INVESTIGATION OF MALARIA PREVALENCE AT NATIONAL THERMAL POWER CORPORATION, SHAKTINAGAR, SONBHADRA DISTRICT (UTTAR PRADESH), INDIA

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Abstract. Malaria in industrial complexes is promoted by extensive mosquitogenic potential generated by excavations and importation of parasite through migratory labor. The National Thermal Power Corporation (NTPC), Shaktinagar, Sonbhadra district was surveyed for malariogenic conditions from 1994 to 1996. The major mosquito breeding sites were drains, storm-water drains, lakes, outside tanks, overhead tanks, sluice-valve chambers, ornamental tanks, wells, pit wells and water reservoirs, etc. *Anopheles culicifacies* was the major vector of malaria in this area. Sibling species identification of *An. culicifacies* revealed that species C predominated during the transmission season and responsible to transmit malaria. Insecticide susceptibility tests against *An. culicifacies sl* showed that *An.culicifacies* population was 100% susceptible to malathion, fenitrothorn and deltamethrin while it was found 44% resistant to DDT. The malaria cases recorded in 1994, 1995 and 1996 were 847, 590 and 409 respectively. *In vitro* study on *P. falciparum* cases showed that 41, 70, 50% of the isolates tested were resistant to chloroquine in 1994, 1995 and 1996 respectively while an *in vivo* follow-up study showed 20-30% *P. falciparum* cases resistant to chloroquine. An integrated approach involving alternate vector control measures along with judicious use of insecticides has been suggested to bring down malaria in industrial complexes.

INTRODUCTION

In the wake of accelerated activities under the five year developmental plans, major ecosystems have seen radical changes. At micro level, developmental projects have emerged as well-characterized paradigms. One such paradigm is industrial malaria. Malaria in such paradigm is promoted by extensive mosquitogenic potential generated by excavations and importation of parasites through migratory labor (Pattanayak *et al*, 1984). The dimension of this type of malaria is further compounded by other requirements of the projects, particularly water management practices. The National Thermal Power Corporation (NTPC), Shaktinagar, Sonbhadra district, UP, India showed a spurt of total malaria cases in district from 2,421 cases in 1979 to 11,455 cases in 1980 with high preponderance of *P. falciparum* (Anonymous, 1986). NTPC, Shaktinagar gives contract to private enterprise for mosquito control work of the township and plant area. The strategy comprises using temephos (abate), fenthion (baytex), diesel and emulsifier as larvicides, thermal fogging with malathion 50 EC and pyrethrum (2%) supported by passive case detection and treatment. Malaria incidence continues to be high in this project area even now. Therefore, we surveyed NTPC Shaktinagar from 1994 to 1996 to assess the malariogenic potential of this industrial complex and its surrounding areas. The surveys covered the response of *P. falciparum* to chloroquine and biological characteristics of the major vector *Anopheles culicifacies sensu lato* such as breeding habitats, feeding preference, insecticides susceptibility and the sibling species prevalence of the major vector *An. culicifacies sensu lato* in this area.

MATERIALS AND METHODS

Study area

NTPC, Shaktinagar is situated on the border of Uttar Pradesh and Madhya Pradesh states with a longitude of 84.6 and a latitude of 24.3 and is spread over an area of 3,000 hectares. On the NTPC complex are located a major industrial complex, planned residential colony with 20,000 population and six labor colonies (population 15,000) adjacent to the campus. The total population of the study area is 35,000. The terrain is a combination of foot-hills and plain region, and sub-soil water level in some labor colonies is about 3 m deep. The average rainfall in this area is 800 mm while

the relative humidity ranges from 60 to 90%.

During the study period, a total of five surveys were carried out, in April, September and November, 1994, September and October, 1995 and September-October, 1996.

Epidemiological studies

The medical facilities available include a 80 bed hospital with 26 medical doctors supported with 111 other staff. Of the consumable budget of the hospital $ 2,25,000.00 in 1993-94, 5.8% was spent on malaria diagnostic and treatment. Clinically symptomatic malaria cases were screened microscopically, although many cases were treated on the basis of clinical symptoms. Therefore, the consumption of antimalarials was very high. The yearly consumptions (1993-94) of chloroquine and metakelfin (sulfalene and pyrimethamine) tablets was 64,293 and 4,400 respectively, and that of chloroquine and quinine injections were 1,570 (5 ml amp) and 350 (2 ml amp) respectively.

