INDIAN JOURNAL OF MALARIOLOGY

Volume 33 Number 1

March 1996

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

INDIAN J. MALARIOL.

Quarterly
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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.

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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.

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INDIAN JOURNAL OF MALARIOLOGY

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Volume 33
Number 1
March 1996

Short Note

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Insecticide Susceptibility Status of Some Anophelines in District Bikaner, Rajasthan

S.K. BANSAL and KARAM V. SINGH

Insecticide susceptibility tests were conducted on the adults of four anopheline species namely, *Anopheles annularis, An. culicifacies, An. stephensi* and *An. subpictus* against the diagnostic doses of six insecticides, viz. DDT (4.0%), dieldrin (0.4%), malathion (5.0%), fenitrothion (1.0%), propoxur (0.1%) and permethrin (0.25%) in District Bikaner (Rajasthan). A time dependent effect has been observed with each insecticide. All the four species were found resistant to DDT and dieldrin and susceptible to fenitrothion and permethrin. *An. culicifacies* and *An. subpictus* showed susceptibility to malathion, while further verification for the other two species was required. However, with propoxur *An. annularis* showed resistance, whereas for other three species further studies are required. DDT and dieldrin, the two organochlorines, were found least effective as compared to organophosphates and carbamates.

Keywords: Anophelines, Bikaner, Insecticides, Susceptibility

INTRODUCTION

District Bikaner, in the north-western Rajasthan, is situated in the Thar Desert characterised by sandy dunes, extreme temperatures and least floral and faunal diversity in particular vector mosquitoes[1-3]. Six anopheline species have been identified in District Bikarner[4], of which *An. culicifacies* and *An. stephensi* are considered as primary and *An. annularis* and *An. subpictus* the secondary vectors. The importance of these malaria vectors can only be recognised by the fact that north-western Rajasthan had to pay a

Desert Medicine Research Centre (ICMR), Post Box 122, New Pali Road, Jodhpur - 342 005, India.

Table 1. Per cent mortality and susceptibility status of *An. annularis* and *An. culicifacies* exposed to different insecticides in District Bikaner

Mosquito species	Per cent mortality and susceptibility status					
	DDT (4.0% x 1h)	Dieldrin (0.4% x 1h)	Malathion (5.0% x 1h)	Fenitrothion (1.0% x 2h)	Propoxur (0.1% x 1h)	Permethrin (0.25% x 1h)
An. annularis	36.2* (102/37)	36.7 (98/36)	94.6 (92/87)	100.0 (112/112)	63.0 (100/63)	98.0 (99/97)
	36.2** (R)***	33.4 (R)	94.6 (V)	100.0 (S)	60.9 (R)	98.1 (S)
An. culicifacies	60.6 (94/57)	64.4 (87/56)	98.7 (81/80)	100.0 (105/105)	96.6 (87/84)	98.0 (100/98)
	60.6 (R)	62.0 (R)	98.6 (S)	100.0 (S)	96.4 (V)	98.0 (S)

*Per cent test mortalities; **Per cent corrected mortalities; ***Susceptibility status; R – Resistant (<80%); V – Verification required (80-98%); S – Susceptible (>98%); Values in parentheses indicate sample size (no. exposed/no. dead).

Table 2. Per cent mortality and susceptibility status of *An. stephensi* exposed to different insecticides for different exposure durations

Insecticide (Conc.)	15 min	30 min	45 min	60 min	120 min	LT_{50} and susceptibility status
DDT (4.0%)	–	12.6 (87/11)	–	40.0 (85/34)	50.4 (101/51)	108 min (R)
Dieldrin (0.4%)	–	20.0 (95/19)	–	45.0 (80/36)	56.2 (96/54)	89 min (R)
Malathion (5.0%)	52.6 (112/59)	60.4 (91/55)	84.0 (75/63)	91.3 (103/94)	–	11 min (V)
Fenitrothion (1.0%)	20.5 (78/16)	40.0 (105/42)	70.5 (85/60)	94.6 (93/88)	100.0 (118/118)	31 min (S)
Propoxur (0.1%)	24.0 (96/23)	44.6 (83/37)	64.2 (95/61)	88.8 (98/87)	–	31 min (V)
Permethrin (0.25%)	55.5 (72/40)	78.4 (97/76)	90.2 (92/83)	100.0 (105/105)	–	14 min (S)

R – Resistant (<80%); V – Verification required (80-98%); S – Susceptible (>98%); LT_{50} – Lethal time in which 50% mortality occurs; Values in parentheses indicate sample size (no. exposed/no. dead).

heavy toll after a malaria epidemic during later half of the 1994[5]. Hence, identification of the vector, establishing its role in transmission and finally its control either by biological or chemical means is the basic requirement for planning an effective vector control strategy in an area. The present insecticide susceptibility status of the above four malaria vectors was determined in District Bikaner during 1993-1994 in the study.

MATERIALS AND METHODS

Fully-fed adult females of all the four anophelines namely, *An. annularis*, *An. culicifacies*, *An. stephensi* and *An. subpictus* were collected early in the morning from 15 villages located in four tehsils of District Bikaner. These mosquito species were collected daily for 7-10 days/month from July to October 1993 and again from March to October 1994. Collections were made from human dwellings and cattlesheds with the help of an aspirator supplied by WHO and kept in Barraud cages with cotton pads soaked in 10% glucose solution.

Insecticide tests were conducted on the engorged females (temperature 28±2^{0}C and RH 75-80%) as per the prescribed procedure[6]. DDT (4.0%), dieldrin (0.4%) malathion (5.0%), fenitrothion (1.0%), propoxur (0.1%) and permethrin (0.25%) impregnated papers supplied by WHO were used. In each test 20-25 adults of each species were exposed to the insecticides for different exposure durations. Tubes were kept horizontal while exposing the mosquitoes to pyrethroids[7]. Three to five replicates were used. Per cent mortalities were recorded 24 h after exposure. Whenever, control mortality exceeded 5%, the corrected mortality was calculated by Abbott's formula[8]. LT_{50} values were estimated from the log concentration-probit regression lines as given in probit analysis method[9]. Susceptibility status was determined as per WHO criteria[10] for anophelines.

RESULTS AND DISCUSSION

Results of mortality observed with all the four anopheline species namely, *An. annularis*, *An. culicifacies*, *An. stephensi* and *An. subpictus* after exposure to different insecticides are presented in Tables 1-3. A time dependent effect has been observed with all the insecticides tested. On exposure to DDT and dieldrin it is quite apparent that all the four anophelines are resistant showing that both the above organochlorines are no more effective on these vector species. Resistance to DDT and dieldrin in several anopheline species has also been reported by several authors in different parts of India[10-16]. Deobhankar and Palkar[17] worked out the magnitude of DDT resistance in *An. culicifacies* to approximately 128 fold at LC_{50} level in about 20 yrs in Maharashtra state. High resistance in mosquitoes especially of the culicines towards DDT[18,19] in District Bikaner may perhaps be attributed to its continuous use for last three decades. Similarly a high degree of resistance

Table 3. Per cent mortality and susceptibility status of *An. subpictus* exposed to different insecticides for different exposure durations

Insecticide (Conc.)	15 min	30 min	45 min	60 min	120 min	LT_{50} and susceptibility status
DDT (4.0%)	–	10.5 (86/9)	–	21.4 (103/22)	44.8 (67/30)	158 min (R)
Dieldrin (0.4%)	–	20.0 (80/16)	–	39.1 (92/36)	58.9 (95/56)	68 min (R)
Malathion (5.0%)	64.6 (65/42)	89.0 (109/97)	92.5 (80/74)	100.0 (63/63)	–	11 min (S)
Fenitrothion (1.0%)	14.6 (75/11)	34.3 (64/22)	73.1 (93/68)	96.6 (90/87)	100.0 (115/115)	32 min (S)
Propoxur (0.1%)	28.0 (75/21)	48.2 (83/40)	72.8 (92/67)	84.7 (111/94)	–	27 min (V)
Permethrin (0.25%)	70.6 (92/65)	88.5 (61/54)	95.0 (100/95)	98.0 (100/98)	–	9 min (S)

R – Resistant (<80%); V – Verification required (80–98%); S – Susceptible (>98%); LT_{50} – Lethal time in which 50% mortality occurs; Values in parentheses indicate sample size (no. exposed/ no. dead).

against dieldrin has been observed, which was never used in this area.

Test conducted with malathion and fenitrothion (organophosphates) have indicated a complete susceptibility of all the four anopheline species except an intermediate resistance shown by *An. stephensi* and *An. annularis* with malathion. *An. annularis* has also shown resistance to propoxur, a carbamate insecticide, while a verification is required with rest of the anopheline species. Several anopheline species are quite susceptible to malathion in different parts of India[13-15], while resistance has been indicated in other parts[16,20]. Stratified maps of India showing areas with DDT, HCH and malathion resistance to *An. culicifacies*, the major vector of rural malaria in India, have been prepared by Sharma[21].

Synthetic pyrethroids are highly effective against mosquitoes as larvicides, pupicides, adulticides and repellents[22,23]. Tests with permethrin (0.25%), a synthetic pyrethroid, indicate that all the four anopheline species are fully susceptible in the present investigation. Pyrethroids, therefore, can be the insecticides of choice in future because of their high toxicity to target organisms and low to non-target organisms especially mammals.

Present susceptibility data from the desert lands of north-western Rajasthan with developmental activities due to Indira Gandhi Canal, in the absence of any previous report, may reasonably serve as a baseline for future studies. Further studies on the biochemical mechanisms of insecticide resistance are needed for confirming the variable responses shown by different species.

ACKNOWLEDGEMENTS

Authors are thankful to Dr. R.C. Choudhary, OIC, DMRC, Jodhpur for providing necessary research facilities and to Mr. Mahavir Prasad for his help in the field investigations.

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Indian Journal of Malariology
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Determination of Levels of HCH and DDT in Soil, Water and Whole Blood from Bioenvironmental and Insecticide-Sprayed Areas of Malaria Control

V.K. DUA, C.S. PANT and V.P. SHARMA[a]

Concentrations of HCH and DDT in soil, water and whole blood were determined in two areas under malaria control. These were, (i) bioenvironmental control of malaria at BHEL, and (ii) residual spraying of insecticides in rural and urban area of Bahadrabad PHC of Hardwar district. Mean concentrations of HCH in soil and whole blood samples from BHEL was 2.26 µg/kg and 1.20 µg/l and from Bahadrabad 61.12 µg/kg and 24.3 µg/l respectively. Similarly, the mean concentration of DDT in soil and whole blood from BHEL was 3.68 µg/kg and 4.71 µg/l, while in Bahadrabad 270.51 µg/kg and 38.13 µg/l respectively. HCH and DDT were never detected in any water samples from BHEL area, while the mean concentration of these compounds in water of Bahadrabad area was 0.18 and 0.07 µg/l respectively. Residual level of HCH and DDT were 27 and 73.5 times higher in soil and 20.2 and 8.1 times higher in whole blood samples from Bahadrabad as compared to their corresponding values from BHEL respectively.

Keywords: Blood, DDT, HCH, Soil, Water

INTRODUCTION

Organochlorine insecticides DDT and HCH have been extensively used in India for the control of vector-borne diseases. The effectiveness of DDT and HCH was due to their stability in environment, high solubility in fat and low

Malaria Research Centre (Field Station), BHEL, Ranipur, Hardwar-249 403, India.
[a]Malaria Research Centre, 20-Madhuvan, Delhi-110 092, India.

or negligible solubility in water. However, due to their chemical nature and extensive use in public health, DDT and HCH have become major environmental pollutants. DDT residues have been reported in soil, water, blood and food[1-4]. DDT and HCH were also detected in bovine and human milk[5,6]. As an alternative to spraying, bioenvironmental malaria control strategy was implemented in certain areas with cost-effective and sustainable malaria control. Under this strategy, ecofriendly methods like biological control, source reduction and environmental management were adopted for malaria control[7,8]. In addition to many advantages of the alternate strategy[9], it was summarized that from areas under the bioenvironmental control strategy the environment would be protected from insecticide pollution.

A study has been taken up to compare the levels of DDT and HCH in soil, water and whole blood from areas under malaria control, (i) bioenvironmental, and (ii) insecticide-sprayed methods. The study would have implications in promoting malaria control programme by ecofriendly methods and thus avoiding the problem of environmental contamination. The results of this study are presented in this paper.

