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Persistent Malaria Transmission in Kundam Block, District Jabalpur (M.P.)

NEERU SINGH[1] and V.P. SHARMA[2]

Malaria survey was undertaken in 7 villages of Kundam PHC from August 1987 to July 1988. Epidemiological findings revealed high spleen (20--70), child parasite (40--85) and infant parasite rates (20--75) in the area. *P. falciparum* was the predominant species and unchecked malaria transmission continued almost throughout the year. The study of monthwise distribution of positive cases and the period during which intensive intervention measures were undertaken revealed that despite an extra round of HCH and intensive chemotherapeutic measures, there was no marked decrease in the prevalence of malaria.

INTRODUCTION

Kundam and Bizadandi blocks of Madhya Pradesh form a contiguous area of hilly terrain which is part of a hard core malarious belt with high incidence of *P. falciparum*. Bioenvironmental control strategy is being implemented in Bizadandi block since 1987. Under this project, malaria has been brought under control by non-insecticidal methods of vector control, regular weekly surveillance and prompt chemotherapy (Singh *et al.*, 1989). There is a good deal of routine population movement between the two blocks particularly on market days and festivals. Areas outside the experimental villages had very high incidence of malaria, although residual spraying of insecticides was being done under NMEP to interrupt transmission. This was confirmed by our own investigations in some villages of Kundam block (Singh *et al.*, 1988). As a result, malaria cases from Kundam block were often detected among visitors to Bizadandi block.

The focus of malaria outbreak was fairly widespread involving at least a few PHCs. This alarming situation was communicated to the health department and the project staff was requested to investigate this outbreak. Therefore, systematic studies were taken up to investigate malaria epidemic in some villages, adjoining Bizadandi, in Kundam block. Results of a one year study are reported in this paper.

MATERIAL AND METHODS

Kundam is one of the thirteen blocks of Jabalpur district, M.P. It is located in the southeastern

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Table 1. Incidence of malaria recorded by the NMEPs surveillance in 2 sections of Kundam PHC even study villages were situated in these sections

Sections	1984			1985			1986			1987			
	BSC	+ve	Pf	BSC	+ve	Pf	BSC	+ve	Pf	BSC	+ve	Pf	Mixed
Jamgaon* (1 Study village)	116	1	-	80	6	2	301	2	-	1569	780	481	6
Pipariya** (6 Study villages)	128	-	-	248	9	4	504	-	-	768	45	39	-

*Source NMEP.
**In 1987 surveillance was strengthened with the help of project staff.

Table 2. Spray records of study villages of Kundam PHC*

Villages	1984		1985		1986		1987
	Ist Rd spray	IInd Rd spray	Ist Rd spray	IInd Rd spray	Ist Rd spray	IInd Rd spray	Date of focal spray
Torka**	24.7.84	9.9.84	1.7.85	19.8.85	19.7.86	7.9.86	28.11.87
Sakari	1.7.84	20.8.84	3.7.84	25.8.85	29.6.86	17.8.86	8.11.87
Amera	26.6.84	15.8.84	6.7.85	31.8.85	26.6.86	14.8.86	13.11.87
Andhar	26.6.84	15.8.84	6.7.85	29.8.85	26.6.86	14.8.86	6.11.87
Jamgaon	27.6.84	16.8.84	5.7.85	27.8.85	1&2.7.86	19.8.86	5&6.11.87
Budbudi	2.7.84	21.8.84	4.7.85	26.8.85	30.6.86	18.8.86	9.11.87
Kastara	1.7.84	19.8.84	2.7.85	24.8.85	28.6.86	16.8.86	8.11.87

*Source NMEP.
**Focal spray done on 29.3.85 in Torka village.

Table 3. Mosquito densities (man hour density) in the study villages, Kundam block, Jabalpur

Months 1987-88	Total anophelines	Total vectors	*A. culicifacies*	*A fluviatilis*	*A. subpictus*	*A. annularis*	Others
Aug	157.5	129.00	128.00	1.0	25.00	2.5	1.5
Sep	240.5	225.75	225.75	-	10.75	3.25	0.75
Oct	156.75	146.75	146.0	0.75	1.75	8.0	-
Nov	118.0	70.25	69.5	0.75	6.25	38.5	3.0
Dec	187.75	142.25	142.0	1.25	6.25	31.5	1.50
Jan	144.0	120.3	120.3	-	-	23.6	-
Feb	175.5	159.5	159.5	-	-	0.39	0.5
Mar	177.2	170.8	169.0	1.2	1.8	4.4	0.3
Apr	242.6	232.4	231.4	1.0	2.8	5.4	-
May	102.5	94.25	94.25	-	0.25	1.5	-
Jun	95.71	85.85	85.85	-	7.42	2.14	0.5
Jul	128.75	109.75	109.25	0.5	12.00	6.75	0.25

region of Jabalpur and occupies an area of 971 sq kms. This region has a succession of low hills and shallow valleys of heavy black soil covered with forest and long grass. The population of this block is 74,163 and is spread in 189 villages. This block has the highest *adivasi* population in the district (80% – mainly Gond). The villages are surrounded by scrub jungle intersected by a large number of streams and low rocky hills. The villagers live in small, dingy, mud-plastered huts scattered in the field and forest. The houses are kept warm by keeping a constant fire inside. Domestic animals are also sheltered in the houses. Sanitation of houses and villages is neglected and a large part of population is below the poverty line. Main occupation of the tribals is agriculture, wood cutting and wage labour in forest nurseries or road construction work etc.

Under the Modified Plan of Operation (MPO) of the NMEP all areas with <2 API are not to be sprayed and according to NMEP records the sections in which study villages are located were practically free from malaria before the commencement of the study (Table 1). This was the reason that the 7 study villages were also not sprayed in 1987. These villages were situated inside the forest adjacent to perennial streams. Spray history is given in Table 2.

Anopheline densities were recorded fortnightly. Mosquito collections were made from these villages by a trained insect collector. In each village, 8 catching stations were fixed (4 human dwellings and 4 cattlesheds). Vector susceptibility tests were carried out with the WHO standard kits at frequent intervals during monsoon and post-monsoon period. Blood smears were prepared fortnightly from all fever cases and cases with history of fever. All fever cases were given 600 mg chloroquine presumptive treatment. Blood smears were stained with JSB and examined in the laboratory. Results were made available to the State public health department which provided radical treatment (RT). All malaria cases were given 600 mg chloroquine. *P. vivax* cases were given 15 mg chloroquine for 5 days and *P. falciparum* 45 mg primaquine single dose. Children were given proportionately low doses and pregnant women were not given primaquine. Spleen enlargement was determined by Hackett's method (Christophers *et al.*, 1958) in children of 2–9 years age group.

The first investigations carried out in August 1987, revealed high malaria infection and enlarged spleens indicating hyperendemicity. NMEP started control operations only in October 1987 as a result of severe epidemic which broke out during the months of October–November 1987 in a number of villages of the block (Singh *et al.*, 1988). This followed intensified surveillance during 1987–88 by the NMEP with the help of additional staff from district headquarters. Arrangements were made for prompt radical treatment of all parasite positive cases from October 1987 onwards.

RESULTS AND DISCUSSION

In August 1987 vector densities in all the affected villages (unsprayed at that time) were determined and it was observed that per man hour density (MHD) of *A. culicifacies* ranged from 70–230 while that of *A. fluviatilis* from 0.5 to 1.25. After the application of HCH focal spray in October–November 1987 there was reduction in vector density during November 1987 but thereafter the vector and anopheline densities were maintained at high levels (Table 3).

Insecticide susceptibility tests showed that *A. culicifacies* was resistant to insecticides. Corrected % mortality was 43% on 4% DDT, 24.5% on 0.4% dieldrin and 61.9% on 5% malathion impregnated papers after one hr exposure and 24 hrs post-recovery period. It may be noted that malathion has never been used in public health and no insecticides are used in agriculture because of the primitive nature of agricultural practices. Therefore, resistance to malathion was inexplicable and requires further investigations.

Results of parasitological surveys are given in Table 4. The annual blood examination rate

Table 4. Epidemiological situation of malaria in study villages of Kundam PHC District - Jabalpur (M.P.)

Villages	Pop.	ABER	BSE	Positive cases: Total +ve	Pv	Pf	Mixed	SPR	API
Sakari	577	53.21	307	155	89	65	1	50.49	268.63
Kastara	577	117.68	679	433	153	277	3	63.77	750.43
Jamgaon	837	111.47	933	522	196	315	11	55.95	623.66
Andhar	377	160.74	606	335	134	196	5	55.28	888.59
Torka	513	93.18	478	306	69	230	7	64.02	596.45
Amera	334	88.62	296	160	49	107	4	54.05	479.04
Budbudi	533	35.08	187	97	33	62	2	51.87	181.99

Period of survey Aug to July 1988.

Table 5. Results of parasitological surveys in infants and children of seven villages of Kundam PHC, Distt. Jabalpur (M.P.)

Months 1987-88	Infants (< 1 year)				Children (1 to 10 year)			
	BSE	+ve	Pf	SPR	BSE	+ve	Pf	SPR
Aug	15	5	1	33.33	122	71	15	58.20
Sep	76	45	16	59.21	534	393	175	73.60
Oct	10	2	-	20.00	122	96	54	85.71
Nov	16	12	5	75.00	183	152	112	83.06
Dec	8	5	4	62.50	82	60	49	73.17
Jan	3	1	1	33.33	43	27	17	62.79
Feb	2	-	-	-	69	32	23	46.78
Mar	14	3	-	21.43	46	26	8	56.52
Apr	10	6	-	60.00	86	54	14	62.79
May	13	5	1	38.46	114	61	18	53.51
Jun	15	3	-	20.00	55	26	5	47.27
Jul	18	8	2	44.44	75	33	16	44.00

Note: Total number of Pv cases were 65 in infants and 500 in children. In children 25 cases of mixed infection were observed.

Table 6. Results of spleen surveys of study villages of Kundam block, Jabalpur (M.P.)

Months 1987-88	No. of villages	No. of children examined	No. of children with enlarged spleen	Enlarged spleen rate
Aug-Sep	5	558	265	47.49% (21-67)
Nov-Dec	5	391	195	49.8% (31-73)
Feb-Mar	5	566	262	46.2% (40-60)

(ABER) is generally 10% in routine surveillance, but collection of blood smears from all fever cases resulted in very high ABER which varied from 35 to 160. Similarly, there was very high incidence of both vivax and falciparum malaria as revealed by all the epidemiological indices viz., SPR, SVR and SfR. API varied from 180 to 890. Epidemiological indices in infants and children also revealed very high active transmission (Table 5) and there appeared to be no difference in the transmission of malaria in infants, children or all age groups taken together. Surveys of spleen enlargement in 5 study villages also showed high proportion (46 to 50%) of enlarged spleen (Table 6).

There was, therefore, mounting evidence of very high level of active malaria transmission, although HCH was being sprayed. It may be noted that during 1987 and 1988 an extra round of HCH was sprayed by NMEP and drug distribution was intensified.

The intensity of malaria was so high in these villages that about 80% population had at least one attack of malaria between July to November. Ninety three children of age below 10 years and 107 above 10 years age had 2 attacks of malaria. Similarly, 24 children below 10 years and 26 above 10 years experienced 3 attacks of malaria. There were at least 26 cases who had experienced 4 to 7 attacks of malaria. Whether these cases were relapses, re-infection, or falciparum recrudescence or drug failure etc., were not determined. A record of recrudescence or repeat attack of falciparum malaria in 10 year age group showed that 15 patients reported a second attack within 7 days and another 13 within about 15 days. In the first month itself a total of 58 patients reported second attack of malaria. In subsequent months, repeated attacks of falciparum malaria were recorded from 22, 22, 13, 7, 6, 4, 7, 4 and 5 patients from 2nd to 10th month during the period of active transmission and the possibility of inadequate drug administration or incipient resistance in *P. falciparum* was evident.

It may be noted that vivax malaria cases started increasing from March 1988 showing a peak in April followed by a gradual decline (Table 7). Incidence of falciparum malaria was very low from March 1988 to May 1988 and slowly started increasing in the following months. Even during the height of winter in January the incidence was quite high and declined during February but the proportion of falciparum cases remained high. Thus there were two distinct peaks, *P. vivax* followed by *P. falciparum* i.e., March–April peak followed by falciparum outbreak during the monsoon and post-monsoon months.

The experimental villages were not sprayed in 1987 as the surveillance had broken down and the API was less than 2. The information of malaria outbreak detected by the project staff was used by NMEP and the study villages were sprayed with a special round of HCH during October–November 1987. It may be pointed out that people repeatedly suffered from malaria attacks and some unusual deaths had occurred in this region at the peak of transmission period. The strain of *P. falciparum* was not responding to the NMEP's drug schedule. This was resulting in increased morbidity. The main reasons for this outbreak were:

(i) Withdrawal of HCH spraying in 1987 resulting in the build-up of high vector densities.

(ii) Average rainfall during the year 1987 was 1003 mm which created a large number of breeding sites.

(iii) From 1984–86 two rounds of HCH were sprayed instead of 3, these rounds were incomplete and the coverage and quality of spraying was poor.

(iv) Complete breakdown of active surveillance for a long period resulting in the build-up of cases.

(v) The quality of laboratory services was far below satisfactory levels and there was a 6 to 8 week backlog.

Table 7. Monthwise epidemiological situation of 7 villages of Kundam PHC (1987-88) (Total cases)

Months	BSE	Total +ve	Positive cases			SPR	SFR	Pf
			Pv	Pf	Mix			
Aug 87	335	174	105	67	2	51.94	20.00	38.51
Sep	593	378	179	218	1	63.74	36.76	57.67
Oct	628	418	121	290	7	66.56	46.18	69.38
Nov	507	363	56	301	6	71.60	59.37	82.92
Dec	226	142	9	129	4	62.83	57.08	90.85
Jan 88	146	73	8	60	5	50.00	41.10	82.19
Feb	96	35	2	32	1	36.46	33.33	91.43
Mar	122	51	30	20	1	41.80	16.39	39.22
Apr	202	110	74	34	2	54.46	16.83	30.91
May	235	100	69	30	1	42.55	12.77	30.00
Jun	199	73	41	30	2	36.68	15.08	41.10
Jul	197	71	29	41	1	36.04	20.81	57.75
Total	3486	2008	723	1252	33	57.60	35.92	62.35

Population : 3748.

Under the above circumstances a change in the malaria control strategy is urgently required to interrupt transmission in the tribal belt of Madhya Pradesh. Research should be encouraged on socio-economic aspects of malaria, methods of vector control, drug resistance, agricultural practices and population movement patterns etc., to tackle the deteriorating malaria situation in some of these areas.

