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Letter to the Editor

Note: The editor assumes no responsibility for the statements and opinions expressed by the contributors. This issue is delayed due to unavoidable circumstances.

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Breeding Preferences of *Anopheles culicifacies* in the Rice Agro-Ecosystem in Kheda District, Gujarat

RAJNI KANT[a] and S.D. PANDEY[b]

Breeding preferences of *Anopheles culicifacies*, a principle malaria vector, in the plains of India was studied in the rice agro-ecosystem of Kheda district in central Gujarat. Extensive breeding of this species was found in the rice field channels (20.83 per cent) and in rice fields (5.32 per cent). However, rice nurseries (0.91 per cent) and post-harvested rice fields (2.51 per cent) were less preferred. The species was found in abundance in newly transplanted rice fields and during early months of rice cultivation with a peak prevalence in the non-monsoon (Rabi) season. The breeding of *An. culicifacies* was inversely proportional and negatively correlated ($r = -0.868$; $p < 0.05$) with the height of the plants, whereas it showed a positive correlation ($r = 0.779$; $p < 0.05$) with the distance between plants. Rice fields near the villages supported maximum breeding of *An. culicifacies* (48 per cent) followed by the rice fields, 0.5 to one km away from the human habitation. Co-efficient of association (C_8 index) revealed a positive association of the species with *An. annularis*, *An. pallidus*, *An. subpictus* and *Cx. quinquefasciatus*. However, it was negatively associated with *An. nigerrimus*, *Cx. tritaeniorhynchus* and *Cx. vishnui* sub groups.

Keywords: *An. culicifacies*, Breeding habitat, Rice agro-ecosystem

INTRODUCTION

Malaria has returned as a major public health scourge causing enormous morbidity and mortality and resulting in economic losses as well.[1,2] It continues to inflict about a population of 2–3 million every year despite various control efforts.[3] Of the total malaria cases reported every year in rural and peri-urban areas of India, about 60–70 per cent are transmitted

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

by *An. culicifacies* alone.[4]

Rice is an important food crop of India and the staple food of about 65 per cent of the total population. It is cultivated in an area of 42 million hectare with a production of 110 million tonnes.[5] Due to prolonged water requirement and diverse ecological niches of the habitat, it offers favourable breeding conditions for mosquitoes to proliferate. It is, therefore, of utmost interest to study the breeding behaviour of malaria vector *An. culicifacies* in the rice agro-ecosystem.

MATERIALS AND METHODS

The study was carried out in Kheda district of Gujarat, which has an ample canal irrigation and is best suited for rice cultivation. The topography, climate, cropping pattern, cultivation practices and other features of the district have already been described.[6-8] Twenty-seven rice fields of an average size of 150 sq m in nine villages of three talukas — Anand, Matar and Petlad were selected for this study. At least three rice fields in each village—one close to the village, another 0.5 km away from the village and the third, one km away from the village—were selected and monitored continuously covering four cropping seasons [2 Rabi (Jan–Jun) and 2 Kharif (Jul–Dec)] beginning June 1988. The sampling of larvae/pupae of mosquitoes from nurseries, rice field channels and post-harvested rice fields was done randomly.

Mosquito immatures were collected from each of the selected rice fields and associated habitats at weekly interval using standard dipper (9.5 cm diam; 300 ml capacity) and brought to the laboratory in plastic containers for rearing and adult emergence. On emergence, adults were anaesthetized with ether and were identified using the keys of Christophers[9] and Barraud.[10] Data on the height of the plants, depth of the water, distance between the plants, presence of aquatic vegetation, agro-chemical applied and other biotic and abiotic factors which might influence the mosquito breeding were recorded every week. The data were analysed statistically by applying students's *t-test*, analysis of variance (ANOVA) and correlation co-efficient as described by Zar.[11] Hurlbert's index[12] of association (C_8) was applied to find out the interspecific associations. The statistical significance test was done by the corrected χ^2 formula as indicated by Pielou[13] for approximating a discrete distribution. Fisher's exact test was applied when any expected cell value was ≤ 5.

RESULTS AND DISCUSSION

Fourteen anopheline species were found breeding in the rice fields. Out of the 21,551 adult mosquitoes emerged from the larval/pupal samples, *An. subpictus* (69.19 per cent) was found to be predominant followed by *An. nigerrimus* (9.18 per cent), *An. annularis* (6.86 per cent), *An. culicifacies* (5.52 per cent) and *An. tessellatus* (4.52 per cent). The remaining nine species were present in low proportions. There were wide variations in the composition of different mosquito species during the two cropping seasons and at different stages of growth of the crop (Table 1).

Table 1. Per cent breeding of anophelines in the rice agro-ecosystem

Species	No.	% composition	Season of prevalence	Breeding preference
An. aconitus Donitz, 1902	118	0.54	–	GRC
An. annularis Van der Wulp, 1884	1479	6.86	NM	GRC
An. barbirostris Van der Wulp, 1884	490	2.27	–	GRC, MRC
An. culicifacies Giles, 1901	1191	5.52	NM	NTP
An. fluviatilis James, 1902	6	0.02	–	GRC
An. jamesii Theobald, 1901	6	0.02	–	GRC
An. nigerrimus Giles, 1900	1980	9.18	–	GRC, MRC
An. pallidus Theobald, 1901	291	1.35	–	GRC
An. splendidus Koidzumi, 1920	1	0.004	–	GRC
An. stephensi Grassi, 1899	72	0.33	–	GRC
An. subpictus Grassi, 1899	14912	69.19	M	NTP, GRC
An. tessellatus Theobald, 1901	975	4.52	NM	GRC, MRC
An. vagus Donitz, 1902	20	0.09	–	NTP
An. varuna Iyengar, 1924	10	0.04	–	NTP, GRC
Total	21551	100		

NM — Non-monsoon; M — Monsoon; (–) denotes more or less equal distribution; GRC — Growing rice crop; MRC — Mature rice crop; NTP — Newly transplanted rice fields.

Besides the breeding of malaria vectors *An. culicifacies*, *An. fluviatilis* and *An. stephensi*, breeding of mosquito vectors of Japanese encephalitis *Cx. vishnui* sub group were also found in abundance. *Cx. quinquefasciatus* and *Ae. aegypti* were found occasionally (Table 2).

Extensive breeding of *An. culicifacies* (20.83 per cent) was found in the rice field channels followed by the rice fields (5.32 per cent). However, the species was present in low proportions in the rice nurseries (0.91 per cent) and in the post-harvested rice fields (2.51 per cent). Russell and Rao[14] also noted the breeding of *An. culicifacies* in low proportions in the rice nurseries, which might be due to the reduced interspace between the plants, obstructing the ovipositing females consequently. The dominance of *An. culicifacies* in the rice field channels is attributed to the fresh and slow moving water being devoid of major aquatic vegetation during the early stages of the crop. It has been suggested that rice planted in the rice field channels without obstructing the flow of water may be useful in controlling the breeding of *An. culicifacies* when the plants attain a height of 30 cm or more.[14]

Table 2. Per cent breeding of mosquito vectors in the rice agro-ecosystem

Species	No.	Nursery	RFC	Rice fields	PHRF
Anophelines					
An. culicifacies	1191	0.91	20.83	5.32	2.51
An. stephensi	72	0.73	0.13	0.33	0.33
An. fluviatilis	6	0.18	0	0.02	0
Culicines					
Cx. vishnui group	13122	87.09	86.05	81.44	93.56
Cx. quinquefasciatus	998	8.21	7.99	7.19	0.09
Ae. aegypti	60	0	0.18	0.42	0.19

RFC — Rice field channels; PHRF — Post-harvested rice fields; Figures are per cent composition among respective groups; Total anophelines — 21,551 (547, 715, 18,776 and 1,513 in each habitat respectively); Total culicines — 15,757 (341, 538, 12,858 and 2,020 in each habitat respectively); *Cx. vishnui* sub group includes *Cx. vishnui, Cx. pseudovishnui* and *Cx. tritaeniorhynchus*.

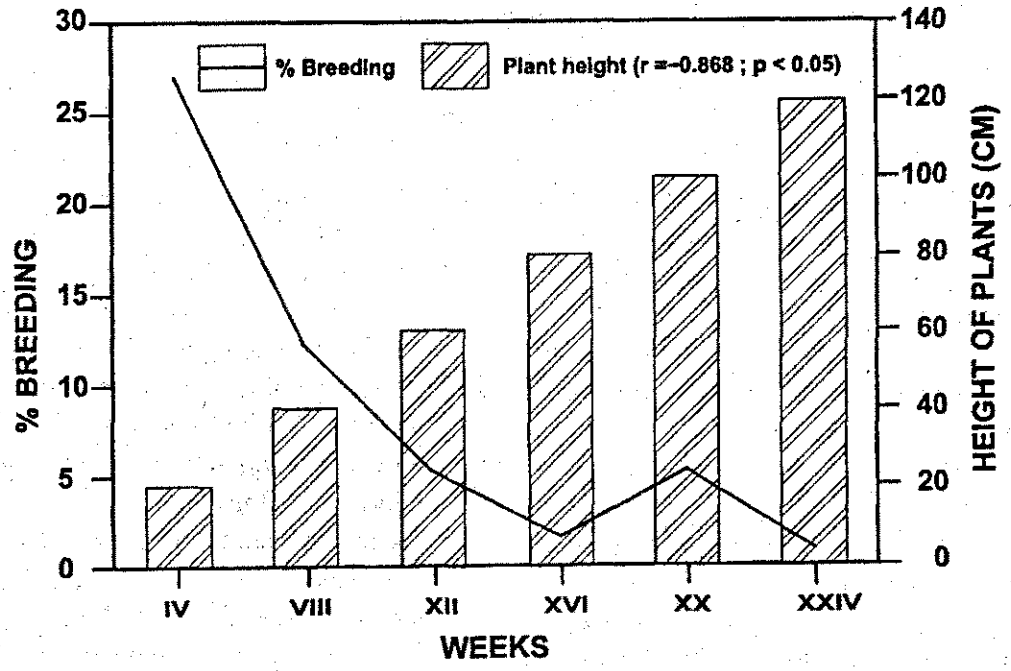

Fig. 1: Per cent breeding of *An. culicifacies* in relation to the plant height

An. culicifacies showed a significant negative correlation with the height of the plants ($r = -0.868$; $p < 0.05$). The per cent breeding of *An. culicifacies* was 27.07 per cent when the plants were less than 20 cm in height, 12.16 per cent when plants measured 21–40 cm and declined further with the increase in the height of the plants (Fig. 1). The dominance of *An. culicifacies* in the early weeks of rice cultivation could be due to sparse plantation and less height of the plants. Russell and Rao[14] noted that the composition of this species decreased when the plants were 37.5 cm tall. The subsequent decline in the compositon in the later half of the crop was due to the increase in the plant height and canopy development resulting in the mechanical obstruction to oviposition.[15] Senior-White[16] also observed that the prevalence of *An. culicifacies* ceases after September. Prasad *et al.*[17] found the breeding of *An. culicifacies* and *An. subpictus* only in the early months (Jun–Aug) of rice cultivation.