MATERIALS AND METHODS

Study area

Soil, water and whole blood samples were collected from Bharat Heavy Electricals Limited (BHEL), Ranipur, Hardwar. BHEL is spread over an area of 25 sq km with a population of 70,000. Insecticide spraying had been stopped in this complex as bioenvironmental methods were implemented to control malaria by Malaria Research Centre (ICMR) since July 1986. An adjoining area of BHEL comprising rural and urban parts of Bahadrabad PHC of Hardwar district was selected as control for comparison (Fig. 1). In this area DDT and HCH were being sprayed for malaria control by the Health Department of the state government during the monsoon season extending from July to September. The yearly usage of HCH during 1987-1992 was 9.0, 4.6, 0.5, 0.6, 1.2 and 1 MT respectively, while DDT was sprayed only in 1991 with a total consumption of 6.5 MT for mosquito control programme in the Bahadrabad area.

Sample collection

Soil: Twenty-eight soil samples were collected from the residential sites covering playgrounds, lawns and gardens of bioenvironmental (BHEL, n=14) and insecticides sprayed (Bahadrabad, n=14) areas during October-November 1991. 250 g soil was scratched from the surface (10 x 10 sq cm) up to 5 cm depth from each site and collected in polyethylene bags. The samples were brought to the laboratory, dried and kept at room temperature till analysed.

Water: Ground water was the only source of water supply in BHEL town-

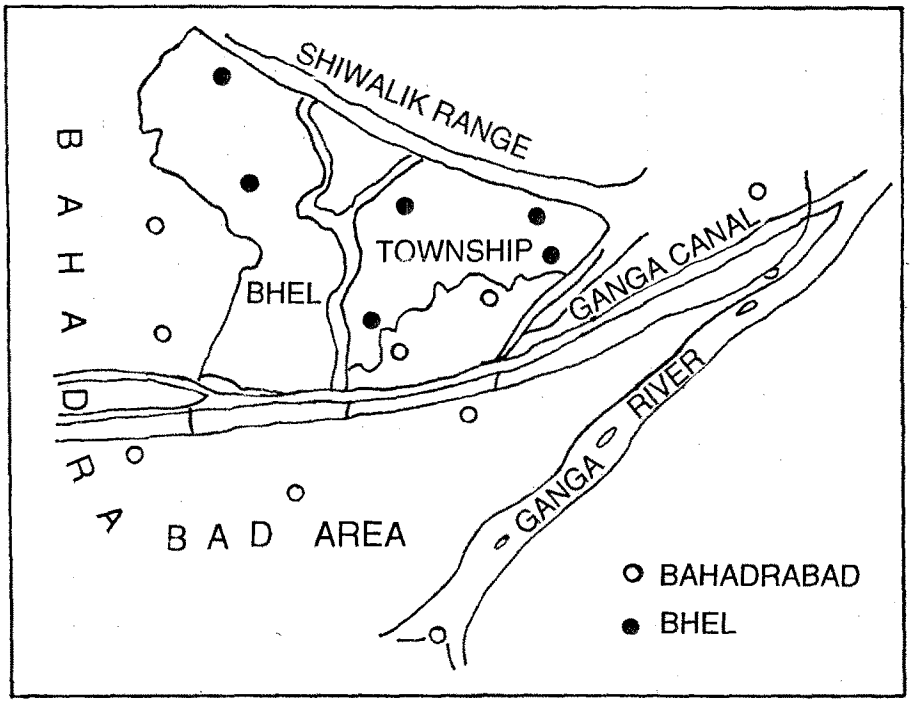

Fig. 1: Map showing the sample sites of BHEL (bioenvironmental) and Bahadrabad (insecticide-sprayed) area

ship, while water from Ganga River was used for drinking purposes in the insecticide sprayed area. Ten water samples (1000 ml) were collected in glass bottles from taps from BHEL (n=5) and Bahadrabad (n = 5) during October 1991. The glass bottles were checked before sampling for insecticide contamination. Samples were brought to laboratory and extracted immediately.

Whole blood: Thirty-six whole blood samples (1 ml) were collected intravenously in oxilated vials from the patients visiting BHEL hospital and district hospital dispensaries located at the BHEL and Bahadrabad PHC in the months of October-November 1992. Nineteen blood samples were from the patients residing in BHEL township, whereas 17 were residents of Bahadrabad PHC area. All the patients were in 18-65 years age group. Samples were stored at 4°C till analysis was done.

Extraction and clean up

Soil: Fifty gm dried soil sample was extracted thrice with 50 ml methanol (AR) by shaking in orbital mixture for 4 h. The extract was filtered on Whatman No. 1 filter paper and concentrated to 1 ml.

Water: One litre water sample was extracted thrice with 50 ml n-hexane (AR) for 10 min in a separatory funnel and n-hexane portion was pooled, dried over anhydrous sodium sulphate, filtered and concentrated to 1 ml.

Whole blood: One ml blood sample was extracted thrice with 5 ml n-hexane by Vortex mixing for 15 min and centrifuged for 15 min at 1500 rpm to break the emulsion. The n-hexane layer was filtered, pooled and concentrated to 1 ml on Vortex evaporator.

All concentrated extracts of various samples were cleaned up with anhydrous sodium sulphate – alumina column and eluted with n-hexane:benzene (40:60 v/v). The eluant was evaporated to 1 ml using Vortex evaporator and stored at 4°C.

Analysis

Samples were analysed for alpha, gamma, beta and delta-isomers of HCH, o,p'-,p,p'-DDE and DDT residues on Hewlett-Packard 5890A gas chromatograph fitted with Ni^{63} electron capture detector on 5% silicon OV-17 coated on Gas Chrom Q (80-100 mesh) packed glass column. Nitrogen (flow @ 120 ml/min) was used as carrier gas with injector 210°C, oven 190°C and detector 220°C temperatures. The identification of DDT and HCH residue peaks were cross checked on another column 5% DEGS coated with Gas Chrom Q (100-200 mesh) glass column[10]. The identity of DDE and HCH residues in different samples was further confirmed by Gas chromatography-Mass spectrometry (GC-MS). The minimum quantity which could be detected by this method was 0.1 ng of HCH isomers and DDT metabolites. The detection limit for soil, water and blood were 0.02 µg/kg, 0.002 µg/l and 0.003 µg/l respectively.

RESULTS AND DISCUSSION

The average (n = 5) percentage recoveries of DDT and its metabolites and HCH isomers in soil, water and whole blood were more than 80% in all cases. The residue levels reported in this paper have not been corrected for recoveries. All analyses were carried out on 5% OV-17 column using nitrogen gas (flow @ 120 ml/min) as carrier gas. A prominent peak at m/e 282 due to molecular ion of HCB (hexachloro benzene) in the mass spectrum of GC-MS analysis confirmed the presence of HCH residues in the samples, while the m/e peak at 316 due to molecular ion of DDE identified DDE residues in the samples[11].

Table 1 shows the residue levels of HCH isomers, DDT and its metabolites in soil samples taken from (i) BHEL (bioenvironmental methods), and (ii) Bahadrabad (under DDT/HCH spray). Gamma-HCH was 44% in BHEL and 89% in Bahadrabad of total HCH present in particular area. Similarly, p,p'-DDT and p,p'-DDE contributed 14.9 and 48% in BHEL and 59.8 and 28% in

Table 1. Concentrations (μg/kg) of HCH and DDT residues in soil

Insecticides	BHEL	Bahadrabad
α-HCH	0.28±0.07* (ND-1.00)	2.84±0.80 (0.50-9.70)
γ-HCH	1.00±0.27 (ND-3.10)	54.69±28.3 (4.30-362)
δ-HCH	0.97±0.18 (ND-2.00)	3.58±1.73 (ND-23.0)
Total HCH	2.26±0.55 (0.3-4.0)	61.12±29.2 (5.1-362.9)
o,p'-DDE	1.07±0.38 (ND-5.00)	5.27±1.10 (ND-11.9)
p,p'-DDE	1.78±0.60 (ND-8.80)	76.91±24.5 (9.6-324)
o,p'-DDT	0.27±0.14 (ND-2.00)	26.44±15.3 (ND-218.1)
p,p'-DDT	0.55±0.23 (ND-3.30)	161.9±104 (4.6-1500)
Total DDT	3.68±0.74 (ND-9.60)	270.5±34.3 (21.1-1833)

*Mean±SE; ND < 0.02 μg/kg; Figures in parentheses are ranges.

Bahadrabad respectively of total DDT present. High values of standard error of mean for HCH and DDT residues have been observed which indicated wide distribution of these residues among the soil samples. The use of HCH and DDT in Bahadrabad was responsible for higher gamma-HCH and p,p'-DDT as compared to BHEL, where bioenvironmental measures were in operation for six years and no HCH or DDT was sprayed. This fact is further supported by higher concentration of DDE in BHEL as compared to DDT which is due to degradation of DDT to DDE with time. The concentration ranges with reference to their mean values were higher in Bahadrabad as compared to BHEL due to their regular use. HCH and DDT were detected in all soil samples from BHEL area in spite of the fact that none were used in this area in the last six years due to implementation of bioenvironmental methods of malaria control[7]. This might be due to long persistance and aerial transport of these insecticides[12]. Bevenue[13] has also reported that DDT and its metabolites can persist in soil for many years. Pillai[1] have detected DDT residues in soil ranged from 0.01 to 2.61 mg/kg from Delhi which is very high as compared to our results in BHEL (<0.02-9.60 μg/kg) and similar to Bahadrabad (21.1-1833 μg/kg).

Results of water analysis for HCH and DDT residues are given in Table 2. Results revealed that no HCH and DDT were detected in any water samples from BHEL township. The township is situated in the rocky foothill of Shiwalik range. It is surrounded by forest and agriculture land is less and non-irrigated. Though the presence of HCH residues in rain water was reported

Table 2. Concentrations (µg/l) of HCH and DDT residues in water samples

Insecticides	BHEL		Bahadrabad	
α-HCH	ND	–	0.06±0.02*	(0.04–0.10)
γ-HCH	ND	–	0.05±0.01	(0.04–0.06)
δ-HCH	ND	–	0.07±0.03	(ND–0.15)
Total HCH	–	–	0.18±0.05	(0.08–0.25)
o,p'-DDE	ND	–	0.01±0.00	(0.006–0.02)
p,p'-DDE	ND	–	0.01±0.00	(0.003–0.02)
o,p'-DDT	ND	–	0.01±0.00	(ND–0.02)
p,p'-DDT	ND	–	0.04±0.02	(ND–0.07)
Total DDT	–	–	0.07±0.03	(0.01-0.12)

*Mean±SE; ND < 0.002 µg/l; Figures in parentheses are ranges.

Table 3. Concentrations (µg/l) of HCH and DDT residues in human blood

Insecticides	BHEL		Bahadrabad	
α-HCH	0.80±0.01*	(0.03-0.29)	5.36±2.10	(0.04-31.25)
β-HCH	1.09±0.09	(0.30-2.57)	13.6±3.86	(0.19-42.85)
γ-HCH	0.03±0.01	(ND-0.27)	5.31±3.50	(ND-59.14)
Total HCH	1.20±0.13	(0.44-3.16)	24.3±8.65	(0.25-129.3)
o,p'-DDE	ND	–	4.23±1.67	(ND-17.45)
p,p'-DDE	4.71±1.50	(ND-25.00)	33.9±6.36	(4.33-81.9)
Total DDT	4.71±1.50	(ND-25.00)	38.13±7.4	(4.33-90.7)

*Mean±SE; ND < 0.003 µg/l Figures in parentheses are ranges.

from BHEL[14] but it could not reach ground level up to the detection limit (0.002 µg/l). However, mean concentrations of HCH and DDT in sprayed area were 0.18 and 0.07 µg/l respectively. The levels of gamma-HCH and p,p'-DDT were 26.6 and 57% of total HCH and DDT present. The run-off water from surface soil, extensive use of insecticides in malaria control programme and atmospheric contamination are responsible for their presence in Ganga water thereby detected in water samples of Bahadrabad area. However, the concentrations of gamma-HCH and total DDT do not exceed the

maximum permissible limit reported by WHO[15] for gamma-HCH (3 μg/l) and DDT (1 μg/l). Agarwal *et al.*[16] showed that Yamuna water in Delhi has DDT residues in appreciable quantities. Ganga water was also found contaminated with different insecticides of varying degree (Ganga Action Plan, ITRC, Lucknow).