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Gametocytocidal and Sporontocidal Activity of Some Standard Antimalarials on *P. berghei* (NK 65) Infection *M. natalensis*

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Three standard antimalarials pyrimethamine, primaquine and quinine were tested against *P. berghei* (NK 65) for gametocytocidal and sporontocidal action. Pyrimethamine at 7.5, 3.25, 2.5 and 1.25 mg/kg., primaquine at 15, 10 and 7.5 mg/kg, quinine at 225, 168.5, 140.62 and 112.5 mg/kg were given orally to infected *M. natalensis* for 3 consecutive days i.e., day 6, 7 and 8 post inoculation of sporozoites. To assess sporontocidal action batches of mosquitoes (*A. stephensi*) were fed on infected-treated and control animals on day 8. Mosquitoes were dissected on day 10 post infective feed to compare the number of oocysts from control groups. Pyrimethamine at 2.5, primaquine at 10 and quinine at 140.62 mg/kg had total sporontocidal action. When mosquitoes were fed on animals treated with 2.5 mg/kg of pyrimethamine and 10 mg/kg of primaquine, very few oocysts were developed and sporozoites from these mosquitoes were not infective to *Mastomys*. When mosquitoes were fed on *Mastomys* treated with 140.62 mg/kg of quinine, no oocyst was observed.

INTRODUCTION

Gametocytes constitute a significant part of the life cycle of malaria parasites. These stages do not develop further if they are not ingested by a susceptible mosquito vector through blood meal. Ultimately, the gametocytes develop into sporozoites and the mosquito becomes infective. The objective of this study was to break the sporogonic cycle by a chemotherapeutic agent. For this purpose, we must either kill the gametocytes or sterilize the sexual forms so that their ability for further development is reduced.

There are very few antimalarials available which are potentially gametocytocidal or sporontocidal. In order to find out new gametocytocidal and sporontocidal antimalarials it is imperative to standardise screening method for candidate compounds. In case of *Plasmodium berghei* (NK 65), Rastogi *et al.* (1983) observed that gametocytes were most infective only for a particular period i.e., 7th and 8th day of post inoculation of sporozoites in *Mastomys natalensis*. This communication reports the results of the effect of standard antimalarials on gametocytes of *P. berghei* (NK 65) in *M. natalensis*.

MATERIAL AND METHODS

Parasite – *P. berghei* (NK 65) was received from

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Table 1. Effect of pyrimethamine on parasitaemia

Dose mg/kg	N	Days/X ± SE						
		6	7	8	10	12	14	16
7.5	9	1.49±0.01	0.62±0.08	0.006±0.002	0.00±0.00	-	-	-
3.25	9	0.67±0.08	0.03±0.02	0.00 ±0.00	-	-	-	-
2.50	15	2.15±0.17	2.08±0.24	1.37 ±0.14	0.40±0.13	1.80±0.54	5.34±1.25	8.64±1.92
1.25	9	1.69±0.08	2.07±0.11	2.63 ±0.13	4.32±0.15	7.51±0.20	13.36±0.36	19.41±0.41
Control	10	1.93±0.15	3.28±0.19	5.30 ±0.23	8.77±0.65	13.15±1.18	17.89±1.99	27.64±1.92

N = No. of animals; X = Mean per cent; SE = Standard error.

Table 2. Effect of pyrimethamine on gametocytes

Dose mg/kg	N	Days/X ± SE						
		6	7	8	10	12	14	16
7.5	9	0.26±0.04	0.07±0.01	0.00±0.00	0.00±0.00	0.00±0.00	-	-
3.25	9	0.22±0.03	0.04±0.01	0.00±0.00	0.00±0.00	-	-	-
2.50	15	0.31±0.03	0.36±0.05	0.36±0.05	0.23±0.06	0.27±0.06	0.22±0.07	0.20±0.09
1.25	9	0.20±0.02	0.28±0.01	0.27±0.04	0.33±0.04	0.47±0.03	0.58±0.03	0.60±0.03
Control	10	0.25±0.03	0.52±0.03	0.68±0.03	0.93±0.07	0.51±0.05	0.70±0.04	0.95±0.11

N = No. of animals; X = Mean percent; SE = Standard error.

the Department of Biochemistry, PGI, Chandigarh, in 1979.

Vector – NICD, Delhi, strain of *Anopheles stephensi* was used as vector.

Host – Eight weeks old female *Mastomys* (GRA Giessen strain) were used for this study. Each animal was inoculated with 1 x 10^4 sporozoites (i.v.) on day 0.

Drug used – Pyrimethamine, primaquine and quinine.

Mastomys harbouring initial gametocytaemia on day 6, post inoculation of sporozoites were used. Five infected animals were taken for each experiment of which 3 were treated with the drug concerned and 2 were kept as untreated control. The drugs were administered orally for three consecutive days i.e., on day 6, 7 and 8.

Pyrimethamine – Four dose schedules, 7.5, 3.25, 2.5 and 1.25 mg/kg x 3 days were tried.

Primaquine – Three dose schedules, 15, 10 and 7.5 mg/kg x 3 days were tried.

Quinine – Four dose schedules, 225, 168.5, 140.62 and 112.5 mg/kg x 3 days were tried.

Criteria for assessment of drug activity

Effect on asexual and sexual forms in the vertebrate host

(i) No. of parasites : in treated-infected and infected-control animals.

(ii) No. of gametocytes : in treated-infected and infected-control animals.

Effect on sporogonic cycle

(i) No. of oocysts : in mosquitoes fed on treated-infected and control-infected animals.

(ii) Infectivity of sporozoites.

The blood smears of both treated and control animals were made from tail blood from day 6 post-sporozoite inoculation from the day of drug administration. These were stained with Giemsa in the usual way and examined for parasitaemia and gametocytaemia.

Infective feeding: Two day old fasting mosquitoes were fed on treated and untreated (control) infected animals on day 8. These were kept at 19 ± 1° C temperature and 80–85% RH. Mosquitoes from both the groups were dissected on day 10 post-infective feed in 0.6% saline to assess the number of oocysts on the midgut.

To assess the infectivity of sporozoites, salivary glands of mosquitoes were isolated in RPM1 1640 on day 16. These were crushed in RPM1 and centrifuged at 1000 rpm for 1 min. The coarse particles settled down and the supernatant containing sporozoites was used for infecting the healthy *Mastomys*. In the untreated controls parasitaemia always appeared between D4 and D6.

RESULTS

Effect on parasites

Pyrimethamine

Pyrimethamine at doses of 7.5 and 3.25 mg/kg cleared the parasites from the blood by day 8–9. At 2.5 mg/kg parasitaemia gradually decreased till day 10 post-treatment. Parasitaemia started increasing from day 12. At 1.25 mg/kg a slight decrease in parasitaemia was observed in comparison to control animals. In control animals parasitaemia increased throughout the course of infection (Table 1).

It is apparent from Table 2 that at 7.5 mg/kg and 3.25 mg/kg gametocytes disappeared from the blood by day 8 and day 9. At 2.5 and 1.25 mg/kg, the drug exerted a gametocytocidal effect; the number of parasites became less on D7, 8, 9 and 10 in comparison to those of control groups.

Table 3. Effect of primaquine on parasitaemia

Dose mg/kg	N	Days/X ± SE						
		6	7	8	10	12	14	16
15.0	15	2.26±0.14	1.55±0.21	0.13±0.07	0.00±0.00	-	-	-
10.0	9	1.89±0.14	1.06±0.13	0.52±0.14	0.01±0.01	1.47±0.23	4.57±0.44	12.16±0.96
7.5	10	2.24±0.28	2.26±0.38	2.18±0.29	2.26±0.19	6.06±1.03	11.76±1.36	20.21±1.96
Control	6	1.75±0.34	3.03±0.47	3.83±0.54	7.67±0.72	11.55±1.02	19.85±0.32	30.85±0.83

N = No. of animals; X = Mean per cent; SE = standard error.

Table 4. Effect of primaquine on gametocytaemia

Dose mg/kg	N	Days/ X ± SE						
		6	7	8	10	12	14	16
15.0	15	0.33±0.03	0.18±0.04	0.007±0.007	0.00±0.00	-	-	-
7.5	10	0.44±0.09	0.44±0.20	0.34±0.05	0.32±0.06	0.44±0.07	0.57±0.04	0.79±0.06
10.0	9	0.33±0.03	0.20±0.03	0.06±0.02	0.00±0.00	0.32±0.06	0.44±0.04	0.54±0.06
Control	6	0.27±0.05	0.52±0.06	0.67±0.04	1.03±0.04	0.52±0.03	0.72±0.02	1.12±0.10

N = No. of animals; X = Mean per cent; SE = Standard error.

Primaquine

A dose of 15 mg/kg of primaquine cleared all the parasites from the blood by day 9. At 10 mg/kg dose, parasitaemia decreased till day 10 followed by an increase. At 7.5 mg/kg dose the parasitaemia remained constant till day 10 showing an increasing trend from day 12 (Table 3). It is clear from Table 4 that at 10 mg/kg a significant decrease was observed in the number of gametocytes in comparison to control group. The gametocytes disappeared from the blood by day 9 and day 10. At 7.5 mg/kg also the number of gametocytes decreased. In all the untreated-control animals the gametocytaemia always remained high.

Quinine

Table 5 shows the effect of the drug on parasitaemia, when 225 mg/kg was administered, parasitaemia decreased sharply after treatment and no parasite was observed from day 10 till day 12. On day 14 the parasites started increasing again. At 140.62 and 112.5 mg/kg significant decrease in parasitaemia was observed in comparison to control group.

Table 6 shows that 225 mg and 168.5 mg/kg of quinine cleared all the gametocytes from blood by day 8. At 140.62 mg/kg, number of gametocytes declined sharply till day 10. Although, at 112.5 mg/kg, the number of gametocytes was less in comparison to control it did not show a decreasing trend.

Assessment of sporontocidal action of the drugs

It is obvious from the results that when 7.5 mg and 3.25 mg/kg of pyrimethamine, 15 mg/kg of primaquine and 225 and 168.5 mg/kg of quinine were administered, parasites disappeared from the blood by day 8 and day 9. Therefore, mosquitoes were fed on animals treated with 2.5 mg and 1.25 mg/kg of pyrimethamine, 10 mg/kg and 7.5 mg/kg of primaquine and 140.62 mg/kg and 112.5 mg/kg of quinine when parasites were present in the peripheral blood.

Pyrimethamine

(i) *No. of oocysts at 2.5 mg/kg*: Table 7 summarises the results of all the 7 experiments. It is clear that the pattern of development of oocysts in all the 7 experiments was similar. The number of oocysts observed was 4.5 ± 3.3 in mosquitoes fed on treated animals whereas in control groups the number of oocyst was 74.17 ± 35.23 (Table 11).

Infectivity of sporozoites: 1×10^4 sporozoites when inoculated into healthy *Mastomys* failed to establish the infection.

(ii) *No. of oocysts at 1.25 mg/kg*: Oocysts were less in mosquitoes (7.71 ± 5.71) which were fed on treated animals than untreated control group 72.5 ± 32.4 (Tables 8 and 11)

Infectivity of sporozoites: A total of 13 *Mastomys* were inoculated with 1×10^4 sporozoites (i.v). All the *Mastomys* became patent by D4 – D6.

Primaquine

(i) *Number of oocysts*: The results with 7.5 mg and 10 mg/kg of primaquine have been presented in Table 9. When 7.5 mg/kg was tried, the number of oocysts decreased in all the experiments in comparison to the control group. At 10 mg/kg a remarkable decline in number of oocysts was found. It is clear from Table 5 that in mosquitoes fed on animals treated with 7.5 mg/kg, the number of oocysts was 35.92 ± 17.34, and at 10 mg/kg the oocysts were 0.68 ± 0.69 in comparison to control group (73.0 ± 26.27).

(ii) *Infectivity of sporozoites*: At 7.5 mg/kg the sporozoites were infective to *Mastomys*. The oocysts, at 10 mg/kg were degenerated showing highly vacuolated cytoplasm and several clumps of pigment. Sporozoites were not developed in these oocysts.

Quinine

It is evident from Table 10 that at 112.5 mg/kg, the

Table 5. Effect of quinine on parasitaemia

Dose mg/kg	N	Days/ X ± SE						
			7	8	10	12	14	16
225.0	9	1.69±0.11	0.81±0.12	0.12±0.04	0.00±0.00	-	0.03±0.03	0.79±0.72
168.5	9	1.81±0.13	0.75±0.17	0.22±0.10	0.00±0.00	-	-	-
140.62	9	2.00±0.08	1.03±0.11	0.44±0.10	0.45±0.15	2.71±0.40	6.24±0.43	12.79±0.38
112.50	9	1.97±0.16	1.47±0.16	0.62±0.16	1.06±0.24	4.58±0.68	10.50±0.85	20.32±1.04
Control	9	1.60±0.17	2.93±0.31	4.38±1.31	9.53±0.40	15.53±0.41	23.10±0.94	32.45±1.52

N = No. of animals; X = Mean per cent; SE = Standard error.

Table 6. Effect of quinine on gametocytaemia

Dose mg/kg	N	Days/ X ± SE						
		6	7	8	10	12	14	16
225	9	0.30±0.03	0.09±0.02	0.00±0.00	-	-	-	-
168.5	9	0.31±0.04	0.12±0.03	0.001±0.001	0.00±0.00	-	-	-
140.62	9	0.29±0.04	0.36±0.03	0.22±0.06	0.07±0.02	0.22±0.03	0.49±0.04	0.82±0.70
112.50	9	0.26±0.10	0.30±0.08	0.35±0.10	0.54±0.08	0.60±0.17	0.54±0.16	0.61±0.14
Control	9	0.23±0.04	0.42±0.04	0.63±0.42	1.00±0.10	0.58±0.05	0.68±0.05	1.11±0.20

N = No. of animals; X = Mean per cent; SE = Standard error.

Table 7. Sporontocidal action of pyrimethamine

Expt. No.	Control			Mosquitoes fed on animals treated with 2.5 mg/kg		
	P	G	N Median/ range	P	G	N Median/ range
1.	5.0	0.8	92 (34-107)	2.1	0.6	9 (5-17)
2.	3.9	0.8	55 (29-109)	1.6	0.6	2 (1-5)
3.	6.2	0.7	78 (38-151)	1.6	0.6	2 (1-8)
4.	4.6	0.9	70 (55-148)	2.0	0.2	2 (1-5)
5.	3.4	0.7	72 (37-150)	1.4	0.4	4 (1-7)
6.	4.3	1.0	74 (29-187)	2.0	0.2	2 (1-4)
7.	3.0	0.5	41 (17-101)	1.6	0.3	5 (2-9)

P = Per cent parasitaemia; G = Per cent gametocytaemia; N = No. of oocysts.

Table 8. Sporontocidal action of pyrimethamine

Expt. No.	Control			Mosquitoes fed on animals treated with 1.25 mg/kg		
	P	G	N Median/ range	P	G	N Median/ range
1.	3.7	0.9	68 (31-191)	2.3	0.2	9 (5-17)
2.	3.4	0.7	73 (39-131)	3.0	0.3	11 (2-23)
3.	4.1	0.8	72 (27-102)	2.6	0.3	6 (1-26)
4.	5.0	0.7	79 (39-98)	2.0	0.5	5 (2-9)
5.	4.2	0.8	56 (19-103)	1.9	0.4	4 (2-12)

P = Per cent parasitaemia; G = Per cent gametocytaemia; N = No. of oocysts.

