though they are expensive if used daily[4-6]

Recently, Sharma *et al.*[7] reported a new technique of repelling mosquitoes using smouldering deltamethrin-impregnated ropes. The technique prevented entry of mosquitoes and provided more than 90% protection at a dosage of as low as 80 ppm for 10-12 h in open rooms against *An. culicifacies*, a principal vector of malaria. The commonly used formulation in mats and coils throughout the world, however, is esbiothrin. Ropes impregnated with esbiothrin at different dosages were evaluated against mosquitoes in Ghaziabad district villages of Uttar Pradesh. The results of this study are reported in this paper.

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MATERIALS AND METHODS

Esbiothrin® is an improved isomeric composition of allethrin series and consists essentially of esters of chrysanthemic acid and allethrolone. The chemical name of esbiothrin is [(±)-3-allyl-2-methyl-4-oxocylopent-2-enyl-(+)-*trans*-chrysanthemate]. It is a viscous oil and technically it contains *d-trans*-chrysanthemate of *d*-allethrolone and *d-trans*- chrysanthemate of *l*-allethrolone in the ratio of 72:21 with *cis* isomers at 2% maximum. It is manufactured by Roussel UCLAF, Paris, and claimed by manufacturers to be a quick knockdown agent particularly effective against mosquitoes when used in coils and mats. Technical esbiothrin (93%) was obtained through the courtesy of Roussel Pharmaceuticals India Ltd.

Jute ropes of 0.9 cm diameter were selected because of their availability in rural areas and also because of uninterrupted burning quality. The ropes were impregnated with 125, 250, 500 and 1000 ppm esbiothrin i.e. ml/L, in kerosene oil, dipped in the required concentration of the insecticide for half an hour, dried in shade for 48 h, and stored in cardboard boxes until use.

An iron wire mesh cylinder of 1 m length and 0.04 diameter was fabricated to prevent fire hazards in huts. The treated rope was inserted from the top exposing about 3 in. of the lower portion to facilitate burning of the rope (Fig. 1). The top portion of cylinder was provided with a hook for hanging the rope at a convenient point inside the room. The rope was burnt uninterrupted from dusk to dawn to prevent the entry of mosquitoes.

Ramgarh village in Dadri PHC and Jadaonpur village in Dhaulana PHC, District Ghaziabad, were selected for evaluation of the rope because the area has a very high density of *An. culicifacies*. Experiments were conducted in human dwellings and cattlesheds of (4 × 3 m size rooms with open windows and doors). Control and experimental rooms were switched periodically to minimize bias. For each concentration of esbiothrin-treated

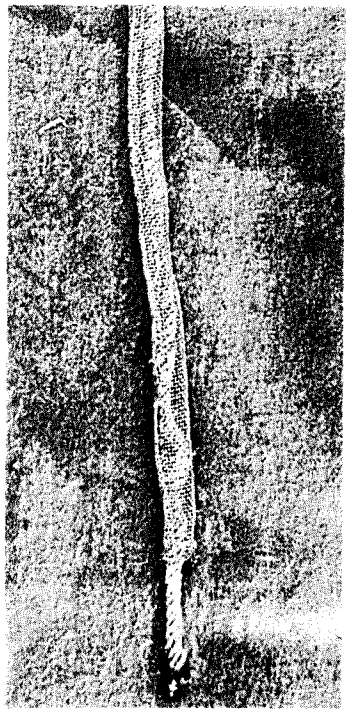

Fig. 1 : Smouldering of esbiothrin-impregnated rope inserted in iron (mesh) cylinder.

rope three types of controls were maintained – normal rope, kerosene-dipped rope and no rope. Adult mosquitoes found in the control and experimental rooms were collected for 15 min at an interval of 1 h from 1900 to 0600 hrs. In addition to this man-biting collections were also made indoors and outdoors in experimental and control structures, which were located at about 125 m away from each other. The mosquitoes which landed on human bait were collected by an Insect Collector with the help of suction tube and torch from 1800 to 0600 hrs. Mosquitoes collected at hourly intervals from each room were identified and reconfirmed in the laboratory with the help of a hand lens. The data from both the villages were pooled for different dwellings and the percentage protection was calculated on the basis of mosquitoes collected in the room without rope. Similarly, the percentage protection of normal and kerosene-treated ropes was calculated

and compared with the results from esbiothrin-impregnated ropes.

RESULTS AND DISCUSSION

Length and weight of rope, and quantity of kerosene and esbiothrin used in the impregnation of ropes are given in Table 1. In the preparation of jute rope containing 125 to 1000 ppm (0.125 to 1.0 ml) technical esbiothrin, 93% was used per kg of rope weight.

Results of burning esbiothrin-impregnated rope in human dwellings and cattlesheds are given in Table 2. The smoke from normal rope and kerosene-impregnated rope also produced some repellent effect on mosquitoes but there was considerable variation in the protection provided by these two types of ropes. The per cent protection with normal and kerosene-impregnated rope varied from 30 to 40 but against mosquitoes it was directly proportional to the concentration of esbiothrin in the rope. At 125 ppm the protection was about 80% and at 250 ppm it was about 90%. Increase of dosage to 500 ppm increased the protection marginally with a further marginal increase at 1000 ppm. The per cent protection was marginally better against anophelines in comparison with culicines, and esbiothrin-impregnated ropes were found effective both in human dwellings and cattlesheds. The treated ropes were tested in cattlesheds because people in this area sleep there from the onset of rains and throughout the winter season. The 500 ppm dosage was good enough against all mosquito species and provided a consistent level of protection. This has been corroborated by statistical analysis of the relative efficacy of different dosages both in cattlesheds and human dwellings. The 'p' values were < 1.2822, 1.5751, 1.5765 and 1.304 against *An. culicifacies,* total anophelines, *Culex quinquefasciatus* and total mosquitoes respectively, when per cent protection was compared with 500 and 1000 ppm dosages. However, the difference was significant ($p > 2.6892$) at 1% level when per cent protection was compared at 250 ppm dosage as between culicines and anophelines. There was no significant difference at 10% level ($p < 0.6193$) when per cent protection in cattlesheds and human dwellings was compared.

The data for whole night collection are pooled and the average protection of eight replicates from dusk to dawn with different species at different dosages of esbiothrin-impregnated rope are given in Table 3. The per cent protection by and large was consistent at different hours and dosages except at the lowest dosage (125 ppm). The variation was from 84.6 to 98.5 in *An. culicifacies* in human dwellings as against from 81.4 to 98.3 in cattlesheds. The variation was more pronounced against *An. annularis, An. subpictus* and *Culex*

Table 1. Weight and length of jute rope and esbiothrin quantity required with kerosene for impregnation at different dosages

Rope weight (kg)	Rope length (m)	Kerosene oil quantity required for impregnation (l)	Esbiothrin quantity (ml) required to mix in kerosene oil at different dosages (ppm)			
			125	250	500	1000
1	36.2	1.5	0.125	0.25	0.5	1
2	72.0	2.3	0.250	0.50	1.0	2
3	108.2	3.5	0.375	0.75	1.5	3
4	144.5	4.6	0.500	1.00	2.0	4
5	180.3	5.8	0.625	1.25	2.5	5
10	359.6	11.5	1.250	2.50	5.0	10

Table 2. Protection from mosquitoes provided by smouldering esbiothrin-impregnated ropes

Dose (ppm)	Type of rope	Protection (%) in houses*			
		An. culicifacies	Anophelines	*Cx. quinquefasciatus*	Total mosquitoes
125	Treated	84.6 – 98.5	78.5 – 98.4	51.8 – 84.6	75.7 – 93.3
	Kerosene	18.5 – 60.2	27.8 – 68.2	10.0 – 64.7	29.9 – 63.7
	Normal	10.9 – 2.50	21.7 – 30.3	7.6 – 45.4	24.1 – 32.7
250	Treated	91.3 – 100.0	86.5 – 99.2	72.2 – 100.0	87.2 – 97.2
	Kerosene	0.0 – 66.7	7.3 – 58.5	4.0 – 55.5	7.6 – 63.1
	Normal	5.5 – 42.7	19.2 – 48.7	8.0 – 41.1	18.5 – 42.1
500	Treated	95.0 – 100.0	94.6 – 100.0	84.0 – 100.0	93.3 – 100.0
	Kerosene	41.5 – 71.9	49.2 – 76.9	12.5 – 60.5	49.1 – 69.7
	Normal	11.5 – 34.8	20.4 – 36.6	12.5 – 69.6	23.2 – 37.2
1000	Treated	90.5 – 100.0	94.7 – 100.0	84.3 – 100.0	93.2 – 100.0
	Kerosene	25.9 – 66.9	31.9 – 63.8	16.6 – 48.0	34.2 – 61.3
	Normal	20.9 – 65.1	27.7 – 64.4	0.0 – 52.9	26.3 – 61.8
		Protection (%) in cattlesheds*			
125	Treated	81.4 – 98.1	85.6 – 98.3	4.0 – 67.8	77.3 – 92.2
	Kerosene	13.2 – 64.7	35.0 – 60.7	9.3 – 79.3	30.3 – 59.0
	Normal	16.8 – 39.4	14.7 – 35.5	13.4 – 35.7	16.5 – 33.1
250	Treated	90.4 – 99.1	89.5 – 97.7	11.1 – 96.8	86.1 – 97.2
	Kerosene	17.1 – 38.4	27.9 – 42.9	22.0 – 58.1	25.7 – 68.8
	Normal	11.4 – 42.7	6.4 – 27.2	2.5 – 56.0	5.6 – 36.7
500	Treated	91.6 – 100.0	88.3 – 99.4	59.5 – 100.0	83.6 – 99.5
	Kerosene	39.8 – 70.1	42.5 – 70.8	20.0 – 56.0	40.4 – 66.6
	Normal	28.0 – 46.5	23.5 – 42.5	6.2 – 35.8	24.0 – 42.8
1000	Treated	98.4 – 100.0	97.8 – 100.0	81.8 – 100.0	96.9 – 100.0
	Kerosene	3.6 – 62.4	11.5 – 60.1	13.7 – 34.2	11.8 – 53.4
	Normal	1.1 – 15.7	6.4 – 25.0	6.4 – 28.5	8.0 – 22.9

*Average of eight replicates.

Note : Protection is calculated by subtracting total mosquitoes in experimental room from the control and divided by control and multiplied by 100.

quinquefasciatus particularly at dawn (0500 to 0600 hrs) in both human dwellings and cattlesheds. Similar results were obtained by earlier workers with deltamethrin-impregnated ropes[7] However, the influx of mosquitoes particularly at dawn was not observed for *An. culicifacies* at higher dosages (500 and 1000 ppm) of esbiothrin-impregnated rope either in human dwellings or in cattlesheds. The per cent protection against *An. culicifacies* at dawn (0500 to 0600 hrs) was 91.3, 98.0, 98.9 at 250, 500 and 1000 ppm dosages respectively in human dwelling as against 90.4, 93.3 and 99.09 respectively in cattlesheds. *An. annularis, An. subpictus* and *Culex quinquefasciatus* were found less susceptible in comparison to *An. culicifacies* and required high dosages, i.e. 500 or 1000 ppm, to check sudden influx of the population of this species in human dwellings and cattlesheds. Statistical analysis also revealed that there was no significant difference when whole night per cent protection of *An. culicifacies* was compared with figures for *An. annularis* ($p < 0.4896$), *An. subpictus* ($p < 0.4373$) and total anophelines ($p < 0.1688$) at 10% level with 500

Table 3. Entries of mosquitoes in human dwellings and cattlesheds with and without smouldering rope

Dose (ppm)	Species	No. of adults collected in 8 nights from 1900 – 0600 hrs					
		Human dwellings		% protection range	Cattlesheds		% protection range
		E	C		E	C	
125	*An. culicifacies*	60	853	84.61 – 98.5	90	1251	81.40 – 98.30
	An. annularis	5	164	69.20 – 100.0	9	114	80.00 – 100.0
	An. subpictus	47	446	60.00 – 100.0	54	557	75.30 – 100.0
	Total anophelines	112	1463	78.50 – 98.4	153	1922	85.60 – 98.30
	Culex quinquefasciatus	110	342	51.80 – 84.60	188	368	4.00 – 67.80
	Total mosquitoes	222	1805	75.70 – 99.30	341	2290	77.30 – 92.20
250	*An. culicifacies*	33	976	91.30 – 100.0	52	1277	90.40 – 99.10
	An. annularis	38	112	28.50 – 100.0	28	92	20.00 – 100.0
	An. subpictus	14	448	88.80 – 100.0	22	593	91.00 – 100.0
	Total anophelines	85	1536	86.50 – 99.28	102	1962	89.50 – 97.70
	Culex quinquefasciatus	34	252	72.20 – 100.0	75	331	11.10 – 96.80
	Total mosquitoes	119	1788	87.10 – 97.40	177	2293	86.10 – 97.20
500	*An. culicifacies*	16	960	95.00 – 100.0	50	1367	91.60 – 100.0
	An. annularis	3	112	85.70 – 100.0	32	140	63.60 – 100.0
	An. subpictus	10	391	92.50 – 100.0	15	567	89.30 – 100.0
	Total anophelines	29	1463	94.60 – 100.0	97	2074	88.30 – 99.40
	Culex quinquefasciatus	22	283	84.00 – 100.0	37	350	59.50 – 100.0
	Total mosquitoes	51	1746	93.30 – 99.30	134	2424	83.60 – 99.50
1000	*An. culicifacies*	14	1192	90.50 – 100.0	12	1691	98.40 – 100.0
	An. annularis	1	55	90.00 – 100.0	5	125	66.60 – 100.0
	An. subpictus	2	616	98.00 – 100.0	5	903	96.60 – 100.0
	Total anophelines	17	863	94.70 – 100.0	22	2719	97.80 – 100.0
	Culex quinquefasciatus	13	244	84.30 – 100.0	20	367	81.80 – 100.0
	Total mosquitoes	30	2107	93.20 – 100.0	42	3086	96.90 – 100.0

E = Experimental rooms with smouldering esbiothrin-impregnated rope; C = Control rooms without rope.

ppm dosage. However, this difference was significant at 5% level ($p > 2.0362$) as between *An. culicifacies* and *Culex quinquefasciatus*.