Concentrations of HCH and DDT residues in whole blood from two areas are given in Table 3. Traces of gamma-HCH were found in both study areas, while p,p'-DDT was not detected in any sample. DDT concentrations reported in BHEL and Bahadrabad area are lower than the earlier reports from Delhi, Lucknow and Jaipur population[2,17,18] and similar to Ahmedabad[3]. The presence of HCH and DDT in human blood from BHEL might be due to dietary uptake of insecticides contaminated vegetables, fruits, food, bovine milk and other eatables. The average daily intake of HCH and DDT by Indians were estimated to be 115 and 48 mg/person respectively[19].

A comparison of HCH and DDT residues in insecticidal sprayed and bio-environmental areas is given in Fig. 2. It is clear that the levels of HCH and DDT in soil samples from Bahadrabad are 27 and 74 times higher and in whole blood, 20 and 8 times higher as com-

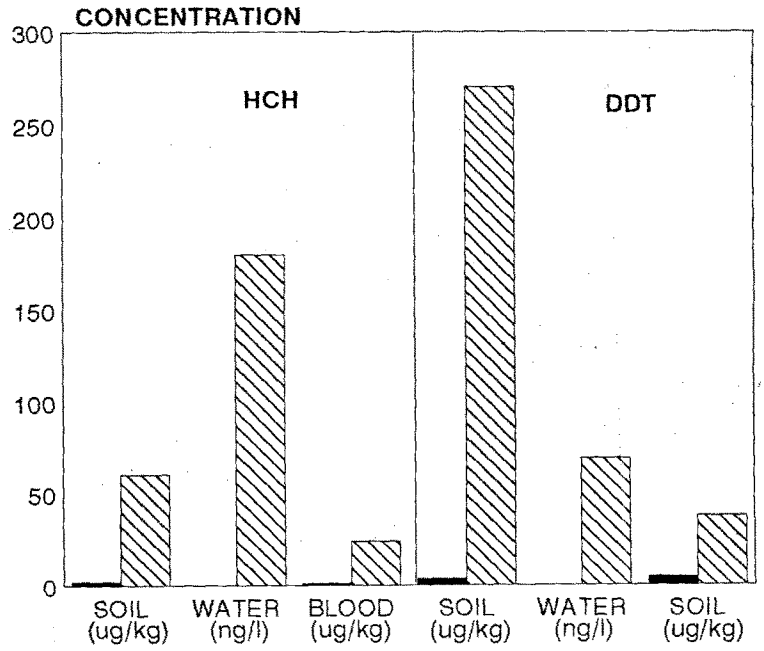

Fig. 2: Comparison of HCH and DDT in BHEL and Bahadrabad

pared to their corresponding values from BHEL. Chand *et al.*[18] have reported 2 and 5 times higher concentration of HCH and DDT residues in experimental than control populations respectively. No HCH and DDT was detected in water from BHEL, while their mean concentrations in sprayed areas were 0.18 and 0.07 µg/l respectively. Battu *et al.*[5] reported that the level of DDT residues in bovine milk from the DDT-sprayed area were 4-12 times higher than their corresponding levels from HCH-sprayed area and HCH level in bovine milk from the HCH-sprayed area were 2-11 times higher than the DDT-sprayed area.

Statistical comparison of the residual level of DDT in soil and whole blood from two different strategic areas showed the significant difference in the mean values [t (soil) = 1.126, $p < 0.05$; t (blood) = 4.603, $p < 0.001$]. Significant differences were also observed for HCH residues in soil and blood from BHEL and Bahadrabad [t (soil) = 2.060, $p < 0.05$; t (blood) = 2.785, $p < 0.01$].

The present study clearly indicates that the levels of HCH and DDT in BHEL area with bioenvironmental methods of malaria control were significantly lower in soil, water and whole blood samples as compared to Bahadrabad area, where HCH and DDT were used to control malaria.

ACKNOWLEDGEMENTS

We thank Mr. Rajesh Kumar Mittal for help in collection of different samples. The authors express appreciation to Mr. R.S. Bhardwaj for typing the manuscript.

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Indian Journal of Malariology
Vol. 33, March 1996, pp. 16-20.

Hut-Scale Trial of Pyraclofos against Malaria Vectors in Malkangiri District of Orissa

S.S. SAHU

A hut-scale trial of pyraclofos 50% EC applied as an indoor residual spray at 1 gm/sq m was carried out against malaria vectors. *Anopheles fluviatilis* and *An. culicifacies* in Kandhaguda village of Malkangiri district, Orissa. Bandhaguda village was kept as control. Pyraclofos was effective in reducing the vector density by 80 - 96% after 24 h of spray, but the density increased within three weeks. Parity rates were reduced only for three weeks as measured by diurnal indoor resting collection. Contact bioassays on mud wall showed pyraclofos has a residual life of only three weeks. No adverse effect was noticed among villagers or spraymen. Cockroaches were found to be very sensitive to this compound.

Keywords: *An. culicifacies*, *An. fluviatilis*, Hut-scale trial, Insecticide, Pyraclofos

INTRODUCTION

Pyraclofos an organophosphorus compound with a chemical name, O-1-(4-chlorophenyl)-4 pyrazolyl-O-ethyl-S-pypopyl-phosphorothioate was made available in 50% EC, through World Health Organization, as a part of WHO programme for evaluation and testing new insecticides[1]. A hut-scale trial was undertaken to evaluate the efficacy of this compound against *An. fluviatilis* and *An. culicifacies*, known malaria vectors in Malkangiri district of Orissa and the results are summarized here.

Study area

Malkangiri district (erstwhile Koraput district) is a hilly forested area with

Vector Control Research Centre, Medical Complex, Indira Nagar, Pondicherry - 605 006, India.

scattered villages inhabited by tribals. It has been highly endemic for malaria predominated by *P. falciparum*[2]. *An. fluviatilis* is the main vector, while *An. culicifacies* also plays an important role[3]. Streams, rivers and terraced paddy fields are the main breeding habitats[4].

In village Kandhaguda a total of 24 huts and 6 cattlesheds were selected for the trial. Another village Bandhaguda having 40 holdings and 10 cattlesheds was kept as control. Both the villages are situated in same ecotype. Transmission was perennial with a peak vector density and malaria incidence in the month of November-December in both the villages. Density of *An. fluviatilis* was comparatively higher in Kandhaguda, but the trend was similar in both the villages.

MATERIALS AND METHODS

The compound was evaluated at the target dose of 1 gm/(ai)/sq m during the first week of October 1991. All precautionary measures were undertaken during the spray operation.

Day time indoor resting collections were made before and after spray and at weekly intervals to monitor the density of *An. fluviatilis* and *An. culicifacies* in both villages throughout the study period (July-December 1991). Six human dwellings and three cattlesheds in each village were fixed as catching stations. In each station with oral aspirator and torch light 10 min were spent for collection. All the mosquitoes were dissected to determine the age using Polovodova's method.

Contact bioassays were carried out on mud walls 24 h after the spray and subsequently on Day 15, 23 and 38 after spray using standard methods. Blood fed wild caught *An. fluviatilis* from near by villages were exposed for 1 h and mortality was recorded after 24 h.

RESULTS AND DISCUSSION

The per man hour densities of *An. fluviatilis* and *An. culicifacies* are shown in Figs. 1-3. *An. fluviatilis* was collected in low numbers in cattlesheds throughout the study period. Hence, for analysis, density in human dwelling was only considered. In sprayed village, the density of *An. fluviatilis* was 47.0 before spray and reduced to 2.0 (97.2% reduction) after 24 h. At the end of third week the density increased to 15.5% and at the end of seventh week it was 18.5%. In control village, pre-spray density of *An. fluviatilis* was 6.0 which remained unchanged after 24 h and there was a slight increase to 7% by the end of seventh week.

Before spray, the man hour density of *An. culicifacies* in sprayed village was 15.0 and 102.0 in human dwellings and cattlesheds respectively. After 24 h, the density decreased to 3.0 and 20.0 respectively. But at the end of third week, the density increased to 5.0 and 47.0 in both the structures. In

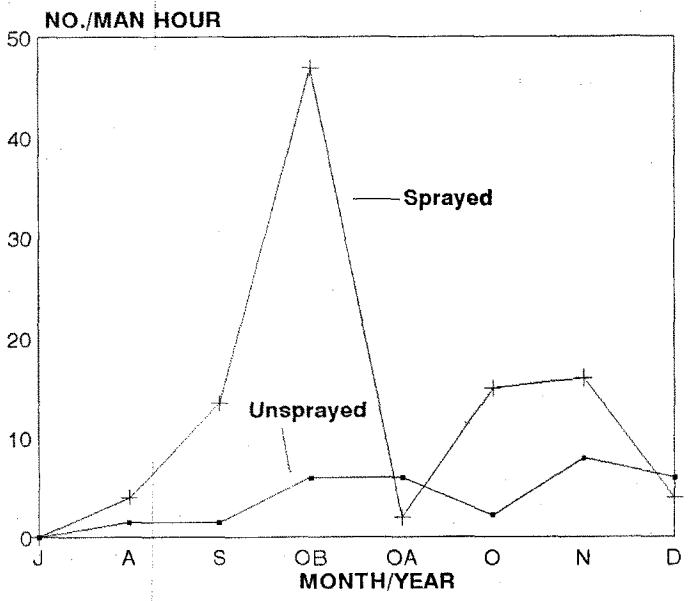

Fig. 1: Indoor resting density of *An. fluviatilis* in human dwellings

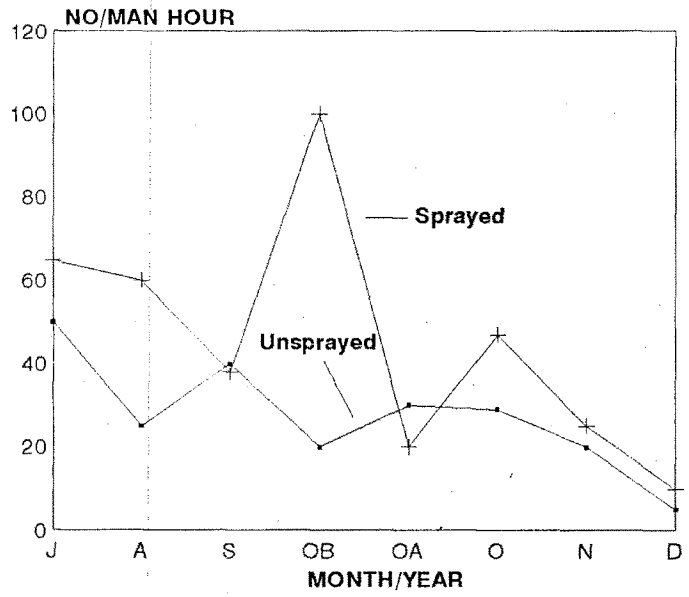

Fig. 2: Indoor resting density of *An. culicifacies* in cattlesheds

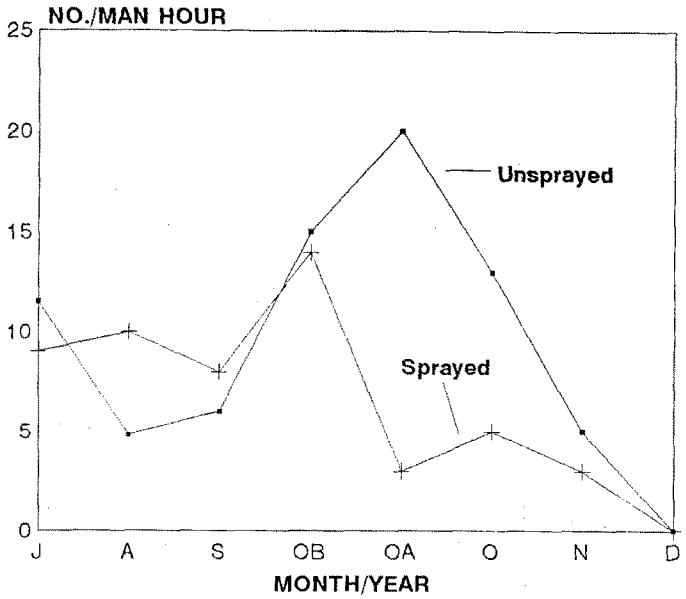

Fig. 3: Indoor resting density of *An. culicifacies* in human dwellings

the control village, the per man hour density of *An. culicifacies* in human dwelling and cattleshed was 16.0 and 14.0 respectively, before the spray period which increased to 20.0 and 30.0 after 24 h and remained almost same till the end of third week.

