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In-vivo and *In-vitro* Sensitivity of *Plasmodium falciparum* to Chloroquine in Chennai (Tamil Nadu), India

VIRENDRA K. DUA, P.K. KAR, N.C. GUPTA, INDRANIL KAR[a] and V.P. SHARMA[b]

In-vitro and *in-vivo* susceptibility of *Plasmodium falciparum* to chloroquine were conducted at Chennai city, India. Eighteen (60%) out of 30 cases showed resistance in *in-vitro* study. EC_{50} of resistant and sensitive cases were 0.40 and 0.24 µmol chloroquine/l blood respectively, while EC_{90} were 2.64 and 0.84 µmol chloroquine/l blood respectively. *In-vivo* tests identified 24 cases (40%) as resistant (23 RI and 1 RII) and 36 (60%) as sensitive out of 60 cases. Eight isolates which were found resistant with *in-vitro* tests showed sensitive behaviour to chloroquine treatment assessed by *in-vivo* studies.

Keywords: Chennai, Chloroquine, Drug sensitivity, *Plasmodium falciparum*

INTRODUCTION

Chennai city accounts for 45-55% of the total malaria incidence recorded in Tamil Nadu state of India. More than 60% of the total *P. falciparum* cases are from Chennai city. Ghosh *et al.*[1] have studied *in-vitro* sensitivity of six *P. falciparum* cases, while Venkatesan and coworkers[2] found high level of chloroquine resistance in one *P. falciparum* case in Chennai. Recently[3] one more case of chloroquine resistance was found in Vellore city by *in-vitro* test and

Malaria Research Centre (Field Station), Sector III, BHEL Complex, Ranipur, Hardwar-249 403, India.
[a]Malaria Research Centre (Field Station), 1304, Anna Nagar (West), Mogappair, Chennai-600 050, India.
[b]Malaria Research Centre, 20-Madhuvan, Delhi-110 092, India.

referred as imported case from Chennai. We have carried out *in-vivo* and *in-vitro* response of 67 *P. falciparum* patients to chloroquine in Chennai to know the extent of chloroquine resistance. Results of the study are presented here.

MATERIALS AND METHODS

The study was carried out in Sowcarpet and its adjoining areas spread over to six corporation divisions namely, 53-55 and 86-88 due to high *P. falciparum* cases. The total population of the area was 90,000 and the study was performed in the month of December 1993 to January 1994.

In-vivo test (WHO 28-day extended test) for chloroquine sensitivity was started with 67 selected patients (mean weight 47.5 kg; age group 15-50 yr) with history of no reinfection and after ascertaining that no chloroquine had been taken during illness by examination of urine for the chloroquine excretion[4]. Each patient received a total dose of 1500 mg (25 mg/kg body weight) of chloroquine base (600, Day-0; 600, Day-2; and 300, Day-3). Blood smears were collected on D0, D2, D7, D14, D21 and D28 and, whenever the patient complained of fever after the completion of the prescribed dosage. Asexual parasites were examined from Giemsa-stained smears. Chloroquine-resistant cases were treated with a single does of sulfadoxine (1000 mg) plus pyrimethamine (50 mg) combination (Rimodar, Anglo-French Drug Company, Bombay). All positive cases were also given 45 mg primaquine as a single dose for radical cure. Parasite density was determined in all the cases on D0 and D2 for assessing the level of resistance.

Micro *in-vitro* tests for chloroquine were conducted with infected blood samples collected from patients in pre-dosed micro-culture plates supplied by WHO and the procedure for incubation and staining of pre- and post-incubation smears were the same as described earlier[5]. A test was considered valid when at least 10 per cent schizont maturation was observed in post-incubation control wells. Schizont maturation at 16 pmol of chloroquine per well and above was considered an indication of resistant. Minimum inhibitory concentration (MICs) of the drug were assessed by microscopic examination of post-incubation smears. The results of *in-vitro* tests were analysed by probit analysis of log-dose response test[6].

RESULTS

In-vitro test

The results of *in-vitro* tests are given in Table 1. Out of 40 samples tested, schizont maturation in control wells occurred in 30 (70%) samples. The minimum inhibitory concentrations in 12 samples were 8 pmol or less, thus showed sensitivity to chloroquine, while 18 samples (60%) showed resistant behaviour with their MICs ranged from 16 to 64 pmol. All the 30 samples were

Table 1. *In-vivo* and *in-vitro* sensitivity of *P. falciparum* to chloroquine in Chennai

Sex/ Age	*In-vivo* test		*In-vitro* test (CQ) MIC (pmol)
	CQ	SDX-PY	
M 25	RI (D21)	S	–
M 18	S	–	–
M 24	RI (D21)	S	NG
M 18	S	–	–
M 17	S	–	–
M 40	S	–	–
M 21	S	–	–
M 28	RI (D28)	S	–
M 20	RI (D21)	S	NG
M 20	RI (D28)	S	NG
M 20	S	–	R (16)
M 24	RI (D21)	S	R (16)
M 34	S	–	–
M 38	S	–	–
M 50	S	–	–
M 41	S	–	–
F 20	RI (D28)	S	NG
F 20	RI (D14)	S	NG
M 18	S	–	R (32)
M 15	S	–	S (4)
M 42	S	–	–
M 15	RI (D21)	S	R (16)
M 39	S	–	S (4)
M 38	S	–	S (8)
M 18	S	–	S (8)
M 45	S	–	R (16)
M 25	RI (D14)	S	R (64)
M 20	RI (D21)	S	R (64)
M 22	RI (D28)	S	R (32)
M 26	S	–	R (16)
M 30	S	–	R (16)

contd...

Table 1. *(contd.)*

Sex/ Age	*In-vivo* test		*In-vitro* test (CQ) MIC (pmol)
	CQ	SDX-PY	
M 26	S	–	R (32)
M 31	S	–	–
M 29	S	–	S (8)
M 26	RI (D14)	S	S (8)
M 48	RI (D14)	S	R (16)
M 29	RI (D21)	S	–
M 23	S	–	–
M 29	S	–	–
M 27	RI (D21)	S	–
M 17	S	–	–
M 15	RI (D21)	–	R (32)
F 44	S	–	NG
M 45	S	–	S (8)
F 22	S	–	R (16)
M 32	RI (D14)	–	NG
M 15	S	–	S (4)
M 21	S	–	R (16)
M 24	S	–	NG
M 25	RI (D21)	S	NG
M 18	S	–	S (8)
M 26	S	–	S (8)
M 25	S	–	NG
M 20	RI (D14)	S	–
M 30	S	–	S (8)
M 20	S	–	S (8)
M 19	RI (D7)	S	R (32)
M 19	RI (D14)	S	R (64)
M 23	RI (D28)	S	R (32)
M 24	RI (D28)	S	–

S — Sensitive; R — Resistant; CQ — Chloroquine; SDX-PY — Sulfadoxine-Pyrimethamine; NG — No growth, not tested; D — Day of recrudescence of parasites; MIC — Minimum inhibitory concentration.

further assessed by a probit analysis using the log-dose response test to know the degree of sensitivity and effective concentration (EC) from the grouped data. The drug concentrations and inhibition of schizont maturation of sensitive and resistant isolates are given in Fig. 1. The effective concentrations of sensitive isolates i.e., EC_{50} and EC_{90} were 0.24 and 0.80 µmol chloroquine/l blood respectively, while 18 resistant isolates were grouped according to their level of resistance. The average EC_{50} and EC_{90} in resistant isolates were 0.40 and 2.64 µmol chloroquine/l blood.

In-vivo test

Sixty cases were successfully followed up for 28-days from a total of 67 cases. 24 (40%) cases were found resistant, while 36 (60%) cases were sensitive to chloroquine (Table 1). All 24 resistant cases were further classified into three categories namely, seven cases in RI early resistance where parasite reappeared between D7 and D21, 16 RI delayed cases, where parasites reappeared between D21 and D28, while one case was identified as RII level of resistance. In RII resistant case, the parasite densities on D0, D2 and D7 were 2500, 500 and 1500/µl blood respectively which showed that parasitaemia was less than 25% on D2 but did not disappear after chloroquine treatment. It may be noted that parasitaemia disappeared in all RI resistant cases within seven days after initiation of chloroquine treatment. Eight isolates which were found resistant by *in-vitro* test, showed sensitive behaviour to chloroquine treatment during *in-vivo* follow-up.

DISCUSSION

Chloroquine resistance in *P. falciparum* cases in Chennai was detected by *in-vivo* and *in-vitro* tests. *In-vitro* results showed 60% isolates as resistant. Ghosh *et al.*[1] have found 80% resistance by *in-vitro* test, however, their sample size was very small. *In-vivo* results implied that only 40% cases did not respond to chloroquine treatment and thus were resistant to chloroquine. Moreover, most of these cases were of RI level except one RII level resistant case out of 60 cases followed-up for *in-vivo* test which was in contrary to earlier reports[2,3] showing high degree of chloroquine resistance in Chennai. It may be noted that their observations were based only on one or two cases. Investigation of all resistant cases showed that most of them were indigenous and belonged to the same area since more than one year. This implied that the resistant *P. falciparum* foci was present in Sowcarpet area and could not be removed due to lack of proper monitoring and treatment.

The analysis of sensitive and resistant isolates with *in-vitro* test indicated the heterogeneity of parasite population. The probit analysis of log-dose concentration at which schizont maturation

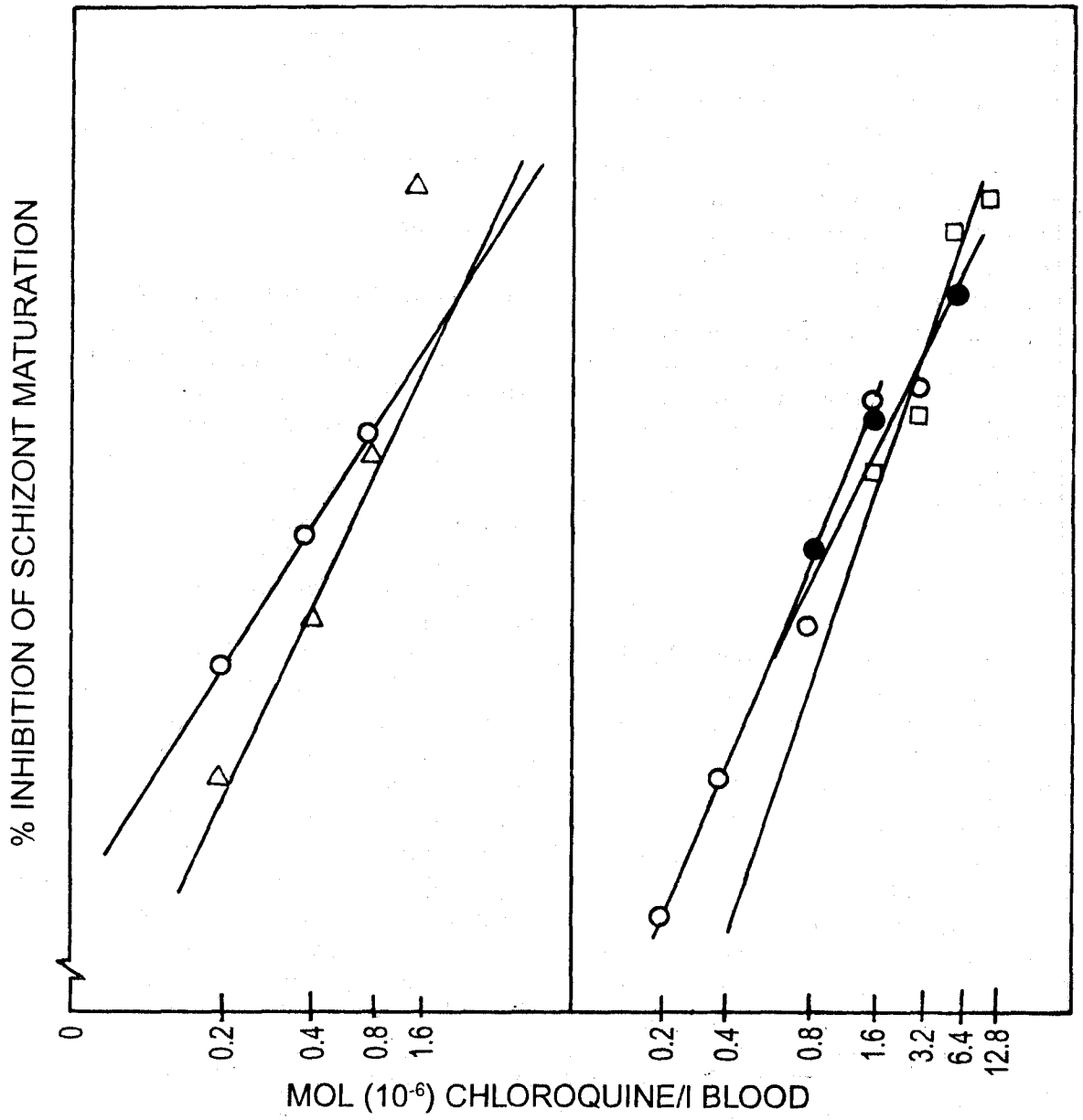

Fig. 1: Analysis of *in-vitro* tests for chloroquine sensitivity** of *P. falciparum* in Chennai (Tamil Nadu), India