Impact of impregnated rope on biting rate of mosquitoes

Since man-mosquito contact is essential for transmission of the disease, studies were also made to evaluate the biting rate on human bait with 125, 250, 500 and 1000 ppm esbiothrin-impregnated ropes. Eight nights' collections were pooled, and the average number of female mosquitoes which landed on indoor and outdoor baits are given in Table 4. The table shows that man-mosquito contact particularly with *An. culicifacies* is not very high even in control. However, on the basis of bait exposed to the impregnated rope and without rope, the average number of mosquitoes landed/night/bait on *An. culicifacies*/bait/night was 1.0, 0.75, 0.0 and 0.0 at 125, 250, 500 and 1000 ppm dosages respectively, as against 6.7, 7.7, 4.7 and 4.8 on bait sitting indoor where no rope was burnt. In outdoor conditions the average number of *An. culicifacies*/bait/night was 0.12, 0.62, 0.0 and 0.0 respectively at similar dosages as against 4.37, 5.0, 2.25 and 2.12 respectively in control baits. However absolute protection was not observed against *Culex quinquefasciatus* even at higher dosages. The average number on control bait varied from 23.5 to 36.4 indoors and from 18.3 to 38.7 outdoors on bait sitting in place where no rope was burnt. The average number on bait exposed to esbiothrin rope varied from 0.12 to 2.6/night indoors and from 0.25 to 3.12 in outdoor conditions at different dosages. Biting activities in indoor and outdoor conditions at 500 and 1000 ppm of both *An. culicifacies* and *Culex quinquefasciatus* are shown in Fig. 2 (a,b). It was revealed that the biting peaks of *An. culicifacies* and *Culex quinquefasciatus* occurred at 2400 to 0100 hrs as against 2300 to 2400 hrs in *Culex quinquefasciatus* in both indoor and outdoor conditions. In general, biting activities were higher indoors than outdoors in both anophelines and culicines. Regardless of indoor and outdoor conditions, baits seated at a distance of 3 m from esbiothrin-impregnated smouldering rope were fully protected from *An. culicifacies* bites. Biting activity was also reduced drastically against *Culex quinquefasciatus*.

The iron mesh cylinder not only provided safety from fire but also reduced the burning rate of the rope without affecting its efficacy. This improvisation further reduced the cost of the rope, as only 1 m rope was required for the whole night as against 1.2 m rope without the cylinder. The study has shown that esbiothrin-impregnated ropes are

Table 4. Mosquitoes collected landing on human baits seated in a room with and without smouldering esbiothrin-impregnated rope

Species		Dosage of esbiothin (ppm) and average no. of mosquitoes* landed on experimental (E), Control human baits (C) from 1800 to 0600 hrs							
		125		250		500		1000	
		E	C	E	C	E	C	E	C
An. culicifacies	Indoor	1.00	6.75	0.75	7.72	0	4.75	0	4.87
	Oudoor	0.12	4.37	0.62	5.0	0	2.25	0	2.12
Culex quinquefasciatus	Indoor	2.62	23.5	2.25	36.25	0.12	27.0	0.12	29.0
	Outdoor	1.12	23.75	3.12	38.75	0.37	18.37	0.25	28.37
Total mosquitoes	Indoor	3.62	30.25	3.0	44.0	0.12	31.75	0.12	33.87
	Outdoor	1.24	28.12	3.75	43.37	0.37	20.62	0.25	30.5

* Average of 8 night collections.

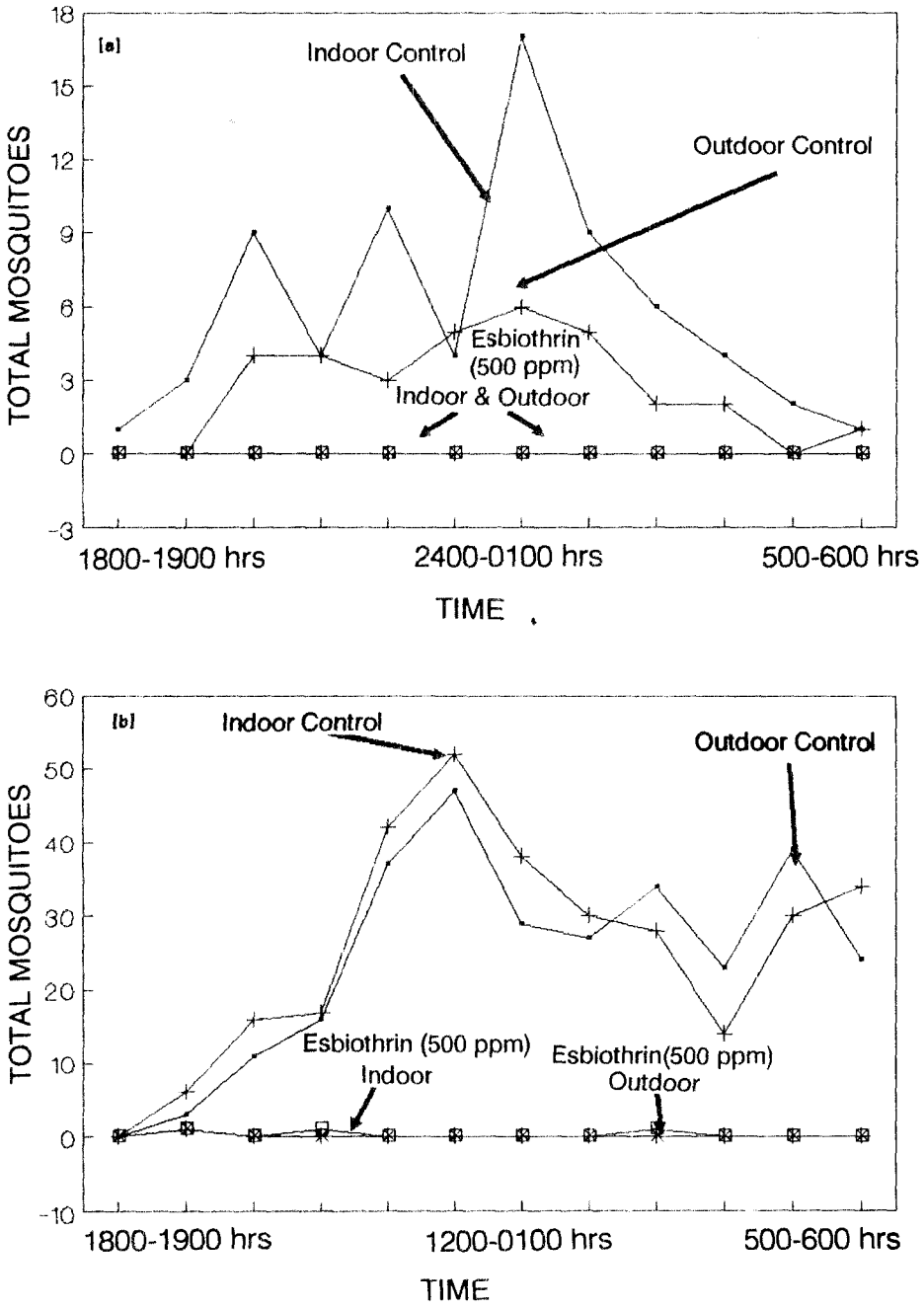

Fig. 2: Mosquitoes collected on human baits exposed to ropes treated with 500 ppm esbiothrin and allowed to smoulder all night. Collections were made from 1800 to 0600 hrs. Figure shows hourly collection and data pooled for 8 nights of (a) *An. culicifacies* and (b) *Cx. quinquefasciatus*. See almost complete absence of man-biting mosquitoes in rooms with treated ropes.

highly effective in preventing the entry of culicines and anophelines in open rooms and cattlesheds when ropes soaked in 500 and 1000 ppm esbiothrin in kerosene are burnt. The smoke from smouldering rope provided more than 96 and 98% protection at 500 and 1000 ppm respectively against anophelines in human dwelling from dusk to dawn. The protection was quite consistent throughout the night and no influx of mosquitoes was observed at these dosages. Since p values at 500 and 1000 ppm are $p > 3.0159$ and $p > 3.0195$ (indoors) and $p < 1.9943$ and $p > 2.015$ (outdoors), respectively against *An. culicifacies*, a lower dosage (500 ppm) is recommended for esbiothrin-impregnated rope on a large scale. The cost of the impregnated rope, calculated by Sharma *et al.*[7], is about 50 paise (US $0.03) per night/room, and hence esbiothrin-treated rope is the cheapest method of personal protection particularly in rural areas in comparison with commercially available repellents, viz. oil, cream, coils and mats. In addition to high cost, they do not provide adequate protection against mosquitoes[8] and some of them require uninterrupted supply of electrical power. The esbiothrin-impregnated rope may be appropriate for use in areas under the influence of *An. dirus, An. maculatus, An. minimus, An. fluviatilis* and *An. culicifacies*. According to an estimate, 30% of total malaria cases and 60% of falciparum cases are recorded in the tribal populations of the country residing under different geoepidemiological forest belts. Esbiothrin-impregnated ropes may provide very effective protection in these backward and neglected areas. It will also be an effective tool to protect labour population in urban slums.

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Sensitivity Status of *Plasmodium falciparum* to Chloroquine, Amodiaquine, Quinine, Mefloquine and Sulfadoxine/ Pyrimethamine in a Tribal Population of District Sundargarh, Orissa

S.K. GHOSH*, R.S. YADAV* and V.P. SHARMA†

In a malaria-endemic area of Orissa, wherein chloroquine has been in use for over thirty years, 58.3% (14/24) *P. falciparum* cases did not respond to single dose chloroquine (10 mg base/kg) in *in-vivo* test. With standard dose (25 mg base/kg) 31.2% cases (10/32) showed resistance, i.e. at RI (15.6%), RII (9.4%) and RIII (6.2%) levels. Standard dose was superior in response to the single dose therapy [$p < 0.05$; $\chi 2$ (df 1) = 4.11]. Out of eight isolates tested *in vitro,* two showed resistance to chloroquine, five to sulfadoxine/pyrimethamine (SP) but all were sensitive to amodiaquine, quinine and mefloquine. Whereas the standard dose of chloroquine would be a better option in general, in resistant cases, SP, quinine and mefloquine offer an alternative drug choice. The implications of drug resistance in a malaria-control programme and the need to revise drug policy in India are discussed.

INTRODUCTION

In Sundargarh district (situated in the north-western Orissa) malaria exists in hyperendemic form and constitutes a major public health problem. A recent study by Yadav *et al.*[1] has revealed that nearly 80% of all malaria cases are *Plasmodium falciparum* cases and the rest, *P. vivax* and *P. malariae* cases. Sundargarh, with a population of 1.5 million (in 1988), is a tribal area and is located in a forested hilly terrain. Under the National Malaria Eradication Programme (NMEP) the use of antimalarial drugs and indoor spray of residual insecticides have been adopted for over 30 years to control the disease in this area. The NMEP drug policy prescribes the use of a single dose regimen of chloroquine (600 mg base, adult dose) for presumptive treatment in all those areas having chloroquine-sensitive *P. falciparum* malaria, but a single dose of amodiaquine (600 mg base) in resistant areas. For radical treatment a further regimen of 600 mg base chloroquine plus 45 mg primaquine is prescribed and in resistant cases, 1000 mg sulphalene plus 50 mg pyrimethamine and 45 mg primaquine. Quinine is the drug of choice in severe and complicated *P. falciparum* cases.

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Occurrence of chloroquine-resistant *P. falciparum* has been reported in different parts of India by Sharma[2], and in some areas of Orissa by Guha *et al.*[3] A case of sulfalene/pyrimethamine resistance was also reported from Delhi[4]. In Sundargarh, where the present study was carried out, except for a solitary report in 1984 of the presence of RII level chloroquine resistance[5], there is no information on the sensitivity of *P. falciparum* to other antimalarial drugs in use.

Thus, in order to determine the efficacy of chloroquine against *P. falciparum* and sensitivity of this species of parasite to other antimalarial drugs, *in-vivo* and micro *in-vitro* tests against chloroquine and micro *in-vitro* tests against amodiaquine, quinine, SP and mefloquine were carried out. The study was part of an applied field research project on malaria in the tribal population of Sundargarh.

MATERIALS AND METHODS

P. falciparum cases were identified through active case detection in the resident tribal population of the villages around Rourkela city in Sundargarh district by examining thick and thin blood smears. Urine examination for the possible presence of 4-aminoquinolines using Dill and Glazko test[6] and for sulpha compounds using Lignin[7] test were carried out. The final selection of cases was done according to the WHO-prescribed procedure[8]. Informed consent of the patients, or guardians in the case of children, to participate in the study was taken. Parasite density was determined by counting the number of asexual parasites per 1000 leucocytes in thick smear and then calculations were made for 8000 leucocytes per μl of blood.

In-vivo **test**: An extended *in-vivo* study was conducted during the dry hot season from March to June 1990 for evaluating sensitivity to chloroquine according to the standard WHO method. Two regimens of chloroquine in tablet form (chloroquine phosphate, 150 mg chloroquine base per tablet) were given orally: (i) 10 mg chloroquine base per kg body weight in a single dose, and (ii) standard regimen of 25 mg chloroquine base per kg body weight over a three-day period (10 mg/kg, 10 mg/kg and 5 mg/kg respectively). Although the NMEP drug policy prescribes a total dose of 1200 mg chloroquine (600 mg presumptive and 600 mg radical) in sensitive falciparum cases, in practice, the radical dose is administered in a large number of cases after a long period, sometimes a few weeks later. Hence, a majority of falciparum cases detected during active surveillance under primary health care system get 600 mg chloroquine only as effective dose. Therefore, we intended to find out the actual response of a single dose treatment in *P. falciparum* versus the standard dose. Consumption of chloroquine was strictly monitored and the presence of chloroquine in body fluid was confirmed by urine test. Parasite counts were made daily from Day 0 to Day 7 and then on Day 14, 21 and 28. In patients showing reduced sensitivity (non-response) to chloroquine the test was terminated and the patients were treated with a single dose of SP (Malocide, Torrent Labs. Pvt. Ltd.) and followed for additional 7 days.

In-vitro **test**: Micro *in-vitro* test was carried out in the month of July 1990 for testing the sensitivity to chloroquine, amodiaquine, quinine, mefloquine and SP following the standard micro-technique[9] using WHO test kit (Mark II). Two hundred μl of blood, obtained aseptically by finger prick from each patient, was added to separate sterile plastic vials containing 1.8 ml of liquid culture medium (RPMI-1640, LPLF with lyophilized L-glutamine) and shaken gently. Wells in the predosed flat-bottom tissue culture plates with serial concentrations in picomol of chloroquine (0,1,2,4,8,16,32,64), amodiaquine (0,0.25,0.5, 1,2,4,8,16), quinine (0, 4, 8, 16, 32, 64, 128, 256), mefloquine (0, 2, 4, 8, 16, 32, 64, 128), SP (0, 10, 30, 100, 300, 1000, 3000, 10000 in 80:1 ratio) were charged each with 50 μl aliquots of the blood-medium mixture. The plates were gently shaken, placed in a candle jar and incubated at 37.5 $\pm$0.5°C. The incubation period (maturation of rings to schizonts) was determined by periodic

examination of the residual culture concurrently incubated in the plastic vials. Smears from post-incubated culture were air-dried, stained with JSB stain and the number of schizonts (showing three or more nuclei) per 200 asexual parasites was determined. Culture samples showing at least 10% schizont maturation in control wells were considered valid. The minimum inhibitory concentrations (MICs) of all the drugs were recorded from the results of the post-incubation smear examinations. Thus, schizont maturation at $\geq$ 8 pmol of chloroquine, $\geq$ 4 pmol of amodiaquine, = 256 pmol of quinine, $\geq$ 64 pmol of mefloquine and $\geq$ 1000/12.5 pmol of SP indicated resistance[10].

