INDIAN JOURNAL OF MALARIOLOGY

Volume 32 **Number 3**

September 1995

MALARIA RESEARCH CENTRE
Indian Council of Medical Research
22-Sham Nath Marg
Delhi-110 054

INDIAN J. MALARIOL.

Quarterly

Year of Revival: 1981

SUBSCRIPTION RATE

Annual	India	Rs. 75.00*
	Other countries (including airmail postage)	$ 20.00

*25% discount would be admissible to individual subscribers on annual basis.

Subscription may be sent by a bank draft or postal order only, payable at Delhi to the Director, Malaria Research Centre, 22-Sham Nath Marg, Delhi-110 054.

The 'Indian Journal of Malariology' is indexed by 'BIOSIS', 'Drugs and Pharmaceuticals Current Indian Titles', 'Index Medicus', 'Indian Science Abstracts', 'Review of Applied Entomology', 'Protozoological Abstracts', 'Quarterly Bibliography of Major Tropical Diseases' and it is selectively abstracted by 'Tropical Diseases Bulletin'. This Journal is also accessible on the CAB Computer Database and MEDLINE.

INDIAN JOURNAL OF MALARIOLOGY

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Number 3
September 1995

Short Note

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Status of Chloroquine Resistance in *Plasmodium falciparum* in Tripura

R.K. DASGUPTA, S.P. MISRA and J. NANDI

In-vivo chloroquine resistance studies on *P. falciparum* were conducted in 1981 and 1988 in South district of Tripura. Resistance status to chloroquine has been gradually changing over the seven years period. A single case at RIII level was detected in 1988, and RI level of resistance had shown a increasing trend.

Keywords: Chloroquine resistance, *P. falciparum*, Tripura

INTRODUCTION

Chloroquine resistance in *Plasmodium falciparum* has been recorded in several countries of southeast Asia including India[1-3]. Over the years resistance to chloroquine has considerably increased in frequency and degree in Bangladesh and resistance to Fansidar has also been reported from the Chittagaon hill tracts[4]. With the presence of high degree of resistance in neighbouring Bangladesh which surrounds the state of Tripura, it was deemed necessary to conduct *in-vivo* chloroquine resistance studies to understand the sensitivity status of the drug and changes over a time period in the area. This paper compares the observations made in 1981 and 1988 in order to highlight the shift in resistance status.

MATERIALS AND METHODS

Two studies were conducted in Santir and Natun Bazar PHCs of South district of Tripura in 1981 and 1988 re-

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spectively. The district was selected because of intense *P. falciparum* transmission and the presence of Chakma refugees from Bangladesh. The study areas are hilly and forested with numerous seasonal streams traversing and have similar malaria epidemiology.

Study was conducted as per the procedure recommended by WHO[5] for a standard 28-day extended test but no attempts other than advising the study cases to sleep under bednets were made to prevent reinfection. Fever surveys were carried out in selected villages and those found positive for *P. falciparum* on blood smear examination with minimum parasitaemia of 500 asexual parasites per cu mm were selected for the study. All serious, complicated cases and those with the history of intake of antimalarials in the past fortnight or evidence of drug intake confirmed by urine test[6] were excluded. The study cases thus selected were administered orally 25 mg/kg/body weight of chloroquine under supervision over three days i.e. 10 mg/kg/body weight on Day 0 and Day 1 followed by 5 mg/kg body weight on Day 2. Blood smears were collected every day for the first 7 days followed by smears on Day 14, 21 and 28 and then stained with 5% Giemsa for 30 min at 7.2 pH. *In-vivo* response to the drug was monitored by counting the asexual forms to 300 leucocytes and then computing the density to 8000 leucocytes that is per cu mm of blood. Interpretation of the response to the drug is as per WHO (loc.cit.).

RESULTS

The epidemiological data not only indicates stability but also intensity of malaria transmission which is similar in the two study areas and therefore comparable (Table 1). In Santir Bazar PHC 33 cases (19 males and 14 females) were studied in 1981 having a mean parasitaemia of 9299/cu mm (range 500-70,000). In 1988 in Natun Bazar only 14 cases (10 males and 4 females) having a mean parasitaemia of 27,460/cu mm (range 3093-59,386) could be followed up.

The *in-vivo* response to chloroquine in various age-groups in both studies is

Table 1. Malaria incidence of study areas in South district of Tripura

Year	Santir Bazar				Natun Bazar			
	ABER	API	SPR	SfR	ABER	API	SPR	SfR
1984	18.9	29.5	15.6	14.9	15.0	29.2	19.4	17.3
1985	11.8	14.8	12.5	11.0	20.4	34.1	16.7	15.7
1986	12.1	18.5	15.2	14.1	18.6	31.7	16.8	14.3
1987	13.7	17.1	11.7	10.2	13.7	18.7	13.6	10.5

Table 2. *In-vivo* response to chloroquine in 1981 and 1988 in South district of Tripura

Age-group	Santir Bazar (1981)					Natun Bazar (1988)				
	S	RI	RII	RIII	Total	S	RI	RII	RIII	Total
1-5	4	2	0	0	6	0	1	0	1	2
6-10	7	5	1	0	13	1	1	0	0	2
11-15	3	0	1	0	4	1	1	0	0	2
> 16	7	3	0	0	10	4	4	0	0	8
Total	21 (63.6)	10 (30.3)	2 (6.1)	0 (0)	33 (100)	6 (42.9)	7 (50.0)	0 (0)	1 (7.1)	14 (100)

S — Sensitive; RI, RII, RIII — Resistance levels; Figures in parentheses are in per cent.

given in Table 2. Out of 33 cases studied in 1981, 36.4 per cent were found resistant, whereas in 1988, 57.1 per cent of the 14 cases were resistant. The difference was not significant ($p > 0.05$, df 1). There was neither significant difference in resistance status among similar age-groups nor among different age-groups in the same study. Though in 1981 two cases (6.1 per cent) were resistant at RII level and only one case (7.1 per cent) was resistant at RIII level in 1988, the shift to higher level is not statistically significant ($p > 0.05$).

In 1981 the mean parasite clearance time (MPCT) among susceptible cases was 2.48 days but in 1988 it was only two days. The mean parasite recrudescence time (MPRT) in 1981 and 1988 was 23 days and 20.2 days respectively.

DISCUSSION

Comparison of the two studies conducted seven years apart in two nearby eco-epidemiologically similar areas indicate that resistance to chloroquine was gradually building up. Though statistically the status has remained the same but caution is definitely required as RIII resistance was detected in one case. The degree and frequency of chloroquine resistance in the adjacent Mizoram state is also increasing. Rosenberg and Maheswary[7] have also reported emergence of chloroquine resistance from four bordering districts of Bangladesh which they suspected was there since 1970.

The MPRT observed in the study may not indicate the true values since reinfection could not be ruled out. However, in majority of the RI cases, 60 and 83.3 per cent in 1981 and 1988 respectively recrudesced after 14 days suggestive of a gradual increase in resistance.

Misra and Dhar[8] incriminated *An. minimus* the principal vector in Tripura but the role of *An. dirus* cannot be ruled out as these vectors have been

observed to play a significant role in nearby states of Assam and Nagaland[9,10]. This is important as chloroquine-resistant *P. falciparum* have been reported to be more infective, producing large number of oocysts in *An. dirus*[11].

As resistance in Tripura is still of low degree it is imperative that vigilance for drug failure be maintained and all measures need to be intensified to detect and eleminate all resistance cases with appropriate therapy.

ACKNOWLEDGEMENTS

The authors are thankful to the members of the *P. falciparum* monitoring teams of the Directorate of NMEP and State Health Authorities of Tripura for extending all help and cooperation to conduct the studies.

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Indian Journal of Malariology
Vol. 32, September 1995, pp. 93-98.

Role of Health Education in Schoolchildren with Particular Reference to Malaria

P.G. BHATI, RAJNI KANT, H.C. SRIVASTAVA, V.S. MALAVIYA and P.K. PUJARA

Knowledge and effect of health education in schoolchildren of primary, secondary and higher secondary classes were evaluated. In schoolchildren exposed to different health education activities such as live demonstrations, slide shows and when both events were grouped together a high average of correct score (47.88%) in comparison to that of control group (26.56%) was noticed. Exposed population could answer most of the questions correctly and supported the role of community participation with the help from Government Departments to control malaria. Involvement and role of schoolchildren in disease vector control programme is discussed in this paper.

Keywords: Health education, Malaria, Schoolchildren

INTRODUCTION

Malaria is a major public health problem in India and many other countries of the world. Most of the disease control programmes during 60's and 70's were vertically organized without paying much attention to community participation, which resulted in several setbacks[1]. Apart from this, drug resistance in parasite and insecticide resistance in mosquito vectors were major obstacles[2,3]. Success in such control programme largely depends on awareness and education among the people regarding disease. Health edu-

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cation is an important tool within the community to enhance knowledge of the disease transmission, treatment and intervention measures designed to control vectors[4].

Socio-economic status, culture and behaviour of the people are few hurdles in health education. Under such circumstances school seems to be an ideal place for health education, where children can devote time to gain health related knowledge and may further extend the same to other members of the family and community up to grass root level[5]. An attempt was made in the present study to evaluate knowledge and the effect of health education on schoolchildren to ensure their involvement in community health programmes.

MATERIALS AND METHODS

Many science exhibitions and simple experimental demonstrations were organized by Sharda Mandir School, Nadiad in December 1992 and Anand Primary Education Society, Anand in February 1993 to propagate scientific knowledge and its uses in daily life. Large number of participants like students, their parents and local residents took active part and Malaria Research Centre was requested for the demonstration of mosquito, malaria and its control programmes that provided an opportunity to study the role of student power in health programmes. During exhibition, posters pertaining to malaria parasite, mosquito, malaria transmission and control strategies in English and local language were displayed. Live demonstration of aquatic stages of mosquitoes, adult, malaria parasite and larvivorous fishes were also included. Slide shows were arranged to explain the knowledge, practices and environmentally safe methods to control malaria. Twelve students of Anand Arts College, Anand (who were trained previously) were included in our programme with the staff of the Centre. During science exhibition, the visiting students of all age-groups and education were grouped together in batches of 15-20 students and half an hour was devoted to each group to show and explain every event displayed. In order to assess the effect of health education and knowledge gained by the participants, a questionnaire (8 questions) was circulated among 489 students of primary, secondary and higher secondary classes selected from the experimental group those who were exposed at least once to health camp (235) or slide shows (103) and from those who attended both the events (151) on random basis. To compare the results 79 students who had not been to any exhibitions were also screened for their health awareness regarding the disease. A mean correct score was calculated from the correct answers, by giving 12.5 marks to each question. Results obtained were analysed statistically.