*Log/probit regression lines for percentage inhibition of schizont maturation at different chloroquine concentrations for sensitive (Left panel : o–o n = 3, Δ–Δ n = 9) and resistant isolates (Right panel : o–o n = 9, ● – ● n = 6, □–□ n = 3); **Comparison of sensitive (up to a drug concentrations of 8 pmol or 1.6 x 10^{-6} mol chloroquine/l blood) and resistant isolates showing schizont maturation inhibition (at or above a drug concentration of 16 pmol or 3.2 x 10^{-6} mol chloroquine/l blood).

occurred indicated that nine isolates were of low-level resistance (MIC = 16 pmol chloroquine/l blood), while nine were of high degree of resistance with six cases MIC of 32 pmol and three cases MIC of 64 pmol. Moreover, 9 (30%) sensitive cases were in the proximity of resistant level i.e., MIC value of 8 pmol.

All chloroquine resistant cases responded well to sulfadoxine-pyrimethamine combination implied that *P. falciparum* in Chennai is sensitive to sulfa-pyrimethamine treatment. Therefore, there is no need to give quinine therapy as reported earlier[2,3] except in cases of emergency because quinine should be prescribed as a life-saving regimen in cerebral malaria cases only[7].

Our study showed the presence of chloroquine resistant *P. falciparum* malaria in Chennai city but only 40% cases were non-respondent to chloroquine treatment and mostly of RI level. Sulfa-pyrimethamine combination worked well in all resistant cases and should be recommended in *P. falciparum* resistant cases. Therefore, there is an urgent need for the prompt case detection and proper treatment of *P. falciparum* cases otherwise the resistance will be precipitated much faster in a higher degree and also to other antimalarials.

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Mosquito Breeding and Resting in Treeholes in a Forest Ecosystem in Orissa

R.S. YADAV, V.P. SHARMA[a] and S.K. CHAND[b]

During a longitudinal study in the deciduous monsoon forest in northwest Orissa, 16 species belonging to Genera *Anopheles* (2), *Culex* (3), *Aedes* (8), *Armigeres* (1), *Orthopodomyia* (1) and *Toxorhynchites* (1) were found breeding in the treeholes, while 20 species including disease vectors *An. culicifacies, Cx. quinquefasciatus, Ae. albopictus, Cx. tritaeniorhynchus* and *Cx. vishnui* were found resting. The study showed that so far malaria vectors have not exploited the breeding potential of treeholes but *Aedes albopictus*, vector of dengue/ dengue haemorrhagic fever in Asia, was one of the main species breeding and resting in the treeholes. The paper describes seasonality, interspecific association and some new species breeding/resting in treeholes in Orissa.

Keywords: Interspecific association, Malaria, Mosquito breeding, Treeholes

INTRODUCTION

Owing to a large-scale deforestation in north western Orissa in recent years, the forest cover has dwindled to a great extent and presently only one third area has forest cover. This has introduced ecological changes and has necessitated reassessment of breeding and resting pattern of mosquitoes in the changed scenario. We recently reported the indoor resting pattern of mosquitoes in this area[1], and this paper describes results of study on the breeding and resting of mosquitoes in tree-holes.

Malaria Research Centre (Field Station), Civil Hospital, Nadiad-387 001, India.
[a]Malaria Research Centre, 20-Madhuvan, Delhi-110 092, India.
[b]Malaria Research Centre (Field Station), Medical College Building, Jabalpur-482 003, India.

MATERIALS AND METHODS

The study was conducted in a forest ecosystem in District Sundargarh, Orissa. The area is a part of Garhjat hills in the eastern plateau with an average elevation of 400-500 m. The forest on hills is deciduous monsoon type with a predominance of Sal (*Shorea robusta*)[2]. Other major tree species are Cadam (*Anthocephalus cadamba*), Safflower (*Carthamus tinctorius*), Plumberry (*Eugenia jambolana*), Mango (*Mangifera indica*), Jackfruit (*Artocarpus integrifolius*), Tamarind (*Tamarindus indica*), Mahuva or Mahul (*Madhuca longifolia*), Guava (*Psidium guajava*) and Banyan (*Ficus benghalensis*). The climate is tropical monsoon type and the total rainfall during 1990 and 1991 was 1448 and 1322 mm, respectively. Broadly, seasons prevailing in this area are described earlier[1] Although, it rained in almost all months, the monsoon season extends from June to September (Fig. 1). Based on the meteorological data in Rourkela town the mean monthly temperature ranged from 20.4 to 37.3°C.

Four villages in the forest namely, Kaliaposh, Barsuan, Sanramlohi and

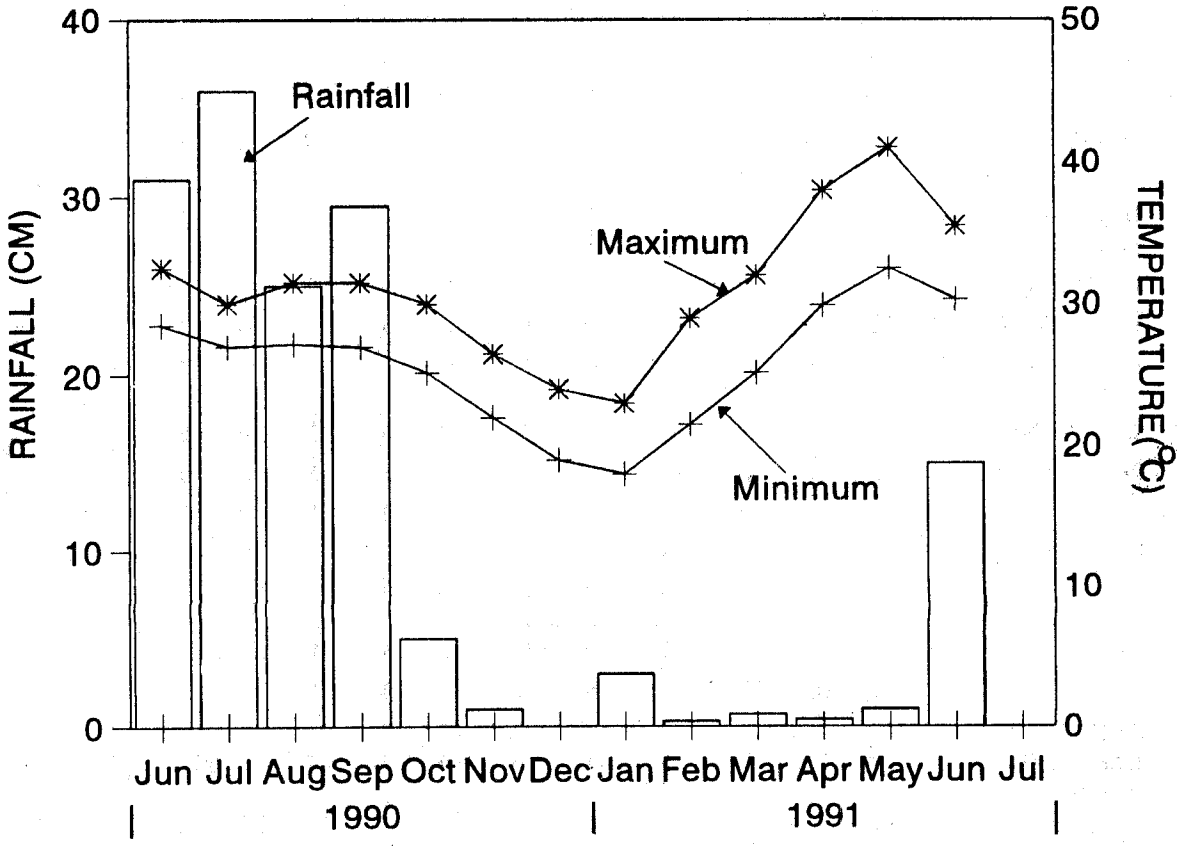

Fig. 1: Mean monthly temperature and distribution of rainfall in the study area

Table 1. Mosquito breeding in the treeholes (July 1990–June 1991)

Species	Area	Number of adults emerged from samples of immatures												
		Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total
An. subpictus	V	0	4	0	0	0	0	0	0	0	0	0	0	4
An. vagus	F	1	0	0	0	0	0	0	0	0	0	0	0	1
Cx. mimulus	V	1	0	0	0	0	0	0	0	0	0	0	0	1
Cx. brevipalpis	F	18	17	0	43	40	76	0	33	30	0	33	25	315
	V	0	27	30	14	1	0	0	0	0	0	0	0	72
Cx. nilgiricus	F	15	4	0	3	5	2	0	0	0	0	0	0	29
	V	2	5	8	1	2	0	0	0	0	0	0	0	18
Ae. albopictus	F	19	11	0	13	21	10	38	21	13	0	73	68	287
	V	47	51	8	19	16	0	0	10	6	0	0	5	162
Ae. lophoventralis	F	6	1	0	9	0	0	0	0	0	0	0	0	16
	V	3	15	5	0	0	0	0	0	0	0	0	0	23
Ae. thomsoni	F	3	2	0	1	0	0	0	0	0	0	6	4	16
	V	0	1	0	0	0	0	0	0	0	0	0	0	1
Ae. vittatus	F	4	1	0	4	2	0	0	0	0	0	0	0	11
	V	6	4	0	0	0	0	0	0	0	0	0	2	12
Ae. albolateralis	V	0	0	1	0	0	0	0	0	0	0	0	0	1
Ae. flavopictus	V	1	0	0	0	0	0	0	0	0	0	0	0	1
Ae. dissimilis	F	12	0	0	0	0	0	0	0	0	0	0	0	12
Ae. pseudotaeniatus	F	0	0	0	0	0	0	0	0	1	0	3	8	12
Ar. obturbans	F	0	0	0	0	0	0	0	0	35	0	1	2	38
Or. anopheloids	F	0	0	0	53	20	10	2	47	1	0	0	0	133
	V	0	0	0	0	9	0	0	0	0	0	0	0	9
Tx. splendens	F	12	2	0	6	1	1	2	2	0	0	0	0	26
	V	2	1	3	0	0	0	0	0	0	0	0	0	6
Total	F	90	38	N	132	89	99	42	103	80	0	116	107	896
	V	62	108	55	34	28	0	0	10	6	0	0	7	310
Treeholes with breeding/No. checked	F	20/67	16/75	N	12/35	14/31	11/35	8/18	16/24	11/20	0	15/27	12/25	145/347
	V	17/73	31/73	17/62	3/10	4/26	0/1	0/1	4/6	1/1	0	0	1/4	79/256

V — Villages; F — In the forest away from villages; N — Not surveyed.