Sensitivity of *P. falciparum* to chloroquine

***In vivo* test:** The WHO 28-day extended test for chloroquine sensitivity was carried out on selected patients with a history of no reinfection during the study, after ascertaining that no chloroquine had been taken during illness by examination of urine for the chloroquine excretion (Lelijveld and Kortmann, 1970). Each patient received a total dose of 1,500 mg (25 mg/kg body weight) of chloroquine base (600 mg day-0, 600 mg day-01 and 300 mg day-02) followed by a single dose of 45 mg primaquine. Absorption of chloroquine was also confirmed by the urine test on day 2. Blood smears were collected on days 0, 2, 7, 14, 21, 28 whenever the patient complained of fever after the completion of prescribed doses. Asexual parasites were examined by Giemsa-stained smears. Chloroquine resistant cases were treated with a single dose of sulfalene (1,000 mg) plus pyrimethamine (50 mg) combination (Metakelfin^{R}) along with primaquine (45 mg).

In vitro test: Micro i*n vitro* tests for chloroquine were conducted with infected blood samples collected from selected patients in pre-dosed microculture plates supplied by WHO and the procedures for incubation and staining of pre- and post- incubation smears were the same as described elsewhere (Bruce-Chwatt, 1981). A test was considered valid when at least 10% schizont maturation was observed in post-incubation control wells (Draper *et al*, 1985). Schizont maturation at 8 pmol of chloroquine was considered an indication of resistance (Tyagi and Tiwari, 1990). Minimum inhibitory concentrations (MICs) of the drug were assessed by microscopic examination of post-incubation smears. The results of *in vitro* tests were analyzed by probit analysis of log-dose response test (Grab and Wernsdorfer, 1983).

Entomological studies

Indoor resting adult mosquito densities were monitored by searches with flash light and aspirator in NTPC township and the five labor colonies : i) Kota Basti, ii) Chilka Tand, iii) Balia Nullah, iv) Harbhava and v) Khadia. The collections were made between 06.00 and 08.00 hours. Mosquitos from each dwelling were kept separately in test tubes and identified. Densities per man hour of searching of total mosquitos, anophelines and major vector species were calculated. Anopheline larvae collected from different breeding habitats were brought to the laboratory, reared and identified after adult emergence.

Susceptibility tests of *An. culicifacies* to different insecticides, were conducted following standard procedures (WHO, 1982). DDT, fenitrothion and deltamethrin impregnated papers were obtained from WHO, while malathion papers were prepared at the Malaria Research Center following standard procedure. Full-fed wild-caught females of *An. culicifacies* were used in the tests, and 40 to 50 mosquitoes were exposed for 60 minutes to each insecticide tested. The data obtained on mortality in different replicates was corrected using Abbot's formula (WHO, 1975).

Anopheles culicifacies collected from the field were brought to the laboratory and ovaries from half-gravid females were removed and fixed in modified Carnoy's fixative (1:3 glacial acetic acid to methanol). The samples were stored in a refrigerator. Polytene chromosomes were prepared according to the method described by Green and Hunt (1980) and observed under compound microscope. The identification of *An. culicifacies* sibling species was carried out as reported earlier (Subbarao *et al*, 1988b). The stomach blood of freshly fed or half-gravid females were smeared on filter paper (Whatman No.1) for the determination of blood meal source. Blood meal samples were assayed by gel diffusion technique (Collins *et al*, 1986).

RESULTS

Malaria cases recorded in NTPC, Shaktinagar hospital from 1989 to 1996 are given in Table 1. During this period, the slide positivity rate (SPR) ranged from 6.53 to 11.42 with predominance of *P. vivax*. However, an increase in the proportion of *P. falciparum* was noticed in the last three years. A follow-up of 323 cases recorded at NTPC hospital revealed that 67 (20.7%) cases were from the township while 256 (79.25%) cases belonged to surrounding areas.