RESULTS AND DISCUSSION

Forty-one *P. falciparum* cases were selected for single dose *in-vivo* chloroquine test; however, only 24 cases could be followed. The results of the test are summarised in Table 1. Asexual parasitaemia disappeared by Day 4 in 10 (41.7%) out of 24 cases (s. no. 15 to 24) and remained absent for the full follow-up period of 28 days, thereby showing sensitivity to chloroquine. In the remaining 14 cases (58.3%), parasitaemia was either continuously or intermittently present during the follow-up period, thereby showing resistance to the drug at a single dose regimen.

In extended *in-vivo* test with the standard dose of chloroquine (25 mg base per kg), out of 56 cases selected, 32 could be followed up (Table 2). Out of the 32 isolates, 22 (68.8%) were found fully sensitive to chloroquine (s. no. 11 to 32). In the rest, 5 isolates (15.6%) were resistant at RI level (s. no. 6 to 10), 3 (9.4%) were at RII level (s. no. 3 to 5) and 2 (6.2%) at RIII level (s. no. 1 and 2).

A total of 12 *P. falciparum* isolates were tested separately for micro *in-vitro* drug sensitivity (Table 3). Out of 12 isolates, 8 showed normal growth in control wells. Two isolates were found resistant to chloroquine as the parasites did not grow at the chloroquine concentrations of 16 and 32 pmol per well, respectively. MIC for one isolate was at the break point of 8 pmol, whereas the remaining five isolates were highly sensitive to chloroquine (MIC $\leq$ 4). Out of 8 isolates tested *in vitro* for sensitivity to SP, 3 were fully sensitive, whereas 5 did not give satisfactory response (MIC $\geq$ 3000/37.5 pmol). One out of the 5 cases was, however, highly resistant (MIC = 10,000/375 pmol). Both the chloroquine-resistant isolates also showed *in-vitro* cross-resistance to SP. However, all the eight isolates showed normal sensitivity to amodiaquine, quinine and mefloquine as the MICs were far lower than the break point concentrations.

When the chloroquine non-responders at the end of *in-vivo* study were given a single-dose Malocide (SP), the clinical and parasitological responses were satisfactory up to the next 7-day observation period.

In early 1950s *P. falciparum* strains in India were highly sensitive to single-dose chloroquine treatment, i.e. 600 mg single dose. Singh *et al.*[11] in 1953, working in Nainital terai in north India, observed total clearance of *P. falciparum* parasites in 122 cases within 72 h of single-dose chloroquine treatment (600 mg, adult dose). Similar observations were made by Roy *et al.*[12] in 1977 in Karnataka. They found that the presumptive treatment with a single-dose chloroquine (600 mg) was fully effective against *P. falcipaurn*. However, a study in Koraput and Sambalpur districts of Orissa revealed that a single-dose chloroquine treatment failed to clear parasitaemia in 3.8% and 12% cases, respectively[13]. But no resistant case was found in Balangir district. In 1989 Mohapatra *et al.*[14] detected a failure rate of 34.3% with single-dose chloroquine in Koraput district in southern Orissa.

In our study, chloroquine at a dosage of 10 mg base per kg body weight failed to clear asexual parasitaemia in 58.3% cases, thereby indicating a further diminished efficacy of the drug. In 41.7% cases it was fully sensitive and the mean parasite

Table 1. Single dose *in vivo* chloroquine (10 mg base per kg) sensitivity of 24 isolates of *P. falciparum*

S. No.	Age/ Sex	Weight (kg)	Asexual parasitaemia (per μl blood)										Parasite response
			D0	D1	D2	D3	D4	D5	D6	D7	D14	D21	D28	
1.	2½/F	9	10850	4350	5190	19740*								NR
2.	16/M	30	62500	6120	15270	12230	32590*							NR
3.	30/M	43	25950	5120	3760	2830	4190	6320	890	110	6750*			NR
4.	7/M	19	65770	5180	360	4930	5420	8550	25740*					NR
5.	15/M	39	18110	6780	1550	340	580	400	790*					NR
6.	20/M	39	10920	2330	2760	7420*								NR
7.	5/F	12	4230	660	0	0	2120*							NR
8.	4/F	13	14320	22550	2060	500	0	0	0	0	0	9840*		NR
9.	18/M	38	64860	525	0	0	0	42120*						NR
10.	6/M	14	950	220	0	0	0	0	780*					NR
11.	45/F	32	4255	1050	0	0	0	0	210	0	4000*			NR
12.	5/M	13	1670	750	0	0	0	0	0	0	8250*			NR
13.	5/F	14	520	0	0	0	0	0	0	500	14750*			NR
14.	8/M	25	32660	0	0	0	0	0	0	0	10320*			NR
15.	45/M	28	1430	1150	1280	375	0	0	0	0	0	0	0	S
16.	19/M	36	1520	3340	520	270	0	0	0	0	0	0	0	S
17.	20/M	42	610	32140	2100	0	0	–	0	0	0	0	0	S
18.	26/F	26	36645	0	775	0	0	0	0	0	0	0	0	S
19.	40/F	36	2370	470	0	0	0	0	0	0	0	0	0	S
20.	48/M	54	26670	8620	0	0	0	0	0	0	0	–	0	S
21.	2½/M	9	10750	9830	0	0	0	0	0	0	0	0	0	S
22.	6/F	26	7560	0	0	0	0	0	0	0	0	0	0	S
23.	15/M	40	7820	0	0	0	0	0	0	0	0	0	0	S
24.	6/F	15	28140	0	0	0	0	0	0	0	0	0	0	S

(–) = Smears not available; NR – Non-response; S – Sensitive; *Test terminated and the patient treated with Malocide.

Table 2. *In vivo* **chloroquine (25 mg base per kg) sensitivity of 32 isolates of** *P. falciparum*

S. No.	Age/ Sex	Weight (kg)	Asexual parasitaemia (per μl blood)										Level of resistance
			D0	D1	D2	D3	D4	D5	D6	D7	D14	D21	D28	
1.	12/F	30	16570	8830	10190	12200*								RIII
2.	22/M	49	97300	4390	18670*									RIII
3.	2½/F	9	32500	25000	1260	3250	1240	220	3100	9980*				RII
4.	30/F	44	4680	930	440	2250*								RII
5.	25/F	35	11750	1300	290	400	550	490	360	270*				RII
6.	18/M	42	52775	8150	16560	1530	0	0	0	0	17880*			RI
7.	25/F	35	8580	6920	850	0	0	0	0	0	640	1225*		RI
8.	12/F	27	68200	32680	15700	0	0	0	0	0	80	7120*		RI
9.	20/F	42.5	27560	8930	1470	0	0	0	0	0	5630*			RI
10.	10/F	34	2800	18230	580	0	0	–	0	0	0	190	550*	RI
11.	45/F	28	1630	1920	1240	500	0	0	0	0	0	0	0	S
12.	10/F	25.5	6750	920	1000	1280	0	0	0	0	0	–	0	S
13.	4/M	12	22180	15840	4290	560	0	0	0	0	0	0	0	S
14.	32/F	48	15720	23270	3550	830	0	0	0	0	0	0	0	S
15.	25/M	40	670	5490	285	0	0	0	0	0	0	0	0	S
16.	25/M	40	1700	1235	580	0	0	0	0	0	0	0	0	S
17.	13/M	41	4350	2670	1630	0	0	0	0	0	0	0	0	S
18.	45/F	36	6550	16470	250	0	0	0	0	0	0	0	0	S
19.	15/F	36	89420	42730	1780	0	0	0	0	0	0	0	0	S
20.	22/F	43	9350	10850	370	0	0	0	0	0	0	0	0	S
21.	2/F	11	5190	2750	380	0	0	0	0	0	0	0	0	S
22.	18/M	41	18570	12550	0	0	0	0	0	0	0	0	0	S
23.	45/F	30	38660	6820	0	0	0	0	0	0	0	0	0	S
24.	25/M	45	880	540	0	0	0	0	0	0	0	0	0	S
25.	9/F	22	30650	420	0	0	0	0	0	0	0	0	0	S
26.	16/M	45	36150	23430	0	0	0	0	0	0	0	0	–	S
27.	7½/M	20	7740	3980	0	0	0	0	0	0	0	–	0	S
28.	3/F	9.5	18920	0	0	0	0	0	0	0	0	0	0	S
29.	10/F	29	5970	0	0	0	0	0	0	0	0	0	0	S
30.	3/M	8	7500	0	0	0	0	0	0	0	0	0	0	S
31.	60/M	51	8000	0	0	0	0	0	0	0	0	0	0	S
32.	25/M	44	850	0	0	0	0	0	0	0	0	0	0	S

(–) = Smears not available; R – Resistant; S – Sensitive; *Test terminated and the patient treated with Malocide.

Table 3. Micro *in-vitro* sensitivity of 8 isolates of *P. falciparum* to chloroquine, amodiaquine, mefloquine and sulphadoxine/pyrimethamine

S. No.	Age/Sex	Asexual parasitaemia (per μl blood)	MICs (in pmols)				
			Chloro-quine	Amodia-quine	Quin-ine	Meflo-quine	SP
1.	28/F	4250	4	0.5	16	4	300/ 3.75
2.	26/F	2180	4	1	16	2	300/ 3.75
3.	18/M	6320	1	2	64	8	3000/ 37.5*
4.	19/F	8160	16*	1	32	4	3000/ 37.5*
5.	25/M	10830	1	1	32	4	10000/ 375*
6.	1/F	55540	1	2	16	2	30/ 0.375
7.	6/M	63770	8	4	64	8	3000/ 37.5*
8.	65/M	4750	32*	4	64	4	3000/ 37.5*

Indication of resistance is schizont growth at: ≥8 pmol (chloroquine); ≥4 pmol (amodiaquine); = 256 pmol (quinine); ≥ 64 (mefloquine); and ≥1000/12.5 pmol (SP); * Showing resistance.

clearance time was 2.3 days. The response of chloroquine at the standard dose of 25 mg base per kg body weight was, however, found superior to that of the single dose regimen [$p < 0.05$; χ^2 (df 1) = 4.11]. The drug was found fully sensitive to 68.8% strains and the mean parasite clearance time was 2.4 days.

The presence of chloroquine-resistant strains was further confirmed through *in-vitro* tests in which 2 out of 8 isolates tested showed resistance. All the 8 isolates tested *in-vitro* were found sensitive to amodiaquine, quinine and mefloquine. In an earlier study in Madras and Jabalpur, chloroquine-resistant strains were found *in vitro* sensitive to mefloquine[15].

Five out of 8 isolates were *in vitro* resistant to SP, whereas three were sensitive. SP resistance had earlier been reported from southeast Asian countries[16], Africa[17,18] and Delhi[4]. However, all the chloroquine non-responders/resistant cases treated with Malocide (SP) at the termination of *in-vivo* test responded satisfactorily at the end of the 7-day observation period. Since the *in-vitro* test system provides baseline sensitivity of the strains to the drugs, our observations clearly indicated the presence of SP-resistant *P. falciparum* strains in the area. Therefore, it appears that the longer duration of *P. falciparum* infection during extended *in-vivo* test resulted in acquired immunity by the hosts and when the chloroquine-resistant cases were given SP in the end, host immunity might have synergised the action of SP in producing a sensitive response. Similar observations were recorded by Mohapatra *et al.*[14], who found *in-vitro* chloroquine-resistance in *P. falciparum* isolates but full sensitivity in *in-vivo* tests. Therefore this is a subject for further study as it has been observed that there was no definite correlation between the sensitivity of *P. falciparum* as measured by *in vivo* and *in vitro* methods[19].

Two chloroquine-resistant isolates had cross-resistance to SP. Cross-resistance, however, is known to exist in Thai strains also[20].

It is now certain that precipitation of resistance has posed a serious problem to the therapeutic dependence on chloroquine in Sundargarh. Strict monitoring is therefore needed, especially in migratory population, to organise measures for

checking further spread of resistant strains. Our study showed a relative superiority of standard dose of chloroquine to the single dose. Therefore, under primary health care system, efforts should be made for providing the standard dose and the gap between presumptive and radical dosages should be narrowed so as to improve the efficacy of the drug. As a result, it will be possible to provide timely clinical relief to patients as well as to arrest incipient resistance to chloroquine. Resistant foci need to be identified and diffused.

Where facilities for blood test are not available or are inadequate, clinical judgement should be exercised in delineating non-responders so as to provide alternative therapy for cure. This might necessitate dissemination of information on simple 'sentinel' tests for clinicians and health workers. Indiscriminate use of SP needs to be checked, especially in *P. vivax* and *P. malariae* cases. It is a matter of relief that all the *P. falciparum* isolates, including chloroquine- and SP-resistant ones, showed normal sensitivity to amodiaquine, quinine and mefloquine. Mefloquine is not in use at present in India and when introduced, it should be judiciously regulated. Sulfadoxine/Pyrimethamine (SP) and quinine continue to be the alternative drugs of choice. Since WHO does not warrant the use of amodiaquine as a first-line substitute for chloroquine, there is a need to revise the national drug policy on the use of amodiaquine for presumptive treatment of malaria.

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Malaria Epidemic in Baniyani Village, District Farrukhabad (U.P.)

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Many deaths were recorded in village Baniyani of Talgram PHC of District Farrukhabad, U.P. during August to November 1991, which is the malaria transmission season in this area. Integrated measures, like one-round spraying of DDT and HCH, six-round fogging of malathion and six-time application of Baytex in mosquito breeding sites, were adopted by the Health Department of the U.P. government to avert an epidemic. Investigations carried out by the Malaria Research Centre during November and December 1991 showed low mosquito densities and larval positivity but very high incidence of malaria in the village. No malaria case was recorded by the surveillance worker of the Health Department of the state government before the outbreak of the disease. However, high slide falciparum rate (51.57), child parasite rate (40.0), infant parasite rate (66.66) and spleen rate (82.90) in the village clearly indicated hyperendemic conditions in the area and hence deaths recorded in the village during the malaria transmission period could be due to malaria only. Major factors responsible for the deaths due to malaria in the village were poor surveillance, faulty diagnosis, and low literacy and socio-economic status of the villagers.

INTRODUCTION

Several sporadic malaria epidemics have been reported from Uttar Pradesh in the recent past[1-4]. Many deaths occurred in Baniyani village of Talgram Primary Health Centre (PHC) of District Farrukhabad during August to November 1991, which is the malaria transmission period in this area. Suspecting the deaths to be due to malaria epidemic, we carried out an investigation in this village to find out the probable cause of deaths and the incidence of malaria. The results of this study are discussed in this paper.

