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Short Note

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Malaria in Stone Quarry Area of Faridabad Complex (Haryana)

S.N. SHARMA*

An analysis of five-year (1987-91) epidemiological data showed that the stone quarries contributed, on an average, 38.7% of malaria cases to the total of Faridabad Town and 11.6% to that of Faridabad district. Various malariometric indices like Annual Parasite Incidence (API), Annual Blood Examination Rate (ABER), Slide Positivity Rate (SPR) and Slide falciparum Rate (SfR) were analysed to assess the endemicity of disease, and attempts were made to compare them with the malaria parameters of the town and then of the district. Migrant population working as labour in stone quarries resides in areas conducive to malaria and hence the need for intensive control measures.

Keywords: Malaria, *P. falciparum*, Stone quarry

INTRODUCTION

Faridabad district lies in the extreme southeast corner of Haryana state, the district coming into existence in 1979 after the split of Gurgaon district. The district comprises five community development blocks and has a population of 6,48,558. It has varied geophysical features with undulating land and alluvial plains. A number of small hill-ranges dot the south-western part of the district. Faridabad Complex constitutes the border areas with Delhi which comprises 75% hilly terrain where a number of stone quarries are operating. There is heavy water logging during rainy season because of the region's topography.

No agriculture is done in the hilly terrain of the border belt. The climate in Faridabad district is arid to semi-arid. The hottest months are May and June and the coldest

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are December and January. The rainy season starts in late July and continues till September. The average temperature ranges from 46°C in summer to 2°C in winter and the mean relative humidity is 75% in August-September and lowest (*c.* 32%) in June.

Very few studies have been carried out at district level to assess the epidemiological status of malaria. This study attempts to find the possible association of malaria with quarrying in Haryana-Delhi border belt.

MATERIALS AND METHODS

The Faridabad complex area has been divided into 39 sectors for epidemiological studies and for anti-larval operations under the Urban Malaria Scheme. Active surveillance was carried out fortnightly and the malaria-positive cases detected were given radical treatment. Malariometric surveys were carried out as per Black's manual[1]. The studies were conducted on fortnightly basis for five years (1987-91). The data were analysed to assess the contribution to the malaria cases by this area alone to the complex and the district. The incidence of malaria in quarry area was compared with that of Faridabad Town and Faridabad district as a whole to find if malaria was associated with quarrying activity so that appropriate disease control measures could be suggested. Data on migratory labourers were collected monthly from the records of contractors.

The quarry area includes the villages Barkhal, Ankhir, Anantpur, Ghedakhor and Surajkund Jhuggies where a large contingent of labour from other parts of the country migrates annually for building construction and stone-crushing work. According to records, about 25% of the population of this area migrates annually.

RESULTS AND DISCUSSION

The Urban Malaria Scheme (UMS) is under operation at Faridabad Complex and stone-quarry areas at the border of Delhi. But the rural areas of Faridabad district come under the Modified Plan of Operation under NMEP. Under UMS, all the water sources are regularly treated with larvicides such as MLO, Baytex, Temephos and Paris Green at weekly intervals. The survey showed that the main water sources were wells, tanks, drains, ponds, overhead tanks, pits, water reservoirs and canals. The main mosquito-breeding habitats were wells, tanks and seepage ponds in the stone-quarry areas, constituting 27.6% of the total breeding sources of Faridabad Complex.

A major portion of migrants belongs to Orissa, Bihar, Gujarat, Madhya Pradesh, Rajasthan and Uttar Pradesh while the rest belong to other nearby places. About 65% of migrants visit their native places once a year and after staying for 2 to 3 months, they carry back chronic malaria with them. Studies carried out in the sample pocket of Barkhal and Surajkund Jhuggis, where stone quarry work was in progress, showed that a high proportion of migrants had clinically diagnosed malaria and splenic enlargement. The data with regard to migrants are under analysis.

Malariometric indices, viz. API, ABER, SPR and SfR, for the years from 1987 to 1991 are given in Table 1. Comparative endemicity of malaria in Faridabad (1987-91) has been shown in Table 2.

During 1976, API of this district (a part of Gurgaon district at that time) was at peak, i.e. 57.1 per thousand population. After the successful implementation of MPO, API was brought down to 1.4 by 1987. This index showed a further decreasing trend till 1991 except in 1990 when it was 1.9. ABER has been considerably good. It remained at a maximum of 17.3 per cent in 1988 and further increased till 1990.

The Faridabad Complex also presented with a low malaria endemicity like the general trend in the district. ABER ranged between 11.1 and 14.1%. There was a declining

Table 1. Malaria incidence in District Faridabad, Faridabad Complex and Stone-quarry area (1987-91)

Year	BSE	Total malaria cases	*Pf* cases	ABER	API	SPR	SfR
		(A) Faridabad district (Population: 16.3 million)					
1987	221052	2220	95	15.8	1.4	0.9	0.1
1988	262951	2056	434	17.3	1.4	0.8	0.2
1989	247827	1805	236	16.0	1.7	0.7	0.1
1990	226873	3061	682	14.9	1.9	1.4	0.3
1991	189259	2055	261	10.7	1.3	1.2	0.1
		(B) Faridabad Complex (Population: 6.5 million)					
1987	76015	918	65	11.8	1.4	1.2	0.1
1988	86101	1083	309	14.1	1.7	4.2	1.2
1989	89665	587	106	13.8	0.7	0.7	0.1
1990	85020	390	106	13.1	0.5	0.5	0.1
1991	72397	228	27	11.1	0.3	0.3	0.3
		(C) Stone-quarry area (Population: 0.7 million)					
1987	9887	314	42	14.8	4.7	3.2	0.4
1988	11673	549	103	17.4	8.2	4.7	0.9
1989	8205	169	29	12.2	2.5	2.1	0.4
1990	6871	149	47	10.3	2.2	2.2	0.7
1991	4663	60	13	7.1	0.9	1.3	0.3

Table 2. Comparative endemicity of malaria in Faridabad (1987-91)*

Areas	Malaria cases	*Pf* cases
Faridabad District	11197	1708
Faridabad Town	3206	613
Quarry area	1241	234
Contribution of quarry area to the malaria incidence of		
(a) Town	38.7%	38.2%
(b) District	11.6%	13.7%

*Based on data in Table 1.

trend in malaria incidence from 1987 to 1991. However, in quarry areas malaria endemicity was relatively higher than in Faridabad Complex as well as in the district.

The malaria cases contributed by stone-quarry area were about 38.7% of the total incidence in Faridabad Complex and 11.6% of the total incidence in Faridabad district. The *Pf* incidence in stone-quarry areas constituted 38.2% of the total *Pf* cases in Faridabad Complex and 13.7% of the total *Pf* cases in Faridabad district over the five-year average (Table 2). The results point to the conclusion that foci of malaria parasite in stone-quarry areas are due to this parasitic load in the community.

In 1991, ABER and API in quarry areas decreased to 7.1 and 0.9% respectively, pointing to poor surveillance and in turn to under-reporting. According to a study conducted in Delhi by Sethi *et al*[2]., 91 out of 701 (12.8%) immigrants had fever clinically diagnosed as malaria at the time of survey while among the native population 45 out of 646 (7%) had such a history. The difference was statistically significant. It is evident that the conditions like low temperature, high humidity, presence of vector, pathogen (human reservoir) and susceptible population around the settlements in the stone quarries become congenial for malaria transmission in post-monsoon months. During rains, innumerable rocky pits dug up as a consequence of quarrying are filled with water, resulting in mosquito breeding and transmission of infection.

Sharma *et al.*[3] suggested that since the settlements of migratory people were found in the vicinity of well-planned authorised colonies all over Delhi, these hutment dwellers not only suffered but also constituted an important source of infection to others.

We infer that stone-quarry areas are more malaria-prone than the Faridabad Town or the remaining areas of the district. It is likely that quarrying has brought about certain ecological and demographic changes in the area which might have supported high malaria transmission or importation of cases through labour migration. Delhi has a short prevalence of chronic malaria in the migratory population[4]. Kondrashin[5] has also reviewed the role of migration in India and its effect on the epidemiology of malaria.

Thus, top priority to control malaria in this area is needed. Emphasis should be on action for screening the migratory population (early diagnosis and treatment). Radical treatment of all malaria cases may also lead to considerable reduction of parasite reservoir.

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Prevalence and Seasonal Distribution of Anopheline Fauna in District Bikaner (Rajasthan)

S.K. BANSAL* and KARAM V. SINGH*

A survey was carried out during 1989-91 to study the prevalence and distribution of anopheline mosquitoes in 12 villages located in all the 4 tehsils of Bikaner district. Six species, viz. *Anopheles subpictus* (34.7%), *An. stephensi* (33.3%), *An. culicifacies* (18.0%), *An. annularis* (12.1%), *An. pulcherrimus* (1.1%) and *An. barbirostris* (0.8%), were collected. *An. stephensi* was present throughout the year and the other species were present during the monsoon and post-monsoon periods. During the peak winter period (Dec-Jan) only *An. stephensi* was present and in low density. *An. culicifacies* made its appearance only during the spring season and continued up to the middle of November. *An. subpictus*, *An. pulcherrimus*, *An. barbirostris* and *An. annularis* were found only during the monsoon and post-monsoon periods. *An. subpictus* was the most abundant species during the monsoon, and so was *An. stephensi* during the spring season in indoor habitats.

Keywords: Anopheline fauna, *An. subpictus*, *An. stephensi*

INTRODUCTION

The resurgence of malaria in recent years has evoked considerable interest in the ecology of malaria vectors in India. Mapping of the distribution and relative abundance of vectors in the resurgence era still remains a primary consideration since man-made changes in the ecosystem have altered the species balances. These changes may have brought about an ecological succession of biological species which may have direct bearing on disease transmission. The construction of Indira Gandhi canal in

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north-western parts of Rajasthan may be one of the important factors for the change in the vector population of the area, especially of the Japanese encephalitis (JE) vectors[1,2].

Bikaner district, a part of north-western Rajasthan, is situated in a typical Thar desert and is characterized by sandy dunes, scanty vegetation and extreme conditions of temperature. The district's geographical and environmental conditions may have an impact on the prevalence and seasonal distribution of anopheline mosquitoes. Although several anopheline surveys have been undertaken in different parts of the country[3-5], yet a detailed survey has not been so far made in this part of the Thar desert. Therefore, we carried out an anopheline survey in 12 villages of District Bikaner during 1989-91 to determine the prevalence, distribution and species composition during different seasons.

MATERIALS AND METHODS

District Bikaner comprises 4 tehsils, viz. Bikaner, Loonkaransar, Nokha and Kolayat. Twelve villages (3 from each tehsil), viz. Dholera, Kalasar, Katriyasar (Bikaner), Beethnok, Kodamdesar, Inda Ka Bala (Kolayat), Kaloo, Loonkaransar, Manera (Loonkaransar), and Desalsar, Maiyasar, Gajsukhdesar (Nokha), were selected for the survey.

Mosquitoes were collected from each village during both dusk and dawn hours from four habitats, viz. indoors (human dwellings and cattlesheds), outdoors (cracks and crevices of pond surroundings near the villages), inside walls of both open and wood-covered wells and clay pitchers (inner walls) kept inside/outside the houses. Mosquitoes were collected with the help of a suction tube as prescribed by the WHO and kept in Barraud cages and test tubes, place- and habitat-wise. Water samples from ponds, wells, pitchers and cemented tanks were taken to observe different stages of mosquito immatures. Adults were reared and identified in the laboratory. Relative humidity and temperature were recorded for each habitat during both dusk and dawn hours.

Specimens were transported to the laboratory and identified with the help of the keys of Christophers[6] and Wattal and Kalra[7]. The density (per man hour, MHD) of each species was calculated according to time of collection and habitats.

RESULTS AND DISCUSSION

A total of 1559 anopheline mosquitoes, representing 6 species, were collected (Table 1). *An. subpictus* was the predominant species (34.7%), followed by *An. stephensi* (33.3%), *An. culicifacies* (18.0%), *An. annularis* (12.1%), *An. pulcherrimus* (1.1%) and *An. barbirostris* (0.8%). Of these, *An. culicifacies* and *An. stephensi* have already been incriminated as primary vectors of malaria in rural and urban areas respectively in different parts of the country, while *An. annularis* is a vector of local importance. *An. subpictus* has recently

Table 1. Per cent distribution and relative abundance of anopheline mosquitoes during different seasons in district Bikaner

Mosquito species	No. of mosquitoes collected	Per cent relative abundance	Per cent distribution of anophelines			
			Monsoon and post-monsoon (Jul-Oct)	Winter (Nov-Feb)	Spring (Mar-Apr)	Summer (May-Jun)
An. stephensi	519	33.3	37.4 (194)	2.6 (13)	41.6 (216)	18.4 (96)
An. subpictus	541	34.7	84.5 (457)	15.5 (84)	0.0	0.0
An. culicifacies	281	18.0	66.1 (186)	3.8 (11)	9.5 (27)	20.6 (57)
An. annularis	189	12.1	100.0 (189)	0.0	0.0	0.0
An. pulcherrimus	17	1.1	100.0 (17)	0.0	0.0	0.0
An. barbirostris	12	0.8	100.0 (12)	0.0	0.0	0.0

Figures in parentheses are the number of mosquitoes collected during a particular season of the species.

been incriminated as a suspected vector of malaria in coastal villages of south India[8] and Bastar district of Madhya Pradesh[9]. In our studies this was the most prevalent species found during monsoon and post-monsoon season (up to November) with a peak density of 36 MHD in indoor habitats during September (Fig. 1). However, it was completely absent from December to June, showing that it is only a monsoon-breeding species in this region. *An. annularis, An. pulcherrimus* and *An. barbirostris* were recorded only during this season. The last two anophelines were collected only from cracks and crevices of pond surroundings in very low density, thus showing their exophilic nature.