RESULTS AND DISCUSSION

Impact of different health education activities on mean correct score in dif-

Table 1. Results of different health education activities on respondents of different education and sex groups

Category	No. of respondents		Mean % correct score ± SD	
	Experimental	Control	Experimental	Control
Education ($\chi^2 = 5.57$; df = 1; $p < 0.05$)				
Primary	234	41	44.51 ± 20.75	23.27 ± 11.43
Secondary	229	38	50.78 ± 23.92	33.92 ± 24.02
Higher Secondary	26	–	53.36 ± 31.33	–
Sex ($\chi^2 = 3.61$; df = 1; $p > 0.05$)				
Male	196	47	50.13 ± 24.58	32.66 ± 17.87
Female	293	32	46.36 ± 22.00	21.29 ± 11.91
Activity				
Health Camps	235	–	43.89 ± 22.63	–
Slide Shows	103	–	50.90 ± 22.69	–
Both	151	–	51.95 ± 23.30	–
Total respondents	489	79	47.88 ± 23.12	26.56 ± 16.00

Mean age ± SD: 12.92 ± 2.0 (Experimental), 13.26 ± 1.77 (Control).

ferent education and sex groups is given in Table 1. The mean±SD age of the 489 students of the experimental group was 12.92±2.0 and 79 students of the control group was 13.26±1.77 yrs. On analysing the data it was found that a high mean correct score (47.88±23.12) was obtained in experimental group compared to control (26.56±16.0). The higher secondary students yielded maximum mean correct score (53.36±31.33) owing to their better knowledge and grasping power in comparison to that of lower classes. The results were also found statistically significant (χ^2=5.57; $p<0.05$). It has been observed in earlier findings as well that the knowledge regarding malaria is increased with education[6]. It was also noticed that males (50.13±24.58) responded better compared to females (46.36±22.0). Aikins *et al.*[6] found similar results that men were more knowledgeable about the correct cause of malaria than women. Comparison of activities revealed that the respondents who attended both the activities namely slide shows as well as health camps scored more (51.95± 23.30) in comparison to respondents with individual activity, probably due to repeat of similar act by both the means.

Most of the experimental population was aware about the transmission of malaria by mosquitoes but only 13.84%

could provide the correct answer that it is transmitted by female *Anopheles* mosquito only. On the contrary, only 7.1% control group could answer the same correctly. About 54.72% population subjected was aware about the symptoms of malaria and 42.41% knew the different control activities, whereas 23.29% students could even name the malaria parasite correctly. About 91.42% students correctly answered that mosquitoes lay eggs in water followed by the time required for the development of eggs to become adults (72.08%) and the purpose of blood smear collection (51.64%). About 54.72% subjected population was aware about the symptoms of malaria and 42.41% knew the different control activities. 23.29% students could name the malaria parasite correctly. However, the response of control population was comparatively poor (Table 2).

Most of the respondents (73.07%) from control group felt that the responsibility of malaria control lies with the Government only, whereas the experimental group was of the opinion that it is a joint responsibility of public as well as Government ($\chi^2 = 37.97$; $p < 0.001$) (Table 3). From the studies it was found that imparting health education to the students was very fruitful as they gathered better knowledge and yielded good response in comparison to the respondents of control group. The voluntary involvement of 12 students of Anand

Table 2. Study of awareness about mosquito and malaria among schoolchildren

Questions asked	% respondents answering correctly	
	Experimental (n=489)	Control (n=79)
(1) Malaria is transmitted by	13.84	7.1
(2) Where do mosquitoes lay their eggs?	91.42	71.4
(3) Time required for the development of an egg to adult	72.08	19.6
(4) Enumerate various mosquito control activities	42.41	26.7
(5) Why blood smear is prepared and who dose it?	51.64	30.3
(6) Name of the commonly used antimalarial drugs	34.94	21.4
(7) Symptoms of malaria	54.72	39.3
(8) Name malaria parasite	23.29	7.1

Table 3. Study of awareness regarding responsibility shared by various agencies in malaria control

Agency	Experimental (%)	Control (%)
Government	65 (21)	19 (73.07)
Public	100 (31.64)	6 (23.07)
Both	104 (32.91)	1 (3.84)
Others	47 (14.87)	–
Total respondents	316 (100)	26 (100)

$\chi^2 = 37.97$; df = 2; $p < 0.001$; Respondents who didn't answer were excluded.

Arts College, Anand was an example of utilising students' power in our programme. In Pondicherry, discussion and community seminars were routinely undertaken by staff of the Vector Control Research Centre. They also conduct health campaigns in village schools and several science clubs have been initiated to encourage student to take some responsibility in vector control[7]. Similarly, using health education as an important tool for the use in health services and the control of communicable diseases like malaria with main emphasis on its all possible methods such as health camps, lived demonstrations, group meetings, door-to-door visits, exhibitions, slide shows, video shows, and use of mass-media for creating awareness and generating scientific understanding produced promising results in Kheda district of Gujarat[3,4,8].

Health care agencies in developing countries often face fiscal and staffing problems when they wish to conduct research and programme evaluation or develop new curriculum and educational material. In such situation the involvement of students in the programme may be extremely useful. Health education is a fundamental right of every child and is inextricably linked to educational achievement, quality of life and economic productivity. By acquiring health-related knowledge, values, skills and practices students can lead a healthy life and may further disseminate the same among their communities. This will accelerate the process of creating health awareness among the larger population that will make health programmes more sustainable through community participation. Though some aspects related to mosquito bionomics and malaria transmission are already covered in school-course curriculum[9], it requires to be updated with the addition of more information and emphasis on practical demonstrations of vector-borne diseases and their controls. This goal can be achieved if we have the will. However, participatory approach in effective health education to school-

children need further investigation to determine its potential in the intersectoral malaria control strategy.

ACKNOWLEDGEMENTS

Authors are highly thankful to Dr. S.K. Sharma and Mr. R.M. Bhatt for their valuable help. We extend our sincere thanks to Mr. S.M. Banerjee, Mr. Alok Kulshreshtha and other staff members for their kind support. Thanks are also due to Mr. Shashi Kant Chinchole, M.P. Urological Hospital, Nadiad for statistical assistance.

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Indian Journal of Malariology
Vol. 32, September 1995, pp. 99-103.

Use of Neem Oil as a Mosquito Repellent in Tribal Villages of Mandla District, Madhya Pradesh

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A field study was carried out to evaluate the mosquito repellent action of neem (*Azadirachta indica*) oil in tribal forested villages of District Mandla. Various concentrations of neem oil mixed in coconut oil (1-4%) were applied to the exposed body parts of human volunteers. Results revealed 81-91% protection during 12 h period of observation from the bites of anopheline mosquitoes. Neem oil is an indigenous product and a practical solution to curtail mosquito nuisance.

Keywords: *An. culicifacies*, *Azadirachta indica*, Malaria, Mosquito repellent

INTRODUCTION

The intensity of malaria transmission in a community depends on the degree of man-vector contact[1]. For protection from mosquito bites, different types of mosquito coils/mats containing synthetic pyrethroids are widely used throughout the world[2]. However, there are reports that prolonged use of these devices may be harmful[3] and requires standardization and quality control[4]. In an attempt to develop a cost-effective, and socially acceptable method for protection against biting insects, neem oil have been used recently in Delhi[5,6]. In Mandla the terrain is rocky, undulating and villages are situated in forested area. The inhabitants of these villages are mostly tribals of various ethnic origin and they have

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their own socio-cultural habits and habitats. Hence, it would be of interest to study the effectiveness of neem oil against mosquito bites in endemic area.

MATERIALS AND METHODS

Two villages were selected in Bizadandi PHC (23° N latitude 80° 10' E longitude) of District Mandla. These are broken forest villages and situated in the valley traversed by perennial streams and inhabited by Gond tribe (90%). There are numerous breeding sites, producing two potential malaria vector species i.e. *Anopheles culicifacies* and *Anopheles fluviatilis*[7]. Baseline data collected during 1992 revelaed that average man hour density of *An. culicifacies* ranged from 100-200. Annual parasite incidence ranged from 200-300 with preponderance of *Plasmodium falciparum* (60-70%). Prevalence of *An. fluviatilis* was recorded mainly during post-monsoon period.

Neem oil was diluted in coconut oil[5] to make 1, 2, 3 and 4% concentrations. 5 ml of each oil mixture was applied on face, arms and legs of each human volunteer before 1800 hrs and simultaneously coconut oil was applied to other human volunteers for comparison as control. Collections were made from 1800 to 0600 hrs for thirty nights between April and September 1993 (5 nights in each month). Human volunteers having different concentrations of neem oil (one for each concentration) occupied different localities in the same village. Volunteers with neem oil or coconut oil were allowed to lie on cots in separate rooms exposing their face, arms and legs. The test and control volunteers and their rooms were rotated to avoid any bias resulting from the host/experimental room. Mosquitoes landing on the exposed body parts were collected by an insect collector using a torch and suction tube. Per cent protection was calculated.

$$\% \text{ protection} = \frac{\text{No. of mosquitoes caught (Experimental-control)}}{\text{No. of mosquitoes caught in control group}} \times 100$$

For data analysis the species considered was *An. culicifacies* that comprised majority among anophelines caught.

RESULTS AND DISCUSSION

Results revealed (Table 1) that neem oil produced a strong repellent action on anophelines. The per cent protection from mosquito bites was 81 and 90.9% with 3 and 4% neem oil respectively for 12 h (Fig. 1). As the per cent protection with 1% and 2% neem oil was below 80%, the data has not been included in the table.