Table 9. Sporontocidal action of primaquine

Expt. No.	Control			Mosquitoes fed on animals treated with					
				7.5 mg/kg			10 mg/kg		
	P	G	Median Range/ N	P	G	Median Range/ N	P	G	Median Range/ N
1.	3.5	0.8	81 (47-148)	2.4	0.4	35 (19-67)	1.2	0.2	1 (1-2)
2.	3.7	0.7	73 (56-105)	1.0	0.2	24 (9-38)	0.8	0.1	1 (1-2)
3.	5.6	0.7	66 (32-149)	3.0	0.5	43 (21-7)	0.9	0.1	1
4.	4.5	0.5	59 (39-97)	2.5	0.7	39 (17-63)	0.2	0.1	1
5.	4.7	0.7	67 (24-104)	2.6	0.7	25 (17-64)	0.7	0.08	-

P = Per cent parasitaemia; G = Per cent gametocytaemia; N = No. of oocysts.

Table 10. Sporontocidal action of quinine

Expt. No.	Control			Mosquitoes fed on animals treated with					
				112.5 mg/kg			140.62 mg/kg		
	P	G	N Median/ range	P	G	N Median/ range	P	G	N Median/ range
1.	3.6	0.6	66 (49-91)	1.6	0.5	28 (15-40)	0.5	0.2	0
2.	3.5	0.6	62 (25-101)	0.6	0.3	21 (13-28)	1.0	0.3	0
3.	4.1	0.5	49 (21-79)	0.7	0.4	35 (19-41)	0.5	0.2	0
4.	4.8	0.6	58 (32-113)	0.7	0.3	23 (16-38)	0.6	0.2	0

P = Per cent parasitaemia; G = Per cent gametocytaemia; N = No. of oocysts.

Table 11. Sporontocidal action of pyrimethamine, primaquine and quinine

Drug	Dose mg/kg	N	X ± SE	P value	Infectivity of sporozoites +/-
Pyrimethamine	2.5	41	4.5 ± 3.33		-
	Control	54	74.17 ± 35.23	<0.01	+
	1.25	35	7.71 ± 5.71	<0.01	+
	Control	37	72.5 ± 32.41		+
Primaquine	10.0	25	0.68 ± 0.69		-
	7.5	38	35.92 ± 17.34	<0.01	+
	Control	40	73.0 ± 26.27	<0.01	+
Quinine	140.62	30	-		
	112.50	30	27.47 ± 9.06	<0.001	+
	Control	32	60.50 ± 22.80	<0.01	+

N = No. of mosquitoes; X = Mean no. of oocysts; SE = Standard error.

number of oocysts were less in comparison to those in control group and at 140.62 mg/kg, no oocyst was observed. At 112.5 mg/kg number of oocysts observed were 27.47 ± 9.06. In mosquitoes fed on untreated animals the number was 60.50 ± 22.80 (Table 11).

DISCUSSION

Although there are a number of blood schizontocidal drugs, very few are effective against gametocytes, particularly mature gametocytes of all the species of human malaria parasites. In order to interrupt the transmission cycle ideal antimalarials with gametocytocidal action are needed. The present study was conducted to standardise the methods using a gametocyte productive rodent model. *P. berghei* (NK 65) is similar to human malaria parasites in many ways and results obtained in this host-parasite model can be compared to those of human parasites (Carter and Diggs, 1977). Hence three standard antimalarials – pyrimethamine, primaquine and quinine were tested against *P. berghei* (NK 65) in *M. natalensis* to assess their gametocytocidal and sporontocidal activity. It is evident that 2.5 mg/kg of pyrimethamine is not able to eliminate gametocytes completely from the blood and gametocytaemia was significantly higher ($P < 0.05$). This suggests that the drug was not able to kill all the gametocytes. However, it deteriorated their infectivity in such a way that though oocysts were developed, sporozoites could not retain their infectivity. A lower dose of pyrimethamine i.e., 1.25 mg/kg could reduce the number of oocysts significantly ($P < 0.01$) in mosquitoes fed on treated animals but it did not have any deleterious effect on the infectivity of sporozoites. Omer *et al.* (1974) reported that a single dose of pyrimethamine (3 mg base/day) had marked sporontocidal action as early as 15 mins after drug administration in *P. cynomolgi* infection.

Primaquine at 10 mg/kg had gametocytocidal/ sporontocidal action. These oocysts were degenerated and no sporozoites were developed in them. In case of human malaria parasites, *P. vivax* and *P. falciparum* (Young, 1959) reported that 30 mg of primaquine weekly either in a single dose or in 2 doses and 5 or 3 mg/day doses usually exerted sporontocidal action and prevented transmission of malaria. Rieckmann *et al.* (1968) also reported that in case of chloroquine resistant *P. falciparum*, primaquine exerted a marked sporontocidal and gametocytocidal action at a single dose of 45 mg of primaquine base. In the present study quinine had complete sporontocidal action at 140.62 mg/kg.

It may thus be inferred from the results obtained in the present study that standard antimalarials – primaquine, quinine and pyrimethamine can kill gametocytes upto a certain dose level, beyond which they are unable to exert any direct gametocytocidal action. But even at lower doses they can damage the gametocytes in such a way that their proper development is hampered, making the mosquitoes non-inefective.

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Ultra Low Volume (ULV) Malathion Application as a Supplementary Malaria Control Measure in Two Villages of South Arcot District, Tamil Nadu

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Ultra low volume (ULV) ground application of technical malathion as a supplementary malaria control measure was carried out in two riverine villages of South Arcot district, Tamil Nadu which had high persistent transmission since 1975. Malaria incidence was reduced to one fifth in villages under ULV malathion as against a 50% drop in the control village. The cost of ULV spray alone came to Rs. 1.07 per capita per year. ULV can be an effective supplementary measure in rural areas with specific problems.

INTRODUCTION

Ultra low volume (ULV) ground application of technical grade malathion or fenitrothion has been used successfully for the control of vectors of Dengue Haemorrhagic fever in small suburbs of Bangkok, Thailand (Pant *et al.*, 1971; 1973; Pant, 1974) and for Japanese Encephalitis in northern Thailand (Pant and Jatanasen, 1971). This technology has also been used for the control of malaria vectors (Hobbs, 1976) in central Java, Indonesia (Pradhan *et al.*, 1979) and in Malaysia, (Hii, 1980). In recent years, ULV application of malathion has been practised in some urban areas of India (Singh *et al.*, 1982; Biswas *et al.*, 1982).

Thenpennai riverine tract in the North and South Arcot districts of Tamil Nadu has been known for persistent malaria transmission since 1972 (Tewari *et al.*, 1984). As the vector *A. culicifacies* became resistant to DDT/HCH, malathion is used for spray in this area since 1977. Though the vector is still susceptible to malathion desired results were not achieved due to poor spray coverage and a certain amount of exophily of the vector *An. culicifacies* (Mani *et al.*, 1984). The anti-larval operations in the shallow riverbed and chemotherapy also did not yield successful results. The present study was taken up to find out whether the ULV cold aerosol spray as supplementary measure could bring about some reduction in the incidence of malaria cases. The

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Table 1. Tests of susceptibility of malathion carried out with *A. culicifacies*

Month & Year	Insecticide and concentration	Period of exposure	No. of replicates	No. of females exposed	% Mortality (corrected using Abbott's formula)
June 1982	Malathion 5%	60 Mins	5	96	100
	Control	60 Mins	1	20	10
	Malathion 5%	30 Mins	10	200	100
	Control	30 Mins	2	40	0

Table 2. Bioassay results with adult *An. culicifacies*

Distance	Machine used	No. of *An. culicifacies* dead after 24 hrs exposure	No. exposed	% mortality (corrected by Abbott's formula)
Indoor				
1.5m	Fontan	245	253	96.2
1.5-3.0m	–	206	255	91.0
Control	–	17	98	17.4

Table 3. Malaria positive cases in experimental and control villages

Name of village	Year	Population	ABER	API	SPR	No. of cases			Total
						Pv	Pf	Mixed	
Moongilthuraipattu	1979 & 80	4658	26.9	41.75	6.09	368	13	2	383
	1981 & 82	5215	28.9	19.42	2.60	176	8	3	187
P. Pudur	1979 & 80	1686	51.75	59.8	5.46	135	77	3	215
	1981 & 82	2145	27.0	11.1	1.17	36	2	1	39

results of the pilot study are presented in this paper.

MATERIAL AND METHODS

Two villages, Porasappattu and Pudur, separated by a few hundred meters only were taken up for supplementary ULV spray. Moongilthuraipattu, another riverine village two kms upstream was kept as control. But for ULV spray, other control operations were similar in these two areas.

ULV operations were carried out from Februrary 1981 to the end of 1982 in the study areas after which it was not considered necessary to continue ULV spray since the incidence of malaria cases dropped down to a low level. First six rounds were given at weekly intervals. Subsequently, ULV was applied when the vector density was two or more per man hour or when any indigenous malaria case was reported. A total of 17 and 13 cycles of ULV were given in 1981 and 1982 respectively.

Two portable backpack Fontan® machines were used in the operations with a third as standby. Technical grade malathion was used and the discharge rate, using nozzle size 0.5, was 25 ml/min. The spraymen walked around each house directing the nozzle of the machine at an angle of 45^{o} towards the eaves, finally standing in the open doorway for about 20 secs. The entire operation took about 2–3 mins. Treatment was carried out in the early mornings from 0600–0700 hrs.

Entomological study consisted of hand catch (aspiration) of mosquitoes for 15 mins per house from 0800–1000 hrs at fortnightly intervals. The density was worked out as the average number per man hour. During 1981, mosquito collection was carried out in both the ULV as well as the control village, but in 1982 due to administrative difficulties, it was confined to the ULV village only. Susceptibility to malathion was tested by standard WHO technique by Regional Entomological Team, Cuddalore.

RESULTS

Entomological evaluation

An. culicifacies, the known vector of the area was found to be susceptible to Malathion as per WHO technique (Report on Thiruvannamalai project, Unpublished data). Results are given in Table 1. Bioassays of caged laboratory reared mosquitoes placed in the houses showed high mortality after treatment of houses with ULV malathion (Mani *et al.*, 1987). The results of bioassays are given in Table 2.

Epidemiological evaluation

The population, ABER, API, SPR and plasmodial species from 1979–82, for both the ULV and the control villages are presented in Table 3 and Figs. 1–2. In the study village malaria cases came down to 39 in 1980 as against 215 in 1979. In the control village, for the same block of two years, it was 383 and 187.

Mass blood survey was conducted during 1982. There was only one case out of 1139 blood smears collected in the ULV sprayed as against 26 cases out of 2931 blood smears in the control villages.

DISCUSSION

It must be admitted that the variety of control measures applied simultaneously (e.g., indoor residual spray, antilarval measures, chemotherapy and ULV) in an integrated approach makes the interpretation of data somewhat difficult. However, the impact is so impressive in the ULV village that it can be attributed to ULV application, since all the other control measures adopted were the same. In the study area malaria incidence was reduced to one fifth as against a 50% reduction in the control village (Table 3). It is worth mentioning that active case detection was carried out every week with a view to maintain a strict watch for new cases.

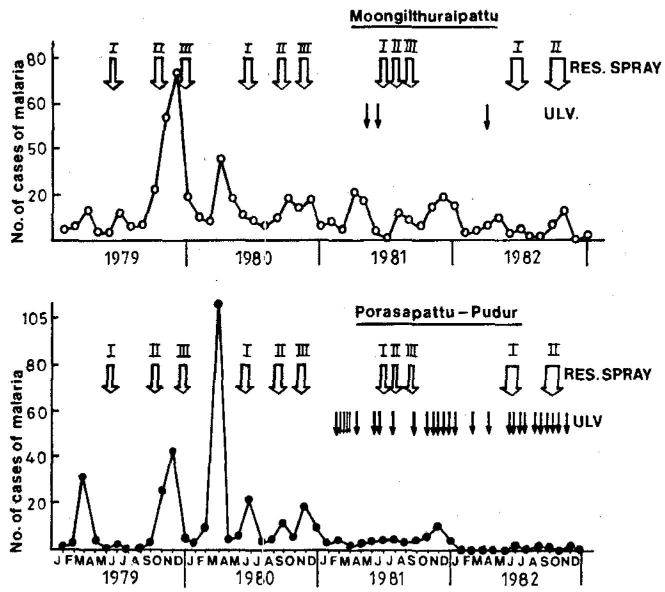

Fig.1: **Malaria incidence and control measures.**

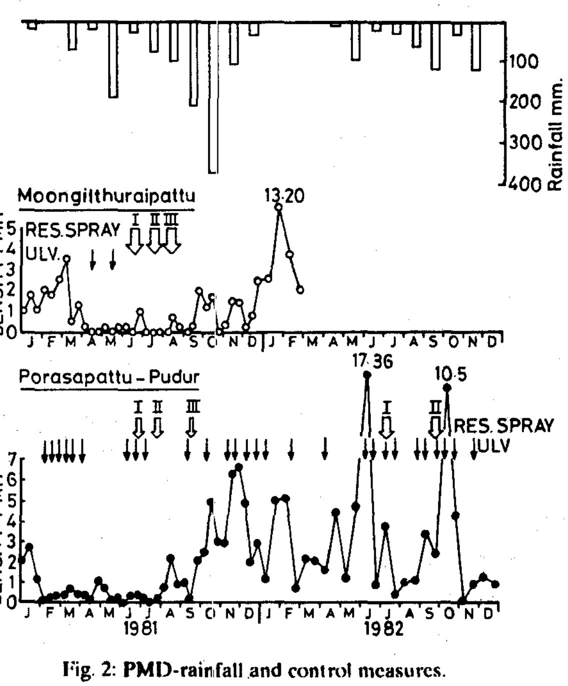

Fig. 2: **PMD-rainfall and control measures.**

Data also showed that the reduction of vector density was consistent when ULV was applied at weekly intervals. The method of monitoring the vector density at fortnightly intervals is not appropriate, since ULV has no residual life. Further ULV application would have killed the adult vectors present at the time of application only, leaving chances for emergence of an adequate population to maintain adult density which may help to sustain transmission.

Antilarval measures were continued without much result in the riverbed pools. Anti-adult spray with malathion would have controlled transmission because of vector susceptibility to this insecticide but coverage was poor due to indifferent public participation. ULV on the other hand was much more acceptable to the public and the coverage was 100%. Here it scores over the other traditionally used control measures.

Cost analysis showed that a total of Rs. 5481.00 was incurred for 17 and 13 cycles of ULV in 1981 and 1982. This includes the cost of insecticide, and petrol for running the ULV machine but excludes transportation charges and labour charges for 357 houses scattered over seven hectares, housing 2145 people. The per capita cost came to Rs. 2.13 for 2 years or Rs. 1.07 per year on an average. Under Indian conditions, the cost is on the higher side for this supplementary operation. Bruce-Chwatt (1985) has also brought out certain drawbacks of ULV (i) lack of uniformity of dosage of the insecticide and of coverage, (ii) high initial and recurring cost, (iii) shortage of trained staff for maintenance and repair, and (iv) contamination of non-target areas.