An. stephensi and *An. culicifacies* are the only two species which were observed during all the four seasons. Two population peaks (Fig. 1) were observed in *An. stephensi* during the year, the first during April and the second during September. The presence of *An. stephensi* throughout the year suggests how ecologically this species has acclimatized itself even to the desert conditions. Similarly, *An. culicifacies*

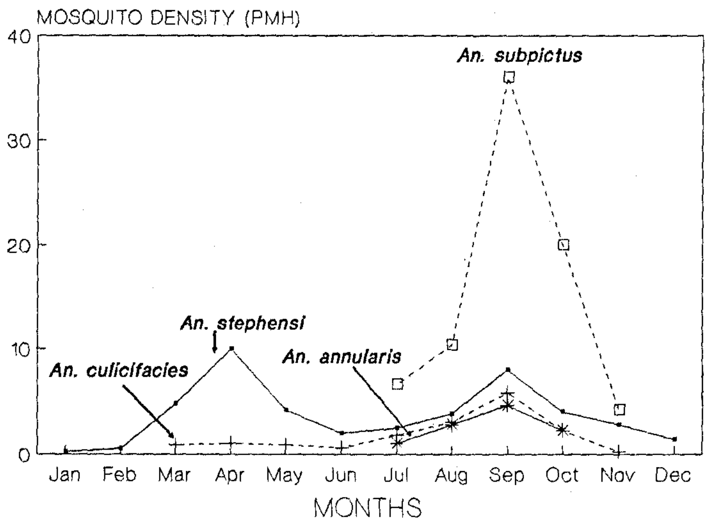

Fig. 1: Density of indoor resting anophelines during dawn hours

Table 2. Species-wise density of different anophelines during dusk and dawn collections in four habitats of district Bikaner

Season	Mosquito species	Dawn				Dusk			
		Pond*	Well*	Pitchers	Indoor*	Pond*	Well*	Pitchers	Indoor*
Monsoon and Post-monsoon	*An. stephensi*	1.7	1.2	1.5	4.8	1.1	1.5	1.3	4.0
	An. subpictus	2.7	2.5	1.1	18.1	1.7	1.3	1.4	11.5
	An. culicifacies	2.1	1.7	1.2	3.2	1.7	1.5	1.0	2.5
	An. annularis	1.8	4.0	0.5	2.7	1.1	0.9	1.3	4.3
	An. pulcherrimus	1.0	-	-	-	0.5	-	-	-
	An. barbirostris	0.6	-	-	-	0.5	-	-	-
Winter	*An. subpictus*	-	-	-	4.2	-	-	-	3.2
	An. stephensi	-	-	-	1.2	-	-	-	-
	An. culicifacies	-	-	-	0.4	-	-	-	0.3
Spring	*An. stephensi*	1.3	1.5	-	7.4	1.5	1.6	2.0	4.3
	An. culicifacies	-	-	1.0	0.9	0.7	-	-	-
Summer	*An. stephensi*	2.0	-	-	3.1	-	-	1.5	1.8
	An. culicifacies	-	1.3	2.0	0.8	-	1.5	-	1.0

All values are expressed as numbers of mosquitoes collected per man hour density (MHD) and are the averages of 6-12 observations; *Pond — cracks and crevices, Well — side walls, Indoor — Cattleshed and human dwelling.

was present during spring and summer in wells and pitchers with a population peak in September during monsoon season. Probably these two species might be responsible for the endemicity of malaria in this region. Verma *et al.*[10] also studied different mosquito vector species in Jaipur, Jodhpur and Bikaner districts, and Tyagi and Verma[11] in Ganganagar district of Rajasthan and observed that *An. stephensi*, *An. culicifacies* and probably *An. subpictus* are the main anopheline species responsible for malaria transmission in this region. Tyagi and Verma[11] incriminated *An. culicifacies* as the vector of malaria by showing the presence of oocyst stage of malarial parasite in the gut.

Generally, maximum density of all anophelines except *An. pulcherrimus* and *An. barbirostris* was observed in indoor habitats during both dusk and dawn hours (Table 2). However, the density during dusk hours is relatively less, owing probably to their various activities like swarming and feeding. High densities in indoor habitats, particularly of malaria vectors, show their endophilic nature; however, *An. pulcherrimus* and *An. barbirostris* were observed only from pond surroundings, showing their exophilic nature. Pond surroundings are the only ecological niche where all the six anopheline species were present. RH was more during dawn hours than during dusk hours (Fig. 2a and b). Maximum RH was observed in September (74%) and minimum during June (37%). In contrast to RH, temperature recorded was higher during dusk hours than during dawn hours with a maximum in June (41°C) and minimum in January (12°C). These variations of temperature and RH in a particular habitat and season have a great impact on the prevalence and seasonal distribution of different species. The findings of the study would be of considerable use

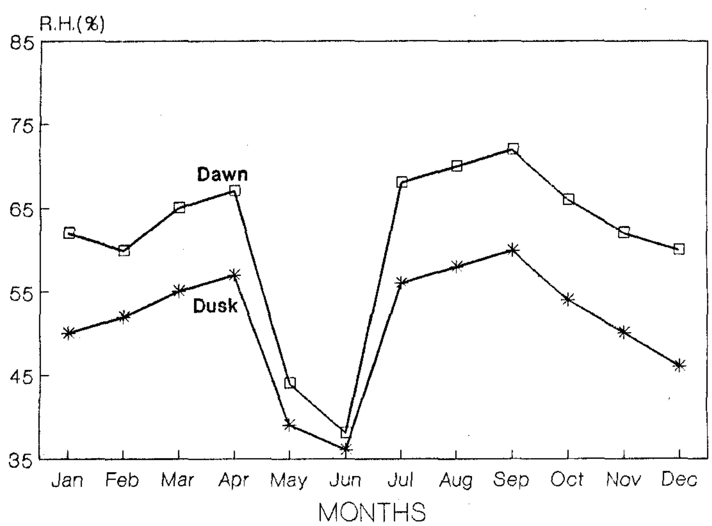

Fig. 2a: **Monthly and diurnal fluctuations in RH in indoor habitats**

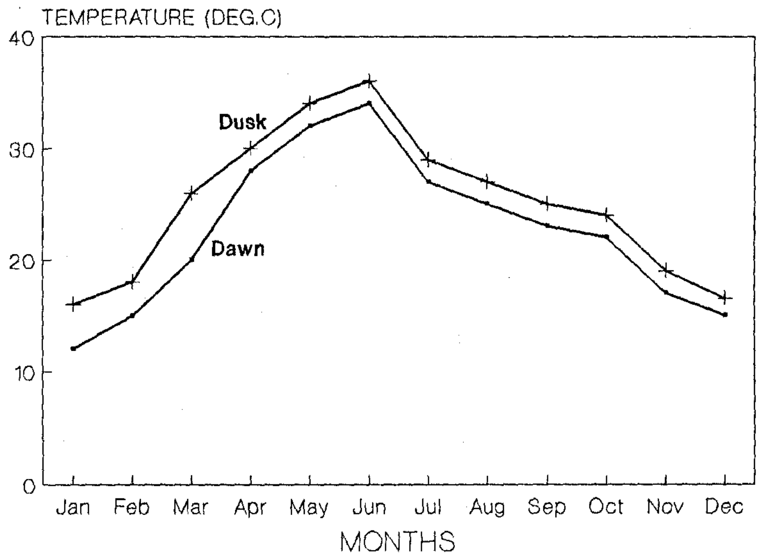

Fig. 2b: Monthly and diurnal fluctuations in temperature in indoor habitats

in formulating control strategies through malariogenic stratification methodologies.

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Oxidative Stress and Antioxidant Defence Mechanism in *Plasmodium vivax* Malaria before and after Chloroquine Treatment

KUMUD SARIN*, AJAY KUMAR+, ANIL PRAKASH++ and ARUN SHARMA++

The protection of *Plasmodium vivax*-parasitized red blood cells (PRBCs) against activated forms of oxygen was examined in relation to the non-parasitized and chloroquine-treated red blood cells. Increased parasitaemia was found to be accompanied with a decrease in the activities of enzymes of the glutathione system, namely glutathione peroxidase (GPx), glutathione reductase (GRx) and glutathione S-transferase (GTr) in the red blood cells (RBC) lysates. In contrast, however, the total amount of reduced glutathione (GSH) and the content of water-soluble antioxidant vitamin C was increased 2-3 fold over those of control RBCs. Chloroquine-treated red cells contained enzyme activities and antioxidant contents (GSH, vitamin C) comparable to those of control and non-parasitized red cells. Our results therefore indicate the oxidative stress experienced by RBCs during *P. vivax* infection and that this infection is accompanied with changes in the antioxidant defence system of the host, which are restored to near normal levels after treatment with chloroquine.

Keywords: Chloroquine, Glutathione, Malaria, *Plasmodium vivax*

INTRODUCTION

Vivax malaria is characterized by a prolonged course, intermittent fever, anaemia, splenomegaly and a tendency towards a relapsing course with repeated paroxysms, which may last several months or years[1]. During the respiratory burst, phagocytic cells such as polymorphonuclear leucocytes, monocytes and macrophages release superoxide ions

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(O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-)[2]. These oxygen-derived free radicals and also the tumour necrosis factor are shown to kill the human malarial parasite *P. falciparum in vitro*[3]. The protection of erythrocytic damage from these oxidants is brought about by the oxidant/ antioxidant defence system, viz. the glutathione system. It is thus apparent that the inefficiency of the glutathione defence system may make erythrocytes more vulnerable to oxidant damage and inhibit parasite growth. Most malarial research has been aimed at obtaining information about RBC susceptibility to oxidative stress during malarial infection and has mostly concentrated on oxidation/reduction status in PRBCs[4] but the effect of malarial infection on the whole cascade of antioxidant defence mechanism is yet to be explored.

To understand the role of glutathione and its related enzyme system in *P. vivax* infections, we have for the first time compared the activities of glutathione cascade enzymes viz. GR, GPx and GTr, in parasitized and nonparasitized human erythrocytes. We have also compared the contents of antioxidant molecules, GSH and vitamin C, to postulate a role for these molecules in *P. vivax* infection. The study has been conducted simultaneously in the erythrocytes of control patients and in the chloroquine-treated patients after 5 days. Our results are consistent with those of the exposure of nonparasitized RBC to oxidative stress and show that with increased parasitic load, complex changes occur in the antioxidant defence mechanism of the PRBCs and following treatment the defence system enzymes are restored to the levels of control and nonparasitized RBCs.

MATERIALS AND METHODS

The study was taken up in the Shankargarh district of Allahabad (U.P.) having a large number of ponds and quarries with a high intenstiy of *P. vivax* transmission. Blood samples were obtained from the patients who reported to the Malaria Clinic at the Malaria Research Centre. They were screened microscopically for the presence of malarial parasite. Age- and sex-matched control samples were obtained from the same area. Blood from *P. vivax*-infected patients of either sex was collected in heparinized tubes when the parasitaemia was 2-3% and the parasites were multinucleated schizonts before and after the treatment with chloroquine. The treatment consisted of an oral dose of 600 mg of chloroquine on the first day. Blood was centrifuged at 800 x g for 10 min. Plasma and buffy coat were removed and parasitized red cells were separated by percoll gradient[5]. Parasitized red cells were washed thrice with normal saline and lysed with 5 mM phosphate buffer pH 8.0. The lysed suspension was washed and centrifuged at 7000 x g for 30 min. The uppermost layer or haemolysate was separated for enzyme activities and content of antioxidant molecules. Erythrocytes from normal patients, also processed like the parasitized erythrocytes, served as controls.

Assay of glutathione and glutathione metabolizing enzymes

Haemoglobin in lysates was estimated by cyanomethaemoglobin method[6] to determine the progress of anaemia. Erythrocyte-reduced glutathione (GSH) was estimated according to the method described by Beutler *et al.*[7], and expressed as µM/ml of RBC. Vitamin C was estimated according to the method of Zannoni *et al.*[8] Glutathione reductase (EC 1.6.4.2)[9], glutathione peroxidase (EC 1.11.1.9)[10] and glutathione S-transferase (EC 2.5.1.18)[11] were estimated spectrophotometrically. Protein was estimated according to the method of Lowry *et al.*[12], using the bovine serum albumin as standard. Statistical significance was evaluated by student *t*-test between healthy controls and malarial infected red cells.

RESULTS

The aim of the study was to assess the susceptibility of *P. vivax*-infected PRBCs to oxidative stress in relation to intraery-throcytic growth and development of the parasite. The parasitized as well as non-parasitized RBCs were separated by percoll density gradient centrifugation. The parasitized RBCs were at the trophozoites and schizont stages. Leucocytes were never found in parasitized RBCs and were always maintained at less than 0.5% contamination.

To test whether the increased parasite load influenced the antioxidant defence mechanisms of the host RBCs, we examined the activities of enzymes involved in the reactive oxygen species (ROS) degradation and also estimated antioxidants, namely reduced glutathione and vitamin C (Table 1). We observed that infected cells had 2-3 times higher amounts of antioxidants than the control and uninfected RBCs. However, chloroquine-treated cells contained comparable amounts of glutathione and vitamin C as those in the controls.

In contrast, however, the activities of all the three enzymes, namely glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione S-transferase (GTr), decreased progressively in the infected red cells. The activities of these enzymes were slightly increased in the uninfected cells as compared to those of the control (Table 2). When the cells of chloroquine-treated patients were compared with the control cells

Table 1. Concentration of glutathione and vitamin C in uninfected, *P. vivax*-infected and chloroquine-treated human red cell lysates

Sl.No.	Group	n	GSH, µg/ml RBC	Ascorbic acid, µg/mg protein
1.	Control	25	192.52±86.34	0.49±0.15
2.	Uninfected cells	25	191.68±62.10	0.59±0.21
3.	Infected cells	26	462.90±129.40*	1.21±0.42*
4.	Chloroquine-treated cells (600 mg)	12	205.41±52.26	0.53±0.16

*$p < 0.05$; n — Number of cases.

Table 2. Activities of GSH related enzymes in uninfected, *P. vivax*-infected and chloroquine-treated human red cell lysates

Sl. No.	Group	n	Glutathione reductase		AC	GPx	GST
			with FAD	w/o FAD			
1.	Control	25	11.35±4.09	8.23±2.60	1.5±0.5	44.19±10.42	13.11±4.16
2.	Uninfected cells	25	13.02±3.07	8.08±3.58	1.8±0.6	47.30±11.4	15.56±5.51
3.	Infected cells	26	6.42±2.32*	4.43±1.84	1.6±0.5*	33.25±11.52	5.43±1.76
4.	Chloroquine-treated cells (600 mg)	12	11.79±3.13	8.60±2.42	1.5±0.5	47.81±9.00	15.35±4.67

*$p < 0.05$; n — Number of cases.

the activities of all these enzymes were comparable to those of the control and uninfected cells. The mechanism by which this increase in the enzyme activities is affected by treatment of chloroquine is still not clear.

DISCUSSION

Evidences that uncompensated oxidative stress is exerted on RBCs and ROS are believed to cause intraerythrocytic death of the malarial parasite[13]. These ROS are also thought to inhibit parasite growth not directly but indirectly in host RBCs in which the protective mechanisms against ROS is insufficient. It has been postulated that oxidative stress on PRBCs is because of the methaemoglobin and sulfhaemoglobin accumulation and also probably because of H_2O_2 production by the parasite[13]. A number of antimalarials, namely primaquine and qinghaosu, are thought to exert their action at least in part via the redox reaction pathways.

The steady decrease in the activities of the enzymes in the PRBCs as the parasite load on RBCs is increased shows that the enzymes may be of erythrocytic origin. However, despite the decrease in the activities of glutathione reductase, the glutathione content increased in infected cells as compared to that of the control cells. Therefore, we believe that a significant portion of the glutathione is associated with the parasite and the enzymes are of host cell origin. In conjunction with the decrease in the activity of glutathione reductase, the increase in the glutathione synthetic enzymes, namely glutamate dehydrogenase and r-glutamyl cysteine synthetase, might also be responsbile for the increase in the contents of GSH. A number of drugs that inhibit the activity of GSSG peroxidase and GSH reductase have been shown by a number of workers to be effective antimalarials[14-16].

Haemoglobin present in RBCs is protected against oxidation by H_2O_2 as long as GSH serves as an electron donor for GSH peroxidase. When GSH is oxidized faster than it can be regenerated, catalase and haemoglobin compete with each other for the excess of H_2O_2, leading to the formation of methaemoglobin. Our results thus imply that cystolic compartment of PRBCs is not under oxidative stress under experimental conditions. The observation was also made that the contents of vitamin C, the important water-soluble antioxidant, increased 3-4 fold above the normal levels in the parasitized RBCs (Table 1) irrespective of the degree of parasitaemia (data not shown). However, the levels of vitamin C were comparable to those of the controls following chloroquine treatment. It has been shown that vitamin C is transported into erythrocytes in the oxidized form followed by reduction to ascorbate in a GSH-dependent reaction[17,18].

We therefore conclude that the host RBC, particularly the host plasma membrane, is reasonably well protected against oxidative stress. The increase in vitamin C in parasitized RBCs from *vivax* malaria patients might be a similar response and is consistent with earlier suggestions that host tissues may come under free radical-induced oxidative attack as the disease progresses. These observations show that exposure to free radicals-induced oxidative damage increases vitamin C and GSH in plasma and RBCs. The redox status of vitamin C seems to be intimately linked to the availability of GSH in these circumstances[18]. This is strengthened by earlier observations of Clark *et al*[19].