The per cent breeding of *An. culicifacies* in relation to plant distance is depicted in Fig. 2. Extensive breeding of *An. culicifacies* (50 per cent) in the rice fields occurred when the mean plant distance was 26–30 cm and it was 10.63 per cent when the plant distance was 21–25 cm, the per cent breeding reduced further with the reduction in the plant distance due to growth of the crop. The per cent breeding was positively correlated with the distance between plants ($r = 0.779$; $p < 0.05$). *An. culicifacies* is believed to perform a hovering dance before ovipositing.[15] The reduced interspace between the plants create hindrance for oviposition and the resultant decline in the breeding of this species during growing and mature stages of the rice crop occurs. Freeborn[18] observed more anopheline larvae in sparsely planted stands of rice in central California than in those with luxuriant growth. Similarly, Chambers *et al.*[19] noted a decrease in *An. quadrimaculatus* larval population in Louisiana rice fields as the plant density increased. Mogi[20] credits dense and tall rice growth with a resultant decline in the growth of micro-organisms upon which *Cx. tritaeniorhynchus* larvae depend.

The appearance of aquatic vegetation on the water surface was found unfavourable for the breeding of *An. culicifacies*. The maximum breeding of this species occurred in the absence of major aquatic vegetation (8.32 per cent) followed by floating (3.87 per cent), erect (3.86 per cent) and submerged (1.10 per cent) vegetation. Rice fields in close proximity to the villages were more conducive to *An. culicifacies* breeding (48 per cent) followed by rice fields which were 0.5 km (29 per cent) and one km (23 per cent) away from the villages.

Seasonal abundance of the species indicate that the breeding of *An. culicifacies* was more pronounced in the non-monsoon season to that of the monsoon season. But the species preferred to breed in abundance in the early months (Jan–Mar and Aug–Sep) of both the Rabi and Kharif crops. The difference in per cent breeding of *An. culicifacies* in the rice fields during the two seasons was found statistically significant ($F = 6.63$; $p < 0.05$). A thick growth of plants and the shade and appearance of aquatic vegetation on the water surface in the later

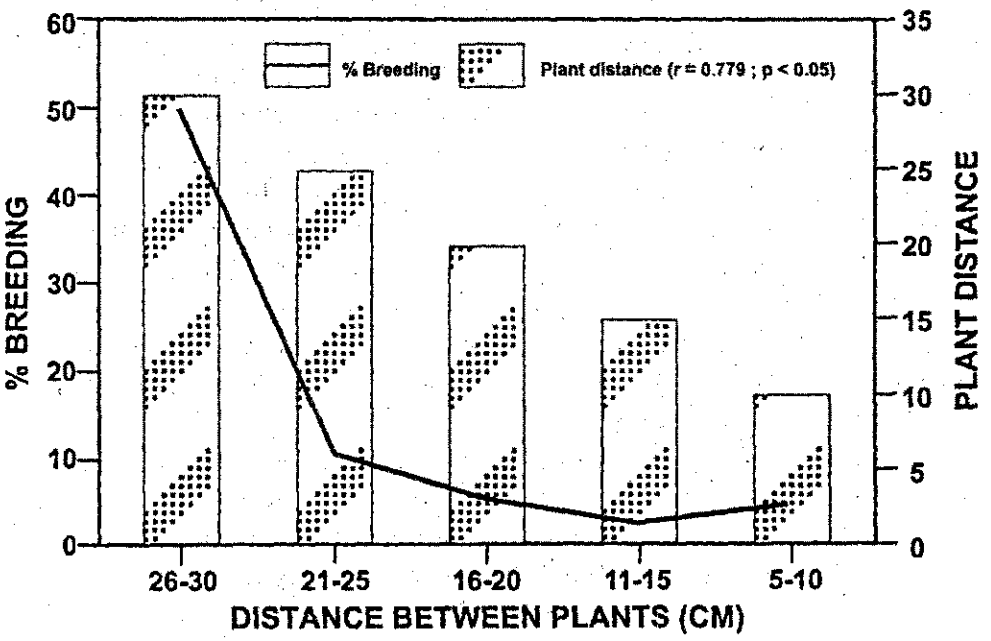

Fig. 2: Per cent breeding of *An. culicifacies* in relation to plant distance

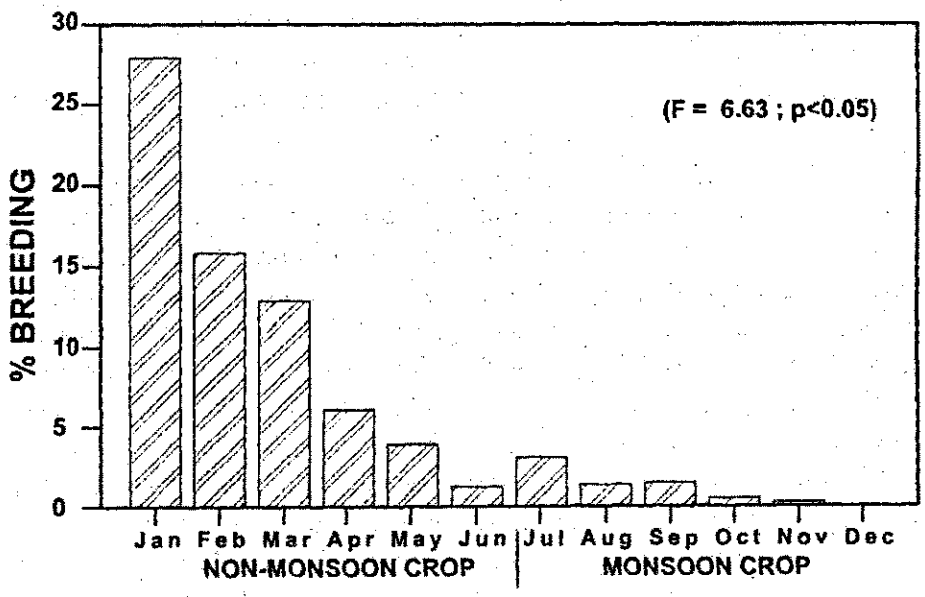

Fig. 3: Seasonal prevalence of *An. culicifacies* in the rice fields

stages of the rice crop was unfavourable for *An. culicifacies* breeding (Fig. 3) and supported species such as *An. nigerrimus*, *An. barbirostris*, *An. tessellatus* and *An. annularis* to proliferate.

In Kheda, the *An. culicifacies* exhibits a bimodal pattern of prevalence with two clear peaks in the months of March and August.[21] The first peak is attributed to well-established rice cultivation as other breeding sources become scarce and being a fresh water biotype, the rice field habitat provides a better ecological condition for *An. culicifacies* to proliferate. Although, due to unfavourable temperature and humidity conditions its longevity and survival seems to be affected, which limits its potential for malaria transmission in that season, unlike the favourable conditions during the monsoon.

Co-efficient of association (C_8 index) of *An. culicifacies* with other mosquitoes is given in Table 3. *An. culicifacies* showed a significant positive association with *An. annularis*, *An. pallidus*, *An. subpictus* and *Cx. quinquefasciatus*. However, it was negatively associated with *An. nigerrimus*, *Cx. tritaeniorhychus* and *Cx. vishnui* sub group. The association of *An. culicifacies* with *An. annularis* in the rice fields has been reported in the previous observation as well.[22] A positive association between species may occur due to common preference for a particular breeding habitat by both the species at the same time. Negative association results due to interspecific repulsion or variation in preference for a particular habitat.

Since rice agro-ecosystem is a complexity of interactions between several biotic and abiotic

Table 3. Co-efficient of association (C_8 index) of *An. culicifacies* with other mosquitoes

Species	Non-monsoon (Rabi) crop		Monsoon (Kharif) crop	
	Joint occurrences	C_8 index	Joint occurrences	C_8 index
An. annularis	43	0.293*	–	–
An. nigerrimus	9	– 0.650*	5	– 0.702*
An. pallidus	–	–	4	0.240^{+}
An. subpictus	78	0.134*	30	0.060**
Cx. quinquefasciatus	60	0.211*	3	0.353^{+}
Cx. tritaeniorhynchus	2	– 0.893*	–	–
Cx. vishnui sub group	–	–	17	– 0.322^{+}
Total (+) ve	108	–	47	–
Total (–) ve	453	–	365	–

*$p < 0.001$; **$p < 0.01$; $^{+}p < 0.05$; *Cx. vishnui* sub group also includes *Cx. pseudovishnui*; Insignificant associations have been excluded from the table.

factors, and malaria is an exclusively local and focal phenomenon governed by a number of ecological factors, a greater understanding on the breeding behaviour of *An. culicifacies* may be useful for designing appropriate control strategy to control the vector population in the rice field habitats.

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Effect of Arteether on Electrocardiogram in the Patients of Falciparum Malaria — A Preliminary Study

D.K. KOCHAR[a], ASHISH JOSHI[a], BRIJ VALLABH[a], C.B. MEENA[a], B.L. KUMAWAT[a] and NEETI JAIN[a]

The effect of intramuscular arteether (150 mg daily for 3 days) on electrocardiogram was studied in 16 patients having falciparum malaria. This included three patients with cerebral malaria, three patients with jaundice (S. bilirubin > 3 mg per cent) and ten patients with uncomplicated malaria. Five patients had tachycardia prior to arteether treatment. The mean RR interval before starting the treatment was 0.59 sec which increased progressively — 0.60, 0.68 and 0.69 sec on Day 1, 2 and 3 respectively after starting the treatment. There was no significant difference statistically in the mean corrected QT interval ($p > 0.05$), PR interval ($p > 0.05$), QRS duration ($p > 0.05$) and diastolic BP ($p > 0.05$) before starting and at the end of treatment on Day 3. One patient had developed first degree heart block (PR 0.24), while another patient had prolonged QTc (0.48 sec) on Day 3. Subsequent ECG of both these patients was normal on Day 5. The profile of ECG changes was same in the patients of uncomplicated and complicated malaria. Absence of any significant effect on BP and ECG changes precludes the significant effect of arteether on the cardiovascular system when compared to quinine which may cause hypotension, arrhythmia and QTc prolongation.