It is concluded that ULV appears to be a good supplementary measure for this hard core area of persistent transmission which has defied all other strategies in the past.

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Evaluation of *Bacillus sphaericus* to Control Breeding of Malaria Vectors

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Bacillus sphaericus formulations (Solvay liquid 2362 and Abbott granules 2297) were tested in the laboratory and field for the control of mosquito breeding. Results of laboratory evaluation revealed that both formulations had good larvicidal activity against *Culex quinquefasciatus*, @ 0.04 ml and 1 gm/sq m dosages respectively. Higher dosages @ 5 ml liquid or 5 gm/sq m granules were required to control *A. stephensi* and *A. subpictus*. *A. culicifacies* required still higher dosages of Solvay (25 ml/sq m) to achieve same levels of control whereas Abbott granules were not effective even at 20 gm/sq m.

INTRODUCTION

The problems presented and created by the spraying of insecticides in malaria control has made it imperative to develop alternate methods of vector control. Biocides offer many advantages over chemicals i.e., high specificity, biodegradability, safety to environment, low cost and in some cases recycling properties. These characteristics make the biocide an important and useful tool in integrated vector control programmes. Of the many biological control agents, *Bacillus sphaericus* was evaluated in the laboratory and field for its value in the control of *inter alia Anopheles culicifacies*, the principal vector of rural malaria in the country. Results of various studies on the application of *B. sphaericus* have so far revealed that the formulations were highly effective against *Culex* mosquitoes and not so much against the anophelines (Mulligan *et al.*, 1978; Singer, 1980; Davidson *et al.*, 1981; Mittal *et al.*, 1985; Gardner *et al.*, 1986). Studies were, therefore, taken up on potential *B. sphaericus* formulations for their efficacy and persistence in the laboratory and field against *A. culicifacies*, *A. stephensi* and *Culex quinquefasciatus*. Results of this study are reported in this paper.

MATERIAL AND METHODS

The formulations of *Bacillus sphaericus* viz., Solvay liquid and Abbott granules (ABG 6185) were obtained through the courtesy of WHO for this study. Laboratory evaluation of these formulations was carried out against colonized larvae of *Anopheles culicifacies*, *Anopheles stephensi*, *Anopheles subpictus* and *Culex quinquefasciatus*. The mosquito larvae were reared in the laboratory as per procedures described by Ansari *et al.* (1977; 1978). Tests were conducted in enamel trays (15 x 20 cms) containing 500 ml tap water and 100 mg larval food. Twenty five III instar larvae of a par-

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Table 1. Laboratory evaluation of *Bacillus sphaericus* 2362 (Solvay formulation)

Concentration (ml/sq m)	Corrected % mortality					
	A. culicifacies		*A. stephensi*		*Cx. quinquefasciatus*	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
25.0	92	100	82	100	100	-
5.0	16	62	70	100	100	100
1.0	00	10	46	74	100	100
0.2	00	00	68	86	100	100
0.04	-	-	-	-	100	100
0.008	-	-	-	-	85.3	87.6

Note: Experiments were conducted in enamel trays (15x20 cms) containing 500 ml water.

Table 2. Laboratory evaluation of *Bacillus sphaericus* Abbott granule formulation (ABG-6185)

Concentration (gm/sq m)	Corrected % mortality							
	A. culicifacies		*A. stephensi*		*A. subpictus*		*Cx. quinquefasciatus*	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
20	29.3	48.4	-	-	-	-	-	-
10	13.3	28.8	-	-	-	-	-	-
5	4.0	13.0	97.3	100	73.3	91	100	-
1	2.6	8.0	62.6	90.6	53.3	80	100	-
0.2	-	-	-	-	-	-	92	97.3

Note : Tests were conducted in enamel trays (15x20 cms) containing 500 ml water.

ticular species were introduced in each tray and biocide applied on the water surface. The Solvay liquid concentrate was tested by adding one ml of the serially diluted formulation into each tray, while Abbott granules were weighed and applied directly in enamel trays. The experiments were repeated three times with a concurrent control. Larval mortality was recorded at 24 and 48 hrs post exposure period. Corrected mortality was determined according to Abbott's formula which is as follows:

$$\text{Corrected \% mortality} = \frac{\text{\% test mortality} - \text{\% control mortality}}{100 - \text{\% control mortality}} \times 100$$

A survey was carried out to map suitable breeding sites of *Culex* and *Anopheles* species in and around Delhi. Rain water pools were the seasonal breeding sites while unused wells and drains were permanent breeding places. The emulsified formulation was diluted in water and sprayed with the help of stirrup pumps and granules were broadcast manually. Treatment was made at pre-calculated dose rates of the biocide and applied on water surface on an area basis. Dipper was used to estimate immature densities and per cent reduction in III and IV instar was calculated using the formula described by Mulla (1971).

$$R\% = 100 - \frac{C1}{T1} \times \frac{T2}{C2} \times 100$$

where

C_1 = Number of larvae in control pre-treatment
C_2 = Number of larvae in control post-treatment
T_1 = Number of larvae in treated pre-treatment
T_2 = Number of larvae in treated post-treatment

Field trials were carried out for 2 years in 1985 and 1986 during the post-monsoon period from September to November.

RESULTS AND DISCUSSION

Laboratory evaluation

Results of laboratory tests are given in Tables 1 and 2. Both Solvay liquid and Abbott granule formulations of *Bacillus sphaericus* produced high mortality in *Culex quinquefasciatus*. Application of Solvay formulation in enamel trays @ 0.04 ml/sq m has produced 100% mortality of *Culex quinquefasciatus* III instar larvae. Similar mortality in *A. culicifacies* and *A. stephensi* was obtained @ 25 ml and 5 ml/sq m dosages in 48 hrs post-treatment (Table 1). Similarly Abbott granules produced 100% mortality in *C. quinquefasciatus* @ 1 gm/sq m whereas 90.6, 80 and 8.0% mortality was produced in *A. stephensi, A. subpictus* and *A. culicifacies* in 48 hrs respectively (Table 2). Results indicated that both Solvay and Abbott granule formulations had variable activity against different species of mosquitoes and that in comparison to Abbott granules, Solvay formulation produced marginally higher mortality in *Culex* and *Anopheles* larvae.

Field evaluation

Field trials were carried out in pools, wells and drains to determine optimum dosage which could provide long-term control of *Culex* and *Anopheles* spp. Results of this study are given in Tables 3–5.

Pools

Results given in Table 3 show that Solvay liquid and Abbott granules produced >90% control of *Culex quinquefasciatus* immatures for 3–4 weeks even at a dose as low as 2.5 ml and 5 gm/sq m. However, the activity was evident for 1–2 weeks against anopheline immatures with similar dosage of Solvay and Abbott granule formulations in pools dominated by *A. subpictus* and *A. stephensi*. In pools where *A. culicifacies* was predominant, 80% control was observed @ 20 ml/sq m for only one week. This confirms the laboratory observations that *A. culicifacies* is refractory in comparison to *C. quinquefasciatus, A. subpictus* and *A. stephensi*.

Wells

Unused wells supporting *Culex* breeding were used for the field trials. The surface area of wells ranged from 1.4 to 4.8 sq m and the depth was 1.5 to 3 m. Results revealed that treatment of wells @ 20 ml and 5 gm/sq m with Solvay and Abbott granule formulations respectively produced drastic reduction in larval density (Table 4). The larvicidal activity of both Solvay and Abbott compounds persisted for at least 9 weeks.

Drains

The surface area of drains used for the control of *Culex* breeding was 67.5 and 167.1 sq m. A dosage of 20 ml/sq m Solvay formulation provided over 95% control of *Culex* larvae for more than six weeks (Table 5), but was effective only for one week in the control of *Anopheles* breeding. Abbott granule formulation was not tested against *Anopheles* in drains because of the non-availability of biocide.

B. sphaericus is a complex of aerobic spore forming bacterial strains found throughout the world in soil and aquatic environments. Of the 186 strains isolated from mosquitoes and black flies, only 45 strains can be considered true pathogens for mosquito larvae. Earlier studies were focussed on strain 1321 (SS II–1) isolated in India, but recently strains 1593, 2362 and 2297 were found more toxic and, therefore, research emphasis has favoured the formulation of these strains (de-Barjac *et al*., 1985). During the present studies, two formulations viz., one from Abbott laboratories (Abbott granules ABG 6185) and Solvay & Co. (Solvay liquid) were used in laboratory and field in the control of mosquito breeding. Results of laboratory evaluation revealed that Solvay and Abbott formulations

Table 3: Evaluation of *Bacillus sphaericus* against larvae of *Anopheles* and *Culex* spp. in pools under natural conditions

Dosage sq. m	Average larval density per ten dips; III and IV instar (% reduction after treatment)													
	Culex spp.							*Anopheles* spp.						
	No. of replicates	Pretreatment count	48 hrs	72 hrs	1 wk	2 wk	3 wk	No. of replicates	Pretreatment count	48 hrs	72 hrs	1 wk	2 wk	3 wk
							Solvay							
2.5ml	2	43.1	30 (57.91)	21.0 (74.9)	7.2 (92.4)	5.6 (91.4)	3.6 (93.4)	2	4.6	0.1 (97.8)	0 (100)	0.2 (96.3)	1.4 (42.5)	1.4 (33.4)
5ml	2	39.5	10.8 (83.46)	4.5 (94.15)	1.7 (98.03)	3.4 (94.21)	0.8 (98.4)	2	3.4	0 (100)	0 (100)	0 (100)	2.25 (0)	-
10ml	1	172	1.2 (99.3)	0.4 (99.9)	0.4 (99.9)	1.8 (99.5)	0 (100)	3	5.25	0 (100)	0 (100)	0 (100)	0.2 (92.8)	7.8 (0)
20ml	3	50.7	0.26 (99.7)	0.13 (99.96)	0.06 99.96	0 (100)	0 (100)	4	14.35	0.15 (99)	0 (100)	0.2 (98.8)	0 (100)	5.5 (19.4)
20ml*			-	-	-	-	-	2	6.3	1.75 (73.5)	2.25 (73.5)	1.12 (83.5)	10.35 (1.9)	-
						Abbott granules								
5gm	6	40.06	0.65 (98.65)		2.3 (95.8)	0.05 (99.9)	1.8 (94.2)	6	55.04	0.1 (99.8)		1.26 (99.18)	1.75 (95.5)	7.2 (72.2)
10gm	3	4.86	0 (100)		0 (100)	0 (100)	dried	7	24.62	0.21 (99.11)		1.2 (98.2)	0.66 (96.2)	dried -

Figures in parentheses indicate % reduction based on untreatred control.
* *Anopheles* population consisted mainly of *A. culicifacies*.

Average surface area of the pools
(8)# Solvay formulation -*Culex* spp. = 27.2 sq m (4.6-107)
(13)# Solvay formualtion-*Anopheles* spp. = 19.12 sq m (1.1-107)
(9)# Abbott granules -*Culex* spp. = 46.06 sq m (9-100)
(13)# Abbott granules -*Anopheles* spp. = 36.27 sq m (9-100).

[illegible]

Dosage/ sq m	No. of repli-cates	Pretreat-ment counts	Average larval density per five dips; III & IV instar (% reduction after treatment)									
			72 hrs	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	7 wk	8 wk	9 wk
Solvay												
10ml	3	62.8	0.4 (99.2)	0.3 (99.67)	0 (100)	0.1 (99.26)	0 (100)	0.3 (95.5)	1.2 (83.8)	0.3 (93.9)	1.6 (59.9)	1.3 (96.9)
20ml	3	274.5	1.9 (99.1)	1.0 (98.3)	0.1 (99.6)	0.5 (99.1)	1.2 (97.4)	3.7 (87.5)	1.33 (93.05)	1.5 (93.05)	3.73 (97.83)	8.3 (95.7)
Abbott granule												
5gm	1	34.6	0 (100)	0 (100)	0 (100)	0 (100)	0.4 (98.52)	2.6 (88.53)	5.4 (76.4)	29.0 (30.8)	46.0 (53.8)	75.0 (52.5)
10gm	2	156.5	0 (100)	0.1 (99.5)	0.1 (99.9)	0 (100)	0.7 (99.4)	3.1 (96.97)	3.9 (96.23)	51.1 (73.05)	35.4 (92.1)	90.0 (87.41)

Figures in parentheses indicate % reduction based on untreated control.
Average surface area 2.5 to 4.6 sq m (9 wells).

Table 5 : Evaluation of *Bacillus sphaericus* to control *Anopheles* and *Culex* breeding in drains

Dosage/ sq m	No. of repli-cates	Average larval density per dip; III & IV instar (% reduction after treatment)												
		Culex spp.									*Anopheles* spp.			
		Pretreat-ment counts	72 hrs	1wk	2wk	3wk	4wk	5wk	6wk	7wk	Pretreat-ment count	72 hrs	1wk	2wk
Solvay														
5ml	1	19.5	8.5 (77.4)	2.9 (45.5)	6.3 (73.4)	15.1 (42.3)	6.3 (78.3)	11.8 (59.1)	8.3 (80.7)	13.8 (64.85)	3.2	0.2 (90.05)	0 (100)	3.7 (0)
10ml	2	13.7	0.7 (99.4)	0.2 (99.1)	1.4 (92.5)	0.1 (99.5)	3.4 (83.3)	6.4 (68.4)	12.2 (59.7)	39.3 (0)	2.4	0 (100)	0 (100)	1.4 (6.6)
20ml	3	42.1	0.5 (99.4)	0.3 (96.6)	1.6 (72.2)	1.4 (97.3)	1.8 (97.1)	2.8 (95.5)	2.0 (97.8)	5.9 (93)	9.4	0 (100)	0 (100)	2.5 (57.4)
Abbott granule														
5gm	1	42.6	2.2 (94.3)	2.1 (96.2)	21.0 (52.03)	9.0 (82.6)	17.0 (73.8)	34.0 (23.6)	53.0 (0)	46.0 (0)	-	-	-*	
10gm	1	433.5	5.0 (98.7)	3.2 (99.4)	23.0 (94.8)	5.0 (99.04)	3.1 (99.5)	3.7 (99.1)	21.6 (94.2)	46.2 (81.6)	-	-	-	

**Anopheles* spp. was not encountered.
Figures in parentheses indicate % reduction based on untreated control.
Average surface area of the drains used for solvay formulations was 167.1 sq m (6 drains) and for Abbott formulation was 67.5 sq m (2 drains).

were more effective in the control of *Culex* as compared to *Anopheles* spp. Similar results were obtained in field trials in pools, wells and drains. The present laboratory and field observations on the susceptibility of isolates 2362 and 2297 against *Culex* and *Anopheles* larvae are comparable with those of Mulla (1986) and Majori *et al.* (1987), who reported that *Culex quinquefasciatus* was more susceptible than *A. albimanus, A. quadrimaculatus* and *A. gambiae* to primary powder of *Bacillus sphaericus* 2362 and 2297 isolates.

