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Short Notes

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

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Anti-neutrophil Cytoplasmic Antibodies (ANCA) in Malaria

VANDANA PRADHAN[a], S.S. BADAKERE[a], U. SHANKARKUMAR[a], Y.S. IYER[a], K. GHOSH[a] and D. KARNAD[b]

Various autoantibodies like anti-nuclear antibodies (ANA), anti-double stranded DNA (anti-dsDNA), anti-histone antibodies (AHA), anti-neutrophil cytoplasmic antibodies (ANCA), anti-myeloperoxidase (anti-MPO), anti-proteinase3 (anti-PR3) and anti-lactoferrin (anti-LF) antibodies were studied in 173 acute hospitalised patients suffering from malaria of which 160 patients had *P. falciparum* and remaining 13 had *P. vivax* infection. Standard methods like indirect immunofluorescence (IIF) microscopy along with Confocal microscopy and ELISA were used for identifying and quantifying the autoantibodies and IIF patterns on PMN and HL-60 cells were studied for ANCA classification. Also HEp-2 cells were used for ANA detection, while estimation of anti-dsDNA, AHA, anti-MPO, anti-PR3 and anti-LF were tested using ELISA. Sera from malaria patients showed prominent immunofluorescence staining patterns where 23.8% cases had ANA in *P. falciparum* group as compared to 15.4% in *P. vivax* group and ANCA was found to be present in 20% in *P. falciparum* and 15.4% in *P. vivax* group. An interesting observation was that, of the total ANCA positives, 59% had p-ANCA, 5.9% had c-ANCA and 44.1% of the cases showed the 'atypical' or X-ANCA pattern. When p-ANCA positivity was compared with c-ANCA positivity among these patients, a good statistical correlation was noted with OR = 16, $\chi^2 = 16.43$, EF = 0.46 and p-value = 5.037E 0.5. ELISA showed 31.2% anti-MPO and 6.2% anti-PR3 in *P. falciparum* cases while the two ANCA positive cases in *P. vivax* had anti-MPO. Anti-LF was found to be present in 40.6% cases. Neither the *P. falciparum* nor *P. vivax* contained autoantibodies with specificities similar to the characteristic lupus autoantibodies such as double stranded DNA (dsDNA). ANCA positivity develops in some types of malarial infection also with the presence of various autoantibodies which is important from a clinical point of view and should be carefully evaluated in those geographic areas where malaria is endemic. It also alerts us to the fact, whether in cases of repeated malarial infections in susceptible individuals, vasculitic disorders, which through ANCA pathways develop, could lead to renal and other complications.

Keywords: Anti-neutrophil cytoplasmic antibodies (ANCA), Anti-nuclear antibodies (ANA), Autoantibodies, Malaria

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INTRODUCTION

Malaria now seems to be endemic in many parts of the world and is returning even to those countries from which it had been eradicated. Acute malaria is quite common in developing countries and even developed countries now have to deal with the problems associated with malarial infection because of extensive population movement from endemic areas. It is also a matter of great concern in the fast developing metropolitan cities in India, which are being trade and commercial centres, attract a large floating population. Nearly two million cases of malaria occur every year in India. WHO data indicate that over 40% of the world's population is under risk and about 300 million suffer from malaria every year, with 1 to 3 million deaths annually.[1] There is a rapid rising trend of *P. falciparum* infection from 20–40% and the ratio of *P. falciparum* to *P. vivax* malaria infection varies significantly throughout the country. The incidence, morbidity and mortality due to malaria infection are increasing and could also be attributed to drug resistance in strains of malarial parasite and resistance of vectors to insecticides.

Autoantibodies of many different types could be present in the sera of patients infected with malarial parasites. The autoantibodies detected include anti-lymphocyte antibodies, anti-RBC antibodies, anti-heart, anti-thyroid and anti-gastric parietal cell,[2–4] as well as anti-nuclear and anti-smooth muscle antibodies.[5–7] Even autoantibodies to intermediate filaments of the cytoskeleton were also identified in 82% of acute malaria patients by immunofluorescence[8] and circulating immune complexes were also reported in acute malaria patients.[9] Occurrence of autoantibodies of various specificities to neutrophil cytoplasmic granules (ANCA) have also been reported in patients with acute malaria indicating their possible role in vascular inflammation which may contribute to the pathogenic process in malaria.[10–12]

Objectives of this study were: to identify and quantitate various autoantibodies like anti-nuclear antibodies (ANA), anti-double stranded DNA antibodies (anti-dsDNA), anti-histone antibodies (AHA) and also anti-neutrophil cytoplasmic antibodies (ANCA) showing perinuclear, cytoplasmic and the rare 'X' ANCA patterns and to correlate by ELISA their subspecificities to myeloperoxidase (MPO), proteinase 3 (PR3) and lactoferrin (LF) in 173 acute malaria patients; and to study the presence and specificities of ANCA with clinical complications like renal, hematological, and hepatobiliary and CNS manifestations along with the control group in *P. falciparum* and *P. vivax* malaria.

MATERIALS AND METHODS

In this study we included 173 hospitalised acute malaria cases, of these 160 were *P. falciparum* infected cases and remaining 13 were infected with *P. vivax*. Age group of the patients ranged from 13–70 years with a mean of 29.9 ± 11.1 years in the *P. falciparum* group, whereas age group ranged from 15–80 years with a mean of 48.1 ± 14.9 years in *P. vivax* group. The diagnosis was based on the clinical examination of the patients and confirmed by the presence of plasmodia seen in thick peripheral blood smears stained with Giemsa. The parasite densities of

all these cases were 2% but anti-malarial antibody profile could not be obtained in them. A detailed proforma sheet was maintained to record the clinical manifestations of the patient. The presence of either cerebral, renal, hepatobiliary or hemolytic manifestations were carefully recorded as complicated malaria. All patients were on standard treatment of a 14 day course of quinine.

This study was conducted with the Ethical Committee permission and the appropriate written consent was obtained from each patient. Blood (4–5 ml) was aseptically collected from each patient and the separated serum was divided in two parts and frozen at –80°C until use. The serological investigations were completed within a month of blood collection. An indirect immunofluorescence test was used for the detection of anti-nuclear antibodies (ANA) using cultured HEp-2 cells as a substrate,[13–15] and various patterns were noted by indirect immunofluorescence microscopy (IIFM). The cut-off for ANA was at a 1: 20 dilution for positivity.

The initial identification of anti-neutrophil cytoplasmic antibodies (ANCA) was done by IIF test which was considered as the 'Gold Standard' for ANCA testing[16–18] using human polymorphonuclear cells (PMN) and human promyelocytic leukemic cells (HL-60) obtained from National Centre for Cell Sciences (NCCS), Pune, and maintained in a continuous culture and harvested at log phase of growth. The cells were used to prepare a cytospun substrate using a cytocentrifuge (Hettich Universal, 16 A, Germany) and one set of the slides was fixed with chilled 96% ethanol and the other with formalin, before treating with patient's sera. Slides were probed using fluovescien isothiocynate (FITC) tagged polyvalent anti-human globulin sera. The cut-off for positivity was at a 1: 20 dilution and the fluorescence patterns were noted using a fluorescence microscope (Nikon, Optiphot II, Japan) and microphotography was done using an automated photography system (Nikon, AFX IIA, Japan). Various ANCA patterns such as perinuclear (p-ANCA), cytoplasmic (c-ANCA) and atypical (X-ANCA) were evaluated. Positive control sera were obtained from ANCA investigators from Germany, Denmark and Hongkong and were used as and when required. All the positive sera were diluted further by double dilution technique and the same test protocol was followed to know the titration end point—the highest dilution of test serum showing a positive result. Commercially available imported ELISA kits were used to detect ANCA specificities like anti-myeloperoxidase (anti-MPO) and anti-proteinase3 (anti-PR3) using 1:50 diluted sera. A cut-off value for identifying positives and negatives was used according to manufacturer's instructions. ELISA methods for the detection of anti-dsDNA, anti-histone and anti-lactoferrin were standardised in our laboratory using the standard ELISA protocols.[19–21] The positive and negative control sera required for each assay were obtained from Novamed, Israel. An ELISA was also standardised for the detection of ANCA specificities other than anti-MPO and anti-PR3, using an 'α-granule' preparation.[22] Optical densities (OD) of end point absorbance results were read using a 405 nm filter on ELISA reader (Titertek, USA). The OD values above the NHS ± 3SD—OD > 0.45 was considered as cut-off and OD above this range was con-

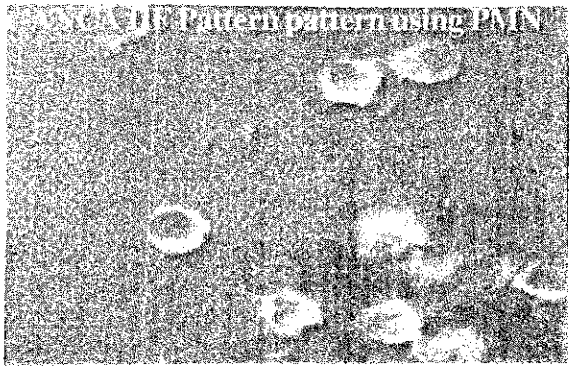
sidered as positive. A control group comprising of 100 age and sex matched normal healthy blood donors without previous history of malaria were included in this study.

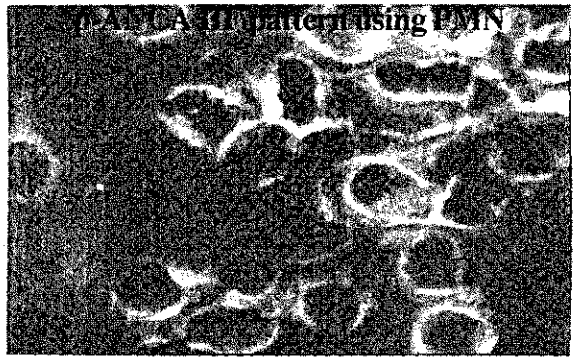
RESULTS

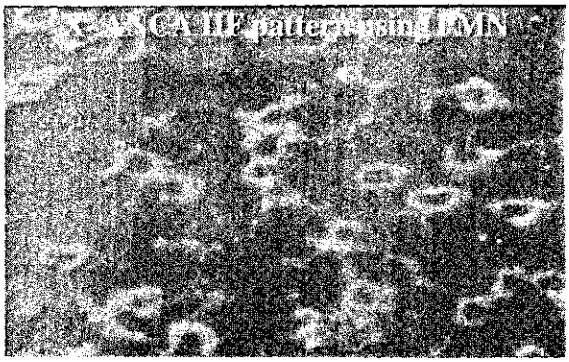
Out of 160 *P. falciparum* infected patients, 38 patients had complicated malaria and 122 were without any complications. In the 38 complicated malaria cases various clinical manifestations were observed — 31 patients (81.6%) had renal complications like nephrotic syndrome or hemoglobinuria, 20 patients (52.6%) had hepatobilliary manifestations like hyperspleenism and/or clinical jaundice, and 14 patients (36.8%) showed hematological abnormalities such as anaemia, severe thrombocytopenia or acute intravascular hemolysis. There was an overlap of clinical manifestations also and there was no patient with cerebral manifestations.

ANA positivity was found to be 23.8% (38/160) in *P. falciparum* group as compared to 15.4% (2/13) in *P. vivax* group (Table 1). Controls had a low incidence of 4% positives. None of the cases had anti-dsDNA antibodies, while there were 10 cases showing AHA of which 8 were in *P. falciparum* with complications, where 6 had renal and 2 had hematological complications.