Bardramlohi of Bisra block were included in the study. Survey was done in a perimeter of 200 m of the villages as well as in an uninhabited site about two km away from the villages in the forest. Holes in tree trunks up to 3 m above the ground were searched. Day resting mosquitoes in the hollow tree trunks and larvae/pupae from the rain filled treeholes were collected once a month from July 1990 to June 1991. Mosquito immatures were collected using a dropper and dipper with the help of a flashlight. Adults were caught using an aspirator. Species of the adult mosquitoes caught as well as those emerged from immatures in the insectary were identified and recorded. Depth of water and size of the holes were also measured. The water pH was measured using a digital pH meter.

RESULTS AND DISCUSSION

Mean size of the treeholes was– length: 19±14 cm (range 2-70 cm); width: 11±7.4 cm (range 1-40 cm); and depth: 11.8±7 cm (range 2-45 cm). The pH ranged from 6.2 to 9.2. During the study 79 (31%) out of 256 samples taken from the treeholes in the villages were found positive for mosquito breeding (Table 1). Similarly, out of 387 hollow trunks checked, mosquitoes were found resting in 74 (19.1%). In an uninhabited forest, larval breeding was recorded from 41.8% (145/347) treeholes, whereas adults were caught from 21.8% (92/421) trunks. Although breeding or resting was associated with 45 tree species, major supporting species in and around the villages were Guava, Kusum, Sal, Mango, Mahuva and Peepal, and inside the forest were Cadam, Sal, Kusum, Mahuva and Peepal.

A total of 16 mosquito species belonging to six Genera namely, *Anopheles* (2), *Culex* (3), *Aedes* (8), *Armigeres* (1), *Orthopodomiya* (1) and *Toxorhynchites* (1) were recorded breeding in the treeholes (Table 1). Eight species namely, *Cx. brevipalpis, Cx. nilgiricus, Ae. albopictus, Ae. lophoventralis, Ae. thomsoni, Ae. vittatus, Or. anopheloids* and *Tx. splendens* were found breeding both in the villages as well as in the forest. While breeding of 4 species namely, *An. subpictus, Cx. mimulus, Ae. albolateralis* and *Ae. flavopictus* was restricted to the villages, another four species namely, *An. vagus, Ae. dissimilis, Ae. pseudotaeniatus* and *Ar. obturbans* were recorded only from forest. Based on total mosquito emerged from larval/pupal samples, the most abundant species were *Ae. albopictus* (37.2%), *Cx. brevipalpis* (32.1%) and *Or. anopheloids* (11.8%). Breeding of *Ae. albopictus* and *Cx. brevipalpis* was recorded almost all through the year. Breeding of most species (13/16) was recorded during June to February covering monsoon and winter seasons. During peak summer (March-May) only six species were breeding.

Adults of 20 species belonging to six Genera namely, *Anopheles* (5), *Culex*

(8), *Aedes* (4), *Armigeres* (1), *Orthopodomyia* (1) and *Toxorhynchites* (1) were collected from the hollow tree trunks (Table 2). Of these, 11 species namely, *An. culicifacies, An. vagus, Cx. brevipalpis, Cx. quinquefasciatus, Cx. tritaeniorhynchus, Cx. nilgiricus, Cx. bitaeniorhynchus, Cx. vishnui, Cx. sinensis, Ae. albopictus*, and *Ae. thomsoni* were recorded both from the villages and forest. *Culex fuscanus* and *Ar. kuchingensis* were found only in the villages, whereas remaining six namely, *An. theobaldi, An. maculatus, Ae. lophoventralis, Ae. vittatus, Or. anopheloids* and *Tx. splendens* were recorded from the forest only. Based on total collections the major species resting in the treeholes were *Cx. brevipalpis* (54.7%), *Ae. albopictus* (14.4%) and *Cx. tritaeniorhynchus* (9.5%).

The mosquitoes tend to rest in treeholes mostly during monsoon and the number of species declined thereafter in summer. Notable among the vector species resting outdoors in treeholes were *An. culicifacies, Cx. quinquefasciatus, Cx. tritaeniorhynchus* and *Cx. vishnui.*

There were 10 species namely, *An. culicifacies, An. theobaldi, An. maculatus, Cx. quinquefasicatus, Cx. tritaeniorhynchus, Cx. bitaeniorhynchus, Cx. vishnui, Cx. sinensis, Cx. fuscanus* and *Ar. kuchingensis* which rested as adult but did not breed in the treeholes. There were another six species namely, *Cx. mimulus, Ae. albolateralis, Ae. flavopictus, Ae. dissimilis, Ae. pseudotaeniatus* and *Ar. obturbans* which were found breeding but did not rest in the treeholes.

Among the major treehole breeding species, degree of association (+1 to -1) was calculated after Cole as described by Service[3] by taking into account of the frequencies of co-occurrences in treeholes (Table 3). There was one positive association between *Cx. brevipalpis* and *Or. anopheloids* (0.4±0.16). There were three negative associations namely, *Ae. albopictus* with *Or. anopheloids* (-0.52±0.18) and *Tx. splendens* (-0.57±0.17), and *Cx. brevipalpis* with *Ae. lophoventralis* (-0.53±0.25). Among all other major co-occurrences there was partial repulsion.

The pH of water in the treeholes ranged from 6.2 to 9.2. In 80% of the samples with larval breeding the water was alkaline. The adaptive range of pH for major treehole breeders was: *Cx. brevipalpis* (6.5-8.6), *Cx. nilgiricus* (6.7-8.6), *Ae. albopictus* (6.2-9.2), *Ae. lophoventralis* (6.7-8.3), *Ae. vittatus* (6.7-9.2), *Or. anopheloids* (6.5-8.7), and *Tx. splendens* (6.6-8.6).

There are several reports on the breeding and resting of mosquitoes in the treeholes in different areas of India[4-8]. Breeding of *Ae. aegypti* in treeholes was reported in Calcutta[9], Mettupalaiyam[10], Poona[11], Pondicherry[12] and Vellore[13] cities. In Nilgiri hills searches of treeholes yielded larvae of *Ae.*

Table 2. Adult mosquitoes caught resting from the treeholes (July 1990–June 1991)

Species	Area	Number of adults caught												
		Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total
An. culicifacies	F	1	2	0	0	0	0	0	0	0	0	0	0	3
	V	0	4	0	2	1	0	0	0	0	0	0	0	7
An. vagus	F	0	2	0	0	0	0	0	0	0	0	0	0	2
	V	1	1	0	0	0	0	0	0	0	0	0	0	2
An. subpictus	V	1	0	0	0	0	0	0	0	0	0	0	0	1
An. theobaldi	F	0	0	0	1	0	0	0	0	0	0	0	0	1
An. maculatus	F	0	0	0	1	0	0	0	0	0	0	0	0	1
Cx. brevipalpis	F	0	7	0	12	21	48	7	27	7	9	0	15	153
	V	0	15	8	15	7	2	3	2	2	8	3	2	67
Cx. quinque-fasciatus	F	13	0	0	0	1	1	1	0	0	0	0	0	16
	V	1	1	0	2	0	0	0	0	0	0	0	0	4
Cx. tritaenior-hynchus	F	1	0	0	0	1	1	0	0	0	0	0	0	3
	V	17	0	12	0	6	0	0	0	0	0	0	0	35
Cx. nilgiricus	F	6	5	0	0	0	0	0	0	0	0	0	0	11
	V	15	1	0	0	0	0	0	0	0	0	0	0	16
Cx. bitaenior-hynchus	F	2	0	0	0	0	0	0	4	0	0	0	0	6
	V	1	0	0	0	0	0	0	0	0	0	0	0	1
Cx. vishnui	F	0	0	0	1	0	0	0	0	0	0	0	0	1
	V	0	0	5	0	0	0	0	0	0	0	0	0	5
Cx. sinensis	F	0	1	0	2	0	0	0	0	0	0	0	0	3
	V	0	1	8	0	0	0	0	0	0	0	0	0	9
Cx. fuscanus	V	1	0	2	0	0	0	0	0	0	0	0	0	3
Ae. albopictus	F	9	3	0	0	8	4	2	1	3	9	1	4	44
	V	1	4	5	0	3	0	0	0	0	0	0	1	14
Ae. thomsoni	F	4	11	0	0	1	0	0	0	0	0	0	1	17
	V	8	0	0	0	0	0	0	0	0	0	0	0	8

contd...

Table 2. (*contd.*)

Species	Area	Number of adults caught												
		Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total
Ae. lophoventralis	F	0	3	0	1	1	0	0	0	0	0	0	0	5
Ae. vittatus	F	0	0	0	0	2	0	0	0	0	0	0	0	2
Ar. kuchingensis	V	0	0	3	0	0	0	0	0	0	0	0	0	3
Or. anopheloids	F	0	0	0	1	0	0	0	0	0	0	0	0	1
Tx. splendens	F	6	2	0	0	0	0	0	0	0	0	0	0	8
Total	F	42	36	N	19	35	44	10	32	10	18	1	20	277
	V	46	27	43	19	17	2	3	4	2	8	3	3	175
Holes with adults/No. checked	F	12/ 73	11/ 74	N	14/ 45	12/ 47	16/ 73	4/ 19	8/ 52	5/ 11	6/ 12	1/ 5	3/ 10	92/ 421
	V	21/ 80	13/ 82	19/ 81	6/ 29	5/ 28	1/ 30	1/ 12	1/ 9	1/ 14	3/ 7	1/ 8	2/ 7	74/ 387

V — Villages; F — In the forest away from villages; N — Not surveyed.

Table 3. Coefficients of association between major treehole breeding species

Species	*Ae. albopictus*	*Cx. brevipalpis*	*Tx. splendens*
Cx. brevipalpis	−0.36 ± 0.09		
Cx. nilgiricus	−0.34 ± 0.24		
Ae. vittatus	−0.27 ± 0.02		
Ar. obturbans	−0.34 ± 0.32		
Ae. lophoventralis	−0.17 ± 0.18	−0.53 ± 0.25	
Tx. splendens	−0.57 ± 0.17	−0.19 ± 0.2	
Ae. thomsoni		−0.23 ± 0.39	
Ae. pseudotaeniatus		0.18 ± 0.27	
Or. anopheloids	−0.52 ± 0.18	0.4 ± 0.16	−0.1 ± 0.4

albopictus, *Ae. thomsoni*, *Cx. fatigans* and *Armigeres* sp but no *Ae. aegypti*[10]. In a canal irrigated area in Gujarat Srivastava[14] reported the breeding of *An. subpictus*, *Cx. quinquefasciatus* and five *Aedes* species in treeholes.

In Orissa a study in Koraput district[15] reported resting of *An. aconitus*, *An. annularis*, *An. fluviatilis*, *An. jeyporiensis* and *An. maculatus* in treeholes, while other[16] from Koraput and Phulbani districts reported both breeding and rest-

ing of *An. culicifacies* and *Ae. aegypti*, besides resting alone of *An. barbirostris*, *An. fluviatilis*, *An. jeyporiensis*, *An. nigerrimus*, *Cx. tritaeniorhynchus* and *Cx. vishnui*. The present paper thus, reports for the first time breeding of 16 species listed in Table 1 and resting of 17 species listed in Table 2 (all except Sl. No. 5, 8 and 11) in treeholes in Orissa.

In the study area *Ae. albopictus* was the most common breeder in treeholes like Nilgiri hills and Gujarat. Since dengue cases have been reported from Ispat General Hospital in Rourkela town in 1993, there is a need to keep vigil on occurrence of dengue in rural areas, particularly at project/mining sites in the forest, as *Ae. albopictus* is reported to transmit dengue/dengue haemorrhagic fever in Asia[17]. In the present study, breeding of malaria vectors *An. culicifacies* and *An. fluviatilis* was not recorded which suggests that so far the species have not adapted to treeholes in the area.