Keywords: Arteether, Electrocardiogram, Falciparum malaria

INTRODUCTION

Artemisinin and its analogues comprise a rapidly expanding chemotherapeutic arsenal in the ongoing global efforts to combat the resurgent threat of *P. falciparum* malaria. Out of various formulations of artemisinin compounds, oil-based preparations — artemether and arteether

[a]Department of Medicine, Neurology Division, S.P. Medical College, Bikaner–334 003, India.

are of particular importance because of their rapid action on asexual stages of the parasite. Their use is indicated in the patients of severe falciparum malaria, particularly in chloroquine resistant cases.[1] Common side-effects of these compounds include gastrointestinal (GI) upsets, dose related decrease in neutrophil, reticulocyte counts and neurotoxicity in the form of gait disturbances, incoordination, convulsions and respiratory depression.[2,3] However, the information available on its side-effects is very limited and is chiefly based on animal studies. We investigated the effect of intramuscular arteether on the electrocardiogram in the patients of falciparum malaria and the results of the preliminary study are reported here.

MATERIALS AND METHODS

The present study was conducted at an academic hospital in Rajasthan in the patients of falciparum malaria admitted in classified malaria wards. Only smear positive (demonstration of asexual forms of parasite in peripheral blood smear) cases of *P. falciparum* malaria were included. The study group included both male and female (non-pregnant) patients with no history of anti-malarial treatment within 24 hours prior to admission. Patients with concurrent heart disease and prior ECG changes were excluded from the study. All the patients were treated with intramuscular arteether at the dose of 150 mg daily for 3 days. Serial 12 lead electrocardiogram was recorded just before starting the treatment and daily after three hours (peak plasma concentration) of injection of arteether. In between, the patient was kept on continuous monitoring. Blood pressure was measured in all the patients just before and after three hours of arteether injection. Various ECG parameters — PR, QRS, RR and QTc intervals were calculated. These changes in the ECG and BP were compared before and after the completion of treatment. The data were analysed statistically by student's *t-test* and the data in text and table denote the mean ± SD values.

Table 1. Changes in ECG intervals and diastolic BP in patients of falciparum malaria (n = 16) treated with arteether

ECG interval (in sec)	Before arteether treatment	After arteether treatment			p-value
		Day 1	Day 2	Day 3	
Mean RR	0.59 ± 0.09	0.60 ± 0.12	0.68 ± 0.12	0.69 ± 0.09	0.017
Mean PR	0.14 ± 0.02	0.14 ± 0.02	0.14 ± 0.03	0.14 ± 0.03	0.799
Mean QRS	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.111
Mean QTc	0.41 ± 0.07	0.40 ± 0.04	0.38 ± 0.05	0.43 ± 0.04	0.334
Mean diastolic BP	77 ± 11	81 ± 7	80 ± 13	80 ± 12	0.689

RESULTS

The study group (n = 16) included an equal number of males and females, aged between 21–64 years, out of which, three patients had cerebral malaria, three had jaundice (S. bilirubin > 3 mg per cent) and ten had uncomplicated malaria. Five cases had sinus tachycardia (pulse rate 110–130/min) prior to arteether treatment. The mean RR interval before starting treatment was 0.59 sec. After starting treatment, the mean RR interval increased progressively — 0.60, 0.68 and 0.69 sec on Day 1, 2 and 3 respectively. The mean PR interval, QRS duration, QTc and diastolic BP before and after every dose of arteether were within normal limits (Table 1). All the changes observed in the ECG parameters and BP were statistically insignificant ($p > 0.05$) except changes in RR interval before and on Day 3 of treatment, which was significant ($p < 0.05$). One patient developed first degree heart block (PR 0.24) and another patient had prolonged QTc (0.48) on Day 3. There was no significant difference in the ECG profile in all the three groups of patients — uncomplicated, cerebral malaria and the patients with jaundice.

DISCUSSION

Artemisinin derivatives are very potent antimalarials against multidrug resistant falciparum malaria. These compounds are replacing quinine in the areas with quinine resistant parasites and demonstrate faster parasite clearance time and higher survival rate than that of quinine.[4] Serious side-effects of these compounds have not yet been reported in humans in therapeutic doses. As these drugs will have an increasing role in the treatment of multidrug resistant malaria, it is important to carefully assess the possibility of cardiovascular side-effects. It was shown in an animal study that artesunate (another derivative of artemisinin) can decrease the blood pressure at a dose of 320 mg/kg but not at the dose of 160 mg/kg or below.[5] However, in our study hypotension was not seen in any patient treated with arteether.

In a multicentric trial, Asthana *et al.*[5] observed transient heart block in one out of the 82 patients treated with artesunate and three out of the 39 cases treated with artemether. In the present study, one patient (uncomplicated malaria) had first degree heart block (PR 0.24) on Day 3. Possibly, arteether suppresses the conducting system of heart as shown earlier in animal experiments,[6] but further studies are needed in this regard. The progressive increase in RR interval after starting arteether treatment in our study reflects improvement, possibly in the condition of patients with the subsidence of tachycardia.

The cardiovascular side-effects of quinine in the form of QTc prolongation and hypotension are well-known.[7] Other serious side-effects—multiple ventricular partial blocks (VPB) and ventricular fibrillation have also been reported.[8] Prolongation of QTc has also been observed with the use of artemisinin compounds. Karbwang *et al.*[9] have shown prolongation of QTc in two patients out of 31 treated with intramuscular artemether in Thailand. They have also observed non-specific T-wave changes (six patients), right bundle branch block (RBBB) and second degree atrio ventricular (AV) block (one

patient). In our study, one patient had prolonged QTc on Day 3 of arteether treatment. Probably arteether might have some membrane stabilizing effect like quinine (in myocardial repolarization), but the possibilities of electrolyte imbalance like hypokalemia, hypocalcemia and the rest could also be the important causative factors.

Based on these preliminary observations, it can be concluded that arteether does not have any serious cardiovascular side-effects when compared to quinine, which may cause hypotension, arrhythmia and QTc prolongation. However, further elaborative studies are essential.

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Field Studies on the Sensitivity and Specificity of an Immunochromatographic Test for the Detection of *Plasmodium falciparum* Malaria in Tribal Areas of Orissa

S.K. SHARMA[a], P.K. TYAGI[a], M.A. HAQUE[a] and K. PADHAN[a]

A rapid immunodiagnostic test developed by an Australian Biotechnology company for the diagnosis of *Plasmodium falciparum* in the peripheral blood has been evaluated in the field for its sensitivity, specificity and efficacy in comparison to microscopic examination. The results showed that the test's sensitivity, specificity and efficacy were 98.2, 96.9 and 97.5 per cent respectively. The positive and negative predictive values of the test were 96.4 and 98.4 per cent respectively. The test when compared to the conventional microscopy did not show any statistically significant difference suggesting that the two diagnostic methods are equally good. The test performed did not show cross-reactions with other parasite species. It is a simple and rapid field diagnostic method, which does not require any expensive laboratory equipment or skilled personnel.

Keywords: Immunochromatographic test kit, Malaria diagnosis, Orissa, Tribals

INTRODUCTION

Recently, an Australian Biotechnology company (ICT Diagnostics) has developed a new method for the detection of *P. falciparum* (*Pf*) using Immunochromatographic technology (ICT). The test kit offers an *in vitro* immunodiagnostic test for the detection of circulating antigen, specific for *P. falciparum* in whole blood. The test uses two antibodies, which are specific to *Pf* histidine rich protein-2 (*Pf* HRP-2) antigen. The antibody and

[a]Malaria Research Centre (Field Station), Civil Township, Rourkela–769 004, India.

antigen reaction results in the appearance of a pink line in *P. falciparum* positive blood samples, whereas no such line appears in the blood samples negative for falciparum malaria. The test is considered to be a simple and rapid field diagnostic method for the detection of *P. falciparum*.[1] The Malaria Research Centre field station at Rourkela has carried out trials in different areas by following a common protocol. The results of field evaluation on the sensitivity and specificity of ICT malaria *Pf* test kit in two tribal districts of Orissa are reported here.

MATERIALS AND METHODS

The ICT malaria *Pf* test kits were procured from M/S. ICT Diagnostics, Australia. The test kit and the methodology was the same as described earlier.[2,3] The kit was tested randomly on patients with clinical symptoms of malaria reported at malaria clinics of Rourkela and Keonjhar. Evaluation was double blind and simultaneously thick and thin blood smears were prepared for each patient tested with ICT malaria *Pf* kit. The blood smears were stained with Jaswant Singh and Bhattacharjee (JSB) stain and examined under a compound microscope. The parasite density of *P. falciparum* was calculated as per the standard procedure.[4] Microscopic examination was conducted without prior knowledge of the result of ICT test for the corresponding sample. The negative and positive slides that did not match the result obtained by the ICT test were re-examined for confirmation of the results.

Intensity of colour of the test line in samples positive in the ICT was noted and graded from +1 to +3 — +1 (faint), +2 (readable with ease), +3 (intense). The known blood samples infected with other parasite species of malaria and filaria in the region were also tested with the ICT kit to rule out the possibility of cross-reaction to other species.