ANCA testing by immunofluorescence microscopy showed that PMN was a better substrate for ANCA detection as compared to HL-60, as in the p-ANCA group (Table 2), of the 15 cases detected by PMN only 13 cases were picked up using HL-60 cells and in the X-







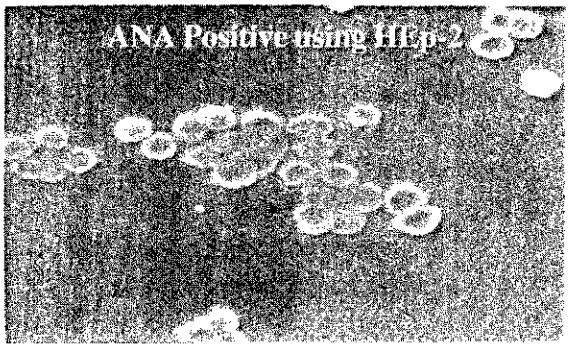


Table 1. Autoantibodies in *P. falciparum* and *P. vivax* acute malaria

Type of malarial infection	No. tested (173)	ANA* (40)	AHA (10)	ANCA (34)	Anti-MPO (12)	Anti-PR3 (2)	Anti-LF (13)
P. falciparum complicated	38	12	8	18	10	2	6
Renal	31	8	6	10	7	2	5
Hematological	14	2	2	5	0	0	1
Hepatobiliary	20	2	0	3	3	0	0
Uncomplicated	122	26	2	14	0	0	7
P. vivax	13	2	0	2	2	0	0
Controls	100	4	0	3	3	0	0

*Anti-dsDNA was not seen in any of the cases.

Table 2. ANCA by immunofluorescence microscopy

Type of malarial infection	No. tested	No. +ve By IIF	p-ANCA*		c-ANCA*		X-ANCA	
			PMN	HL-60	PMN	HL-60	PMN	HL-60
P. falciparum	160	32	15	13	2	2	15	10
P. vivax	13	2	2	2	0	0	0	0
Total	173	34	17(50%)		2(5.9%)		15(44.1%)	

*Patterns of immunofluorescence changed from perinuclear to granular cytoplasmic after formalin fixation p-ANCA vs c-ANCA (OR = 16; χ^2 = 16.43; EF = 0.46; p-value = 5.037E0.5).

ANCA group of the 15 detected by PMN, 10 were detected using HL-60 cells. ANCA were found to be present in 20% of *P. falciparum* and 15.4 % of *P. vivax* groups while controls showed 3% positivity for ANCA. Among 34 ANCA positive patients (50%) had p-ANCA, 2 patients (5.9%) showed classic c-ANCA pattern, while the atypical X-ANCA or the 'snowdrift' pattern though rare was more commonly seen in 15 patients (44%). When p-ANCA positivity was compared with c-ANCA positivity among these patients, a good statistical correlation was noted with OR = 16, χ^2 = 16.43, EF = 0.46 and p-value = 5.037E 0.5. All IIF ANCA positive patients were also confirmed by ELISA (Table 3) and it was seen that in *P. falciparum* group, there were 10 anti-MPO, 2 anti-PR3 and 13 anti-LF while a lower incidence of only 2 cases in *P. vivax* was seen having anti-MPO, whereas anti-PR3 and anti-LF were absent. Most of the antibodies were IgG type, only 4 in *P. falciparum* group being

Table 3. ANCA testing by ELISA

Type of infection (n)	No. IIF positives	α-granules				Anti-MPO	Anti-PR3	Anti-LF
		Positives	IgM	IgG	IgG+M			
P. falciparum (160)	32 (20%)	32	4	28	–	10	2	13
P. vivax (13)	2 (15.4%)	2	–	2	–	2	–	–
Total (173)	34 (19.4%)	34	4	30	–	12	2	13

(n) = No. of cases.

IgM-anti-MPO and there was no anti-IgG+M ANCA. The cases with renal involvement showed 32.2% ANCA of which majority had anti-MPO positivity and in those with hematological complications, 35.7% ANCA positivity was seen, while a low incidence of 15% was seen in malaria with hepatobiliary manifestations. While the uncomplicated malaria cases mostly with *P. vivax* infection had a low incidence of only 4.9% ANCA positivity.

DISCUSSION

Various autoantibodies have been reported in patients with malarial infection.[1,2,4,12] We have observed an incidence of 23.8% ANA positivity in *P. falciparum* cases as compared to 15.4% in *P. vivax* infected cases. Incidence of ANA positivity in malaria cases from some countries like west Africa was much lower 16%, while in Thai patients it was 35%[23] and in a mixed group of Caucasians, Asians and Africans it was 39.5%.[7] Phanuphak *et al.*[24] encountered 35 cases of complicated malaria comprising of 19.1% of the total tested and observed 51.4% ANA positivity, while in the uncomplicated cases 43.9% were ANA positive. The ANA positive patients did not show presence of anti-dsDNA. Similar observations were also reported by Phanuphak *et al.*[24]. The development of ANA could be either due to cross reaction with the nuclear antigens of the malarial parasite or with the host cells such as hepatocytes altered by the malarial infection, or it could be due to the general phenomenon of polyclonal B cell activation by mitogen. Some common antibodies seen in SLE like anti-dsDNA were found to be absent which probably suggests that the inducing stimuli are different for malaria and SLE.

An incidence of 20% positivity of ANCA in acute *P. falciparum* and 15.4% in *P. vivax* malaria was observed. A lower incidence of ANCA 13.3% was observed by Wenisch *et al.*[11] in acute *P. falciparum*. Yahya *et al.*[12] had studied 93 acute malaria cases and observed 50.5% positivity of ANCA by IIF, of which 46% showed atypical or X-ANCA. It was also

observed a higher incidence of ANCA (47.4%) in *P. falciparum* patients having clinical complications.

We have also observed 50% patients showing p-ANCA, 5.9% showing c-ANCA and 44.1% showing X-ANCA by immunofluorescence using PMN, though HL-60 showed a lower incidence due to the fact that PMN contain primary as well as secondary cytoplasmic granules while HL-60 contain only the primary granules. Though the IIF technique is a good screening technique, the specificity to different cytoplasmic antigens can only be determined by ELISA. However, the α-granules ELISA used in this study is known to detect antibodies against many neutrophil cytoplasmic antigens and a positivity in such an ELISA indicates the presence of autoantibodies to some antigens which could also be other than to MPO and PR3.

ELISA showed 31.2% anti-MPO and 6.2% anti-PR3 in *P. falciparum* cases while the two ANCA positive cases with *P. vivax* had anti-MPO, and anti-LF were seen in 40.6% patients. Esnault *et al.*[21] have mentioned that all anti-LF positive sera, but not anti-MPO positive sera, also show ANA with specificities for histone and have commented that this could be a subgroup of patients with systemic vasculitis, or these patients have systemic vasculitis without histological evidence of immune complex deposits. Hypothetically anti-LF antibodies could have a pathogenic importance by counteracting the anti-inflammatory effect of Lactoferrin thus aggravating and prolonging the inflammatory process.[25]

Probably ANCA also develops after an infection or an inflammatory stimulus, as an antigen nonspecific immunological derangement in malarial infection is also known to cause autoantibody formation. Therefore, the presence of ANCA could be a secondary response and our observations indicate that malaria infection may induce or predispose the development of such autoantibodies as a secondary immune response. The four cases of IgM ANCA seen in the present study indicate a recent sensitisation and development of autoantibodies to MPO.

We can also summarise that in a malaria endemic area when evaluating a patient with suspected vasculitic disorder, due consideration should be given to the fact that ANCA positivity could be a serological reaction related to the malarial infection. Vasculitic lesions related to falciparum malaria had also been described previously in pre ANCA era[26,27] in malaria and it is quite possible that in at least some of these cases, ANCA with immune complexes might have played a role in its etiopathogenesis but also it needs to be seen whether repeated malarial infection in a susceptible individual can also produce vasculitic disorders through the production of ANCA pathway leading to glomerulonephritis or post infectious encephalomyelitis. It could be also interesting to see how long these autoantibodies would persist with out reinfection and also to see whether the antibody negative cases would remain so with recurrent infections.

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Prevalence of Asymptomatic Malaria Parasitaemia amongst Pregnant Women

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Two hundred and forty-six apparently healthy pregnant women aged 19–40 years, without symptoms were recruited (147 recruited during the dry season and 99 recruited during the rainy season) for the present study. Blood examinations for malaria parasites, *Plasmodium falciparum* specific-IgG concentration and serological reactivity with *P. falciparum*-histidine rich protein-2 (HRP-2) antigens were conducted on all the pregnant women during the dry and rainy seasons of the year. During the dry season, 109 (74%) of the recruited pregnant women without symptoms had *P. falciparum* parasitaemia, while 79 (80%) of the recruited pregnant women without symptoms had *P. falciparum* parasitaemia during the rainy season. However, the *P. falciparum* malaria parasites density was significantly raised during the dry season compared with that of in the rainy season ($p<0.05$). Serological analysis with *P. falciparum* histidine rich protein-2 antigen (HRP-2) showed 108 (73%) and 71 (77%) of the pregnant women without symptoms as seropositive during the dry and rainy seasons respectively. The *P. falciparum* specific-IgG concentration was similar during both seasons in the HRP-2 seropositive pregnant women without symptoms ($p>0.05$). The results showed no seasonal tide in the incidences of asymptomatic *P. falciparum* parasitaemia; however, the significantly raised parasitaemia during the dry season may suggest possible increased parasites tolerance. The *P. falciparum* specific-IgG concentration during both seasons may not be the primary effector mechanism offering tolerance in asymptomatic parasitaemia in pregnant women.

Keywords: Antibody, HRP-2, Parasitaemia, Prevalence, Seasons

INTRODUCTION

In malaria endemic areas, naturally acquired immunity against malaria is not sterile but is usually associated with parasitaemia.[1–3] This protective immunity is responsible for the high prevalence rate of asymptomatic malaria parasitaemia in adults without symptoms residing in

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malaria endemic areas.[3] Studies of asymptomatic malaria parasitaemia in malaria endemic areas have been focused on children,[4–8] and non-pregnant adults[2, 9–12] with no appreciable study of such in pregnant women without symptoms. Similarly, the pattern of transmission of malaria over a year in Nigeria and other endemic areas had been classified as peak and low transmission seasons based on reports from studies of symptomatic malaria but not due to studies from asymptomatic malaria. Therefore, the present study was designed to screen for the presence of asymptomatic malaria parasitaemia and malaria protective antibody amongst asymptomatic pregnant women and to observe if the pattern of its transmission is similar to that already reported from clinical cases of malaria. In pursuit of this study, pregnant women with no sign and symptom of malaria reporting at the Antenatal clinic,Obstetric and Gynaecology Department, University College Hospital, Ibadan, Nigeria were randomly recruited for the study during both the rainy and dry seasons in Nigeria.

MATERIALS AND METHODS

Subjects: Pregnant women without any sign and symptom, reporting for Antenatal clinic at Obstetric and Gynaecology Department, University College Hospital, Ibadan, Nigeria were recruited for the present study. The mean age (±SD) of the pregnant women were (30±10 years). One hundred and forty-seven of the pregnant women were recruited during the dry season (November to march) while ninety-nine of the pregnant women were recruited during the rainy season (September to October). After careful examination, blood samples were collected from the pregnant women for malaria parasite screening, *P. falciparum* specific-IgG estimation and *P. falciparum* histidine rich protein (HRP-2) seroprevalence. The pregnant women gave informed consent and the UI/UCH Board of Ethical Committee, Postgraduate Institute for Medical Research, College of Medicine, UCH, Ibadan, approved the study design.

Asymptomatic malaria parasites screening

This was done by microscopic examination of Giemsa stained thick blood film for malaria parasite detection as described by Rooth *et al.*[13] The malaria parasite density was reported as number of parasites per micro litre of blood.