In vitro test

The results of i*n vitro* tests from 1994 to 1996 are given in Table 2. The number of samples tested having schizont maturation in control wells in 94, 95 and 96 were 44, 31 and 26 respectively. 41% in 94, 70.9% in 95 and 50% in 96 had MICs above 8 pmol thus showed resistance to chloroquine. All samples were 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 Table 3. The effective concentrations of sensitive isolates (EC50) were 0.22, 0.19 and 0.26 ($x10^{-6}$) mol/l while for resistant isolates were 0.34, 0.42 and 0.50 ($x10^{-6}$) mol/l in 1994, 1995 and 1996, respectively.

In vivo test

32, 22 and 18 *P. falciparum* cases were followed successfully up to 28 days for *in vivo* chloroquine sensitivity test in 1994, 1995 and 1996 respectively, and 24 cases (25%) in 1994, 17 (22.7%) in 1995 and 15 (16.6%) in 1996 were found resistant to chloroquine (Table 4). One case each in 1995

Table 1
Malaria cases recorded in NTPC, Shaktinagar.

Year	Blood smears examined	*Pv*	*Pf*	Mixed (*Pv* + *Pf*)	Total	SPR
1989	6,900	578	88	03	669	9.6
1990	4,218	297	65	01	363	8.6
1991	4,085	235	120	02	357	8.7
1992	4,295	309	87	02	398	9.2
1993	3,965	303	94	13	410	10.3
1994	7,628	358	470	19	847	11.10
1995	4,856	345	161	-	506	10.42
1996	6,257	280	129	-	409	6.53

Source : CMO, NTPC; *Pv* = *Plasmodium vivax*; *Pf* = *P. falciparum*; SPR = Slide positivity rate

Table 2
In vitro study of *P. falciparum* to chloroquine at NTPC, Shaktinagar.

Year	No of isolate tested	MIC (pmol)[a]						% Resistance cases
		2	4	8	16	32	64	
September 1994	30	3	4	11	7	3	2	40.0
November 1994	14	2	-	6	6	-	-	42.8
September 1995	31	1	4	4	5	8	9	70.9
September 1996	26	2	7	4	7	2	4	50.0

[a]MIC above 8 pmol was considered as resistance.

Table 3
Analysis of *in vitro* tests for chloroquine sensitivity of *P. falciparum* at NTPC, Shaktinagar.

Year		Percentage inhibition of schizont maturation at following drug concentrations (x 10^{-6} mol/l)							Effective concentration (x 10^{-6}mol/l)	
		0.2	0.4	0.8	1.60	3.20	6.40	12.80	EC50	C90
1994	S	49.75	82.53	91.58	99.30	100	100	100	0.22	0.46
	R	40.31	64.91	80.78	92.18	97.51	98.83	100	0.34	1.08
1995	S	52.88	86.65	94.73	99.71	100	100	100	0.19	0.48
	R	35.25	63.27	80.86	90.55	96.19	99.59	100	0.42	1.12
1996	S	46.69	83.43	96.15	99.51	100	100	100	0.26	0.52
	R	29.72	54.33	77.06	92.19	96.59	98.34	99.82	0.50	1.76

S = Sensitive; R = Resistant

Table 4
In vivo study of *P. falciparum* to chloroquine at NTPC, Shaktinagar.

Year	No. of cases followed	Sensitive	Resistant			%
			RI	RII	RIII	
1994	32	24	8	-	-	25
1995	22	17	4	1	-	22.7
1996	18	15	2	1	-	16.6

Table 5
Mosquito breeding sites surveyed at NTPC, Shaktinagar.