The adult mosquitoes did not rest in those treeholes which retained water, possibly because little dark space was left above the water surface in the holes thereby rendering the exposed area unsuitable for resting. The adults rested only in shaded places in hollow trunks. Outdoor resting of *An. culicifacies* and *Cx. quinquefasciatus* which are otherwise considered as predominantly endophilic species, suggests either a possible shift to outdoors due to irritant effect of indoor residual spraying of DDT for last 35 years, or the presence of a small population naturally resting outdoors during favourable climatic conditions.

The study thus showed that the treeholes supported the breeding of several mosquito species but the malaria vectors in the area namely *An. culicifacies* and *An. fluviatilis* have so far not exploited the breeding potential of treeholes in the forest ecosystem in northwest Orissa although a small population of the former tend to rest in them, and *Ae. albopictus* was the main species breeding in treeholes.

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Malaria in Pregnancy in Nigerians: Seasonality and Relationship to Splenomegaly and Anaemia

O.A. EGWUNYENGA, J.A. AJAYI[a] and D.D. DUHLINSKA-POPOVA[a]

The effect of malaria parasitaemia on spleen size and anaemia in 1,905 pregnant women in Jos Plateau highlands, Bauchi Savannah plains and Ethiope river basin of Nigeria was evaluated. The overall spleen rates in Jos Plateau, Bauchi and Ethiope were 15, 23, 16.33 and 10.71% respectively. Higher cases of palpable spleen were detected in pregnant women than non-pregnant controls. Spleen rates also showed seasonal variation, but not very significant. Malaria prevalence rates were higher than spleen rates. In all three study sites, parasitaemic pregnant women had significantly lower haemoglobin values than malaria negative mothers, especially among primigravids. However, there was no constant association between higher parasite density and splenomegaly, since few cases of enlarged spleens were also recorded among subjects with low parasitaemia. Severe anaemia was predominant among parasitaemic pregnant women with high spleen classes.

Keywords: Anaemia, Malaria, Nigerians, Pregnancy

INTRODUCTION

The pattern of malaria in Nigeria is largely hyperendemic to holoendemic[1]. Of the estimated 50 million people affected each year, children and women constitute the major group. The occurrence and clinical consequence of malaria on materno-foetal relationship have been a focus of attention in many endemic areas[2-7].

Some of these reports have highlighted splenomegaly and anaemia as possible

Zoology Department, Delta State University, Abraka, Nigeria.
[a]Applied Parasitology Research Laboratory, Department of Zoology, University of Jos, Nigeria.

clinical complications of malaria in pregnancy. The incidence of anaemia and splenomegaly in malaria parasitaemic pregnant women have been explained on the basis haemolysis of red blood cells[8] and their subsequent clearance in the spleen by the lymphoid-macrophage system.

Although the Nigerian reports[9,10] from western Nigeria indicate that splenomegaly and anaemia may occur in pregnant women, there is lack of adequate information on the influence of seasonality, parity and intensity of infection on splenomegaly and anaemia in malaria parasitaemic pregnant women. In addition the occurrence of anaemia in relation to splenomegaly in malarious pregnant women needs to be investigated.

This study was undertaken to determine the seasonal incidence of malaria in women in three climatically distinct regions of Nigeria; the effect of intensity of infection on anaemia and splenomegaly; and the relationship between anaemia and splenomegaly in malarious parturient women in the study area.

MATERIALS AND METHODS

Study area

The study was carried out in public hospitals in Bauchi, Jos Plateau and Ethiope areas of Nigeria (Fig. 1). These sites were chosen to reflect the epidemiological influence of rainfall patterns, altitude, climate and types of settlements on endemicity of malaria.

Bauchi, which is located between longitude 9-12°E and latitude 9-13°N is a part of the Sahelian region, usually hot in the month of April with temperature ranging from 40-50°C, and cold in December with temperature as low as 12°C. The rainy season is short between June and September. Maximum annual rainfall is about 1250 mm. The inhabitants are predominantly farmers. Human settlement is semi-urban.

Jos Plateau is an urban settlement located at latitude 10.6°N and longitude 9°E. The area is one of the highest points in Nigeria (1,948 m above sea level). The rainy season lasts for seven months, May-October, with an average rainfall of about 1,500 mm. The average minimum and maximum temperature range is 9-32°C. Although majority of the inhabitants are engaged in agricultural activities, tin and columbite mining also employs a significant proportion of the population. Mining and quary activities have resulted in semi-permanent water bodies in various parts of the metropolis.

Rural communities around Ethiope River selected for the study are located between latitude 5°N-6°S and longitude 5.5°E-6.5°N. The vegetation is tropical rainforest. There is abundant rainfall for most of the year; about 8-9 months with a peak in July-October. The yearly average rainfall is over 2000 mm. Dry

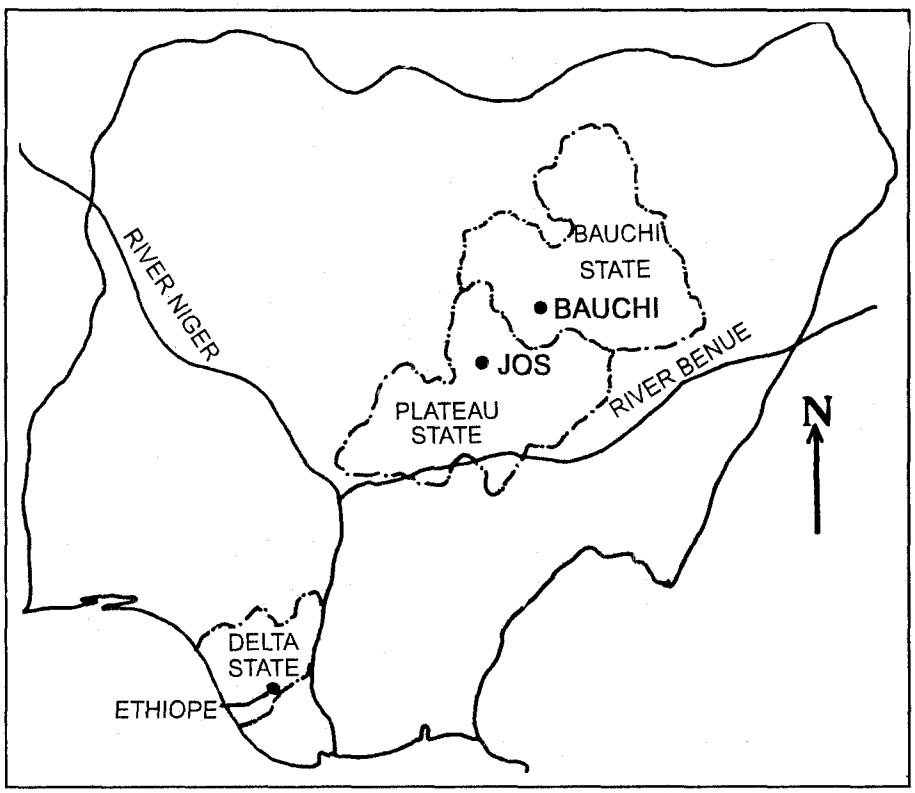

Fig. 1: Map of Nigeria showing location of the study sites

season remains from November-February.

Subjects and blood examination

A total of 3,355 subjects comprising 1,905 pregnant women and 1,450 non-pregnant were investigated between February 1994 and January 1995. The number of pregnant women coming from each area were 830, 652 and 423 in Bauchi, Jos Plateau and Ethiope respectively. The breakdown of non-pregnant subjects with respect to study sites in 650 in Bauchi, 400 in Jos Plateau and 400 in Ethiope.

Patient's history relating to age, location, whether pregnant or not pregnant, gestational age, parity and whether or not the subject has been on malaria chemoprophylaxis were obtained. These information were further confirmed from the hospital records with the cooperation of the medical records staff.

Thick and thin blood films were made from each blood sample and stained by Field's and Giemsa techniques respectively[11]. Thick films were examined under 100x oil immersion objective and density of parasitised red blood cells

Table 1. Seasonal prevalences of malaria and spleen rates

Season	Bauchi Savannah				Jos Plateau	
	% prevalence		% spleen rate		% prevalence	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant
Feb-Apr	23.0 (186)	14.20 (160)	14.31	8.40	23.60 (178)	20.51 (98)
May-Jul	21.43(238)	9.60 (146)	12.58	6.25	23.03 (105)	21.30 (105)
Aug-Oct	25.0 (208)	15.84 (170)	18.60	7.41	32.67 (150)	26.47 (140)
Nov-Jan	19.70(198)	10.80 (174)	15.46	4.52	15.70 (159)	9.66 (93)
Overall	22.29(830)	12.61 (650)	15.24	6.65	23.62 (532)	19.50 (436)

Number of subjects given in parentheses.

was estimated by counting trophozoites concomitantly with white blood cells (WBC) in each field and positive smear recorded as a ratio of trophozoites per 200 WBC[7]. Parasite densities were calculated by multiplying the number of trophozoites per 200 WBC by average WBC count of maternal population. Thin films were examined only if thick films were positive to identify *Plasmodium* species.

Blood samples were also collected from freely flowing capillary blood and the packed-cell volume measured with a microhaematocrit centrifuge and heparinised capillary tubes.

The haemoglobin concentration of each blood sample was also determined using the technique described by Dacie and Lewis[12].

Spleen examination

Spleen examination by palpitation was carried out on all 3,355 subjects as described by Bruce-Chwatt[13]. The degree of enlargement of the spleen was classified from 0, indicating normal spleen to 5, indicating very enlarged spleen, palpable beyond the symphysis pubis[14]. Spleen rates were then determined from data collected.

RESULTS

Malaria occurred in pregnant subjects from the three study sites to varying degrees (Table 1). The pattern of malaria in Bauchi and Jos Plateau was similar with pregnant subjects showing higher rates of infection than non-pregnant controls, but this was statistically significant ($p<0.05$) only in Bauchi. In Ethiope non-pregnant women were more infected than pregnant subjects, but this was not significant ($p>0.05$).

Comparison of seasonal prevalence in Jos Plateau and Bauchi reveal that no significant difference occurred in the rate of infection, except in Ethiope

in pregnant and non-pregnant women in the study area

Jos Plateau		Ethiope			
% spleen rate		% prevalence		% spleen rate	
Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant
16.10	10.30	5.66 (106)	8.50 (103)	3.28	0
14.20	9.61	18.37 (98)	23.45 (101)	10.61	2.5
26.61	20.50	27.78 (108)	31.61 (93)	24.15	16.2
8.40	6.82	7.20 (111)	12.44 (103)	4.81	0.97
16.33	11.81	14.66 (423)	19.0 (400)	10.71	4.91

where significant difference ($p<0.05$) was observed in infection rate between the period of high rainfall (August-October) and period of low rainfall (February-April). Infection rates were significantly ($p<0.05$) higher than spleen rates in the three sites studied.

Data on mean haemoglobin values in relation to malaria infection show that in all study sites and for both parities, parasitaemic pregnant women had significantly lower haemoglobin values than their malaria negative counterparts, particularly among primigravidae (Table 2). Larger spleens consistently occurred among subjects with low haemoglobin values. This was more among primigravidae where all subjects in the largest spleen class (5) were severely anaemic (Hb conc. less than 8 gm/dl). Splenomegaly generally increased with increasing parasite density (Table 3). However, there was no constant association between higher parasite density and splenomegaly, since enlarged spleens were recorded even with very light infections (<100 parasites/cu cm of blood) in Bauchi. In addition cases of normal spleen were recorded in Jos Plateau and Ethiope among subjects with high parasitaemia ($\leqslant$ 10,000 parasites cu/cm of blood).

Mean packed cell volume (PCV) values decreased with increases in parasite density (Table 3). This decrease was constant and more pronounced among primigravid mothers. Anaemia (PCV $\leqslant$ 30%) occurred among all primigravids in the study sites with more than 100 parasites cu/cm of blood. In contrast, anaemia did not occur in all multigravidae regardless of the intensity of infection.