Estimation of *P. falciparum* specific-IgG concentration

The *P. falciparum* specific-IgG concentration was measured by indirect enzyme-linked immunosorbent assay (ELISA) technique as described in malaria IgG CELISA kit (Cellabs Pvt. Ltd., Australia). About 100 µl of diluted (1 in 100) samples, reference positive and negative controls were added into *P. falciparum* antigen coated wells. This was followed by incubation at 37°C for 60 min, washing with phosphate buffered saline-tween solution, and addition of 100 µl of conjugate antibody with further incubation at 37°C for 60 min. The amount of colour formed after the addition of the enzyme substrate was proportional to the concentration of *P. falciparum* specific-IgG in the respective samples.

Detection of *P. falciparum* histidine rich protein-2

The *P. falciparum* malaria antigen-histidine rich protein-2 (HRP-2) was detected based on indirect enzyme-linked immunosorbent assay (ELISA) technique as described in malaria CELISA kit (Cellabs Pvt. Ltd., Australia). About 100 µl of freeze thawed blood samples; reference positive and negative controls were added into monoclonal anti-*P. falciparum* histidine rich protein-2 coated micro wells. This was followed by incubation at 37°C for 60 min, washing with phosphate buffered saline-tween solution, and addition of 100 µl of conjugate antibody with further incubation at 37°C for 60 min. The colour formed after the addition of the enzyme substrate indicates the presence of malaria antigen in the blood samples under investigation.

Statistical analysis

The prevalence of malaria parasitaemia amongst the asymptomatic pregnant women was expressed as percentage.The level of significance of the parametric variables were assessed using the Student's t-test while chi-square was used to determine the level of significance for the non-parametric variables. The p-values < 0.05 were considered significant.

RESULTS

The incidence rates of *P. falciparum* malaria parasite during the dry and rainy seasons were 74 and 80% respectively. There is just a 6% difference in incidence rate between the two seasons (Table 1). The malaria parasites density expressed as number of parasites per microlitre of blood was raised in asymptomatic pregnant women with positive malaria blood films during the dry season rather than during the rainy season ($p<0.05$).

Furthermore, the seroprevalence test for *P. falciparum* histidine rich protein-2 in pregnant women showed that 73 and 77% were seropositive for this antigen during the dry and rainy seasons respectively. There is 4% difference

Table 1. Incidence rates and malaria parasites density in asymptomatic parasitaemic and aparasitaemic pregnant women during the dry and rainy seasons

Varaibles	Asymptomatic parasitaemic incidence rate (%)	Asymptomatic parasitaemic malaria parasites density (Parasites/µl of blood)	Aparasitaemic incidence rate (%)
Dry season (n = 147)	74 (n = 109)	450 ± 343 (n = 109)	26 (n = 38)
Rainy season (n = 99)	80 (n = 79)	367 ± 280 (n = 79)	20 (n = 20)
p-value	6 (p > 0.1)*	< 0.05	6 (p > 0.1)*

6 = * Per cent difference in incidence rate between the dry and rainy season.

Table 2. HRP-2 seroreactivity and *P. falciparum*-IgG concentration in HRP-2 seropositive (asymptomatic parasitaemic) and HRP-2 seronegative (aparasitaemic) pregnant women during the dry and rainy season

Varibales	HRP-2 seropositive (Asymptomatic parasitaemic)	HRP-2 seronegative (Aparasitaemic)	$^{+}$Pf IgG level in HRP-2 sero-positive (Asymptomatic parasitaemic)	$^{+}$Pf IgG level in HRP-2 sero-negative (Aparasitaemic)	$^{+}$p-value
Dry season (n = 147)	73%	27% (n = 39)	0.56 ± 0.15 (n = 108)	0.65 ± 0.21 (n = 39)	<0.05
Rainy season (n = 99)	77% (n = 71)	23% (n = 21)	0.58 ± 0.18 (n = 79)	0.52 ± 0.23 (n = 21)	>0.05
p-value	4 (p > 0.1)*	4 (p > 0.1)*	>0.05	<0.05	

4 =*Per cent difference in HRP-2 reactivity; $^{+}$Comparison between both groups.

in seroprevalence between the two seasons (Table 2). However, the *P. falciparum* specific-IgG concentration was similar in HRP-2 seropositive asymptomatic pregnant women during both the dry and rainy seasons of malaria transmission ($p>0.05$). The *P. falciparum* specific-IgG concentration was higher in HRP-2 seropositive asymptomatic pregnant women than in the HRP-2 seronegative pregnant women during the dry season of malaria transmission ($p<0.05$), but such difference in *P. falciparum* specific-IgG concentration was not noticeable during the rainy season of malaria transmission ($p>0.05$).

DISCUSSION

The prevalence of asymptomatic malaria parasitaemia amongst pregnant women was uniformly spread throughout the year in Nigeria. Thus suggesting that seasons did not affect the prevalence of asymptomatic malaria parasitaemia as suggested for symptomatic malaria. The 4–6% difference in incidence rate of asymptomatic malaria parasitaemia amongst pregnant women between the dry and rainy seasons did not indicate any seasonal peak in incidences of asymptomatic malaria parasitaemia amongst pregnant women.

However, the malaria parasites density was significantly different during both the seasons. The raised malaria parasites density during the dry season may indicate higher tolerance to malaria parasites during this period or prolonged retention of infection. The likely reason for the difference in parasites density in asymptomatic pregnant women between both the seasons is not known. Although studies by Krajden *et al.*[14] and Frank *et al.*[15] have reported that untreated malaria infections can persist for a considerable period of time reaching 18 months

even in the absence of exposure to re-infection.

Achidi *et al.*[2] in their study on asymptomatic parasitaemic blood donors observed significantly raised malaria parasite prevalence during the peak (rainy) season but a significantly lowered malaria parasites density during the same season of malaria transmission in Nigeria. The report on malaria parasites density by Achidi *et al.* agree with that of the present study, however, the report on prevalence is in variance with that of the present study. The difference in prevalence rate may be attributed to different sample populations used for the separate studies and the possible role of the physiologic impact during pregnancy could play. This could suggest existence of separate prevalence rate for different population sets within the country. The seroprevalence of HRP-2 was similar to that of malaria parasites density prevalence amongst the pregnant women for both seasons. Thus suggesting no seasonal peak in asymptomatic parasitaemia and possible usefulness of HRP-2 for malaria screening.

Studies elsewhere had successfully used HRP-2 for *P. falciparum* screening.[2,3,16–19] The *P. falciparum* specific-IgG concentrations in the HRP-2 seropositive pregnant women during both seasons of malaria transmission are similar. This may suggest that the protective antibody concentration required to maintain asymptomatic state in malaria parasites infection is fairly constant. However, it seems likely that for the same level of protective antibody, malaria parasitaemia tend to be greater during the dry (November–April) season in Nigeria.

Finally, the incidence of asymptomatic malaria pariasitaemia and *P. falciparum* specific-IgG concentration in the pregnant women did not show any seasonal variation. Thus, factors enhancing higher parasites density tolerance during the dry seasons in pregnant women needs investigation.

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Operational Feasibility and Bio-efficacy of Alphacypermethrin (Fendona) Treated Jute Curtains to Control Urban Malaria in a Slum Settlement of Delhi, India

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Operational feasibility and bio-efficacy of alphacypermethrin treated curtains was evaluated in slum settlements of Andrews Ganj, New Delhi. Jute curtains treated with alphacypermethrin @ 100 mg/m^2 were fixed on windows, doors and eves before on set of transmission and evaluations were carried out for two years. Entomological evaluation revealed that jute curtains treated with alphacypermethrin in Slum-I resulted drastic reduction in daytime indoor resting mosquitoes such as *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. Similarly, malaria incidence also reduced in the treated curtain used area when compared to that of in untreated curtain and without curtain areas. Bioassay tests on *An. stephensi* and *Cx. quinquefasciatus* showed that alphacypermethrin could produce > 70 per cent mortality up to six months in case of *An. stephensi*—a principal urban malaria vector and hence two rounds of treatment are sufficient in an year to protect inhabitants from malaria. The study indicates alphacypermethrin treated curtains could curtail malaria transmission in slum settlements and is operationally feasible in small houses and is also cost-effective.

Keywords: Alphacypermethrin, *An. culicifacies,* Insecticide treated curtains, Malaria control

INTRODUCTION

Insecticide impregnated bednets and curtains are of growing concern as personal and community protection measures against anopheline vectors in most malaria regions of the world.[1] Insecticide treated curtains (ITCs) is the most appropriate and feasible method to contain mosquito menace in urban environment in terms of usage and social acceptability.[2] Few studies

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were carried out both in laboratory and field using various insecticides such as deltamethrin, cyfluthrin, lambdacyhalothrin, permethrin, etc. and different types of curtains such as jute, cotton, polyester, nylon, etc. and demonstrated their usefulness in reducing the malaria morbidity and mortality.[2-6] Trials carried out in India and China with different synthetic pyrethroids provided encouraging results.[7] Insecticide treated mosquito curtains are of great use in urban and peri-urban areas involving the community to reduce the reliance on chemically hazardous indoor residual spraying carried out in the blanket manner. Very few reports are available on the efficacy of alphacypermethrin in controlling mosquito nuisance. In view of this the present study was undertaken to evaluate the bio-efficacy of a new synthetic pyrethroid—alphacypermethrin commonly known as Fendona against *An. stephensi* which is a principal vector of urban malaria in India and its impact on malaria incidence. Results of two years field trials are reported in this paper.

MATERIALS AND METHODS

Study area

JJ cluster slums I, II and III located in Andrews Ganj area of south Delhi at a distance of about 18 km from the Centre were selected for the present study. The population of JJ cluster slums I, II, and III was 1350, 1500 and 1410 distributed in 225, 250 and 235 huts respectively, made up of brick wall/mud wall with either iron sheet roof or thatched roof. These dwellings were 3 x 2 x 3 m structures with one window and a main door. Human dwelling huts without window had eves for ventilation. The study sites were closely monitored for a period of seven months and experimental sites were selected for testing the efficacy of alphacypermethrin. Slum-I was taken as experimental and treated jute curtains were fixed on doors, windows and eves of the huts/houses. In Slum-II only plain jute curtains were fixed. In Slum-III no curtains were used.

Methodology

Standard procedure was used for the treatment of jute curtains, which is briefly described below.

Insecticide and type of curtain: Alphacypermethrin 10 per cent SC formulation as per World Health Organization specifications (WHO/IS/98.1.3) was obtained through the courtesy of M/s.Cyanamid Agriculture Limited. Jute cloth (5.5 x 5.5 mm mesh hole size with 322 horizontal x 288 vertical/m^2 with a weight of 160 g/m^2) used in slum area was also supplied by the sponsoring agency.

Treatment of hessian (jute) curtain by dipping method: Absorption rate of the fabric was calculated using water in a bucket and the mean (10 replicates) absorption rate was found to be 350 ml/m^2. Later the target dose and volume of the solution required for 1 m^2 jute curtain was calculated by following formula.

$$\text{Volume required} = \frac{\text{Target dose x surface area x 100}}{\text{\% solution of the insecticide}}$$

Procedure

Dry and clean curtain piece (1 m^2) was put in a known quantity of water-insecticide solution to achieve the target dose. The cloth was squeezed and released repeatedly so that the solution reaches evenly to the entire fabric. After treatment, the fabric was dried under shade. Drying was done in two stages. In the first stage of drying, fabric was laid down on non-absorbent surface such as polythene sheet to become semi-dry for two hours, thereafter suspended on line to let it air dry. For control, the jute curtain of same measurement was soaked in tap water and dried as per the procedure described above.