MATERIALS AND METHODS

Study area: Farrukhabad district, situated between 79 and 80° longitude E and 27 and 28° latitude N, adjoins the districts of Shahjahanpur, Kanpur, Etah and Mainpuri. The district has 16 PHCs. Five main rivers, namely Ganga, Ramganga, Kali Nadi, Esary and Pandu, pass through the district. The annual average rainfall is 62.5 mm and the temperature varies from a minimum of 6°C to a maximum of 44°C. The epidemic occurred in the villages of Talgram PHC. Many deaths were reported from the village Baniyani, situated about 4 km from the sub-PHC Gur-

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Table 1. Blood smear collections by NMEP worker from Baniyani village† during Jan to Dec 1991

Month	No. of blood slides collected	Positive for malaria			Total	Deaths
		P. vivax	*P. falciparum*	Mixed		
Jan	8	0	0	0	0	0
Feb	0	0	0	0	0	0
Mar	0	0	0	0	0	0
Apr	20	0	0	0	0	0
May	25	0	0	0	0	0
Jun	21	0	0	0	0	0
Jul	37	0	0	0	0	1
Aug	53	0	0	0	0	5
Sep	76*	0*	0*	0*	0*	8
Oct	2134	197	128	0	325	10
Nov	585	49	78	1	128	8
Dec	275	4	31	0	35	0

*38 slides were cross-examined, 16 were positive for *P. falciparum* (SfR = 42.10); 2 were positive for *P. vivax* (SvR = 5.26); SPR = 47.37; †Population = 1295.

sahayganj. The population of the village, according to the latest survey carried out by the Adult Education Department of U.P. government, is 1295 (males 795, females 500). Ninety per cent of the villagers are illiterate. Their main profession is agriculture, and the main crops of the area are wheat, maize and rice. About 90% of the villagers are also engaged part time in the production of bidis locally made cigarettes (tobacco wrapped in tendu leaves). A number of villagers visit Satna district of Madhya Pradesh during the months of April and May to bring the leaves required for bidi production.

Control operations by NMEP: Over the previous ten years or so no antimosquito or antilarval measures had been undertaken in Baniyani. However, following the recent epidemic, one-round spraying of 75% DDT was done on 10-11 October 1991. According to the record of the Health Inspector, 50% HCH was also sprayed on these dates. Six rounds of fogging with 5% or 6% malathion were also done. Antilarval measures were undertaken by applying six rounds of Baytex in the ponds and other water collections in the village.

After the occurrence of 14 deaths in the village, antimalarial drugs (chloroquine, primaquine and metakelfin) were distributed extensively in October 1991 by the Health Department of U.P. government, under the Fever Radical Treatment (FRT) and Mass Radical Treatment (MRT) programmes, and the distribution was still being continued. Surveillance was also geared up. Two tablets of metakelfin were distributed by the employee welfare units of the bidi factory to patients who were not responding to chloroquine.

MRCs malaria surveys: Rapid fever surveys were carried out from 20 October to 19 December 1991 on five different occasions and presumptive treatment was given. All the malaria-positive cases were referred to PHC for radical treatment. Infor-

mation on deaths and malaria situation was collected from PHC and villagers.

Spleen surveys: Spleen surveys were carried on 4 and 12 December 1991 among 70 school children of the village. Enlargement of spleen was noted[5].

Entomological survey by MRC: A preliminary entomological survey was carried out to find out the various breeding places in and around the village and their positivity for mosquito larvae. Adult mosquitoes were also caught by suction-tube method to find out the species of vector mosquitoes and their density.

Table 2. Death record of village Baniyani based on different sources

Source of information	No. of deaths	Major reasons
PHC record	32*	Hyperthermia
		Pneumonia
		Diarrhoea
		Shock
		Malaria*
Press reporter	50	Malaria
Villagers	45	Shivering fever
Local physicians	45-50	Malaria

*Two cases were confirmed as suffering from *P. falciparum* malaria.

RESULTS

Malaria record: Records of malaria cases for the last five years were not available in PHC. However, we could collect the malaria record for only 1991 from the PHC. Data on monthly collections of blood smears by their surveillance worker from Baniyani during January to December 1991 are given in Table 1. There was a continuous increase in the collection of slides in subsequent months, indicating an increase in the fever cases (20 in April and 76 in September). A total number of 240 blood smears were collected during January to September 1991 but none was positive for malaria parasite (PHC record), whereas in the month of October 1991 there was a drastic increase in the collection of blood smears (2134) and their positivity (total 325, *P. vivax*-197 and *P. falciparum*-128).

Monthwise data on deaths in this village are also given in Table 1. According to PHC records the first death was reported from this village in July. The patient was a 9-year-old girl, suffering from fever for many days. There were 5 deaths in August, 8 in September, 10 in October and 8 in November 1991. All the villagers had suffered from fever some time or the other. Meanwhile the local press made a hue and cry which attracted the attention of high officials of the health department. The number of deaths reported varied with the source of information (Table 2). However, according to the Gram Pradhan (village head), 45 deaths had occurred in this village.

Entomological survey: There are 12 small ponds in the village (none in agricultural field), 33 wells (31 in village, 2 in field) and one minor drain around the village. There is no river or canal nearby. The area is not water-logged. None of the 10 ponds and 7 wells, checked for mosquito breeding, were positive for mosquito larvae. However, mosquito breeding was encountered in the water collections in the rice field and tube-well tank near the village. The density of mosquito larvae was 78 and 241 per 5 dips respectively in rice field and tube-well tank (Table 3).

Three anopheline species, viz. *An. culicifacies*, *An. annularis*, *An. subpictus*, were found in the village. *An. culicifacies* seems to be the malaria vector. The man-hour density (MHD) of *An. culicifacies* was 2 (Table 4).

Parasitological findings: A total number of 113 slides were collected on different days during November-December 1991, of which, 98 were positive for *P. falciparum* (Table 5). None was

Table 3. Larval positivity of mosquito breeding sites

Type of breeding site	No. of breeding sites checked	Breeding sites found +ve	Larval density/5 dips	
			Culex	Anopheline
Pond	10	0*	0	0
Waste water street	4	0*	0	0
Small ditch	2	0*	0	0
Rice field†	2	1	0	78
Waste water (Tube-well)	2	2	10	241
Well	7	0	0	0

*Six rounds of Baytex application was done by Health Deptt.; † Harvested rice fields with patchy collection of water from tube wells.

Table 4. Mosquito density in Baniyani

Species	MHD
An. culicifacies	2
An. annularis	11
An. subpictus	3
Culex	16

Note: In spite of spraying of one round each of DDT and HCH, six rounds of malathion fogging and six rounds of application of Baytex in ponds/drains, etc.

positive for *P. vivax*. A survey of children of 2-9 years age group of the local primary school showed a very high spleen rate (82.9%). The average enlarged spleen rate was 1.9. The child parasite rate was also very high (40.0 SfR). Blood smears of only three infants could be collected, of which two were positive for *P. falciparum*.

Malaria survey in other villages, nearby as well as in the villages of other PHCs, is under way to find out incidence of the disease in the district.

Table 5. Slide positivity for malaria in Baniyani

Date of MRC survey	Age group	No. of blood slides collected	Cases positive for		SPR	*Pf*%
			P. vivax	*P. falciparum*		
20.11.91	All ages*	27	0	21	77.8	100
26.11.91	All ages*	45	0	18	40.0	100
03.12.91	All ages*	34	0	20	58.8	100
04.12.91	Children† 2-9 years	52	0	20	38.5	100
19.12.91	All ages*	14	0	11	78.5	100
19.12.91	Children†	18	0	8	44.4	100
	Total	190	0	98	51.57	100

* Fever survey; † School survey.

DISCUSSION

The results of a parasitological survey carried out by us demonstrated high incidence of malaria in this village. Only *P. falciparum* was encountered. It is reasonable to assume that the unusual deaths recorded in the village could be exclusively due to *P. falciparum*. This finding is supported by the records of PHC: (i) all the deaths occurred during July to November, the transmission season of malaria; (ii) the increase in fever cases directly corresponds with the number of deaths in the village; and (iii) the occurrence of high rate and grade of splenomegaly in children also indicates epidemic conditions. Similarly, detection of infection in infants (66.66%) confirms the active transmission of the disease during this period but this was not detected by the PHC staff. Poor surveillance and faulty examination of peripheral blood smears could be the major factors responsible for the epidemic and mortality. This conclusion is substantiated by the fact that all the 76 blood slides collected from fever cases during September 1991 were declared negative by the local microscopist, whereas on cross-examination of 38 slides, 18 were found positive, 16 for *P. falciparum* and 2 for *P. vivax*. The respective values of SPR and SfR were 47.37 and 42.10.

Thereafter, a drastic increase in the blood smears and malaria positivity was recorded, indicating the clear-cut breakdown in surveillance as well as faulty diagnosis during the earlier months. There is still a problem with the species identification as no *P. vivax* case was recorded during the MRC survey in November and December 1991, whereas 53 cases were found positive for *P. vivax* by the PHCs microscopist.

According to the Gram Pradhan, 45 deaths (officially 32 deaths) occurred in Baniyani during three months. Out of 32 officially declared deaths, only 2 cases who died in mid-November 1991 were shown to be due to malaria. This could be due to *P. falciparum* infection.

The measures used by the NMEP for the control of malaria epidemic in the village also indicate the severity of malaria problem as spraying of one round each of 75% solution of DDT and 50% of HCH was done whereas there was no history of spraying of any insecticide in the area for the previous 10 years. For the first time, six rounds of fogging and application of Baytex in ponds/drains were carried out in a rural area to control the breeding of vector. This could be the reason for negativity of ponds and drains for mosquito larvae. However, the high density of mosquito larvae in the rice field and tube-well tank of the village indicates that a high density of anopheline may have persisted in the village during or before the spraying of insecticides.

On the basis of results we can conclude that all the unusual deaths recorded in the village might be due to malaria. The high incidence of malaria in the village provides proof that the epidemic occurred owing to breakdown in surveillance, faulty diagnosis of cases, nonspraying of insecticide for the previous 10 years, immigration of the people of this area to malarious areas of Madhya Pradesh, illiteracy and poor socio-economic conditions of the villagers. These points have already been discussed in the case of malaria epidemic[6]. To overcome all these problems, it is necessary to undertake proper surveillance, to provide better laboratory training of technical staff, to impart health education to villagers, and to screen migratory labourers. Above all, receptivity of the area for malaria should be reduced by bioenvironmental intervention methods[4,7,8].

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Fishes of District Sundargarh, Orissa, with Special Reference to their Potential in Mosquito Control

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An extensive fish fauna survey was carried out in Sundargarh, a malaria-endemic district in Orissa, during 1988 to 1990 to identify and evaluate the indigenous larvivorous fishes for mosquito control. In all, 57 species belonging to 19 families under 6 orders were found in the local water bodies. On laboratory evaluation against anopheline and culicine larvae, six potential larvivorous fishes, viz. *Aplocheilus panchax, Oryzias melastigma, Oreochromis mossambicus, Gambusia affinis, Danio (B.) rerio* and *Esomus danricus* were selected. Feasibility of mass multiplication of these fishes in village ponds for operational use is being studied.

INTRODUCTION

Mosquito control using biocontrol agents has received ample attention in recent years. Among all the biological control agents, larvivorous fishes which offer excellent potential in malaria control have been studied extensively[1,2]. Although exotic species of fish like *Gambusia affinis* and *Poecilia reticulata* have been widely used in India in malaria control for long, there has been as much emphasis on identification, evaluation and operational use of the indigenous larvivorous fishes[3-5]. Hence, an extensive fish fauna survey was carried out in District Sundargarh in an effort to identify and evaluate the probable biological control agents for mosquitoes in the district, which has remained meso-to hyperendemic for malaria for long[6].

MATERIALS AND METHODS

A survey of fish fauna was carried out from August 1988 to May 1990 in District Sundargarh in north-western Orissa. All the 17 blocks of the district (area 9700 km^2) were extensively covered (Fig. 1) and various major water bodies, viz. ponds, reservoirs, streams, stream-bed pools, wells, paddy fields, rivers and bunds (ponds with open end to receive surface run-off water), were surveyed. Fishes were captured using nylon dragnets of mesh size 2 mm. Some of the fishes were also collected from the catches of the local fishermen fishing at the sites surveyed, especially from river systems. One species (*Oryzias melastigma*) was, however, specially brought from the coastal lagoons of District Ganjam in Orissa for evaluation.

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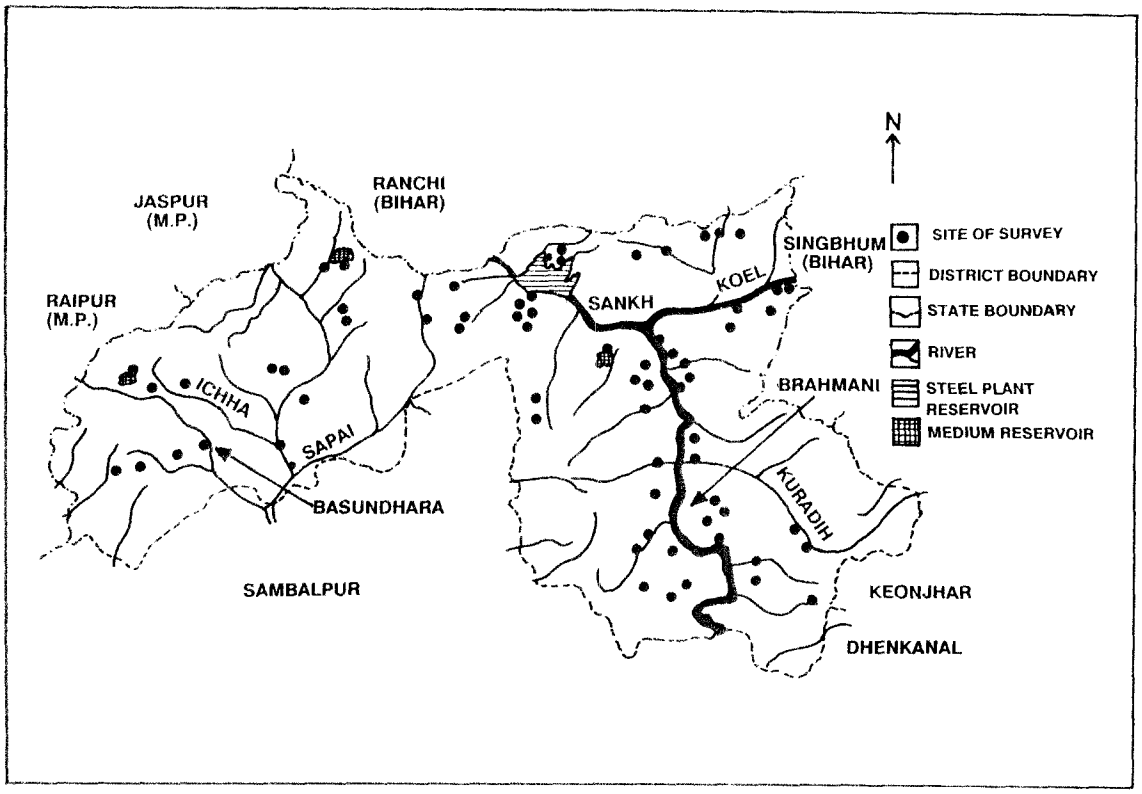

Fig. 1: Map of District Sundargarh showing sites of fish fauna survey.