The erythrocyte content of ascorbate has also been found to be about six times higher than normal at late stages of *P. vinckei* infection[18]. The source of this did not seem to be *de novo* synthesis in the liver. We, however, could not rule out the possibility of synthesis by the parasite itself. However, parasitized RBCs had gained the ability to take up reduced ascorbic acid more rapidly than control RBCs.

The utilization of exogenous NADPH for reduction of parasite GSSG to GSH via GSH reductase indicates a metabolic dependence of the parasite upon the host cells and also the acceleration of pentose phosphate pathway in infected cells. Our results also suggest a mechanism whereby G-6-PD deficiency may be protective against malaria infection. The utilization of NADPH by both the host erythrocytes and the malarial parasite would overwhelm the limited ability of G-6-PD-deficient red cells to regenerate NADPH[20]. A few-fold increase in erythrocyte NADPH + NADH/NADP + NAD ratio has been reported in *P. berghei* infection[21]. The resultant decrease in cellular GSH may predispose the infected erythrocytes to premature, oxidant-induced destruction. Accumulation of GSSG on the other hand is also inhibitory to the parasite protein synthesis.

Thus, our observations of decrease in GSH and ascorbic acid and increase in GSH metabolizing enzymes following chloroquine treatment might be a consequence of increased utilization of host cell NADPH by infected red cells leading to accumulation of GSSG, which inhibits parasite protein synthesis. The reliance of malarial parasite on host cell NADPH and GSH redox system enzymes may therefore represent a novel site of action of antimalarial drugs and may provide a new insight into the mechanism of action of antimalarial drugs which are believed to act at least in part via reactive oxygen species production.

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Anopheline Fauna and Malaria Incidence in Changlang District (Arunachal Pradesh)

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Data on malaria incidence and prevalence as well as on abundance of anopheline species collected in Jairampur, Arunachal Pradesh, show that *P. falciparum* contributed 27.59% of the total positive cases, most of these being recorded during July to September. Of the twelve anopheline species recorded, *Anopheles maculatus* was the most predominant comprising 54.32% of the total collection. Other common species were *An. peditaeniatus, An. philippinensis* and *An. kochi* in order of decreasing proportion. *An. dirus*, the known vector, was collected in the whole-night human bait catches (indoor) but in low numbers. *An. philippinensis* is implicated as another potential vector, and changes in anopheline/ vector fauna are attributed to ecological succession.

Keywords: Anopheline fauna, Malaria incidence, Vector bionomics

INTRODUCTION

Arunachal Pradesh, formerly North East Frontier Agency (NEFA), had been under DDT spray operations carried out by NMEP/ NMCP since 1953. However, some areas are still known to be endemic, particularly for drug-resistant *P. falciparum*[1]. There have been sporadic reports on malaria incidence and short-term entomological surveys of various parts of the state have been carried out[2-5]. Our study was undertaken in some areas under Nampong and Monmao circles of Changlang district of Arunachal Pradesh with the objective to collect data on malaria incidence, anopheline fauna, relative abundance of various species and their feeding behaviour. Data included in this paper are for the study period from November 1989 to September 1990.

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Table 1. Malaria incidence in villages of Nampong and Manmao circles of Changlang district, Arunachal Pradesh

Month/ Year	ACD			PCD			Total			Indices			
	BSC/E	+ve	*Pf*	BSC/E	+ve	*Pf*	BSC/E	+ve	*Pf*	SPR	SfR	*Pf*%	BER
Nov 89	35	6	1	0	0	0	35	6	1	17.14	2.86	16.67	1.2
Dec 89	25	5	1	0	0	0	23	5	1	21.74	4.35	20.00	0.8
Jan 90	30	4	0	0	0	0	30	4	0	13.33	0.00	0.00	1.0
Feb 90	20	3	0	45	9	1	65	12	1	18.46	1.54	8.33	2.2
Mar 90	50	1	0	84	7	0	134	8	0	5.97	0.00	0.00	4.5
Apr 90	43	5	0	91	7	0	134	12	0	8.96	0.00	0.00	4.5
May 90	93	2	1	143	7	2	236	9	3	3.81	1.27	33.33	7.9
Jun 90	133	4	2	193	23	1	326	27	3	8.28	0.92	11.11	10.9
Jul 90	177	3	1	259	18	10	436	21	11	4.82	2.52	52.38	14.6
Aug 90	147	5	2	240	11	5	387	16	7	4.13	1.81	43.75	12.9
Sep 90	104	3	1	118	22	12	222	25	13	11.26	5.86	52.00	7.4
Total	855	41	9	1173	104	31	2028	145	40	7.15	1.97	27.59	67.6

Population = 3000.

Table 2. Anopheline fauna and per cent composition of species in Changlang district, Arunachal Pradesh (February to September 1990)

Sl.No.	Species	No. collected	Percentage
1.	*Anopheles (Cellia) annularis*	12	0.77
2.	*An. (Anopheles) barbirostris*	23	1.48
3.	*An. (Cellia) dirus*	24	1.55
4.	*An. (Anopheles) gigas*	10	0.64
5.	*An. (Cellia) jamesii*	1	0.06
6.	*An. (Cellia) jeyporiensis*	1	0.06
7.	*An. (Cellia) kochi*	129	8.31
8.	*An. (Cellia) maculatus*	843	54.32
9.	*An. (Anopheles) peditaeniatus*	266	17.14
10.	*An. (Cellia) philippinensis*	183	11.79
11.	*An. (Cellia) tessellatus*	6	0.39
12.	*An. (Cellia) vagus*	54	3.48
	Total	1552	100.00

MATERIALS AND METHODS

District Changlang lies in the southern part of Arunachal Pradesh, sharing an international border with Myanmar to the east. This is a foothill area with large forestations traversed by rivers, rivulets and streams. The villages are scattered, thinly populated and difficult to approach and become unreachable by road during floods. The inhabitants are largely tribal aborigines with some migratory populations of Napalese origin. The climate is hot and humid for most part of the year with heavy rainfall from May to September.

Active surveillance was undertaken at weekly intervals in order to obtain data on malaria incidence in 29 villages under Nampong and Monmao circles. The total population of these villages was about 3000. A malaria clinic was established in Jairampur dispensary serving as passive case detection agency. Mosquito collections from the fixed catching stations were made fortnightly. With the help of a suction tube, mosquitoes were collected between 1800 and 2000 hrs from cattlesheds and human dwellings. CDC miniature light traps were also operated between 1800 and 0500 hrs by placing the traps in mixed dwellings (tribals stay in 'chang ghar' or 'moshang', made of bamboos without mud plastering under which they prefer to keep cattle, poultry etc.; mixed dwelling habit is very common among tribals). Morning collections were also made in human dwellings. In addition, whole-night collections were made using human bait indoors and outdoors. Host blood meal analysis was done for some mosquito species by counter-current immunoelectrophoresis at the Malaria Research Centre, Delhi. Mosquitoes were identified using the keys of Wattal and Kalra[6] and of Chirstophers[7].

RESULTS

Malaria incidence

Data for five years (1985-89) from the Directorate of National Malaria Eradication Programme showed that villages under Jairampur health sub-centre were endemic for malaria with *P. falciparum* constituting more than half of the positive cases except in 1986 when it was about 36%. Slide positivity rate (SPR) ranged from 6.56% in 1986 to 19.02% in 1988. The annual parasite incidence (API), calculated per thousand population, varied from 14.84 to 50.10. Malaria-positive cases occurred throughout the study period but %*Pf* was highest during July to September and for the remaining period, *P. vivax* was the predominant species (Table 1). For the study period as a whole (11 months), SPR, SfR and %*Pf* were 7.15, 1.97 and 27.59 respectively.

Mosquito fauna and relative abundance

Twelve anopheline species were recorded from all sources, of which *An. maculatus* (54.32%) was the most predominant followed by *An. peditaeniatus* (17.14%), *An. philippinensis* (11.79%) and *An. kochi* (8.13%) (Table 2). *An. vagus, An. dirus, An. barbirostris, An. annularis, An. gigas, An. tessellatus, An. jeyporiensis* and *An. jamesii* were also recorded but in low numbers. Most of these species were captured

Table 3. Man hour density of anopheline species collected by suction tube from cattlesheds and human dwellings in evening hours, Changlang district, Arunachal Pradesh (February to September 1990)

Species	Feb		Mar		Apr		May		Jun		Jul		Aug		Sep	
	No.	MHD	No.	MHD	No.	MHD	No.	MHD	No.	MHD	No.	MHD	No.	MHD	No.	MHD
An. annularis	0	0.00	2	0.04	0	0.00	0	0.00	0	0.00	0	0.00	9	0.64	0	0.00
An. barbirostris	0	0.00	0	0.00	0	0.00	0	0.00	1	0.02	1	0.02	1	0.07	1	0.10
An. dirus	0	0.00	1	0.02	0	0.00	0	0.00	1	0.02	0	0.00	1	0.07	0	0.00
An. gigas	1	0.01	1	0.02	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
An. kochi	2	0.02	10	0.27	18	0.60	12	0.44	0	0.00	6	0.17	25	1.78	18	1.80
An. maculatus	23	0.27	134	2.97	280	10.37	155	5.74	15	0.40	47	1.34	21	1.50	8	0.80
An. peditaeniatus	10	0.11	15	0.33	27	1.00	21	0.77	16	0.43	6	0.17	18	1.28	8	0.80
An. philippinensis	0	0.00	1	0.02	0	0.00	0	0.00	0	0.00	1	0.02	15	1.07	10	1.00
An. vagus	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	51	3.64	51	5.10
Total	36	0.41	164	3.67	325	11.97	188	6.95	33	0.87	61	1.72	141	10.05	96	9.60

MHD Man-hour density.

from cattlesheds by suction tube during evening collections and from whole-night collections in mixed dwellings by using CDC traps. *An. jeyporiensis, An. jamesii* and *An. tessellatus* were recorded only in CDC trap collections, though in very few numbers. Collection of mosquitoes in the morning hours was not satisfactory. The tribals burn *chullahs* in the very early morning for cooking and this makes the whole house very smoky, and as a result no mosquitoes are found in the house during morning hours.

In hand-catch collections made during evening hours from cattlesheds and human dwellings, *An. maculatus* was found throughout the study period but more abundantly during March to May (Table 3). *An. kochi* and *An. peditaeniatus* were also collected throughout the study period but in fewer number. *An. philippinensis* and *An. vagus* were more prevalent in the beginning of August. *An. gigas* was recorded only in February and March (winter months), and *An. barbirostris* from June to August though in fewer number. *An. annularis* was recorded only in March and August. *An. dirus* was recorded rarely.

In CDC trap collections, *An. maculatus, An. peditaeniatus,* and *An. philippinensis* were captured in good numbers from mixed dwellings while all other species were recorded in fewer number, barring *An. annularis* which

Table 4. Records of CDC light trap collections in Changlang district, Arunachal Pradesh (February to September 1990)

Month	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Total	Percentage
No. of traps	10	10	9	7	9	5	9	3	62	of total
Species	No.	No.	No.	No.	No.	No.	No.	No.	No.	collection
An. barbirostris	1	1	1	1	0	14	1	0	19	0.49
An. dirus	0	0	0	2	4	1	0	0	7	0.18
An. gigas	7	1	0	0	0	0	0	0	8	0.20
An. jamesii	0	0	0	0	0	1	0	0	1	0.02
An. jeyporiensis	0	0	0	0	0	1	0	0	1	0.02
An. kochi	8	9	1	0	1	21	15	1	56	1.44
An. maculatus	5	9	71	10	0	6	48	7	156	4.01
An. peditaeniatus	9	25	10	64	1	18	17	3	147	3.78
An. philippinensis	0	2	0	0	6	7	43	65	123	3.16
An. tessellatus	0	0	0	0	0	3	3	0	6	0.15
An. vagus	1	0	0	0	0	0	1	1	3	0.08
Culicines	478	769	58	689	176	612	475	102	3359	86.44
Total	509	816	141	766	188	684	603	179	3886	100.00

Table 5. Biting densities of anopheline mosquitoes recorded in Changlang district, Arunachal Pradesh (June to September 1990)

Species	Total No. of man baits	No. of biting per man per night	No. collected during the period (hrs)												Total	% of total collect-ions
			1700-1800	1800-1900	1900-2000	2000-2100	2100-2200	2200-2300	2300-2400	0000-0100	0100-0200	0200-0300	0300-0400	0400-0500		
An. dirus	11 (I)*	1.09	-	-	1	-	-	-	3	3	3	1	1	-	12	4.41
	9 (O)+	0.22	-	-	1	-	-	-	-	-	1	-	-	-	2	0.74
An. philippinensis	11 (I)	1.45	-	-	1	-	-	7	4	3	-	-	1	-	16	5.88
	9 (O)	3.00	-	-	-	1	6	4	6	-	4	4	2	-	27	9.92
An. maculatus	11 (I)	0.55	-	-	-	-	1	2	2	1	-	-	-	-	6	2.21
	9 (O)	0.67	-	-	2	2	1	1	-	-	-	-	-	-	6	2.21
An. annularis	11 (I)	0.09	-	-	1	-	-	-	-	-	-	-	-	-	1	0.37
	9 (O)	0.00	-	-	-	-	-	-	-	-	-	-	-	-	0	0.00
An. peditaeniatus	11 (I)	0.18	-	-	-	-	-	1	-	-	1	-	-	-	2	0.74
	9 (O)	0.44	-	-	-	-	1	-	1	-	1	1	-	-	4	1.47
An. barbirostris	11 (I)	0.00	-	-	-	-	-	-	-	-	-	-	-	-	0	0.00
	9 (O)	0.11	-	-	-	-	-	-	-	-	-	1	-	-	1	0.37
Culicines	11 (I)	11.64	-	16	6	13	12	16	13	13	20	7	12	-	128	47.05
	9 (O)	7.44	-	16	-	3	14	11	5	4	5	6	3	-	67	24.63
Total		26.88	-	32	12	19	35	42	34	24	35	20	19	-	272	100.00

*Based on 7 man-nights (indoor); +Based on 6 man-nights (outdoor).

was not entrapped (Table 4). A total of 3886 mosquitoes were collected in 62 night traps during the 8-month study period. Out of these, 527 were anophelines constituting 13.6% of total collections. On an average, 62.6 mosquitoes were collected per trap of which 8.5 were anophelines. Culicines constituted 60-94% of all collections except those in April, in which *An. maculatus* was the predominant species.

Feeding behaviour

Of the twelve anopheline species recorded, 6 species, viz. *An. dirus, An. philippinensis, An. maculatus, An. annularis, An. peditaeniatus* and *An. barbirsotris,* were collected over human bait in whole-night catches (Table 5). *An. dirus* fed mostly indoors from 2300 to 0400 hrs with a man-biting rate (MBR) of as high as 1.09 and the species was rarely recorded outdoors. *An. philippinensis* fed more frequently outdoors than indoors from 2100 to 0400 hrs. There was no variation in the feeding behaviour of *An. maculatus* whether indoors or outdoors from 1900 hrs to midnight. *An. annularis, An. peditaeniatus* and *An. barbirostris* were recorded in low numbers. Of the total mosquito species captured over human bait, culicines were most abundant and fed throughout the night. Of the potential vectors of malaria in north-eastern region of India, *An. philippinensis* was collected mostly by CDC traps and in whole-night collections on human bait. To determine the host feeding preference, a few blood meal samples of *An. philippinensis* collected by CDC traps in mixed dwellings were subjected to host blood meal analysis and were found to be overwhelmingly anthropophilic. Out of the 41 blood meals examined, 40 (97%) were human-positive.