Entomological evaluation

Daytime indoor resting density of mosquitoes was monitored fortnightly in experimental and control areas with the help of suction tube and flashlight for 15 min in selected huts. Collected specimens were identified in the laboratory and the man hour density (MHD) was calculated. Adults collected from experimental and control areas were dissected for gut and gland infection of the malaria parasite.

Epidemiological evaluation

Active fever cases were detected by door-to-door surveillance on weekly basis. All positive cases were given radical treatment as per NAMP schedule. Data were pooled monthly and SPR, SfR, cases/000 and *Pf*/000 were calculated as per standard formulae.

Laboratory bioassays

Bioassay tests were performed on 3–5 days old blood-fed female mosquitoes following WHO standard bioassay procedures. The treated jute curtains were fixed on cardboard with the help of drawing pins. Plastic cones as supplied by WHO, were fixed with adhesive tape on treated fabric of net. Fifteen female mosquitoes were released in each cone. Mosquitoes were put in cone with the help of suction tube and exposed for 15 min. Later they were kept in holding tubes vertically and a cotton wool swab soaked in 10 per cent glucose solution was plugged in the opening. In the next 24 hours a vigil was kept and the mortality of the mosquitoes, if any, was recorded as a post-exposure sequel. All the tests were replicated 10 times and carried out at 25 ± 2°C and 70 ± 10 per cent relative humidity. Untreated jute curtains were used concurrently as control. Tests were repeated when control mortality was 20 per cent or more. Corrected mortality was calculated as per Abbott's formula described hereunder.[8]

$$\text{Corrected \% mortality} = \frac{\text{\% observed mortality} - \text{\% control mortality}}{100 - \text{\% control mortality}} \times 100$$

Laboratory bioassay experiments were performed at monthly intervals to assess the effect of storage of nets after treatment with the insecticides. The experiments were carried out continuously for about 24 months. Field bioassay tests were performed fortnightly at the onset of first and second treatments.

RESULTS

Impact of alphacypermethrin treated jute curtains on mosquito density

Man hour densities of different mosquitoes obtained in different slums is shown in Table 1 and MHD of *An. stephensi*, a principal malaria vector is plotted in Fig. 1. It is revealed from the table that predensity of different mosquitoes was more or less comparable in all the three slum settlements, but installation of treated jute curtains in slum-I resulted drastic reduction in daytime indoor resting density of all mosquitoes particularly *An. stephensi*. The mean monthly density of *An. stephensi* was 2.2 before introduction of curtains, and reduced to nil immediately after introduction and remained nil up to March 2000. Similarly, the density of *Ae. aegypti* was 33 before fixing the curtains, which reduced to two by the end of the year. The predensity of *Cx. quinquefasciatus* was 106.2 and reduced to 11.2 within five months. Similar trend was observed after the second treatment. Contrary to this, the density of all mosquito species was much higher in slum-II (untreated jute curtain area) and without curtain area.

Impact of alphacypermethrin treated jute curtains on malaria incidence

Prevalence of malaria in slum-I, II and III is shown in Table 2 and cases/000 and *Pf*/000 are plotted in Fig. 2. It is clear from the data that malaria cases were more or less same in all the three slum localities before the installation of curtains. Introduction of alphacypermethrin treated curtains in slum-I resulted in drastic reduction in malaria cases. The mean monthly SPR

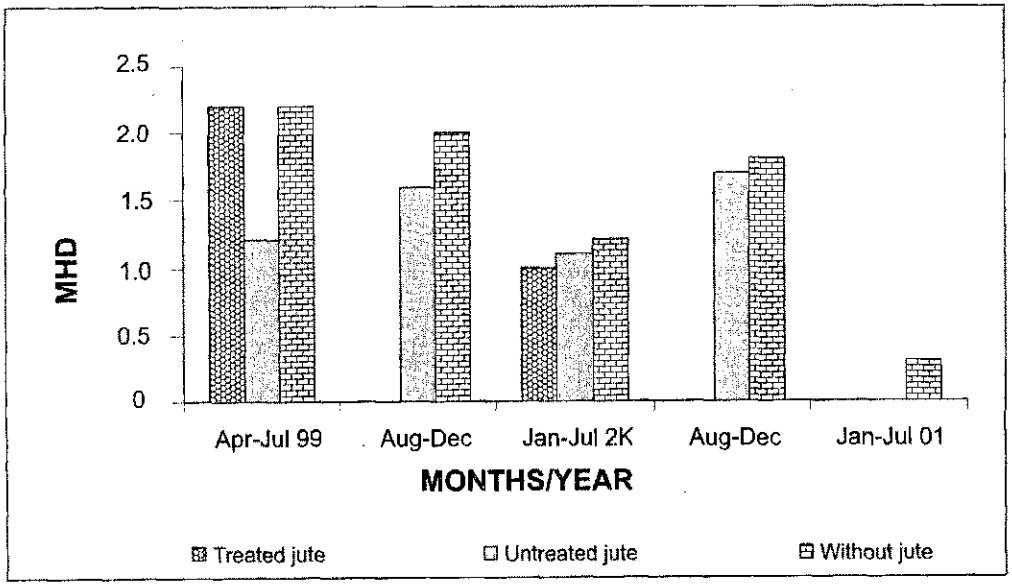

Fig.1: Man hour densitiy of *An. stephensi*, an urban malaria vector in control and experimental slums

Table 1. Density of adult mosquitoes per structure in the

Month/ Year	Treated jute cloth (Slum-I)				Untreated jute cloth (Slum-II)	
	Anopheles	*An. stephensi*	*Aedes*	*Culex*	*Anopheles*	*An. stephensi*
Pre-treatment density						
Apr 1999	3±0.3	2±0.2	40±2	210±5	2±0.2	1±0.1
May	4±0.5	3±0.2	55±2.5	65±2.5	3±0.2	2±0.2
Jun	2±0.2	2±0.2	9±0.5	40±2	1±0.1	1±0.1
Jul	2±0.2	2±0.2	28±4	110±5	3±0.3	1±0.1
Total/Av.	11(2.7)±1.2	9(2.2)±0.8	132(33)±13	425(106)±0.05	9(2.2)±0.2	5(1.2)±0.1
Post-treatment density						
Aug (I treatment)	0	0	3±0.2	4±0.5	4±0.5	2±0.3
Sep	0	0	5±0.5	10±1	5±0.5	3±0.2
Oct	0	0	2±0.2	11±1.5	3±0.2	2±0.2
Nov	0	0	0	22±2	1±0.1	1±0.1
Dec	0	0	0	9±0.5	0	0
Total/Av.	0(0)	0(0)	10(2)±0.9	56(11.2)±0.5	13(2.6)±1.3	8(1.6)±0.1
Jan 2000	0	0	0	22±1	0	0
Feb	0	0	0	40±5	0	0
Mar	0	0	0	70±5	1±0.1	1±0.1
Apr	2±0.2	2±0.2	12±1	90±5	3±0.5	2±0.2
May	3±0.2	2±0.2	18±1	120±10	1±0.1	1±0.1
Jun	1±0.3	1±0.1	4±0.5	45±2.5	2±0.2	2±0.2
Jul	3±0.5	2±0.1	22±1	60±5	3±0.3	2±0.2
Total/Av.	9(1.2)±0.6	7(1)±0.5	56(8)±3.5	447(63.8)±3.5	10(1.4)±0.8	8(1.1)±0.4
Aug (II treatment)	0	0	0	1±0.1	3±0.5	2±0.2
Sep	0	0	1±0.1	0	4±0.5	3±0.3
Oct	0	0	2±0.2	3±0.2	2±0.2	2±0.1
Nov	0	0	1±0.1	6±0.5	0	0
Dec	0	0	0	11±1.5	0	0
Total/Av.	0(0)	0(0)	4(0.8)±0.4	21(5.2)±2.1	9(2.2)±1.2	7(1.7)±0.3
Jan 2001	0	0	0	24±2	0	0
Feb	0	0	0	36±2	0	0
Mar	0	0	0	52±4	0	0
Total/Av.	0(0)	0(0)	0(0)	112(37.3)±0	0(0)	0(0)

Figures in parentheses indicate average density of mosquitoes. Jute curtains were treated with alphacyper-

slums of Andrews Ganj, New Delhi

Month/ Year	Untreated jute cloth (Slum-II)		Without jute cloth (Slum-III)			
	Aedes	*Culex*	*Anopheles*	*An. stephensi*	*Aedes*	*Culex*
Pre-treatment density						
Apr 1999	30±2	180±10	4±0.5	3±0.3	20±2	172±6
May	45±2.5	80±5	5±0.5	3±0.3	35±2.5	55±2.5
Jun	12±1	32±4	1±0.1	1±0.1	7±0.5	28±2
Jul	35±2.5	80±5	3±0.3	2±0.2	15±1.5	72±4
Total/Av.	122 (30.5)±3	372 (93)±6	13 (3.2)±1.2	9 (2.2)±0.7	77 (19.2)±1.5	327 (81.7)±4.5
Post-treatment density						
Aug (1 treatment)	44±2	70±5	6±1	4±0.5	51±4.5	76±2.6
Sep	60±5	90±5	7±0.5	3±0.3	72±4	110±4.5
Oct	72±4	70±5	4±0.5	2±0.2	80±5	65±3.8
Nov	50±3	80±5	2±0.2	1±0.1	40±2	84±4.8
Dec	9±0.5	30±3	0	0	12±1	42±2.4
Total/Av.	235 (47)±10.5	340 (68)±23	19 (3.8)±2.2	10 (2)±0.7	255 (51)±2.5	377 (75.4)±4.5
Jan 2000	0	32±4	0	0	0	36±2
Feb	0	48±4	0	0	0	56±8
Mar	0	90±5	1±0.1	1±0.1	0±0	120±10
Apr	18±1	110±5	2±0.2	2±0.2	20±2	132±6
May	24±5	130±5	2±0.2	2±0.2	28±2	140±10
Jun	9±0.5	55±2.5	1±0.1	1±0.1	11±1.5	50±5
Jul	27±1.5	70±5	4±0.5	3±0.3	30±3	84±6
Total/Av.	78 (11.1)±2	535 (76.4)±22.5	10 (1.4)±0.3	9 (1.2)±0.3	89 (12.7)±8.5	618 (88.2)±11
Aug (II treatment)	35±2.5	55±2.5	5±0.5	3±0.5	40±3	35±2.5
Sep	45±2.5	70±5	6±1	2±0.2	52±4	55±7.5
Oct	70±5	80±5	8±1	4±0.5	65±2.5	60±5
Nov	15±1.5	90±5	1±0.1	0	18±1	84±8
Dec	2±0.2	72±4	0	0	2±0.1	50±5
Total/Av.	167 (33.4)±1.7	367 (73.4)±21.5	20 (4)±0.4	9 (1.8)±1.2	177 (35.4)±10.7	284 (56.8)±2
Jan 2001	0	28±2	0	0	0	32±1
Feb	0	34±3	0	0	0	38±1
Mar	0	70±5	1±0.1	1±0.1	0	80±5
Total/Av.	0 (0)	132 (44)±10	1(0.3)±0.1	1(0.3)±0.1	0 (0)	150 (50)±5

methrin @ 100 mg/m^2 on 1-8-1999 and 26-7-2000.