Table 1 shows parous rates of indoor resting *An. fluviatilis* and *An. culici-*

Table 1. Parous rate of *An. fluviatilis* and *An. culicifacies* in sprayed and control villages (1991)

Month		*An. fluviatilis*		*An. culicifacies*	
		Kandhaguda	Bandhaguda	Kandhaguda	Bandhaguda
Aug		20.0 (5)	0 (1)	31.9 (20)	75.0 (28)
Sep		46.2 (39)	0 (1)	48.4 (64)	54.5 (11)
Oct	24 h/B	59.1 (22)	50.0 (2)	61.6 (18)	50.0 (4)
	24 h/A	0.0 (1)	75.0 (4)	30.7 (13)	50.0 (18)
	3 wks/A	13.1 (23)	100.0 (3)	27.1 (37)	56.7 (37)
Nov		47.4 (59)	42.8 (28)	40.9 (44)	59.5 (47)
Dec		66.6 (6)	43.8 (16)	45.4 (11)	44.4 (9)

A – After; B – Before; Figures in parentheses indicate number dissected.

Table 2. Bioassay results with *An. fluviatilis*

Sl. No.	Days after spray	No. of mosquitoes released		Corrected mortality (%)
		Control	Test	
1	1	30	45	96
2	15	Expt. discarded due to control mortality		
3	23	20	30	100
4	30	30	45	19

facies. Post-spray parous rates in the sprayed village decreased significantly by third week, whereas in unsprayed areas, there was no significant difference between pre- and post-spray period.

Results of bioassays on mud surfaces (presented as per cent mortalities of averages of three consecutive tests) are shown in Table 2. Mortality was above 90% until three weeks after spray, which reduced to 19 per cent by the end of fifth week.

Thus, the study shows that the indoor residual application of pyraclofos could reduce the density of vectors up to three weeks only.

ACKNOWLEDGEMENTS

The author is grateful to Dr. V. Danda, Director, Vector Control Research Centre, Pondicherry for providing facilities. He is also grateful to Dr. P.K Das, Senior Deputy Director and Dr. P. Jambulingam, Senior Research Officer for their guidance and encouragement. The assistance rendered by the technical staff of VCRC field station, Malkangiri is also gratefully acknowledged.

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Indian Journal of Malariology
Vol. 33, March 1996, pp. 21-29.

Malaria Survey in Tarajulie Tea Estate and Adjoining Hamlets in Sonitpur District, Assam

V. DEV

Malaria survey in Tarajulie tea estate (TE) and its adjoining hamlets revealed that *Pf* was the predominant parasite species (79%) and morbidity was alarming. Malaria positive cases were recorded in all age groups including infants. However, morbidity was much pronounced in the hamlets than among garden dwellers. Mostly *Anopheles minimus* were recorded in the day resting collections from human dwellings (indoor) and in overnight man biting catches in the hamlets. From these collections, *An. minimus* was incriminated and the sporozoite infection rate was found to be 4.23 per cent. *An. minimus* were not recorded in the garden premises. Thus morbidity in the garden population was attributed to the movement of labourers to and fro between hamlets and the garden premises. The hamlets served as reservoirs for malaria infection.

Keywords: *Anopheles minimus*, Malaria survey, *Plasmodium falciparum*

INTRODUCTION

The tea estates (TE) which form the economic backbone of Assam and provide employment to nearly two million population are reporting high incidence of malaria. Some of these tea estates particularly, those located along the forest fringes of Bhutan border and Arunachal Pradesh are highly malarious. Tarajulie is one such tea estate which had malaria epidemic in 1991. As many as 14 deaths were recorded due to *Pf* (S. Gogoi, personal communication). In view of this a malaria survey was conducted beginning from 1st

Malaria Research Centre (ICMR), Field Station, Sonapur, Kamrup, Assam-782 402, India.

May till 15 June 1992 in Tarajulie tea estate and three of its adjoining hamlets (*bastis*). Results of the survey are elaborated in this paper.

Topography

Tarajulie tea estate, District Sonitpur, Assam have plantation spreading over an area of 492 ha with adjoining government reserve forests along Arunachal border to the north. There are several scattered and thinly populated hamlets in the reserve forest. The total garden population is 3781 comprising mainly of labour force with few executives. The garden is surrounded by rivers on eastern and western side, and there are streams which originate from the hills of Arunachal Pradesh and pass through the hamlets and the garden premises. The permanent labour force resides in labour quarter lines numbered as 1 to 8 (3 and 5 not existing). Each quarter has 2 to 3 rooms made of brick and cement with asbestos roofing and a false ceiling. The rooms are provided with windows but were often kept closed, thus rooms were generally dark inside. Many of the quarters have attached cattleshed madeup of split bamboos with thatched roofing. The minimum and maximum temperatures varied between 9 to 32.5°C respectively. The annual rainfall varies from 2500 to 3000 mm with pre-monsoon showers in April/May, and maximum precipitation occurs during June to October. Weather remains hot and humid throughout the year except from November to February (winter).

MATERIALS AND METHODS

To determine malaria prevalence, besides passive case detection, mass blood surveys were carried out in garden labour lines and adjoining hamlets namely, Tengabil, Kalabil and Lutera in which the movement of garden labourers was frequent. Blood smears were taken from all fever cases visiting malaria clinic and various age groups (both febrile and afebrile) in mass blood surveys. Blood smears (both thick and thin) were stained with JSB stain, and were examined for malarial parasites. All malaria positive cases were administered antimalarials as per NMEP drug policy.

For adults density, indoor day time resting collections were made during 0900 to 1200 hrs in garden labour lines, and huts in hamlets with the help of aspirator and flash light. Mosquitoes were identified following regional pictorial keys[1]. To ascertain the time of biting whole night human bait catches were made indoor in the huts of adjoining hamlets (four man nights), and in the garden labour line (one man night) between 1800 to 0500 hrs. Anophelines collected during whole night man biting catches and those from day resting collections were dissected for gland in 0.9% saline solution to detect sporozoites. Larval samplings were made from ponds, streams and drains to identify the vector breeding habitat.

RESULTS

Parasitological observations

Passive case detection: Malaria clinic was established in the Tarajulie tea estate and during the period from 1st May to 15th June 1992, 995 blood smears were collected from the garden labour force with those reporting fever. Of these, 322 (32.36%) were malaria positive, and over 79% were *Pf* infections. Malaria positivity were recorded in all age groups including infants (Table 1).

In the corresponding study period 76 blood smears were collected from fever patients those residing in adjoining hamlets namely, Tengabil, Kalabil and Lutera. Malaria positivity was much pronounced in all three hamlets, and it ranged from 53 to 84%, and most of the infections were *Pf* positive (79%) (Table 1).

Mass blood surveys: In mass and contact surveys a total of 985 blood smears were collected from all labour lines representing 28% of the total garden labour population. Of these, 147

Table 1. Passive case detection in Tarajulie tea estate and its adjoining hamlets, Assam

Age group (yrs)	Parasitological indices	Tarajulie (TE)	Tengabil hamlet	Kalabil hamlet	Lutera hamlet	Total
0-1	BSE/E	94	0	1	4	99
	(+)ve	19	0	1	2	22
	Pf	16	0	1	1	18
1-5	BSC/E	292	3	5	10	310
	(+)ve	69	3	1	3	76
	Pf	61	3	1	3	68
5-15	BSC/E	206	3	5	7	221
	(+)ve	77	3	5	3	88
	Pf	64	3	2	3	72
>15	BSC/E	403	7	20	11	441
	(+)ve	157	5	17	9	188
	Pf	115	5	14	5	139
Total	BSC/E	995	13	31	32	1071
	(+)ve	322	11	24	17	374
	Pf	256	11	18	12	297

Table 2. Mass blood surveys in labour lines (LN) of Tarajulie

Source (Pop.)	0-1 yrs						1-5 yrs						5-15 yrs					
	Male			Female			Male			Female			Male			Female		
	BSC	(+)ve	*Pf*	BSC	(+)ve	*Pf*	BSC	(+)ve	*Pf*	BSC	(+)ve	*Pf*	BSC	(+)ve	*Pf*	BSC	(+)ve	*Pf*
Tea estate																		
LN 1 (936)	8	3	3	8	1	1	27	5	4	35	4	4	49	8	8	55	7	7
LN 2 (868)	8	2	2	2	0	0	20	2	2	23	8	8	31	3	3	24	6	5
LN 4 (103)	0	0	0	1	0	0	3	0	0	3	0	0	10	0	0	11	1	0
LN 6 (665)	2	1	1	5	0	0	27	3	3	17	2	2	31	8	8	21	8	8
LN 7 (749)	3	1	1	3	0	0	14	4	3	9	1	1	20	1	1	17	3	3
LN 8 (212)	6	0	0	4	1	1	9	2	2	6	1	1	19	2	2	22	4	4
Hamlets																		
Tengabil (838)	0	0	0	3	1	1	6	3	3	8	4	4	6	3	3	8	3	3
Kalabil (1379)	4	3	3	2	1	1	8	4	4	4	3	3	7	3	3	12	5	4
Lutera (961)	6	1	1	6	2	2	19	5	5	16	5	5	19	8	8	18	4	4

(14.92%) were malaria positive. Malaria cases were prevalent irrespective of sex in all labour lines except 4 where single malaria case was recorded (Table 2).

In the adjoining three hamlets, as many as 374 blood smears were collected of which 111 (29.67%) were malaria positive. Majority (>96%) were *Pf* infections. All hamlets were found to be more malarious than garden labour lines and positivity rate ranged from 23.78 to 42.22% (Table 2).

Entomological observations

Indoor day resting collections: In the indoor day resting collections from human dwellings, *An. minimus* were found to be prevalent only in the hamlets, while *An. culicifacies* were present in both (Table 3). The man hour density (MHD) for *An. minimus* was 2.68 and for *An. culicifacies*, it varied from 0.33 to 1.40 in hamlets. Most of the adult females of both the species were either semigravid or gravid.

tea estate and its adjoining hamlets, Assam

Source (Pop.)	> 15 yrs						Total						Grand total		
	Male			Female			Male			Female			BSC	(+)ve	SPR
	BSC	(+)ve	*Pf*	BSC	(+)ve	*Pf*	BSC	(+)ve	*Pf*	BSC	(+)ve	*Pf*			
Tea estate															
LN 1 (936)	41	6	6	77	5	5	125	22	21	175	17	17	300	39	13.00
LN 2 (868)	29	4	4	37	5	4	88	11	11	86	19	17	174	30	17.24
LN 4 (103)	8	0	0	15	0	0	21	0	0	30	1	0	51	1	1.96
LN 6 (665)	32	5	5	58	10	10	92	17	17	101	20	20	193	37	19.17
LN 7 (749)	35	3	3	58	9	9	72	9	8	87	13	13	159	22	13.84
LN 8 (212)	17	5	4	25	3	2	51	9	8	57	9	8	108	18	16.67
Hamlets															
Tengabil (838)	25	4	4	43	11	11	37	10	10	62	19	19	99	29	29.29
Kalabil (1379)	11	3	3	42	16	15	30	13	13	60	25	23	90	38	42.22
Lutera (961)	41	8	8	60	11	9	85	22	22	100	22	20	185	44	23.78

Whole night collections: During the whole night human bait collections in the hamlets (4 man nights), *An. minimus* was the most predominant species, and man biting rate (MBR) was as high as 13.25. *An. minimus* fed throughout night but most of the feeding occurred between midnight onwards till 0400 hrs. However, within the garden premises (one man night), not even a single anopheline landed over human bait.