Fish specimens were brought to the laboratory for identification, evaluation and subsequent preservation. The specimens were identified by the standard method. Later, identification of most of these fishes was also got confirmed by the Zoological Survey of India, Calcutta.

Evaluation of predatory potential : Three specimens of each species of fish were released individually in glass containers (20 × 17 × 20 cm size) containing chlorine-free tap water up to 15 cm depth. After releasing a sufficient number of late III and early IV instar mosquito larvae (range 100 - 2000) the containers were covered with pieces of net. The average number of mosquito larvae consumed per fish per day was found out and the experiment was repeated on 3 more consecutive days. Most of the fish species were evaluated both against anopheline and culicine larvae separately. The number of larvae consumed by a fish per gram body weight per day, taken as predatory index, was calculated. Some of the rarely available species which died during transportation and those species which were not found suitable due to bigger size could not be evaluated for predatory efficacy.

RESULTS AND DISCUSSION

Fifty-seven species of fishes belonging to 19 families under 6 orders as listed in Table 1 were recorded from the area. Thirty-six species of fish were found in rivers followed by 30 species in streams, 25 in bunds, 23 in ponds, 21 in reservoirs,

Table 1. Fish fauna of District Sundargarh

Order	Family	Fish species	Max. length recorded (mm)	Mouth position	Habitats	Local availability
(1)	(2)	(3)	(4)	(5)	(6)	(7)
Osteoglossiformes	Notopteridae	1. *Notopterus notopterus* (Pallas)	152 (609)	Terminal	3, 5	+
Clupeiformes	Clupeidae	2. *Gonialosa manmina* (Ham.)	80 (279)	Terminal	5	+
Cypriniformes	Cyprinidae					
		Subfamily – *Abramidinae*				
		3. *Chela (C.) cachius* (Ham.)	65 (102)	Terminal	3, 4, 5	+
		4. *Chela (C.) laubuca* (Ham.)	60 (89)	Terminal	2, 3, 4, 5	+ +
		5. *Salmostoma bacaila* (Ham.)	115 (177)	Terminal	1 to 5	+ +
		Subfamily – *Rasborinae*				
		6. *Barilius barila* (Ham.)	73 (102)	Terminal	3, 4, 5	+ +
		7. *Barilius barna* (Ham.)	72 (125)	Terminal	5	+
		8. *Barilius bendelisis* (Ham.)	120 (153)	Terminal	5	+
		9. *Danio (D.) aequipinnatus* (McCl.)	88 (152)	Terminal	4, 5	+
		10. *Danio (D.) dangila* (Ham.)	70 (150)	Terminal	4	+
		11. *Danio (B.) rerio* (Ham.)	48 (50)	Terminal	1 to 7	+ + +
		12. *Esomus danricus* (Ham.)	90 (127)	Terminal	1 to 7	+ + +
		13. *Rasbora daniconius* (Ham.)	84 (203)	Terminal	1 to 6	+ +
		Subfamily – *Cyprininae*				
		14. *Amblypharyngodon mola* (Ham.)	68 (75)	Terminal	1, 2, 3	+ +
		15. *Aspidoparia morar* (Ham.)	75 (177)	Inferior	5	+
		16. *Catla catla* (Ham.)	302 (1828)	Terminal	1, 2	+
		17. *Cirrhinus mrigala* (Ham.)	230 (914)	Inferior	1	+
		18. *Cirrhinus reba* (Ham.)	205 (304)	Inferior	2 to 5	+
		19. *Ctenopharyngodon idella* (Val.)	435 (1200)	Terminal	1	+
		20. *Cyprinus carpio communis* (Linn.)	350	Inferior	1	+
		21. *Garra gotyla gotyla* (Gray)	130 (152)	Inferior	3, 4	+
		22. *Hypophthalmichthys molitrix* (Val.)	250 (814)	Terminal	1	+
		23. *Labeo boga* (Ham.)	100 (304)	Inferior	4	+
		24. *Labeo boggut* (Sykes)	150 (190)	Inferior	5	+
		25. *Labeo calbasu* (Ham.)	250 (914)	Inferior	2	+
		26. *Labeo dero* (Ham.)	157 (254)	Inferior	2	+

contd...

Table 1. Fish fauna of District Sundargarh *(contd.)*

(1)	(2)	(3)	(4)	(5)	(6)	(7)
		27. *Labeo rohita* (Ham.)	350 (1000)	Inferior	1, 2	+
		28. *Osteobrama cotio cotio* (Ham.)	130 (152)	Terminal	2, 4, 5	+
		29. *Puntius amphibius* (Val.)	85 (150)	Inferior	2,4	+
		30. *Puntius conchonius* (Ham.)	98 (127)	Terminal	5	+
		31. *Puntius gelius* (Ham.)	40 (50)	Terminal	2	+
		32. *Puntius sarana sarana* (Ham.)	155 (304)	Terminal	2, 5	+
		33. *Puntius sophore* (Ham.)	87 (127)	Terminal	1 to 5	+ +
		34. *Puntius ticto ticto* (Ham.)	68 (100)	Terminal	1 to 6	+ +
	Cobitidae	35. *Lepidocephalous (L.) berdmorei* (Blyth)	87 (90)	Inferior	1 to 5	+ +
		36. *Noemacheilus savona* (Ham.)	78	Inferior	4, 5	+
Siluriformes	Bagridae	37. *Mystus bleekeri* (Day)	120 (135)	Terminal	4, 5	+
		38. *Mystus seenghala* (Ham.)	145	Terminal	4, 5	+
	Siluridae	39. *Ompok bimaculatus* (Bloch.)	165 (457)	Terminal	5	+
		40. *Ompok pabda* (Ham.)	155 (172)	Terminal	5	+
	Schilbeidae	41. *Pseudeutropius garua* (Ham.)	65 (600)	Terminal	5	+
	Amblycipitidae	42. *Amblyceps mangois* (Ham.)	46 (125)	Terminal	4	+
	Claridae	43. *Clarias batrachus* (Linn.)	160 (450)	Terminal	1, 4	+
	Heteropneustidae	44. *Heteropneustes fossilis* (Bloch.)	155 (304)	Terminal	2, 4	+
Atheriniformes	Belonidae	45. *Xenentodon cancila* (Ham.)	205 (304)	Terminal	3, 4, 5	+
	Oryziatidae	46. *Oryzias melastigma* (McCl.)	32 (38)	Superior	1	+
	Cyprinodontidae	47. *Aplocheilus panchax* (Ham.)	64 (89)	Superior	1, 4, 5, 6	+ +
	Poecilidae	48. *Gambusia affinis* (Biard & Giard)	48	Superior	1	+ +
Perciformes	Centropomidae	49. *Chanda nama* (Ham.)	65 (76)	Terminal	2, 3, 5	+
	Cichlidae	50. *Oreochromis mossambicus* (Peters)	160 (230)	Terminal	1	+ +
	Gobiidae	51. *Glossogobius giuris* (Ham.)	140 (304)	Terminal	1 to 5	+ +
	Channidae	52. *Channa orientalis* (Schn.)	165 (203)	Terminal	1 to 6	+ +
		53. Channa marulius (Ham.)	150 (1219)	Terminal	3, 5	+
		54. *Channa punctatus* (Bloch.)	240 (304)	Terminal	1 to 6	+ +
Mastacembeli-formes	Mastacembelidae	55. *Macrognathus aculeatus* (Bloch.)	188 (380)	Inferior	1, 2	+
		56. *Mastacembelus armatus armatus* (Lacepede)	190 (600)	Inferior	3, 4, 5	+
		57. *Mastacembelus pancalus* (Ham.)	170 (177)	Inferior	1 to 5	+ +

1. Ponds; 2. Bunds; 3. Reservoirs; 4. Streams; 5. Rivers; 6. Wells; 7. Paddy fields; + + + = Abundant; + + = Moderate; + = Less/Rare. Figures in parentheses are the maximum reported size of the fishes (mm), after Sen (1985)[8].

7 in paddy fields and 2 in wells. Eight species, viz. *Danio (B.) rerio, Esomus danricus, Puntius ticto ticto, Puntius sophore, Rasbora daniconius, Channa punctatus, Channa orientalis* and *Glossogobius giuris,* were found in ponds, bunds, reservoirs, streams and rivers.

The position of the mouth is one of the important characteristics to determine the larvivorous capability of a fish. Among 47 species evaluated for predatory potential in laboratory (Table 2), three species had superior mouth position, 38 had terminal mouth and the remaining 16 species had inferior mouth position. Based on the predation rate and other characters, these fishes may be broadly categorized as under :

Category I : Fishes with high predatory index and small size are surface feeders because of their superior mouth position and show an overall high potential in mosquito control. These are *Oryzias melastigma, Aplocheilus panchax* (indigenous spp.) and *Gambusia affinis* (exotic sp.).

Category II : Fishes with terminal mouth, which are sub-surface to surface feeders, have good potential in mosquito control. These are *Danio (B.) rerio, Esomus danricus* and the exotic but locally well-adapted and fast-breeding tilapias *(Oreochromis mossambicus).* In this category the following fishes may also be included, which have moderate to limited potential owing to their limited availability, probably due to limited breeding efficiency : *Barilius barila, Barilius bendelisis, Chela (C.) laubuca, Puntius ticto ticto, Chela (C.) cachius, Rasbora daniconius, Danio dangila, Puntius sophore, Chanda nama, Salmostoma bacaila, Danio (D.) aequipinnatus, Puntius gelius* and *Puntius amphibius.*

Category III : These are mostly carps with food value. They have terminal/inferior mouth position and have a limited potential for use in mosquito control, i.e. they can be used up to fingerling stage (Table 2, s. no. 12-14 and 16-23).

Category IV : This comprises mostly carnivorous fishes with less to rare local availability, probably owing to their low breeding efficiency or survival, which are least suitable for use in larval control (Table 2, s. no. 1, 15, 28-36 and 42-47).

Based on our observations important larvivorous fishes are further described as under :

Table 2. Consumption rate of mosquito larvae by different fishes

Order	Family	S. No.	Fish species	Length range (mm)	Average weight (g)	Spp. of mosquito larvae	Average no. of larvae consumed per fish per day	Predatory index*
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Osteoglossiformes Cypriniformes	Notopteridae Cyprinidae	1.	*Notopterus notopterus*	76-88	2.98	*Cx.*	79	26.5
		Subfamily—*Abramidinae*						
		2.	*Chela (C.) cachius*	28-29	0.21	*An.*	151	719.0
						Cx.	34	161.9
		3.	*Chela (C.) laubuca*	47-53	1.25	*An.*	260	208.0
				50-53	1.32	*Cx.*	199	150.8

contd...

Table 2. Consumption rate of mosquito larvae by different fishes *(contd.)*

(1)	(2)	(3) (4)	(5)	(6)	(7)	(8)	(9)
		4. *Salmostoma bacaila*	78-81	2.92	*An.*	423	144.9
			50-51	1.05	*Cx.*	149	141.9
		Subfamily – *Rasborinae*					
		5. *Barilius barila*	38-44	1.25	*An.*	72	57.6
					Cx.	32	25.6
		6. *Barilius bendelisis*	96	8.05	*An.*	600	74.5
		7. *Danio (D.) aequipinnatus*	68	2.5	*An.*	956	382.4
					Cx.	192	76.8
		8. *Danio (D.) dangila*	36-43	0.55	*An.*	276	501.8
			26-43	0.38	*Cx.*	67	176.3
		9. *Danio (B.) rerio*	24-30	0.25	*An.*	96	384.0
					Cx.	26	104.0
		10. *Esomus danricus*	47-51	1.05	*An.*	401	381.9
			53-65	1.81	*Cx.*	36	19.9
		11. *Rasbora daniconius*	58-64	2.04	*An.*	104	51.0
					Cx.	35	17.2
		Subfamily – *Cyprininae*					
		12. *Catla catla*	65	2.05	*An.*	377	183.9
					Cx.	256	124.9
		13. *Cirrhinus mrigala*	67	2.61	*An.*	291	111.5
					Cx.	143	54.8
		14. *Cirrhinus reba*	90	7.25	*An.*	1357	187.2
					Cx.	558	77.0
		15. *Garra gotyla gotyla*	80-95	7.9	*Cx.*	6	0.8
		16. *Hypophthalmichthys molitrix*	74	2.62	*An.*	328	125.2
		17. *Labeo boga*	94-97	7.51	*An.*	948	126.2
					Cx.	479	63.8
		18. *Labeo boggut*	83	6.04	*An.*	324	53.6
		19. *Labeo calbasu*	23-25	0.36	*An.*	33	91.7
		20. *Labeo dero*	73	3.87	*An.*	349	90.2
		21. *Labeo rohita*	54	1.63	*An.*	233	142.9
					Cx.	106	65.0
		22. *Osteobrama cotio cotio*	81	3.85	*An.*	624	162.1
					Cx.	388	100.8
		23. *Puntius sarana sarana*	110	16.45	*An.*	992	60.3
					Cx.	580	35.3
		24. *Puntius amphibius*	50-54	1.27	*An.*	225	177.2
					Cx.	159	125.2
		25. *Puntius gelius*	30	0.24	*An.*	87	362.5
					Cx.	67	279.2

contd...

Table 2. Consumption rate of mosquito larvae by different fishes *(contd.)*

(1)	(2)	(3) (4)	(5)	(6)	(7)	(8)	(9)
		26. *Puntius sophore*	70-91	6.71	*An.*	687	102.4
					Cx.	267	39.8
		27. *Puntius ticto ticto*	44-50	1.73	*An.*	139	80.3
					Cx.	116	67.1
	Cobitidae	28. *Lepidocephalous (L) berdmorei*	76-87	3.95	*An.*	36	9.1
					Cx.	6	1.5
		29. *Noemacheilus savona*	40-43	0.49	*An.*	17	34.7
					Cx.	10	20.4
Siluriformes	Bagridae	30. *Mystus bleekeri*	50-81	1.45	*An.*	107	73.8
	Siluridae	31. *Ompok bimaculatus*	65	1.47	*An.*	412	280.3
					Cx.	164	111.6
		32. *Ompok pabda*	101	14.35	*An.*	488	34.0
	Amblycipitidae	33. *Amblyceps mangois*	43-48	0.78	*An.*	5	6.4
	Claridae	34. *Clarias batrachus*	97	5.65	*An.*	1880	332.7
					Cx.	1006	178.1
	Heteropneustidae	35. *Heteropneustes fossilis*	50-67	0.98	*An.*	462	471.4
					Cx.	325	331.6
Atheriniformes	Belonidae	36. *Xenentodon cancila*	61	0.57	*An.*	56	98.2
					Cx.	52	91.2
	Oryziatidae	37. *Oryzias melastigma*	22-27	0.12	*An.*	72	600.0
			17-27	0.07	*Cx.*	30	428.6
	Cyprinodontidae	38. *Aplocheilus panchax*	35-43	0.91	*An.*	161	176.9
					Cx.	59	64.8
	Poecilidae	39. *Gambusia affinis*	30-34	0.46	*An.*	89	193.5
					Cx.	37	80.4
Perciformes	Centropomidae	40. *Chanda nama*	36-42	0.53	*Cx.*	22	41.5
	Cichlidae	41. *Oreochromis mossambicus*	35-38	0.78	*An.*	371	475.6
					Cx.	147	188.5
	Gobiidae	42. *Glossogobius giuris*	71	3.22	*An.*	116	36.0
					Cx.	67	20.8
	Channidae	43. *Channa orientalis*	61	2.51	*An.*	452	180.1
					Cx.	259	103.2
		44. *Channa marulius*	125	14.41	*An.*	1319	91.5
					Cx.	1360	94.4
		45. *Channa punctatus*	50-61	1.55	*An.*	382	246.5
					Cx.	175	112.9
	Mastacembelidae	46. *Macrognathus aculeatus*	140-153	9.67	*An.*	148	15.3
					Cx.	12	1.2
		47. *Mastacembelus pancalus*	147-152	9.51	*An.*	250	26.3
			58-84	1.42	*Cx.*	36	25.4

* No. of larvae consumed per gram body weight per day; *An.* – *Anopheles*; *Cx.* – *Culex*.