DISCUSSION

Changlang (formerly under Tirap district) is known to be endemic particularly for chloroquine-resistant falciparum malaria[1]. Misra[2] reported *P. falciparum* as the predominant species constituting 65.8% while *P. vivax, P. malariae* and mixed infections accounted for 15.5%, 16.1% and 2.6% respectively. Our study, as also the NMEP data, showed the absence of *P. malariae.* Furthermore, the proportion of *Pf* cases showed declining trends.

There is ample reason to believe that Arunachal Pradesh had been under continuous ecological changes because of deforestation and migration of population which might have direct or indirect bearing on the mosquito fauna and disease transmission. Misra[2] in 1956 recorded 6 species in human dwellings during winter months, of which *An. minimus* comprised 9.7% of the total catch, and was incriminated as the vector of malaria. Sen *et al.*[3] in 1973 recorded 14 species during February to November 1969, of which, *An. b. balabacensis* (*An. dirus*) comprised nearly 60% of the total catch in the man-biting collections and was incriminated as the vector. *An. minimus* was not recorded by Sen *et al.*[3] Malhotra *et al.*[4] in 1987 recorded 18 species during July and August in cattlesheds/human dwellings, of which *An. maculatus* was the most predominant; *An. minimus* was not recorded. Surprisingly, Dutta and Baruah[8] in 1987 incriminated *An. minimus* during their collections in April and May 1986. In our investigations, 12 anopheline species were recorded, of which *An. maculatus* was the most predominant but *An. minimus* was not found, in concurrence with the observation of Malhotra *et al*[4]. *An. dirus*, which was once reported to be the most predominant species[3,9], was recorded in fewer numbers only in man-biting collections (Table 5).

From the results it appears that distribution of *An. minimus* and *An. dirus* is patchy and changing owing to forest-related activities of man in forest/forest-fringe settlements in the state. In the adjoining state of Assam, *An. minimus* has been incriminated as the major vector, the other being *An. dirus*[10]. In addition to these vectors, the role of *An. philippinensis*, particularly in Arunachal Pradesh, cannot be ruled out as it has been found to be highly anthropophilic with high man-biting rate particularly outdoors (Table 5). Besides, this species had been documented as the vector of malaria in Assam[11,12]. It appears that *An. philippinensis* is establishing as a major species owing to increased paddy cultivation by removing jungle growth, thereby disrupting the ecological niche for *An. dirus*.

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Seasonality of Indoor Resting Mosquitoes in a Broken-Forest Ecosystem of North-Western Orissa

S.K. CHAND*, R.S. YADAV* and V.P. SHARMA+

The seasonal prevalence and indoor resting habits of mosquitoes in a broken-forest ecosystem of Orissa, which is known to be endemic for malaria and bancroftian filariasis, have been studied. A total of 15 anopheline spp., 9 *Culex* spp. and one each of *Aedes, Armigeres* and *Mansonia*, were collected. Major species with perennial occurrence were *Anopheles culicifacies, An. subpictus, An. annularis, An. vagus, An. pallidus, An. nigerrimus, Culex quinquefasciatus* and *Cx. tritaeniorhynchus*. These species rested more in cattlesheds than in human dwellings, except *Cx. quinquefasciatus* which was abundant in human dwellings. The prevalence and indoor resting pattern of different species in relation to seasons and other factors were also investigated.

Keywords: Bionomics, Broken-forest, Mosquitoes, Orissa plateau

INTRODUCTION

The eastern plateau in India has continued to be meso- to hyper-endemic for malaria for a long time. Previous studies on mosquitoes and malaria transmission were conducted in the late thirties in the Singhbhum hills[1] and in the early forties in the Hazaribagh ranges covering some localities of the present state of Orissa[2]. Since then considerable ecological changes have taken place in the area. Recent studies conducted in District Sundargarh (Orissa), located in the Garhjat hills in eastern plateau, have confirmed high-malaria endemicity[3,4]. Hence the malaria control programme in the region needs full entomological support and, therefore, we have carried out a study on

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the seasonality of mosquitoes to reassess the vector potential in the area in changing ecological scenario.

Study area

The study was conducted in District Sundargarh in Garhjat hills of Orissa from 1989 to 1990. The district is situated between longitudes 83°32' E and 85°22' E and between latitudes 21°35' N and 22°32' N and has a general elevation of 400-500 m above sea level. The plateau formations have undergone deforestation and excessive soil erosion and are interspersed with deciduous wet forest with a predominance of Sal (*Shorea robusta*) trees. Large broken-forest patches have been created in the process. The resultant forest-cleared areas are now being used for rice cultivation. Rice is the principal crop of the area, which is mostly monsoon-dependent. Irrigation facilities are poor. The forest cover in the district has dwindled — from 4569 sq km (47%) in 1975-76 to 3746 sq km (36%) in 1985-86 — and the area under rice cultivation has increased from 235,000 ha in 1975-76 to 261,000 ha in 1989-90[5]. Local population is predominantly aboriginal and lives in small villages comprising tiny hamlets which are often situated near streams or rivers.

Seven index villages, viz. Barsuan in Bisra block, Timjore and Sonaparbat in Lathikata block and Padampur, Ushera, Lanjiberna and Baniguni in Kuarmunda block of the district, were selected for the study. Three of these villages are situated on the banks of streams, one is about 1.5 km away from a stream, and three are on the banks of rivers Sankh and Brahmini. All the study villages were within a radius of 40 km from Rourkela city. During the study period only Padampur village received DDT spray in July 1989.

Meteorological data

The area is characterized by a tropical climate and receives high rainfall due to south-western monsoon and retreating north-eastern monsoon[6]. According to the meteorological data acquired locally, the annual rainfall was 1048 mm in 1989 and 1448 mm in 1990. The area had a high mean relative humidity (> 60%) except from February to May in 1989 and in April 1990. The mean monthly temperature ranged from a low of 19.3°C in January to a high of 36.7°C in May. The meteorological data given in Fig. 1 show that the area experiences broadly three seasons, viz. (i) summer, which starts with a short spell of spring in mid-February and extends to a relatively dry and hot climate until mid-June; (ii) monsoon season from mid-June to mid-October characterized by moderate temperature and high humidity; and (iii) winter season from mid-October to mid-February with a relatively low temperature and high humidity.

MATERIALS AND METHODS

Mosquito collections for 15 min. each were made on a fortnightly basis in the morning from four cattlesheds (two random and two fixed in each village) and four human dwellings using an aspirator and torch light. Human dwellings were huts made of mud-plastered walls and floors having earthern-

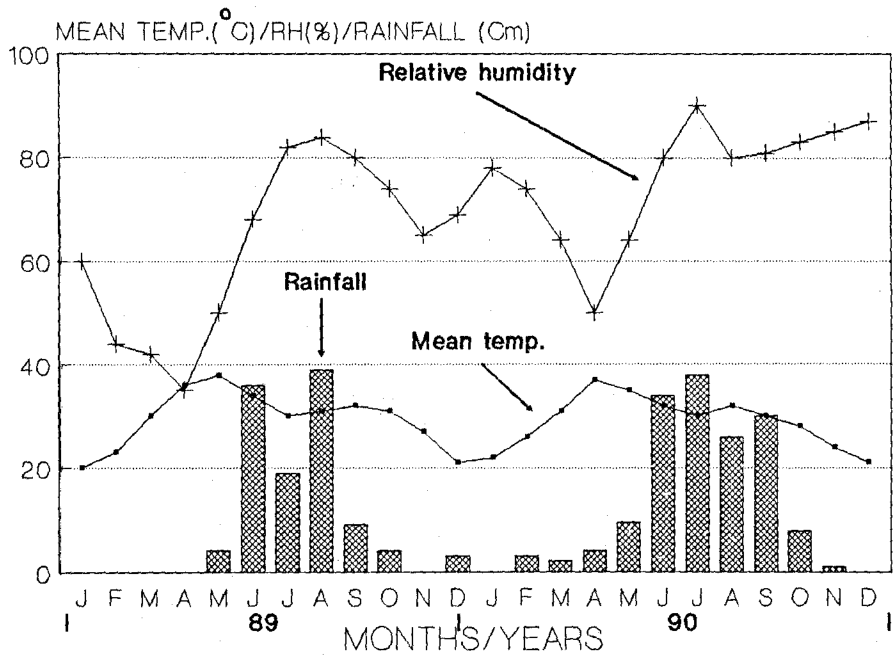

Fig. 1: Meteorological pattern of the study area

tile roofs and often with small verandahs. Cattlesheds were relatively open structures with separate roofs and enclosures, and were often close to human dwellings. Mosquitoes were anaesthetized with ether, species were identified and their man-hour densities recorded. Graphical analysis of data was done using a microcomputer.

RESULTS AND DISCUSSION

During the two-year study a total of 15 anopheline species, 9 *Culex* species and one each of *Aedes*, *Armigeres* and *Mansonia* species, were recorded. Major species among anophelines were: *An. culicifacies, An. subpictus, An. vagus, An. annularis* and *An. pallidus,* while major species among culicines were *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus*. Although malaria vector species *An. culicifacies* and *An. annularis* and filariasis vector *Cx. quinquefasciatus* are reported to be resistant to DDT in the area[7], residual sprays are likely to influence indoor densities of mosquitoes due to excito-repellent action. However, since there was no marked variation in indoor density of mosquitoes due to DDT spray in Padampur village in July 1989, the data for this village were pooled with those of other villages for seasonality analysis. Prevalence of the species found in abundance and resting indoors is projected in Figs. 2-8. All major species preferred cattlesheds to human dwellings for resting, except *Cx. quinquefasciatus* which rested more in hu-

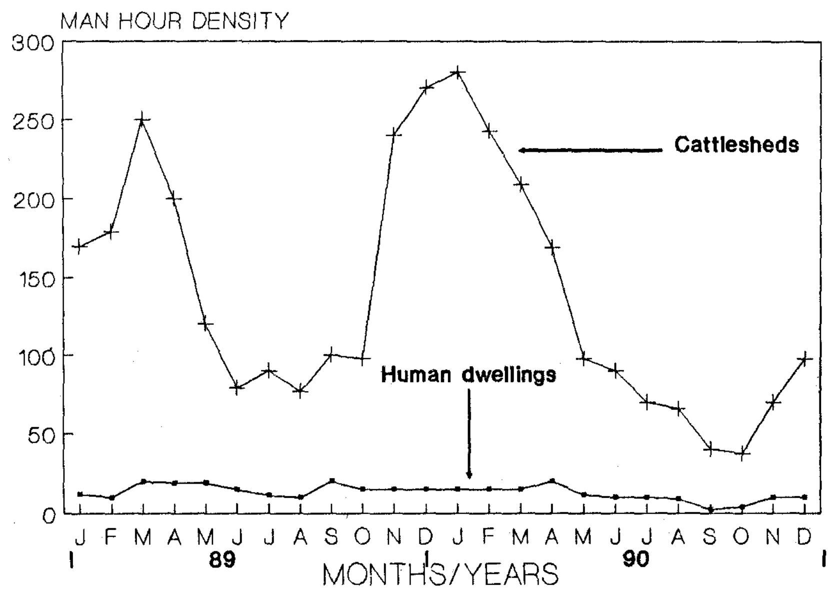

Fig. 2: Seasonal prevalence of *An. culicifacies*

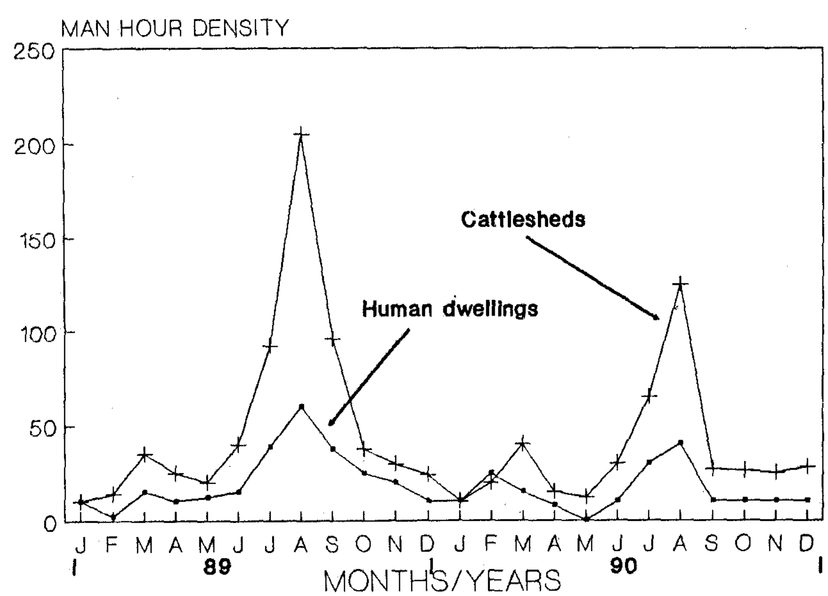

Fig. 3: Seasonal prevalence of *An. subpictus*

man dwellings. The number of anophe-lines caught in cattlesheds was seven times as much as in human dwellings.

An. culicifacies, a known malaria vector in the area, was abundant throughtout the year. Highest density was recorded during spring, i.e. early part of the summer season (Fig. 2). The density declined as summer approached, reaching a low figure in June on the onset of monsoon. During poor monsoon interspersed with lean periods the density either ascended marginally beyond June as seen in 1989 or continued to decline due to continuous heavy rains as seen in 1990. From November onwards it ascended continuously, attaining a peak in the next spring. Although density in cattlesheds was higher than in human dwellings as observed in previous studies[8], there was no difference in the pattern of seasonal prevalence between the two.

In northern India, a study near Delhi revealed that *An. culicifacies* was prevalent during the whole year. However, highest density was recorded in September with another spring peak in April-May[9]. In an irrigated area in southern India the species was prevalent round the year coinciding with the season of irrigation from June to November and was not related to temperature, humidity and rainfall[10].

Contrary to these observations, the density reached a peak in the study area in spring when the fields were largely fallow. A relatively low density was attained during monsoon which was also the paddy season. Streams and rivers were the major breeding habitats of *An. culicifacies* during inter-monsoon periods, while during monsoon other habitats such as rain pools, paddy fields and hoof prints, supported breeding. Apparently, therefore, density remained low during monsoon due to flushing effect in streams and rivers, which in turn depended largely on the frequency and amount of rainfall. From November onwards, when the receding water created numerous pools in these habitats, vector population built up was fast reaching a peak in the following spring.

An. subpictus was the next most abundant anopheline species after *An. culicifacies*. However, during July and August it was most abundant among all anophelines (Fig. 3). This was contrary to the observations made in a study in irrigated, non-irrigated and riverine areas in Gujarat where *An. subpictus* was the most predominant species[11]. Although *An. subpictus* was perennial, it showed two peaks in a year: a lower one in March with the approach of spring season and a higher one in August amidst monsoon due to the creation of widespread breeding habitats, as the species is known to prefer a wide range of habitats[11,12].

Another species closely related to *An. subpictus* in the pattern of seasonality was *An. vagus* (Fig. 4). Its population built up with two distinct peaks, one in March and another in August. Generally from February to April and again from July to October it had a comparatively higher prevalence.