During the study it was seen that 63% *An. culicifacies* feed after midnight on the neem oil treated volunteers. However, no such feeding preference was recorded on volunteers with coconut oil. It appears that the deterrent effect of

Table 1. Results of neem oil as repellent against anophelines based on 30 nights collection (April-September 1993)

Observation time (hrs)	Species*	No. of anophelines caught			
		3% neem oil (Mixture)	Coconut oil	4% neem oil (Mixture)	Coconut oil
1800-1900	*An. culicifacies*	0	0	0	0
	Anophelines	0	0	0	0
1900-2000	*An. culicifacies*	1	2	0	1
	Anophelines	1	2	0	3
2000-2100	*An. culicifacies*	0	6	1	5
	Anophelines	0	9	1	6
2100-2200	*An. culicifacies*	1	10	0	5
	Anophelines	1	12	0	5
2200-2300	*An. culicifacies*	2	7	0	7
	Anophelines	2	7	0	10
2300-2400	*An. culicifacies*	2	8	1	3
	Anophelines	3	11	1	3
2400-0100	*An. culicifacies*	1	10	0	4
	Anophelines	1	10	0	4
0100-0200	*An. culicifacies*	2	8	0	6
	Anophelines	2	9	0	7
0200-0300	*An. culicifacies*	2	11	1	6
	Anophelines	2	15	1	7
0300-0400	*An. culicifacies*	1	4	2	6
	Anophelines	1	4	2	7
0400-0500	*An. culicifacies*	2	0	0	3
	Anophelines	2	0	0	3
0500-0600	*An. culicifacies*	0	0	0	0
	Anophelines	0	0	0	0
Total	*An. culicifacies*	14	66	5	46
	Anophelines	15	79	5	55
% protection	*An. culicifacies*	78.8		89.1	
	Anophelines	81.0		90.9	

*Seven anopheline species viz. *An. fluviatilis, An. annularis, An. theobaldi, An. subpictus, An. vagus, An. pallidus* and *An. turkhudi* were found in small numbers.

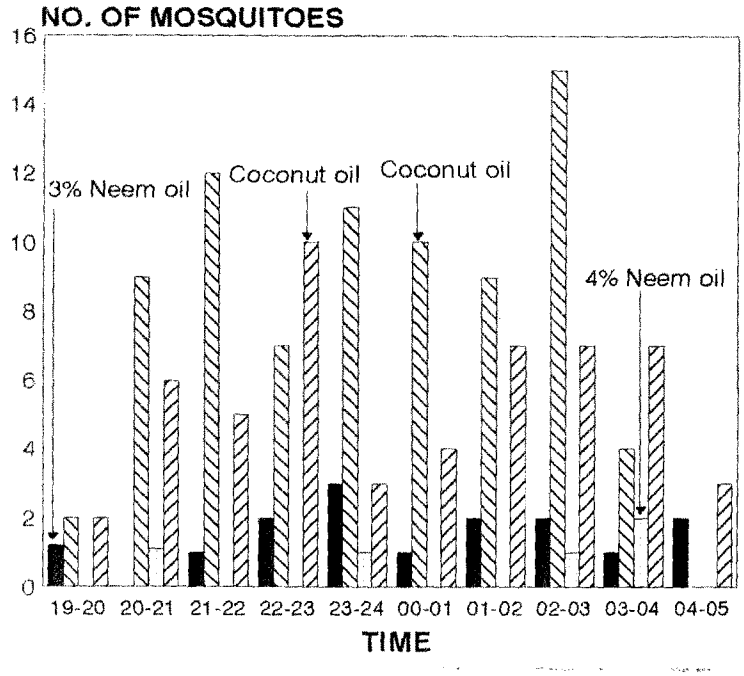

Fig. 1: Hourly collection of mosquitoes from volunteers having 3 and 4% concentration of neem oil

neem oil on exposed body parts started declining gradually after 5-6 h. The abdominal condition of mosquitoes shows that comparatively more *An. culicifacies* caught on neem oil volunteers were unfed (26%) as against 6.4% caught on control volunteers. This shows that neem oil also produced a feeding inhibition among mosquitoes.

Since *An. culicifacies* is a known zoophilic species[1], the man-biting rate is low but overall results indicate that neem oil is an effective repellent against this species. Similar results were obtained in Delhi[5]. Ninety per cent protection provided by the neem oil is of significance among alternative methods of vector control. Since the tribals have forest based economy[8], they often visit forest and other places for their livelihood. Neem oil can provide protection from mosquito bites to these tribals at any place. Further, application of oil on exposed body parts is a practice among tribals hence, socio-cultural practices also will enhance usage of neem oil for malaria control.

ACKNOWLEDGEMENTS

Excellent technical assistance rendered by the field staff of MRC (FS), Jabalpur is thankfully acknowledged.

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Indian Journal of Malariology
Vol. 32, September 1995, pp. 104-111.

Relative Efficacy of Various Oils in Repelling Mosquitoes

M.A. ANSARI and R.K. RAZDAN

Field studies were carried out to determine the relative efficacy of repellent action of vegetable, essential and chemical base oils against vector mosquitoes. Results revealed that essential oils viz. *Cymbopogan martinii martinii* var. *Sofia* (palmarosa), *Cymbopogan citratus* (lemon grass) and *Cymbopogan nardus* (citronella) oils are as effective as chemical base oil namely mylol. These oils provide almost complete protection against *Anopheles culicifacies* and other anopheline species. Per cent protection against *Culex quinquefasciatus* ranged between 95-96%. Camphor (*C. camphora*) oil also showed repellent action and provided 97.6% protection against *An. culicifacies* and 80.7% against *Cx. quinquefasciatus*. Vegetable oils namely mustard (*B. compestris*) and coconut (*C. nucisera*) showed repellent action, however the efficacy of these oils was not much pronounced against *Cx. quinquefasciatus*. Results of statistical analysis revealed significant difference between vegetable and essential oils ($p<0.01$) against tested species of mosquitoes. Essential oils were found marginally superior in repellancy than camphor and mylol ($p<0.01$) against *An. culicifacies* and *Cx. quinquefasciatus*.

Keywords: *An. culicifacies*, *Cx. quinquefasciatus*, Repellent, Vegetable oils

INTRODUCTION

Since time immemorial, oils extracted from plants are being used for repelling mosquitoes in several countries[1]. Traditional repellents not only provide protection against mosquito bites, they also curtail malaria transmission. The

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efficacy of turmeric, gingili and mustard oil was demonstrated as early as 1947 against *An. fluviatilis*. Lower spleen rate was observed in women who smear their bodies with these oils[2]. Similarly, oils of aromatic grasses of the genus *Cymbopogan* (Graminae) that comprises about 40 species have shown variable degree of repellent action against mosquitoes. Among these oils *C. nardus* (L.), *C. winterianus* Jowitt (citronella oil), *C. citratus*, *C. flexuosus* (lemon grass oil), *C. martinii martinii* var. *Sofia* and *C. martinii* var. *Motia* (palmarosa oil) are well-known repellents and have been used as base in creams and oils marketed in India and abroad. These are as effective as DEET against sandflies and mosquitoes[3,4]. Recently, neem oil extracted from seeds of *Azadirachta indica* has shown repellent action against mosquitoes[5]. In view of growing concern about safety of chemical based repellents, interest is revived in oils extracted from plants as repellent for mosquitoes. Studies were therefore carried out to evaluate relative efficacy of promising oils extracted from plant in repelling mosquitoes. Results of the study are presented in this paper.

MATERIALS AND METHODS

Samples of citronella oil (*C. nardus* (L.) Rendle), lemon grass oil (*C. citratus* D.C. Stapf.), palmarosa oil (*C. martinii martinii* var. *Sofia*) were obtained from the Division of Plant Genetic Resources, Indian Agricultural Research Institute, New Delhi. Camphor oil (*Cinnmonium camphora*) was obtained from Japan Bottle House Exporter, Manufacturers and Importers of Perfume, Delhi, mylol oil form Boots Pharmaceuticals Ltd., Bombay, coconut oil (*Cocos nucisera*) of Marico Industries Ltd., Bombay and mustard oil (*Brassica compestris*) from Kanodia Mills, Delhi.

Study area

Experiments were carried out in Dehra village of Dhaulana PHC, located near Ganga Canal on Mussoorie-Dhaulana Road, southeast of Delhi at a distance of 45 km. The village is inhabited by 7000 people distributed in 1400 houses. *An. culicifacies*, a principal vector of malaria, is prevalent in high number throughout the year and responsible for maintaining active malaria transmission in the village. *Cx. quinquefasciatus* is predominant species and considered a serious nuisance in the village. *An. annularis*, a secondary vector of malaria was also prevalent throughout the year but *An. subpictus* was prevalent only during monsoon and post-monsoon season. Occasionally, specimen of *An. nigerrimus*, *An. stephensi*, *An. pulcherrimus*, *Cx. vishnui* and *Mansonia* spp were also encountered.

Human volunteers as baits were used in this study. Baits were asked to sit or relax at a distance of 5 m in a row from dusk to dawn with uncovered fore-arms, face and feet up to ankle. One ml oil of each type was applied on face, hand and feet. They were not al-

lowed to wash hands, face and feet. All were given food and water during the study period by other volunteers. Untreated (control) bait was run concurrently. Eight human baits were used per night. Female mosquitoes landed on treated and untreated baits were collected hourly throughout the night by trained Insect Collectors and identified with the help of handlens and reconfirmed in the laboratory. Insect collectors were rotated at an interval of 4 h to avoid slackness and bias. Site of each type of bait was interchanged to prevent bias. Relative efficacy was tested for 20 nights at weekly interval from August to December 1993. Per cent protection and average protection time was calculated according to standard procedure described earlier[6]. SPS software computer package was used for statistical analysis.

RESULTS AND DISCUSSION

Number of female mosquitoes landed on treated and untreated baits in twenty night collections from dusk to dawn is given in Table 1. Results revealed variable degree of protection against different oils and mosquitoes. Of seven oil tested, mylol, *C. martinii martinii* var. *Sofia* (palmarosa) and *C. citratus* (lemon grass) provided absolute protection against *An. culicifacies* Complete protection was also obtained against total anophelines. However, protection against *Cx. quinquefasciatus* and total mosquitoes ranged from 95-96%. *C. nardus* (citronella oil) has also provided 99.5% protection for anopheline and 95% for *Culex quinquefasciatus* and total mosquitoes. Protection obtained with camphor oil was 97.6, 93.8, 80.7 and 81.1% against *An. culicifacies*, total anophelines, *Cx. quinquefasciatus*

Table 1. Relative efficacy of different oils in repelling mosquitoes

Oils	% protection based on landing rate of females on treated and untreated volunteers			
	An. culicifacies	Total anophelines	*Cx. quinquefasciatus*	Total mosquitoes
B. compestris	85.9±13.3	86.7±18.8	60.8±19.8	61.7±15.2
Cocos nucisera	87.5±11.6	86.3±10.5	63.8±11.1	64.6±15.0
Cinnmonium camphora	97.6±4.5	93.8±9.0	80.7±10.3	81.1±11.4
Mylol	100±0.0	100±0.0	95.5±1.6	95.6±2.5
C. nardus	99.2±3.6	99.5±3.2	95.7±1.3	95.8±2.7
C. martinii var. *Sofia*	100±0.0	100±0.0	96.8±1.8	96.9±2.0
C. citratus	100±0.0	100±0.0	96.8±3.4	97.0±2.8

Per cent protection based on 20 nights collection from dusk to dawn.