Vector incrimination: From the day resting and man biting collections, four anopheline species, i.e. *An. annularis*, *An. culicifacies*, *An. minimus* and *An. varuna* were dissected for gland to detect sporozoites and ovaries for parity. Of these, only *An. minimus* was found positive for sporozoite (Table 4). Of 142 *An. minimus* dissected, six were found gland positive and parity rate was over 50%. Out of these, four were found sporozoite positive in the day resting collections made in Lutera and Tengabil hamlets (2 each), and remaining two were found in man biting whole night collection in Kalabil hamlet.

Table 3. Man hour density (MHD) of *An. minimus* and *An. culicifacies* and their physiological condition of females in Tarajulie tea estate (TE) and adjoining hamlets, Assam

Sl. No.	Species	Tarajulie TE		Hamlets		Abdominal condition			
		No. collected	MHD	No. collected	MHD	UF	FF	SG	G
1.	*An. minimus*	0	0.00	118	2.68	4	11	69	34
2.	*An. culicifacies*	7	0.33	62	1.40	4	8	38	19

UF – Unfed; FF – Fully-fed; SG – Semigravid; G – Gravid.

Table 4. Sporozoite and parity rate in indoor day resting and whole night anopheline catches in Tarajulie tea estate (TE) and adjoining hamlets, Assam

Sl. No.	Species	Vector incrimination			Parity				
		Total dissected	No. gland (+)ve	Sporozoite rate (%)	Total dissected	NP	1P	2P	3P	% parous
1.	*An. annularis*	76	0	0.00	53	29	19	5	–	45.28
2.	*An. culicifacies*	68	0	0.00	35	10	18	7	–	71.43
3.	*An. minimus*	142	6	4.23	90	43	40	7	–	52.22
4.	*An. varuna*	3	0	0.00	2	1	1	–	–	50.00

Breeding surveys: Eight different anopheline species were recorded breeding in ponds, streams and drains. The vector species, i.e. *An. minimus* and *An. culicifacies* were recorded in streams.

DISCUSSION

In the pre-DDT era, over 30% of the hospital attendance were reported due to malaria in endemic tea estates, and the parasite density ranged from 13 to 69%. Majority of the infections were due to *P. falciparum* (70%); *P. vivax* accounted for 20%, *P. malariae* for 5%, and 3% were mixed (*Pv* + *Pf*)[2-4] infections. With the advent and subsequent application of DDT as residual insecticide, there was a continuous decline in malaria morbidity in tea estates. Since then, residual insecticides (mainly DDT) are in great demand for malaria containment programme.

However, in the light of present data (Table 1), it is evident that morbidity due to malaria is alarming and has reached the level of pre-DDT era. Malaria positivity in infants indicated that transmission was active and man/vector contact was extremely high. Mass blood surveys in the garden labour force and adjoining hamlets revealed that a good proportion of population were

parasite carriers which served as reservoirs for transmission (Table 2). However, it was noted that morbidity due to malaria was much pronounced in the hamlets (non-intervention area) as opposed to labour lines located within the garden premises (intervention area). Besides, there being regular 2 to 3 rounds of DDT spray, fogging and antilarval operations in the garden premises (S. Gogoi, personal communication), all labour lines were malarious except line no. 4 (the protection force for the garden manager). Consequently, a survey was conducted in the garden labour force to study their migration pattern between garden labour lines (controlled area) *vis-a-vis* adjoining hamlets (uncontrolled area). From the survey report (Table 5), it was evident that many of the permanent labour force had their own agricultural plot/

Table 5. Malaria incidence in Tarajulie tea estate (TE) due to migration between labour lines and adjoining hamlets

Parameters	Labour line number					
	1	2	4	6	7	8
1. No of houses surveyd	34	43	11	31	9	26
2. No. having land in *basti*	19	18	4	14	6	13
3. No. of family visiting *basti*						
Daily	6	5	1	8	3	5
Weekly	3	4	2	2	3	2
Monthly	4	5	1	1	0	0
Seasonally	6	1	0	3	0	3
4. Migration						
Family	3	6	2	7	2	0
Parents	3	3	1	1	2	5
Individual	13	6	1	6	2	5
5. Per cent using bednets	59	53	82	97	66	53
6. DDT spray coverage in the TE (%)	70	100	91	100	77	93
7. No. of family halting for night	8	5	1	11	3	5

hut in the hamlets to supplement their income. Migration was frequent, often with whole family coupled with night halts except line no. 4 (wherein a lone family was halting). Since the intervention measures in the hamlets were irregular and inadequate the latter served as the site for acquiring malaria infection. This was further substantiated from the entomological findings.

In the day resting collections, *An. minimus* (the principal vector) were collected only from hamlets and none from the garden labour lines (Table 3). In addition, in the whole night human bait catches in hamlets, *An. minimus* was the most predominant species with man biting rate as high as 13.25, while no anopheline species were collected over human bait in the garden premises. It was interesting to note that while *An. minimus* is known to be an endophilic species in the northeast region of India[5], a contradiction was noticed between day resting density and man biting rate in the present study. The data suggest that certain proportion of population of *An. minimus* is exophilic in character.

The site of acquiring infection was further affirmed by the vector incrimination data (Table 4). *An. minimus* was found sporozoite positive from the day resting collections and whole night human bait catches (all from the hamlets). *An. minimus* which was once believed to have disappeared from Assam[6], were also recorded in other parts of the State, and were incriminated[7].

It is evident that the hamlets are the source to maintain malaria transmission with huts (resting sites) located along the perennial slow flowing streams (breeding sites) with good proportion of population as parasite carriers (source of blood meal and gametocytes). In face of intense transmission and high man/vector contact, insecticide impregnated bednets were proposed as personal protection method for malaria containment based on the promising results documented in similar terrain elsewhere[8].

ACKNOWLEDGEMENTS

The author is thankful to the Director, Malaria Research Centre and Dr. V.S. Orlov, WHO for his advice and encouragement to initiate such an investigation. Thanks are also due to Dr. D.S. Choudhury for cross-checking slides on his visit to the tea estate. We are grateful to Assam Branch Indian Tea Association (ABITA), Dr. S. Gogoi, Senior Medical Officer, and the Manager, Tarajulie TE for their support and full cooperation. Technical assistance of Mr. S. Phookan and other MRC staff is duly acknowledged.

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Indian Journal of Malariology
Vol. 33, March 1996, pp. 30-40.

Mosquito Breeding in Relation to Aquatic Vegetation and Some Physico-Chemical Parameters in Rice Fields of Central Gujarat

RAJNI KANT, S.D. PANDEY[a] and S.K. SHARMA

Mosquito breeding in relation to aquatic vegetation and certain physico-chemical parameters was studied in rice fields of Kheda district in central Gujarat. A total of 14 anopheline and 15 culicine species were encountered in close association with different types of aquatic vegetation in different proportions. Among anophelines, *Anopheles annularis, An. nigerrimus, An. subpictus* and *An. tessellatus* were of general distribution and were found associated with each aquatic vegetation. *An. culicifacies* showed poor association with most of the aquatic weeds. Maximum number of anophelines were found associated with algae. *Culex vishnui* sub-group predominated among culicines and showed frequent association with *Ceratophyllum, Hydrilla* and algae. Physico-chemical parameters also exerted some impact on mosquito larval population.

Keywords: Aquatic vegetation, Mosquitoes, Rice fields

INTRODUCTION

Rice agro-ecosystem represents a complex interaction between various biotic and abiotic factors, which determine the breeding and prevalence of a number of mosquito species including disease vectors. Due to standing water in rice fields, a variety of plant species grow, which provide food and shelter to different mosquito species and offer favourable conditions for oviposition

Malaria Research Centre (Field Station), Civil Hospital, Nadiad-387 001, India.
[a]Christ Church College, Kanpur-208 001, India.

and subsequent larval development. The surface canopy of these plants may reduce the effectiveness of natural predators and thus enhance survival of mosquito larvae.

The type and density of aquatic vegetation in larval habitats may also affect the abundance of mosquito larvae through their effect on water temperature, surface characteristics, water chemistry and predation rate[1]. Sen[2], Neogy and Kachroo[3], Hall[4], Chandler and Highton[5], Rejmankova *et al.*[6] and Victor *et al.*[7] studied the mosquito breeding in relation to aquatic vegetation in different breeding habitats. Mosquito breeding in rice agro-ecosystem, species succession and seasonal prevalence has already been studied[8,9]. Present study was attempted to find out the role of different aquatic vegetation in the breeding and abundance of various mosquito species in rice fields, as a vast agricultural area is subjected to rice cultivation which directly or indirectly affect the incidence of prevailing vector-borne diseases.

MATERIALS AND METHODS

Studies were confined to Kheda district in central Gujarat which has an extensive canal irrigation for rice cultivation a staple food crop in this district. The topography, climate, rainfall, cropping pattern and cultivation practices in the area have already been described previously[8-11]. Usually two crops of rice namely, non-monsoon *rabi* and monsoon-dependent *kharif* are grown in the area. Cultivation practices continue almost round the year due to variation in seeding transplanting and harvesting time at different places in different revenue areas (talukas). Twenty-seven rice fields in nine villages of three talukas, i.e. Anand, Matar and Petlad were selected for the study owing to ample irrigation facilities in these areas. Observations were made from three rice fields in each village. All the rice fields were monitored at weekly intervals for mosquito larval abundance and the occurrence of weed species for a period of two years covering four cropping seasons beginning from June 1988. Random samples were also collected from the rice fields of adjoining talukas as well to ensure bigger sample size. A standard larval (enamel) dipper (9.5 cm dia m and 300 ml capacity) was used for collecting the mosquito larvae. About 10-15 dips were taken along the perimeter of each rice field. Immatures collected were counted instar-wise and brought to the laboratory in plastic containers for adult emergence. Emerged adults were identified with the help of Christophers[12] and Barraud[13] keys.

Samples of floating and submerged aquatic vegetation occurring in the vicinity of rice plants were also collected every week along with the larvae (when larval density in association with each type of vegetation was > 10/dip) and identified[14]. Some uncommon plant species were confirmed at the Depart-

Table 1. List of aquatic plants found in rice fields

Plant species	Group	Nature
1. Algae		
(a) *Cladophora glomerata*	Green algae	Free floating
(b) *Hydrodictyon*	Green algae	
(c) *Pithophora oedogonia*	Green algae	
(d) *Spirogyra pratensis*	Green algae	
(e) *Anabaena ambigua*	Blue-green algae	
(f) *Aphanocapsa montana*	Blue-green algae	
(g) *Lyngbya*	Blue-green algae	
(h) *Nostoc*	Blue-green algae	
(i) *Oscillatoria limnatica*	Blue-green algae	
(j) *Phormidium tenue*	Blue-green algae	
(k) *Phacus acuminatus*	Euglenoids	
(l) *Gomphonema montanum* var. *commutatum*	Diatoms	
2. *Azolla pinnata*	Pteriodophyte	Floating
3. *Ceratophyllum demersum*	Dicotyledons	Submerged
4. *Eichhornia crassipes**	Monocotyledons	Floating
5. Grasses *(Cynadon dactylon)*	Monocotyledons	Erect
6. *Hydrilla verticillata*	Monocotyledons	Submerged
7. *Ipomea aquatica*	Dicotyledons	Erect, creeping
8. *Lemna minor*	Monocotyledons	Floating
9. *Marsilea* species	Pteriodophyte	Erect

*Infestation of *E. crassipes* (water hyacinth) in few rice fields occurred due to drift of hyacinth plants along with water from adjoining infested ponds.

ment of Biosciences, Sardar Patel University, Vallabh Vidyanagar and J & J College of Science, Nadiad. Association of each aquatic weed with different mosquito species was studied.

Water samples of the same rice fields were also collected and brought to the laboratory for physico-chemical analysis. However, separate samples were collected for determination of dissolved oxygen. All the samples were analysed following standard methods as described by Trivedi and Goel[15]. The pH of the samples was recorded in the field using Qualigen field pH paper and fur-

ther it was checked in the laboratory by Systronic digital pH meter. Dissolved oxygen was determined by Winkler's iodometric method. The samples were collected in 250 ml glass stoppered bottles and after adding the required reagent a known volume was titrated with standard thiosulphate solution using starch as indicator. Phenolphthalein alkalinity (PA), total alkalinity (TA), carbonates and bicarbonates were estimated by titrating the samples with a strong acid using phenolphthalein and methyl orange as an indicator for PA and TA, respectively. Values of carbonates and bicarbonates were computed from these two types of alkalinities. Chloride content of the sample was determined by argentometric method by titrating with standard silver nitrate using potassium chromate indicator. The hardness was measured by titrating the sample with EDTA solution using eriochrome black T as an indicator. Calcium was also measured as per EDTA method using murexide as an indicator, whereas magnesium was calculated by substracting the calcium hardness from the total hardness.