In Orissa, *An. annularis* is considered to be a species of secondary importance in

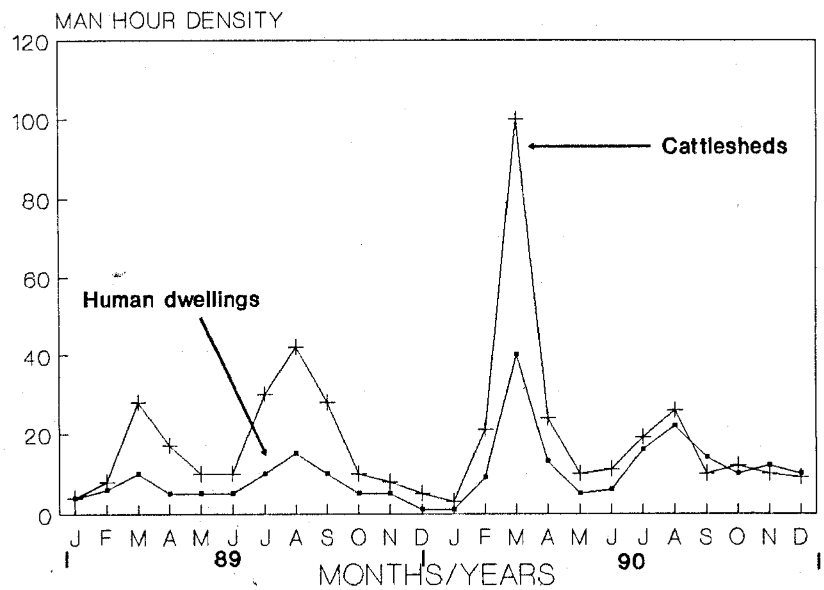

Fig. 4: Seasonal prevalence of *An. vagus*

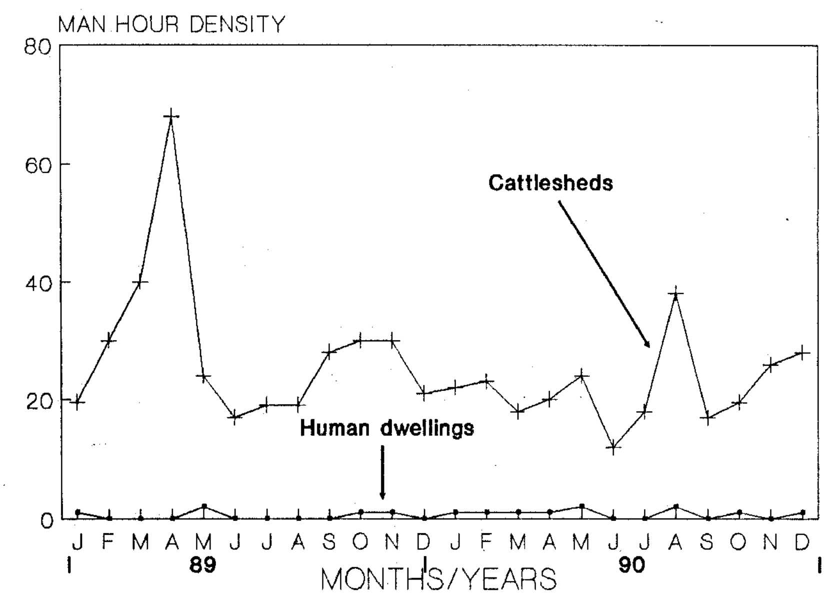

Fig. 5: Seasonal prevalence of *An. annularis*

malaria transmission. In the study area, major breeding habitats of this species were pools, paddy fields, streams and ponds. The species occurred throughout the year in cattlesheds with little preference to human dwellings (Fig. 5). During 1989 the density attained two peaks — one in April and another in October. The following year, however, the first peak was attained in May and the second in August as heavy rains in September appear to have checked any further rise in the density beyond August. Hence, a mixed pattern was seen. By and large, spring and winter were the favourable seasons.

An. pallidus and *An. nigerrimus* were collected in good numbers from cattlesheds from August to January and from September to December, respectively, with autumnal peaks around October when monsoon receded and winter started (Fig. 6). In other months *An. pallidus* was recorded in very low numbers while *An. nigerrimus* was rare.

Among culicines, *Cx. quinquefasciatus* transmits bancroftian filariasis in Orissa. It prefers to breed in waste-water collections near houses, wells and hand-pumps, disused wells, domestic containers, cowdung pits filled with rainwater and impounded waters used for bathing and other purposes. The species had a high density throughout, but was more abundant in human dwellings than in cattlesheds (Fig. 7). Broadly there

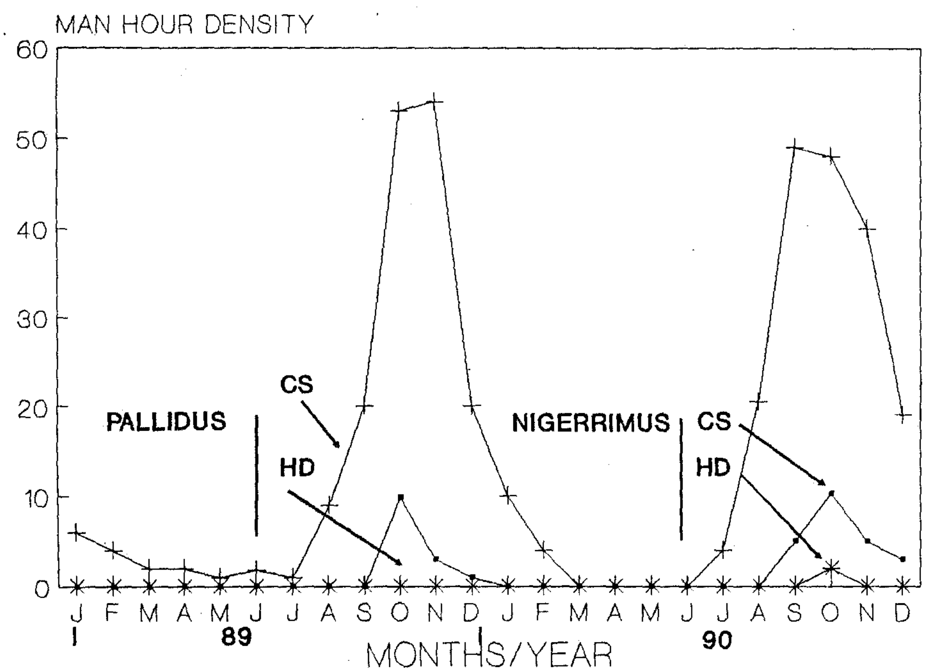

Fig. 6: Seasonal prevalence of *An. pallidus* and *An. nigerrimus*

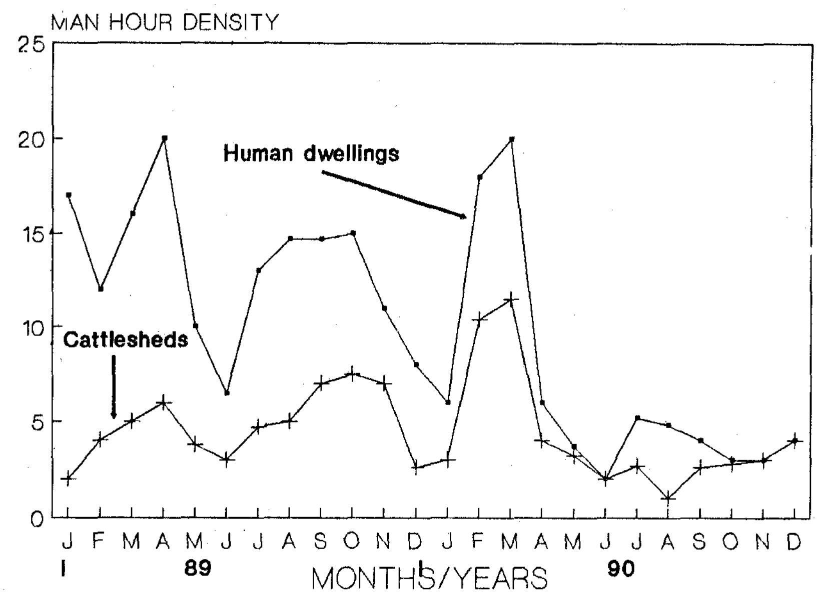

Fig. 7: Seasonal prevalence of *Cx. quinquefasciatus*

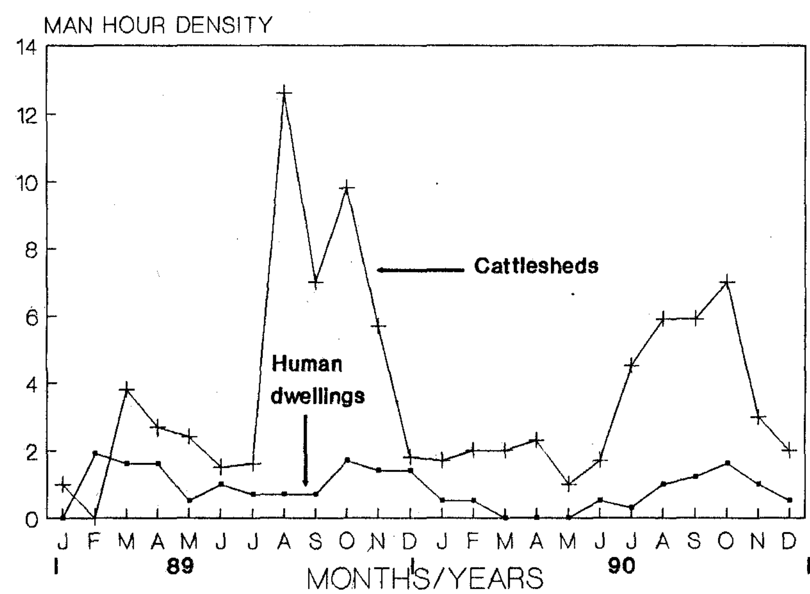

Fig. 8: Seasonal prevalence of *Cx. tritaeniorhynchus*

were two high-density periods, i.e. from February to April and again from July to November. While low precipitation facilitated a gradual build-up of population during the first year, continuous heavy rains caused a temporary setback in the population build-up the following year. June and December represented low-density months. In villages near Delhi the population of *Cx. fatigans* ascended in February and March, reached a peak in April and descended from May[13].

Cx. tritaeniorhynchus also showed two high peaks in density in cattlesheds, i.e. one during March-April and another from August-November (Fig. 8). Density usually coincided with the periods of paddy cultivation during monsoon season as the mosquito breeds in paddy fields.

The remaining 19 species were present in very low man-hour densities (< 3.5). Generally they were recorded indoors from the later part of the monsoon through winter except for *Cx. bitaeniorhynchus* which was collected in most of the months though in low numbers. Among these species, eight, viz. *An. aconitus, An. fluviatilis, An. jeyporiensis, An. splendidus, An. varuna, Cx. bitaeniorhynchus, Cx. gelidus* and *Cx. vishnui*, were collected from both cattlesheds and human dwellings, one (*Cx. nilgiricus*) from human dwellings only and the remaining ten (*An. barbirostris, An. jamesii, An. ramsayi, An. tessellatus, Cx. brevipalpis, Cx. fuscanus, Cx. whitmorei, Ae. pipersalatus, Ar. kuchingensis* and *Mansonia* spp.) only from cattlesheds. Among these, *An. fluviatilis* is a known vector of malaria and in forested villages it had high indoor densities as seen in other studies. However, from larval collect-ions made from broken-forest villages during a study on bioenvironmental control of malaria, very few specimens of *An. fluviatilis* and other anophelines, viz. *An. aconitus, An. jeyporiensis, An. varuna, An. jamesii, An. ramsayi* and *An. tessellatus*, emerged. It therefore appears that the low breeding potential and a possible preference for outdoor resting[14] might be responsible for the low indoor densities of these species. This aspect, however, needs further study.

We conclude that seasonal distribution of mosquitoes may vary in time and space depending upon environmental conditions and availability of breeding habitats. Although capture of indoor resting mosquitoes alone does not provide real estimates of mosquito population, it still provides a working assessment of the population dynamics of indoor resters[15]. Since the health programme is currently seized of the ever-increasing problem of mosquito-borne diseases in Orissa state, we hope that our study which updates information on the seasonality of mosquitoes would be useful in strengthening the strategy for control of mosquito-borne diseases in the broken-forest ecosystem of Garhjat hills.

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Role of *An. culicifacies* and *An. stephensi* in Malaria Transmission in Urban Delhi

S.N. SHARMA*, SARALA K. SUBBARAO+, D.S. CHOUDHURY+ and K.C. PANDEY++

Studies on adult densities, vector incrimination and breeding habitats of *An. culicifacies* and *An. stephensi* were carried out during June 1984 to May 1986 in three different ecological areas of urban Delhi. Both *An. culicifacies* and *An. stephensi* prevailed throughout the year in peri-urban areas with higher densities during the post-monsoon months. In south and northwest areas, low densities of these vector species were recorded. *An. culicifacies* was found naturally infected with sporozoites in peri-urban and south areas, whereas *An. stephensi* showed gland infection in south areas only. Out of 827 blood smears collected, 302 were positive for malaria parasites. Of the positives, *P. vivax* formed 48.3% and *P. falciparum* 51.7%. Peri-urban areas showed a higher number of malaria cases than south and northwest areas.

Keywords: *An. culicifacies*, *An. stephensi*, Urban malaria

INTRODUCTION

Malaria has played a vital role in shaping the history of Delhi. In 1911 Delhi was declared the new Imperial Capital City in place of Calcutta. The original site for the capital city selected near Dhaka village in north Delhi was rejected owing to its highly malariogenic conditions. As a result, New Delhi Capital City was constructed because of decreased malarial rate[1]. *Anopheles culicifacies* and *Anopheles stephensi* were incriminated malaria vectors of Delhi[1-3]. *An. culicifacies* was found to breed mainly in the riverine belt of

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Yamuna and *An. stephensi* in wells of the 'walled city'. While *An. stephensi* was successfully controlled by closing wells, the strategy for controlling *An. culicifacies* consisted in major or minor engineering operations and source reduction methods during the antimalaria operations launched in Delhi in pre-DDT era (1936-45). Considerable success in the control of both *An. culicifacies* and *An. stephensi* was achieved during 1936-39. However, the regional epidemic was contri-buted only by *An. culicifacies*[2].

During DDT era Delhi UT was divided into 11 wards (8 urban and 3 rural). Urban Delhi comprises 8 wards and rural Delhi 3 wards, both coming under the jurisdiction of Municipal Corporation of Delhi (MCD). However, the control strategy differs in the two areas. In the urban area, the strategy consisted in anti-larval measures and passive case detection (PCD). In the rural area, the strategy consisted in spraying and both ACD (active case detection) and PCD.

Despite efforts made in the past for the control of malaria the disease is still prevalent, the number of malaria cases varying from area to area in Delhi[3,4]. The problem was accentuated by the incessant growth of the city in a haphazard manner with a heterogeneous population and occupational differentiation. All these factors led to a change in the topography of Delhi which might have resulted in a change in the bionomics of the vector mosquitoes. Our study was therefore aimed at establishing the role of *An. culicifacies* and *An. stephensi* in malaria transmission in different areas of urban Delhi. The findings may help devise a better control strategy against the disease.

MATERIALS AND METHODS

Study sites

Three sites, viz. peri-urban, south Delhi, and northwest Delhi localities, were chosen for entomological and parasitological surveys.

Peri-urban: The localities selected were Wazirabad Mandirwala, Gopalpur, Timarpur and Mukherji Nagar Bandh. These localities in north Delhi lie close to the river Yamuna (Fig. 1). Only 550 houses with a total population of 2500 from the above localities were studied. In the periphery of these localities rural-like conditions prevail. Apart from river Yamuna, the other mosquito-breeding areas were roadside drains, collections in depressions near leaking water-supply pipes, sewage pools and overhead tanks. In Wazirabad and Gopalpur, a majority of the houses contained at least one cattleshed.

South Delhi: Kalkaji, Humayunpur and Sarojini Nagar were selected in south Delhi area. From these, only 800 houses with a total population of 4500 were surveyed. These are semi-urban localities surrounded by typical urbanized areas. Here the water is supplied through pipes and is mostly stored in overhead tanks. There is a fairly dense green belt around with a large number of pits in which rain-water accumulates.