Table 2. Average protection time (in hours) with various oils against different mosquitoes based on twenty nights collection

Oils	*An. culicifacies*	Total anophelines	*Cx. quinquefasciatus*	Total mosquitoes
B. compestris	7.6	6.7	2.9	2.1
Cocos nucisera	10.2	8.3	1.8	1.8
Cinnmonium camphora	10.6	9.2	4.3	4.3
Mylol	11.0	11.0	5.3	5.3
C. nardus	10.9	10.9	6.3	6.3
C. martinii var. *Sofia*	11.0	11.0	6.5	6.5
C. citratus	11.0	11.0	7.3	7.3

and total mosquitoes respectively. Mustard and coconut oils did not provide same degree of protection.

Average protection time provided against different mosquito species is given in Table 2. Results revealed variable degree of protection time by each oil against different mosquitoes. Mylol, palmarosa and lemon grass oils provided 11 h protection against *An. culicifacies* and total anophelines. However, only 5-7 h protection was observed against *Cx. quinquefasciatus* and total mosquitoes. In anopheline prolonged protection time was observed in comparison to culicines. The average protection time with citronella, camphor, coconut and mustard oils for anopheline was 10.9, 9.2, 8.3 and 6.7 h respectively as against 6.3, 4.3, 1.8 and 2.1 h respectively for total mosquitoes.

Hourly landing of female mosquitoes from dusk to dawn and per cent protection obtained with different oils and mosquitoes are shown in Figs. 1-2. Results revealed that mosquito bites throughout the night with varying biting peaks. Similarly, there was also variation in protection with different oils and mosquitoes from dusk to dawn. The per cent protection from dusk to dawn with mustard oil was ranging from 53.8 to 100, 46.6 to 100 with coconut oil, 86.0 to 100 with camphor oil, and 93 to 100 with *C. nardus* oil against *An. culicifacies* (Fig. 1). However, absolute protection from dusk to dawn against *An. culicifacies* and anophelines were provided with mylol, palmarosa and lemon grass oils. The per cent protection was ranging from 49.8 to 73.7 with mustard oil, 54 to 83.3 with coconut oil, 60.6 to 100 with camphor, 90.7 to 100 with mylol oil, 91.6 to 100 with citronella, 92.6 to 100 with *C. martinii* oil and 89.7 to 100 with lemon grass against *Cx. quinquefasciatus* (Fig. 2).

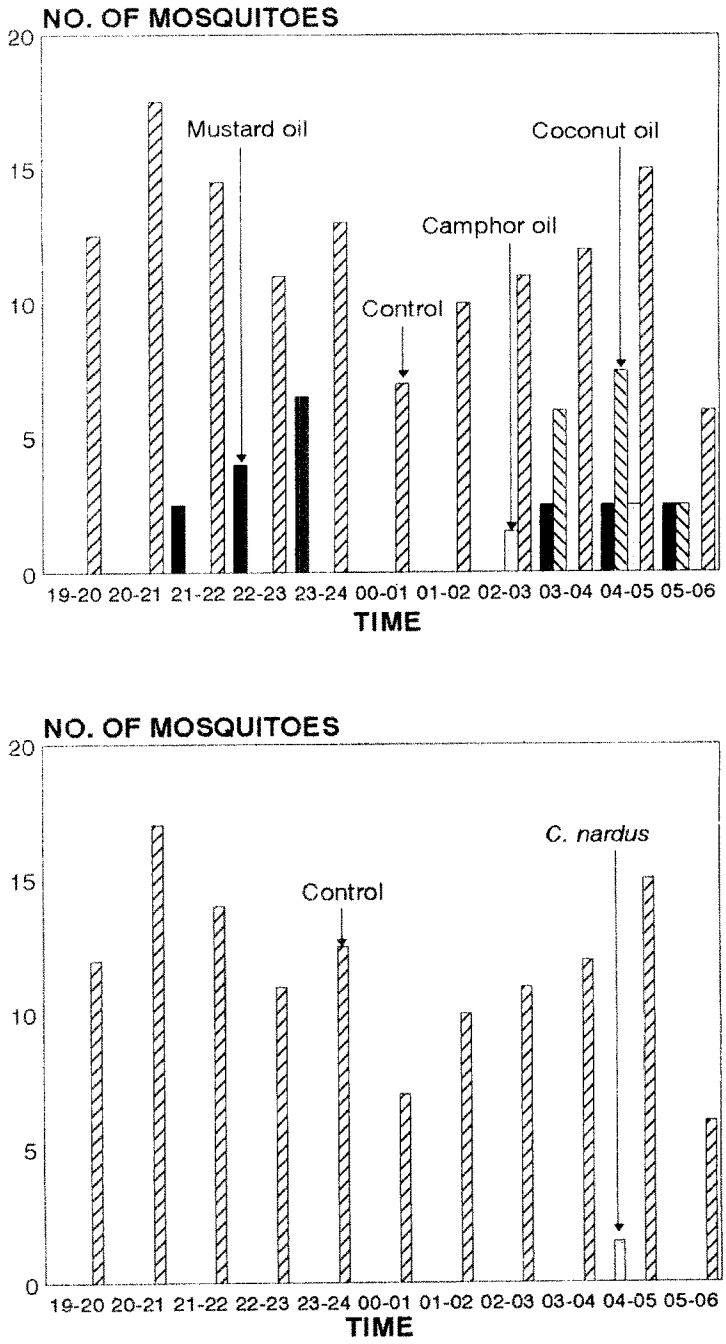

Fig.1: Landing rate of female *An. culicifacies* on treated and untreated human baits with various oils

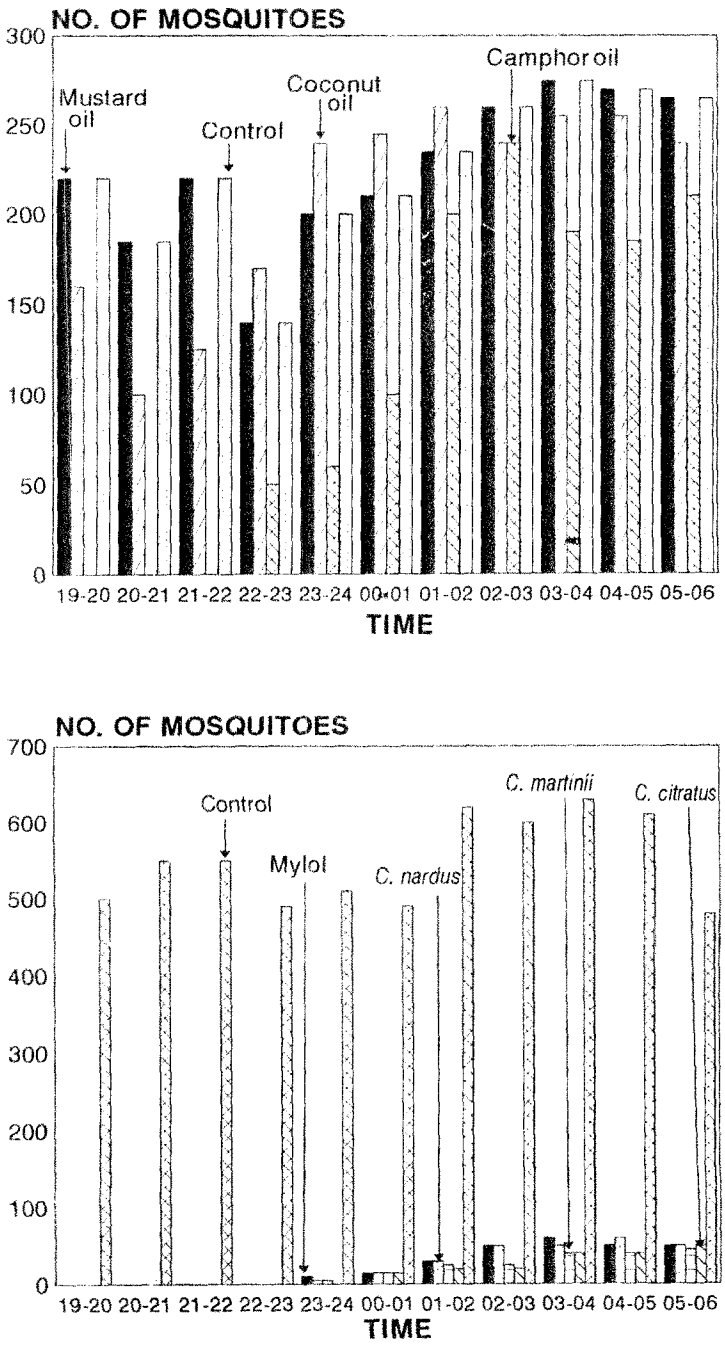

Fig. 2: Landing rate of female *Cx. quinquefasciatus* treated and untreated human baits with various oils

Statistical analysis of results revealed that there is a significant difference in repellent action with oil of genus *Cymbopogan* and vegetable oils ($p<0.01$ to 0.002). Significant difference ($p<0.0002$ to 0.003) were observed, when relative efficacy of camphor was compared with mustard and coconut oils. Similarly, there was significant difference in relative efficacy of camphor and essential oils against *Cx. quinquefasciatus*. *C. nardus* and mylol oil efficacy was more or less comparable against *An. culicifacies* ($p>0.1$) but mylol oil was marginally superior against *Cx. quinquefasciatus* ($p<0.004$). However, *C. martinii* and *C. citratus* oils were marginally superior to mylol ($p<0.001$) against *An. culicifacies* and *Cx. quinquefasciatus* ($p<0.16$). No significant difference ($p>0.161$ to 0.4) was observed against any species of mosquitoes, when relative efficacy of *C. nardus*, *C. martinii* and *C. citratus* was compared.