DISCUSSION

The higher prevalence of malaria infection in pregnant subjects than non-pregnant controls in Bauchi and Jos Plateau is consistent with the findings of other workers in Africa[2,15-17]. The contrasting situation in Ethiope can

Table 2. Mean haemoglobin values ($gdl^{-1} \pm SD$) in pregnant women and their relationship to spleen size

Locality/Parity	Mean haemoglobin values ($gdl^{-1} \pm SD$)					
	Malaria (+)ve	Malaria (-)ve	p*	Spleen size (Hackett's classification)		
				0	1-4	5
Bauchi						
Primigravidae	9.6±1.1	10.6±0.6	0.001	10.1±0.9	8.5±1.1	6.9±1.9
Multigravidae	10.3±1.6	11.2±0.2	0.001	10.6±0.6	9.4±1.3	8.6±1.5
Jos Plateau						
Primigravidae	9.8±1.5	11.0±1.3	0.001	10.8±1.5	8.9±1.2	7.1±1.3
Multigravidae	10.8±1.2	11.3±1.2	0.001	11.2±1.0	9.6±1.6	8.8±1.4
Ethiope						
Primigravidae	8.8±1.8	10.2±1.9	0.001	10.2±1.9	8.2±1.5	6.7±1.8
Multigravidae	10.4±1.2	11.6±1.5	0.001	11.6±1.5	9.7±1.1	9.2±1.6

*Student's *t*-test used to compare mean Hb values.

Table 3. Mean parasite density (peripheral blood) in relation to per cent spleen class and mean packed cell volume values (%) of parasitaemic pregnant subjects

Parasite density (No./cu cm of blood)	% in each spleen class			Mean PCV (% ± SD)	
	0	1-4	5	Primigravidae	Multigravidae
Bauchi					
<100	51.9	1.08	0	30.6±1.6	34.8±1.3
101-1,000	18.4	4.3	0	28.8±1.5	33.6±1.4
1,001-10,000	8.1	4.3	1.08	28.2±1.2	31.8±1.5
>10,000	1.6	6.5	2.7	25.8±1.5	31.2±1.5
Jos Plateau					
< 100	63.6	0	0	30.0±1.4	34.5±1.4
101-1,000	16.9	0.7	0	28.2±1.6	33.9±1.6
1,001-10,000	10.4	1.9	0	27.9±1.4	32.4±1.2
>10,000	0	3.9	2.6	26.7±1.3	31.8±1.2
Ethiope					
<100	61.3	0	0	31.2±1.3	34.8±1.1
101-1,000	17.7	0	0	30.3±1.4	33.6±0.9
1,001-10,000	4.8	3.2	0	29.4±1.1	33.0±1.2
>10,000	0	6.5	6.5	28.8±1.5	32.4±1.6

be attributed to the situation in the local hospitals where all parasitaemic pregnant women on first antenatal visit are routinely placed on chemoprophylactic drugs.

Although malaria prevalence and spleen rates were higher during the peak wet season (August-October), the occurrence was not markedly seasonal, except in the Ethiope area. The pattern of malaria in Bauchi and Jos Plateau is typical of urban and suburban malaria, with reduced fluctuations in prevalences with season. Therefore, although prevalence of infection and spleen rates increased at the peak rainfall, it was not significant.

The pattern in Ethiope is clearly different. Here transmission is seasonal; mainly in the rainy season (May-July and August-October) (Table 1). This is typical of rural malaria in rainforest regions of Africa. Similar results were obtained by McGregor[18] in rural Gambia, where malaria transmission was high at the peak rainy season between May and November and falls to negligible levels during dry season. High infection and spleen rates in the wet season result not only from increased number of infections but also due to decrease in recovery rate of infection in pregnancy[19]. Parasitaemia in the dry season may be due to a small proportion of wet season primary infections which persist through the dry season also[20,21].

Lower mean haemoglobin levels of malaria positive mothers when compared to non-parasitaemic subjects support earlier findings by Fleming *et al.*[8] in Zaria, where malaria is the major cause of anaemia during pregnancy. Table 2 illustrates the fact that anaemia becomes increasingly severe with increase in the size of the spleen. Subjects with spleen class up to 5, especially among primigravids showed average haemoglobin concentration of about 7.0 g/dl. Since anaemia may also develop in pregnant women with or without splenomegaly (Table 2) especially in primigravidae, the role of other causes of anaemia such as iron or folic acid deficiency needs to be quantified. The significant reduction in mean haemoglobin concentration in relation to increase in spleen size confirms that spleno-megaly is an important cause of anaemia, especially among primigravidae (Table 2) .

In this study, the largest spleens (5) were recorded among subjects with heavy parasitaemia (>10,000 parasites/cu cm of blood) leading to anaemia in primigravidae. Thus severe anaemia and splenomegaly are associated with heavy parasitaemia. However, most of the cases of splenomegaly (1-4 spleen classes) occurred among subjects with moderate infection (<10,000 parasites/cu cm of blood), which also led to anaemia in primigravids (Table 3). This therefore suggests that haemolysis of parasitised red blood cells alone can not account for all cases of anaemia.

A final conclusion from this study is that although considerable variation occur in malaria infection and spleen

rates among pregnant women in the study area, the pattern of associated complication of splenomegaly and anaemia are quite similar.

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Field Evaluation of *Bacillus sphaericus*, H5a5b and *B. thuringiensis* var. *israelensis*, H-14 against the Bancroftian filariasis Vector *Culex quinquefasciatus*, Say in Chennai, India

INDRANIL KAR, ALEX EAPEN, K. JOHN RAVINDRAN[a], R.K. CHANDRAHAS, N.C. APPAVOO[b], A.V. SADANAND[b] and B. DHANRAJ[c]

Fortnightly application of *Bacillus sphaericus* (strain B101, serotype H5a5b) and *B. thuringiensis* var. *israelensis* (strain 164, serotype H-14) in two different waterways of Chennai @ 1 g/sq m surface area has resulted in significant reduction in both immature and adult densities of *Culex quinquefasciatus* Say. The use of these biolarvicides as biocontrol agents is suggested in the urban areas to control mosquitoes in general.

Keywords: *Bacillus sphaericus*, *B. thuringiensis* var. *israelensis*, Chennai, *Culex quinquefasciatus*

INTRODUCTION

Culex quinquefasciatus is one of the major nuisance mosquito and a known vector of bancroftian filariasis in Chennai. The city's waterways such as Coovum, Adyar Rivers, Buckingham, Railway Canals and Otteri Nullah are some of the major sources of its breeding. All these waterways carry sewage

Malaria Research Centre (Field Station), 1304, Anna Nagar (West), Mogappair, Chennai-600 050, India.
[a]Malaria Research Centre (Field Station), Epidemic Diseases Hospital, Bangalore-560 038, India.
[b]Directorate of Public Health and Preventive Medicine, Govt. of Tamil Nadu, Chennai-600 006, India.
[c]Municipal Corporation of Chennai, Rippon Building, Chennai-600 003, India.

and waste-water. In addition, the choked storm water drains also facilitate the proliferation of mosquito breeding.

Chennai is under National Filaria Control Programme (NFCP) since 1972. The city shows a decreasing trend in the incidence of bancroftian filariasis (Table 1) but still the number of cases reported every year is a cause for concern. Various insecticides namely, larvicidal oils, fenthion temephos etc. are applied at weekly intervals to control mosquito density in the city. Concern about environmental contamination associated with the use of these pesticides and resistance in target species have stimulated interest in alternate methods of mosquito control. In recent times biological control of mosquitoes with larvivorous fish and entomopathogenic organisms are receiving increased attention. Among the

Table 1. Incidence of Bancroftian filariasis in Chennai

Year	Blood smear collected	Microfilaria (+)ve	Manifestation	Microfilaria rate	Manifestation rate	Endemicity rate
1973	4889	415	–	8.5	–	8.5
1974	44002	3299	498	7.5	1.1	8.6
1975	45617	1741	96	3.8	0.2	4.0
1976	29543	1268	38	4.3	0.1	4.4
1977	35519	2069	152	5.8	0.4	6.3
1978	29961	1646	106	5.5	0.4	5.9
1979	34807	1606	152	4.6	0.4	5.1
1980	43834	1926	203	4.4	0.5	4.9
1981	44892	1768	227	3.9	0.5	4.4
1982	62318	1639	387	2.6	0.6	3.3
1983	68332	1375	402	2.0	0.6	2.6
1984	87579	988	538	1.1	0.6	1.7
1985	102594	1546	594	1.5	0.6	2.1
1986	117096	1529	518	1.3	0.4	1.8
1987	117716	944	402	0.8	0.3	1.1
1988	115384	1041	372	0.9	0.3	1.2
1989	114050	642	204	0.6	0.2	0.7
1990	113895	359	188	0.3	0.2	0.5
1991	111752	238	147	0.2	0.1	0.3
1992	121001	163	103	0.1	0.1	0.2
1993	120154	343	274	0.3	0.2	0.5
1994	121772	290	135	0.2	0.1	0.4

Source: Chennai Corporation.

pathogens the endotoxin producing bacterial strains of *Bacillus sphaericus* Neide and *B. thuringiensis* var. *israelensis* hold promise for mosquito control programmes. These two biolarvicides are completely safe for the non-target organisms[1,2]. Earlier studies with *B. sphaericus* and *B. thuringiensis* have shown effectiveness in the control of mosquitoes[3-5] In India various formulations of these two biolarvicides have been tested against immatures of different mosquito species under both laboratory and field conditions[6-12]. Dua *et al.*[13] have achieved successful control of mosquito breeding with Bactoculicide (*B. thuringiensis* H-14) in industrial scraps. Therefore, a study was undertaken in Chennai to supplement the current antilarval measures against *Cx. quinquefasciatus*. A large-scale field evaluation of Spherix (*B. sphaericus*, strain B101, serotype H5a5b)* and Bactoculicide (*B. thuringiensis* var. *israelensis*, strain 164, serotype H-14)* formulations were conducted under regular operations of mosquito control programme of Chennai Corporation. This paper reports the findings of the study.

MATERIALS AND METHODS

Study area

Chennai city is situated along the eastern coast of India between 13°-4'N latitude and 80°-15'E longitude with an area of 173.53 sq km and 3.8 million population (1991 census). It has warm and humid climate with mean temperature and humidity ranging from 36.5 to 20.8°C and 84 to 65% respectively. It receives both southwest and northeast monsoon with an average rainfall of about 1215.4 mm every year.

A study was undertaken in Coovum River and Otteri Nullah with the application of Spherix and Bactoculicide formulations respectively (Fig. 1) to control mosquito breeding. The railway canal was selected as the control area. The areas selected were surveyed, mapped, divided into zones and subzones for the purpose of application and evaluation. All the breeding sources except potable water sources located within one km on either side of the major water bodies were treated with biolarvicides. The main waterways brought under application were:

Coovum River: It includes seven km long stretch from Anna Nagar to bridge near Connemera Hotel, subdivided into 5 zones (viz. A, B, C, D and E) and 29 subzones comprising 50,000-60,000 sq m sprayable surface area. The area is very vast and at places inaccessible to carry out spray operations due to thorny bushes at the banks. From the upstream the water during its course towards sea becomes more and more polluted due to addition of sewage and other waste water from different sources. The margin is not well de-

*The biocides were manufactured by the Berdsk Plant of Biological Preparations, Russia and supplied under the trade name of Spherix and Bactoculicide through the courtesy of Ministry of Health and Family Welfare, Government of India, New Delhi.