Table 2. Incidence of malaria in

Month/Year	Slum-I Treated hessian jute (Pop.: 1350)						Slum-II Untreated hessian jute (Pop.: 1500)		
	BS	BER	SPR	SfR	Cases/000	Pf/000	BS	BER	SPR
Pre-treatment									
Apr 1999	30	2.2±0.1	6.6±0.4	0	1.4±0	0	30	2±0.2	6.6±1.4
May	35	2.5±0.1	2.8±0	0	0.7±0	0	35	2.3±0.05	5.7±0.15
Jun	15	1.1±0.1	0	0	0	0	25	1.6±0.1	0
Jul	32	2.3±0.15	12.5±1.5	0	2.9±0	0	37	2.4±0.1	5.4±0.4
Total/Av.	112	8.2±0.3	6.2±0.4	0	5.1±0.3	0	127	8.4±0	4.7±0.05
Post-treatment									
Aug (I treatment)	5	0.3±0.05	0	0	0	0	20	1.3±0.06	20±2
Sep	6	0.4±0	0	0	0	0	15	1±0.03	40±2.6
Oct	5	0.3±0.05	0	0	0	0	13	0.8±0.5	15.3±1
Nov	0	0	0	0	0	0	10	0.6±0.01	10±0
Dec	0	0	0	0	0	0	10	0.6±0	0
Total/Av.	16	1.1±0.05	0	0	0	0	68	4.5±0.05	19.1±0.9
Jan 2000	0	0	0	0	0	0	12	0.8±0.1	0
Feb	0	0	0	0	0	0	8	0.5±0	25±0
Mar	24	1.7±0.15	4.1±0	0	0.7±0	0	20	1.3±0.05	15±3.5
Apr	40	2.9±0.15	2.5±0	0	0.7±0	0	60	4±0.15	1.6±0
May	60	4.4±0.15	1.6±0	0	0.7±0	0	80	5.3±0.35	2.5±0.3
Jun	40	2.9±0.15	0	0	0	0	60	4±0	0
Jul	28	2±0.05	0	0	0	0	80	5.3±0.15	1.2±0
Total/Av.	192	14.2±1	1.5±0	0	2.2±0	0	320	21.3±0.3	2.8±1
Aug (II treatment)	12	0.8±0.1	8.3±0	0	0.7±0	0	100	6.6±0.3	7±0.3
Sep	20	1.4±0.1	5±0	0	0.7±0	0	96	6.4±0.15	4.1±0.15
Oct	4	0.2±0	0	0	0	0	34	2.2±0.1	14.7±2
Nov	0	0	0	0	0	0	22	1.4±0.1	0
Dec	0	0	0	0	0	0	12	0.8±0.1	0
Total/Av.	36	2.6±0	5.5±0	0	1.4±0	0	264	17.6±0	6±0
Jan 2001	0	0	0	0	0	0	12	0.8±0.2	0
Feb	9	0.6±0	11.1±0	0	0.7±0	0	13	0.8±0.1	0
Mar	12	0.8±0.1	0	0	0	0	15	1±0.1	6.6±0
Total/Av.	21	1.5±0.1	4.7±0	0	0.7±0	0	40	2.6±0.1	2.5±0

Jute curtains were treated with alphacypermethrin @ 100 mg/m^2 on 1-8-1999 and 26-7-2000.

slums of Andrews Ganj, New Delhi

Month/Year	Slum-II Untreated hessian jute (Pop.: 1500)			Slum-III Untreated hessian jute (Pop.: 1410)					
	SfR	Cases/000	Pf/000	BS	BER	SPR	SfR	Cases/000	Pf/000
Pre-treatment									
Apr 1999	0	1.3±0	0	30	2.1±0.07	10±2.7	0	2.1±0.3	0
May	0	1.3±0	0	40	2.8±0.15	2.5±0	0	0.7±0	0
Jun	0	0	0	35	2.4±0.2	0	0	0	0
Jul	0	1.3±0	0	37	2.6±0.1	8.1±2.1	2.7±0	2.1±0.3	0.7±0
Total/Av.	0	4±0	0	142	10±0.35	4.9±0.35	0.7±0	4.9±0.35	0.7±0
Post-treatment									
Aug (I treatment)	5±0	2.6±0	0.6±0	30	2.1±0.05	16.6±2.2	6.6±0.4	3.5±0.3	1.4±0
Sep	13.3±0.8	4±0	1.3±0	38	2.6±0.1	13.1±1.9	5.2±0.2	3.5±0.3	1.4±0
Oct	7.7±0	1.3±0	0.6±0	35	2.4±0.1	8.5±2.1	5.7±0.5	2.1±0.3	1.4±0
Nov	0	0.6±0	0	15	1±0.2	20±2.8	6.6±0	2.1±0.3	0.7±0
Dec	0	0	0	14	0.9±0	0	0	0	0
Total/Av.	5.8±1.6	8.6±0.3	2.6±0.3	132	9.3±0.05	12.1±0.2	5.3±0.6	11.3±0	4.9±0.3
Jan 2000	0	0	0	28	1.9±0.04	7.1±0.5	0	1.4±0	0
Feb	25±0	1.3±0	1.3±0	36	2.5±0.05	2.7±0	2.7±0	0.7±0	0.7±0
Mar	0	2±0.3	0	40	2.8±0.15	0	0	0	0
Apr	0	0.6±0	0	64	4.5±0.15	4.6±4.6	0	2.1±0.3	0
May	0	1.3±0	0	80	5.6±0.4	2.5±0.3	0	1.4±0	0
Jun	0	0	0	72	5.1±0.3	2.7±0.3	0	1.4±0	0
Jul	0	0.6±0	0	100	7±0.4	1±0	0	0.7±0	0
Total/Av.	0.6±0	6±1	1.3±0	420	29.7±2	2.6±0.2	0.2±0	7.8±0.3	0.7±0
Aug (II treatment)	2±0.2	4.6±0.3	1.3±0	88	6.2±0.3	3.4±0.8	0	2.1±0.3	0
Sep	1±0	2.6±0	0.6±0	102	7.2±0.4	6.8±0.2	1.9±0.2	4.9±0.3	1.4±0
Oct	2.9±0	3.3±0.3	0.6±0	90	6.3±0.3	10±0	3.3±0.7	6.3±0.3	2.1±0.3
Nov	0	0	0	57	4±0.1	5.2±1.4	1.7±0	2.1±0.3	0.7±0
Dec	0	0	0	24	1.7±0.1	4.1±0	4.1±0	0.7±0	0.7±0
Total/Av.	1.5±0	10.6±0	2.6±0	361	25.6±0.1	6.3±0.3	1.9±0.8	16.3±0.3	4.9±1
Jan 2001	0	0	0	30	2.1±0.1	0	0	0	0
Feb	0	0	0	42	2.9±0.1	4.7±0	0	1.4±0	0
Mar	0	0.6±0	0	50	3.5±0.05	2±0	0	0.7±0	0
Total/Av.	0	0.6±0	0	122	8.6±0.4	2.4±0	0	2.1±0	0

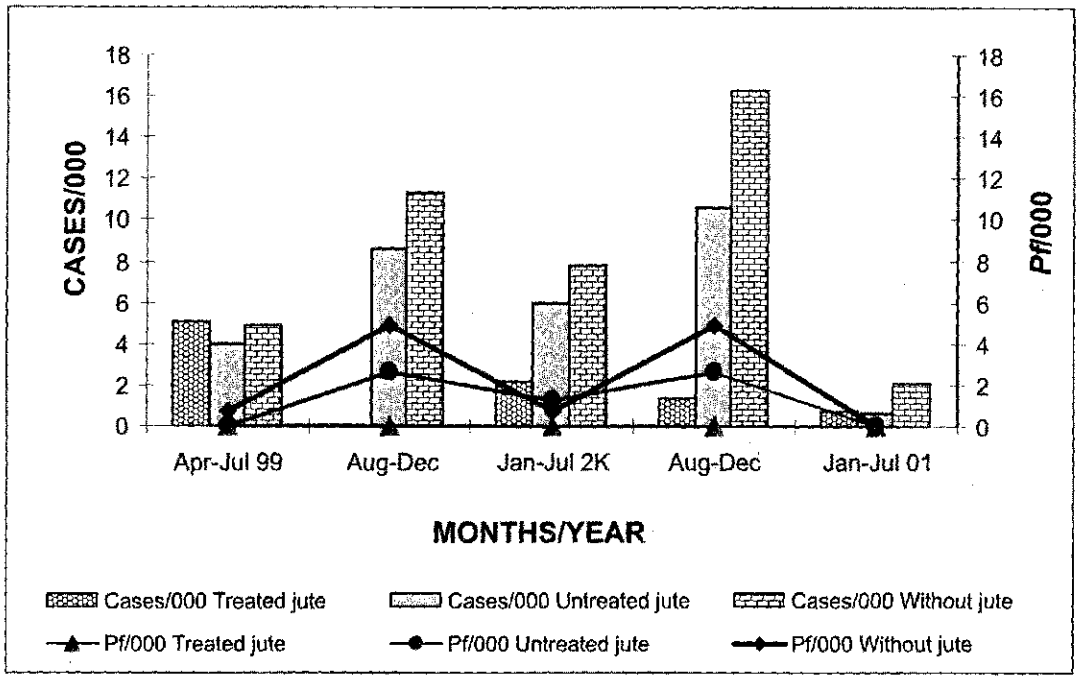

Fig. 2: Cases/000 and *Pf*/000 in control and experimental slums

before installation of curtain in slum-I was 6.2 and it immediately reduced to nil after installation, whereas in the untreated curtain and without curtain areas malaria transmission continued. It was interesting to note that not a single case of *P. falciparum* malaria was recorded throughout the study in slum-I suggesting thereby that malaria transmission was completely interrupted.

Bioassay tests

Results of bioassay tests on *An. stephensi* and *Cx. quinquefasciatus* revealed that alphacypermethrin treated jute curtains could produce >70 per cent mortality against *An. stephensi* up to six months and against *Culex* up to two months. Hence, two treatments are sufficient in an year to control mosquito nuisance.

DISCUSSION

The results of the present study indicated that insecticide treated curtains were quite effective in reducing the indoor resting density of *An. stephensi* in the experimental area when compared to that of in untreated and without curtain areas. A significant reduction in indoor densities of *An. stephensi* and other mosquitoes in the slum area where treated curtains were used can be attributed to the killing action of treated curtains[7] or both killing and repellent action. The treated curtains might have prevented the mosquito entry and the rested mosquitoes might have

also been killed on contact with the treated curtains.

The installation of alphacypermethrin treated curtains also produced desirable impact on malaria incidence. The substantial reduction in cases/000 and *Pf*/000 clearly indicates that treated curtains were highly effective in reducing malaria incidence as evidenced by all epidemiological indicators in the treated curtain used area when compared to that of untreated and without curtain areas. The use of insecticide-treated curtains is more appropriate compared to indoor residual spraying in terms of safety to inhabitants as mass use of treated curtains on sustained basis at recommended dosage does not pose any hazard either to those treating or using curtains as there is less human contact.

In view of this, it is safe to conclude that use of alphacypermethrin treated curtains was found to be operationally feasible, cost-effective, socially acceptable and produced desired impact both on vector density and malaria incidence in slum areas. The cost per hut with the present technology is quite cheaper than indoor residual spraying with DDT, malathion and other synthetic pyrethroids. Based on present findings and global recommendations the use of synthetic pyrethroid treated curtains could be promoted with appropriate partners for effective malaria control in urban areas.

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HLA Associations in *P. falciparum* Malaria Patients from Mumbai, Western India

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In the present study, HLA associations among the cohort of 171 severe *P. falciparum* malaria patients were compared with that of 101 normal sex, age and ethnically matched control samples. All these individuals lived in Mumbai in an area of low and seasonal *P. falciparum* transmission. HLA A, B, DRB1 and DQB1 antigens were serologically (A and B) and molecularly (DRB and DQB) determined using isolated lymphocytes and genomic DNA following the microlymphocytotoxicity assay and PCR-SSP techniques. Significant differences were observed between patients with malaria and controls in the following groups of alleles: A3, B27, B49, DRB1*04, and DRB1*0809 were increased, while A19, A34, B18, B37, and DQB1*0203 were decreased. HLA B49 and DRB1*0809 were found to be positively associated with the complicated severe malaria patients (OR=13.88; $p < 0.0001$). HLA A19, B5 and B13 were protective in patients with high parasite index (>2%). These observations revealed the importance of ethnic background, which has to be taken into consideration while developing an ideal malaria vaccine. Further, when compared to HLA associations of other world populations the present study indicates the relative importance of different HLA alleles that may vary in different populations.