Northwest Delhi: The localities selected in northwest Delhi area were Prem Nagar, Nehru Nagar and Dayabasti railway colony. From

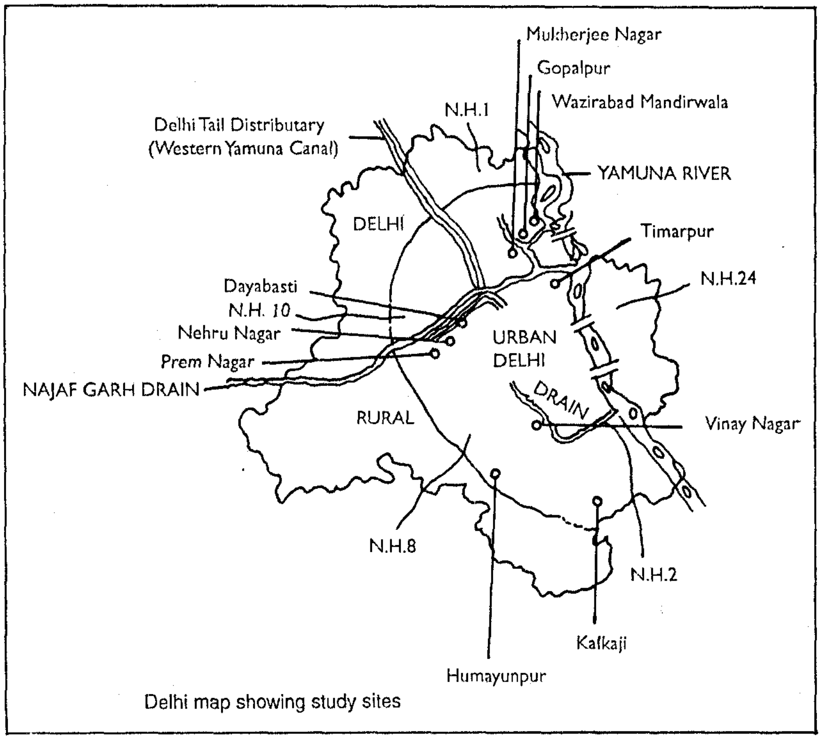

Fig. 1: Delhi map showing study sites

these localities only a population of 4000 people dwelling in 750 houses was surveyed Nehru Nagar and Prem Nagar are situated in the ridge area. The ridge has many pits, where water accumulates, and a number of borrow-pits near the railway tracks. Railway quarters have overhead tanks. There are also a few wells in these localities. Water-supply pipes existed only in Dayabasti railway colony. A cement pipe manufacturing factory (Hume Pipes) near Dayabasti railway station had special structures to store water for soaking pipes. A water channel passes through the area. All the above-mentioned types of breeding sites made the area mosquitogenic.

Entomological surveys

These comprised larval and adult collections, sporozoite detection, and parasitological surveys.

Larval collections: In view of the known breeding habitats of two vectors, viz. *An. stephensi* (domestic breeder) and *An. culicifacies* (feral breeder), larval searches were made indoors and outdoors to detect the active breeding sites of these two species. Various types of water collections in each locality were searched for mosquito breeding in different seasons of the year. When a particular breeding site was found positive for anophelines, maximum number of larvae were collected from it and brought to the laboratory for rearing.

Adult collections: To correlate larval and adult populations, hand collections of adult mosquitoes were also made by suction tube method in each area in the morning (0600 to 0900 hrs). Indoor resting mosquitoes were collected once a week from June 1984 to January 1986 and later, once a month up to May 1986. Searches for mosquito collections were made in various types of habitats like human dwellings, cattlesheds and mixed dwellings for getting maximum number of specimens. To study fluctuations in the prevalence of mosquitoes in each area, per man hour densities were calculated using the standard procedure.

Sporozoite detection: Specimens of *An. culicifacies* and *An. stephensi* were separated from the field-collected mosquitoes. To detect sporozoites, salivary glands of the vector species were dissected in normal saline. Whenever sporozoites were detected they were fixed in formaline, dried and stained with Leishman stain[5].

Anopheles culicifacies has been identified as a complex of 4 sibling species[6]. In our study no efforts were made to identify the sibling species; hence the study dealt with *An. culicifacies sensu lato.*

Similarly *An. stephensi* eggs were not examined to study the ridge number on egg float, which is characteristic of those ecologically identified in this species[7].

Parasitological surveys

To understand the pattern of malaria prevalence in each area, active fever surveys were carried out weekly only in the selected population of each locality as mentioned earlier. Week after week the same people residing in fixed localities were screened for malaria parasites.

Blood smears from persons reporting fever were collected on microslides. A presumptive treatment with 600 mg chloroquine base for adults and proportionate doses for children and infants were given immediately after the collection of blood smears. The smears were examined for malaria parasites after staining with JSB stain[8]. No radical treatment was given to the positive cases.

RESULTS AND DISCUSSION

Entomological surveys

Breeding habitats

An. culicifacies: The results of intradomestic (indoor) and peridomestic (outdoor) larval surveys are given in Table 1. River-bed pools and water collections near leaky pipes were the most preferred breeding places of *An. culicifacies*. In peri-urban areas, water percolating from sewage pools accumulated on roadsides. Out of 34 searches made in such water collections, *An. culicifacies* was found to breed in three searches. There was no breeding inside the sewage pools. This may be due to the accumulation of highly organic matter in them which was reported inhibitory to *An. culicifacies* breeding by Russell and

Table 1. Breeding sites of *An. culicifacies* and *An. stephensi* in the study areas of Delhi

Types of breeding source	No. of surveys made	No. of times breeding detected	
		An. culicifacies	*An. stephensi*
Intradomestic (Indoors)			
Water storage tanks for soaking bricks	16	0	1
Water storage tanks for soaking cement pipes	10	0	7
Cisterns and overhead tanks	20	0	2
Water containers for cattle	18	0	3
Peridomestic (Outdoors)			
Water collections near railway tracks	8	0	3
Water collections in parks	7	0	1
Water collections near leaking water-supply pipes	18	18	18
Temporary rain-water collections in open fields	7	4	2
Pits in kitchen gardens	27	0	4
Drains	14	3	4
Water collections near sewage pools	34	3	8
Pools in Yamuna river bed	46	37	11
Water collections in and around tail distributary of western Yamuna canal	15	0	6

Rao[9] in south India. The other breeding habitats of *An. culicifacies* in peri-urban areas were rain-water collections in the open and drains carrying no sullage water. Such drains had been constructed during development work on National Highway No. 1. No breeding occurred in the well-known Nazafgarh drain, which carried heavy sullage water. Our observations fairly agree with those of Senior-White[10] in that heavy breeding of *An. culicifacies* occurred in river-bed pools even before the commencement of rainy season and the onset of monsoon only increased the number of breeding places.

In south Delhi areas, *An. culicifacies* breeding was recorded in a storm-water drain during September and October. Breeding occurred in slow water current constantly flowing on its margins. No breeding was recorded in other breeding sites like overhead tanks, pit collections and intradomestic containers. No breeding of *An. culicifacies* was encountered in northwest areas.

An. stephensi: Breeding of *An. stephensi* in peri-urban areas was found in pit collections near leaking water-supply pipes, river-bed pools and on banks of Yamuna, seepage

Table 2. Densities of *An. culicifacies* in different areas of Delhi

Year		Month											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
							Peri-urban area*						
1984	No.	-	-	-	-	-	26	34	21	45	79	23	2
	MHD	-	-	-	-	-	6.5	6.8	7	11.3	19.8	11.5	0.5
1985	No.	0	1	7	4	3	1	14	65	124	156	133	43
	MHD	0.0	0.3	1.4	1	0.6	0.3	3.5	13	41	39	26.6	10.8
1986	No.	37	7	41	53	12	-	-	-	-	-	-	-
	MHD	7.4	7	20.5	17.7	3	-	-	-	-	-	-	-
							South area**						
1984	No.	-	-	-	-	-	0	12	22	66	60	0	0
	MHD	-	-	-	-	-	0.0	0.5	0.6	2.3	2.2	0.0	0.0
1985	No.	0	0	0	0	1	0.0	2	16	71	2	0	0
	MHD	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.8	3.4	0.1	0.0	0.0
1986	No.	0	0	0	3	0	-	-	-	-	-	-	-
	MHD	0.0	0.0	0.0	1.0	0.0	-	-	-	-	-	-	-
							Northwest area+						
1984	No.	-	-	-	-	-	0	0	1	5	0	0	0
	MHD	-	-	-	-	-	0.0	0.0	0.3	1.3	0.0	0.0	0.0
1985	No.	0	0	0	0	0	0	2	0	0	2	0	0
	MHD	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.4	0.0	0.0
1986	No.	0	0	0	0	0	-	-	-	-	-	-	-
	MHD	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-

*Well-defined monsoon season and perennial breeding; **Temporary breeding during monsoon only to support *An. culicifacies*; +Extra temporary breeding sites which do not support *An. culicifacies* even during monsoon period.

water near stagnant water collections, rainwater collections in depressions, cisterns and waste-water collections near houses. Overhead tanks in peri-urban areas were not searched for want of facilities. The breeding places observed in the northwest areas were tanks constructed either on ground or on roofs, water collections near houses or railway lines, stagnant water in the distributary of western Yamuna canal, pit collections in the Aravali hills and small collections in kitchen gardens and parks. In south areas, breeding of *An. stephensi* was found in a drain, overhead tanks and pit collections in green belt.

Almost all types of water collections were suitable for *An. stephensi* breeding. Prior to 1936 wells were considered to be the principal source of *An. stephensi* breeding in Delhi[1,10]. But during the extensive antimalaria work carried out in Delhi after 1936, wells were closed and pumps installed. However, a few wells were still found in northwest areas but no breeding was found in them during our surveys. As a result of closure of wells, tanks were constructed either on ground (cisterns) or on roofs of houses (overhead tanks) and these tanks were positive for *An. stephensi* breeding. These changes in breeding habits resulted in a focal outbreak of malaria in Delhi[11]. The new breeding places of *An. stephensi* found in our surveys were big water reservoirs used for curing newly built cement pipes in the factories. The other species found breeding in such water collections were *An. annularis* and *An. subpictus*.

Breeding of *An. stephensi* was associated with *An. culicifacies* only in outdoor water collections.

Adult densities

The densities (MHD) of *An. culicifacies* recorded in three study areas are summarised in Table 2. In peri-urban areas, a total of 931 female specimens were collected and the densities varied from 0.5 to 19.8 in 1984, from 0 to 41 in 1985 and from 3 to 20.5 in 1986. In south areas, 255 specimens were collected and the densities were very low, the maximum being 3.4 in September 1985. In northwest areas, *An. culicifacies* was almost absent except for a few specimens (10) collected during July to October.

An. culicifacies was found throughout the year in peri-urban areas. It showed well-defined monsoon and spring peaks of prevalence. But in the other two areas this species showed only monsoon peaks. The two peaks of *An. culicifacies* prevalence recorded in this study were similar to those generally found in northwest India.

The densities (MHD) of *An. stephensi* are summarised in Table 3. In peri-urban localities, 1046 specimens were collected and the MHD ranged between 3.3 and 20 in 1984, between 0.8 and 35.5 in 1985 and between 3 and 22.2 in 1986. The total number of specimens collected in south areas was 1717 and MHD ranged between 0.6 and 19.5 in 1984, between 0 and 5.5 in 1985; only 3 specimens in April and 6 in May were recorded in 1986. A total of 232 specimens was collected in northwest areas and MHD varied from 0 to 5 in 1984, from 0 to 7 in 1985 and from 0 to 4 in 1986. Except for 3 specimens collected during February 1985 in south area, *An. stephensi* was totally absent both in south and

Table 3. Densities of *Anopheles stephensi* in different areas of Delhi

Year		Month											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
							Peri-urban area*						
1984	No.	-	-	-	-	-	73	100	19	29	15	8	13
	MHD	-	-	-	-	-	18.3	20.0	6.3	7.3	3.8	4.0	3.3
1985	No.	3	23	66	142	137	3	29	34	15	66	63	27
	MHD	0.8	5.6	13.2	35.5	27.4	1	7.3	6.8	5.0	16.5	12.6	6.8
1986	No.	65	3	8	16	89	-	-	-	-	-	-	-
	MHD	13.0	3.0	4.0	5.3	22.2	-	-	-	-	-	-	-
							South area**						
1984	No.	-	-	-	-	-	134	470	452	318	85	3	8
	MHD	-	-	-	-	-	6.0	19.5	13.2	11.3	3.0	0.6	1.0
1985	No.	0	3	0	7	19	10	94	44	26	24	6	5
	MHD	0.0	0.8	0.0	0.7	1.4	1.3	5.5	2.2	1.2	1.0	1.0	1.0
1986	No.	0	0	0	3	6	-	-	-	-	-	-	-
	MHD	0.0	0.0	0.0	1.0	1.0	-	-	-	-	-	-	-
							Northwest area+						
1984	No.	-	-	-	-	-	20	10	5	18	4	3	0
	MHD	-	-	-	-	-	5.0	2.5	1.3	4.5	0.8	1.5	0.0
1985	No.	0	0	0	22	15	14	31	21	10	18	22	12
	MHD	0.0	0.0	0.0	5.5	5.0	3.5	6.2	7.0	3.3	3.6	5.5	2.4
1986	No.	0	0	0	3	4	-	-	-	-	-	-	-
	MHD	0.0	0.0	0.0	3.0	4.0	-	-	-	-	-	-	-

*Area constituting vast number of outdoor breeding sites; **Area constituting traditional domestic breeding sites and outdoor habitats; +Area constituting more domestic breeding sites and less outdoor habitats.

northwest areas during January to March. However, in peri-urban areas the densities ranged between 3 and 13.2 during the same period (Table 3).

In peri-urban areas, *An. stephensi*, like *An. culicifacies*, prevailed throughout the year. High densities were recorded during April and May. Entomological studies carried out by Malaria Research Centre during 1983-84 showed higher densities of *An. stephensi* in June. The difference in prevalence may be due to the well-known erratic behaviour of *An. stephensi*.

In south areas, high densities were recorded from July to September. In northwest areas densities were low throughout the year but the pattern of prevalence during April to November was similar to that in the peri-urban areas.

Sporozoite rate

The results of salivary gland dissections for sporozoites in *An. culicifacies* are given in Table 4. In peri-urban and south areas, mosquitoes harbouring sporozoites were found in September and October 1985. No infected specimens were detected in northwest areas.

Based on the results of adult densities and gland dissections, it may be inferred that *An. culicifacies* has no role in malaria transmission in this area. In peri-urban areas the mosquitoes harbouring sporozoites were found in those months when the adult densities were the highest. This supports the view of Rao[12] that *An. culicifacies* is comparatively a weak vector because of its mainly zoophilic habit. But the species makes up for this deficiency by occurring in very high densities.

All the previous studies on incrimination of *An. culicifacies* in Delhi had been carried out mainly in the area adjoining the river Yamuna[1,13,14]. Our study covering south Delhi areas showed that *An. culicifacies* has a role in malaria transmission in these areas also.

The results of gland dissection of *An. stephensi* are given in Table 5. In peri-urban and northwest areas no sporozoite positivity was detected in this species. Though no positivity was found in the above areas, yet, due to high prevalence and favourable breeding sites, it might be contributing to malaria trans-

Table 4. Salivary gland dissections of *Anopheles culicifacies*

Study area	1984		1985		1986	
	No. dissected	No. +ve	No. dissected	No. +ve	No. dissected	No. +ve
Peri-urban	200	0	519	3*	150	0
South Delhi	160	0	92	1**	3	0
Northwest	6	0	4	0	0	0

*One in September (sporozoite rate, 0.8%) and two in October (sporozoite rate, 1.4%); **One in September (sporozoite rate, 1.4%).