Persistence of biocide formulation was directly proportional to the dosage used and results were consistent in successive weeks at higher concentrations. The efficacy of both formulations was more or less the same in clear and polluted water. In fact longest activity (9 weeks) was observed in unused wells which were highly polluted. This may be due to proliferation of bacterium from floating dead larvae on water surface or retention of particles (spores) for prolonged time (Lacey, 1985). It was further observed that larvicidal activity persisted in clear water for a prolonged time as compared to a habitat infested with vegetation. This is also in conformity with Goldberg *et al.* (1977) who reported negative effect of natural aquatic microflora on larvicidal activity of *B. sphaericus*. The study showed that the two *Bacillus sphaericus* formulations were promising biocides at this stage of development. These formulations can be used successfully in bioenvironmental control to eliminate mosquito breeding of *Culex* spp. and *A. subpictus* for several weeks. It may be pointed out that the Centre has successfully demonstrated malaria control by using non-insecticidal methods in Gujarat and the strategy is being tested in 10 different ecological sites (Sharma, 1986). Any formulation which shows broad spectrum activity against the commonly encountered mosquitoes like *Culex* and *Anopheles* spp. with recycling properties can become a potential tool in the bioenvironmental control strategy.

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Chloroquine Sensitivity of *P. falciparum* in Koraput district, Orissa

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Malaria is a persistent problem in the tribal dominated Koraput district of Orissa state. *Plasmodium falciparum* is the predominant parasite species accounting for about 90% of the infections in the locality. Chloroquine sensitivity of *P. falciparum* was carried out in 4 Primary Health Centres (PHCs) and Muran project area in the district. A total of 139 cases were subjected to *in vivo* extended test, of which resistance was detected in 11 at RI and in 2 at RII level. One case out of 16 subjected to standard 7 day *in vivo* test showed resistance at RII level. Micro *in vitro* test was done for 17 cases of which 8 were found to be resistant. Majority of cases of resistance were from Malkangiri PHC and Muran project areas. The implications of the findings are discussed.

INTRODUCTION

Koraput district of Orissa state (17°, 50'N and 20°, 3'N latitude and 81°, 27'E and 84°, 10'E longitude) with a predominant tribal population, has been endemic for malaria since many decades (Perry, 1914; Senior White, 1938). During the period 1981-1986, annual parasite incidence (API) ranged between 14.8 to 27.9 and over 85% of the cases were due to *P.falciparum* infection (NMEP, unpublished data). A total of 80 deaths due to malaria was also recorded during this six year period by the NMEP. Detailed scientific studies have not been carried out on the epidemiology of persistent malaria in the district, since the launching of the National Eradication Programme in 1958. The Vector Control Research Centre (VCRC) has opened a branch in the district since 1986 for in-depth studies on all aspects of malaria and to develop appropriate control strategy. Chloroquine resistant strains of *P. falciparum* have been detected in many parts of northeastern India including all the 13 districts of Orissa (Guha *et al.*, 1979b; Pattanayak *et al.*, 1979; WHO, 1982a; WHS, 1987). The majority of *P. falciparum* cases in Orissa state are from Koraput district and there is only one report of RI resistance by *in vivo* test from the district (WHS, 1987). The present study reports the chloroquine sensitivity status of *P. falciparum* in the district carried out both by *in vivo* and micro *in vitro* techniques.

MATERIAL AND METHODS

Study area

All the 42 Primary Health Centres (PHCs) in Koraput district are malarious and API in 1986

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Table 1. Malaria situation in study PHCs of Koraput

Year		Primary Health Centres			
		Lamptaput	Mathili	Borigumma	Malkangiri
1984	API	36.92	11.39	45.07	20.90
	Pf%	92.21	91.17	95.8	94.83
1985	API	31.52	10.51	21.53	28.79
	Pf%	86.88	95.22	94.04	95.09
1986	API	31.40	12.65	15.18	86.59
	Pf%	86.30	89.90	92.00	99.70

ranged between 3.6 to 86.6 (NMEP unpublished data). The study area included four PHCs and an irrigation project site at Muran. The malaria situation in the PHC areas for 3 years (1984–1986) is shown in Table 1. The irrigation project site of Muran was chosen because a mass blood survey by VCRC in 1986, showed that 45% (375/833) labourers were positive for malaria parasite and *P. falciparum* accounted for 94.7% of these infections (VCRC, Annual Report 1987–88).

Preliminary study

Chloroquine is available to the *P. falciparum* cases in the community in three different dosage schedules as described below.

1. A dosage of 600 mg chloroquine base is given as presumptive treatment at the time of blood smear collection. The positive cases were given an additional 600 mg chloroquine with 45 mg primaquine (adult cases) for radical cure.

2. In inaccessible areas, a single dose of 600 mg base of chloroquine and 45 mg of primaquine is being given presumptively at the time of blood smear collection.

3. Some cases of schedule 1 receive only 600 mg chloroquine base given presumptively, as the radical treatment is not administered due to logistic problems (non-availability of drug/patient/staff etc.).

A total of 70, 43 and 35 *P. falciparum* patients were treated with dosage schedules 1, 2 and 3 respectively. They were followed for persistence of parasitaemia on the 7th day of their receiving the drug/drugs (for cases in schedule 1, 7th day was calculated from the administration of second dose of 600 mg chloroquine and 45 mg primaquine).

In vivo study

In vivo extended test was done in all the 4 PHC areas mentioned in Table 1. *In vivo* standard test was carried out in Muran project area. The case selection and test were carried out as per WHO procedure (WHO, 1973). In the extended test 149 persons were included, of whom 139 (93.3%) were followed up upto 28 days. In the standard test, 16 out of 20 persons included were followed upto 7 days.

In vitro study

Blood samples collected from 17 *P. falciparum* cases (11 from Borigumma PHC area and 6 from Muran project area), were subjected to micro *in vitro* chloroquine sensitivity test by using WHO kit following standard procedure (WHO, 1982b). The chloroquine sensitivity plates, charged with the sample were incubated at 37° to 38° C for 24 hrs.

The blood smears collected for the preliminary *in vivo* and screening for *in vitro* test were stained with Giemsa stain (3% Giemsa, in buffered distilled water, pH 7.2 for 50 mins). Both thick and thin smears were examined and parasites were counted against 8,000 WBC. The post culture *in vitro* test slides were stained with Romonowsky I and II supplied with the kit.

The standard Dill and Glazko technique was employed to test the presence of chloroquine in urine before and after chemotherapy in all patients as per test requirements (Lelijveld and Kortmann, 1970).

RESULTS

The preliminary study showed the presence of asexual parasites in 18.6% (13/70), 9.3% (4/43), 34.3% (12/35) cases using dosage schedules 1, 2 and 3 respectively on the 7th day following drug/drugs administration.

The results of *in vivo* study are presented in Table 2. Of the 139 cases followed upto 28 days, 11 showed resistance at RI/S level and 2 cases at RII level. Apart from these another case of RII was detected but followup was discontinued from 14th day as the patient became seriously ill, necessitating treatment with alternative drugs. Cases resistant to chloroquine were detected in all PHC areas except Borigumma. One case of RII was detected by the standard *in vivo* test at Muran project area. Of the 17 blood samples subjected to micro *in vitro* test, 8 showed growth of schizonts at above 5.7 pico mol chloroquine concentration indicating resistance (Table 3). Six of the resistant cases were from Muran project area and 2 from Borigumma PHC area.

All the resistant cases detected by both *in vivo* and *in vitro* tests, were treated with 1000 mg sulphamethopyrazine and 50 mg pyrimethamine (Metakelfin, Walter Bushnell).

DISCUSSION

The preliminary studies showed that chloroquine failed to clear parasitaemia in 9.3% to 34.3% of cases using different drug schedules indicating possibility of prevalence of drug resistant strains in the locality. Previous study by Guha *et al.* (1979a) in Koraput district had shown that the dosage of 600 mg of chloroquine failed to clear *P. falciparum* in 3.8% of cases. The present study (1987'88) showed the persistence of parasitaemia in 34.3% of cases with the same dosage (schedule 3 of present study). This indicates the reduced efficacy of chloroquine in the same dosage schedule over the years.

The chloroquine sensitivity studies by *in vivo* and *in vitro* methods showed that 9.4% of cases in the former and 47% in the latter are resistant to the drug. Epidemiological investigation revealed that all the resistant cases were indigenous. Majority of cases were from Malkangiri and Muran areas. In Borigumma PHC area, though no resistant cases could be detected by the *in vivo* method, two cases showed resistance by *in vitro* technique. Available reports indicate that there is no definite correlation between the sensitivity status of *P. falciparum* as measured by *in vivo* and *in vitro* methods (Schapira *et al.*, 1988).

The resistance of *P. falciparum* to chloroquine in the locality could be due to introduction of a resistant strain from outside or due to natural selection. There is a large refugee settlement (over 5,00,000 population) in Malkangiri area and these people who are originally from East Bengal of undivided India (present Bangladesh), make frequent visits to eastern parts of the country (Bengal, Assam etc.), where resistant strains are known to exist (Sharma, 1984). In Muran project area there is a large congregation of local labourers and migrant labourers come from other states like Bihar, Kerala, Tamil Nadu, Andhra Pradesh, Maha-

Table 2. Results of *in vivo* chloroquine sensitivity test in Koraput district

S. No.	Area	Period of test	D0	D1	D2	D3	D4	D5	D6	D7	D14	D21	D28	Remarks
A. *Extended* test (28 days)														
1.	Bori-gumma	July 1986	33/33	25/33	9/33	0/33	0/33	0/33	0/33	0/33	0/33	0/33	0/33	S=33
2.	Lamta-put	Sep & Oct 1986	38/38	21/38	7/37	3/36	3/35	1/35	0/35	0/35	5/35	4/35	4/35	S=29 RI/S=6
3.	Malkan-giri	Oct & Nov 1986	38/38	30/38	20/38	12/38	8/38	6/37	3/36	3/37	4/36	4/34	5/34	S=28 RI/S=4 RII=2*
4.	Mathili	March & April 1987	40/40	27/40	12/38	4/37	0/37	0/37	0/37	0/37	0/37	0/37	1/37	S=36 RI/S=1
	Total	Jul 1986 to April 1987	149/149	103/149	48/165	19/144	11/143	7/142	3/141	3/142	9/141	8/139	10/139	S=126 RI/S=11 RII=2
B. *Standard test (7 days)*														
1.	Muran project area	June 1987	20/20	17/20	9/19	3/16	1/16	1/16	1/16	1/16	-	-	-	S=15 RII=1
	Grand Total	July 1986 to June 1987	169/169	120/169	57/165	22/160	12/159	8/158	4/157	4/158	9/141	8/139	10/139	S=141 RI=11 RII=3

Numerator-No. of cases positive; Denominator-No. of cases examined.
*Apart from these one case of RII was discontinued from day 14th onwards (See text).

Table 3. Results of *in vitro* test (Period covering Dec. 1987 to Apr. 1988)

S. No.	Case code	Parasite per cu mm blood	Pico mol concentration of chloroquine								Remarks
			A Control	B 1 P. mol.	C 2 P. mol.	D 4 P. mol.	E 5.7 P. mol.	F 8 P. mol.	G 16 P. mol.	H 32 P. mol.	
Borigumma PHC Area											
1	KH-1	4200	196	98.50	14.80	0.00	0.00	0.00	0.00	0.00	S
2	PP-1	13040	197	95.40	56.30	16.80	2.50	1.50	2.00	0.50	R
3	4KP	6080	30	93.30	–	10.00	10.00	0.00	–	–	R
4	12 F/KP	19800	189	96.80	94.70	0.00	0.00	0.00	0.00	0.00	S
5	1 F/C	8800	175	90.30	8.00	0.00	0.00	0.00	0.00	0.00	S
6	2 F/C	16360	187	94.70	85.60	2.70	0.00	0.00	0.00	0.00	S
7	6/AM	1840	167	93.40	79.00	11.90	0.00	0.00	0.00	0.00	S
8	65 F/R	30320	111	95.40	45.00	0.00	0.00	0.00	0.00	0.00	S
9	72 F/R	840	57	87.70	26.30	0.00	0.00	0.00	0.00	0.00	S
10	73 F/R	1000	98	87.70	40.80	0.00	0.00	0.00	0.00	0.00	S
11	68 F/R	1160	87	96.50	90.80	5.70	0.00	0.00	0.00	0.00	S
Muran project area											
1	6	1440	64	112.00	95.00	106.00	73.40	87.50	0.04	0.00	R
2	20	2520	82	51.20	59.30	59.30	41.40	34.10	4.87	–	R
3	11	20720	189	98.40	96.20	96.20	93.60	91.50	67.70	1.05	R
4	9	36120	145	–	91.70	91.70	96.50	91.00	72.40	71.00	R
5	5	36240	64	81.20	70.30	70.30	65.60	64.00	4.70	0.00	R
6	27	15680	25	60.00	76.00	48.00	20.00	16.00	8.00	0.00	R

rashtra and Madhya Pradesh (VCRC, Annual Report, 1987-88). The fact that a majority of the resistant cases were from these two areas indicate probable introduction of resistant *P. falciparum* strain from outside. However, the possibility of establishment of the resistant strain due to natural selection cannot be ruled out due to the following reasons. The parasite in these areas has been subjected to chloroquine pressure for a long time since the drug is easily available to people due to deliberate liberalization for the purpose of control. The administration of drug in inadequate dosage schedules as recorded in the present study, could also have enhanced the process of natural selection of resistance in the locality.

The spread of this resistant strain to other receptive areas within the district and outside is of great concern considering the fact that there is substantial degree of human movement for developmental purposes. Role of human movement in the spread and persistence of malaria cannot be over emphasized (Prothero, 1984; Rajagopalan *et al.*, 1986).

As majority of *P. falciparum* cases were found to be sensitive by *in vivo* method, an immediate change to an alternate drug is not warranted, but chloroquine should be administered in the dosage recommended for radical cure (1500 mg base for adults).

Metakelfin (sulfalene and pyrimethamine) has been recommended as the alternate drug in treating resistance cases in this area (according to PHC Medical Officers), which was employed in present study also. However, this drug must be employed judiciously as resistance to it has already been detected in India (Choudhury *et al.*, 1987). In the present circumstances quinine could also be an alternative drug.

There is no surveillance in developmental project areas at present under the national programme in Koraput district. Since the migration of labourers from other states to several project areas like Muran is going to continue for developmental reasons, it is essential to establish monitoring centres at focal points to prevent spread of malaria especially drug resistant strains.