RESULTS AND DISCUSSION

One hundred twenty-five tests were performed with the immunochromatographic (ICT) test kit, out of which five were invalid, because the control line did not appear. Table 1 shows the results from ICT malaria *Pf* test kit and conventional microscopic examination. Out of 120 valid tests, 55 were positive for *P. falciparum* and the corresponding microscopic examination results were: *Pf* — 48; Mix *Pf*+ *Pv* — 3; *Pf* + *Pm* — 3 and one negative. The patient who was negative for malaria in microscopic examination revealed that he had already taken chloroquine on the advice of a Medical Practitioner before reporting to the malaria clinic. A microscopic examination of the remaining 65 slides of persons which were negative for *P. falciparum* with ICT test kit showed two slides positive for *P. falciparum*, eight for *P. vivax* and 55 slides negative for malaria. The two patients who were positive for *P. falciparum* by microscopic examination and tested negative with ICT kit had moderate level of parasitaemia — > 5000 parasites/µl. The results showed that the test sensitivity, specificity and efficacy were 98.2, 96.9 and 97.5 per cent respectively. The positive and negative predictive values of the test were 96.4 and 98.4 per cent respectively. Previous field studies on the kit in different areas have also reported similar results.[2,3] The mar-

Table 1. Results of field trial of the ICT malaria *Pf* test kit against microscopic examination

Results of ICT kit	Microscopic examination					Total
	Pf	*Pv*	Mix		Negative for malaria	
			Pf+Pv	*Pf+Pm*		
Positive	48*	0	3*	3*	1[+]	55
Negative	2**	8[++]	0	0	55[++]	65
Total	50	8	3	3	56	120

*No. positive with both microscopic examination and ICT – 54; **No. negative with ICT but microscopically positive – 2; [+]No. negative microscopically but positive for ICT – 1; [++]No. negative with both microscopic examination and ICT – 63.

ginal difference between the results of the ICT test kit and microscopic examination was not statistically significant ($\chi^2 = 0.017$; $p > 0.05$); and it can be concluded that both the tests are equally good.

The parasite density was calculated, for all *P. falciparum* positive slides, to study whether there was any positive correlation between the ICT test line intensity and the parasite density (Table 2). Results indicate that a majority of the samples with parasite density < 500 parasites/μl exhibited +1 (faint) test band intensity on the ICT card although five samples with < 500 parasites/μl showed +2 (readable) band intensity. Three samples with parasite density between 500–5000 parasites/μl showed an intensity of +1 on the ICT card. The remaining samples with parasite density between 500–5000 parasites/μl showed a band intensity of +2 (readable) and +3 (intense). A majority of the samples with a parasite density > 5000 parasites/μl showed a line intensity of +3 (intense) on the ICT card, however, three samples with parasite density > 5000 parasites/μl also showed a line intensity of +2 (readable) on the test card. Among all the samples, the lowest measurable parasite density calculated was 40 parasites/μl and the highest was 36,000 parasites/μl. A correlation graph (Fig. 1) was plotted to study the

Table 2. Results of ICT band intensity and parasite density

Parasite density per μl	No. of cases showing different grades of band intensity		
	Faint (+1)	Readable (+2)	Intense (+3)
< 500	12	5	0
500 – 5000	3	10	5
> 5000	0	3	16

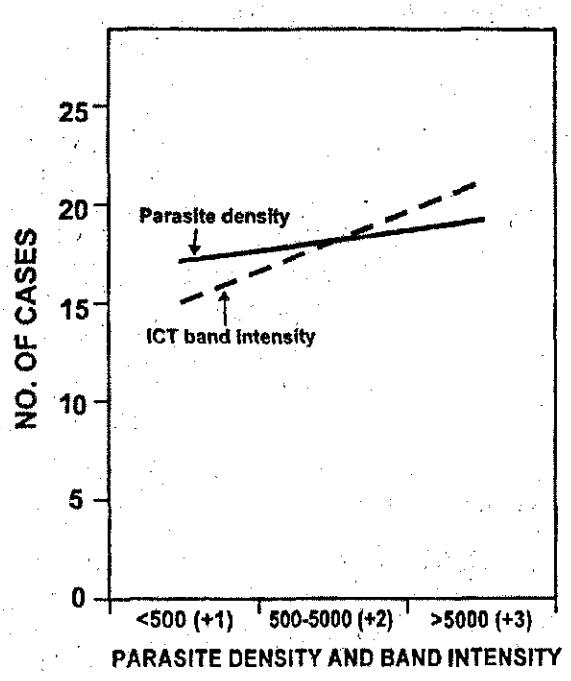

Fig. 1: Correlation between ICT band intensity and parasite density

relationship between parasite density and ICT band intensity instead of calculating the correlation co-efficient as one of the variables (ICT band intensity), which were not represented in absolute numbers. Both the lines representing parasite density and ICT band intensity showed a similar trend, which indicates a positive correlationship between them.

During the microscopic examination of blood slides, two out of three mixed infections of *P. falciparum* and *P. malariae* were declared positive for *P. malariae* only and the corresponding result of ICT test was also positive. Initially it was interpreted that the kit was perhaps showing cross-reaction to *P. malariae*, but a re-examination of these two blood smears revealed that both these cases were of mixed infection—*P. malariae* and *P. falciparum* and the parasite density of *P. falciparum* was low at the sub-microscopic level, which was not noticed during the initial microscopic examination. The tests were also performed on known samples of *P. vivax* (n = 8), *P. malariae* (n = 5) and microfilaria (*mf*) of Bancroftian filariasis (n = 2); and the results were negative, indicating that the ICT test kit did not show any cross-reaction to other parasite species.

The study showed that the ICT malaria *Pf* test kit is reliable due to its high specificity and sensitivity when compared to blood film examination. The only drawback is that the test is positive even after elimination of the viable *P. falciparum* parasites following radical treatment with antimalarial drugs. This is because of the circulating *Pf* HRP-2 antigen in the blood up to a certain time period even after treatment. The ICT test is useful in diagnosing partially treated unconfirmed cases, where microscopy is of little help. Previous field studies on the efficacy of ICT test kit in different regions have demonstrated the usefulness of this test kit.[5,6] Presently the ICT kit is being used for early and easy diagnosis of *P. falciparum* malaria. Although the kit is useful in all epidemiological paradigms, it may assume special significance in highly endemic areas where the prevalence of asymptomatic carriers is very high. Such populations can be very easily screened using the ICT test kit and, therefore, the parasite load in the community can be checked. The ICT test kit is useful in tribal areas characterized by high incidence of *P. falciparum* malaria, which are located in remote and poorly accessible parts of the country.

The test was found to be very simple and easy to perform even by unskilled personnel. The test is valuable in early diagnosis of complicated falciparum malaria, which is often confused with other diseases like viral encephalitis and meningitis. Early diagnosis and treatment will help in reducing the malaria transmission, consequently preventing morbidity and mortality caused due to malaria.

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Raised Serum Thiobarbituric Acid Reactive Substance Levels in Malaria

M.K. MOHAPATRA[a], K.N. PADHIARY[a], B.V. PUROHIT[a], K.K. SAMAL[a], S.K. TRIPATHY[a] and P.K. PADHY[a]

To study the extent of serum lipid peroxidation in malaria, 62 patients of falciparum malaria (18 uncomplicated and 44 complicated), 15 patients of vivax malaria and 25 healthy controls were enrolled in this study. The extent of serum lipid peroxidation was evaluated by estimating serum thiobarbituric acid reactive substances (TBARS) colorimetrically. The mean serum TBARS levels were 1.5 ± 0.29, 1.21 ± 0.2 and 3.58 ± 1.35 nmol/ml in controls, vivax malaria and falciparum malaria patients respectively. The TBARS level was significantly more in complicated falciparum malaria patients (4.2 ± 1.03 nmol/ml) than uncomplicated falciparum malaria patients (2.01 ± 0.61 nmol/ml). The TBARS level was also more in patients who died (4.82 ± 0.64 nmol/ml) when compared to the survivors (2.92 ± 1.05 nmol/ml).

Keywords: Falciparum malaria, Lipid peroxidation, TBARS levels

INTRODUCTION

Free radicals are chemical species possessing an unpaired electron having independent existence. They are very reactive in the biological system.[1] Of them, radical derivatives of oxygen are very important. These oxygen free radicals alongwith non-radical oxygen derivatives are collectively called as reactive oxygen species (ROS).[1] ROS damage all the major biomolecules — proteins, carbohydrates, nucleic acids lipids and contribute to the pathogenesis of many acute and chronic health problems.[2]

[a]Department of General Medicine, M.K.C.G. Medical College Hospital, Berhampur–760 004, India.

ROS are generated either accidentally by the electron "leakage" from electron transport chains or deliberately by the activation of neutrophils.[1] Apart from bacteria, the malaria parasites also generate abnormally large quantities of free radicals within the red blood cells (RBC) and also extracellularly as a result of host's immune response.[3] This may be responsible for the extensive tissue damage observed in falciparum malaria. The present study, therefore, was designed to observe the extent of lipid peroxidation by estimating thiobarbituric acid reactive substances (TBARS) in the patients of vivax and falciparum malaria with different complications.

MATERIALS AND METHODS

The present study was carried out at the M.K.C.G. Medical College Hospital, Berhampur from January 1997 to December 1998. A total of 62 consecutive cases of *P. falciparum* and 15 consecutive cases of *P. vivax* malaria were enrolled in this study. Twenty-five age and sex matched healthy subjects were taken for comparison. Patients and control subjects who received antimalarials within 15 days, antioxidants within one month and had evidence of rheumatoid arthritis, ischaemic heart disease and diabetes mellitus were excluded. All the patients were admitted to the hospital and pre-informed consent was taken either from the patient or from the relatives. The parasitological evaluation was done before enrollment by examining the blood smear stained with Giemsa stain. The classification of severe and complicated falciparum malaria was done according to the criteria described by WHO.[4] After a detailed clinical examination, before initiation of drug therapy, blood was collected for routine laboratory investigations and for the present experiment.

TBARS estimation was done colorimetrically by the method of Satoh.[5] For this, 0.5 ml of serum was taken and to it 2.5 ml of trichloroacetic acid was added. It was centrifuged at 3500 rpm for 10 min, the supernatant was decanted and the precipitate was washed with sulfuric acid. Then, 2.5 ml of sulfuric acid was added and stirred thoroughly. Next, 3.0 ml of thiobarbituric acid (TBA) was added and heated in the boiling water bath for 30 min and was then cooled. Again, 4.0 ml of *n*-butanol was added and chromogen was extracted by centrifugation at 3000 rpm for 10 min. The supernatant was pipetted into a clean test tube and its abosorbance was measured at 532 nm in a spectrophotometer. The absorbance was noted and the TBARS concentration was calculated from standard curve. 1, 1, 3, 3–tetrahydroxy propane (Wako Pure Chemicals, Japan) was used as standard. Data in the text denote mean $\pm$ SD values. Statistical analysis was performed by student's *t-test* and Pearson's rank correlation formula.