The study revealed that essential oils extracted from aromatic grasses particularly of genus *Cymbopogan* (F. Graminae) have strong repellent action against mosquitoes. The efficacy of these oils is far more superior with vegetable oils such as mustard and coconut oils. In fact the efficacy of these oils is as promising as synthetic repellents like dimethyle phthalate (DMP) and dibutyle phthalate (DBP) main active ingredients of mylol oil. This is in confirmity with the findings of earlier workers who found fresh application of oil is as effective as DMP[7].

Oil extracted from *Cymbopogan nardus* is used as traditional repellent to prevent the mosquito bites in India. This is one of the few natural product that is still used in commercial products of south Asian, European and north American countries. The active ingredient of *C. nardus* oil is citronellal, a monoterpene aldehyde and is being used in commercial repellents. Commercial product based on citronellal have been found as effective as DEET against sand flies and mosquitoes[4].

Similarly the oil extracted from *Cymbopogan martinii martinii* var. *Sofia* and *Cymbopogan citratus* has shown strong repellent action against mosquitoes, when it is used in cream or as pure oil[3]. In view of growing concern of mosquito nuisance and vector-borne diseases in urban and tribal population, the use of plant base oil particularly genus *Cymbopogan* should be promoted to reduce the toxic load of insecticides on environment. Synthetic repellent which are harmful in long-term and do not provide absolute protection also should be replaced with indigenous edible or essential oils. India produces 200 tons of citronella oil, 50-60 MT of palmarosa oil and 1-2 MT of lemon grass oil that is used as raw material in perfume industry. High yielding improved genetic varities are being developed by Plant Genetic Resources (ICAR) that may be cultivated in wasteland to increase the production[8,9]. It may be pointed out that essential oils because of aroma and non-stickyness are highly appreciated

in both rural and urban areas and if grown in wasteland, the production of these oils can increase several folds, that can be used in both urban and rural areas in controlling vector-borne diseases. Therefore, there is an urgent need to carry out systematic research for using different fractions of essential oil and its constituents to evolve an indigenous, cost-effective and environment friendly methods for mosquito and flies control. Field trials also should be carried out particularly in endemic area to evaluate operational feasibility and dermal toxicity over a long period specially to infants and children.

ACKNOWLEDGEMENTS

Authors are grateful to Dr. V.P. Sharma, Director, MRC for encouragement and facilities provided to complete the study. Authors are also grateful to Shri. N.L. Kalra, Consultant, MRC for critically going through the manuscript. Thanks are also due to Dr. M.L. Maheshwari for providing literature and samples of essential oils. Technical help rendered by Sh. S.N.S. Kachhawaha, Sh. K.C. Pushap, Sh. C.S. Sahota, Sh. Janak Singh, Sh. Daulat Ram, Sh. M.D. Tewari and Sh. B.D. Sati is gratefully acknowledged.

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Indian Journal of Malariology
Vol. 32, September 1995, pp. 112-118.

A Study on Insecticide Resistance in *Anopheles fluviatilis* and *Anopheles culicifacies* to HCH and DDT in the Malkangiri District of Orissa

S.S. SAHU and K.P. PATRA

The susceptibility status of malaria vectors to HCH and DDT was studied in Malkangiri district which has been highly endemic for malaria since many decades. *An. fluviatilis* is the major malaria vector and *An. culicifacies* is a secondary vector in the area. HCH is being used in malaria control programme from 1972 onwards. Before to that, DDT was used. This study reports that *An. fluviatilis* has developed resistance to HCH and is susceptible to DDT. This is the first confirmed report of resistance of this species to HCH in Malkangiri district of Orissa. *An. culicifacies* was found to be resistant to both the insecticides. Extensive use of pesticides in agriculture and indoor residual spraying of HCH for malaria control might have led to the development of resistance in these malaria vectors.

Keywords: *An. culicifacies*, *An. fluviatilis*, DDT, HCH, Insecticide resistance

INTRODUCTION

Malkangiri is a newly carved district of Orissa state bordering Andhra Pradesh and Madhya Pradesh. This district was earlier a part of erstwhile Koraput district (Fig. 1). The district has uneven terrain with hills, valleys and forests at different altitudes. Several streams and rivers criss-cross these valleys.

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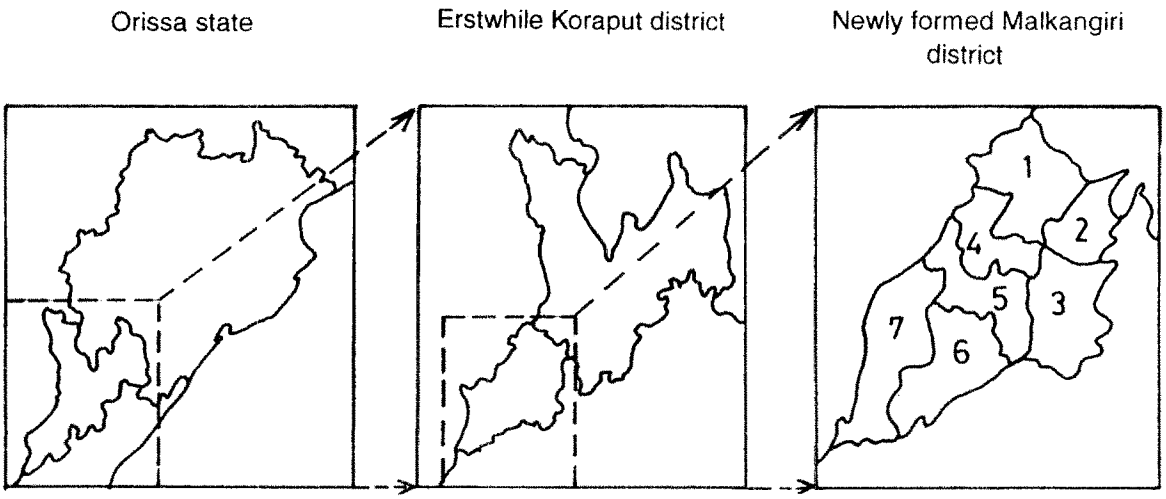

Fig. 1: Study area of Malkangiri district curved out of Andhra Pradesh and Madhya Pradesh; 1 – Mathili PHC; 2 – Khairput PHC; 3 – Kodumulugumma PHC; 4 – Malkangiri PHC; 5 – Korkunda PHC; 6 – Kalimela PHC; 7 – Podia PHC (Test were carried out with *An. fluviatilis* in 2,3,4 & 5 and with *An. culicifacies* in 1,2,3,4 & 5 PHCs).

Based on the topography, villages in this district can be grouped into four different ecotypes viz. hill top, foothill, plain and riverine. The district has a population of 4.5 lakhs of which 52 per cent are settlers (Bangladeshi refugees) and the remaining are native tribals. Earlier studies in this area showed that the foothill and hill top villages are hyperendemic for malaria, whereas the plain and riverine villages are hypoendemic[1]. Out of total malaria positive cases *P. falciparum* accounts for 80 per cent[2]. *Anopheles fluviatilis* is the main vector and *An. culicifacies* plays a secondary role[3]. Both these vectors are endophilic in the area[4].

There are seven Primary Health Centres (PHCs) in the district and all the areas had been receiving two rounds of residual spray of DDT every year from 1958 to 1971 and three rounds of HCH spray from 1972 onwards. In spite of control measures, malaria remains a major health problem in the district.

Development of resistance in malaria vectors to the residual insecticide may be one of the obstacles for effective control. Study on vector susceptibility to residual insecticides in the DDT sprayed areas of erstwhile Koraput district[5] showed that *An. fluviatilis* was susceptible to DDT and HCH. The present study was carried out from April 93 to January 94 in HCH sprayed area of Malkangiri district to determine the susceptibility status of malaria vectors

Table 1. Susceptibility status of *An. fluviatilis* to HCH and DDT in Malkangiri district

PHC	No. of village covered	Date of Experiment	Exposure Time (h)	No. exposed	Corrected mortality (%)	Last spray
HCH						
Malkangiri	5	04.11.93	1	75	24.0	Oct 93
	5	05.11.93	2	70	31.4	
	4	15.11.93	4	80	73.8	
	4	19.11.93	8	60	100.0	
K. Gumma	3	30.11.93	1	60	26.7	Oct 93
	6	02.12.93	2	120	55.0	
	3	07.12.93	4	40	77.5	
	3	08.12.93	8	60	100.0	
Korkunda	8	09.12.93	1	130	41.9	Oct 93
	9	10.12.93	2	130	54.4	
	3	13.12.93	4	80	63.8	
	4	16.12.93	8	60	100.0	
Khairput	5	06.01.94	1	60	26.6	Oct 93
	5	07.01.94	2	60	50.0	
	7	12.01.94	4	60	92.4	
	5	14.01.94	8	60	100.0	
DDT						
Malkangiri	4	08.11.93	1	60	100.0	Oct 93
K. Gumma	2	04.12.93	1	80	100.0	Oct 93
Korkunda	4	17.12.93	1	60	100.0	Oct 93
Khairput	7	08.01.94	1	60	100.0	Oct 93

to the residual insecticide and the results are presented in this paper.

MATERIALS AND METHODS

The insecticide susceptibility tests were carried out following standard procedure[6] with the diagnostic doses of HCH (0.4%) and DDT (4%). Insecticide impregnated papers used in the study were prepared at VCRC following the standard WHO procedure[7]. Fully fed indoor resting females of *An. culicifacies* and *An. fluviatilis* collected during morning hours from different PHC area of Malkangiri district were used in the test. Ten to twenty mosquitoes were exposed with triplicates for one hour to the diagnostic dose of each insecticide. Controls were maintained simultaneously. Mortality after 24 h holding periods in different replicates was cor-

rected using Abbott's formula[6]. Ninety-eight per cent corrected mortality was taken as the indicator of susceptibility and the proportion of the surviving mosquitoes after 24 h of holding periods was taken as the indicator of degree of resistance. Tests were repeated with the increase of exposure period from one to two hours and later four and eight hours to get the increased mortality up to 100% among resistant population.