Keywords: HLA, Mumbai, *P. falciparum* malaria

INTRODUCTION

Our understanding of the host genetic factors that influence susceptibility to and the course of infectious diseases are growing rapidly. Malaria is a global problem that ranks second only to tuberculosis in the total number of human deaths attributable to an infectious agent. It accounts for 300 to 500 million annual infections; estimated to cause 1.5–2.7 million deaths annually with about 90% of malaria associated mortality occurring in sub-Saharan Africa.[1] Most malar-

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ia associated mortality and severe morbidity such as anemia and cerebral malaria are attributable to *P. falciparum*.[2] The genetic basis of susceptibility to malaria has been studied extensively using a variety of approaches. The protective role of several erythrocyte variants is well established. There has been evidence that genes determining a variety of immune responses influence susceptibility to malaria. Further immunogenetic associations with severe malaria have already suggested new approaches for intervention, and the highly polygeneic nature of susceptibility to this disease suggest that the identification and analysis of new susceptible and resistant loci should be worthwhile.[3] Different HLA alleles influence susceptibility to malaria. In a control study involving children in Gambia, HLA B53 and HLA DRB1*1302 were less frequent among children with severe malaria.[4] It has been proposed that the HLA B53 mediated protection may involve effective cytotoxic T cell mediated immunity to liver stage antigen.

In homozygous children of Gambia, a polymorphism in the promoter (-308) region of TNF-α gene, have a seven fold increased risk for death or severe neurologic sequelae to cerebral malaria.[5] Another immune protein, intracellular adhesion molecule (ICAM-1) which is involved in cell to cell communications, influences malaria severity.[6] Malaria is now endemic in many parts of the world and is returning even to those countries from which it has been eradicated. It is endemic in many areas of India, and repeated infection with *P. falciparum* and *P. vivax* occur.[7] The present study aims to determine the HLA associations among a cohort of severe *P. falciparum* malaria patients compared with normal ethnically matched controls from Mumbai, western India.

MATERIALS AND METHODS

Patients and controls: Blood samples from 171 confirmed *P. falciparum* infected malaria patients with symptomatic clinical manifestations such as high parasitaemia (> 5%), hepatic, renal and haematological involvement who were admitted in the Medicine Department, K.E.M. Hospital, were collected and studied for their HLA A, B, DRB1 and DQB1 antigen distribution. The diagnosis of the *P. falciparum* malaria was also confirmed by using an immunochromatographic test that detects both *P. falciparum* and *P. vivax* infection. A detailed questionnaire regarding the patient history, pathological investigations, parasite index, drug regimen, clinical details, other population specific details were recorded and blood was collected after the approval and ethical clearance of the Institute Ethical Committee. The average age group of the patients was 15 to 50 years with 3 : 1 male to female ratio. One hundred and one, age and sex matched healthy individuals living in the same area with similar socio-economic status and ethnic background studied for HLA during the same period comprised the controls. Further the control population had no disease during the collection and was devoid of past history of malaria infection.

HLA typing

Serology: Ten to fifteen millilitres of venous blood (in heparin 50 IU/ml) was collected in a sterile tube from each individual. The lymphocytes were isolated by density gradient centrifugation on Histopaque.[8] HLA A and B locus antigens were identified by NIH two – stage Microlymphocytotoxicity assay.[9] The antisera

were indigenous[10] and commercial (Biotest, Germany; Boehring, Germany; Pelfreez, USA) in origin. A total of 190 antisera were used for defining 17 specificities for HLA A locus, 29 for HLA B locus and 8 for HLA C locus antigens. The typing tray included a minimum of three antisera for each supertypic specificity.

Molecular: Five millilitres of EDTA blood was used for the genomic DNA extraction following the XII IHWC protocol.[11] The HLA DRB1 and HLA DQB1 alleles were determined by PCR-SSP technique (Biosyn kit, U.S.A.). The polymerase chain reaction-sequence specific (PCR-SSP) amplification technique utilises the allele specific primers along with the control primers to identify the respective allele. The DRB1 kit contains 24 sets of sequence specific primers while the DQB1 kit contains 7 sets of sequence specific primers to define the molecular HLA alleles. The amplified products are visualised by the gel electrophoresis and interpreting the positive bands as per the interpretation sheets provided along with the kit.

Statistical analysis: The phenotype frequencies, odds ratio (OR), probability value[12, 13] confidence intervals[14] were estimated using database and computer programmes. Since each individual is tested for several HLA alleles and the same data used for comparing the frequency, it is possible that one of the alleles will by chance deviate significantly. To overcome this error, the p-value is corrected by using of Bonferoni inequality method.[15]

RESULTS

The phenotype frequencies of HLA A, B, DRB and DQB antigens in malaria patients were compared with that of the controls (Table 1). The gel documentation result obtained from the DQB1 gene amplification of a single malaria patient showing the positive bands is presented (Fig.1). A significant increase was observed in the frequency of A3, B27, B49, DRB1*04, and DRB1*0809 among malaria patients when compared to that of controls. However, the observed differences for HLA A10, B21, B22,

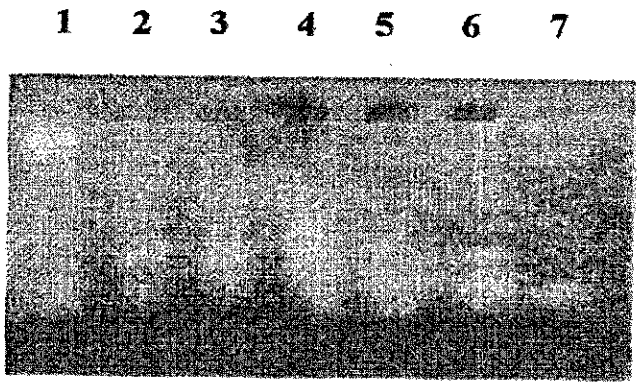

Fig. 1: HLA DQB-SSP typing results of a single patient sample. Lanes 2, 3 and 6 are positive denoting the presence of the respective alleles. Note that the control bands are present in all the lanes above

Table 1. HLA allele distribution in malaria patients compared with that of controls

HLA	Patients[#] (171)	Controls[#] (101)	Odds ratio	p-value
A1	22.20	17.80	1.30	
A2	31.60	29.70	1.09	
A3	25.10	16.80	1.63	<0.05
A9	40.40	39.60	1.03	
A10	7.00	13.90	0.47	
A11	21.10	23.80	0.85	
A19	28.70	38.60	0.64	<0.001
A28	17.00	11.90	1.48	
A34	0.60	3.00	0.25	
B5	26.90	29.70	0.87	
B7	19.90	18.80	1.06	
B8	4.10	3.00	1.28	
B12	22.20	17.80	1.30	
B13	13.50	9.90	1.38	
B15	12.90	16.80	0.73	
B17	11.70	12.90	0.89	
B18	2.90	6.90	0.42	
B21	2.90	12.90	0.89	
B22	4.10	6.90	0.57	
B27	9.40	1.00	7.11	<0.0001
B35	31.00	24.80	1.35	
B37	2.30	5.90	0.39	
B40	30.40	26.70	1.19	
B48	1.20	1.00	0.99	
B49	2.90	1.00	2.21	<0.0001
DRB1*(n=30)				
*04	57.10	11.10	7.29	<0.0001
*0809	71.40	11.10	12.47	<0.00001
*1105	28.60	22.20	1.36	
DQB1*(n=30)				
*0203	28.60	55.60	0.37	<0.0001
*0301	42.90	44.40	0.95	
*0305	14.30	11.10	1.31	
*0401	28.60	22.20	1.36	
*0501	28.60	22.20	0.84	
*06	14.30	22.20	0.69	

[#]Percentage frequencies.

B44, B56, DRB1*1105, DQB1*06 and DQB1*0501 did not remain significant after the p value correction was applied. It was interesting to observe that, in HLA B49 and DRB1*0809 positive malaria patients, among the high risk alleles, had more clinical complications (OR=13.88; $p < 0.00001$). HLA A19 was observed to be reduced significantly (OR=0.64; $p < 0.001$). The comparison of high-risk alleles with the observations of the world populations revealed the importance of ethnic background and indicates that relative importance of different HLA alleles may vary in different populations studied.

DISCUSSION

Malaria patients experience asymptomatic parasitaemia; acute febrile illness (with cerebral damage, anaemia, respiratory distress, hypoglycemia); chronic debilitation (anaemia, malnutrition, nervous system related sequelae) and complications of pregnancy (anaemia, low birth weight, increased mortality). These manifestations in patients, communities and countries reflect intrinsic (human, parasite and mosquito) and extrinsic (environmental, social, behavioural, political and economic conditions, as well as disease control effects) determinants.[16]

The immune mechanism whereby malaria parasites are eliminated by the human host or how they may avoid the immune response are poorly understood. Individuals living in malaria endemic areas gradually acquire immunity. It is well established that this immunity involves both cell mediated and humoral mechanisms and T cells are the major regulators in both these events. The existence of functionally distinct *P. falciparum* specific CD_4 + T cell subsets in humans has been shown in several studies.[17] Studies in Gambia have provided indirect evidence that cytotoxic T lymphocyte (CTL) play a protective role against malaria in humans and using allele-specific HLA class I peptide motifs several peptide epitopes for CTL in pre-erythrocytic *P. falciparum* antigens have been identified in naturally exposed Gambians.[18] Recent findings emphasise the diversity of *P. falciparum* antigens recognised by CD_8 + T cells in humans[19] and supports the inclusion of components from several antigens in CTL inducing vaccines against malaria. Further a panel of HLA A*0201 restricted antigenic peptide epitopes and with specific CD_8 + T lymphocyte responses in mediating pre-erythrocyte stage gene products of *P. falciparum* has been established.[20]

A highly significant positive correlation was found between HLA B35 frequency and malaria in 136 different villages of Sardinia suggesting that malaria has been the selective factor for B35 frequency in Sardinia.[21] Further, several studies in HLA association with *P. falciparum* malaria reveals that different HLA alleles show either a positive association or negative association with malaria in different ethnic populations all over the world.[16, 22–27] Heterogeneity in HLA antigen association for a particular disease in different populations may be due to the linkage of susceptible gene(s) with various different HLA alleles in different populations. It is also possible that the putative disease susceptibility gene lies in the other HLA region[28, 29] or due to synergism and epistasis between HLA antigens.[30] Thus in the present study some sig-

nificant conclusions can be made as (i) HLA B49 and DRB1*0809 alleles are associated with *P. falciparum* malaria patients; (ii) HLA A19 and DQB1*0203 alleles are protective; and (iii) The study reveals that ethnic background is important and indicates that relative importance of HLA alleles may vary in different populations studied.

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Mosquito Fauna and Breeding Habitats of Anophelines in Little Andaman Island, Andaman and Nicobar Islands, India

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A total of 37 species of mosquitoes belonging to nine genera—*Anopheles*, *Aedes*, *Armigeres*, *Culex*, *Harpagomyia*, *Mansonia*, *Orthopodomyia*, *Toxorhynchites* and *Uranotaenia* were collected from Little Andaman Island. Mosquitoes of nine *Anopheles* group of species—*An. barbirostris*, *An. barbumbrosus*, *An. balabacensis*, *An. insulaeflorum*, *An. kochi*, *An. philippinensis*, *An. roperi*, *An. sundaicus* and *An. vagus* were recorded. *An. philippinensis* was found to be the most predominant species. Species-specific breeding preference and association in various types of acquatic habitats have been reported. This is the first record of mosquito fauna of Little Andaman Island.