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Breeding Habitats of Mosquitoes in Goa

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Studies carried out in Goa from 1986 to 1987 revealed immature stages of 43 species of mosquitoes. Breeding habitats were divided into three categories, viz., (i) Ground water habitats consisting of ground pools and tanks, rocky pools, paddy fields and stream beds, yielding 30 species; (ii) Plant habitats consisting of tree holes and fallen coconut shells and leaf sheaths yielding 20 species; and (iii) Domestic/peridomestic habitats consisting of cement tanks, glass, earthen, metallic containers and tyres yielding 11 species. Five species viz., *Anopheles subpictus, Aedes albopictus, Armigeres subalbatus, Culex quinquefasciatus* and *Cx. pallidothorax* were found in all three types of habitats. *Cx. tritaeniorhynchus* preferred to breed in ground pools and paddy fields but was found in domestic cement tanks and ground tanks in adverse season. Majority of the anophelines and culicines were found in ground water habitats. Five out of 11 species of *Aedes* were found in tree holes. *Aedes aegypti* and *Toxorhynchites splendens* were found only in discarded tyres. Three species of the genus *Uranotaenia*, viz., *U. bicolor, U. stricklandi* and *U. campestris* were recorded from stream beds and plant containers.

INTRODUCTION

Mosquito-borne diseases like malaria and Japanese encephalitis are endemic in the State of Goa (Choudhury *et al.*, 1983; Mohan Rao *et al.*, 1983; Narasimhan and Khamre, 1987). In order to gain knowledge on the mosquito fauna and their ecology, studies were undertaken in the state between 1983 and 1987. As a result of these studies, 89 species of mosquitoes were recorded from Goa and the data on the ecology of adult mosquitoes especially of potential vector species have been reported (Kulkarni *et al.*, 1986; Naik *et al.*, 1988). Since the information on the breeding habitats of mosquitoes in Goa is scanty, studies were conducted on these aspects and results are presented in this paper.

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MATERIAL AND METHODS

Immature stages of mosquitoes were collected during 1986–87 from their natural breeding habitats in several localities of Goa. Four surveys, each of about one month duration were undertaken to cover all the seasons. Eggs were collected from oviposition sites with camel hair brushes, and were transferred into glass jars or test tubes containing water from the same oviposition site; the eggs were reared to adults for species identification. Larvae and pupae were collected by means of a dipper or a ladle. These were transferred to white enamel trays containing water from the same breeding sites for rearing into adults. Larval food consisting of yeast powder mixed with pow-

Table 1. Breeding habitats of mosquitoes in Goa

S. No.	Species	Habitats*										
		Ground water				Plant		Domestic/Peridomestic				
		1	2	3	4	5	6	7	8	9	10	11
1.	*Anopheles (Cellia) culicifacies*	-	-	-	+	-	-	-	-	-	-	-
2.	*An. (Cel.) fluviatilis*	-	-	-	+	-	-	-	-	-	-	-
3.	*An. (Cel.) jamesii*	+	-	+	+	-	-	-	-	-	-	-
4.	*An. (Cel.) dirus*	-	+	-	-	-	-	-	-	-	-	-
5.	*An. (Cel.)subpictus*	+	-	+	+	+	-	+	-	-	-	-
6.	*An. (Cel.) tessellatus*	+	-	+	-	-	-	-	-	-	-	-
7.	*An. (Cel.)vagus*	-	-	+	-	-	-	-	-	-	-	-
8.	*An. (Cel.)varuna*	-	-	-	+	-	-	-	-	-	-	-
9.	*Aedes (Christophersiomyia)annulirostris*	-	-	-	-	+	-	-	-	-	-	-
10.	*Ae. (Diceromyia) reginae*	-	-	-	-	+	-	-	-	-	-	-
11.	*Ae. (Finlaya)auriostriatus* var. *kanaranus*	-	-	-	-	+	+	-	-	-	-	-
12.	*Ae.(Fin.) gubernatoris*	-	-	-	-	+	-	-	-	-	-	-
13.	*Ae.(Fin.)niveus* group	-	-	-	-	+	-	-	-	-	-	-
14.	*Ae.(Fin.)pseudotaeniatus*	-	+	-	-	-	-	-	-	-	+	+
15.	*Ae.(Neomelaniconion)lineatopennis*	+	-	-	-	-	-	-	-	-	-	-
16.	*Ae.(Stegomyia)aegypti*	-	-	-	-	-	-	-	-	-	-	+
17.	*Ae.(Stg.)albopictus*	-	+	-	-	+	+	-	+	+	+	+
18.	*Ae.(Stg.)krombeini*	-	-	-	-	-	+	-	-	-	-	-
19.	*Ae.(Stg.)vittatus*	+	+	-	-	-	-	+	-	-	+	+
20.	*Armigeres (Ar.)subalbatus*	-	+	+	-	-	+	-	-	-	-	+
21.	*Culex(Cux.)bitaeniorhynchus*	-	-	+	+	-	-	-	-	-	-	-
22.	*Cx.(Cux.)fuscocephala*	-	-	-	+	-	-	-	-	-	-	-
23.	*Cx.(Cux.)gelidus*	+	-	-	-	-	-	-	-	-	-	-
24.	*Cx.(Cux.)infula*	-	-	+	+	-	-	-	-	-	-	-
25.	*Cx.(Cux.)pseudovishnui*	+	-	+	+	-	-	-	-	-	-	-
26.	*Cx.(Cux.)quinquefasciatus*	+	+	+	+	-	+	+	+	-	-	+
27.	*Cx.(Cux.)tritaeniorhynchus*	+	-	+	-	-	-	+	-	-	-	-
28.	*Cx.(Cux.)whitmorei*	+	-	+	-	-	-	-	-	-	-	-
29.	*Cx.(Culiciomyia) fragilis*	-	-	-	-	-	+	-	-	-	-	-
30.	*Cx.(Cui.)nigropunctatus*	-	-	-	-	-	+	-	-	-	-	-
31.	*Cx.(Cui.) pallidothorax*	-	+	-	+	-	+	+	-	-	+	+
32.	*Cx.(Eumelanomyia)brevipalpis*	-	-	-	+	-	-	+	-	-	-	-
33.	*Cx.(Eum.)khazani*	-	+	-	-	+	-	-	-	-	-	-
34.	*Cx.(Eum.)malayi*	+	-	-	+	-	-	-	-	-	-	-
35.	*Cx.(Lophoceraomyia)minor*	-	+	-	-	-	+	-	-	-	-	-
36.	*Cx.(Lop.)minutissimus*	-	-	-	+	+	-	-	-	-	-	-
37.	*Cx.(Lutzia)fuscanus*	-	-	-	-	-	+	-	-	-	-	-
38.	*Heizmannia (Hez.)viridis*	-	-	-	+	+	+	-	-	-	-	-
39.	*Orthopodomyia flavicosta*	-	-	-	-	+	-	-	-	-	-	-
40.	*Uranotaenia (Pseudoficalbia)bicolor*	-	-	-	-	+	-	-	-	-	-	-
41.	*Ur.(Pfc.)stricklandi*	-	-	-	+	-	-	-	-	-	-	-
42.	*Ur.(Ura.)campestris*	-	-	-	+	-	-	-	-	-	-	-
43.	*Toxorhynchites (Tox.)splendens*	-	-	-	-	-	-	-	-	-	-	+

* Habtats: (1) Ground pools and tanks, (2) Rock pools, (3) Paddy fields, (4) Stream beds, (5) Tree holes, (6) Fallen coconut shells and leaf sheaths of betel-nut trees, (7) Cement tanks, (8) Glass containers, (9) Earthen containers, (10) Metallic containers and Tyres.

dered dog biscuits was provided. Larvae and adults were identified with the help of keys provided by Christophers (1933), Barraud (1934), Peyton (1977), Peyton and Harrison (1979) and Tewari *et al.* (1987). The present taxonomical status of various species was identified from the catalogue by Knight and Stone (1977).

Breeding habitats of different species and genera of mosquitoes have been classified variously in the past (Belkin, 1962; Mattingly, 1969). For the purpose of the present study, the breeding habitats of mosquitoes in Goa were classified into three categories, viz., (i) Ground water habitats consisting of ground pools, tanks, rocky pools, paddy fields and stream beds; (ii) Plant habitats consisting of tree holes and otherparts of plants such as fallen coconut shells and leaf sheaths of betel-nut tree and (iii) Domestic and peridomestic habitats consisting of man- made containers, viz., cement tanks, glass, earthen and metallic containers and tyres. The relevant data on the breeding habitats of mosquitoes in Goa was recorded and analysed.

RESULTS AND DISCUSSION

A total of 43 species of mosquitoes were identified in all the three categories of breeding habitats. Of these, 30 species were found breeding in ground water, 20 in plant and 11 in domestic and peridomestic habitats (Table 1). Five species, viz., *Anopheles subpictus, Aedes albopictus, Armigeres subalbatus, Culex quinquefasciatus* and *Cx. pallidothorax* were found in all the three categories of habitats. Majority of the anophelines and culicines were found in the ground water habitats. *Culex tritaeniorhynchus* was found mainly in the paddy fields and ground pools during monsoon and post-monsoon seasons; in the dry seasons, its larvae were collected in large ground water tanks and cement tanks in domestic habitats. Seven out of 11 species of Aedes and five species of culicines viz., *Cx. fragilis, Cx. fuscanus, Cx. whitmorei, Orthopodomyia flavicosta* and *Uranotaenia bicolor* were exclusively found in plant habitats. Domestic and peridomestic containers searched in the area were mostly man-made. *Aedes albopictus* was found in all types of domestic containers except cement tanks. It was also found in the water collections in fallen coconut shells and leaf sheaths of betel-nut and in rocky pools. In the present study, *Aedes aegypti* and *Toxorhynchites splendens* were collected only in discarded tyres. A number of larvae of *An. subpictus* were found breeding in cement tanks, however, a single larva was found in association with *Heizmannia viridis* in a tree hole. This is not unusual for this species since it is known to breed in every type of breeding place (Rao, 1984).

Mattingly (1969) has reported that the ground water habitats could be permanent or temporary in nature. The permanent habitats like ponds and tanks provide a stable environment to some of the species like *An. subpictus* and *Cx. quinquefasciatus*, while temporary habitats such as ground pools, stream bed pools and paddy fields, provide breeding habitats to species like *Cx. tritaeniorhynchus, Cx. gelidus* and *Cx. pseudovishnui.* In winter or summer, when paddy fields dry up, these species were found breeding either in the lift irrigated paddy fields or in permanent water bodies like large ground water tanks and occasionally in cement tanks.

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A Focus of High Degree Chloroquine Resistant *P. falciparum* in Mandla District (M.P.)

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A study on the bioenvironmental control of malaria was launched in Bizadandi block (Mandla district, M.P.) in May 1986. Besides intervention, using environmental management methods and larvivorous fishes, weekly surveillance and chloroquine administration @ 25 mg/kg body weight was practiced. Studies during 1987 revealed that a large number of *P. falciparum* cases did not respond to the standard anti-malarial treatment. Therefore, systematic 28 day *in vivo* studies were taken up on the follow-up of *P. falciparum* cases after administration of 3 day course of 25 mg/kg body weight as per the WHO procedure. Results revealed a high proportion of drug resistant cases belonging to RI (237), RII and RIII (182) category. *In vivo* studies on the sensitivity to metakelfin showed that some cases were resistant to this drug. There is an urgent need to eradicate this focus before it starts spreading to other areas.

INTRODUCTION

The hilly and forested tribal belts of Madhya Pradesh are highly malarious. Factors contributing to malaria endemicity are difficult terrain, poor communication, a backward population dependent on minor forest produce or petty jobs for their livelihood and extreme poverty. Surveillance and treatment was extremely poor and drug distribution centres (DDCs)/fever treatment reports (FTDs) were not functioning. There was high parasite reservoir in the community (Singh *et al.*, 1988). The villages were under DDT spray, but there were several lacunae in spraying schedules. Initial surveys by the project staff showed that 25% of the houses could not be sprayed due to refusals and many houses (10%) were found locked as the whole family moved out early in the morning in search of jobs. Further, 25% of the houses remained unsprayed by the squads because of the inacessibility of the villages and sparseness of population over difficult terrain. Out of the remaining houses that were sprayed at least one room in each house was unsprayed for fear of spoiling stored food grains. Thus out of total households targeted for coverage, 60% houses were unsprayed and the rest (40%) only partially sprayed. Thus, the impact on malaria transmission was negligible. DDT (50%) was being sprayed by greatly diluting the insecticide formulation which did not produce the required 1gm/sq m deposits on the walls. Consequently there was a high degree of perennial transmission (Singh *et al.* 1988).

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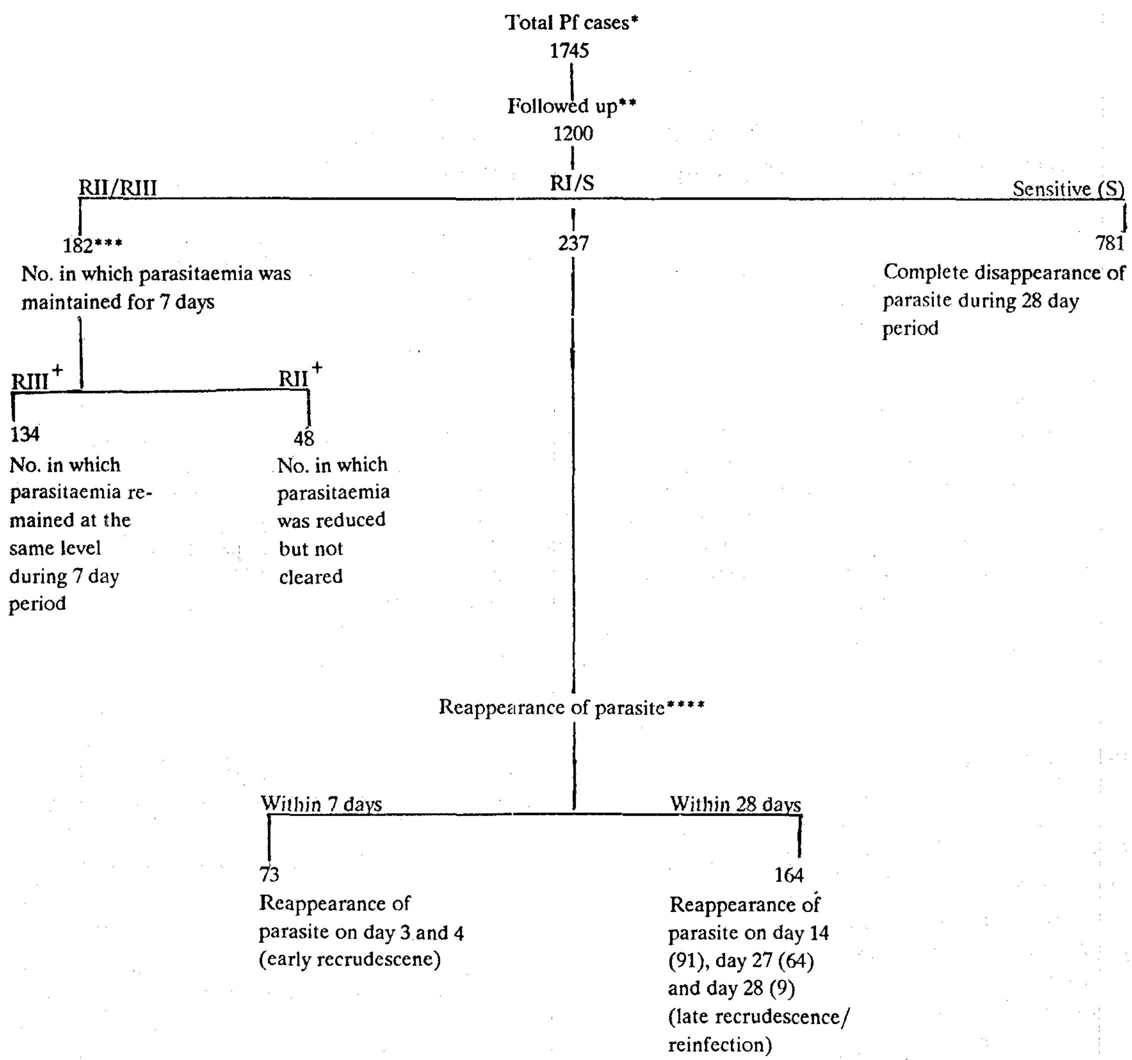
Table 1. Results of WHO *in vivo* test for chloroquine resistance in Gond tribe of Bizadandi block, Distt. Mandla, showing level of resistance (Jul-Dec 1987)



*Patients were given 1500 mg chloroquine (600 mg on D0, 600 mg on D1 and 300 mg on D2).
**Cases did not respond to 1500 mg chloroquine.
***Parasitological follow-up of serious cases is given in Table 2.
****Terminated with second dose of chloroquine (1500 mg).
+Terminated with metakelfin 2nd tablet on 8th day.