Breeding habitats	No. surveyed	No. positive	Positive for				% Positivity
			Anophelines	*Cx*	*Ae*	Mix	
Drains	134	70	26	40	-	4	52.2
Lake	3	3	2	-	-	1	100.0
Pits	141	86	34	17	1	34	49.6
Outside tanks	40	10	4	-	-	6	9.3
Overhead tanks	212	2	-	1	-	1	0.9
Ornamental tanks	6	3	3	-	-	-	50.0
Coolers	58	6	2	3	-	1	10.3
Ponds	6	6	1	1	-	4	100.0
Wells	34	15	13	2	-	-	44.1
Hoof-prints	290	77	32	-	-	45	26.5
Pit wells	35	15	9	-	-	6	42.8
Drums	16	6	-	2	2	2	37.5
Sluice-valve chambers	8	4	4	-	-	-	50.0
Water reservoirs	2	2	2	-	-	0	100.0
Total	1,085	305	132	66	3	104	28.1

Cx = *Culex*, *Ae* = *Aedes*, Mix = Anophelines and Culicines.

and 1996 had RII level resistance while all other cases had RI levels of resistance. The parasite densities of RII level case in 1995 on D0, D2, D7 were 24,000, 4,500 and 2,900/µl blood while of 1996 case were 4,000, 1,440 and 800/µl blood respectively. All resistant cases responded to metakelfin and the parasites cleared within seven days of treatment.

Entomological studies

Extensive surveys of mosquito breeding sites of township, thermal power plant area and labor colonies were carried out and results are presented in Table 5. The main breeding habitats in the township were drains, storm-water drains, outside tanks, overhead tanks, sluice-valve chambers, ornamental tanks and lakes, while in the thermal power plant area, these were underground tanks, drains, low-lying water logged areas, sluice-valve chambers and water reservoirs. The breeding sites in the labor colonies were wells, pit-wells, ponds, borrow-pits and waste water collections due to improper drainage system. Outside tanks, sluice-valve chambers, lakes, factory water reservoirs, wells, pit-wells and water collected in storm water drains were found positive for *An. culicifacies* breeding.

Table 6
Prevalence of different mosquito species in indoor resting collections at NTPC, Shaktinagar[a].

Species	Number	%
An. culicifacies	814	32.60
An. fluviatilis	40	1.60
An. vagus	28	1.12
An. subpictus	872	34.90
An. maculatus	2	0.08
An. splendidus	47	1.80
An. tesselatus	1	0.04
An. aconitus	3	0.12
An. annularis	145	5.81
Total Anophelines	1,973	79.10
Culex quinquefasciatus	522	20.90

[a]Total number of collections were 25 each in 1994, 1995 and 1996.

Ten mosquito species from the genera *Anopheles* and *Culex* were recorded in indoor resting collection. The percent prevalence of each species recorded from NTPC Shaktinagar area are given in Table 6. Of a total of 2,495 mosquitos collected, 1,973 (79.1%) were *Anopheles* and 522 (20.9%) were *Culex quinquefasciatus*. Among *Anopheles*, *An. subpictus* (34.9%) and *An. culicifacies* (32.6%) were most abundant followed by *An. annularis* (5.8%). The other *Anopheles* species were *An. fluviatilis*, *An. stephensi*, *An. vagus*, *An. maculatus*, *An. splendidus*, *An. tesselatus* and *An. aconitus* and their proportion was less than 2% of the total *Anopheles*.

Table 7
Collection of indoor resting mosquitos presented as man hour numbers.

	1994			1995	1996
	April (4)[a]	Sept (5)	Nov (4)	Sept - Oct (4)	Sept - Oct (4)
Total mosquitos	96	81	115	137	84
Total *Anophelines*	61	60	89	124	71
An. culicifacies	37	49	34.7	37.7	20.8

[a]Figures in the parentheses represent number of mosquito collections.

Table 8
An. culicifacies sibling species composition at NTPC, Shaktinagar.

Time of collection	Total identified	Sibling species		
		A	B	C
April 1994	22	9 (40.9)[a]	5 (22.7)	8 (36.4)
September 1994	37	2 (5)	11 (29)	24 (66)
September 1995	31	0	4 (13)	27 (87)
September 1996	39	0	11 (29)	28 (71)

[a]Figures in paretheses represent percentages.

Table 7 shows the collections of indoor resting mosquitos presented as per man hour for total mosquitos, anophelines, and *An. culicifacies*. The abundance of *An. culicifacies* was high in all collections probably because this was the only species which was breeding in permanent sites like tanks, lakes, water reservoirs, wells, pit wells etc.