RESULTS

There were 62 patients (41 males and 21 females) of falciparum malaria and 15 (10 males and 5 females) patients of vivax malaria. The age ranged from 14 to 60 years. Out of the 62 patients of falciparum malaria, there were 18 (29.1 per cent) patients with

Table 1. Serum TBARS in malaria

Groups	No. of cases	TBARS value mean ± SD (nmol/ml)	p-value
Control	25	1.05 ± 0.29	
P. vivax malaria	15	1.21 ± 0.2	< 0.1*
P. falciparum malaria	62	3.58 ± 1.35	< 0.001*
Uncomplicated falciparum malaria (UFM)	18	2.01 ± 0.61	< 0.05
Complicated falciparum malaria (CFM)	44	4.2 ± 1.03	< 0.001
(a) Cerebral malaria	15	3.29 ± 1.05	< 0.001**
(b) Jaundice	9	4.53 ± 0.47	< 0.001**
(c) Acute renal failure	5	3.78 ± 0.52	< 0.001**
(d) Anaemia	4	4.88 ± 0.3	< 0.001**
(e) Pulmonary edema	3	4.67 ± 0.42	< 0.001**
(f) Multiple complications	8	5.29 ± 0.21	< 0.001**

*Comapred with controls; **(a), (b), (c), (d), (e) and (f) compared with UFM.

uncomplicated falciparum malaria (UFM) and 44 (70.9 per cent) with different complications.

The mean TBARS level in the patients of falciparum malaria (3.58 ± 1.35 nmol/ml) was significantly higher ($p < 0.001$) than the controls (1.05 ± 0.29 nmol/ml) and vivax malaria patients (1.21 ± 0.2 nmol/ml). Further, falciparum malaria patients with complications had higher TBARS level (4.2 ± 1.03 nmol/ml) than uncomplicated falciparum malaria patients (UFM) (2.01 ± 0.61 nmol/ml; $p < 0.05$). Patients with multiple complications had higher level of TBARS than patients with a single complication (Table 1).

All the cases of vivax malaria recovered, whereas 14 (22.6 per cent) patients of falciparum malaria died. The mean TBARS level in the patients who died was 4.82 ± 0.64 nmol/ml, which was higher than the survivors (2.92 ± 1.05 nmol/ml; $p < 0.001$).

DISCUSSION

The oxidative destruction of polyunsaturated fatty acids (PUFA) is known as lipid peroxidation.[1] As the PUFAs are the major constituents of the cell membrane, lipid peroxidation directly damages the cell. However, the chain propagation of membrane lipid peroxidation may be terminated by a membrane antioxi-

dant or by an adjoining membrane protein which itself get damaged.[3] Damage to the membrane protein is more injurious than the lipid peroxidation because of the functional role (receptors, ion channels and the rest) of most of the membrane proteins. Apart from the PUFA and proteins of the cell wall, the serum lipoproteins also get peroxidised.[2] The oxidative destruction results in a rise of wide range of compounds, notably aldehydes. Malondialdehyde (MDA) is one of them. Estimation of MDA by the TBA method has been used as an index of oxidative damage because of its sensitivity and simplicity.[6] However, TBA method also measures TBA break-down products and reactions between TBA and various other compounds. Hence, by the TBA method, TBARS concentration is measured which also includes MDA.[6] Since in the method used here, the supernatant after protein precipitation is discarded, any soluble TBARS which might have spilled from the tissues are lost in the supernatant. Therefore, the present method primarily measures peroxidation of lipoproteins.

The present study showed an increased TBARS levels in the patients of falciparum malaria in comparison to controls and vivax malaria patients. The patients with different complications in falciparum malaria had also raised TBARS when compared to UFM. Increased serum lipoprotein oxidation could be an indicator of oxidative stress exerted by soluble oxidants, which are produced by polymorphonuclear leucocytes (PMNS), macrophages and plasma antioxidants.[2] From these observations it can be inferred that the phagocyte induced oxidative stress is increased in falciparum malaria.[7]

Neutrophil derived oxidants appear to be a significant factor in the patients of sepsis with adult respiratory distress syndrome (ARDS) and multiorgan dysfunction syndrome (MODS).[8,9] It has been observed that phagocytosis of parasitised RBC produced soluble oxidants, which induces oxidative damage.[10] Consequently, very high level of TBARS in the patients of malaria with multiple complications can be explained in this line.

Increased haemolysis, which has been attributed as a cause of anaemia and jaundice is invariably present in the patients of falciparum malaria.[4] *P. falciparum* inflicts oxidative stress upon the host red cells which may contribute to haemolysis. Raised erythrocyte TBARS levels and reduction in erythrocyte antioxidants were found in acute falciparum malaria.[11] In the present study, we found high serum TBARS level in the patients with anaemia and jaundice than with only cerebral affection. The extent of tissue damage in falciparum malaria is determined not only by the oxidants but also by the level of different plasma antioxidants.[12]

In the present series, the mortality in falciparum malaria was 22.6 per cent. The significant rise in TBARS level in those who expired when compared to the survivors brings in the possibility of casual relationship between extent of tissue damage and outcome. It has been suggested in earlier reports that oxidative damage could be responsible for se-

verity of the tissue damage observed in malaria infected mice.[13]

In conclusion, enhanced TBARS levels was observed in falciparum malaria with complications and deaths suggest a possible role of free oxygen radical tissue injury in the pathogenesis of complications observed in falciparum malaria.

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Malaria Investigation in District Jodhpur, Rajasthan, during the Summer Season

C.P. BATRA[a], P.K. MITTAL[a], T. ADAK[a] and V.P. SHARMA[b]

Studies were carried out in District Jodhpur of the Thar region of Rajasthan. Epidemiological investigation revealed high slide positivity rate in the canal irrigated area (54.5 per cent), sand dunes area (67.54 per cent), stone quarry area (26.66 per cent) and in the desert plain area (41.5 per cent). Similarly, slide falciparum rates were 7.10, 4.38, 6.66 and 5.6 per cent respectively. Entomological studies showed *An. stephensi* and *An. culicifacies* as major species and their densities ranged between 2 to 14.58 and 0 to 0.9 pmh respectively. Resistance in malaria vectors to insecticides, poor surveillance and suppressive treatment of cases appear to be the factors for persistent transmission in the study area.

Keywords : Ecotypes, Epidemiological investigation, Vector density

INTRODUCTION

Malaria was nearly eradicated from India in the early 1960s but the disease resurged in the 1970s as a major public health problem, even in areas which were at one time free from this disease.[1] The state of Rajasthan, an arid zone, experienced frequent malaria epidemics since 1990s,[2,3] but the epidemic in 1994 was the most severe and recorded 447 deaths in the state. Four districts — Barmer, Bikaner, Jodhpur and Jaisalmer were severely affected, where 108, 94, 69 and 56 deaths respectively were reported.[4] To investigate the ecoepidemiological and entomological factors responsible for the malaria outbreak in these areas, a survey was

[a]Malaria Research Centre, 2 Nanak Enclave, Delhi–110 009, India.
[b]World Health Organization (SEARO), I.P. Estate, New Delhi–110 002, India.

carried out during the month of May 1995 in Jodhpur district, which was one of the most affected districts and the findings are presented in this paper.

MATERIALS AND METHODS

Study area

District Jodhpur is located in the Thar desert, in western part of Rajasthan (Fig. 1). The district has an area of 22,850 sq km with a population of 2.15 million spread over 94/sq km. The north-western parts of the district have typical desert conditions with sandy soil, scanty vegetation and sand-dunes whereas the southern part, which is about 40 km from the western range of Aravalli hills is a rocky terrain with desert conditions, where stone quarrying is very common. The labour engaged in quarrying activities are natives. Irrigation in the north-western areas of the district is through distributaries of the Indira Gandhi Canal (surface irrigation) and in the southern areas, it is through lift irrigation. The average annual rainfall is about 250 mm in the district, but in 1990, a rainfall of 821 mm, in 1992—526 mm and in 1994—470 mm were recorded.[5] During May 1995, the maximum temperature recorded ranged between 41 and 49°C during the daytime, while the minimum temperature recorded was 25 to 29 °C.

A survey was conducted in nine primary health centres (PHCs) of the district, representing four

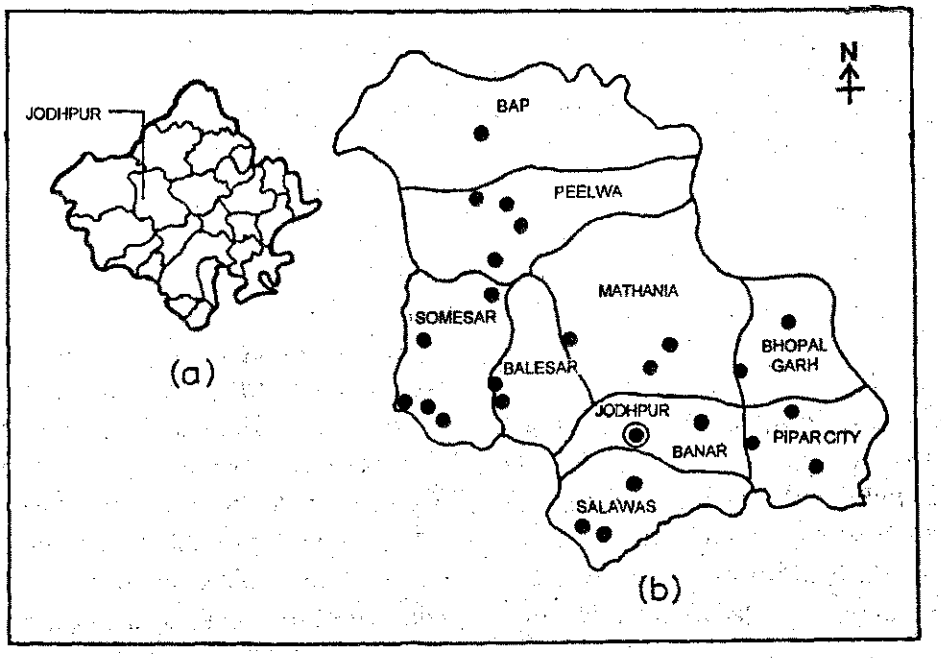

Fig. 1: Location of: (a) Jodhpur district in Rajasthan state; (b) Selected study sites (●) in nine PHC's of District Jodhpur

distinct ecotypes: (i) canal irrigated area, where surface irrigation is through the Indira Gandhi Canal and some areas are in the vicinity of the canal irrigation (PHCs — Bap, Peelwa, and part of Mathania); (ii) sand-dunes area is a vast sand-covered tract and sand hills called 'tibas', which sometimes rise to a height of 90 to 120 m (PHC — Somesar); (iii) stone quarry area which is a short offshoot of the Aravalli hills (PHC — Balesar); and (iv) desert plain area, where the area is a plain with sand-covered tract (PHCs — Salawas, Banar, Bhopalgarh, Pipar and part of Mathania). In each PHC and the distinct ecotype, a minimum of two villages with high and low malaria incidence (based on 1994 epidemiology data of NMEP) was selected for the present survey.