Keywords: Breeding sources, Little Andaman, Mosquito fauna

INTRODUCTION

Our present knowledge of mosquito fauna of the Andaman Islands is atleast two decade old and based on the work of Christophers,[1] Covell,[2] Basu,[3] Krishnan and Halernkar,[4] and Nagpal and Sharma.[5] The areas of Andaman Islands so far surveyed were South Andaman, Middle Andaman and North Andaman. However, there is no report on the mosquito fauna of the Little Andaman though it is in the Andaman group of Islands. In this Island great emphasis was given to agriculture. Large areas have also been deforested and construction of new roads and buildings have come up leading to the new settlements. Interisland ship transportation has increased several times to this Island leading to population migration. The use of insecticides in

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agriculture and DDT in public health may have brought about changes in the ecosystem and in the composition and bionomics of mosquitoes. Das *et al.*[6] reported RII level of chloroquine resistance from Little Andaman Island. Therefore, there was a need for mosquito faunastic studies, particularly anopheline fauna and their breeding preference in Little Andaman and Andaman group of Islands. The study was carried out from January 1998 to February 1999.

MATERIALS AND METHODS

Study area

Little Andman (6 – 14°N latitude and 92–94°E longitude) is situated at a distance of 66 nautical miles away from Port Blair. The Little Andaman is isolated from rest of the Andaman Islands by Duncan passage and from the Nicobar group by 10° channels (Fig. 1). This relatively flat island has the highest height—156 m. About 10 per cent of the total area is of tidal flats, mangrove swamps and beaches and about 63 per cent forms coastal plains. The central and southern portions of the Island are undulating to moderately hilly. The area of Little Andaman is 731.416 km^2. The Island is rich with dense tropical rain forest and natural vegetation. The mangrove forests are wide-spread along the coasts and estuaries of many creeks. Wet cultivation is carried out only in limited area. Only 10% area of the Island is said to be in the human habitation. Most of the area falls under thickly vegetated forest cover. There are two major creeks in the Island—Dugong creek in the northeast of the Island and Jackson creek in the extreme north. Much of the forest area is

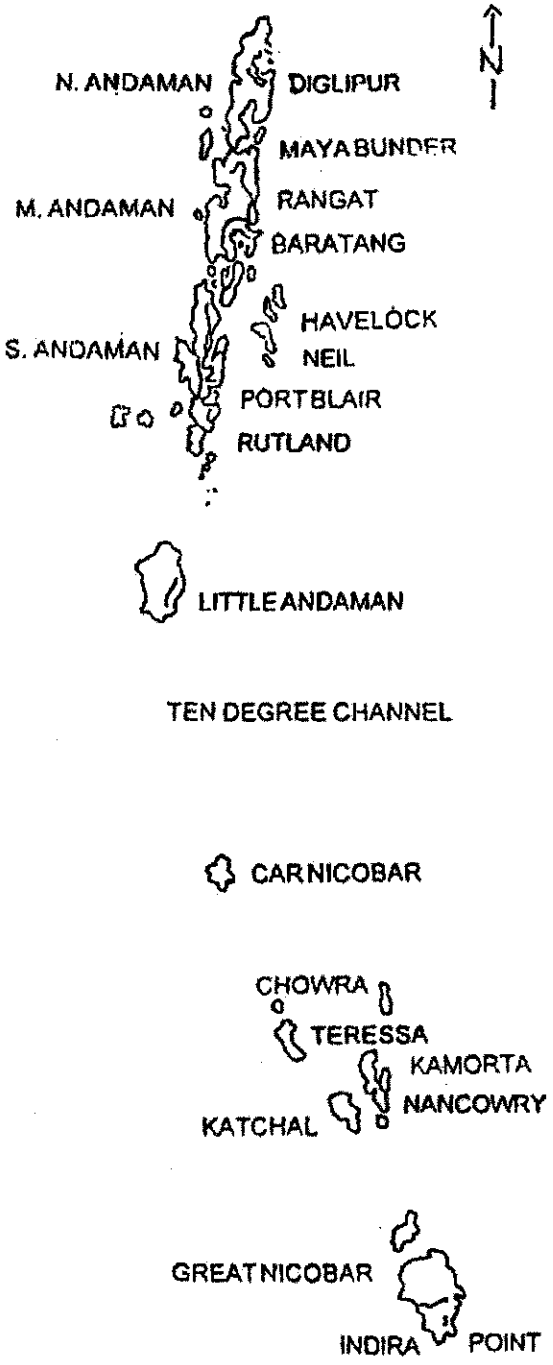


Fig. 1: Map of A&N Islands showing location of Little Andaman Island

still unexplored. Roads are being constructed so as to approach the interior of the forest. The climate is of tropical equitorial type. The temperature varies from 24 to 31°C and relative humidity from 70 to 80 per cent. The Island receives both southeast monsoon from May to October and northeast monsoon from November to April. The average annual rainfall is about 300 cm. The climate of Little Andaman provides an ideal environment for mosquito breeding and proliferation due to high rainfall associated with hot and humid conditions.

Streams, nullahs, creeks, marshy area, mangroves and ponds are the perennial mosquito breeding sources.

The total population of this Island is 12,247 (1991 census) spread over 13 villages. Aborigine tribe constitutes about 10 per cent of the total population and the rest are immigrants from mainland, India. The refugees from Bangladesh have been settled here during 1971–1974. Dugong creek, Jackson creek and South Bay are occupied by Onges and Harmider Bay is occupied by Nicobarese.

Adult mosquito collection

Indoor resting mosquitoes were collected from human dwellings, cattlesheds and mixed dwellings. Outdoor resting mosquitoes were collected from vegetation—Keori bushes (Pandanus) and other plants, coconut tree holes, uprooted stumps, mangrove cutting areas, crab holes, etc. during day time. All night (1700 to 0500 hr) man and cattle biting collections were made in each village for two days—a total of eight whole night collections were made (Table 1).

Larval collection

All possible larval breeding sites—streams, ponds, creeks, mangrove areas, rice fields, cement tanks, used tyres, crab holes, coconut shells, etc. were searched and immatures were collected with the help of dipper (9.5 cm diam and 300 ml capacity pippette and wellnet) and kept in the field laboratory until adult emergence. All newly emerged mosquitoes were anaesthetised and killed by ether and dry mosquitoes packed in a wide mouth plastic vial. In the laboratory the specimens were relaxed and pinned on cork sheet for identification and storage in the entomological boxes. The packed mosquitoes were brought to the Malaria Research Centre, field station, Car Nicobar laboratory for further studies and preservation. The mosquitoes were identified at Malaria Research Centre, Car Nicobar and Delhi, using the keys of Christophers,[7] Barraud,[8] and Nagpal and Sharma[9] and catalogue of Knight and Stone.[10] The location of the villlages where collections were made is given in Fig. 2.

RESULTS AND DISCUSSION

A total of 11,226 mosquitoes belonging to 37 species and nine genera—*Anopheles*, *Aedes*, *Armigeres*, *Culex*, *Harpagomyia*, *Mansonia*, *Orthopodomyia*, *Toxorhynchites* and *Uranotaenia* were collected in the present survey. The number of species and their specimens collected in each genus are given in Table 2. Identification of all the mosquitoes revealed that genus *Anopheles* and *Aedes* comprising of nine species each, *Armigeres* two, *Culex* ten, *Harpagomyia* one, *Mansonia* one, *Ortho-*

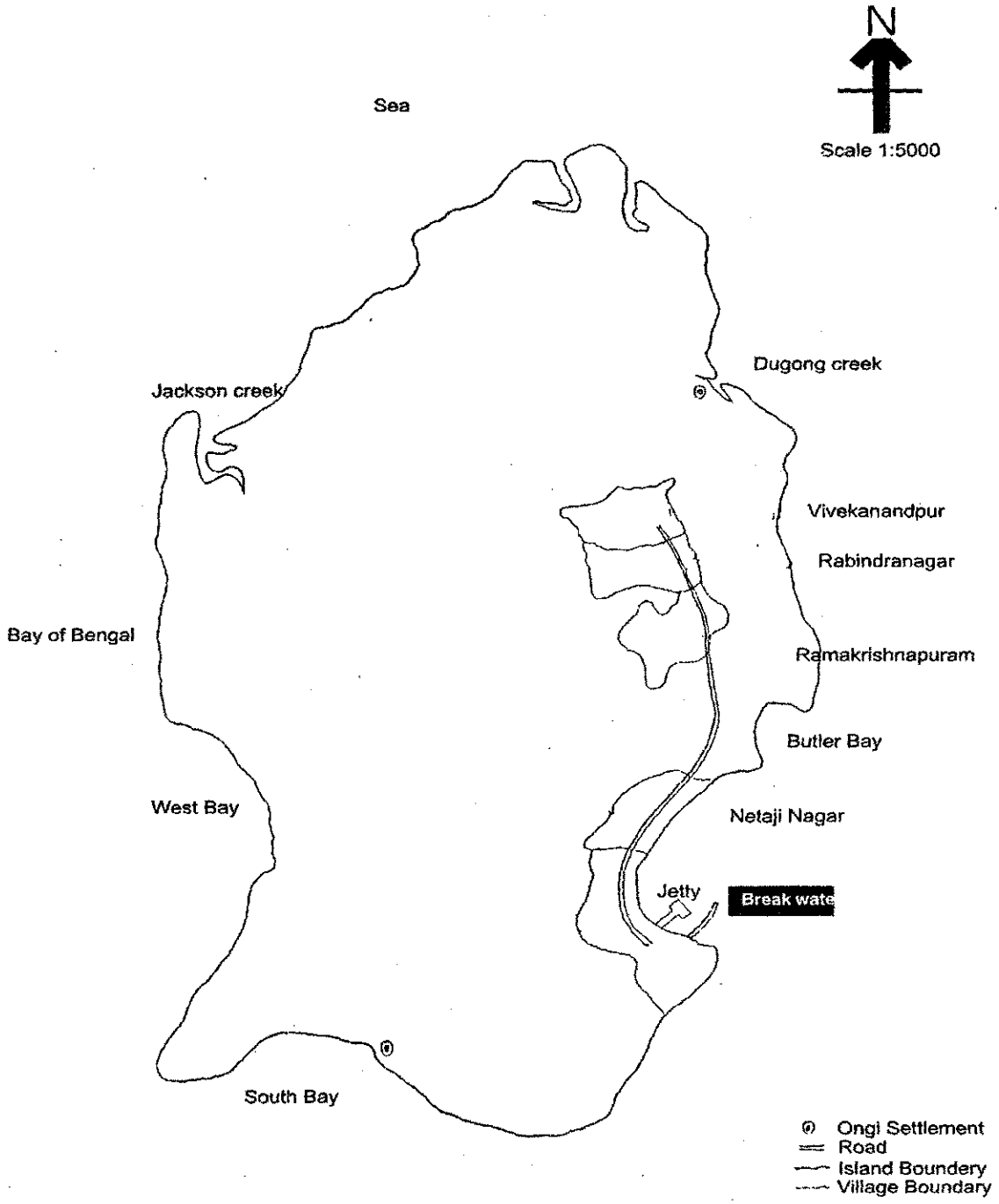


Fig. 2: Map of Little Andaman Island (solid circles on the map indicate sites from where the mosquitoes were collected)