RESULTS AND DISCUSSION

Nine plant species of different genera were encountered in rice fields (Table 1). Algae were found floating on the water surface and sometimes formed an algal bloom which mainly belonged to the group of green and blue-green algae. The presence of water hyacinth (*Eichhornia crassipes*) in some rice fields was also observed at some places due to overflow of pond water infested with hyacinth plant or through irrigation of rice fields by these ponds. The relative abundance of anophelines in relation to presence of different aquatic plants is given in Table 2. *An. annularis, An. nigerrimus, An. subpictus* and *An. tessellatus* were of general distribution and were found associated with each aquatic vegetation although with varying proportions. *An. annularis* was commonly encountered in fields infested with *Azolla* (21.86%) followed by those with *Hydrilla* (11.70%) and grasses (8.33%). *An. nigerrimus* preferred the fields with *Ceratophyllum* (61.48%), *Hydrilla* (34.49%), *Ipomea* (27.22%) and *Azolla* (19.53%). *An. subpictus* showed the most frequent association with filamentous green algae (80.83%) such as *Spirogyra* followed by water hyacinth (76.11%) and weed free fields. *An. tessellatus* was most dominant in association with grasses (22.10%) followed by *Marsilea* (10.79%) and *Ceratophyllum* (8.14%).

An. barbirostris showed maximum association with *Ceratophyllum* (9.62%) followed by *Hydrilla* (8.54%) and *Ipomea* (7.42%). *An. culicifacies* was poorly associated with aquatic vegetation and was not found breeding in association with water hyacinth and *Ceratophyllum*. Its maximum breeding occurred in *Azolla* infested fields (19.89%) which might also be accidental due to lot of patches developed by aquatic insects/animals and presence of fresh water in the fields followed by the fields without any aquatic vegetation (8.32%). Spe-

Table 2. Per cent composition of anophelines in rice fields in relation to aquatic vegetation

Species	Without vegetation	Algae	*Lemna*	*Marsilea*	*Azolla*	*Hydrilla*	*Ipomea*	Grass	Water hyacinth	*Cerato-phyllum*
An. aconitus	0.04	0.23	0.56	0.42	1.25	0.94	2.47	0.36	–	1.48
An. annularis	4.33	5.06	4.15	4.76	21.86	11.70	5.44	8.33	7.46	7.40
An. barbirostris	1.01	1.50	2.45	2.96	4.30	8.54	7.42	6.88	–	9.62
An. culicifacies	8.32	3.02	2.73	4.55	19.89	1.58	0.99	3.62	–	–
An. fluviatilis	0.03	0.02	0.09	–	–	–	–	–	–	–
An. jamesii	–	0.01	–	–	0.17	–	–	–	–	–
An. nigerrimus	4.58	6.17	10.00	11.32	19.53	34.49	27.22	10.50	10.44	61.48
An. pallidus	0.76	0.44	0.56	0.10	0.35	1.26	0.99	–	–	0.74
An. splendidus	–	–	0.09	–	–	–	–	–	–	–
An. stephensi	0.59	0.15	0.09	–	1.25	0.63	–	–	–	–
An. subpictus	73.85	80.83	71.88	64.97	28.49	35.75	52.47	48.18	76.11	11.11
An. tessellatus	6.33	2.40	7.16	10.79	2.68	5.06	2.97	22.10	5.97	8.14
An. vagus	0.06	0.07	0.18	0.10	0.17	–	–	–	–	–
An. varuna	0.06	0.03	–	–	–	–	–	–	–	–
Total adults emerged	6394	8823	1060	945	558	316	202	276	67	135

Mosquito composition is based on immature collection from fixed and random rice fields; (–) Denotes not found.

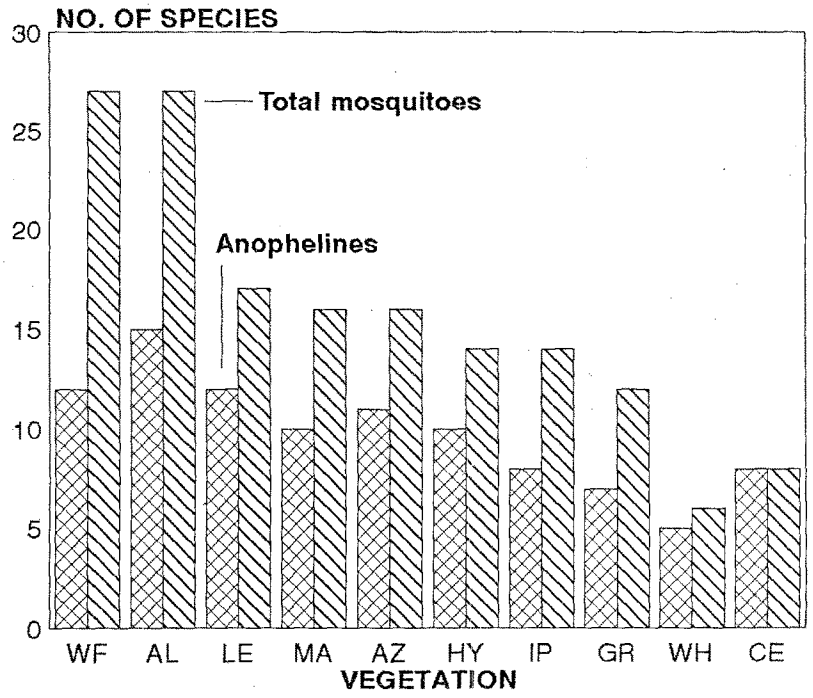

Fig. 1: Number of species associated with each type of aquatic vegetation

cies such as *An. aconitus, An. pallidus, An. stephensi, An. fluviatilis, An. jamesii, An. splendidus, An. vagus* and *An. varuna* were present in very low proportions and the latter five species were not found in association with *Hydrilla, Ipomea, Ceratophyllum,* grasses and water hyacinth. The number of anopheline species found breeding in association with different types of plants were: algae (13); *Lemna* (12); *Azolla* (11) and *Marsilea* (9) (Fig. 1).

The association of *An. annularis* with *Hydrilla* and *Ceratophyllum* plants has been reported in previous observations as well by Sen[2] in lower Bengal. Maximum anophelines were found in association with green and blue-green algae which indicate that algae encourage the mosquito breeding by providing food and shelter to different mosquito species. Chandler and Highton[4] reported that the growth of aquatic plants and algae in rice fields in Kenya encouraged the breeding of *An. ziemanni* and *Cx. poicilipes.* Schaefer *et al.*[16] and Kramer and Garcia[17] also found a positive correlation between the presence of green algae and high number of mosquito larvae. However, Russell and Rao[18] reported the nega-

Table 3. Per cent composition of culicines in rice fields in relation to aquatic vegetation

Species	Without vegetation	Algae	*Lemna*	*Marsilea*	*Azolla*	*Hydrilla*	*Ipomea*	Grass	Water hyacinth	*Cerato-phyllum*
Ae. aegypti	0.70	0.40	0.09	0.21	–	–	0.62	0.22	–	–
Ae. indica	0.02	0.025	–	–	–	–	–	–	–	–
Ae. pallidostriatus	0.02	0.025	–	–	–	–	–	–	–	–
Ae. scatophagoides	0.21	0.10	–	0.21	–	0.24	–	–	–	–
Ae. taeniorhyn-choides	8.05	0.30	1.30	21.57	0.32	–	1.86	4.01	–	–
Ae. vittatus	0.02	–	–	–	–	–	–	–	–	–
Cx. bitaenior-hynchus	0.36	2.34	0.18	0.32	4.46	0.72	1.55	–	–	–
Cx. fuscanus	2.76	1.98	17.80	4.20	1.63	1.93	2.48	6.69	6.25	1.53
Cx. gelidus	0.21	0.75	0.27	–	–	–	–	1.33	–	–
Cx. quinque-fasciatus	10.94	4.50	5.96	4.09	8.16	–	8.07	7.14	–	–
Cx. seniori	0.04	0.07	–	–	0.10	–	0.31	–	–	–
Cx. tritaenior-hynchus	7.88	1.93	12.86	4.31	6.74	0.24	12.73	21.20	25.00	–
Cx. vishnui group	68.68	87.01	61.50	64.72	78.23	96.36	72.36	59.37	68.75	98.46
Fi. chamberlaini	0.02	0.50	–	0.21	0.32	0.24	–	–	–	–
Ma. uniformis	0.04	0.025	–	0.10	–	0.24	–	–	–	–
Total adults emerged	4669	3974	1073	927	919	413	322	448	48	65

Mosquito composition is based on immature collection from fixed and random rice fields; (–) Denotes not found; *Cx. vishnui* group also includes *Cx. pseudovishnui.*

tive impact that macroscopic algae had on several rice field inhabiting mosquitoes in south-eastern Madras. Larvae of *An. culicifacies* and majority of other species were most numerous when macroscopic algae were not present. Extensive growth of aquatic algae on water surface and grass at the edges of rice fields was observed with the declining trend in the breeding of *An. culicifacies* and succession of *An. theobaldi* and *An. splendidus* in Mandla district (M.P.).[19]

Among culicines, three out of six *Aedes* species namely, *Ae. indica, Ae. pallidostriatus* and *Ae. vittatus* were not recorded in association with most aquatic plants except at few occasions with algae, *Lemna, Marsilea, Ipomea* and grasses. *Ae. taeniorhynchoides* showed predilection for *Marsilea* plant (21.57%) followed by weed free fields (8.05%) and grasses (4.01%). *Ae. scatophagoides* was present only in fields infested with algae, *Marsilea* and *Hydrilla. Cx. bitaeniorhynchus* was commonly encountered in association with *Azolla* (4.46%) and green algae (2.34%). It was poorly associated with other aquatic weeds. *Cx. fuscanus* was most prevalent in *Lemna* infested fields (17.80%) followed by grasses (6.69%) and water hyacinth. *Cx. quinquefasciatus* preferred to breed in fields without vegetation (10.94%) followed by *Azolla* (8.16%), *Ipomea* (8.07%) and grasses (7.14%). *Cx. tritaeniorhynchus* was most abundant in fields with water hyacinth (25%) followed by those with grasses (21.20%), *Lemna* (12.86%) and *Ipomea* (12.73%). *Cx. vishnui* subgroup was found to be most predominant mosquito species and showed frequent association with *Ceratophyllum* (98.46%),*Hydrilla* (96.36%), algae (87.01%) and *Azolla* (78.23%). Breeding of *Ficalbia chamberlaini* and *Mansonia uniformis* was found in low proportion in rice fields and preferred the vegetation like *Marsilea, Azolla, Hydrilla* and algae (Table 3). Maximum number of culicines were found in weed-free fields (15) followed by those with algae (14) and *Marsilea* (10).

Most of the culicines were also found associated with algae, however, it was not ascertained which algal species was more conducive or deterrent to mosquito larval abundance. Pitcairn *et al.*[20] observed a positive correlation between the presence of macrophytic algae *Chara* spp and the abundance of *Cx. tarsalis* and *An. freeborni.* Rao[21] reported that Cyanophyceae (blue-green algae) are definitely unfavourable to *An. culicifacies.* The inimical effect of certain other plant species such as *Azolla* and *Lemna* are also reported[22-24]. They prevent the mosquito oviposition and survival of immatures if growing thickly on water surface. In the present investigation, the growth of these two plant species was not much to study the deterrent effect on mosquito immatures.

Physico-chemical parameters varied considerably during different seasons from field-to-field. Dissolved oxygen was recorded maximum during monsoon, which might be due to heavy rainfall.