Bioenvironmental control of malaria was started in May 1986 in this area. During surveillance it was noticed that one or two courses of three day schedule of 1500 mg chloroquine (D0 600 mg,D1 600 mg, D2 300 mg) administered to *P. falciparum* cases failed to clear the asexual parasites. In view of this situation, a study was initiated to obtain a realistic assessment of the effectiveness of chloroquine in the treatment of *P. falciparum* malaria in 40 villages of Bizadandi block. This is the first report of chloroquine resistant *P. falciparum* detected by *in vivo* test method in Mandla district. Results of a one year study are reported in this paper.

MATERIAL AND METHODS

Study area

Bizadandi block (consisting of 80% Gond tribe) consists of rocky foothills, teak forests, perennial and seasonal streams and deep valleys without proper irrigation or drainage.

There are over 125 thinly populated villages (471 sq km). Of these 25 villages have been submerged due to dam construction. Most villages have no communication facilities and remain cut-off during the rainy season for 2–3 months. *A. culicifacies* and *A. fluviatilis* are the main vectors in this area.

The study was carried out in 40 villages of Bizadandi block, district Mandla. In study villages, the following activities were carried out (i) listing of all houses, census of population by age and sex; (ii) weekly blood smear collection from all fever cases and cases with history of fever; (iii) weekly follow-up of all Pf cases and passive slide collection. In addition to this larvivorous fishes were introduced in a large number of breeding sites and engineering methods were used to control mosquito breeding (Singh *et al*., 1988).

Selection of cases

P. falciparum cases were selected for the study. Mixed infections (*P. vivax* and *P. falciparum*), seriously ill patients and those with scanty infection were excluded from the study. Patients were given chloroquine base @ 25 mg/kg body weight i.e., 600 mg on D0, 600 mg on D1 and 300 mg on D2. Chloroquine was given after food and all cases were followed for 28 days. *In vivo* test was carried out according to WHO (1973).

Excretion of chloroquine in the urine was monitored, to ensure absorption of chloroquine in the blood, by Dill and Glazko test. For the treatment of chloroquine resistant *P. falciparum* malaria 2 tablets of metakelfin (sulfalene 1000 mg + pyrimethamine 50 mg) adult dose and 45 mg primaquine was given.

RESULTS

A total of 1745 *P. falciparum* cases were detected in 40 villages of Bizadandi. Of these 1200 cases were followed for 28 days. In 781 cases (65.1%) asexual parasites disappeared by day 7 and were absent for the full period of 28 days. The parasites were sensitive to chloroquine (Table 1). In 237 cases (19.7%) asexual parasites disappeared by day 3 and 4 but reappeared within the period of test (28 days). Of these 237 cases showing RI level of resistance, 91 cases (7.58%) showed reappearance of parasitaemia during the second week, 64 (5.33%) during the third week and 9 cases (0.75%) during the fourth week of study (delayed recrudescence). In 73 cases parasite clearance was noted on day 3 and 4 but parasitaemia reappeared on day 9 (early recrudescence).

Out of 134 cases, asexual parasitaemia was maintained at the same level in 98 cases and increased in 36 cases. In 48 cases, parasitaemia did not clear but was markedly reduced, showing RII level of resistance. Serious cases of falciparum malaria were followed up daily for 7 days after the administration of the full dose of chloroquine @ 25 mg/kg body weight. Parasitological results (of the severest cases) are given in Table 2. Results clearly suggest high degree of chloroquine resistance in all cases (15 cases were RIII and 3 cases were

Table 2. Chloroquine sensitivity of *P. falciparum* (WHO *in vivo* tests) in Bizadandi, district-Mandla

S.No.	Age	Sex	Result	Parasitaemia (Pf rings) per cubic mm*								Level of resis-tance
				D0	D1	D2	D3	D4	D5	D6	D7**	
1.	12	M	PfR	3325	1625	1525	875	1375	1525	1925	2250	R III
2.	6	F	PfR	3325	1500	1800	1925	2525	2625	2900	6225	R III
3.	12	M	PfR	4525	1625	1200	1625	1900	2900	6625	9925	R III
4.	20	F	PfRG	6975	4325	9625	10225	10325	11000	16225	19225	R III
5.	10	F	PfR	6275	1725	1200	775	1600	2225	2975	3800	R II
6.	30	M	PfR	3125	1600	1675	1825	1900	2025	2125	2275	R III
7.	6	F	PfR	1625	925	1425	600	900	1500	3250	8475	R III
8.	13	F	PfR	2225	575	825	1825	1325	1575	1625	1725	R II
9.	12	M	PfR	4450	5325	4725	7900	7775	5375	11625	13625	R III
10.	10	M	PfR	4650	1850	2025	3025	1625	6825	9325	1525	R III
11.	18	M	PfR	1225	15775	18325	4225	1425	6225	1225	8025	R III
12.	8	F	PfR	2225	1025	2275	2975	6225	4225	6375	7325	R III
13.	8	M	PfR	6375	11000	8025	2025	1075	1675	1875	***	R III
14.	40	F	PfR	9425	1525	2700	1025	5325	5425	5025	2925	R II
15.	30	F	PfR	2250	5250	1025	600	4700	2400	2325	2250	R III
16.	13	M	PfRG	3350	1025	1750	7525	10100	16375	15975	2875	R III
17.	30	F	PfR	4275	850	13300	13775	9700	11625	2525	2275	R III
18.	18	F	PfR	2025	1275	8275	1625	4225	7225	11325	3325	R III

+ Only few selected severe cases are presented here for the sake for the sake of brevity.

* Nos. positive for asexual stage.

** Cured with metakelfin.

*** Smear on day 7 not available.

Table 3. Response of *Plasmodium falciparum* to metakelfin in cases that did not respond to chloroquine

S.No.	Age	Sex	D0	D1	D2	D3	D7	D14	D21	1 month
1.	15	M	+	+	+	+	+ PfR			
2.	24	F	+	+	+	+	+ PfR	-		
3.	35	M	+	+	+	-	+ PfR	-		
4.	12	F	+	+	-	-	-	-	* PfR	** PfR
5.	14	F	+	+	+	+	+ PfR	-		
6.	4	F	+	+	+	+	+ PfR	* PfR	** PfR	** PfR
7.	12	F	+	+	+	+	+ PfR	* PfR	-	
8.	20	M	+	+	+	+	+ PfR	* PfR		
9.	11	M	+	+	+	+	+ PfR			
10.	12	M	+	+	+	+	+ PfR	* PfR	** PfR	** PfR

+ *P. falciparum* rings with fever.
* *P. falciparum* rings on and after 14 days, second dose of Metakelfin given (2 Tablets suphalene + pyrimethamine) and 45 mg primaquine.
** *P. falciparum* rings after repeat dose of metakelfin; cases were referred to District/Hospital for complete cure.

RII). There is, therefore, clear evidence of drug resistant malaria outbreak in the area.

All chloroquine resistant cases were treated with metakelfin of which 10 (2.3%) cases did not respond to metakelfin also. In two cases after 72 hrs the patients showed asexual stages but after 7 days they became afebrile and did not show any parasite. A second dose of metakelfin was given to those cases where single dose of metakelfin and 45 mg primaquine failed to bring down fever and parasitaemia. These cases responded to the second dose of metakelfin (no parasitaemia was found) and did not complain of fever during the follow-up period of one month. Only 3 cases did not respond to second dose of metakelfin and can be said to be truly resistant to it (Table 3).

DISCUSSION

P. falciparum has been a major problem in foothill areas and other regions of high transmission. The tribals that inhabit these regions are one of the weakest sections of society and are responsible for a sizeable number of falciparum malaria cases (Sharma, 1984a). In foothill areas, transmission is more or less perennial (Ray and Sharma, 1983). In 1987–88, 23 districts, out of a total of 45 districts, in Madhya Pradesh were affected by an outbreak of falciparum malaria (Source: NMEP Bhopal).

While reviewing the position of chloroquine resistance in *P. falciparum* in India, Sharma (1984b) found RIII level of resistance in 7 out of 55 places reviewed. All these cases were from northeastern parts of India except one in Bastar district (M.P.).

The adjoining villages of Kundam block were not under regular intervention measures according to the MPO policy as the API (prior to an epidemic outbreak) was less than 2 per thousand for several years till 1985–86. While a number of issues are involved in understanding falciparum malaria dynamics and parameters of assessment under the NMEP, the existing information indicates complete lack of surveillance. As a result, the incidence of falciparum malaria rose to extremely high levels and constituted the main parasitic load in the community (Infant parasite rate 48%, Child parasite rate 70% and Spleen rate > 50%; Singh *et al.*, 1988). During 1988, the situation worsened further and there were reports of deaths due to malaria. Investigation into the cause of deaths revealed that there was a total breakdown of surveillance and malaria control was completely ignored (Singh *et al.*, 1988). It is essential that full dosage of an effective antimalarial is administered to *P. falciparum* cases to achieve radical cure in all cases. This alone would reduce the chances of appearance of resistant strains which might occur as a result of administration of repeated small ineffective doses of the drugs.

In another study 5 isolates were collected from Kundam block in January 1987 for laboratory research on the dynamics of chloroquine resistant *P. falciparum*. *In vitro* tests showed a high degree of resistance and one isolate survived even 64 pico moles concentration thus substantiating the above findings of an outbreak of drug resistant malaria. The strains are being maintained *in vitro* (Subbarao, personal communication). Under the National Malaria Eradication Programme all fever cases are given 600 mg chloroquine as presumptive dose. Radical treatment is given after the results of blood slide examination have been received. Generally there is an interval of 4 to 6 weeks in radical treatment which comprises of another dose of 600 mg chloroquine and 45 mg primaquine. Delayed radical treatment and poor surveillance may have precipitated the high degree of resistance in the area. it is notable to mention that construction of a dam started in Bizadandi block in 1980 and work continued for 7–8 years. A large labour force was imported from various malaria endemic areas in the country through contractors (Singh *et al.*, 1988). Although data on malaria incidence in itinerant labour is not available, several studies have clearly shown high rate of falciparum malaria in labour moving from one area to another (Ray, personal communication; Sharma and Sharma, 1988). Most of this population has asymptomatic malaria due to high immunity and poor response to chloroquine. It is likely that the initial focus of drug resistant malaria was seeded via the labour from the dam project or some other similar activity in the region.

There is already evidence of metakelfin resistance in *P. falciparum* which may spread, if transmission is not interrupted immediately. At least ten such cases were recorded (Table 3). Further studies are indicated on micro *in vitro* tests for determining parasite sensitivity against Sulfadoxine/Pyrimethamine (SDX/PYR). Resistance against this drug has already been reported (Choudhury *et al.*, 1987) from Delhi. Resistance to other potential antimalarials (Fansidar) has been reported from different parts of southeast Asia (Black *et al.*, 1982). Rumans *et al.* (1979) reported resistance against Fansidar from Indonesia. Similar reports have also come from different parts of Africa (Hess *et al.*, 1982; Bjorkman and Willcox, 1986). All this, however, shakes the confidence that malariologists have in metakelfin, especially in light of the fact that it is not as cheap and non-toxic in its action as chloroquine.

The present study clearly identified chloroquine resistant malaria foci of RII and RIII types and metakelfin resistance in Bizadandi block by the *in vivo* method. There is a need to revise the drug policy in this area. Follow-up beyond seven days was not of much use as the area was not free from transmission and even if many cases (164) were found positive, no clear conclusions could be reached in view of the possibility of re-infection.

In this area malaria control is difficult and has been further complicated by chloroquine and metakelfin resistance. This may be another major drug resistant belt outside the northeastern part of India. If the foci of drug resistant strains are not delimited fully and eliminated in time, there is a possibility of its widespread dissemination from this epicentre. Therefore, an all out effort should be made to simultaneously monitor and eradicate this focus as was done in Assam, Chandrapur and Mirzapur (Sharma, 1984b). Besides monitoring should also be done to test the response of other antimalarials like pyrimethamine, amodiaquine and mefloquine etc.

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SHORT NOTES

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Bioenvironmental Control of Malaria Linked with Edible Fish Production in Gujarat

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An alternate strategy known as the bioenvironmental control of malaria was initially launched in 1983 in 7 villages of Nadiad taluka, Kheda district, Gujarat. By 1985 the project area was extended to cover the entire Nadiad taluka comprising of approximately 3,50,000 population living in 100 villages (Sharma and Sharma, 1986). Kheda project aimed to demonstrate malaria control by integrating source reduction, minor engineering work, biological control of mosquito breeding, prompt case detection and radical treatment, health education and involvement of communities in vector control and developmental activities. The strategy was developed as a holistic approach to malaria control incorporating income generating schemes with a view to increase the income of the panchayats concerned, so as to to enable them to participate in vector control with their own resources. Two income generating schemes were favoured in the area viz., social forestry and production of edible fishes. Results of a two year study on composite fish culture i.e., production of food fishes along with larvivorous fishes are reported in this paper.

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Mosquito breeding surveys showed that every village has one or more permanent and seasonal ponds. These ponds supported moderate to heavy mosquito breeding and its control was a major activity of the project. In these ponds most of the breeding was confined to the shallow margins and hoof prints at the periphery. In summer heavy mosquito breeding was encountered in innumerable ponds and pools which were well covered by water hyacinth (*Eichornia crassipes*) and produced enormous mosquito populations. Major efforts were, therefore, directed to control mosquito breeding in all ponds.