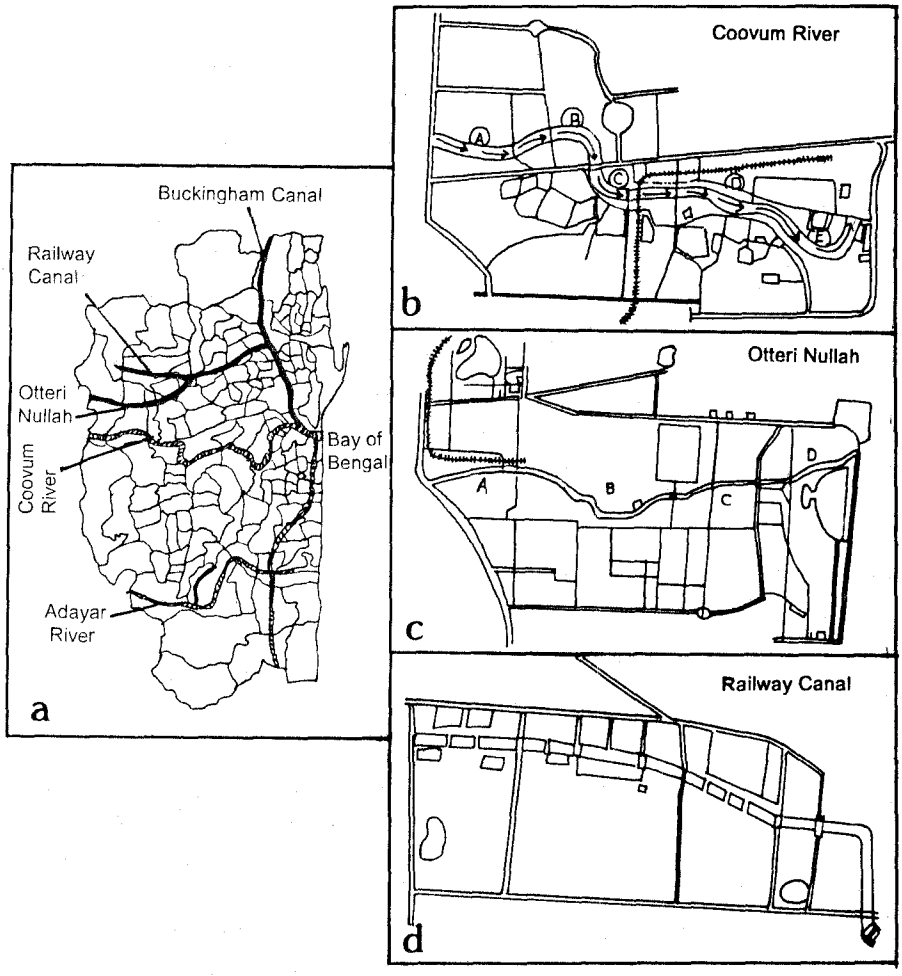

Fig. 1: Maps showing (a) Chennai city's major waterways, (b) Spherix trial area, (c) Bactoculicide trial area, and (d) Control area

fined at certain places. The movement restriction in zone A allows the water to stagnate covering a larger area in the form of a large lagoon. Growth of water hyacinth is also observed at places. Formation of numerous pits and pools during dry season (March-June) leads to the creation of additional sources for mosquito breeding.

Otteri Nullah: It stretches from Anna Nagar West to New Avadi Road (5 km), comprising 4 zones (viz. A, B, C and D) and 12 subzones with approximately 50,000 sq m sprayable surface area. The area also includes a sewage treatment plant, oxidation ponds extending to an area of about one sq km.

The margins are well defined. Certain areas are covered with thorny bushes making it inaccessible for spray opera-

tions. The sewage treatment plant and oxidation ponds were covered with water hyacinth initially that was later removed during the study. The excess sewage from the sewage treatment plant is drained directly into the Otteri through a connecting drain, creating pollution and making it conducive for *Cx. quinquefasciatus* breeding.

Railway Canal: It is a well defined canal and is about 5 km long carrying polluted water. It has about 20,000 sq m sprayable surface area.

Application of biolarvicides

Before the commencement of biolarvicides spray operations in the experimental areas the spray of conventional insecticides (Malariol, Baytex® etc.) was withdrawn. Coovum River and Otteri Nullah were sprayed with Spherix (*Bacillus sphaericus* strain B101, serotype H5a5b) and Bactoculicide (*B. thuringiensis* var. *israelensis* strain 164, serotype H-14) respectively. The fortnightly spray operations in both the sites commenced from March 1993 and continued till February 1994. The biolarvicidal application was confined to 1 m stretch in the waterways along the margin @ 1 g/sq m with the help of knapsack sprayer having flat fan nozzle. The spraying cycle was completed in about five working days each, engaging 6-10 spraymen in Spherix and Bactoculicide trial areas. Thorough deweeding was undertaken before and during the spray operations. Floats/boats were used to reach inaccessible areas.

Evaluation

Both adult and larval densities were measured zonewise in the week following the spraying for evaluating the efficacy of the formulations.

Larval densities were monitored using long handled dippers of 300 ml capacity in both experimental and control areas. Immature sampling was carried out from 50-150 points depending on the surface area in each zone. The larvae collected were brought to the laboratory, separated instarwise, counted and reared at room temperature. The adults emerged were identified using Barraud's[14] key.

The adult mosquitoes were collected by hand catch method using a suction tube and torch light in 8 fixed catching stations per zone (four near and four slightly far away from the waterways). In two zones each in Spherix and Bactoculicide trial areas only four catching stations were fixed due to non availability of proper collection sites.

The application of biolarvicides in experimental areas and evaluation in both the areas continued upto 25 rounds. The percentage reduction of III and IV instar larvae, pupae and adults were calculated as per the formula described by Mulla[15]. A two-way analysis of variance was done to study the seasonwise reduction of larvae and pupae/dip and adult man hour density for both the trial areas in comparison with the control area i.e., summer (April to July, rounds 1-8), pre-monsoon (August to

November, rounds 9-16), post-monsoon (January to March, rounds 20-25).

RESULTS AND DISCUSSION

Immature density

Pre-treatment breeding survey during February/March 1993 revealed high density of *Cx. quinquefasciatus* in both Spherix and Bactoculicide trial areas. The density of III and IV instar larvae/dip was 260 and 160 respectively. The pupal density/dip was 107 and 84 respectively in the two experimental areas. In the control area the density was 75/dip for III and IV instar larvae and 29/dip for pupae.

Impact of Spherix spraying

Spherix application brought about 98.4% reduction in III and IV instar larvae and 95.4% for pupae after first round of spray. During subsequent rounds, the reduction was maintained between 97.0 and 84.1% in case of III and IV instar larvae and between 99.8 and 83.7% for pupae (Table 2, Fig. 2). When zonewise results were analysed it was found that during certain rounds the reduction was not as pronounced in zone C as in other zones. This could be attributed to the operational problems associated with difficult terrain and inaccessibility in the zone.

Impact of Bactoculicide application

The reduction after the first round of Bactoculicide spray was 97.8% for III and IV instar larvae and 93.5% for pupae. Thereafter the reduction was between 99.4 and 82.4% for III and IV instar larvae and 99.6 and 80.3% for pupae during the subsequent rounds (Table 2, Fig. 3). The zonewise analysis revealed a steady control of breeding in zone B and D compared to reduction obtained in zone A and C. This could be due to the accessibility problem in these two zones due to which proper coverage was not possible.

The findings of two-way analysis of variance on the number of larvae/dip showed that overall there was significant difference in larval density/dip ($p<0.001$) between Spherix treated and control areas. Even during different seasons (summer, pre-monsoon and post-monsoon) the difference was significant ($p<0.01$). Similarly, reduction in larval density was also found significant ($p<0.01$) between Bactoculicide and control areas.

The reduction of pupal density, the difference between Spherix trial and control areas, as well as between the seasons was found to be significant ($p<0.001$). Similarly, reduction of pupae/dip was found to be significant between Bactoculicide trial and control areas ($p<0.05$).

Adult density

The per man hour density (MHD) of adult *Cx. quinquefasciatus* was 243 and 260 respectively for Spherix and Bactoculicide trial areas. For control

Table 2. Per cent reduction in larvae, pupae and adult densities in Spherix and Bactoculicide trial areas

Round	Spherix trial area			Bactoculicide trial area			Control area		
	Larvae/ dip	Pupae/ dip	Adults (MHD)	Larvae/ dip	Pupae/ dip	Adults (MHD)	Larvae/ dip	Pupae/ dip	Adults (MHD)
Pre-treatment	260	107	243	160	84	260	75	29	32
1	46	9	112	51	10	86	740	53	32
2	33	4	63	25	7	78	71	15	72
3	3	1	22	3	0.2	69	28	20	51
4	3	2	9	4	4	52	20	7	52
5	2	0.7	14	3	0.3	62	19	5	74
6	3	0.1	31	6	1	88	16	9	94
7	6	1	26	2	2	53	38	20	55
8	52	10	65	16	7	104	483	43	196
9	23	6	54	3	0.7	79	50	24	107
10	13	5	54	7	1	93	71	16	144
11	19	6	78	7	1	87	36	14	115
12	30	8	107	6	3	92	56	17	132
13	29	10	86	4	0.6	86	61	52	86
14	11	5	82	5	1	93	23	10	85
15	15	5	63	10	4	90	32	15	95
16	16	5	89	0.4	0.2	77	30	14	68
17*	0	0	53	0	0	77	0	0	86
18*	0	0	79	0	0	60	0	0	50
19*	0	0	41	0	0	95	0	0	90
20	2	0.4	43	1	0.1	90	12	6	91
21	6	1	60	6	1	89	45	14	201
22	8	2	105	2	0.8	139	30	10	147
23	14	3	132	3	0.7	128	45	10	227
24	22	6	141	2	0.6	117	40	10	187
25	7	6	129	6	1	100	37	11	147

*17, 18 and 19 rounds evaluation was not taken up due to rain.

contd...

Table 2. (*contd.*)

Round	Per cent reduction					
	Spherix area			Bactoculicide area		
	Larvae	Pupae	Adults	Larvae	Pupae	Adults
Pre-treat-ment						
1	98.21	95.39	53.90	97.77	93.49	66.92
2	86.59	92.77	88.47	83.49	83.89	86.66
3	96.91	98.64	94.31	94.98	94.82	83.34
4	95.67	92.26	97.72	90.63	80.27	87.69
5	96.96	96.21	97.50	92.60	98.07	89.68
6	94.59	99.82	95.65	82.42	96.16	88.47
7	95.44	98.64	93.77	97.53	96.55	88.13
8	96.89	93.70	95.72	98.45	94.38	93.46
9	86.73	93.22	93.35	97.19	99.00	90.91
10	94.71	91.53	95.06	95.38	97.84	92.05
11	84.77	88.38	91.06	90.89	97.53	90.68
12	84.54	87.25	89.32	94.98	93.91	91.42
13	86.28	94.78	86.83	96.93	99.61	87.69
14	86.20	86.45	87.29	89.81	96.55	86.53
15	86.48	90.97	91.26	85.35	90.79	88.34
16	84.61	90.32	91.88	99.38	99.58	86.06
17*	-	-	91.88	-	-	88.98
18*	-	-	79.19	-	-	85.23
19*	-	-	94.00	-	-	87.01
20	95.19	98.19	93.77	96.09	99.25	87.82
21	96.15	98.06	96.06	93.75	97.53	94.55
22	92.30	94.58	90.59	96.88	97.24	88.36
23	91.03	91.87	92.34	96.88	97.58	93.05
24	84.13	83.74	90.07	97.66	97.93	92.29
25	86.74	85.22	88.44	92.40	96.86	91.62

*17,18 and 19 rounds evaluation was not taken up due to rain.