Breeding of *An. subpictus* was associated with high dissolved oxygen. Other parameters such as carbonates, chloride, calcium, magnesium and alkalinity were high during non-monsoon period possibly due to well established and undisturbed fields, since no rains occurred during that period (Table 4). These factors supported the vigorous breeding of the maximum number of mosquito species. Water pH remained around 7.0 throughout the cropping season, which was suitable for the breeding of most of the mosquito species. Breeding of *An. nigerrimus, An. tessellatus, An. barbirostris, Cx tritaeniorhynchus, Cx. vishnui* sub-group, *Ae. taeniorhynchoides* and *Ae. scatophagoides* increased with the decline in alkalinity, hardness, calcium and chloride content of water. In Lahore (Pakistan), progressive increase in water conductivity and alkalinity resulted in a quantitative decline in the mosquito fauna of temporary pools. Only more tolerant species like *An. subpictus* and *Cx. tritaeniorhynchus* survived. Increase in alkalinity in water was accompanied by increased turbidity, suspended solids, pH, phosphates, nitrates and sulphates, which also exerted a negative impact on the breeding of most mosquito species[25].

Table 4. Mean values of the physico-chemical parameters of water samples of rice fields

Sl.No.	Parameters*	Rabi crop	Kharif crop
1.	pH	7.06	7.13
2.	Dissolved oxygen	3.90	7.58
3.	Phenolphthalein alkalinity	21.06	17.22
4.	Total alkalinity	318.48	243.05
5.	Carbonates	42.12	34.45
6.	Bicarbonates	276.35	208.60
7.	Chlorides	118.91	72.48
8.	Hardness	196.53	160.58
9.	Calcium	33.76	32.75
10.	Magnesium	27.39	19.21

*Except pH, all figures are in mg/l.

It can be concluded that aquatic vegetation usually growing in rice fields influences mosquito breeding and their abundance varies with the occurrence and intensity of growth of each aquatic plant. Physico-chemical factors also exert some impact on mosquito larval survival and emergence. However, a detailed study on the role of other interrelated factors such as predator-parasite relationship, cultivation practices, emergence rate, etc. is needed for full understanding of the subject.

ACKNOWLEDGEMENTS

Authors are highly thankful to Shri R.M. Bhatt for extending invaluable guidance and constructive suggestions throughout the study. Thanks are also due to Mrs. Bina Srivastava and other staff of entomology and biological control divisions for technical assistance.

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Indian Journal of Malariology
Vol. 33, March 1996, pp. 41-47.

Malaria Parasite Density in Pregnant Women of District Jabalpur, Madhya Pradesh

NEERU SINGH, M.M. SHUKLA and NEENA VALECHA[a]

Twenty-two cases (nineteen *Plasmodium falciparum* and three *P. vivax*) of severe malaria among 200 pregnant women from District Jabalpur, M.P. were studied for malaria parasite density. Almost all of them were found anaemic. The patients were treated with quinine/chloroquine intravenously but seventeen *Pf* cases died within 96 h of admission in hospital. Out of twenty-two, thirteen were primigravidae.

Keywords: Malaria, *P. falciparum*, *P. vivax*, Pregnant women

INTRODUCTION

It is generally agreed that during pregnancy women show an increased prevalence and density of malaria[1]. The complications due to disease are also more pronounced in pregnant than in non-pregnant women[2]. Parasite density in peripheral blood is an important parameter as it reflects the severity of illness with level of immunity and is also easy to determine[3]. The risk of complication due to malaria is roughly proportional to the parasite load[4]. Studies in Malaysia have shown that the death rate in falciparum infections rises steeply as parasitaemia exceeds 1,00,000/µl of peripheral blood[5]. Approximately 1% mortality has been recorded with parasite count < 1,00,000 µl; this increases to more than 50% with counts > 5,00,000 µl. During 1991-92, a very high density of *P. falciparum* and *P. vivax* parasites were found in some pregnant women in District Jabalpur, M.P. The paper describes

Malaria Research Centre (Field Station), Medical College Building, Jabalpur-482 003, India.
[a]Malaria Research Centre, 22-Sham Nath Marg, Delhi-110 054, India.

the results of the blood smear examination of 22 cases of malaria.

MATERIALS AND METHODS

Jabalpur with an area of 10,160 sq km is located in the centre of Madhya Pradesh (pop. 21,98,743) and is a reservoir of intense perennial malaria transmission. Both *Plasmodium falciparum* and *P. vivax* are prevalent with peaks in wet and dry season. As Malaria Research Centre provides prompt diagnosis of malaria, authorities of Government Medical College, Jabalpur approached us in January 1991 and started sending a large number of fever cases for blood smear examination to confirm malaria. Fever cases came from Jabalpur and adjoining tribal areas directly and some were referred by hospitals and clinics. This provided us an opportunity to study parasitological profile in different socio-economic groups as well as from areas of different endemicity. To conduct the study a malaria clinic was established in Obstetrics and Gynaecology Department of Medical College in January 1991 to update the knowledge about the prevalence and density of malaria parasite in pregnant and non-pregnant women.

Women in the reproductive age group (16-45 yrs) with a history of fever were screened for malaria parasites in peripheral blood smears. Thick and thin smears were made from finger prick on the same slide. Thin smears were stained with Giemsa[6] and examined under microscope for the presence of malaria parasites. Results of blood smear were made available within an hour of collection. History of fever, clinical findings, parasite species, treatment given and response to treatment were recorded in each case. Blood group, haemoglobin (gm%) and biochemical investigations for jaundice were also conducted in each case. Per cent parasitaemia was calculated by counting number of asexual parasites per 10,000 erythrocytes.

RESULTS AND DISCUSSION

A total of 1000 pregnant and 1200 non-pregnant women were screened for the presence of malaria parasites during 1991-93. The density of malaria parasites in 22 out of 200 positive pregnant patients and in two out of 140 non-pregnant women was extremely high. Among these *P. falciparum* accounted for 17, *P. vivax* for three and two cases were of mixed infection. The latter two cases were analysed with the *P. falciparum* cases as that was the predominant parasite species. The parasitaemia was between 5.5 to 69% in *P. falciparum* cases and 2-9% in *P. vivax* cases (Table 1). Multiple infection of RBC was very frequent (2.5-50%). In two cases (Sl. No. 1 and 3) 10-12 parasites were recorded in some cells (Fig. 1). Several erythrocytes were seen having 2-4 double chromotin rings. Schizonts and Maurer's dots were present in almost all the cases. The mean density of gametocytes was low, but in two cases high density of crescents was recorded. Out of 18 deaths, 12 were within 48 h

Table 1. Malaria in pregnant/non-pregnant women showing density of parasitaemia, record of sickness, clinical findings, treatment and outcome

Sl. No.	Parasit-aemia (%)	Multiple infection (%)	Treatment received	Blood transfusion (in units)	Clinical findings	Outcome	History of fever (in days)
Pregnant women							
1.	49.4	26.0	Quinine I.V.	1	Unconscious Splenomegaly	Expired within 24 h after admission	3
2.	42.0	27.0	Quinine I.V.	2	Unconscious	Expired within 4 h after admission	–
3.	69.2	50.0	Quinine I.V.	0	Unconscious Splenomegaly Jaundice (Serum bilirubin 4.5 mg%)	Expired within 2 h after admission	7
4.	43.0	15.0	Quinine I.V.	0	Unconscious Splenomegaly Jaundice (Serum bilirubin 3.4 mg%) Hepatomegaly	Expired within 48 h	7
5.	30.0	13.0	Quinine I.V.	0	Unconscious Splenomegaly Jaundice (Serum bilirubin 9 mg%)	Expired within 96 h	4
6.	6.5	3.0	Chloroquine I.V.	0	Still birth	Survived	5
7.	5.5	3.0	Quinine I.V.	1	Unconscious Jaundice	Expired within 72 h	5
8.	6.0	3.0	Quinine I.V.	0	Unconscious Splenomegaly	Expired within 48 h	3
9.	6.0	2.5	Quinine I.V.	1	Unconscious Jaundice	Expired within 72 h	5
10.	30.0*	9.0	Quinine I.V.	1	Unconscious	Expired within 96 h	5
11.	5.5	2.5	Chloroquine I.V.	0	Unconscious Splenomegaly Jaundice (Serum bilirubin 18 mg%)	Expired within 12 h	5

contd....

Table 1. (*contd.*)

Sl. No.	Parasit-aemia (%)	Multiple infection (%)	Treatment received	Blood transfusion (in units)	Clinical findings	Outcome	History of fever (in days)
12.	7.0	3.0	Quinine I.V.	1	Unconscious Abortion Severe anaemia	Expired within 24 h	8
13.	40.2*	18.0	Quinine I.V.	1	Jaundice (Serum bilirubin 9 mg%) Splenomegaly	Expired within 48 h	2
14.	10.0	4.0	Quinine I.V.	1	Unconscious Still birth Pulmonary oedema Severe anaemia	Expired within 72 h	6
15.	8.0	4.0	Quinine I.V.	1	Unconsious	Expired within 48 h	3
16.	8.0	3.5	Chloroquine I.V.	1	Unconscious	Expired within 48 h	4
17.	5.5	2.5	Chloroquine I.V.	0	Unconscious	Expired within 48 h	5
18.	5.5	2.5	Quinine I.V.	2	Unconscious Pulmonary embolism	Expired within 144 h	8
19.	5.5	2.5	Quinine I.V.	1	Unconscious Neonatal death	Expired within 48 h	4
20.**	9.0	4.5	Chloroquine I.V.	2	Unconscious	Survived	2
21.**	3.0	0.0	Chloroquine (1500 mg)	0	Nil	Survived	5
22.**	2.0	0.0	Chloroquine (1500 mg)	0	Nil	Survived	6
Non-pregnant Women							
1.	15.0	3	Quinine I.V.	0	Unconscious Very high fever	Expired after 24 h	8
2.	6.0	2	Chloroquine 1500 mg	0	Unconscious	Expired after 144 h	7

*Mixed infection (*Pv*+*Pf*); **P. vivax cases; *Pf* – 40%; *Pv* – 0.2; *Pf* – 29.96%; *Pv* – 0.12%.

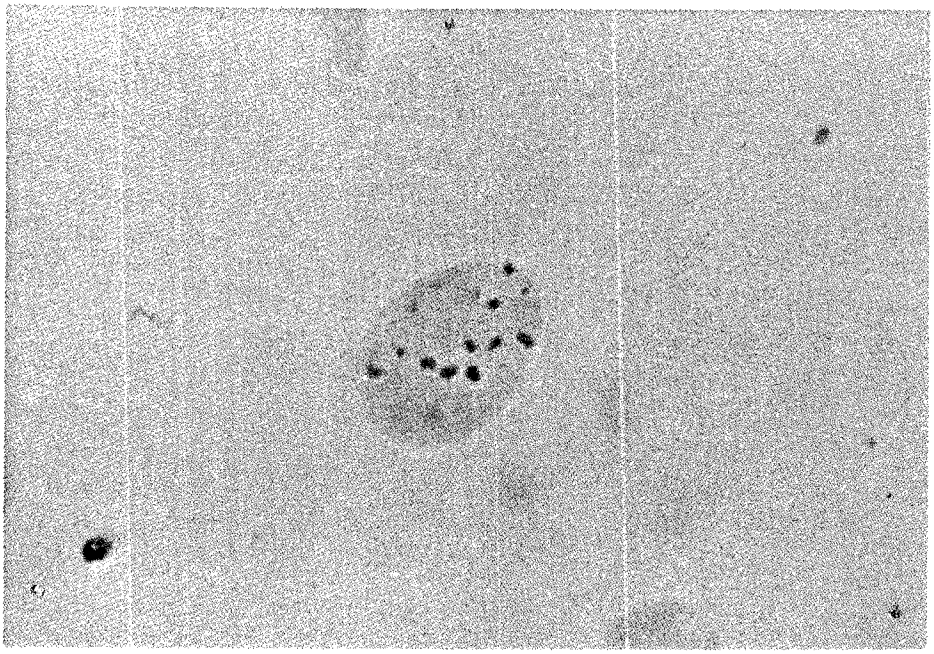

Fig. 1: Multiple infection of RBC

of admission, despite intravenous quinine or chloroquine therapy and blood transfusion (one/two units). Majority of the patients were severely anaemic (Hb 2.0-7.0 gm%).