Aplocheilus panchax **(Lesser top minnow)** : This species measured up to a total length of 64 mm but most of the specimens collected were within the range of 40–50 mm. The fish was more frequently encountered in streams and occasionally found to inhabit rivers and ponds also. During rainy season it was also recorded from paddy fields and other inundated areas connected with streams. It is a hardy and surface-feeding fish and is a year-round breeder. The fish with an average weight of 0.91 g consumed on an average 161 anopheline larvae and in a separate evaluation 59 *Culex* larvae.

Oryzias melastigma **(Estuarine top minnow)** : This has a superior mouth position and is a surface feeder. Fish weighing 0.12 g consumed on an average 72 anopheline larvae per day, whereas another fish weighing 0.07 g consumed 30 *Culex* larvae per day. The fish was originally brought by us in 1990 from the coastal area of Ganjam district in Orissa. Though a delicate fish, it is a year-round breeder and offers great promise for use in mosquito control in rice fields and other similar habitats.

Oreochromis mossambicus **(Tilapia)** : The tilapia, which has been introduced into the area more than 40 years ago, was found in large numbers in many village ponds. It has a terminal mouth and is a voracious feeder. In separate tests, each fish with an average weight of 0.78 g consumed daily 371 anopheline and 147 *Culex* larvae. It has a high breeding potential coupled with parental care. It is a very hardy fish and smaller size fishes have good potential for use as biocontrol agents in certain situations, e.g. polluted water in dung-pits during rainy season.

Danio (Brachydanio) rerio **(Zebra)** : This is a very small, hardy, active and shoaling fish and was found in ponds, wells, streams, rivers, bunds and reservoirs but in large numbers in streams. Specimens collected during the survey measured up to 48 mm in length. They breed profusely during monsoon when they also inhabit paddy fields and small streams in large numbers. It is a surface to sub-surface omnivore with an upwardly turned terminal mouth. Fish weighing 0.25 g consumed each, on an average, 96 anopheline and 26 *Culex* larvae per day.

Danio (Danio) dangila : This is bigger in size, more active and hardy than the zebra fish but is comparatively less abundant. It was encountered only in hilly streams. Specimens collected during the survey measured up to 70 mm. Like the zebra fish it is also a surface to sub-surface feeder with an upwardly turned terminal mouth. Under laboratory conditions, fish with an average weight of 0.55 g consumed 276 anopheline larvae daily; and fish weighing 0.38 g consumed 67 *Culex* larvae daily.

Esomus danricus **(Flying barb)** : This is one of the most abundant fishes encountered in the area in ponds, bunds, reservoirs, streams and rivers. It is also found to inhabit deeper paddy fields and other inundated areas during rainy season. In our survey it measured up to 90 mm in total length. It is a sub-surface feeding omnivore with a terminal mouth. It breeds freely both in flowing and confined waters during rainy season. Fish with an average weight of 1.05 g consumed 401 anopheline larvae per day, while another fish with an average weight of 1.81 g consumed on an average 36 *Culex* larvae per day.

Barilius barila : This is a small, hardy and active fish with a terminal mouth. It is basically a riverine fish and is available in plenty in rivers immediately after the onset of rains. However, it was also found in streams and reservoirs during the survey. The longest recorded length was 73 mm. Fish weighing 1.25 g consumed each, on an average, 72 anopheline and 32 *Culex* larvae per day respectively.

Chela (Chela) laubuca **(Winged rasbora)** : This is an active fish found in bunds, reservoirs, streams and rivers. However, it was more frequently present in streams. The maximum length of the fish recorded during the survey was 60 mm. It is a sub-surface feeder with an upwardly turned ter-

minal mouth. Two hundred and sixty anopheline and 199 *Culex* larvae were consumed per day by fish weighing 1.25 g and 1.32 g respectively.

Chela (Chela) cachius : This was found in reservoirs and rivers but streams seemed to be its natural habitat. It is comparatively bigger in size and is less abundant than *Chela (C.) laubuca*. Specimens collected during survey ranged up to 65 mm. It is a very active fish found swimming on the surface for food and has an upwardly turned terminal mouth. Fish with an average weight of 0.21 g was found to consume on an average 151 anopheline and 34 *Culex* larvae per day respectively.

Rasbora daniconius : This was found in ponds, reservoirs, streams, rivers and paddy fields but not in sufficiently large numbers. It is a sub-surface feeding omnivore with a terminal mouth and breeds during rainy season in flowing as well as confined waters. In separate tests, fish weighing 2.04 g consumed each, on an average, 104 anopheline and 35 *Culex* larvae per day.

Puntius ticto ticto **(Barb)** : A column-feeding omnivore with a terminal mouth, it is one of the abundant fishes in the survey area found in ponds, bunds, reservoirs, streams and rivers. During rainy season it also inhabits deeper paddy fields and other inundated areas. Specimens collected during survey measured up to a maximum length of 68 mm. Under laboratory conditions, fish with 1.73 g weight consumed each, on an average, 139 anopheline and 116 *Culex* larvae per day in different tests.

Large-scale mass multiplication of *Danio rerio, Oryzias melastigma, Gambusia* and *Oreochromis* in disused village ponds has been taken up so that the fishes become available for field trials. It is also proposed to conduct compatibility studies of these fishes with major carps. Since the malaria control programme is already faced with several technical problems such as insecticide resistance in vectors, there has been in recent years an enormous emphasis on bioenvironmental control of malaria[7]. In such a strategy, larvivorous fishes have an important role to play.

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Frequency of ABO Blood Groups, Sickle-Cell Haemoglobin, G-6-PD Deficiency and their Relation with Malaria in Scheduled Castes and Scheduled Tribes of Kheda District, Gujarat

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Frequency of sickle cell in Scheduled Caste and Scheduled Tribe populations was found to be 1.5 and 14.9% respectively, whereas G-6-PD deficiency was 5.9 and 4.2% respectively. Blood group B was dominant in both the communities. A significantly lower frequency of *P. falciparum* malaria was observed among sicklers.

INTRODUCTION

Malaria is a problem in public health in the major part of the world including the Indian subcontinent. It is necessary to understand the human blood polymorphic systems as these are the important biochemical markers in distribution of diseases, including malaria. Some genetic abnormalities of human erythrocytes have been found to decrease their susceptibility to malaria parasites[1]. Luzzatto *et al.*[2] reported the relationship of genetic variants of human red blood cells with malaria in African populations. A few reports on the genetic abnormalities and malaria in Indian populations are also available [3-5]. Although some reports on the genetic abnormalities are available from some Gujarati communities[6-8], the distribution of genetic markers in relation to malaria in Scheduled Castes (SC) and Scheduled Tribes (ST) of Kheda district has not been studied so far. WHO has emphasized the need to study the distribution of different genetic abnormalities in different population groups[9]. The present study was undertaken to find out the distribution of malaria and three genetic markers, viz. G-6-PD deficiency, sickle cell haemoglobin and ABO blood grouping, among the SCs and STs of Kheda district in Gujarat.

MATERIALS AND METHODS

During June 1989 – April 1990 human blood samples were collected from 769 people, belonging to both the sexes and all age groups, with fever at the time of survey or a history of fever within a week. Four main castes, viz. Harijans (Bhangis), Rohits (Chamars), Vankars and Shenvas, among SCs and

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Bhils and Tadvis among STs were surveyed. Blood samples (0.3–0.4 ml) were collected in heparinized vials of 1.5 ml capacity. Kept in ice boxes and brought to the laboratory, these samples were processed for detection of G-6-PD deficiency, sickle cell haemoglobin and blood group. Thick and thin blood smears were also prepared for microscopic examination of malaria parasites. The smears were stained with JSB stain and examined under oil immersion lens for 200 thick film fields before being declared negative.

Screening for G-6-PD enzyme deficiency in males was done by flourescent spot test[10], females being excluded as the test invariably misses heterozygote females. Sickling was examined following Dacie and Lewis[11] method and further confirmed by elect rophoresis as sickle cell trait. ABO blood grouping was done by agglutination slide test. Fisher's chi-square test was used to determine the association of ABO blood group, sickle cell and G-6-PD deficiency with malaria cases.

RESULTS

ABO grouping

Out of 769 blood samples tested, 534 belonged to SCs and 235 to STs (Table 1). The frequency of blood group B was highest (37.3%) followed by A (26.2%), O (25.3%) and AB (11.2%) in SCs, whereas in STs, B group (33.2%) was followed by O (29.8%), A (25.5%) and AB (11.5%). Table 2 shows a total of 88 malaria cases (*Pf* = 61 and *Pv* = 27) recorded from both the communities. Malaria incidence was higher in blood groups O and AB (14.6 and 13.8%) than in B and A (9.7 and 9.5%). However, no significant difference was seen in the distribution of ABO polymorphs among malaria positive and negative samples (χ^2 [3] = 4.14, $p > 0.10$).

Sickle-cell haemoglobin

All the 769 blood samples were tested for sickling and 43 were found positive (Table 1). The fre-

Table 1. Frequency of blood groups, G-6-PD deficiency and sickle-cell in Scheduled Castes and Scheduled Tribes of Kheda district

Castes	Sample size	Blood groups				Sickle-cell	G-6-PD deficiency	
		A	B	AB	O		Sample size*	Deficient
(a) *Scheduled Castes*								
1. Harijans	103 (19.3)	33 (32.0)	31 (30.1)	6 (5.8)	33 (32.0)	1 (0.9)	46	8 (17.4)
2. Rohits	145 (27.1)	40 (27.6)	57 (39.3)	13 (8.9)	35 (24.1)	2 (1.4)	86	1 (1.2)
3. Vankars	260 (48.7)	60 (23.1)	102 (39.2)	39 (15.0)	59 (22.7)	5 (1.9)	143	8 (5.6)
4. Shenvas	26 (4.9)	7 (26.9)	9 (34.6)	2 (7.7)	8 (30.8)	0	11	0
Subtotal	534	140 (26.2)	199 (37.3)	60 (11.2)	135 (25.3)	8 (1.5)	286	17 (5.9)
(b) *Scheduled Tribes*								
1. Bhils	149 (63.4)	39 (26.2)	49 (32.9)	14 (9.4)	47 (31.5)	18 (12.1)	94	2 (2.1)
2. Tadvis	86 (36.6)	21 (24.4)	29 (33.7)	13 (15.1)	23 (26.7)	17 (19.7)	49	4 (8.2)
Subtotal	235	60 (25.5)	78 (33.2)	27 (11.5)	70 (29.8)	35 (14.9)	143	6 (4.2)
Grand total	769	200 (26.0)	277 (36.0)	87 (11.3)	205 (26.7)	43 (5.6)	429	23 (5.4)

Figures in parentheses are percentages; *Male samples only.

Table 2. Malaria cases with blood groups, sickle-cell and G-6-PD deficiency

	Groups	Samples	*Pv*	*Pf*	Total
Blood groups	A	200	7 (3.5)	12 (6.0)	19 (9.5)
	B	277	4 (1.4)	23 (8.3)	27 (9.7)
	AB	87	6 (6.9)	6 (6.9)	12 (13.8)
	O	205	10 (4.8)	20 (9.8)	30 (14.6)
χ^2 [3]			7.55	2.15	4.14
Sickle cell	Sicklers	43	2 (4.6)	0	2 (4.6)
	Non-sicklers	726	25 (3.4)	61 (8.4)	86 (11.8)
χ^2 [1]			0.0001	5.16*	2.84
G-6-PD	Deficient	23	0	1 (4.3)	1 (4.3)
	Non-deficient	406	8 (2.0)	44 (10.8)	52 (12.8)
χ^2 [1]			2.16	1.79	2.33

Figures in parentheses are slide positivity rates; * $p < 0.05$.

quency of sicklers was high in STs (14.9%) and low in SCs (1.5%). Among SCs the percentages in Harijans, Rohits and Vankars were 0.9, 1.4 and 1.9 respectively, whereas no sickle cell was recorded in Shenvas. In STs, high frequency was observed both in Tadvis (19.7%) and Bhils (12.1%). Only two malaria (*Pv*) cases (4.6%) were recorded from the sicklers of both the communities against 86 cases (11.8%) in non-sicklers (Table 2). No *Pf* case was found in sickler samples, which is statistically significant (Table 2, χ^2 [1] = 5.16, $p < 0.05$). Sexwise distribution of sickle-cell trait showed that it was higher in males (62.8%) than in females (Table 3).

G-6-PD Deficiency

Out of the 429 blood samples from males, 23 were found G-6-PD deficient (Table 1). The percentage of G-6-PD enzyme deficiency was higher in SCs (5.9%) than in STs (4.2%) and the frequency among Harijans, Vankars and Rohits was 17.4, 5.6 and 1.2% respectively. No deficieny was observed in Shenvas. The highest percentage was recorded in Tadvis (8.2) and the lowest in Bhils (2.1) of STs. Only one (4.3%) malaria case (*Pf*) was recorded in G-6-PD deficient blood samples against 52 (12.8%) in non-deficient cases. Chi-square test did not reveal any significant difference in the distribution of malaria cases in G-6-PD deficient and non-deficient individuals (Table 2, χ^2 [1] = 2.33, $p > 0.05$).