RESULTS AND DISCUSSION

Out of seven PHCs of Malkangiri district, tests were carried out with *An. fluviatilis* collected from 35 villages of four PHCs and with *An. culicifacies* collected from 44 villages of five PHCs. Tests were not carried out in other PHCs due to non-availability of enough vector species. Results of susceptibility of *An. fluviatilis* and *An. culicifacies* to HCH and DDT are depicted in Tables 1 and 2.

The mortality rate of *An. fluviatilis* ranged from 24-41.9% in the four PHCs with one hour exposure to HCH, indicating that *An. fluviatilis* in the four PHCs areas developed resistance to HCH. With the increase of exposure time, mortality rate increased and at 8 h exposure 100% mortality was observed in all the PHCs.

An. fluviatilis from four PHCs when exposed to DDT for one hour showed 100% mortality, indicating that it is susceptible to DDT. Since the villages in different PHCs, from where the adults were collected for the test are distributed throughout the district, it can be concluded that *An. fluviatilis* is resistant to HCH and susceptible to DDT in Malkangiri district.

An. culicifacies showed higher degree of resistance to both HCH and DDT in all the five PHCs tested, as the mortality rate for one hour exposure ranged from 0 to 6.7. The mortality rate did not increase significantly ($p>0.05$) with increased exposure time except in Malkangiri PHC (4 h exposure to HCH, $\chi^2=18.3$, $p=0.0001$).

Earlier reports on the susceptibility tests carried out on *An. fluviatilis* in India have been a few due to paucity of adults[8]. This species is still susceptible to DDT and HCH in most of the places, though some tolerance to DDT and dieldrin has been reported from Maharashtra and Karnataka[9-11]. Resistance to DDT has been detected in Bihar, Karnataka, Maharashtra and Tamil Nadu[12]. In Orissa, resistance to DDT has been reported in Balasore and Puri districts[13]. The test carried out in the DDT sprayed area of erstwhile Koraput district of Orissa (Jeypore zone) showed that *An. fluviatilis* was susceptible to both HCH and DDT[5]. The present study shows that *An. fluviatilis* has developed resistance to HCH in Malkangiri district. It may be noted that *An. fluviatilis* is endophilic in Malkangiri zone[4]. The areas in Malkangiri district receive HCH spray since 1972. Some villagers also use

Table 2. Susceptibility status of *An. culicifacies* to HCH and DDT in Malkangiri district

PHC	No. of village covered	Date of Experiment	Exposure time (h)	No. exposed	Corrected mortality (%)	Last spray
HCH						
Malkangiri	3	02.04.93	1	30	0	Jul 92
	4	30.04.93	2	30	3.4	
	3	03.06.93	4	45	26.2	
Mathili	6	12.05.93	1	70	0	Jul 92
	3	24.05.93	2	45	0	
	4	14.06.93	4	45	11.1	
Korkunda	4	02.07.93	1	80	2.5	Jul 92
	3	05.07.93	2	80	3.8	
	3	06.07.93	4	80	1.3	
K. Gumma	4	06.08.93	1	80	5.0	Aug 93
	5	25.08.93	2	80	5.0	
	6	01.09.93	4	80	3.8	
Khairput	4	08.09.94	1	80	0	Aug 93
	3	14.09.94	2	80	0	
DDT						
Malkangiri	3	23.04.93	1	30	6.7	Jul 92
	3	18.05.93	2	45	4.4	
	4	07.06.93	4	45	8.9	
Mathili	6	19.05.93	1	90	0	Jul 92
	3	25.05.93	2	45	0	
	3	21.06.93	4	30	10.0	
Korkunda	10	29.06.93	1	80	2.5	Jul 93
	3	30.06.93	2	80	1.3	
	4	08.07.93	4	80	1.3	
K. Gumma	5	16.08.93	1	80	1.3	Aug 93
	5	27.08.93	2	80	3.8	
	5	03.09.93	4	80	5.0	
Khairput	5	10.09.93	1	80	2.5	Aug 93
	4	17.09.93	2	80	1.3	

HCH to smear on the false ceiling of their houses for protection from pests. These practices could have enhanced the development of resistance to HCH in this area.

In *An. culicifacies* resistance to DDT and HCH has been wide spread in the country[9]. Development of cross-resistance in *An. culicifacies* in this area to both the insecticides may be due to its preferential breeding in paddy fields, where chances of exposure to the agricultural pesticides is high. There are reports showing resistance in *An. culicifacies* due to usage of insecticides in agriculture[14-16]. Unlike tribals in Jeypore zone of the present Koraput district[17], the settlers (Bangladeshi refugees) in Malkangiri district use pesticides belonging to chlorinated hydrocarbon, oganophosphate and synthetic pyrethroid groups extensively in both Khariff and Rabi cultivation. Out of 76,130 ha of paddy fields available in the district, 76,000 ha are under paddy cultivation in Khariff and 1500 ha area during Rabi cultivation.

An. fluviatilis is mainly distributed in hilly tract villages[18] and the preferential breeding habitat is stream[19], where the chances of exposure to the agriculture pesticides is relatively less. Finding of resistance to HCH in the major malaria vector *An. fluviatilis*, raises question about the continued use of this insecticide in malaria control programme in this district. It would therefore be recommended to switch back to DDT, as the main vector is still highly susceptible to DDT.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. V. Dhanda, Director, Dr. P.K. Das, Sr. Dy. Director and Dr. P. Jambulingam, Assistant Director, Vector Control Research Centre for guidance and extending all facilities for carrying out the study. The technical assistance rendered by all the staff at VCRC Field Station, Malkangiri is also acknowledged.

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Indian Journal of Malariology
Vol. 32, September 1995, pp. 119-128.

Investigation of Malaria Outbreak in Rajasthan

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Jaisalmer and Barmer districts in the Thar Desert of Rajasthan experienced an unprecedented rains during 1994 leading to an outbreak of malaria. Investigations were carried out at three sites in two districts (i) Pokaran PHC and (ii) Nachana PHC in Jaisalmer district and Dhorimana in Barmer district during November 1994. Epidemiological and entomological studies in Pokaran PHC revealed presence of small foci of stable malaria. These foci were maintained by large bodies of water (ponds/lakes) drained from surrounding areas and spread over 1 to 5 sq km. Slide positivity rate (SPR), slide falciparum rate (SfR) and child spleen rate (SR) were 60.1, 56.9 and 86.9%, respectively with 3 deaths reported. Adjoining villages experienced epidemic reporting deaths fed by the reservoir from these stable foci. *An. culicifacies* and *An. stephensi* were the major malaria vectors.

Nachana PHC recorded stable malaria foci of irrigation malaria due to introduction of Indira Gandhi Canal (IGC). SPR, SfR and SR recorded were 52.35, 50.58 and 80.8%, respectively. Transmission appeared to be maintained by *An. stephensi*, *An. culicifacies* and *An. fluviatilis* in relays. *An. fluviatilis* seems to have established breeding in silted grassy margins of IGC. Dhorimana PHC in border district was also found to be a stable malaria foci although with low malaria indices. However, *An. culicifacies* and expanded breeding potential of *An. stephensi* were the main factors in maintaining malaria endemicity in the region.

Keywords: *An. culicifacies*, *An. stephensi*, Malaria outbreak, Rajasthan

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INTRODUCTION

On the basis of climate and physiography the Thar Desert of Rajasthan is considered to be a hypoendemic area for malaria with high potential of epidemics[1]. Barmer district on the international border of Pakistan recorded a wide spread outbreak, in 1990 with 47 deaths[2]. This epidemic was attributed to high rainfall preceded by prolong drought and build up of *An. culicifacies* population. During 1994, Jaisalmer and Barmer again received heavy rainfall, which resulted in build up of serious epidemic in these areas. A team of Malaria Research Centre carried out a retrospective study in November 1994 in few selected sites in these two districts. The findings of the study are presented in this paper.

Study area

District Jaisalmer and Barmer are situated in west of India, in the heart of Thar Desert of Rajasthan. Study area comprised two site(s) in District Jaisalmer viz. three villages namely Bhaniyana, Lawan and Dhursar with a population of 935, 1783 and 655 respectively under Pokaran PHC and Bhadaria (pop. 817) situated in the command area of Indira Gandhi Canal under Nachana PHC and Dhorimana village with population of 3429 of Godamalani PHC district, Barmer (Fig. 1).

Meteorological data: In Pokaran and Nachana areas temperature ranges between 42-49.2°C during summer (May-June) and between 1.5-9.6°C in winters (November-January). Average humidity was over 50% and rainfall of the study area was 8.4 cm in 1991 and 45.9 cm in 1994 (av. 16.4 cm). This shows nearly three-fold (179.9%) increase from normal rainfall in 1994 which resulted in submergence of 36 villages in floods.

In Dhorimana maximum temperature ranges from 41-49°C during summer and minimum from 5-13.9°C. Rainfall was 32.9 cm in 1990 and 63.55 cm in 1994. The annual normal rainfall recorded is 27.75 cm.

MATERIALS AND METHODS

Insecticidal spray: Data on insecticidal spray was collected and analysed and it was observed in Pokaran PHC, District Jaisalmer since 1991 to 1994, 50% DDT was used in two rounds. The per cent spray coverage was very poor which varied from 2.7 to 88% in cattlesheds and 15.15 to 57.72% in human dwellings.

In Godamalani PHC, District Barmer from 1991 to 1994, 50% DDT was used in two rounds. The per cent coverage varied from 45.5 to 93% in human dwellings and 31.5 to 100% in cattlesheds. The spray operations were carried out as contract job and such a spray was not satisfactory.