METHODOLOGY

Parasitological studies: During the present study, blood smears were collected through door-to-door fever survey (active surveillance) in 23 villages and of reported fever cases in PHCs (passive surveillance). In the mass blood surveys, blood smears were collected in nine villages of different PHCs to ascertain the proportion of afebrile "inapparent" malaria cases. Blood smears were stained with JSB stain[6] and examined microscopically for malaria parasites.

Entomological studies: Mosquitoes were collected from domestic and peridomestic resting places (underground water storage tanks) between 0530 to 0730 hours using suction tubes. Collected mosquitoes were identified individually and only *An. stephensi* and *An. culicifacies*, the known vector species of malaria, were investigated for abdominal condition, vector incrimination and the insecticide susceptibility test by following standard procedures.[7]

The number of ridges on the egg float was also checked in *An. stephensi* to find out the composition of different varieties — type form, variety *mysorensis* and intermediate form.[8,9] A survey of larval breeding was carried out in and around each locality in various cement tanks, underground storage tanks (*tanka*), cemented drains, wells, mud pots (pitcher), pits, ditches, pools and ponds of the study villages and the larval density was estimated.

RESULTS

Parasitological studies : The results of blood smear examination of fever cases from four different ecotypes are presented in Table 1. Out of a total 419 blood smears examined from 9 PHCs, 207 were positive for *P. vivax* and 26 for *P. falciparum* and the SPR was 55.6 and SfR was 6.2. Table 2 shows the results of mass blood survey of four different ecotypes. A total of 950 blood smears were examined and SPR was 9.68 and SfR was 0.73. Among 23 blood smears of infants, 11 were positive for *P. vivax*, and the infant parasite rate (IPR) was 47.8. All the positive infants had low parasitaemia and were in the age group of 9 to 11 months. No *P. falciparum* case was detected among infants.

Entomological studies: Five anophline mosquito species were encountered during the survey and the per cent collection estimated was: *An. culicifacies* (2.33 per cent), *An. stephensi* (42.98 per cent), *An. annularis* (11.54 per

Table 1. Results of fever (active and passive) surveillance in different ecotypes of District Jodhpur

Type of area	Population surveyed	BS collected	*Pv*	*Pf*		Total (+) ve	SPR	SfR
				R	R+G			
Canal irrigated area	18468	211	100	10	5	115	54.50	7.10
Sand-dunes area	9750	114	72	3	2	77	67.54	4.38
Stone quarry area	6225	15	3	1	0	4	26.66	6.66
Desert plain area	11385	79	32	2	3	37	41.57	5.60
Total	45828	419	207	16	10	233	55.60	6.20

R — Ring and G — Gametocyte; BS — Blood smear; SPR — Slide positivity rate; SfR — Slide falciparum rate.

Table 2. Results of mass blood survey in different ecotypes of District Jodhpur

Type of area	Population surveyed	BS collected	*Pv*	*Pf*	Total (+) ve	SPR	SfR
Canal irrigated area	3738	280	23	1	24	8.57	0.35
Sand-dunes area	2333	151	20	3	23	15.23	1.98
Stone quarry area	980	40	2	1	3	7.50	2.50
Desert plain area	6526	479	40	2	42	8.76	0.41
Total	13577	950	85	7	92	9.68	0.73

Table 3. Density of anophelines in villages of different ecotypes of District Jodhpur

Type of area	Per man hour density (No. of mosquitoes)			Anopheline larval density	
	An. stephensi	*An. culicifacies*	*An. subpictus*	Breeding site % positive	Av. larvae/dip
Canal irrigated area	14.58 (175)	0.91 (11)	15.08 (181)	28.4	22.6
Sand-dunes area	12.50 (50)	0.00 (0)	0.50 (2)	19.6	9.0
Stone quarry area	2.00 (2)	0.00 (0)	3.00 (3)	16.6	12.0
Desert plain area	6.18 (68)	0.45 (5)	9.50 (105)	17.3	12.8

cent), *An. subpictus* (42.5 per cent) and *An. pulcherrimus* (0.58 per cent). The per man hour density of *An. stephensi* varied between 2.0 and 14.58 in different ecotypes. *An. culicifacies* was found in low numbers and the average density was 0.91 and 0.45 pmh in canal irrigated and desert plain areas respectively (Table 3).

Sixty specimens of *An. stephensi* and five of *An. culicifacies* were dissected for gut and gland infection but none were found positive. The number of ridges in the egg float in representative eggs collected from nine *An. stephensi*, single female oviposition revealed presence of type form (17–18 ridges) and intermediate form (13–16 ridges). Longevity study of *An. stephensi* using ovarian dilatation revealed a maximum of two gonotropic cycles in 5 per cent of specimens.

Per cent breeding sites positive for anopheline larvae and average larval density per dip are given in Table 3. The percentage of positive breeding habitats was maximum (28.4) in canal irrigated areas and minimum (16.6) in stone quarry areas.

Insecticide susceptibility tests with adult *An. stephensi* from different ecotypes in two replicates (15 blood-fed in each replicate) revealed 30 to 40 per cent mortality against DDT (4 per cent) and 20 to 30 per cent against dieldrin (0.4 per cent) showing a high degree of resistance to these insecticides. However, an exposure to 5 per cent malathion resulted in100 per cent mortality indicating a complete susceptibility to malathion.

DISCUSSION

In Jodhpur district, high SPR–21.96 was recorded during 1975 and in the subsequent years, a low endemicity level was observed in the district with focal and sporadic rise in the incidence of malaria till 1988.[4] There was a gradual rise in the SPR from 0.4 in 1987 to 5.37 in 1994.

In the present study, the SPR in canal irrigated areas (54.5) and sand-dunes areas (67.5) was almost similar, indicating that unusual rainfall (2 to 3 times of average) during 1990–94 provided not only extensive breeding foci of vectors but also made the climate favourable for the transmission of malaria. In the PHCs of canal irrigated area, both *An. stephensi* and *An. culicifacies* were present, while in the PHCs of sand-dunes area, only *An. stephensi* was collected. It may be pointed out that a high average density of *An. stephensi* which ranged from 2.0 to 14.58 pmh in prevailing the high daytime temperature (41–49°C) indicated that species could find safe niches for their survival even under extreme conditions. This observation clearly indicates that *An. stephensi* was breeding and resting in *tankas* which are below the ground level and they were being protected from high temperatures which were prevailing at that time.

In District Jodhpur, resistance in malaria vectors *An. stephensi* and *An. culicifacies* to DDT and dieldrin has been reported.[10] In this study too, *An. stephensi* revealed a resistance to DDT and dieldrin but was found susceptible to malathion. Another important factor which explains the persistence of malaria is the inadequate sur-

veillance mechanism and treatment due to inaccessibility of the area through the PHC system. In such a situation, the malaria patients are given suppressive treatment with intramuscular injection of chloroquine (40–80 mg base) by private practitioners, which is inadequate for complete cure.

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Short Notes

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Susceptibility Status of *Culex quinquefasciatus* Larvae to Fenthion in Delhi—A Note on the Possible Development of Resistance

P.K. MITTAL[a], C.P. BATRA[a] and T. ADAK[a]

Keywords: *Cx. quinquefasciatus*, Fenthion, Larvae, Susceptibility

Mosquito menace in most of the towns and cities of India is extremely high due to the high density of *Cx. quinquefasciatus* which is associated with the disposal of waste water. Extensive breeding of this mosquito is encountered throughout Delhi in stagnant and slow moving polluted water habitats.[1] As a preventive measure to control malaria vectors and mosquito breeding in urban areas, organophosphorus insecticides, such as temephos and fenthion are commonly used as larvicides under the urban malaria scheme in India.[2,3] However, fenthion is used only in polluted water habitats, where *Cx. quinquefasciatus* breeds profusely. Though resistance to temephos and fenthion in *Cx. quinquefasciatus* and other mosquitoes in India have been documented by Pillai[4] and WHO,[5] there is very little information on the susceptibility status in different areas.[6,7] In Delhi, fenthion (Baytex 1000), the EC formulation is most com-

[a]Malaria Research Centre, 2 Nanak Enclave, Delhi–110 009, India.

monly used as a larvicide in polluted water habitats. In one of our recent studies, while conducting a field trial with Baytex-G (fenthion 2% granules) against *Aedes aegypti* in some natural habitats of Delhi, it was observed that the breeding of *Ae. aegypti* was completely eliminated at a dose of 0.1 ppm fenthion, while *Cx. quinquefasciatus* larvae survived well in the same habitat (unpublished data). In view of this observation fenthion was bioassayed against the larvae of *Cx. quinquefasciatus* populations collected from different localities of Delhi.