The results of susceptibility of *An. culicifacies sensu lato* against DDT, malathion, deltamethrin and fenitrothion revealed that *An. culicifacies* of this area were only 44% susceptible to 4% DDT (n=50), and 100% susceptible to 5% malathion (n=40), 0.025% deltamethrin (n=40) and 1% fenitrothion (n=40).

The relative proportions of *An. culicifacies* sibling species in different collections are given in Table 8. The sample size of *An. culicifacies* examined for sibling species was small. However, data suggest that species A, B and C are prevalent in the area. In April, 1994 species A and C were almost in equal proportions constituting 40.9% and 36.4% respectively while species B comprised 22.7% of the total specimens identified. In all September-October collections species C was predominant, 66% in 1994, 87% in 1995 and 71% in 1996, and species A was virtually absent except for 5% in 1994 suggesting that its prevalence was mainly confined to premonsoon months. Species B constituted the remaining population of *An. culicifacies*.

A total of 107 blood meal smears of *An. culicifacies sl* were assayed against human and bovine antisera for host identification. Only one was found positive for human blood while the remaining were positive against bovine antisera, and the human blood index (HBI) was 0.01. HBI results support the proposal that *An. culicifacies* is a zoophagic species.

DISCUSSION

The NTPC, Shaktinagar area was endemic for malaria since the inception of the project and continues to be high even now. Chloroquine resistance in *P. falciparum* was reported in 1980 (Dwivedi *et al*, 1981); the present study confirmed that about 20% *P. falciparum* cases were resistant to chloroquine. Although *in vitro* study showed the resistance levels in all three years were above 40%, the low level of resistance found in *in vivo* follow-up associated with immunity factors.

There are six major vectors of malaria in India namely *An. culicifacies, An. stephensi, An. fluviatilis, An. sundaicus, An. dirus* and *An. minimus*. Only *An. culicifacies, An. stephensi* and *An. fluviatilis* are the reported vector species in the states where NTPC Shaktinagar established (Pattanayak *et al*, 1994). Although *An. subpictus* is widely distributed in most parts of India including northern states, its role as a vector species to transmit malaria has not reported so far. *An. culicifacies* (32.6%) is the most abundant vector species in NTPC Shaktinagar except *An. subpictus* (34.9) while other two vector species *An. stephensi* and *An. fluviatilis* were less than 1% of the total population. Therefore, it is safe to consider *An. culicifacies* as a vector species responsible to transmit malaria in this area.

Among the four sibling species of the *An. culicifacies* complex recorded from India (Subbarao *et al*, 1988a), species A, B and C were found at NTPC Shaktinagar. Species A, C and D have been incriminated as vectors of *P. vivax* and *P. falciparum* malaria (Subbarao *et al*, 1988b, 1992) while species B is not considered as a vector in this part of India. During transmission season (September-October), only species B and C were prevalent at NTPC. Since species B is not a vector therefore it is concluded that *An. culicifacies* sibling species C is responsible for malaria transmission in NTPC Shaktinagar area.

Malaria control in the NTPC project area is usually given to pest control agency on a contract basis with an approximate cost of Rs 10-12 lacs per year. The approach to control malaria consist of application of larvicides using abate, fenthion thermal fogging with malathion as insecticides supported by passive case detection and treatment. Our survey revealed that about 20% cases belonged to township while rest were from surrounding areas where NTPC authority or pest control agency do not take any control measures. In the laboratory bioassay tests, although *An. culicifacies* was found completely susceptible to malathion which is being sprayed in the NTPC area, the number of malaria cases which from township remained same. This could be due to poor coverage during spraying operations and improper case detection and treatment.

Recently, bioenvironmental strategy for control of malaria was found to be feasible, appropriate and cost-effective, particularly for industrial complexes, to tackle malaria problem on the long term basis (Dua *et al*, 1997). The present study

suggests that an integrated approach based on environmental management measures like minor engineering works, use of alternative vector control methods like biolarvicides and larvivorous fishes on the breeding habitats of *An. culicifacies*, along with judicious use of insecticides may be able to control vector population. In addition, active surveillance and prompt treatment of malaria cases with appropriate drugs are suggested to reduce malaria cases in industrial areas. The authority of the project area should also incorporate adjoining areas of the township into malaria control.

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