DISCUSSION

The results do not show any significant correlation between blood groups and malaria disease (Table 2). Joshi *et al.*[5] did not find any significant

Table 3. Sexwise distribution of sickle-cell and malaria cases

Sex	Sample size	Sickle-cell	Malaria cases
Male	429 (55.8)	27 (62.8)	52 (59.1)
Female	340 (44.2)	16 (37.2)	36 (40.9)
Total	769	43	88

Figures in parentheses are percentages.

difference in the distribution of ABO polymorphs among *P. vivax* and *P. falciparum* malaria cases in Delhi. A similar observation was also made by Vasantha *et al.*[4] during malaria epidemic in the tribal population of Dadra and Nagar Haveli. No correlation between blood groups and malaria had been observed in African populations[12]. Gupta and Raichowdhury[3] reported that malaria parasite shares group A antigen and hence is better tolerated by the host's immune system. Athreya and Coriell[13] postulated that blood group B may have an advantage in a malarious region, suggesting a plausible relationship between malaria and blood groups. Singh *et al.*[14], studying 22 genetic polymorphisms of blood, observed a significant association for P and Hp systems.

Sickle-cell anaemia and sickle-cell trait are observed to occur in relatively significant frequencies among the endogamous tribals throughout India except in the northwest and extreme south. In Orissa, Andhra Pradesh, Bihar and Madhya Pradesh the figures for HbS are relatively high among most tribals[15]. The sickle-cell haemoglobin was found in high frequency in the STs and low in SCs of this region (Table 1). Comparable incidence of HbS has been reported among the Bhils from Madhya Pradesh[16]. However, in Rajasthan, low frequency was observed in STs and SCs[17,18].

During our study no *P. falciparum* case was recorded from the sickler samples, which suggests that sickle-cell gene (HbAS) may have a tendency to protect the RBC from *P. falciparum*. Evidences are available that HbS mutation confers a protective value against *P. falciparum* infection[1]. The sickle-cell trait (HbAS) has a survival advantage over homozygous HbA in the malarious areas[19]. Luzzatto *et al.*[20] found increased sickling of parasitized erythrocytes as the mechanism of resistance against malaria in the sickle-cell trait.

The frequency of G-6-PD deficiency was recorded as 5.9 and 4.2 per cent in SCs and STs respectively (Table 1). Sathe *et al.*[16] reported 3.4-4.5% incidence of this enzyme deficiency in Bhils of Jhabua and Ratlam of Madhya Pradesh. Jain *et al.*[17] found 16.3% G-6-PD deficiency in the Bhil tribe of southern Rajasthan. A review of Indian studies on erythrocytic G-6-PD indicates that the deficiencies reported so far are at times conflicting and vary from 0 to 27.06%[1].

In our investigation one *P. falciparum* malaria case was detected out of 23 G-6-PD deficient samples (Table 2). However, in the absence of incidence in heterozygous females it was not possible to correlate the protective role of G-6-PD deficiency against malaria infection. Bienzle *et al.*[21] also reported that the heterozygous state of G-6-PD deficiency (female carriers) has a protective function against malaria.

More detailed studies are needed to determine the frequency of blood group, sickle cell and G-6-PD deficiency and their relationship with malaria in the other population groups, so that the knowledge gained could be utilized for a better understanding of malaria dynamics in different parts of the country.

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Malaria and ABO Blood Groups

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Subjects from Muria gond tribal community (n = 258) as well as from Delhi (n = 100) were classified according to ABO blood groups, and were also assayed for malarial antibodies by ELISA technique. The distribution of ABO blood groups did not differ significantly in Muria gonds and Delhi subjects. Within Muria gonds the observed frequency of ABO blood groups did not differ significantly from the expected values. No significant difference was observed in the rate of seropositivity for malarial antibodies among subjects with different blood groups. Malarial parasitaemia, although observed more in individuals with blood group A, did not differ significantly as compared with other blood groups. We conclude that ABO blood groups do not show differential susceptibility to malaria.

INTRODUCTION

The association of genetic markers with malaria has been the subject of numerous investigations, since Allison[1] demonstrated the protection afforded by sickle-cell haemoglobin against infection by parasite of falciparum malaria. The observation by Miller *et al.*[2] that human erythrocytes lacking the Duffy blood group antigens are refractory to invasion by *P. vivax* parasites indicates the usefulness of studying the association of blood groups with malaria. Wood[3] observed that *Anopheles* mosquitoes, which transmit malarial infection, tend to bite group O and B persons in preference to those having group A. Gupta and Raichowdhury[4] showed that among individuals with malaria, group A was over-represented and group O under-represented. Kagan *et al.*[5] demonstrated that malarial parasites share antigens with blood group A and, hence, are better tolerated by the blood group A host's immune system, leading to greater infection. In a meta-analysis of published reports Singh *et al.*[6] confirmed the higher susceptibility to malaria of individuals with blood group A. Joshi *et al.*[7] reported no correlation between ABO blood groups and malaria in Delhi. Similar observations have been made in Dadar and Nagar Haveli tribal population by Vasantha *et al.*[8] In view of the controversial nature of the topic, we have explored this association in an area hyperendemic for malarial infection as well as in an area with low prevalence of malaria.

MATERIALS AND METHODS

The study was conducted in Geedum and Pharasgaon blocks of Bastar district, central India. These areas are known to be hyperendemic for malaria.

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Annual parasite index for Geedum and Pharasgaon blocks was 24.6 and 18.45 respectively in 1987. A multistage stratified random sampling procedure was used to select 100 households; among these, 258 subjects agreed to participate in the study. The subjects belonged to the Muria gond tribe, which is protoaustraloid in origin[9]. Blood samples were also collected from randomly selected 100 afebrile individuals of the Punjabi community residing in Delhi, which has low incidence of malaria, for comparison. The age group of subjects ranged from 5 to 65 years in both Bastar and Delhi populations.

Blood for determining ABO blood groups was collected in heparinized capillaries, and was analysed in the field itself by the standard agglutination method[10]. Briefly, one volume of whole blood was mixed with one volume of anit-A, anti-B and anti-AB sera (obtained from Decruz Corporation). Readings were taken after 1 h. All negative results were confirmed by examination under microscope.

Diagnosis of malaria was established by preparing thick and thin smears from finger-prick blood, and the parasite was identified by the standard criteria. Additionally, malaria antibodies were analysed by enzyme-linked immunosorbent assay (ELISA). For this purpose, blood was collected on filter paper strips and stored at 4°C and the test carried out at AIIMS hospital, according to the method of Spencer *et al.*[11] with slight modifications. The specificity of ELISA was tested by studying 25 sera from acute *P. falciparum* malaria patients and 25 samples from healthy individuals from Jammu & Kashmir, who were never exposed to malaria in the past. Patients' sera showed an optical density (at 492 nm) of greater than 0.25 in dilutions ranging from 1:16 to 1:256, whereas in normal control sera, the value was less than 0.25 even in 1:16 dilution. Therefore positive titres were considered to be greater than or equal to 0.25 at 1:16 dilution. The reference positive serum always showed an optical density greater than 1 at 1:256 dilution while the reference negative serum showed OD less than 0.25 at 1:8 dilution. Soluble antigen A-18 derived from FCB-2 strain of *P. falciparum* was cross-reacting with antibodies against *P. vivax*. Antigen A-18, positive and negative control sera were supplied by Dr. Espinal of Colombia, South America.

RESULTS

Table 1 shows the distribution of blood groups among Muria gonds as well as Delhi subjects. The observed frequency of ABO blood groups did not differ significantly from the expected frequency, both in Muria gonds and in Delhi subjects, although the two areas have markedly different rates of malarial infection.

Table 2 shows the distribution of malarial antibodies in subjects with different blood groups. The rate of seropositivity was more in subjects

Table 1. Distribution of ABO blood groups in Muria gonds and controls

Blood group	Muria gonds					Delhi				
	No. obs.	No. exp.	%	G.F.	χ^2	No. obs.	No. exp.	%	G.F.	χ^2
A	77	73.5	29.85	0.2562	0.16	25	28.49	25.0	0.2114	0.42
B	86	87.17	33.33	0.2800	0.015	35	33.79	35.0	0.2774	0.04
O	56	59.8	21.70	0.4638	0.24	27	23.18	27.0	0.5112	0.629
AB	39	37.46	15.12	–	0.06	13	14.5	13.0	–	0.16

Muria Gond vs Delhi subjects df – 3; χ^2 = 1.77.

Table 2. Seropositivity for malarial antibodies in subjects with different blood groups

Blood group	Total	Malarial antibodies			
		Positive		Negative	
		No.	%	No.	%
A	77	66	85.7	11	14.3
B	86	65	75.6	21	24.4
O	56	44	78.6	12	21.4
AB	39	29	73.7	10	26.3

No significant difference observed among different blood groups.

with blood group A, but it did not differ significantly from the seropositivity in subjects with blood groups B,O or AB.

Table 3 summarizes the distribution of different blood groups in subjects who had fever at the time of collection of blood (in M.P.) and who were positive for malarial parasites in their blood. The observed distribution of the blood groups did not differ significantly from the expected.

Table 3. Distribution of malaria parasite in different ABO blood types

Blood group	No.	*Pf*	*Pv*	Both
A	31	16	12	3
B	23	13	4	6
AB	7	3	3	1
O	9	4	3	2

Distribution of malaria parasite in different ABO blood types; $\chi^2 = 4.82$.

DISCUSSION

Singh *et al.*[6], reviewing the published data on association of ABO blood groups with malaria, observed a marked excess of blood group A in patients with malaria, as compared with blood groups B and O. However, a detailed analysis of their summary table shows that a significant excess of blood group A over O was observed only in the Italian samples while subjects from the UK, the former USSR, and India (Assam and Gujarat) did not show any significant excess of A over O blood group[12]. On the other hand, a significant excess of blood group B over O was observed in the samples from one area of Italy, and the former USSR (Voroesh), but not in another area of Italy (Sassari), the UK, other areas of the former USSR (Kharkov and Uzbekistan) and India (Assam and Gujarat).

From the data obtained in our study, four lines of evidence can be deduced to support the view that ABO blood groups may not have any significant relationship with malarial infection. First, the distribution of ABO blood groups in the subjects from an area with high prevalence of malaria (Muria gonds) did not differ from that in subjects from Delhi, an area with low prevalence of malaria. Second, within Muria gonds the observed frequency of different blood groups is not significantly different from the expected frequency, which would happen if the ABO blood groups had a differential susceptibility to malarial infection. Third, the rate of seropositivity to malarial antigen did not vary significantly among subjects with different blood groups. Fourth, infection with malarial parasites in peripheral blood films, although more in subjects with blood groups A, did not differ significantly in comparison with subjects with other blood groups. We therefore conclude

that ABO blood groups are unlikely to have any significant role in differential susceptibility to malaria infection.

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SHORT NOTES

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Note on Urban Malaria Vector *Anopheles stephensi* (Liston) in Cochin

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Resurgence of malaria is a major health hazard our country is facing now. Kerala state, which had controlled malaria effectively in the past, is also coming in its grip, with Ernakulam district alone reporting a steady increase from 161 cases in 1985 to 508 in 1990. The NMEP records indicate the persistence of this disease in this district. Among the malaria-positive cases, *P. vivax* was found to be the most predominant in this region (Table 1).

The city of Cochin, the headquarters of this district, is a centre of considerable tourist interest. Because of the mosquito menace in this city, the Vector Control Research Centre has been engaged in an extensive survey to demarcate the mosquito-breeding habitats in the Corporation limits in order to suggest remedial measures. The study indicates that the proliferation of *Anopheles stephensi* coupled with frequent movement of parasite carriers through tourists can lead to focal outbreaks of malaria.

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The Corporation of Cochin covers an area of about 95 km^2, which is divided into east and west zones with prominent physical barriers of backwaters having an average width of 1 km. The study was carried out in the west zone consisting of 10 health circles with a population of 2,26,987 (1981 census).

A systematic door-to-door survey was carried out from May 1990 to April 1991 to enumerate all mosquito-breeding sources in this area. An enamel bowl (15 cm wide and 6 cm deep) with a long handle was used as a dipper for sampling habitats such as cement tanks and other receptacles. In wells, a bucket (21 cm wide and 20 cm deep) was used. Three bucket samples from each well were examined. Immature stages collected were brought to the laboratory and reared up to adults for species identification.

Major mosquito-breeding habitats in this part of the city were found to be cement tanks, wells, cesspools, ponds, canals, drains, septic tanks, water-meter chambers and miscellaneous peridomestic habitats such as tree holes, tree stumps, containers, tyres, mud pots, flower pots, grinding stones, etc. Among these, cement tanks, overhead tanks and wells were found to support the

Table 1. Number of malaria parasite carriers reported in Ernakulam district (1987 to 1991)

Month	1987			1988			1989			1990			1991		
	Total	*Pv*	*Pf**	Total	*Pv*	*Pf**	Total	*Pv*	*Pf**	Total	*Pv*	*Pf**	Total	*Pv*	*Pf**
Jan	15	9	6	19	19	0	27	27	0	29	26	3	36	36	0
Feb	12	10	2	18	18	0	22	22	0	13	11	2	15	13	2
Mar	19	18	1	23	21	2	37	37	0	24	18	6	30	29	1
Apr	18	16	2	23	23	0	29	28	1	37	37	0	31	29	2
May	12	12	0	34	34	0	46	46	0	72	70	2	44	44	0
Jun	13	12	1	48	47	1	67	67	0	69	69	0	57	57	0
Jul	28	28	0	53	52	1	43	43	0	57	56	1	27	26	1
Aug	15	15	0	44	43	1	39	36	3	50	49	1	34	34	0
Sep	23	23	0	62	62	0	39	37	2	40	38	2	40	37	3
Oct	29	28	1	35	33	2	54	49	5	49	49	0	47	45	2
Nov	23	22	1	34	32	2	24	23	1	37	35	2	40	38	2
Dec	21	21	0	26	26	0	34	33	1	31	30	1	30	29	1
Total	228	214	14	419	410	9	46.	448	13	508	488	20	431	417	14

*Mixed (both *Pv* and *Pf*) incorporated with *Pf*; *Pf* – *Plasmodium falciparum*; *Pv* – *Plasmodium vivax*.

Note: Malaria cases from 1979 to 1986 were 208, 196, 225, 236, 228, 233, 161, 206, respectively.

Source: Department of Health Services, Ernakulam.

breeding of *An. stephensi*. Of the 2581 wells and 2128 cement tanks examined, 59 and 39 respectively were found to support heavy breeding of *An. stephensi*. Disused wells were more prone than other habitats. Owing to the availability of piped water supply the number of disused wells is increasing. Focal outbreaks of malaria due to buildup of populations of *An. stephensi* in confined areas are well documented[1-4]. Epidemics of malaria due to *An. stephensi* breeding in urban construction sites and where developmental activities are expanding have been reported[5,6]. Considering the magnitude of vector breeding in this area of high tourist interest, focal outbreaks are a distinct possibility and call for adequate preventive measures and continued vigilance.

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JSB versus Giemsa Stain : An Evaluation

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Diagnosis of malaria is confirmed by detection of parasites under the microscope. Staining is the prime requirement for microscopy as it enhances the clarity and differentiation of the elements of blood and parasites including its various stages. The original idea of Romanowsky for staining of blood films for malaria parasites has been modified and improved by several workers. Each of the methods has its own advantage in staining.