Mosquito larvae collected from different localities of Delhi were brought to the laboratory and reared to adults and the F_1 larvae of these adults were used for bioassay tests. In addition, a laboratory colonised strain of *Cx. quinquefasciatus* from Sonepat, Haryana, being maintained in the laboratory since 1980, without any insecticide selection pressure, was used for comparison of fenthion susceptibility status of different field-collected populations of *Cx. quinquefasciatus*. Six freshly prepared, different concentrations of Baytex 1000 (82.5 per cent w/w. E.C. formulation of fenthion) were used in all bioassay tests, which were performed following the standard WHO procedure for mosquito larvae.[8] These tests were conducted in 500 ml glass beakers, each containing 249 ml of stored tap water and one ml of insecticide solution to achieve the desired concentration and 25 late III instar larvae. Six serially diluted concentrations ranging between 1.0 and 0.0312 ppm were used in these bioassays. Two replicates were used for each concentration and control, and each bioassay was repeated three times. The mortality of the larvae exposed to different concentrations were recorded after 24 hours of continuous exposure by counting dead and moribund larvae and the lethal concentration for 50 per cent (LC_{50}) and 90 per cent (LC_{90}) mortality were determined by log-probit analysis.[9]

Table 1 shows the susceptibility status of *Cx. quinquefasciatus* larval populations against fenthion. The LC_{50} values for different field populations varied between 0.059 to 0.156 ppm, while the LC_{90} values ranged between 0.12 to 0.34 ppm. The LC_{50} and LC_{90} values of fenthion for the laboratory colonised strain of *Cx. quinquefasciatus* were 0.02 and 0.05 ppm respectively. These results clearly show that the LC_{50} values for different field collected populations of *Cx. quinquefasciatus* were significantly different (3 to 8 fold higher) than the LC_{50} value for laboratory colonised strain (as there was no overlapping of 95 per cent fiducial limits), thus indicating the possibility of development of fenthion resistance in field populations of *Cx. quinquefasciatus* in Delhi. This may be due to continuous use of fenthion as a larvicide, resulting in the selection of resistance in *Cx. quinquefasciatus* populations. Further, it may be stated that the application of larvicides is more liable to induce resistance than the adulticides in field,[10] which may be due to the fact that larvae are exposed to toxicants for a longer period resulting in the elimination of all susceptible individuals and permitting the survival of those which are resistant.[11] The resistance to organophosphorus compounds in *Cx. quinquefasciatus* is mainly due to the over production of detoxifying esterases.[4,12] Whether the resistance to fenthion as observed in the present study is also due to similar mechanism, is not clear.

Table 1. Susceptibility of larval populations of *Cx. quinquefasciatus* to fenthion

Collection sites of *Cx. quinquefasciatus* populations	Concentration (ppm)		χ^2 (df)
	LC_{50} (Fiducial limits)	LC_{90}	
Lab. colonised Sonepat strain	0.024 (0.02–0.03)	0.05	0.208 (3)
Localities in Delhi			
Rithala	0.059 (0.05–0.066)	0.192	12.39 (3)
Sarai-Kale-Khan (Nizamuddin)	0.064 (0.058–0.069)	0.122	10.97 (3)
Rani Jhansi Road	0.083 (0.075–0.09)	0.167	9.05 (3)
Jharoda	0.105 (0.096–0.115)	0.286	8.92 (3)
Khanpur	0.107 (0.098–0.115)	0.27	9.40 (3)
Loni Road (Shahdara)	0.1125 (0.09–0.134)	0.342	0.9 (3)
Wazirpur	0.1195 (0.106–0.133)	0.256	32.2 (3)
Pitampura	0.1415 (0.126–0.157)	0.298	9.21 (3)
Sarita Vihar	0.156 (0.147–0.167)	0.311	5.48 (3)

Among different field populations of *Cx. quinquefasciatus*, the Rithala population was most susceptible (LC_{50} value being 0.059 ppm), while the Sarita Vihar population was least susceptible to fenthion (LC_{50} = 0.156 ppm). From the LC_{90} values, it was observed that the Loni road (east Delhi) population was least susceptible (LC_{90} = 0.34 ppm), while the Sarai-Kale-Khan (south-east Delhi) population was most susceptible (LC_{90} = 0.12 ppm). In view of these observations, it is suggested that monitoring of fenthion resistance in *Cx. quinquefasciatus* should be carried out periodically and target dose of field application of fenthion for larval control should be rescheduled. Further, alternative larvicidal agents such as *Bacillus thuringiensis* H-14 may be used in rotation with fenthion. Studies are also required on cross-resistance and to find out the biochemical mechanism of fenthion resistance in *Cx. quinquefasciatus* population in Delhi.

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A Study of Human Genetic Markers in Mewat Region, Gurgaon, Haryana

HEMA JOSHI[a], SARALA K. SUBBARAO[a] and V.P. SHARMA[b]

Keywords: ABO, Rh, G-6-PD, HbS, Hp, Malaria

Human blood polymorphic systems are important biochemical markers in anthropological surveys, especially in relation to disease distribution. G-6-PD deficiency and certain haemoglobinopathies are known to confer a selective advantage to the subjects against falciparum malaria.[1] However, a use of certain antimalarials, such as primaquine and other aminoquinolines, increase the oxidant stress in G-6-PD deficient individuals, thus resulting in haemolytic crisis,[2] which can be fatal if not checked in time. These genetic disorders are inherited from one generation to the next. In an epidemiological survey carried out during November 1996 in the Mewat region of District Gurgaon, Haryana, which had experienced an outbreak of malaria during the post-monsoon season of 1996, a high prevalence of *P. falciparum* (*Pf*) ranging from 53.8–100 per cent in the villages of the region was observed.[3] Sharma *et al.*[4] in their study during 1996, reported about 2,101 fever related deaths and 24 confirmed deaths due to malaria in that region.

A study on the prevalence of human genetic markers in the Mewat region was undertaken with an aim to study the distribution and frequencies of polymorphs of ABO, Rh, haptoglobin, haemoglobin and G-6-PD enzyme in the population and their association with malaria. This region comprises three community blocks (CHCs), namely Nuh, Punhana and Ferozepur Zhirka (approx. 27° N and 70° E). The population of the region is around 0.64 million and the area is about 2000 sq km.

[a]Malaria Research Centre, 22 Sham Nath Marg, Delhi–110 054, India.
[b]World Health Organization (SEARO), I.P. Estate, New Delhi–110 002, India.

Fever surveys were conducted during April 1997 in four villages in each of CHCs Nuh and Ferozepur Zhirka. Both these CHCs are muslim dominated and the people belong to lower economic strata. The two CHCs namely Nuh and Ferozepur Zhirka were the worst affected during the epidemic. About 0.3 to 0.4 ml of blood was collected from each fever case by pricking a finger in heparinized vials. Simultaneously, thick and thin blood smears were also made. Methods used for the collection, storage and analysis of samples were as described earlier.[5,6] Blood smears were stained with JSB[7] and examined for malaria parasites under bright field Leitz Dialux 20 microscope at a magnification of 1000*x*.

In a total of 363 blood samples collected, 49 were positive for *Plasmodium vivax* (*Pv*), while 28 were positive for *P. falciparum* (*Pf*). Table 1 gives the CHC-wise data on slide positivity rate. The trend of transmission was same in the villages of both the CHCs. The slide positivity rate (SPR) and slide falciparum rate (SfR) were 22.0 and 7.8 in CHC Nuh and 19.6 and 7.6 in Ferozepur Zhirka respectively giving a *Pf* per cent of 36.3 in total. Parasitaemia varied from scanty to high (++++) as per grading of Bruce-Chwatt.[8]

A higher *P. vivax* positivity among patients was expected, because in north India spring transmission of *P. vivax* with a small peak during April and May with an interruption caused by dry and hot month of June is common. However, the prominent peak of *P. vivax* transmission occurs during the post-monsoon months of July to September.[9] In an epidemiological survey conducted in the two CHCs during November 1996, malaria indices were 61.8 per cent SPR and 87.2 per cent *Pf*.[3] October-November being the peak transmission period for *Pf* infection in north India, a high percentage of *Pf* was quite expected. But in our survey during April 1997, 36 per cent positivity for *Pf* was recorded, which is unusal. Therefore, it can be presumed that, these cases might be of recrudescence or resistant strain types, because drug and dosage schedule followed in this area was as per the National Drug Policy (Source: NMEP). This finding is supportive to MRC's earlier observation that about 33 per cent of the isolates tested from the area were showing a tolerance to chloroquine by *in vitro* micro test.[10]

Of the 363 samples, 297 samples were typed for ABO and Rh groupings using standard

Table 1. Slide positivity rates of CHCs Nuh and Ferozepur Zhirka

CHC	No. of slides examined	*Pv* (+) ve	*Pf* (+) ve	Total (+) ve	SPR	SfR	*Pf*%
Nuh	232	33	18	51	22.0	7.8	35.5
Ferozepur Zhirka	131	16	10	26	19.9	7.6	38.5
Total	363	49	28	77	21.2	7.7	36.3

antisera of ortho-diagnostics. The CHC-wise analysis of the data on ABO and Rh grouping has shown identical distribution pattern in the villages of both the CHCs. Observed proportion (per cent) of A,B,O and AB blood groups in CHC Nuh were 22.2, 35.7, 30.8 and 9.8 and in CHC Ferozepur Zhirka they were 19.6, 42.9, 27.7 and 7.8 respectively. The proportion of Rh(–) subjects in these two CHCs were 13.0 and 17.8 per cent respectively. These findings indicated the homogenous genetic make-up of the population in both the CHCs. Further, there was no significant difference in the distribution pattern of ABO between malaria positive and negative groups.

Haptoglobin typing was done in a total of 289 samples using polyacrylamide horizontal slab gel electrophoresis.[11] The proportion of non-typable samples (functional ahaptoglobinemia — HpO) was significantly higher among the malaria positive subjects (50 per cent) compared to malaria negative subjects (34.8 per cent). The observed chi-square value for HpO versus non-HpO between malaria positive and negative groups was 11.91 ($p < 0.001$). This reflects a positive association of malaria with HpO and supports our earlier observations.[6,11–12] However, the distribution of common phenotypes namely Hp 1–1, 2–1 and Hp 2–2 did not differ significantly between malaria positive and negative groups. The proportion of the polymorphs Hp 1–1: 1.1 per cent, Hp 2–1: 17.1 per cent and Hp 2–2 : 50.7 per cent were within the normal range as reported for other Indian populations.[13]

Of the 363 samples, 350 were analyzed for G-6-PD deficiency by fluorescent spot test and 343 for haemoglobin variants by CAM electrophoresis.[14] Three samples were found to be G-6-PD deficient and one showed sickle-cell trait (heterozygous condition). Of the three G-6-PD deficient subjects, two were from CHC Nuh giving a prevalence of 0.86 per cent and one (0.76 per cent) was in CHC Ferozepur Zhirka. All the three were male children aged between 5 to 15 years. Of them two were found to be positive for *P. falciparum*, while one was negative for malaria parasite at the time of blood examination. In the two *P. falciparum* positive subjects, parasitaemia was graded low (Pf+). Sickle-cell trait (HbAS) was observed in a boy aged about eight years from the Ferozepur Zhirka CHC. The boy had taken chloroquine prior to our survey and was found negative for malaria parasites.