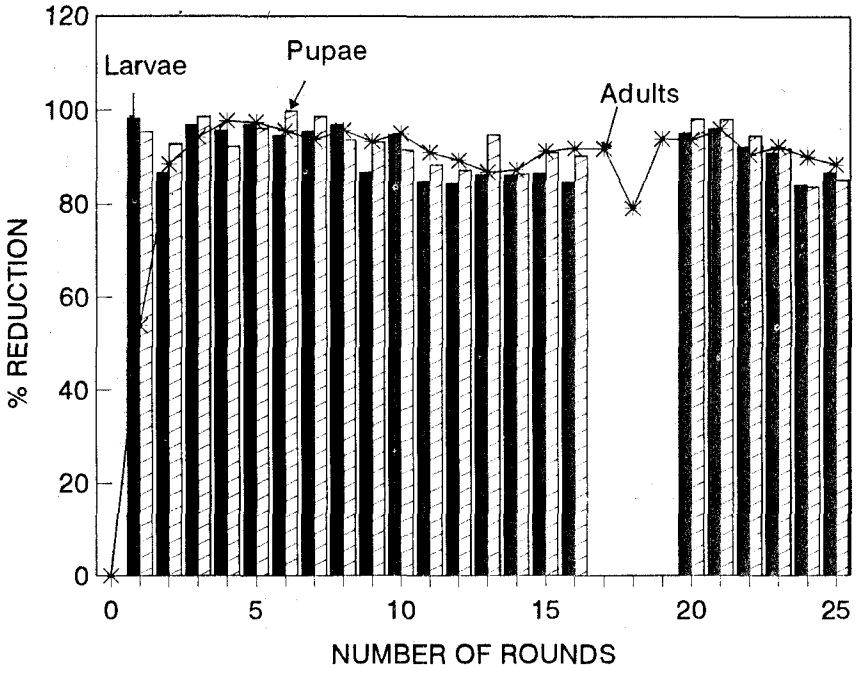

Fig. 2: Percentage reduction of III and IV instar larvae, pupae and adult densities of *Culex quinquefasciatus* in Spherix trial area

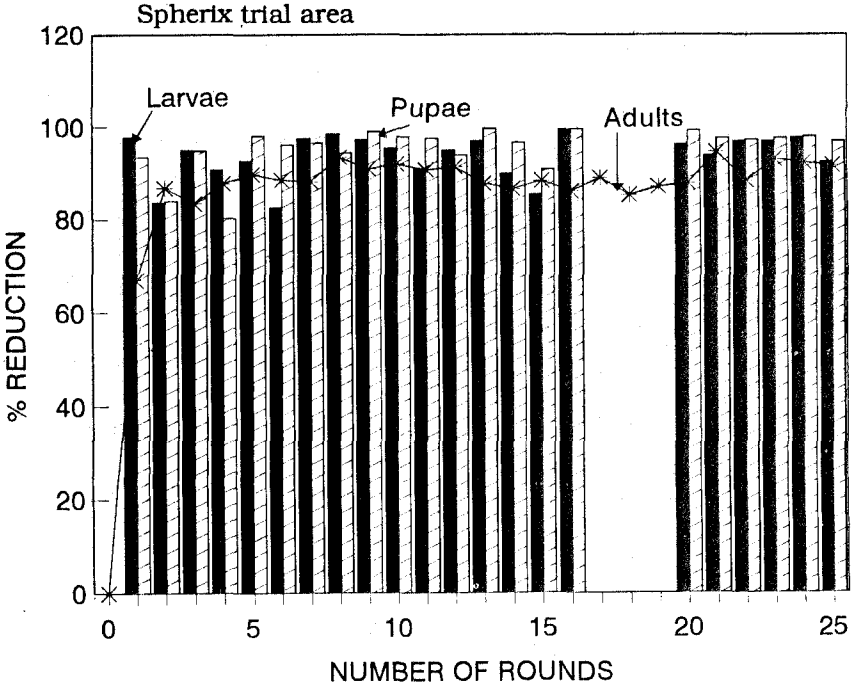

Fig. 3: Percentage reduction of III and IV instar larvae, pupae and adult densities of *Culex quinquefasciatus* in Bactoculicide trial area

area during pre-treatment survey MHD was 32 (Table 2). In comparison to control area 53.9 and 66.9% reduction of adult *Cx. quinquefasciatus* could be observed after the first round of spray of Spherix and Bactoculicide in trial areas respectively. The reduction in density was maintained between 97.7 and 86.8% during the subsequent rounds in the Spherix trial area. Whereas, the percentage reduction was between 94.5 and 85.2% in the Bactoculicide trial area during the subsequent rounds.

Two-way analysis of variance shows significant difference ($p<0.001$) in the adult density in Spherix and Bactoculicide trials and control areas.

Chennai city with warm and humid climate throughout the year along with numerous breeding sources becomes highly conducive for mosquito proliferation during post-monsoon season (MRC unpublished data). There are earlier reports on the usefulness of biolarvicides in the control of urban malaria vector *Anopheles stephensi*, in construction sites and abandoned overhead tanks in Goa[11,12]. In Delhi the advantages of Spherix over chemical larvicides for the control of *Cx. quinquefasciatus* have been reported[16] However, recently reported[17,18] development of resistance in *Cx. quinquefasciatus* against *B. sphaericus* is a major cause of worry. But no information is available on the development of resistance in mosquitoes against *B. thuringiensis* var. *israelensis* under field or laboratory conditions.

An earlier report has shown that both the biolarvicides are economical as compared to commonly used chemical larvicides namely, larvicidal oil, temephos and paris green[19].

The study clearly demonstrates the effectiveness of *B. sphaericus* strain B101, serotype H5a5b and *B. thuringiensis* var. *israelensis* strain 164, serotype H-14 for the control of *Cx. quinquefasciatus* in a metropolitan city. Their regular use to control vectors and other mosquitoes in an urban environment will prove to be a successful ecofriendly alternative for chemical larvicides in future.

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SHORT NOTES

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Field Trial of Bacticide on Larval Populations of Two Species of Vector Mosquitoes in Calcutta

D. BISWAS, S.K. GHOSH, R.N. DUTTA and A.K. MUKHOPADHYAY[a]

Keywords: *Anopheles stephensi*, *Bacillus thuringiensis israelensis*, Bacticide, *Culex quinquefasciatus*, Larvicidal activity

Compared to conventional chemical larvicides, microbial agents are safe and less polluting[1,2]. The concept of mosquito liquidation by microbes is gaining ground in various countries, including India, where mosquito-transmitted diseases, especially malaria, filariasis and Japanese encephalitis are still rampant. Over a period of last two decades, several strains of microbes have been studied in India and abroad, but only two proved highly effective against vector mosquitoes, *Bacillus sphaericus* and *Bacillus thuringiensis israelensis*.

However, of the two, *Bacillus thuringiensis israelensis* seems to be a better choice. According to a report[3], *Culex quinquefasciatus* developed 150-fold resistance against the toxin of *Bacillus sphaericus* after 20-25 rounds of its application in a span of only one year, unravelling the fact that this microbe

Vector Control Unit, Health Department, Calcutta Municipal Corporation, Calcutta-700 014, India.
[a]Regional Office for Health and Family Welfare, Govt. of India, Calcutta-700 029, India.

can never be the weapon of choice for long-term mosquito abatement. On the other hand, the credibility of *Bacillus thuringiensis israelensis* has not been questioned yet and its toxin can be used even against those mosquito larvae which are resistant to *Bacillus sphaericus* and hence different countries are now commercially producing this toxin.

Of different formulations of *Bacillus thuringiensis israelensis*, one is Bacticide, a sharp-smelling, grey-light brown wettable powder produced by the Ministry of Medical Industries, Russia. The Directorate of National Malaria Eradication Programme has procured this microbial preparation for field trial in certain areas of the country, including Calcutta. In this paper we are reporting our preliminary observation on its efficacy against the larvae of *Anopheles stephensi* (vector of malaria) and *Culex quinquefasciatus* (vector of lymphatic filariasis) in an area in Calcutta from August to October 1996. The larvicidal impact of Bacticide on *An. stephensi* larvae was studied in fresh rain-water collections in unused masonry tanks and ground pools. A total of four masonry tanks and six ground pools were selected for this trial. Measuring 0.41-0.74 sq m in area, the masonry tanks were located within the campus of a newly constructed housing complex. These tanks were used for soaking bricks and for other construction purposes. But after the completion of the work, promoters did not care to demolish them, as a result, rainwater accumulated and supported the breeding of *An. stephensi*. As the study-period coincided with the rainy season, temporary collections of rainwater to low-lying ground infested with *An. stephensi* larvae were easily available in nature. The selected ground pools were found in a slum area and they measured 1.5-4.5 sq m in area. Water in both masonry tanks and ground pools was transparent and the level of its pH, as measured by pentype digital pH meter, varied from 5 to 6.5. As the water in these habitats was clear, the degree of its organic pollution was considered low. This assessment was done purely on the basis of visual judgement following the suggestion of an earlier report[4].

As the predominant mosquito species in Calcutta is *Cx. quinquefasciatus*, evaluation of Bacticide against its larvae, too, was deemed necessary and it was carried out in six cement drains, 50-210 m long and 0.3-0.5 m wide. The drains were located in a slum area and had both waste and sewage water collections. They were clogged at several places with domestic wastes, faeces and other wastes, such as rotten leaves, pieces of bricks, broken containers, plastic packets etc., thrown by the slum-dwellers and others. Water in them was still and turbid, and had a slightly alkaline pH in the range of 7 to 8.5. All the drains were heavily infested with the immatures of *Cx. quinquefasciatus*. The water surface areas of all the larval habitats were measured and a 2.5 per cent suspension of Bacticide (prepared before) was thoroughly applied to each of them with

the help of knapsack sprayers at the rate of 20 ml per sq m. Two drains (35 x 0.3 m and 60 x 0.5 m), one masonry tank (1.5 x 0.5 m) and one ground-pool (2.5 x 1 m) were kept untreated to check the natural fluctuation in the larval densities.

For estimation of the larval density before and after the application of Bacticide, average number of III and IV instar larvae together per dip was calculated by taking five dips in masonry tanks and ground pools and 10 dips in drains with the help of a sauce-pan dipper (500 ml capacity). The post-treatment larval counts were taken at an interval of 24 h for three consecutive days, then after seven days and finally after 14 days. From the pre- and post-treatment data, per cent reduction in the larval densities in experimental sites was calculated using the Mulla's[5] formula:

$$\% \text{ reduction} = 100 - \frac{C_1}{T_1} \times \frac{T_2}{C_2} \times 100$$

Where, C_1 and C_2 stand for the larval counts in control sites before and after treatment, while T_1 and T_2 for the counts in experimental sites before and after treatment respectively.

Table 1. Field evaluation of Bacticide at 0.5 g per sq m surface area against the larvae of *Cx. quinquefasciatus* and *An. stephensi* in Calcutta during August to October 1996

Species	Larval habitat	Water pH	Mean larval density (III and IV instars) per dip					
			Pre-treatment	Post-treatment taken after				
				Hours			Days	
				24	48	72	7	14
Cx. quinque-fasciatus	Blocked cement drains	7-8.5	252.2	1.5 (99.5)	0 (100)	0 (100)	169 (35.1)	221 (22.1)
	Control	7	182.6	189 (+3.5)	178 (2.5)	185 (+1.3)	188.5 (+3.2)	206 (+12.8)
An. stephensi	Masonry tanks	5-6.5	21.5	0 (100)	0 (100)	0 (100)	0.5 (97.3)	12.5 (53.5)
	Control	5	8	7 (12.5)	9 (+12.5)	7.5 (6.2)	6.8 (15)	10 (+25)
	Ground-pools	5.5	15	0 (100)	0 (100)	0 (100)	0 (100)	8.5 (62.3)
	Control	5.5	10	8.5 (15)	7 (30)	10.5 (+5)	9.8 (2)	15 (+50)

Figures in parentheses indicate per cent reduction in larval density; Increase in larval density has been denoted by (+).

The results of the trial are presented in Table 1 which shows that Bacticide was equally effective against the larvae of *Cx. quinquefasciatus* and *An. stephensi*, as it yielded cent per cent larval mortality within 24 h. But as far as persistence of its toxicity is concerned, a marked variation was evident. In drains, the larvicidal impact of Bacticide lasted for three days. As a result of this low stability, reduction in the larval density of *Cx. quinquefasciatus* dropped to 35.1 per cent after seven days. In view of this, the use of Bacticide for the abatement of *Cx. quinquefasciatus* in Calcutta, where the mosquito mainly breeds in dirty water collections in a wide variety of places, such as drains, ditches, canals, gully-pits, cesspits and so on, may not be very promising.