Two out of three cases of *P. vivax* having more than 2% parasitaemia had still births. Out of 22 cases of *P. falciparum, P. vivax* and mixed infections, 13 were primigravidae, eight in late pregnancy (7-9 months), four in early pregnancy (3-6 months) and one 4-days after delivery. High fever, jaundice and severe anaemia were predominant clinical features in most pregnant women, a finding that is consistent with those of other reports. Menon[7] analysed the case records of 17 Malaysian women in late pregnancy. Of the 17 cases, three terminated in maternal death, five women had still births and three children were born premature. Pyrexia and anaemia were predominant clinical features. In Nigeria, severe anaemia is responsible for a large proportion of the maternal mortality and morbidity[8].

Although malaira in pregnancy produces serious complications; the mortality in pregnant women with low parasitaemia was significantly less than the growth with high parasitaemia (33% *vs.* 82%) (Table 2). Although 70% (n = 7) patients reported with cerebral symptoms but more than 50% (n = 4) responded to I/V Quinine/Chloroquine therapy. Final outcome varied from ab-

Table 2. Malaria in pregnant women (with low density of parasitaemia), showing record of sickness, clinical findings, treatment and outcome

Sl. No.	Parasitaemia %	Treatment received	Blood transfusion (in units)	Clinical findings	Outcome	History of fever (in days)
1.	3.0	Chloroquine I.V.	0	Abortion	Survived	4
2.	3.0	Quinine I.V.	1	Unconscious Jaundice G.I. breeding	Expired within 48 h	5
3.	2.0	Quinine I.V.	1	Unconscious Splenomegaly	Survived	4
4.	2.0	Quinine I.V.	0	Unconscious	Expired within 120 h	5
5.	2.0	Quinine I.V.	0	Unconscious	Survived	5
6.	1.0	Quinine I.V.	1	Unconscious Severe anaemia	Survived	7
7.	1.0	Chloroquine I.V.	1	Unconscious Abortion	Expired within 96 h	5
8.	1.0	Quinine I.V.	1	Unconscious Severe anaemia	Survived	4
9.	0.4	Chloroquine I.V.	1	Preterm birth	Survived	5
10.	0.1	Chloroquine I.V.	0	Abortion	Survived	1

ortion, preterm deliveries to normal delivery.

Out of 118 positive cases of non-pregnant group only 2 patients presented with high density of *P. falciparum* infection. Both the non-pregnant patients with high parasitaemia of 6 and 15% were admitted in serious condition and died within a week and 2 days of admission respectively.

A parasitaemia of > 5% in *P. falciparum* is classified as severe malaria by WHO[5], while parasite count of more than 2% in *P. vivax* is a rare finding and considered high. Exchange transfusion has been recommended, when the peripheral blood parasitaemia is > 10% with pulmonary, renal and cerebral complications and > 50% in their absence[5,9]. When parasitaemia is > 20%, the prognosis is generally very grave[9].

Fourty years ago, Walton[10] reported that "during pregnancy the ability to limit the number of parasites appears to be lost". Brabin[3] also stated that the development of immunity is reflected by the number of parasites in the blood. It appears that pregnant women from this geographic area remain highly suscep-

tible to malaria throughout pregnancy and puerperium[2]. Therefore, there is an urgent need to elucidate the mechanisms by which pregnancy enhances susceptibility to malaria. This is utmost important for development of control measures.

ACKNOWLEDGEMENTS

Help rendered by the technical staff of Malaria Research Centre, Field Station, Jabalpur is gratefully acknowledged.

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SHORT NOTE

Indian Journal of Malariology
Vol. 33, March 1996, pp. 48-51.

A Note on Present Trend of Chloroquine Sensitivity of *P. falciparum* in Malkangiri District, Orissa

L.K. DAS and S.S. SAHU

Keywords: Chloroquine, *In-vivo* test, Malkangiri, *P. falciparum*

In 1992, the erstwhile Koraput district of Orissa state (17° 50' N and 20° 30' N latitude and 81° 27' E and 84° 10' E longitude) was divided into four districts. Malkangiri is the southernmost of the four districts. It has seven community health centres (CHC) namely, Malkangiri, Korkunda, Podia, Khairput, Kudumulugumma, Mathili and Kalimela. The district is inhabited mostly by Koya tribes and Bengali settlers from former east Pakistan. The area is highly malarious and *Plasmodium falciparum* is the predominant malaria parasite[1]. The slide positivity rate varied from 2.7 to 26.4 during 1987-92 (source: NMEP data). Shortage of chloroquine (CQ) resulting in lack of treatment was one of the major reasons for the persistant malaria in the district. The health department of Malkangiri district have recently opened 570 chloroquine distribution centres through Aganwadi workers at the village-level for malaria control. Earlier studies had shown presence of chloroquine-resistant parasites in this area[2-3]. Hence, there is a need for monitoring chloroquine sensitivity and stratification of areas in the district accord-

Vector Control Research Centre (ICMR), Medical Complex, Indira Nagar, Pondicherry-605 006, India.

ing to the susceptibility status of *P. falciparum* to chloroquine. This would rationalise the use of chloroquine in this area. This report highlights the results of the study carried out during 1994 on the chloroquine sensitivity status of *P. falciparum* in five out of seven CHC areas of the district.

P. falciparum cases were detected by door-to-door fever survey from villages selected at random from five CHC areas of the district. The blood smears collected by finger prick were stained with Giemsa stain (3% Qualigen's Giemsa stain in buffered distilled water, pH 7.2 for 55 min). Both thick and thin smears were examined and parasites were counted against 8000 white blood corpusels (WBCs). Those cases with a parasite count between 400 and 1,00,000/µl of blood were included in the study.

Adult dose of 1500 mg base of CQ (supplied by NMEP, Batch no. 912, Bengal Immunity, MFD, March 1992) was given at the rate of 600 mg base each on Day 0 and Day 1 and 300 mg on Day 2 (all single dosage) as per WHO method[4]. The urine of the selected cases were examined for the presence of CQ before and after chemotherapy as per the techniques of Dill and Glazko[5] and for the presence of sulfonamides by Lignin test[6].

Test procedures for standard 7-day *in-vivo* CQ sensitivity test of *P. falciparum* were followed[4]. Since reinfection can not be ruled out in these villages, cases were not followed up to Day 28. All resistant cases were treated with 1,000 mg sulphamethopyrazine and 50 mg of pyrimethamine (metakelfin, Walter Bushnell) and on Day 7 blood smear was examined to confirm clearance of the parasites.

The number of cases follwed in CHC area and results are given in Table 1. In Kudumulugumma CHC, 32 cases were susceptible to CQ and three were resistant, one each at RII, RI with early recrudescence (ER) and RI/S level. In Khairput CHC, 31 cases were successfully tested, of which 30 were sensitive and one resistant at RII level. In Malkangiri CHC 32 cases were successfully tested in which 31 cases were sensitive and one resistant at RI/S level. All the 31 cases tested in Korkunda CHC were CQ sensitive. Out of 25 cases tested in Podia CHC only one case was resistant at RI/S level.

Earlier studies in the undivided Koraput district by Guha *et al.*[2], in 1979 had shown that administration of 600 mg single dose (adult) of CQ failed to clear *P. falciparum* in 3.8% cases. In 1989, Mohapatra *et al.*[3], showed that there was no parasite clearance in 34.3% cases on Day 7 after 600 mg chloroquine administration. The present study in Malkangiri district showed that only 3.9% *P. falciparum* cases were resistant to chloroquine and only 2(1.3%) cases were resistant at RII level and none at RIII level. Therefore, chloroquine can continue to be the drug of

Table 1. Results of *in-vivo* chloroquine sensitivity test in Malkangiri district

Community Health Centre	Period of study	Days								No. of resistant cases	Resistance status
		0	1	2	3	4	5	6	7		
Kudumulugumma	Dec 93	35/35	25/35	11/35	0/35	3/35	2/35	2/35	2/35	3	S = 32 RI/ER = 1 RI/S = 1 RII = 1
Khairput	Jan 94	31/31	17/31	6/31	2/31	1/31	1/31	1/31	1/31	1	S = 30 RII = 1
Malkangiri	Feb	32/32	9/32	6/32	1/32	1/32	1/32	0/32	0/32	1	S = 31 RI/S = 1
Korkunda	Jul	31/31	8/31	1/31	0/31	0/31	0/31	0/31	0/31	0	S = 31 R = 0
Podia	Oct	25/25	13/25	6/25	1/25	1/25	1/25	0/25	0/25	1	S = 24 RI/S = 1
Total		154/154	72/154	30/154	4/154	6/154	5/154	3/154	3/154	6	S = 148 RII = 2 RI/S = 3 RI/ER = 1

choice for the treatment of *P. falciparum* cases in the district.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. V. Dhanda, Director, VCRC, for providing facilities to conduct this study and to Dr. P.K. Das, Deputy Director (SG) under whose initiative the study was undertaken. The authors are also thankful to Dr. P. Jambulingam, Assistant Director for critically examining the manuscript. The technical assistance rendered by the staff of VCRC field stations of Malkangiri and Jeypore are gratefully acknowledged.

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2.	Periodicity of its publication	Quarterly
3.	Printer's Name Nationality Address	Dr. V.P. Sharma Indian Malaria Research Centre (ICMR) 22-Sham Nath Marg Delhi-110 054.
4.	Publisher's Name Nationality Address	Dr. V.P. Sharma Indian Malaria Research Centre (ICMR) 22-Sham Nath Marg Delhi-110 054.
5.	Editor's Name Nationality Address	Dr. V.P. Sharma Indian Malaria Research Centre (ICMR) 22-Sham Nath Marg Delhi-110 054.
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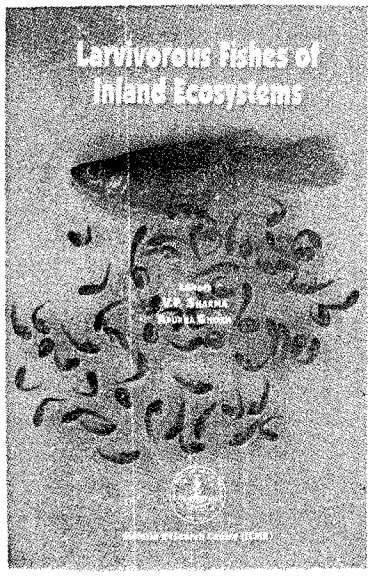

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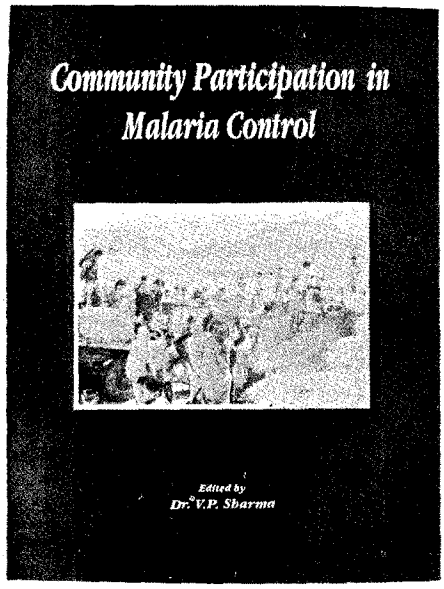

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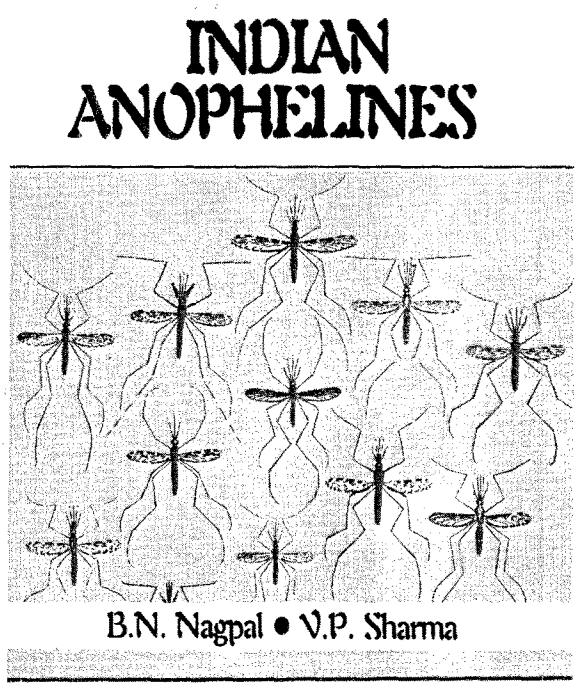

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