Subjects with heterozygosity of these genetic disorders (carriers) are known to limit the parasitaemia, thus preventing complications.[15] Observed low incidence of G-6-PD deficiency and haemoglobin variants in the region may explain the high prevalence of complicated falciparum malaria in this area. Similar findings of low prevalence of G-6-PD deficiency and sickle-cell haemoglobin were reported earlier in the Haryana,[5] Delhi[6] and neighbouring Uttar Pradesh[11] populations. Pant *et al.*[16] have also reported similar low incidence of 1.5 and 1.8 per cent for sickle-cell haemoglobin and G-6-PD deficiency respectively in muslims of Kheda district, Gujarat. On the other hand, a significantly higher incidence of these disorders was observed in tribal populations analyzed from malaria endemic areas

of Uttar Pradesh,[12] Madhya Pradesh and northeastern states (Joshi *et al.*, unpublished data). In general, a high incidence of the above genetic disorders has been reported among tribal groups.[13] The study shows that there was no significant relation between ABO distribution pattern and malaria incidence and there is a low prevalence of G-6-PD deficiency and haemoglobinopathies in the Mewat region of Haryana.

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Susceptibility Status of *An. fluviatilis* and *An. culicifacies* to DDT, Deltamethrin and Lambdacyhalothrin in District Nainital, Uttar Pradesh

S.N. SHARMA[a], R.P. SHUKLA[a] and K. RAGHAVENDRA[b]

Keywords: Pyrethroid, Susceptibility, Vectors

Anopheles fluviatilis and *An. culicifacies* are the major vectors of malaria in the Terai region of District Nainital, Uttar Pradesh.[1] In this district, insecticides (DDT or HCH) were being used as an indoor residual spray under National Anti Malaria Programme (NAMP) to control malaria vectors. Resistance status of *An. culicifacies* and *An. fluviatilis* against dieldrin in the Terai was reported to be 86 and 75 per cent respectively.[2] The HCH spray has been banned since April 1997. Elsewhere in India, synthetic pyrethroids have been found effective against DDT and HCH resistant malaria vectors[3,4] and have also been used to treat bednets.[5] Recently, deltamethrin was used during malaria epidemic in District Gurgaon, Haryana, where *An. culicifacies* was the major vector of malaria and was resistant to DDT and HCH.[6] Synthetic pyrethroids have not yet been introduced in malaria control programme in District Nainital. In this paper, we present the baseline susceptibility levels in these two species to two synthetic pyrethroids — deltamethrin and lambdacyhalothrin compared with that of DDT.

[a]Malaria Research Centre (Field Station), Inderjeet Garden, Bhotia Parao, Haldwani–263 141, India.
[b]Malaria Research Centre, 22 Sham Nath Marg, Delhi–110 054, India.

Indoor resting mosquitoes were collected during the early morning hours from three villages—Jolasal and Haspur, situated in a deep forested area and village Sultan Nagri situated in the forest fringe, by the standard WHO methods using suction tube and flash light[7] and were held in a cloth cage. The mosquitoes were brought to the laboratory at Haldwani for further tests.

Fully-fed and semi-gravid mosquitoes were used to determine the susceptibility levels against insecticide papers impregnated with diagnostic concentration of DDT (4 per cent), deltamethrin (0.025 per cent) and lambdacyhalothrin (0.1 per cent) as per the WHO standard technique.[8] The exposure period was one hour. Results of the tests are given in Table 1. The exposure tubes with pyrethroids impregnated papers were held horizontally to the surface so as to allow the knocked down mosquitoes in contact with the test papers. The average temperature (28.4 °C) and relative humidity during the test ranged from 75 to 91 per cent respectively. Three to six replicates, both in experimental and control, were carried out with each diagnostic concentration of the respective insecticide paper. The total number of *An. fluviatilis* exposed to DDT, deltamethrin and lambdacyhalothrin impregnated papers were 85, 70 and 35 respectively. The number of *An. culicifacies* exposed were 90 to DDT and 30 each to deltamethrin and lambdacyhalothrin papers. After exposure, the holding tubes with the mosquitoes were kept for recovery in a cool and dark place for 24 hours. Later, per cent mortalities were de-

Table 1. Results of insecticide susceptibility test

Period of test	Vector species	Insecticide paper and diagnostic concentration	No. exposed		Mortality within 1 h		Mortality after 24 h		Corrected mortality %
			Exp	Cont	Exp	Cont	Exp	Cont	
March to April 1997	*An. fluviatilis*	DDT (4%)	85	75	0	0	21	3	21.6
		Deltamethrin (0.025%)	70	35	70	0	70	0	100.0
		Lambdacyhalothrin (0.1%)	35	35	35	0	35	0	100.0
July to August 1997	*An. culicifacies*	DDT (4%)	90	90	0	0	1	0	1.1
		Deltamethrin (0.025%)	30	15	30	0	30	0	100.0
		Lambdacyhalothrin (0.1%)	30	30	30	0	30	0	100.0

termined by scoring the dead and alive mosquitoes.

From the results given in Table 1, it is observed that both *An. fluviatilis* and *An. culicifacies* are respectively 78 and 99 per cent resistant to DDT but were completely susceptible to deltamethrin and lambdacyhalothrin. Results of susceptibility tests conducted by Malaria Research Centre, field station at Haldwani during1984–85 in Nainital Terai area revealed 89 and 25 per cent mortality in *An. fluviatilis* against DDT and dieldrin respectively.[2] Their susceptibility tests also showed resistance in *An. culicifacies* to dieldrin (14 per cent mortality). The results further highlighted the fact that despite HCH spray carried out in the area in the early 1980, incidence of malaria was extremely high. About four-fold decrease in mortality from 89 per cent in 1984 to 21 per cent in 1997, indicated an increase in tolerance to DDT in *An. fluviatilis*. Similar tolerance to DDT has been reported in *An. fluviatilis* from Maharashtra.[9] However, complete susceptibility to DDT in *An. fluviatilis* and the resistance to DDT in *An. culicifacies* have been reported from Orissa.[10]

The observed resistance to DDT in these two species did not confer cross resistance to pyrethroids. Highly organochlorine resistant and variable malathion resistant strains of *An. culicifacies* from the states of Gujarat, Karnataka and Maharashtra were found to be susceptible to deltamethrin.[11] On the contrary, a strain of *An. stephensi* from Kasur, Pakistan which was 144-fold resistant to DDT has shown about a 15–20 fold increase in the permethrin tolerance and was suggested to be due to the changes in the nervous system producing lower sensitivity to permethrin.[12]

Thus, indoor residual spray of synthetic pyrethroids can be a viable alternative in controlling malaria in areas, where the vectors have become resistant to the hitherto commonly used public health insecticide like DDT.

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LETTER TO THE EDITOR

Indian Journal of Malariology
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Serial ParaSight-F Test in Patients with Severe Malaria

Dear Editor,

The ParaSight™-F test (Becton Dickinson) is a dipstick test that detects the soluble *Plasmodium falciparum* hitstidine rich protein II (HRP-2) in blood from patients with falciparum malaria. It has a specificity of over 96.5 per cent and sensitivity of over 90 per cent and is being recommended as a diagnostic tool at the peripheral health care level.[1] In India, Kar *et al.*[2] have evaluated the efficacy of this test and shown the specificity and sensitivity of the test to be 100 per cent. Singh *et al.*[3] have shown 93 per cent sensitivity and 92.5 per cent specificity of this test in falciparum malaria. Following its introduction, the scope of the test has been expanded beyond diagnosis in detecting recrudescence. Thai workers have suggested that antigenemia may be detectable up to six days after the last parasite has been killed and, therefore, a positive ParaSight™-F test after Day 14 in uncomplicated malaria indicates persistence of parasites and that these patients should be closely followed for recrudescence.[4] We have previously reported that in patients with chloroquine sensitive uncomplicated *P. falciparum* malaria, the ParaSight™-F test remained positive up to Day 15 in the absence of recrudescence and hence it is postulated that in India, because of escalating chloroquine resistance, clearance of the parasite by chloroquine is slow and hence the HRP-2 antigen may persist for a longer period than in Thailand.[5] The duration of antigenemia in severe malaria and the importance of the ParaSight™-F test in diagnosing recrudescence in these patients is not known. We, therefore, prospectively studied 36 patients with severe malaria (diagnosed on the basis of clinical and laboratory criteria) admitted to the medical intensive care unit of a tertiary referral hospital in Mumbai. All patients received treatment with 480 mg of intramuscular artemether over five days. Peripheral smear examination and ParaSight™-F test

were performed on Days 1 (pre-treatment), 4, 8, 15, 22 and 29. Eight patients died between Day 2 and Day 7; 28 patients completed the study protocol.

All 28 patients (baseline parasitaemia 12.26 ± 20.14 per cent) had ParaSight™-F test positive on Day 1, giving a sensitivity of 100 per cent. The test remained positive in 26/28 patients on Days 4, 8, 15, 22 and 29, although the intensity of the 'pink line' (indicative of a positive test) diminished progressively as the follow-up progressed. Of these 26, recrudescence was observed only in two patients (on Days 22 and 29 respectively) as confirmed by fever and peripheral blood smear positivity. Two out of the 28 patients had negative ParaSight™-F tests with negative peripheral smears on Day 29 (baseline parasite indices of 3.2 and 2.59 per cent). Thus in 93 per cent patients, who had not shown recrudescence, the test remained positive up to Day 29. This may be explained by higher parasitaemia and hence a higher antigen load as well as a deranged macrophage-monocyte system, which is responsible for the clearance of protein antigens from circulation after an attack of severe malaria.[6] We, therefore, conclude that the ParaSight™-F test remains positive for too long in severe malaria and can be used in detecting recrudescence.

Department of Clinical Pharmacology and Medicine
Seth G.S. Medical College and K.E.M. Hospital
Parel, Mumbai–400 012, India.

N.J. GOGTAY
R.N. KOTWANI
D. RAJGOR
A. KANBUR
D.R. KARNAD
N.A. KSHIRSAGAR

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