Ever since their development, both JSB and Giemsa stains are in wide use. In the national malaria control programme in India, JSB is being used for malaria microscopy. However, it has been recommended that Giemsa should replace JSB[1]. Hence a study was undertaken to compare Giemsa and JSB.

Giemsa stain of E. Merck (W. Germany) make was procured and the stock solution was prepared at MRC laboratory according to Bruce-Chwatt's method[2]. The stock solution was supplied to laboratory technicians of eight PHCs of Nadiad taluka of Kheda district in Gujarat. The technicians were asked to prepare blood smears of suspected malaria cases of OPD in duplicate and stain one with Giemsa and the other with JSB, which is routinely prepared in their own laboratories. A pro forma was supplied to laboratory technicians for recording their observations on the clarity of malaria parasite, and leucocytes and the presence of artefacts in smears stained with Giemsa and JSB. Similarly, six laboratory technicians of Malaria Research Centre, Nadiad, were also asked to compare and record the results of staining with the two stains. 2618 blood smears were stained with JSB and Giemsa (1309 smears with each stain) and examined.

In 8 PHCs of Nadiad taluka, blood smears in duplicate from 784 cases were prepared. One was stained with JSB and the other with Giemsa stain by 8 laboratory technicians engaged in malaria microscopy (Table 1, s. no. 1-8). Five out of 8 technicians found leucocytes stained better with JSB, 2 found Giemsa was better and one found no difference between the two. Similarly, with regard to the presence of artefacts, 3 technicians found JSB superior, one Giemsa and 4, no difference. The clarity of malaria parasite was superior with JSB in 2 cases, with Giemsa in one case and there was no difference in the observations of 4 laboratory technicians. Six laboratory technicians of MRC, Nadiad, examined 525 smears stained with each stain and reported insignificant differences in leucocytes and artefacts but found that the clarity of malaria parasites was better with

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Table 1. Results of staining with JSB and Giemsa stains

S. No.	Sample size*	Leucocytes (clear)		Artefacts (few)		Total +ve	Parasite (clear)	
		JSB	Giemsa	JSB	Giemsa		JSB	Giemsa
1.	103	20	80	5	5	3	3	3
2.	100	63	72	32	38	7	5	6
3.	115	115	0	115	0	0	0	0
4.	80	75	1	0	0	13	13	0
5.	100	100	100	95	95	7	7	7
6.	100	89	52	58	78	4	4	4
7.	101	101	81	61	61	6	3	3
8.	85	72	49	63	42	6	6	3
9.	64	45	53	25	35	13	9	12
10.	71	61	19	29	28	9	8	9
11.	100	70	84	25	28	15	7	15
12.	90	63	57	41	34	18	8	16
13.	100	90	75	70	65	8	3	7
14.	100	100	100	45	60	19	15	19
Total	1309	1064	823	664	569	128	91	104
Student's t test		NS*		NS*			NS*	

*Blood smears examined for each stain; NS — Difference not significant ($p > 0.05$).

Giemsa than with JSB (Table 1, s. no. 9-14). However, the overall results and their statistical analysis show insignificant differences in the clarity of parasites, and leucocytes and the number of artefacts. Both the stains, therefore, appear to be equally good regarding the quality of staining, but the one which is otherwise suitable can be used. Boyd *et al.*[3] found JSB stain to be rapid and simple one. To dehaemoglobinize and stain the smear, Giemsa requires about 45 min whereas JSB about 1.25 min.

Jaswant Singh[4] found that JSB stained all the blood elements, including leucocytes, very well and thus aided in differential leucocyte count also. In addition, it stained Leishmania and Trypanosomes. He further observed that preparations treated with JSB stain compared favourably with those stained with any standard preparation like Leishman or Giemsa.

According to Christophers *et al.*[5], JSB stain can be easily prepared, is relatively inexpensive, remains unaltered under tropical conditions, and is extremely fast. Furthermore, blood cells and parasites are clearly and brilliantly differentiated, and the results are less dependent on the pH of the diluting agent than with Giemsa. JSB stain is far superior to Leishman and Giemsa for routine work and is the most rapid stain in malaria investigation. The blood picture obtained with this stain compares favourably with that obtained with the original Romanowsky stain and its various modifications.

According to Shute[6], JSB stain is comparatively inexpensive to produce and when made and used properly it is an excellent stain. Its use for several years in India in most peripheral laboratories has now attained a place which no other available stain has with respect to characteristics like ease

of preparation, economics of use, clarity and brilliancy in staining and above all very rapid staining.

We therefore conclude that JSB stain is better than Giemsa stain in peripheral laboratories for malaria microscopy as it is rapid, inexpensive and convenient to prepare and has as good staining qualities as Giemsa.

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Water Mite (*Arrenurus* sp.) Parasitizing Mosquitoes in District Shahjahanpur, U.P.

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Mosquitoes are vectors of many diseases like malaria, filariasis, Japanese encephalitis and dengue fever. The development of insecticide resistance in vector mosquitoes has posed a serious problem in controlling these diseases. Hence natural parasites of mosquitoes at their breeding sites may prove to be a better tool to control their propagation. Water mites (*Arrenurus* sp.) parasitizing mosquitoes in their natural breeding habitats were reported in India as early as AD 1900. Investigations on mites infesting mosquito species with seasonal changes and intensity of parasitization have been carried out by various workers in different parts of the country[1-4], but no such report is available from Shahjahanpur, Uttar Pradesh. We therefore carried out such a study in Shahjahanpur district in 1990.

Extensive mosquito collections were made from January to December 1990 from different localities and habitats of the district, using suction tube and torchlight. Mite-parasitized mosquitoes were sorted out and identified as per the standard key. Mites attached to different parts of the host were recorded and preserved for identification as per the key of Mullen[5].

Out of 19,685 mosquitoes examined, 812 (4.1%) specimens under 8 species were found parasitized by *Arrenurus* species of water mites (Table 1). Specieswise infestation rate was found to be highest in *An. annularis* (9.5%), followed by *An. splendidus* (6.6%), *An. nigerrimus* (4.2%), *An. culicifacies* (2.3%), *An. subpictus* (1.5%), *An. aconitus* (1.2%), *An. pallidus* (0.8%) and *Culex quinquefasciatus* (0.1%). The results further revealed that about 85% mites were found attached over the head (43.99%) and thorax (40.9%). These two parts of the insect body appear to be the preferred sites of attachment for mites. The remaining 15% mites were found attached on the abdomen (13.7%) and legs (1.28%). Only one mite was found attached on the wing of *An. subpictus*. In our study, only 54.7% mites were found attached to thorax and abdomen, whereas according to Rajput and Singh[3], 85% mites were on these body parts of mosquitoes. The mite infestation rate was 2.7 per mosquito, the highest being 2.8 both in *An. annularis* and *An. subpictus* (Table 1). Our findings are in agreement with those of Rahman *et al.*[1], Rajput and Singh[3] and Saxena and Sharma[2]. In our study the number of mites attached to a single mosquito ranged between 1 and 18, whereas other workers have reported as follows:

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Table 1. Mosquitoes found parasitized by water mites (*Arrenurus* sp.) in Shahjahanpur

S. No.	Mosquito sp.	No. of mosquitoes		Infestation rate (%)	No. of mites recovered from					Total no. of mites	No. of mites / +ve mosquito
		Examined	Parasitized		Head	Thorax	Abdomen	Legs	Wings		
1.	*An. culicifacies*	2920	66	2.3	82	51	9	4	0	146	2.2
2.	*An. aconitus*	652	8	1.2	3	2	6	0	0	11	1.4
3.	*An. annularis*	6483	618	9.5	723	742	248	21	0	1734	2.8
4.	*An. pallidus*	113	1	0.8	0	1	0	0	0	1	1.0
5.	*An. subpictus*	6167	91	1.5	131	84	35	1	1	252	2.8
6.	*An. nigerrimus*	24	1	4.2	0	1	0	0	0	1	1.0
7.	*An. splendidus*	347	23	6.6	17	11	2	1	0	31	1.3
8.	*Cx. quinquefasciatus*	2979	4	0.1	3	1	0	0	0	4	1.0
	Total	19685	812	4.1	959	893	300	27	1	2180	2.7

Table 2. Monthwise incidence of water mites on mosquito species

Month	Mosquito species							
	An. culicifacies		*An. aconitus*		*An. annularis*		*An. pallidus*	
	Examined	Infested %	Examined	Infested %	Examined	Infested %	Examined	Infested %
Jan	109	0.9 (1)	46	0	525	4.8 (25)	0	0
Feb	236	2.1 (5)	155	0.6 (1)	1490	12.1 (180)	1	0
Mar	356	3.7 (13)	46	8.7 (4)	2264	10.9 (246)	0	0
Apr	345	2.0 (7)	67	3.0 (2)	474	10 (47)	0	0
May	267	1.9 (5)	115	0	350	12.6 (44)	2	0
Jun	777	2.4 (19)	1	0	322	8.7 (28)	0	0
Jul	132	0.8 (1)	0	0	50	4.0 (2)	0	0
Aug	259	0.8 (2)	0	0	262	5.7 (15)	0	0
Sep	213	1.4 (3)	38	0	175	5.1 (9)	55	0
Oct	193	2.1 (4)	158	0.6 (1)	457	3.5 (16)	55	1.8 (1)
Nov	10	38 (3)	26	0	62	3.2 (2)	0	0
Dec	23	13.0 (3)	0	0	52	7.7 (4)	0	0
Total	2920	2.3 (66)	652	1.2 (8)	6483	9.5 (618)	113	0.9 (1)

contd...

Table 2. Monthwise incidence of water mites on mosquito species *(contd.)*

Month	Mosquito species							
	An. subpictus		*An. nigerrimus*		*An. splendidus*		*Cx. quinquefasciatus*	
	Examined	Infested %	Examined	Infested %	Examined	Infested %	Examined	Infested %
Jan	2	0	0	0	2	0	190	0
Feb	0	0	1	0	150	0	632	0.2 (1)
Mar	0	0	0	0	150	14.7 (22)	84	1.2 (1)
Apr	14	0	3	33.3 (1)	19	5.3 (1)	596	0
May	287	1.7 (5)	0	0	20	0	511	0.2 (1)
Jun	952	2.6 (25)	0	0	6	0	336	0.3 (1)
Jul	700	2.1 (15)	0	0	0	0	176	0
Aug	2884	1.1 (33)	6	0	0	0	123	0
Sep	897	0.4 (4)	13	0	0	0	177	0
Oct	390	2.1 (8)	1	0	0	0	135	0
Nov	32	0	0	0	0	0	19	0
Dec	9	11.1 (1)	0	0	0	0	0	0
Total	6167	1.5 (91)	24	4.2 (1)	347	6.6 (23)	2979	0.1 (4)

Figures in parentheses indicate total number infested.

Rajput and Singh[3], 1-13; Saxena and Sharma[2], 1-29; and Sarkar *et al.*[4], 1-23. In our study a majority of the mites were found attached ventrally on head and thorax and a few of them attached dorsally and occasionally on lateral portions of the above body parts. This is contrary to the findings of Saxena and Sharma[2] who reported that most of the mites attached laterally between the abdominal tergites. The attachment pattern observed during our study was in conformity with the findings of Rahman *et al.*[1]

Monthwise incidence of water mites on mosquito species is given in Table 2. *An. culicifacies* and *An. annularis* were found parasitized throughout the year. Since *An. culicifacies* is a well-known vector of rural malaria and *An. annularis* is of secondary importance the role of mites in vector control programmes needs to be investigated. *An. aconitus, An. subpictus* and *An. splendidus* were parasitized by the mites mostly during their seasonal abundance. One specimen each of *An. nigerrimus* and *An. pallidus* was found parasitized during April and October respectively. *Culex quinquefasciatus* was found parasitized during February, March, May and June (Table 2). A positive correlation between rainfall and mite infestation was reported by Sarkar *et al.*[4] In our study no such positive correlation was recorded. This is because when the highest rainfall (438.1 mm) was recorded in July 1990 the infestation rate varied from 0 to 4.0, whereas in the month of April, when no rainfall occurred, the infestation rate varied from 0 to 33.3 in different species (Table 2). Further investigation on the species distribution of water mites and their role in the regulation of mosquito population is indicated.

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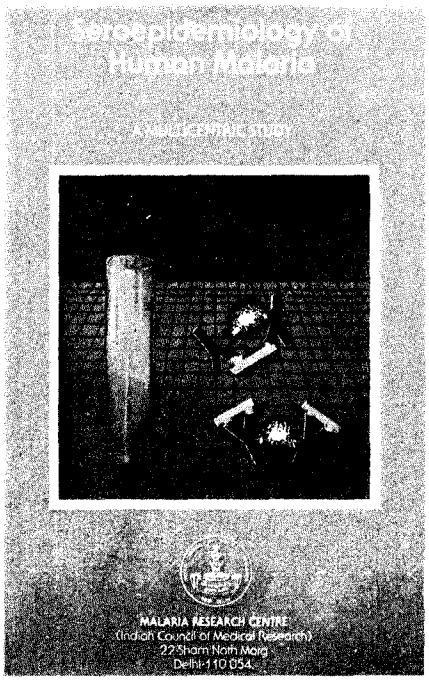

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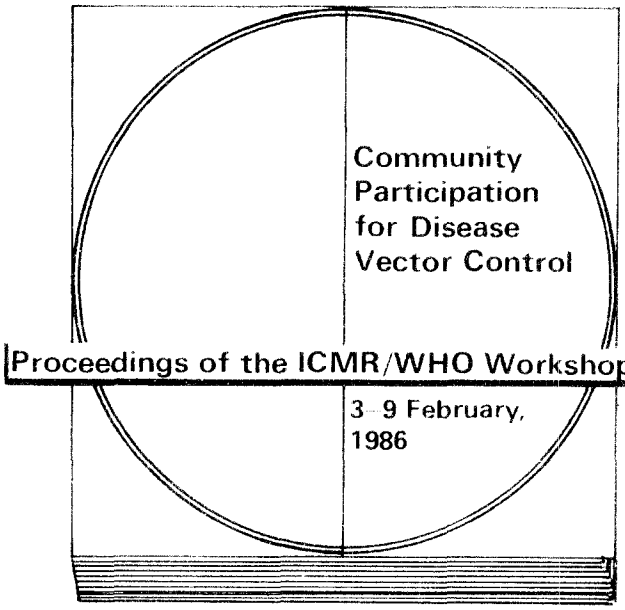

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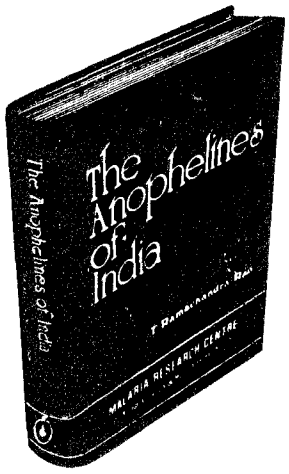

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