Case detection and treatment: Data on case detection, treatment and death records for the study villages were col-

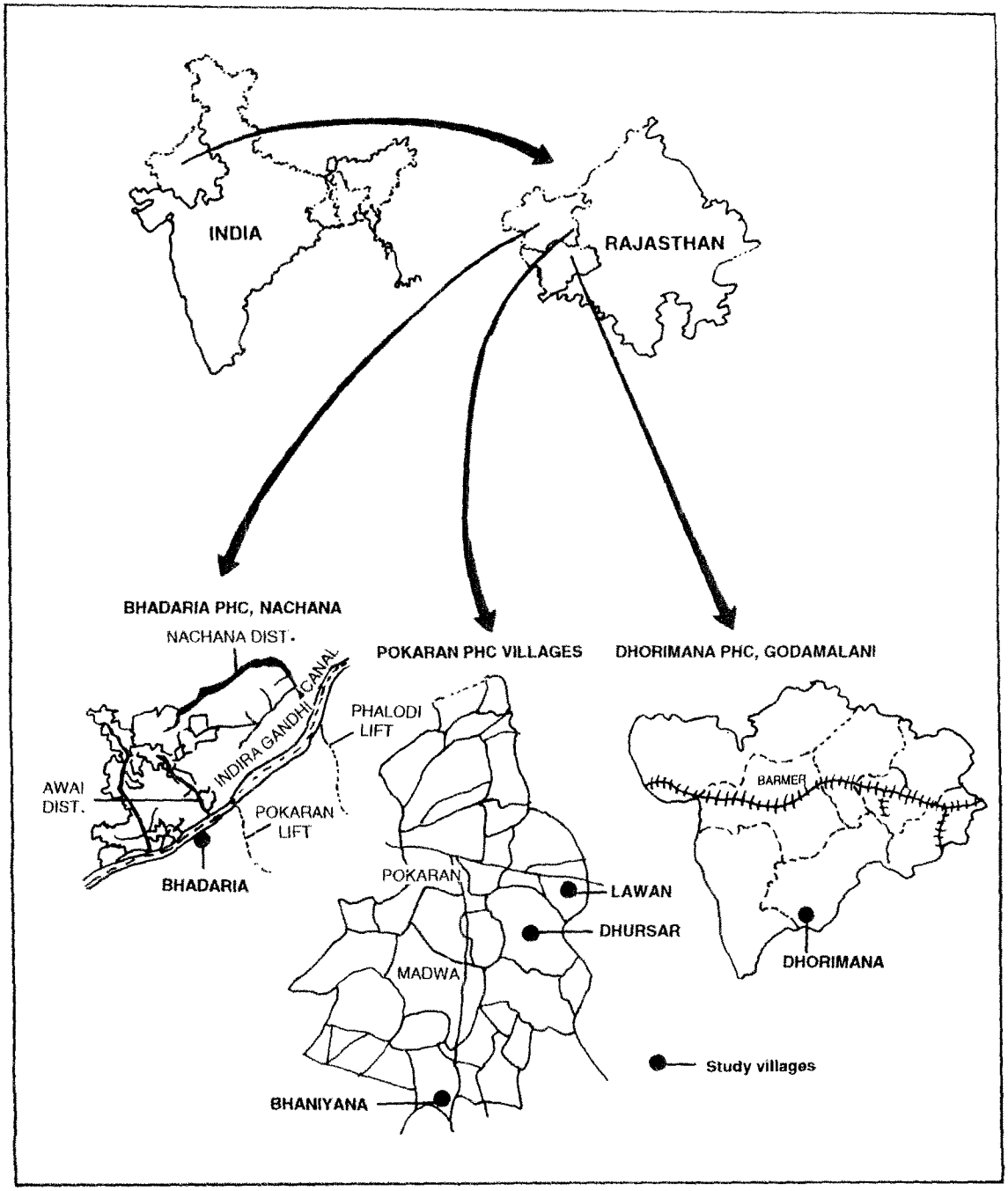

Fig. 1: Study area in Districts Jaisalmer and Barmer (Rajasthan)

lected from the concerned PHC. The active surveillance was made monthly and nearly 60% blood smears were collected from passive agencies. The time lag between the collection of blood slides and examination was within a fortnight and treatment after a week. Visits by health guide and multi-purpose/surveillance workers were irregular. These practices resulted in poor surveillance, delay in blood slide examination and radical treatment.

Mass survey: Mass blood survey was carried out to know the current malaria situation in all the three study areas. Positive cases were given radical treatments as per NMEP schedule.

Spleen survey: Spleen survey among children aged 2–9 yrs in all study areas were made according to Hackett[3] classification to know the endemicity of the disease.

Larval breeding survey: Immature mosquito collections from various potential breeding grounds like ponds, tanks (tankas), canals and its seepages using standard dipper (10 cm diam of 300 ml capacity) were carried out and density of vector species per dip was calculated. Anopheline larvae were reared up to IV instar to confirm the species identification[4].

Adult prevalence: Adult anopheline collections were made from indoor (cattlesheds and human dwellings), outdoor (dung cakes and stones) by standard method from different sites of villages to estimate per man hour density (MHD).

Abdominal condition of vector anophelines: Vectors collected from study villages were screened for abdominal conditions i.e. unfed, fully fed, semi gravid and gravid to understand feeding and resting behaviour.

Man-mosquito contact: Whole night collections of vectors were made from human bait indoors and outdoors to determine man-mosquito contact and biting pattern.

Susceptibility to insecticides: Susceptibility of *An. culicifacies* to DDT (4%), dieldrin (0.4%) and malathion (5%) were determined according to WHO test methods[5].

Parity rate: Parity rate was based upon study of treachial dilations of ovaries[6].

Vector incrimination study: It included dissection of salivary glands for detection of sporozoite in *An. stephensi* and *An. culicifacies* collected from indoors (human dwelling) and outdoors.

RESULTS

Surveillance data and death record for the year 1994 for the three study areas are given in Table 1. Out of 5185 blood smears prepared from 3 villages i.e. Bhaniyana, Lawan, and Dhursar, 1782 were found positive

(SPR 34.37%). In village Bhadaria, a total of 73 blood smears collected, out of which 20 were declared positive (SPR 27.39%). In village Dhorimana, out of 4824 blood slides prepared, 608 were found positive (SPR 12.6%). Two deaths (3 and 12 yr) from village Lawan, one death from village Bhaniyana (9 yr) and no deaths from village Dhursar, Bhadaria and Dhorimana were recorded (Table 1). The above findings reported from PHC data revealed highest incidence of malaria in villages of Pokaran PHC followed by Bhadaria and Dhorimana.

Surveys on mass blood and spleen in November 1994 revealed that out of 734 blood slides prepared from Pokaran PHC villages (Bhaniyana, Lawan and Dhursar), 441 were found positive (SPR 60.1%). In village Bhadaria a total of 170 blood smears were prepared and 89 were declared positive (SPR 52.35%) and in Dhorimana village, out of 354 blood smears made 104 were positive (SPR 28.96%). Malaria incidence was high in Pokaran PHC villages and Bhadaria and lowest in Dhorimana village. The spleen rates varied between 80-86.7% (Table 2).

Table 1. Surveillance data and death record from the three study areas

BSC/E	*Pv*	*Pf*	Mix	Total (+ve)	SPR	SfR	Deaths recorded
Pokaran PHC villages							
5185	666	1109	7	1782	34.37	21.52	3
Bhadaria (IGC area), PHC Nachana							
73	4	16	–	20	27.39	21.9	–
Dhorimana, PHC Godamalani							
4824	311	297	–	608	12.6	6.16	–

Table 2. Results of mass blood and spleen survey of the three study areas

BSC/E	*Pv*	*Pf*	Total	SPR	SfR	Spleen rate
Pokaran PHC villages						
734	23	418	441	60.1	56.9	86.7
Bhadaria (IGC area), PHC Nachana						
170	3	86	89	52.35	50.58	80.8
Dhorimana, PHC Godamalani						
359	17	87	104	28.96	24.23	80.0

Table 3. Results of larval breeding survey

Village/ Breeding sites	Anopheline density per dip Larvae*
Pokaran PHC villages	
Ponds**	0.2
Tanks***	0.08
Bhadaria (IGC area), PHC Nachana	
Tanks**	0.02
Seepage canal***	0.2
Canal****	0.2
Dhorimana, PHC Godamalani	
Tanks***	0.07

*III and IV instar; **An. *culicifacies* and An. *stephensi*; ***An. *stephensi*; ****An. *culicifacies*.

Larval surveys in potential breeding grounds of 3 villages of Pokaran PHC, Bhadaria and Dhorimana were conducted (Table 3). Anopheline density per dip revealed highest density (1.3) in tanks than to ponds (1.0). *An. culicifacies* and *An. stephensi* breeding was encountered in ponds, whereas *An. stephensi* preferred tanks. In village Bhadaria, highest anopheline density per dip was recorded in seepage (2.41), followed by canal (1.0) and tanks (0.02). Breeding of *An. culicifacies* and *An. stephensi* was observed in IG Canal and its seepage respectively. In tanks of village Dhorimana, anopheline density per dip was recorded 1.33 with *An. stephensi* breeding only.

Per man hour density (MHD) of vector anopheline in indoor and outdoor were recorded (Table 4). In Pokaran PHC villages highest density of *An. culicifacies* was recorded from cattlesheds

Table 4. Result of adult density in the three study areas

Village/Vector	Per man hour density (MHD)		
	Indoor		Outdoor (Dung cakes and stones)
	Human dwelling	Cattleshed	
Pokaran PHC villages			
An. culicifacies	5.3	16.3	3.0
An. stephensi	1.3	2.3	2.0
Bhadaria (IGC area), PHC Nachana			
An. culicifacies	5.0	N.A.	0
An. fluviatilis	9.0	N.A.	1.0
An. stephensi	8.0	N.A.	1.0
Dhorimana, PHC Godamalani			
An. stephensi	2.0	4.0	0

N.A. – Not available.

Table 5. Abdominal condition of vector anophelines in the two study areas

Vector anophelines	Average abdominal condition								Total
	Indoor resting				Outdoor resting				
	UF	FF	SG	G	UF	FF	SG	G	
Pokaran PHC villages									
An. culicifacies	1	28	28	8	0	4	2	3	74
An. stephensi	2	1	7	1	0	0	5	1	17
Bhadaria (IGC area), PHC Nachana									
An. culicifacies	0	1	2	2	0	0	0	0	5
An. fluviatilis	1	6	2	0	0	0	1	0	10
An. stephensi	1	2	3	2	1	0	0	0	9

UF — Unfed; FF — Fully fed; SG — Semi-gravid; G — Gravid.

(16.2) than human dwellings (5.3), whereas in outdoor its density was higher (3.0) than *An. stephensi* (2.0). In village Bhadaria, highest density was of *An. fluviatilis* (9.0) followed by *An. stephensi* (8.0) and *An. culicifacies* (5.0). No *An. culicifacies* was collected from outdoor, whereas *An. fluviatilis* and *An. stephensi* density was 1.0 for each species. In village Dhorimana, *An. stephensi* density was recorded highest in cattlesheds (4.0) than in human dwellings (2.0).