However, the persistence of larvicidal activity of Bacticide was of longer duration in masonry tanks and ground pools. The density of *An. stephensi* larvae in these habitats remained almost zero for seven consecutive days after the treatment. Reduction in larval density to the extent of 53.5 per cent in masonry tanks and 62.3 per cent in ground pools was noticed even after 14 days of Bacticide treatment, suggesting that this microbial preparation is highly effective in clean water and its application at an interval of 10 days is expected to yield significant control of stenotopic species of mosquitoes, like *An. stephensi* and *Aedes aegypti* that normally breed in fresh water collections. But in Calcutta, as these two vector species mainly breed in man-made containers in and around human-dwellings[6,7], enforcement of bye-law is now considered to be the only means of controlling them. In this case, Bacticide has, practically, very little role to play. However, during rainy season, when the number of fresh breeding grounds of *An. stephensi* and *Aedes aegypti* increases manifold in nature, this biocide may be used, instead of spraying the traditional chemical larvicides which are reported to be unsafe to non-target organisms.

According to a report, *Bacillus thuringiensis* was found to be highly and almost equally effective at different levels of water pH between 3.5 and 9.5[8]. In the present study, variation of water pH was in the range of only 5 to 8.5 and hence it cannot be linked with the bioefficacy of Bacticide. The shorter period of its efficacy in drains, as noticed in the present trial, was possibly due to the organic pollution of the water. This finding is also in conformity with an earlier report which suggests greater reduction in the persistence and field efficacy of *Bacillus thuringiensis israelensis* due to its inactivation by organic materials, as well as its adsorption on soil particles and organic mattes in the water[9].

As far as the acceptability of Bacticide is concerned, it may not be a popular biocide because of its strong smell which causes discomfort to the spraymen and also makes them reluctant to use it. Another drawback of this formulation is its low water solubility. Hence some modifications are neces-

sary to get optimum benefits of this biocide.

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Distribution of Three Genetic Markers and Malaria in Other Backward Castes of Kheda District, Gujarat

C.S. PANT and H.C. SRIVASTAVA

Keywords: ABO system, G-6-PD deficiency, Sickle-cell haemoglobin

India's rich heritage and varied culture evolved through centuries has great importance in genetic studies related to various castes, ethnic, religious and linguistic groups, which have retained their separate identity through generations. Some genetic abnormalities of human erythrocytes have selective advantages over malaria parasite namely, sickle-cell haemoglobin and G-6-PD deficiency against *P. falciparum* and duffy-negative antigen against *P. vivax*. Certain drugs especially antimalarials cause haemolytic anemia in G-6-PD deficient individuals. World Health Organisation (WHO) has emphasized the need to study the distribution of different genetic abnormalities in various population groups[1]. The distribution of G-6-PD deficiency, sickle-cell haemoglobin and ABO blood groups (genetic markers) in various groups of population such as Upper caste Hindus, Muslims, Christians, Scheduled castes and Scheduled tribes have been reported earlier from Kheda district, Gujarat[2-4]. The present study reports the distribution of these genetic markers and malaria among some of the Other backward castes (OBCs) of Kheda district.

The study was conduct in Kheda district, Gujarat during 1990-1993. Blood

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

samples were collected from different designated villages of Kheda district and malaria clinic operating at Malaria Research Centre, Nadiad. Blood samples were collected from the persons having clinical symptoms of malaria or history of fever in previous week. Methods used for sample collection and analysis were same as described by Pant *et al.*[2]

A total of 1287 human blood samples (male 645; female 642) collected from both sexes of all age groups, were categorized as OBCs who are socially and economically backward and come under *Bakshi Panch* Commission of the Gujarat Government. Out of 1287 samples 163 malaria cases were detected of which 48 were *P. vivax* and 115 *P. falciparum*. The caste-wise distribution of ABO blood groups, sickle-cell haemoglobin and G-6-PD enzyme deficiency is given in Table 1.

Among the 1287 study subjects 23.5% had blood group A, 38.4% group B, 9.9% group AB and 28.1% blood group O. Similar trend was also found in other caste groups of Kheda population[2-4] A significant difference was found in the distribution of ABO polymorphs among OBCs sub-groups (χ^2 = 132.360; df = 42; $p < 0.001$). High malaria incidence was found in blood group AB (18.7%) followed by group A (15.2%), group O (11%) and group B (10.7%) (Table 2). Chi-square test revealed the significant association of ABO system with malaria (χ^2 = 8.548; $p < 0.01$). Similar observations had also been made in earlier studies[2,4]. Gupta and Raichowdhuri[5] reported that malaria parasite shares group A antigen and hence is better tolerated by host immune system. Athreya and Coriell[6] reported that blood group B has an advantage in a malarious region. However, no correlation has been found between ABO blood groups and malaria in some Indian and Colombian populations[7,8]

Out of 1287 blood samples screened for sickle-cell haemoglobin, only 7 (0.5%) were found positive. Highest frequency of sickle-cell gene was found in Thakarda (4.7%) followed by Bhoi (2.9%) and Koli patel (1.9%). No sickling was found in other sub-groups. Among other population of Kheda district, the sickle-cell frequencies were 14.9% in Scheduled tribes, 1.5% in Muslims and Scheduled castes and 1.3% in Rajputs[2-4] The frequency of sickle-cell anemia and sickle-cell trait in Indian population varies from 0-30%, it is relatively high among tribals throughout India except, in the northwest and extreme south. The occurrence in other population is sporadic[9] During the present investigation no malaria case was detected from sickler samples which suggested that sickle-cell gene (HbAS) might have a tendency to protect RBCs from malaria parasite. Luzzatto *et al.*[10] reported increased sickling of parasitized erythrocytes as a mechanism of resistance against malaria. Evidences are also available that HbS mutation confers a protection against *P. falciparum* infection either due to deoxy-HbS aggregates within RBC interfering with intra-eryth-

Table 1. Caste-wise distribution of ABO blood groups, sickle-cell haemoglobin and G-6-PD deficiency

Other backward castes	Sample size	Blood group				Sickle-cell	G-6-PD deficiency	
		A	B	AB	O		Male sample	No. deficient
Vaghari	329	63 (19.1)	176 (53.3)	37 (11.3)	53 (16.1)	0 –	177	13 (7.3)
Bharwad	140	38 (27.1)	40 (28.6)	16 (11.4)	46 (32.9)	0 –	58	5 (8.6)
Gadvi	135	23 (17.0)	44 (32.6)	4 (3.0)	64 (47.4)	0 –	69	0 –
Koli patel	101	31 (30.7)	24 (23.8)	15 (14.8)	31 (30.7)	2 (1.9)	47	4 (8.5)
Barot	90	26 (28.9)	38 (42.2)	7 (7.8)	19 (21.1)	0 –	41	0 –
Suthar	87	24 (27.6)	37 (42.5)	2 (2.3)	24 (27.6)	0 –	44	7 (15.9)
Thakarda	85	18 (21.2)	28 (32.9)	10 (11.8)	29 (34.1)	4 (4.7)	49	0 –
Rabari	49	14 (28.6)	13 (26.5)	5 (10.2)	17 (34.7)	0 –	16	0 –
Valand	49	8 (16.3)	23 (47.0)	6 (12.2)	12 (24.5)	0 –	23	1 (4.3)
Ode	41	14 (34.1)	9 (22.0)	7 (17.1)	11 (26.8)	0 –	20	0 –
Rawal	39	14 (35.8)	9 (23.1)	5 (12.9)	11 (28.2)	0 –	21	1 (4.7)
Prajapati	36	9 (25.0)	14 (38.9)	4 (11.1)	9 (25.0)	0 –	21	1 (4.7)
Bhoi	34	2 (5.9)	20 (58.8)	1 (2.9)	11 (32.4)	1 (2.9)	14	1 (7.1)
Luhar	30	11 (36.7)	5 (16.7)	3 (10.0)	11 (36.6)	0 –	14	0 –
Others (6 small groups)	42	8 (19.1)	14 (33.3)	6 (14.3)	14 (33.3)	0 –	31	0 –
Total	1287	303 (23.5)	494 (38.4)	128 (9.9)	362 (28.1)	7 (0.5)	645	33 (5.1)

Figures in parentheses indicate percentage.

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

Groups		Samples	*Pv*	*Pf*	Total
Blood groups	A	303	10 (3.3)	36 (11.9)	46 (15.2)
	B	494	19 (3.8)	34 (6.9)	53 (10.7)
	AB	128	11 (8.6)	13 (10.1)	24 (18.7)
	O	362	8 (2.2)	32 (8.8)	40 (11.0)
Sickle-cell	Sicklers	7	0 –	0 –	0 –
	Non-sicklers	1280	48 (3.7)	115 (10.1)	163 (12.7)
G-6-PD	Deficient	33	2 (6.1)	0 –	2 (6.1)
	Non-deficient	612	26 (4.2)	62 (10.1)	88 (14.3)

Figures in parentheses indicate percentage.

rocytic schizogony or by initiating sickling of RBCs with developed schizonts and their subsequent removal by macrophages.

Of 645 male subjects screened for G-6-PD deficiency, 33 (5.1%) subjects were found deficient (Table 1). The per cent frequency of enzyme deficiency was highest in Suthar (15.9) followed by Bharwad (8.6), Koli patel (8.5), Vaghari (7.3), Bhoi (7.1), Prajapati (4.7), Rawal (4.7) and Valand (4.3). G-6-PD deficiency was also found in SC (5.9%), ST (4.2%), Christians (5.9%), Muslims (1.8%) and Upper caste Hindus (3.8%) of the same study area[2-4].

Most of the findings of G-6-PD deficiency in India, are available in caste groups and tribals which vary from 0-30%[11]. High incidence of malaria (14.3%) was found in non-deficient subject (Table 2). No *P. falciparum* case was detected from deficient subjects, which was also statistically significant ($\chi^2 = 3.886$; $p<0.05$). In absence of incidence of heterozygous females, it was not possible to correlate the protective role of G-6-PD deficiency against malaria infection, however, there are evidences regarding the protective role of heterozygous females against malaria. Bienzle *et al.*[12] have reported that heterozygous state of G-6-PD defi-

ciency (female carrier) plays a protective function against malaria.

We may hence conclude that these genetic markers have certain relationship with malaria. Therefore, such studies are important in different population groups and could be utilized for better understanding of malaria dynamics in the ethnic population to plan safe anti-malaria drug schedule.

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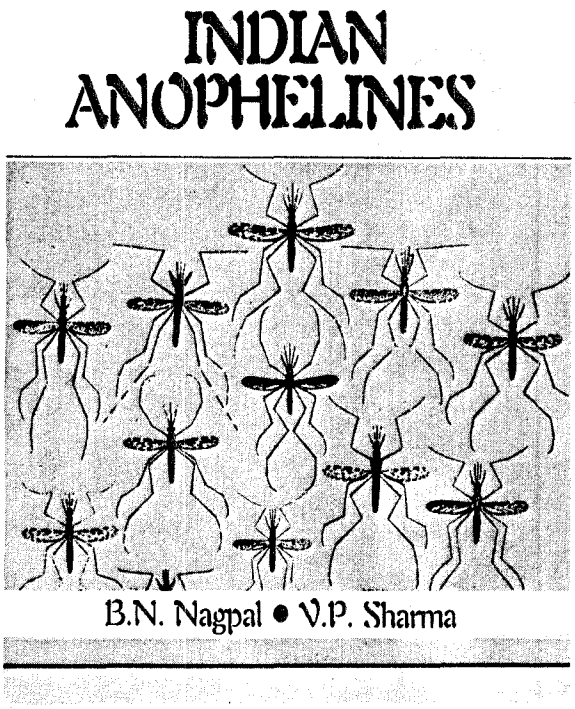

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