A survey of fish fauna revealed the presence of 27 varieties of indigenous fishes, of which 14 species were larvivorous. Of these fishes Guppy (*Poecilia reticulata*) was found in the wild and had maximum potential as a biological control agent (Sharma *et al*., 1987). This fish, a native of South America was introduced in India in 1908 for the control of mosquito breeding (Jhingran, 1985). In the experimental villages, hatcheries were established in small and medium size ponds for decentralized guppy production. Fish were periodically removed from these hatcheries and introduced in wells, ponds and other water collections to control mosquito breeding. Village workers are encouraged to replenish guppies in all water bodies

Table 1. Data on stocking of fishes and prawns in experimental ponds

Village pond	Size in hectare	1985 (Aug & Sept)		1986 (Oct & Nov)				
		Major carp FR	Juvenile prawn	Common carp FR	Silver carp FG	Grass carp FG	Major carp FG	Juvenile prawn
1. Bamroli	1.27	40700	20000	675	702	1350	5000	-
2. Dantali	1.40	-	-	-	-	-	2000	43000
3. Davda	3.00	50875	-	6480	5850	3375	21590	-
4. Dumral	3.14	45000	20000	3147	3780	3375	10000	-
5. Khuntaz	2.91	17555*	-	2290	2250	1688	9430	-
6. Pij	4.12	-	-	7605	8213	5738	30000	25000
Total	15.84	152300	40000	20197	20795	15526	78020	68000

* Includes 1830 fingerlings; FR—Fry; FG—Fingerlings.

Table 2 : Fish production in experimental ponds

Village Pond	Normal production rate 1975-85 (ten yrs)	Experimental ponds 1986 (First year)		Experimental ponds 1987 (Second year)		Total for 2 years	
	Total income (in Rs)	Total yield (in kg)	Total income (in Rs)	Total yield (in kg)	Total income (in Rs)	Total yield (in kg)	Total income (in Rs)
1. Bamroli	-	380	2064	460	2415	840	4479
2. Davda	10379	4973	44566	7240	66083	12213	110649
3. Dumral	10000	1615	12937	3551	25698	5166	38635
4. Khuntaz	-	3385	18875	2417	21307	5802	40182
5. Dantali	-	-	-	1315	11375	1315	11375
6. Pij	-	-	-	3415	32520	3415	32520
Total	20379	10353	78442	18398	159398	28751	237840

Average yield/hectare/year = 1099 kg; Average income/hectare/year = Rs. 9091.

periodically; as a result this fish can be found in almost all stored/stagnant waters in Nadiad taluka.

At present there are approximately 20 million guppies in 94 hatcheries which are maintained by the Project staff. These fishes are held in reserve for field use. Often large numbers are required for the control of mosquito breeding in rice-fields for which no other suitable method is yet available. A few ponds were utilized for composite fish culture of edible fishes with guppies.

In 1985 eight village ponds were taken from village panchayats for food fish culture. All weed fishes were removed by repeated netting. Iron wire nets were fitted at outlets wherever required. These ponds were stocked with indian major carps (*Labeo rohita*, *Cirrhinus mrigala* and *Catla catla*) in August and September 1985 (Table 1). Fish seed of Indian major carps were procured from the Department of Fisheries, Government of Gujarat, Lingda and were transported either in open containers for short distances or in oxygen filled polythene bag containers to minimise the mortality. Juvenile prawns were procured from estuarine region of Narmada river in Bharuch district, Gujarat and were introduced in the ponds of village Bamroli and Dumral in 1985. Four ponds located in Finav, Dadusar, Dantali and Sapla had to be abandoned due to large-scale poaching followed by drying up of ponds due to water scarcity.

In October 1986, six ponds were taken up for composite fish culture and exotic fishes like *Hypophthalmichthys molitrix* (Silver carp), *Ctenopharyngodon idella* (Grass carp) and *Cyprinus carpio* (Common carp) were stocked (Table 1). The seeds of exotic fishes were bought from a private firm of Calcutta. They were conditioned and transported by train. It took about 70 hrs, from the time of conditioning and packaging to their introduction in the ponds. A maximum of 10% mortality was observed in some containers average being only 7%. Seeds of major carps were procured from Fisheries Department, Government of Gujarat, Anand and Lingda. In addition, juvenile prawns were also introduced in Dantali and Pij village ponds as was done in 1985.

Soil analysis of these ponds was done to ascertain the physicochemical condition of the soil. On the basis of the results inorganic fertilizers were applied at the rate of 200 kg/ha/yr. The mixture applied comprised of equal amounts of urea, superphosphate, ammonium sulphate along with a cattle feed marketed under the trade name 'Amuldan' (grain 15–25%, oil cake 15–30%, rice bran 20–30%, molasses 10–15%, salt 4%, vitamins and mineral traces) produced by Kheda District Cooperative Milk Producers Union Limited, Anand. This was also used as a fertiliser, at the rate of 250 kg/ha/yr, at fortnightly intervals alternately with cow dung to obtain maximum growth of plankton.

To prevent poaching, night watchmen were hired and patrolling was supervised by panchayat members. This helped in reducing thefts. Two poaching parties were handed over to the police and many were driven away. Besides this, village panchayat members did occasional night patrolling. The growth of fish was observed after periodic netting in experimental ponds. All the experimental ponds were harvested after 8 months in 1986 and 12 months in 1987.

Results of fish production in experimental ponds are given in Table 2. In 1986 four ponds having an area of 10.32 hectares gave a total yield of 10353 kg which generated an income of Rs. 78,442 by auction. In 1987 number of experimental ponds stocked were six with a total area of 15.84 hectares. Total fish yield in 1987 was 18398 kg which was auctioned for Rs. 1,59,398. Besides this, the ponds served as permanent hatcheries for large-scale production of guppies. About 20 lakh guppies were collected from the six ponds and distributed at various places in the experimental villages. By averaging two years' yield the fish production comes to 1099 kg/ha/yr. In terms of money the ex-

Table 3 : Fish production in Kheda district (Government Fisheries)

Year	No. of ponds	Area (hectare)	Stocking of seeds	Total production (in kg)	Yield per hr. (in kg)	Income (in Rs.)	Income per hr. (in Rs.)
1982-83	43	115.5	2,06,000 FR 3,08,673 FR	28495	246	1,89,400	1640
1983-84	33	84.92	4,13,328 FG	33021	388	2,85,992	3368
1984-85	21	53.18	4,77,155 FG	18586	349	1,77,068	3330
1985-86	19	43.18	4,25,250 FG 10,000 FR	14739	339	1,36,668	3150
1986-87	18	42.40	2,64,000 FG	16519	389	1,57,436	3713
Total	134	339.38	18,88,406 FG	1,11,360		9,46,564	

FR - Fry; FG - Fingerlings; Average yield/hectare/year = 328 kg; Average income/hectare/year = Rs. 2789; Fish production per year = 22270 kg; Source: Department of Fisheries, Anand.

perimental ponds gave a gross average income of Rs. 9091/ha/yr. The total amounts received by Davda and Dumral panchayats, through auction of fishes from their village ponds in the previous ten years period were Rs. 10,379 and Rs. 10,000 respectively and there was no income from fishery in the other villages for that period (Table 2).

An extensive survey was carried out to explore maximum potential of food fish culture in Nadiad taluka. In the taluka there are 201 perennial and 472 seasonal ponds. The area of these ponds is 481 and 424 hectares respectively. According to a conservative estimate the perennial ponds of Nadiad taluka alone could give a gross income of over Rs. 43 lakhs at a rate of Rs. 9091/ha/yr. An estimate was made on the potential of edible fish production in Nadiad taluka along with guppies. Table 3 provides data on fish production by the Government Fisheries Department in Nadiad taluka. Average fish production by the Fisheries Department was Rs. 328 kg/ha which gave an income of Rs. 2789/ha/yr. This is about one third the yield achieved by the project staff (Table 2).

Experience gained in the two year period showed that 201 ponds can generate an income of Rs. 43,72,771 @ Rs. 3015 /ha/yr, the net income generated as a result of fish production alone could be Rs. 29,22,556. After deducting the expenditure @ Rs. 3015/ha/yr, the net estimated income is little over Rs. 29 lakhs (Table 4). Total expenditure incurred for stocking and maintenance of the experimental ponds for a two year period is Rs. 78,875. Out of this, the main heads are seeding (Rs. 46,882), harvesting (Rs. 17,600) and maintenance (Rs. 14,393). Total income generated during the 2 year period was Rs. 2,37,840. After deducting expenditure of Rs. 78,875 the net income available to village panchayats was Rs. 1,58,965. Panchayats in consultation with Malaria Research Centre diverted this money for mosquito control by source reduction methods as briefly described below.

(1) In Bamroli two small parks covering an area of 3108 sq. m were developed.

(2) In Dumral earthwork using a bulldozer was undertaken to deepen the pond for food fish culture. The excavated soil was used for levelling the nearby low-lying ground.

(3) In Khuntaz a 141 m long brick wall was constructed to protect the village from flooding.

Table 4 : Estimated income from fish culture in Nadiad taluka

1.	Total no. of village ponds in which food fish culture can be undertaken	201
2.	Total area of ponds	481 hectare
3.	Estimated income @ of Rs. 9091 per hectare per year	Rs. 43,72,771
4.	Estimated expenditure	Rs. 14,50,215
	Estimated net income per year	Rs. 29,22,556

(4) In Davda a playground of 7300 sq m with a barbed wire fence was prepared. In the same village an underground drainage system (415 m) was laid.

(5) In Dantali earthwork was done and a 1558 sq m park was prepared. The park was fenced and trees were planted at the periphery.

All the above activities resulted in large-scale elimination of breeding sites and also contributed towards better and healthier environment.

From the above study it can clearly be seen that perennial ponds should be used for food fish production run on scientific lines. Further, it is suggested that small seasonal ponds could be used for rearing of fish seed, which would generate additional income and result in savings on transportation. These activities will also generate employment locally. Sharma and Sharma (1986) estimated that the cost of bioenvironmental control of malaria in Nadiad taluka was Rs. 18 lakhs based on the actual expenditure incurred during 1985–86. Thus, it can safely be stated that the income generated from 201 perennial ponds of Nadiad taluka would be sufficient to finance the bioenvironmental control of malaria in Nadiad taluka and also undertake other developmental activities.

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Indirect Fluorescent Antibody (IFA) test for Malaria in Pregnant and Non-pregnant Women

S.L. SHOLAPURKAR[1], R.C. MAHAJAN[1], A.N. GUPTA[1] and R.N. PRASAD[1]

The resurgence of malaria in most tropical countries has made it imperative to explore the epidemiology of malaria and modify the strategy for its control. Studies from African countries have revealed that pregnant women are more prone to severe attacks of malaria, probably because of the breakdown of acquired immunity (Lawson, 1967; McGregor, 1984). There are hardly any studies on this aspect from the Indian subcontinent where transmission of malaria is unstable. The present study was carried out prospectively between June 1984 and November 1985 in the Nehru Hospital attached to the Postgraduate Institute of Medical Education and Research, Chandigarh.

All pregnant women with history of fever were screened for malaria by peripheral blood smear examination and positive cases were recruited in the study group (S_1). Twenty two non- pregnant women from childbearing age group of 15–45 years, positive for malaria parasite, were also randomly included during the study period (S_2). In the control group, 80 normal pregnant womer without any infection, equally distributed in different trimesters of pregnancy and puerperium (C_1) and 20 normal non-pregnant women (C_2) were randomly selected.

Five ml blood samples were collected as soon as the patients came under our care and serum was stored at -20°C till use. Antimalarial antibodies were detected by indirect fluorescent antibody test (Mahajan *et al.*, 1982) using *P. knowlesi* schizonts as antigen.

Out of the 78 pregnant women with malaria recruited in the study group (S_1) and 22 non-pregnant women with malaria (S_2) approximately half were nulliparous and the remaining half were parous. *P. vivax* infection was present in 58 cases while 42 cases had *P. falciparum* infection. Mean age of subjects in study and control groups did not vary significantly.

Taking positive titres as 1:40 and above according to our laboratory standards, only 7.5% of pregnant controls (MP negative) and 20% of non-pregnant controls had positive titres. This difference was not statistically significant ($p > 0.05$ by χ^2 test). Further, 83% pregnant malaria cases and 86% of non-pregnant malaria cases had positive titres, the

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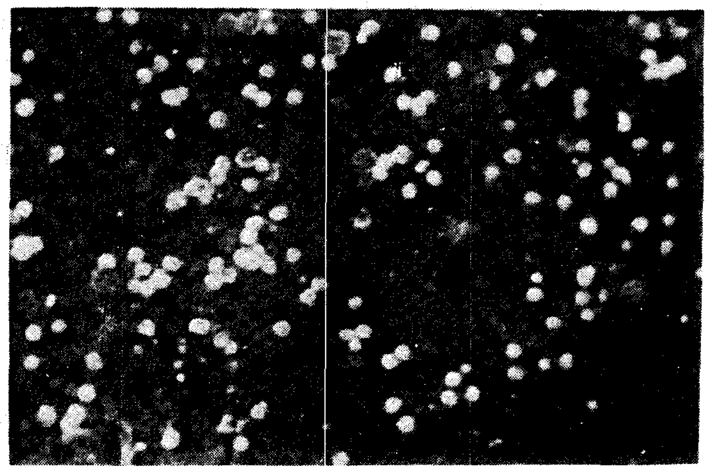

Fig.1: Indirect fluorescent antibody test showing positive immuno-fluorescence.

Table 1. IFA reactivity in pregnant patients with malarial infection (*P.vivax and P. falciparum*) according to period of gestation and parity

	Weekly reactive IFA < 1:160	Strongly reactive IFA ≥ 1:160	Total
1st trimester	6	4* (40%)	10
2nd trimester	11	5* (31.2%)	16
3rd Trimester	22	9* (29%)	31
Puerperium	12	9* (43%)	21
Total	51	27	78
Nulliparas and primigravidas	22	16** (43%)	38
Para 1	25	15** (38%)	40

* Difference not satistically significant ($p > 0.05$ by χ^2 test.)
** One delivery.

difference again being not statistically significant ($p > 0.05$ by χ^2 test). But when malaria cases were divided into strongly reactive (IFA > 1:160) and weekly reactive (IFA < 1:160) there was a much smaller number (40%) of strongly reactive cases in pregnant malarious group (Fig. 1) as opposed to those in non-pregnant malarious group (59%). This difference was statistically significant ($p < 0.05$ by χ^2 test). IFA reactivity was not different in nulliparas and multiparas. IFA titres were lower in 2nd and 3rd trimesters compared to 1st trimester and puerperium (Table 1). Similar results were reported by Bray and Anderson (1979) and Campbell *et al.* (1980). Thus, it can be surmised that impaired antibody production during pregnancy could account for increased severity of malaria in pregnant women.

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