Abdominal condition of vector anophelines revealed highest percentage of fully fed and semi-gravid. In village Bhadaria, *An. culicifacies*, *An. fluviatilis* and *An. stephensi* feeding and resting were recorded higher in indoor than outdoor. Abdominal condition of the vectors was not studied from village Dhorimana (Table 5).

Man-mosquito contact study in village Bhaniyana PHC Pokaran was conducted. Biting density per human bait per night was 1.0 in indoor and average biting was recorded 0.5 for *An. culicifacies*. Vector susceptibility study to DDT (4%), dieldrin (0.4%), malathion (5%) against *An. culicifacies* revealed that after 24 h of exposure period the mortality rate was 30, 20 and 100% respectively. No deaths were recorded in control (Table 6). Parity rate study was conducted in a village Bhaniyana against *An. fluviatilis*. A total of 50 females were dissected showing 32% nulliparous and 68% parous. During study period a total of 83 *An. culicifacies* and 5 *An. stephensi* were dissected for gut and gland infection and none found positive for malaria parasite.

Table 6. Results of *An. culicifacies* susceptibility test to various insecticides in Bhaniyana village, PHC Pokaran

Insecticides	Concentration (%)	No. exposed	Mortality after 24 h exposure period	% mortality
DDT	4	10	3	30
Dieldrin	0.4	10	2	20
Malathion	5	10	10	100
Control	–	10	0	0

DISCUSSION

Investigations revealed that Rajasthan (Thar Desert), which is a well-recognized epidemic-prone area has established small foci of stable malaria, a fact so far overlooked. The stability of these foci are due to low-lying terrain features, where water gets accumulated in small lakes/ponds (ranging from 1-5 sq m) from its catchment area. Availability of this water leads to formation of green pastures, and ensure stability of human population. This is reflected in Pokaran PHC, where three villages viz. Bhaniyana, Lawan and Dhursar were maintaining high spleen rate among children of 2-9 yrs of age. High vector breeding potential of both *An. culicifacies* and *An. stephensi* in ponds and tanks were recorded[2]. There appears to be a herd immunity in the community as only one death due to malaria in Bhaniyana, two in Lawan and none in Dhursar (PHC records) were recorded. However, the villages situated at the periphery of these stable foci namely Mandwa and Pokaran reported seven deaths in the villages. It appears that whenever there is heavy rainfall in Rajasthan, breeding potential of *An. culicifacies* increases manifold. Reservoir of infection is provided by the migrants from this stable foci leading to rapid epidemic build up. The epidemic cycles (5-10 yrs) revolve around these stable foci.

Another dimension of the problem is the emergence of irrigation malaria and its entrenchment as foci of stable malaria. This has resulted due to introduction of Indira Gandhi Canal (IGC), which has its own ecological and epidemiological consequences. Studies carried out at Bhadaria, a highly affected village in the command area of IGC under Nachana PHC revealed high spleen rate 80.8% (SPR 52.35% and SfR 50.58%) among children of 2-9 years. These parameters show recurring transmission in the area.

Transmission appears to be maintained by three vector species. Extensive vector potential for breeding of *An.*

culicifacies was provided by water-logged swamps and canal due to lack of drainage of the command area. High density of *An. fluviatilis* (9) is a recent phenomenon. Species appear to have drifted from Aravali ranges[7] through Loni and Gurra rivers that traverse Jaisalmer district. The grassy silted margins of flowing waters of IG Canal seems to have provided the breeding habitats for the species. It is likely that the extended transmission is maintained by *An. stephensi* in spring, *An. culicifacies* during monsoon and *An. fluviatilis* during post-monsoon period. Further indepth studies on the subject is highly desirable to verify the dynamics of transmission.

Studies in Dhorimana PHC, Godamalani in Barmer district revealed that the area is comparatively less endemic compared to Pokaran PHC and irrigation command area of IGC. Although spleen rate was high, but SPR and SfR were 28.96% and 24.23% respectively. Community tanks and water reservoirs have provided additional breeding potential of the species.

Records of malaria deaths revealed that there were few deaths from the study areas and only two deaths were reported from village Loharwan, a satellite village of this foci.

Entomological information on the vector species reveals that all the three vector species as reflected are endophilic and endophagic. Intensity of resting in partly filled tanks was observed to be more rather than in fully filled tanks. Information on parity rate and man-mosquito contact indicates that the vector species have adequate longevity and contact with the community to maintain extended transmission.

Susceptibility test carried out with *An. culicifacies* at discriminating doses indicated that the species is resistant to DDT and dieldrin but continue to be susceptible to malathion. No suitable test could be carried out with *An. stephensi* and *An. fluviatilis* due to lack of specimen.

ACKNOWLEDGEMENTS

Authors are highly grateful to Dr. V.P. Sharma, Director and Shri N.L. Kalra, Consultant, Malaria Research Centre for their guidance, advice and active interest in planning the study and preparation of manuscript. Grateful acknowledgements are expressed to Dr. R.C. Mathur, Dr. J.S. Rathore, Jaisalmer; Shri Rajendra Singh, Government Agriculture Research Centre, Dr. Raghubir Singh, Nachana; Shri M.K. Pareekh, Dr. V.D. Jyetha, Shri Avardan, Pokaran; Dr. Girdhar Bhoomia, and Miss Kalpana, Barmer for their help in providing data for the study. The technical assistance rendered by Shri J.C. Sharma, Shri C.S. Bisht and Shri C.S. Mahara, MRC Field Station, Haldwani is highly appreciated.

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SHORT NOTE

Indian Journal of Malariology
Vol. 32, September 1995, pp. 129-131.

Incrimination of *Anopheles minimus* as a Vector of Malaria in Karbi Anglong District of Assam

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Keywords: *Anopheles minimus*, Assam, Incrimination, Malaria, Sporozoite

Topogeography of Greater Assam of pre-DDT era comprised all the present states of northeast had many ecotypes inhabiting different ethnic tribal groups. Many studies aimed at incrimination of malaria vectors prominently brought out *An. minimus* as the primary vector[1,2]. However, after the start of DDT spray in 1958, under NMEP, the species showed transient disappearance[3]. After the withdrawal of DDT spray, the species again surfaced in it erstwhile endemic areas and re-established malaria endemicity. Meanwhile, Greater Assam underwent political upheavals and seven small states were carved out leaving alone Assam with plain areas. Recently, the role of *An. minimus* in the transmission of malaria was confirmed in District Kamrup and the areas of Nowgong and Dibrugarh. Present investigations were carried out in one of the ethnic district viz. Karbi Anglong, where *P. falciparum* shows resistance to chloroquine.

A malaria survey was carried out in Khotkhoti area of Karbi Anglong district of Assam during March to June 1993. The study area is a heterogeneous terrain with foothills, forests and

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inhabited by tribals like Kachari, Karbi, with a few settlers from Nepal and ex-tea garden labours.

A total of 703 blood slides of fever cases were examined and out of these 122 cases were positive for malaria (80.33 per cent cases of *P. falciparum*, 18.04 per cent of *P. vivax* and 1.60 per cent of mixed infections of both *Pf* and *Pv*) showing slide positivity rate (SPR) and slide falciparum rate (SfR) of 19.06 and 13.9 per cent respectively for this period.

Table 1 shows the record of mosquitoes collected in the indoor resting sites from human dwellings and the results of dissection of salivary glands for malaria parasite. The results of dissection revealed the presence of sporozoites in the salivary glands of 10 specimens of *An. minimus* collected from human dwellings showing sporozoite rate of 4.06 per cent. Thus, *An. minimus* has been incriminated as a vector of malaria for the first time in Khotkhoti area of Karbi Anglong district, Assam. The detection of malarial infections in a good number of specimens of *An. minimus* is worthy of serious consideration because there is every possibility of dissemination of malaria infection to large human population of the area investigated. The predilection for indoor day resting of the vector *An. minimus* was evident.

Although in earlier studies, *An. minimus* was thought to have disappeared from the Terai region of Uttar Pradesh[4] and from north-eastern region of India[3], but in recent studies, *An. minimus* has been again incriminated from the north-eastern region of India[5-8]. The present study also reveals there are still some areas in Assam, particularly the foothill areas as well as the areas bordering other hill states with poor or no DDT spray coverage experiencing a persistent type of malaria, where

Table 1. Indoor resting collection of anopheline mosquitoes from human dwellings and results of dissection for malaria parasites

Species	No. collected	MHD	No dissected*	Gland (+)ve	Sporozoite rate (%)
An. aconitus	26	1.44	24	0	0.00
An. annularis	6	0.33	6	0	0.00
An. jeyporiensis	12	0.67	10	0	0.00
An. minimus	249	13.83	246	10	4.06
An. philippinensis	3	0.17	2	0	0.00

MHD — Man hour density; *Only live mosquitoes were dissected.

An. minimus is playing vectorial role for transmission of malaria.

ACKNOWLEDGEMENTS

Excellent technical assistance received from the staff of Malariology Section, RMRC, Dibrugarh is gratefully acknowledged.

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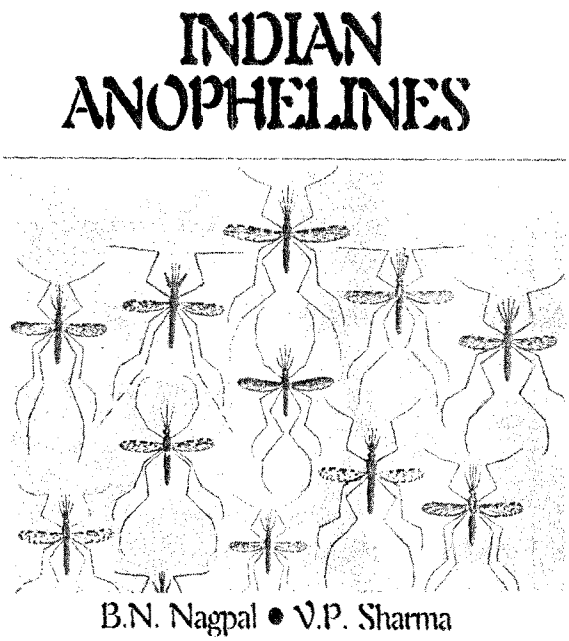

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