Table 5. Salivary gland dissections of *Anopheles stephensi*

Study area	1984		1985		1986	
	No. dissected	No. +ve	No. dissected	No. +ve	No. dissected	No. +ve
Northwest	56	0	157	0	7	0
Peri-urban	253	0	588	0	181	0
South Delhi	1470	1*	238	1**	9	0

*Positive mosquito was found in August with a sporozoite rate of 0.2 %; **Positive mosquito was found in October with a sporozoite rate of 4.2%.

mission to some extent there. In south area, sporozoite-positive mosquitoes were found with a sporozoite rate of 0.2 in August 1984 and 4.2 in October 1985. This establishes that *An. stephensi* plays a definitive role in malaria transmission in south areas.

Although *An. stephensi* is an established urban vector, its role in malaria transmission in urban Delhi varied from time to time, and up to mid-1930s a certain proportion of malaria in Delhi was positively due to *An. stephensi*[1,10]. Thereafter, the extensive antimalaria work launched in 1936 eliminated the most potential breeding sites of *An. stephensi*. As a result, this species played no role in the transmission of urban malaria in Delhi and only *An. culicifacies* was considered important in the autumn outbreak of 1942[2]. After mid-1960s the species was once again considered as a vector in Delhi city[11]. Pattanayak *et al*[3]. also showed the importance of *An. stephensi* in malaria transmission in south Delhi.

Parasitological surveys

The result of fever surveys are given in Table 6. A total of 827 blood smears was collected in all the areas. Of these, 302 were positive for malaria parasites, 146 of *P. vivax* and 156 of *P. falciparum*. No mixed infection was found in these collections.

During June 1984 to May 1985 a total of 587 (269 in peri-urban + 99 in south and 219 in northwest areas) blood smears were examined. Of these, 237 (142 in peri-urban + 33 in south and 62 in northwest areas) were found positive for malaria parasites. In positive slides, 100 (44 in peri-urban + 19 in south and 37 in northwest areas) showed *P. vivax* and 137 (98 + 14 + 25 respectively in above areas) for *P. falciparum* infections. The area-wise slide positivity rate (SPR), slide falci-parum rate (SfR), annual blood examination rate (ABER) and annual parasite incidence (API) during the above period were found as follows: 52.8, 36.4, 10.8 and 56.8 in peri-urban; 33.3, 14.1, 2.2 and 7.3 in south area; and 28.3, 11.4, 5.5 and 15.5 in northwest areas respectively.

Out of 240 (87 in peri-urban + 42 in south and 111 in northwest areas) blood smears examined during June 1985 to May 1986, 65 (33 + 7 + 25 respectively in above areas) were found

Table 6. Results of examination of blood smears collected from fever cases in different areas of urban Delhi

Month/ year	Peri-urban areas*						South areas**						Northwest areas+					
	BSE	Positive			SPR	SfR	BSE	Positive			SPR	SfR	BSE	Positive			SPR	SfR
		Pv	*Pf*	Total				*Pv*	*Pf*	Total				*Pv*	*Pf*	Total		
Jun 1984	10	3	0	3	30.0	0.0	5	2	0	2	40.0	0.0	6	3	0	3	37.5	0.0
Jul	12	3	2	5	41.7	16.7	15	4	1	5	33.3	6.7	10	4	1	5	50.0	10.0
Aug	5	1	1	2	40.0	20.0	14	3	0	3	21.4	0.0	17	5	0	5	29.4	0.0
Sep	33	6	18	24	72.7	54.5	9	2	0	2	22.2	0.0	24	5	2	7	29.0	8.3
Oct	96	11	51	62	64.6	53.0	35	5	10	15	42.9	28.6	45	10	11	21	46.6	24.4
Nov	19	1	11	12	63.0	57.9	12	1	3	4	33.3	25.0	10	0	3	3	30.0	30.0
Dec	16	1	5	6	37.5	31.3	4	0	0	0	0.0	0.0	6	0	2	2	33.3	33.3
Jan 1985	8	0	5	5	62.5	62.5	0	0	0	0	0.0	0.0	23	3	4	7	30.4	17.4
Feb	10	1	3	4	40.0	30.0	0	0	0	0	0.0	0.0	19	0	1	1	5.3	5.3
Mar	17	1	2	3	17.6	11.8	2	0	0	0	0.0	0.0	21	1	0	1	4.8	0.0
Apr	23	7	0	7	30.4	0.0	2	1	0	1	50.0	0.0	19	5	0	5	26.3	0.0
May	20	9	0	9	45.0	0.0	1	1	0	1	100.0	0.0	17	1	1	2	11.7	5.9
Total (1984-85)	269	44	98	142	52.8	36.4	99	19	14	33	33.3	14.1	219	37	25	62	28.3	11.4

contd...

Table 6. (*contd.*)

Month/ year	Peri-urban areas*						South areas**						Northwest areas+					
	BSE	Positive			SPR	SfR	BSE	Positive			SPR	SfR	BSE	Positive			SPR	SfR
		Pv	*Pf*	Total				*Pv*	*Pf*	Total				*Pv*	*Pf*	Total		
Jun 1985	3	2	0	2	66.6	0.0	0	0	0	0	0.0	0.0	7	4	0	4	57.1	0.0
Jul	9	2	0	2	22.2	0.0	3	1	0	1	33.3	0.0	15	2	1	3	20.0	6.6
Aug	27	7	6	13	48.1	22.2	5	1	0	1	20.0	0.0	9	3	0	3	33.3	0.0
Sep	7	1	2	3	42.8	28.6	14	3	0	3	21.4	0.0	16	5	1	6	37.5	6.3
Oct	6	1	2	3	50.0	33.3	5	1	0	1	20.0	0.0	8	0	0	0	0.0	0.0
Nov	6	1	3	4	66.6	50.0	8	0	1	1	12.5	12.5	5	0	0	0	0.0	0.0
Dec	4	1	0	1	25.0	0.0	1	0	0	0	0.0	0.0	10	1	0	1	10.0	0.0
Jan 1986	4	1	0	1	25.0	0.0	2	0	0	0	0.0	0.0	6	0	1	1	16.6	16.6
Feb	2	0	0	0	0.0	0.0	4	0	0	0	0.0	0.0	7	1	2	3	42.9	28.5
Mar	3	1	0	1	33.3	0.0	0	0	0	0	0.0	0.0	10	2	0	2	20:0	0.0
Apr	10	2	0	2	20.0	0.0	0	0	0	0	0.0	0.0	8	2	0	2	25.0	0.0
May	6	1	0	1	16.6	0.0	0	0	0	0.0	0.0	0.0	10	0	0	0	0.0	0.0
Total (1985-86)	87	20	13	33	37.9	14.9	42	6	1	7	16.7	2.4	111	20	5	25	22.5	4.5

Population — *2500, **4500, +40,000; *Pv* — *Plasmodium vivax*; *Pf* — *Plasmodium falciparum*; SPR — Slide Positivity Rate; SfR — Slide falciparum Rate.

positive for malaria parasites. The positive slides contained 46 (20 in peri-urban + 6 in south and 20 in northwest areas) *P. vivax* and only 19 (13 + 1 + 5 in respective areas) for *P. falciparum* infections. The area-wise SPR, SfR, ABER and API during the same period were as follows: 37.9, 14.9, 3.5 and 13.2 in peri-urban; 16.7, 2.4, 0.9 and 1.6 in south areas; and 22.5, 4.5, 2.8 and 6.3 in northwest areas respectively.

Overall results showed a higher number of malaria cases in peri-urban areas than in the other two areas. The reasons for higher incidence of malaria cases in peri-urban areas than in south and northwest areas are the different ecological conditions prevailing in these areas. The localities mentioned in peri-urban areas are located near river Yamuna, which has high receptivity for malaria. The same view was held by Kalra and Sharma[4].

The slide positivity rates in peri-urban and south areas of our study showed a different pattern from those observed by Pattanayak *et. al.*[3] In their study, the SPR was 14.8 in Civil Lines areas, which included Wazirabad and Timarpur (area located in the vicinity of Yamuna river), while in New Delhi south area it was 21.3. The above investigators, however, did not carry out active surveys in these areas and their observations were based on the results obtained from the malaria clinics of each area.

Seasonal prevalence

The results of blood smears examined in three areas are summarised monthwise in Table 6. *P. vivax* cases were predominant from April to August and from July onwards there was an increase in *falciparum* incidences. In September the two species were found almost in equal proportions. Thereafter, *P. vivax* cases declined and the trend continued up to the end of March. Incidence of *P. falciparum* was low from March to June. *P. falciparum* incidence touched the lowest limit during April and June.

A similar prevalence of *P. vivax* and *P. falciparum* was also observed by Chaudhury[15] in patients who attended the malaria clinic of Malaria Research Centre, Delhi.

Our study showed a high rate of morbidity due to malaria prevalence in urban population. The morbidity was due to *P. vivax* and *P. falciparum* as no cases of *P. malariae* were observed.

Both entomological and parasitological surveys in our study have established that even in changed ecological situations, *An. culicifacies* and *An. stephensi* continued to play significant roles in the transmission of urban malaria in Delhi.

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Use of Kerosene Lamp Containing Synthetic Pyrethroids to Repel Mosquitoes

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An indigenous and appropriate method of personal protection was developed and tested in rural areas. It consists of an ordinary kerosene lamp made of tin with a regulator to adjust the wick. Different concentrations of esbiothrin, a synthetic pyrethroid, were mixed in kerosene and allowed to burn in living rooms and cattlesheds from dusk to dawn to observe hourly entry of mosquitoes. The tin lamp (100 ml capacity) without chimney provided the most efficient protection from mosquito bites. The protection varied from 84.2 $\pm$ 8.2 to 97.8 $\pm$ 2.8. Maximum protection was observed against *An. culicifacies*. Esbiothrin kerosene lamp (0.01%) lighted in living room provided 99.7% protection to exposed human baits seated at a distance of 1 m from the lamp. The technique is simple, cost-effective and does not require electricity and can be used both for illumination and repelling mosquitoes in remote and inaccessible areas.

Keywords: Esbiothrin, Malaria control, Mosquito repellents

INTRODUCTION

Malaria is present in 102 countries, causing disease in 100 million population and 1 to 2 million deaths each year. In India about 2 million new malaria cases are reported annually by the National Malaria Eradication Programme[1]. In addition to malaria, large parts of the country are endemic for filariasis and surveys done till 1980 have revealed that about 304 million people live in filaria-risk areas, 22 million have circulating microfilaria (mf), and 16 million suffer from the disease manifestations[2]. Dengue epidemics are encountered in urban areas[3] and now with the introduction of rural water supply, dengue epidemics have started visiting rural areas[4]. Japanese encephalitis (JE) epidem-

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ics are increasing in geographical areas mainly in the rice growing tracts with 40-50% case fatality rates[5]. Besides, mosquito nuisance throughout the country and almost round the year is very high, making the life of the people miserable. Most of the mosquito nuisance is man-made owing to environmental degradation and often developmental activities.

The spraying of residual insecticides in rural areas and antilarval measures in urban areas are producing diminishing returns because of multiple resistance among rural vectors and poor performance in urban areas. Therefore, personal protection methods such as coils, bednets, repellents, etc. have a crucial role particularly under Primary Health Care approach.

The Malaria Research Centre, where development work on alternative and cheap strategies for vector control is under progress, has successfully demonstrated the use of insecticide-impregnated ropes for curtailing rural malaria as a cheap and effective alternative to mats which are relatively expensive and require electricity. Smouldering of esbiothrin-impregnated ropes throughout the night provided good protection from mosquito bites and also from the transmission of malaria[6].

In an effort to develop an indigenous and low-cost personal protection method, kerosene lamps which contain low concentrations of synthetic pyrethroids were evaluated. Since synthetic pyrethroids such as allethrin and esbiothrin have a long history of use as repellents and are marketed all over the world. There is no health risk involved in their use in lamps, as only the technique of vaporizing the chemical has been simplified. Our paper deals with the results of studies on (i) selection of a lamp that would be cost-effective in terms of kerosene consumption, and (ii) protection provided from mosquito bites.

MATERIALS AND METHODS

Study area

Jadoanpur (Dhaulana PHC) and Ramgarh (Dadri PHC) villages of Ghaziabad district, Uttar Pradesh, were selected for the study. Jadoanpur village (population 693; houses 127) is located on the Massouri and Gulawthi road southeast of Delhi at about 36 km from Delhi; Ramgarh village (population 1137; houses 155) is located on the Delhi-Bulandshahar road at about 45 km. These villages are rural in character with ponds, puddles, drains, canal irrigation and a variety of other mosquito-breeding habitats. Mosquito density is very high almost throughout the year. Although bulk of the mosquito populations comprises *Culex quinquefasciatus,* yet the malaria vector *Anopheles culicifacies* is found in very high densities. Both villages have high malaria transmission — API in Jadoanpur was 23 and in Ramgarh 15.8 in 1990.

So far, there is no evidence of transmission of filaria or Japanese encephalitis. Since 1980 the villages have been sprayed with 3 rounds of HCH (0.2 g/m^2). Although spraying was patchy and in many years even two rounds were not sprayed, yet even in years when spraying was good the impact on mosquito nuisance was transient and malaria transmission was not interrupted. Villagers do not use any personal protection methods. Case detection and treatment is carried out under the multipurpose workers (MPW) scheme at fortnightly intervals by the state health department.

Ordinary kerosene lamps were used in the study. The lamps were made of tin with a regulator to adjust the wick. Lamps of different sizes and capacities with or without the provision of a glass chimney are easily available in the local market.

Initially tests were done in one village to select the most cost-effective kerosene lamp. Later, preliminary experiments were taken up both in the living rooms (human dwelling) and in cattlesheds in which 0.05%, 0.075% and 0.1% concentrations of esbiothrin in kerosene were used to observe hourly entry of mosquitoes. One room in each human dwelling and cattleshed were held as control where kerosene lamp was used, and one room and one cattleshed were held for comparison (control without lamp) in each village. Mosquitoes were collected from both the villages concurrently at weekly intervals for 5 nights, i.e. from 10 living rooms and 10 cattlesheds. At weekly intervals, experimental rooms were alternated with control rooms and vice-versa.

In the same villages human baits were used indoors for ascertaining the landing rates of mosquitoes. The arms and legs of the baits were exposed and mosquitoes which landed on the exposed body parts were collected by volunteers with the help of suction tube and torch from 1800 to 0600 hrs. Volunteers belong to Malaria Research Centre and are well trained in mosquito collections. In each village there was one volunteer in a room without lamp. Therefore, a total of 10 baits in each category were used during 5 night collections at weekly intervals.

RESULTS AND DISCUSSION

Table 1 gives the results of experiments conducted to select the optimum size of the lamp and kerosene consumption as against protection from mosquitoes. Esbiothrin was mixed in kerosene to get 0.05% and 0.1% concentrations and these lamps were lighted in the rooms in villages from 1800 to 0600 hrs inside the rooms. The results showed that 100 ml capacity tin lamps without chimney provided the best protection from mosquitoes. Maximum protection was observed in *Anopheles culicifacies*. In general, protection from anophelines was better than from culicines but the overall protection from mosquitoes was 83.4 ± 6.6 at 0.05% and 88.9 ± 6.2 at 0.1% esbiothrin in kerosene.

Table 2 gives the results of 100 ml lamp without chimney at various concentrations of esbiothrin in the living rooms and cattlesheds. The study was carried out at weekly intervals, and rooms with and without lamp were alternated each week to minimize the mosquito density variations due to preferred resting of mosquitoes for a particular structure. At 0.05% esbiothrin concentration, protection from mosquitoes was about 95% and did not increase significantly by increasing dosage to 0.075% or 0.1%.

Table 1. Results of tests to select kerosene lamp of optimum size in relation to protection from mosquitoes and kerosene consumption

Type of lamp and study period	Esbiothrin conc. (%) in kerosene	Kerosene consumed ml/12 h	No. of rooms used for night collection	*An. culicifacies*			Total anophelines			*Cx. quin.*			Total mosquitoes		
				E	C	% pro.	E	C	% pro.	E	C	% pro.	E	C	% pro.
Tin lamp (100 ml) with chimney (Nov-Dec 1990)	0.05	65	5	17	56	69.6	81	265	69.4	69	126	45.2	150	391	61.6
	0.1	65	3	3	28	89.2	14	123	88.6	33	92	64.1	47	215	78.1
	Kerosene only	65	3	24	34	29.4	149	177	15.8	67	84	20.2	216	261	17.2
Tin lamp (150 ml) with chimney (Jan-Feb 1991)	0.05	160	10	75	430	82.6	143	881	83.8	527	1820	71.0	670	2701	75.2
	0.1	160	10	14	421	96.7	43	709	93.9	245	1120	78.1	288	1829	84.3
	Kerosene only	160	2	7	9	22.2	19	40	52.5	51	86	40.7	70	126	44.4
Tin lamp (600 ml) with chimney (Mar-Apr 1991)	0.1	250	15	30	396	92.4	49	590	91.6	302	1176	74.3	351	1766	80.0
	Kerosene only	250	7	128	188	31.9	172	312	44.7	313	490	36.1	485	802	39.5
Tin lamp (100 ml) without chimeny (Jun-Jul 1991)	0.05	75	5	56	448	87.5	151	944	84.0	94	534	82.4	245	1478	83.4
	0.1	75	5	9	417	97.8	74	996	92.5	124	789	84.2	198	1485	88.9
	Kerosene only	75	3	213	308	30.8	448	647	30.7	233	309	24.6	681	956	28.7

Note: Number of mosquitoes are the total numbers collected against each dosage on different nights; % pro. — per cent protection from mosquito bites in living rooms; *Cx. quin.* — *Culex quinquefasciatus.*

$$\% \text{ protection} = \frac{\text{Number of bites in control room - Number of bites in experimental room}}{\text{Number of bites in control room}} \times 100$$

Table 2. Total number of mosquitoes collected by suction tube in room with lamp without chimney but containing esbiothrin in kerosene

Conc. (%) (Place of collection)	Species	Jadoanpur village			Ramgarh village		
		Expt.	Cont.	%protection	Expt.	Cont.	%protection
0.05	*An. culicifacies*	8	228	96.4	13	240	94.5
(HD)	Total anophelines	60	618	90.2	44	684	93.5
	Culex	129	372	65.3	19	317	71.2
	Total mosquitoes	189	990	80.9	135	1001	86.5
0.05	*An. culicifacies*	31	151	87.6	26	251	89.6
(CS)	Total anophelines	499	2061	75.7	455	2061	77.9
	Culex	398	1103	63.9	359	1103	67.4
	Total mosquitoes	897	3164	71.6	814	3164	74.2
0.075	*An. culicifacies*	15	311	95.1	11	308	96.4
(HD)	Total anophelines	161	1954	91.7	129	1800	92.8
	Culex	111	309	64.0	129	353	63.4
	Total mosquitoes	272	2263	87.9	258	2153	88.0
0.075	*An. culicifacies*	49	548	91.0	58	611	90.5
(CS)	Total anophelines	275	1991	86.1	347	2516	86.2
	Culex	133	388	65.7	121	363	66.6
	Total mosquitoes	408	2379	82.8	468	2879	83.7
0.1	*An. culicifacies*	8	272	97.0	3	284	98.9
(HD)	Total anophelines	51	586	91.2	13	652	98.0
	Culex	109	402	72.8	60	347	82.7
	Total mosquitoes	160	988	83.8	73	999	92.6
0.1	*An. culicifacies*	15	252	94.0	16	252	93.6
(CS)	Total anophelines	245	2082	88.2	295	2082	85.8
	Culex	226	1006	77.5	210	1006	79.1
	Total mosquitoes	471	3088	84.7	505	3088	83.6

Expt.— Rooms with 100 ml capacity esbiothrin in kerosene; Cont. — No lamps used, normal conditions in village with various light systems.

Note : Mosquitoes were collected in 2 villages simultaneously and collections were made at weekly intervals on 5 nights, i.e. 10 rooms in human dwellings (HD) and 10 cattlesheds (CS). At weekly intervals experimental rooms in Jadoanpur village were alternated with experimental rooms in Ramgarh village and vice-versa with concurrent control.

Table 3. Total number of mosquitoes that landed on human baits in rooms with esbiothrin-kerosene lamps

Study period	Conc. of esbiothrin %	*An. culicifacies*			*Culex quinquefasciatus*			Total mosquitoes		
		Expt.	Cont.	P	Expt.	Cont.	P	Expt.	Cont.	P
Jul-Aug 1991	0.05	3	34	91.1	23	135	82.9	29	199	85.4
Nov-Dec 1991	0.075	4	155	97.4	17	524	96.7	10	309	96.7
Jul 1991	0.1	0	50	100.0	1	264	99.6	1	355	99.7

P —% protection; Total of 5 night collections at each dosage from 1800 to 0600 hrs.

Also, the method was more effective against anophelines than against culicines, but the density of even *Culex* mosquito was about 1/10th of the normal population found in control rooms without lamp.

Table 3 gives the results of mosquitoes collected while landing on human baits at three esbiothrin concentrations. Very few mosquitoes landed on the exposed body parts in rooms with lamps. Although a few mosquitoes did land at 0.05 and 0.075% concentrations, yet at 0.1% concentration no *An. culicifacies* was collected from the exposed body parts, except one *Culex quinquefasciatus*.

Table 4 gives the effectiveness of lamps containing 3 dosages of esbiothrin, and human baits allowed to sit at 1, 2 and 3 m away from the lamp. As the distance from the lamp increased, more mosquitoes started landing on the bait. Protection from mosquitoes decreased as the distance from the lamp increased, maximum protection being provided within 1 m of the lamp.

To reduce reliance on insecticides, alternative methods of malaria control are being developed. One age-old method for the protection from mosquitoes and malaria is the use of bednets. In recent years, bednets have been impregnated with synthetic pyrethroids and this method is becoming very popular almost throughout the world, and when applied in certain areas, malaria transmission has been interrupted[7]. In India deltamethrin-impregnated bednets were tried in endemic villages in Assam and there was reduction in mosquito vector densities and transmission of malaria[8]. In these areas, *An. minimus* is the vector responsible for maintaining high transmission and stable malaria conditions. Although the peak biting period of *An. minimus* is midnight, yet biting may take place up to 2100 or 2200 hrs before the bednets are put to use. In *An. culicifacies* the biting rhythm varies seasonally. Besides, studies on *An. culicifacies* also revealed that the biting rhythm of *An. culicifacies* varies with the sibling species (A,B,C and D) while A and B populations prefer to bite after 2200 hrs, most of the C and D populations prefer to bite before 2100 hrs. Hence people may contract malaria

Table 4. Total number of mosquitoes landing on human bait sitting at various distances from esbiothrin-kerosene lamps

Study period/ Conc. % of esbiothrin/	*An. culicifacies*			Anophelines			*Culex quinquefasciatus*			Total mosquitoes		
Distance from lamp	Expt.	Cont.	P	Expt.	Cont.	P	Expt.	Cont.	P	Expt.	Cont.	P
Aug 1991												
0.05												
1 m	1	18	94.4	3	30	90.0	12	144	91.6	15	174	91.3
2 m	8	88	90.9	31	264	88.2	21	100	79.0	52	364	85.7
3 m	9	62	85.4	41	222	81.5	28	100	72.0	69	322	78.5
Oct-Nov 1991												
0.075												
1 m	1	24	95.8	2	28	92.8	2	40	95.0	4	68	94.1
2 m	2	28	92.8	4	44	90.9	9	48	81.2	13	92	85.8
3 m	10	98	89.7	19	161	88.1	25	83	69.8	44	244	81.9
Aug 1991												
0.1												
1 m	0	18	100.0	2	30	93.3	5	144	96.5	7	174	95.9
2 m	4	100	96.0	16	264	93.9	35	260	86.5	51	524	90.2
3 m	7	108	93.5	29	292	90.0	60	416	85.5	89	708	87.4

Expt. Experimental; Cont. — Control; P — % protection; Total of 3 night collections at each dosage from 1800 to 0600 hrs (Aug-Nov 1991)

(S.K. Subbarao 1992, personal communication) before entering into the bednets, thus seriously questioning the utility of bednets.

In stable malaria areas where the sporozoite rates are very high, even occasional bites may be enough to maintain transmission within the communities. Thus, there is need to find an ideal solution to the problem of personal protection from mosquito bites preferably for the whole night. In addition, the method should be simple, cost-efffective, highly adaptable under varied local situations and easily accessible to the common man residing even in remote areas. Ansari *et al.*[6] reported that insecticide-impregnated ropes when allowed to smoulder can be used to repel mosquitoes either throughout the night or for the period when bednets are not in use. Another suitable substitute could be kerosene lamps in which low concentrations of synthetic pyrethroids are mixed with kerosene. Synthetic pyrethroids such as allethrin or esbiothrin have been used almost all over the world and their use as repellents is considered safe. This view is supported by the fact that there have been no reports of health hazards associated with their use. Therefore, use of such lamps would be quite safe from the health point of view as only the technique of vaporizing the chemical has been simplified.

Ansari *et al.*[9] evaluated various mosquito repellent devices marketed in India and found that although most devices successfully prevented mosquito nuisance yet the repellent action of each device was variable and there was no device that could ensure 100% protection against various mosquito species tested.

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SHORT NOTE

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Screening of *Coptis teeta* Wall. for Antimalarial Effect: A Preliminary Report

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Keywords: Antimalarial, *Coptis teeta*, *P. berghei*, *P. falciparum*

The increase in isolates of *P. falciparum* resistant to chloroquine and conventional antimalarials has created a serious problem for the control and eradication of malaria[1,2].

The use of indigenous plant products for the treatment of malaria is a popular practice amongst the tribes of the north-eastern India. One of the most commonly used plant species for the treatment of malaria and many other febrile diseases is *Coptis teeta* Wall. (Ranunculaceae). According to practitioners of herbal medicine in Arunachal Pradesh the water extract of the rhizome possesses antimalarial properties. We evaluated the antimalarial property of this plant.

The plant material was collected from the reserve forest of Mayodia hills (*c.* 8000 ft above sea level), 56 km from Roing, Arunachal Pradesh. The plant was identified by the Horticulture Extension Officer at Roing.

The rhizomes were washed, dried and coarsely powedered, and the powder was extracted in water in a Soxhlet. The extracts were concentrated and lyophilized. Extracts of different concentrations were prepared at the time of the experiment. Cultures of *P. falciparum in vitro*, employing the candle jar method[3], and *P. berghei* (K 175) in mice were maintained in the laboratory.

The culture of *P. falciparum* was set with an initial count of 1% parasitized erythrocytes in tall glass vials in approximately 2.5 ml of RPMI 1640 complete medium. The

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Table 1. *In vitro* antimalarial activity of crude water extract of *Coptis teeta* against *P. falciparum*

	Control	Conc. of crude water extract (μg/ml)			
		66	132	660	1320
Schizonts that matured, No.	58	3	1	0	0
Schizonts that matured, %	-	5.17	1.72	0	0
Schizonts inhibited, %	-	94.83	98.28	100	100

No. of schizonts that matured are the average of four.

Table 2. *In vivo* antimalarial activities of crude water extract of *Coptis teeta* against *P. berghei* in mice

Control		Conc. of crude water extract mg/kg/day x 4											
a	b	3		10		30		100		300		1000	
		a	b	a	b	a	b	a	b	a	b	a	b
43.39		48.26		48.21		47.69		48.56		48.28		**	
51.66		48.39		47.38		48.22		48.03		47.96		**	
49.08	48.82	47.70	48.08	48.13	47.82	48.11	48.25	48.63	48.18	*		**	
48.31		48.11		46.80		48.22		47.41		*		**	
46.0		47.92		48.61		48.17		48.26 .		*		**	

*One mouse died on D2 and two on D3; **All five mice died on D2; a — % parasitaemia; b — Average % parasitaemia.

culture was treated, in quadruplicate, with selected concentrations of the extracts, this being the experimental, and the untreated, the control. After 24 h of incubation, blood smears were prepared and stained with Giemsa stain[4]. Percentage maturation of schizonts against control was recorded.

For monitoring the *in vivo* antimalarial activity, the 4-day suppressive test of blood schizontocidal action against *P. berghei* in mice was used[5]. Random-bred Swiss albino mice weighing 20 $\pm$ 2 g were selected and five mice in each cage were set up for experiment. The infected erythrocytes were injected intraperitoneally (10^7-10^8 parasitized red cells) on Day 0. The extracts of different concentrations of rhizomes were administered orally 3 h after inoculation. Further doses of the extract were given on Days 1, 2 and 3. On Day 4 tail blood smears were prepared, stained and per cent parasitaemia was recorded against the control group of mice.

The *in vitro* antimalarial activities against *P. falciparum* are given in Table 1. The table shows that the inhibition is proportional to the dose and that higher concentrations completely inhibit the parasitaemia.

Table 2 gives *in vivo* antimalarial activities against *P. berghei* in mice. Increase in concentration did not suppress the parasitic growth. The crude water extract of *Coptis teeta* was active *in vitro* against *P. falciparum*, with an IC_{50} value of about 8.8 µg/ml, but was not active against *P. berghei* in mice.

In a previous study, protoberberin alkaloids were monitored for *in vitro* antimalarial activity against *P. falciparum* and *in vivo* against *P. berghei* in mice[6]. None of the alkaloids was active *in vivo* but they showed *in vitro* activity. Berberin and its derivatives are the major constituents of *Copits teeta*, and our study corroborated the findings of the previous report. The antimalarial activities of different fractions of *Coptis teeta* are being evaluated.

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Corrigendum

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Insecticide Susceptibility Status of *Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti* in Panaji, Goa by Thavaselvam *et al.*

Page 78-Reference 4

Please read as

Subbarao, S.K., V.P. Sharma, K. Vasantha and T. Adak (1984). Effect of malathion spraying on four anopheline species and the development of resistance in *An. stephensi* in Mandora, Haryana. *Indian. J. Malariol.*, **21**: 109-114.

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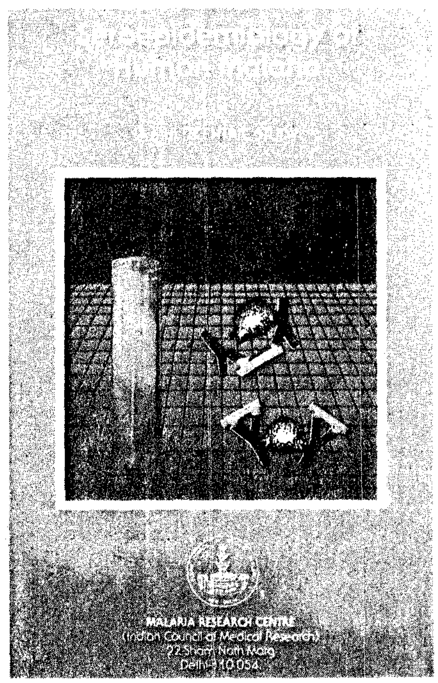

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