Table 1. Adult mosquito collection sites in Little Andaman Island

Species collected	Mosquito collection sites/Structures								
	Indoor			Outdoor					
	Cattle-sheds	Human dwel-lings	Mix dwel-lings	Pandan-uslarum	Coconut dry leaves	Coconut tree holes	Grasses/ small plants	Bushes inside jungle	Deep jungle of stream site
An. barbirostris	+	–	+	–	+	–	–	+	–
An. barbumbrosus	+	–	+	–	+	–	–	+	–
An. balabacensis	+	–	+	–	+	–	–	+	–
An. insulaeflorum	–	–	–	–	–	–	–	+	–
An. kochi	+	–	+	–	+	–	+	+	–
An. philippinensis	+	+	+	–	+	–	+	+	–
An. roperi	+	–	+	–	+	–	+	+	–
An. sundaicus	+	–	+	–	+	–	+	+	–
An. vagus	+	+	+	–	+	–	+	+	–
Ae. jamesi	+	–	+	+	+	–	–	+	–
Ae. aegypti	+	+	+	+	–	+	–	+	–
Ae. albopictus	+	+	+	+	–	+	–	+	–
Ae. albolateralis	+	–	+	+	–	–	–	–	–
Ae. andamanensis	–	–	–	–	–	–	–	+	–
Ae. edwardsi	+	–	+	+	–	+	–	–	–
Ae. malayensis	+	–	–	+	–	+	–	+	–
Ae. niveus	–	+	–	–	–	+	–	–	–
Ae. scutellaris	+	–	+	+	+	+	–	–	–
Ar. kuchingensis	+	–	–	–	–	–	–	–	–
Ar. subalbatus	+	–	–	–	–	–	–	–	–
Cx. quinquefasciatus	+	+	+	+	–	+	+	+	–
Cx. vishnui	+	+	–	–	–	–	–	–	–
Cx. sitiens	–	–	–	–	–	–	–	+	–
Cx. tritaeniorhynchus	+	–	+	–	–	–	–	–	–
Cx. fragilis	–	–	–	+	–	–	–	–	–
Cx. pallidothorax	–	–	–	–	–	–	+	–	–
Cx. malayi	+	–	+	–	–	–	–	–	–
Cx. gelidus	+	–	+	–	–	–	–	–	–
Cx. fuscanus	–	–	–	–	–	–	–	+	–
Cx. minor	+	–	–	+	–	–	–	–	–
Harpagomyia	–	–	–	+	–	–	–	–	–
Mansonia	+	–	–	–	–	–	–	–	–
Toxorhynchites	–	–	–	–	–	+	–	–	–
Uranotaenia	–	–	–	–	–	+	–	–	–

+ Present; – Absent.

podomyia and *Toxorhynchites* two species each and *Uranotaenia* one species (Table 2).

Among the nine anopheline species collected, the most prevalent species were *An. philippinensis* (41.37%) followed by *An. kochi* (24.78%), *An. vagus* (18.92%), *An. sundaicus* (6.15%) and *An. barbirostris* (2.63%). The other species were collected in small number. Among Culicinae most dominant genus was *Culex* and was represented by *Culex quinquefasciatus* (93.81%) followed by *Cx. fragilis* (6.21%) and *Cx. vishnui* (4.90%), the most prevalent species in the genus *Aedes* was *Ae. aegypti* (39.31%) followed by *Ae. albopictus* (33.94%) and *Ae. malayensis* (19.02%).

Larval ecology

Habitat-wise per cent composition of *Anopheline* species found in the samples collected from the different breeding habitats have been summarised in Table 3 and Fig. 3. It was observed that creeks supported the maximum breeding of *An. sundaicus* followed by ponds and seepage water of dam. The species were found breeding in both brackish and fresh water. Profuse breeding of *An. sundaicus* was observed in the brackish water with free floating and submerged algae. Putrefying mass of algae and vegetation provide the ideal condition for the breeding and growth of *An. sundaicus*. Covell and Singh[10] also made the similar observations while working in Chilka lake. Breeding of *An. philippinensis* was observed in streams, nullahs, rice fields, jungle pools, ditches, seepage water of dams and ponds in association with algal growth and vegetation like chara, hydrilla and spirogyra. The shaded stream with algal growth and vegetation in deep jungle supported the maximum breeding of *An. philippinensis*. Rao[11] also made similar observation and found that the species was known to breed in a variety of breeding habitats with all types of algae and vegetation except lemna. The breeding of *An. philippinensis* was not observed in creeks and near mangrove area where little salinity is observed. It was often found breeding in association with *An. sundaicus*, *An. vagus* and *An. barbirostris* and occasionally with *An. insulaeflorum*.

The breeding of *An. vagus* was observed in seepage water of dam, ditches, mangroves, creeks, marshy area, jungle pools, pits, streams and forest nullahs. The breeding of *An. vagus* in the above places was associated with muddy water except seepage water of dam.

Seepage water of dam, creek, streams, marshy area, forest nullahs, mangroves, ditches, jungle pools and rice fields supported the breeding of *An. barbirostris*. Shaded streams inside the deep jungles, forest nullahs, marshy area, plant roots associated with putrefying leaves in the streams were the breeding places of *An. insulaeflorum*. Pits, jungle pools and rice fields supported the breeding of *An. kochi*. Shaded stream margins inside deep jungle and jungle pools supported the breeding of *An. barbumbrosus*. Shaded streams were found to be preferred breeding sites of *An. roperi*.

Bait collection

All night human and animal bait collections in the Hutbay forest area, Kichard basti, Netaji

Table 2. List of mosquitoes collected from Little Andaman Island

Species collected	Total specimens
Anopheles (An.) barbirostris Van der Wulp, 1884	135
An.(An.) barbumbrosus Strickland and Chowdhury, 1927	92
An.(An.) balabacensis Baisas, 1936	88
An.(An.) insulaeflorum Swell and Swell, 1919	36
An.(Cellia) kochi Doenitz, 1901	1268
An.(An.) philippinensis Ludlow, 1902	2117
An.(An.) roperi Reid, 1950	97
An.(Cellia) sundaicus Rodenwaldt, 1925	315
An.(An.) vagus Doenitz, 1902	968
Aedes (Stegomyia) aegypti Linnaeus, 1762	1351
Ae.(Stegomyia) albopictus Skuse, 1894	1165
Ae.(Stegomyia) albolateralis Theobald, 1908	75
Ae.(Stegomyia) edwardsi Barraud, 1923	157
Ae.(Verrallina) andamanensis Edwards, 1922	8
Ae.((Aedimorphus) jamesi Edwards, 1914	12
Ae.(Stegomyia) malayensis Colless, 1962	653
Ae.(Finiya) niveus Ludlow, 1903	4
Ae.(Stegomyia) scutellaris Walker, 1959	11
Armigeres (Ar.) kuchingensis Edwards, 1915	272
Ar.(Ar.) subalbatus Coquillett, 1898	288
Culex (Cu.) fragilis Ludlow, 1903	124
Cx.(Lutzia) fuscanus Wiedemann, 1828	4
Cx.(Cx.) gelidus Theobald, 1901	14
Cx.(Eu.) malayi Leicester, 1908	42
Cx.(Lophoceraomyia) minor Leicester, 1908	12
Cx.(Cu.) pallidothorax Theobald, 1905	11
Cx.(Cx.) quinquefasciatus Say, 1823	1673
Cx.(Cx.) sitiens Wieldmann, 1828	7
Cx.(Cx.) tritaeniorhynchus Giles, 1901	11
Cx.(Cx.) vishnui Theobald, 1901	98
Harpagomyia genurostris Leicester, 1908	2
Mansonia (Mansonioides) annulifera, Theobald, 1901	8
Orthopodomyia anopheloides Giles, 1903	18
Orthopodomyia flavithorax Barraud, 1927	35
Toxorhynchites (Toxorhynchites) edwardsi Barraud, 1924	20
Toxorhynchites (Toxorhynchites) splendens Wiedemann, 1819	33
Uranotaenia atra Theobald, 1905	2
Total	11226

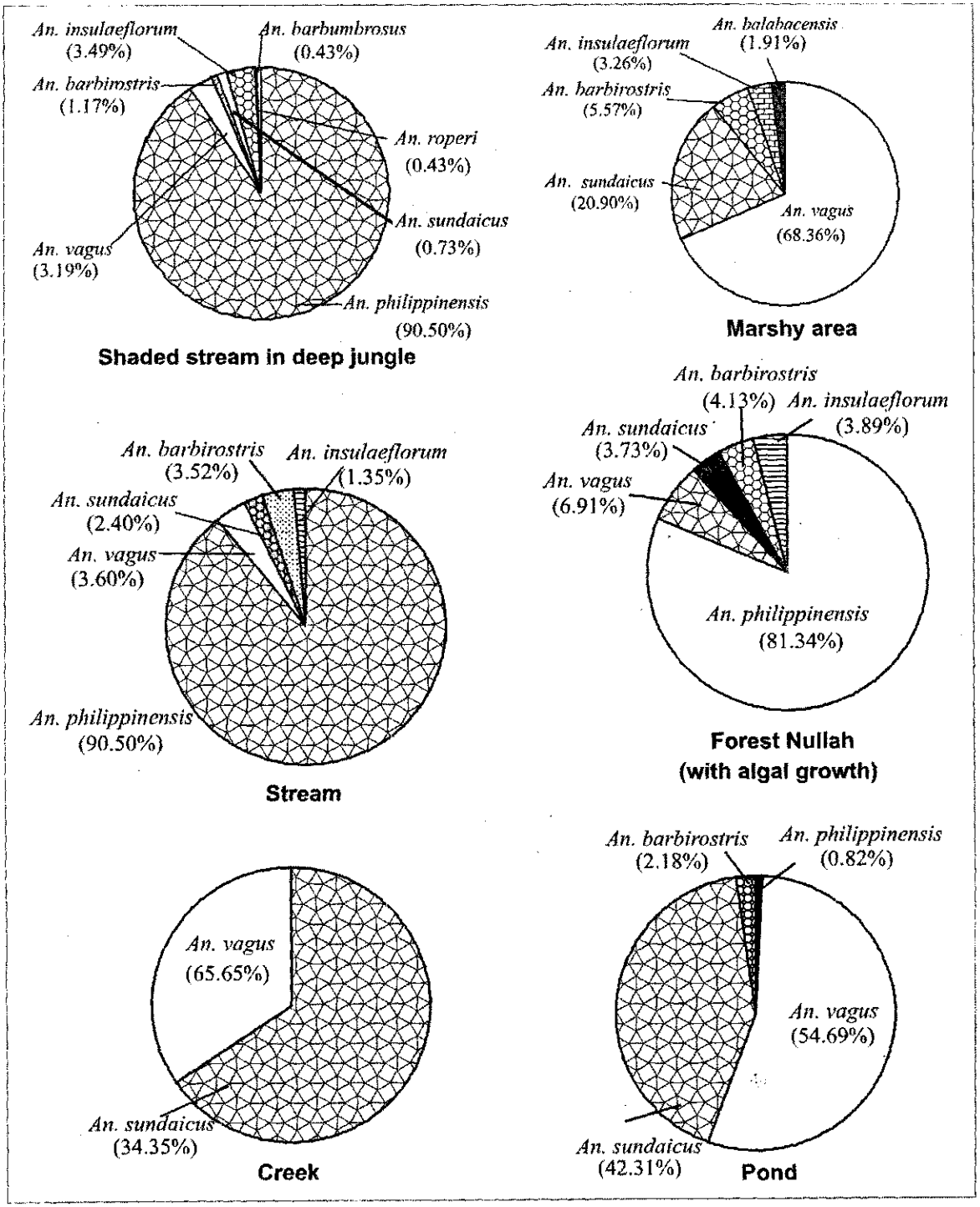
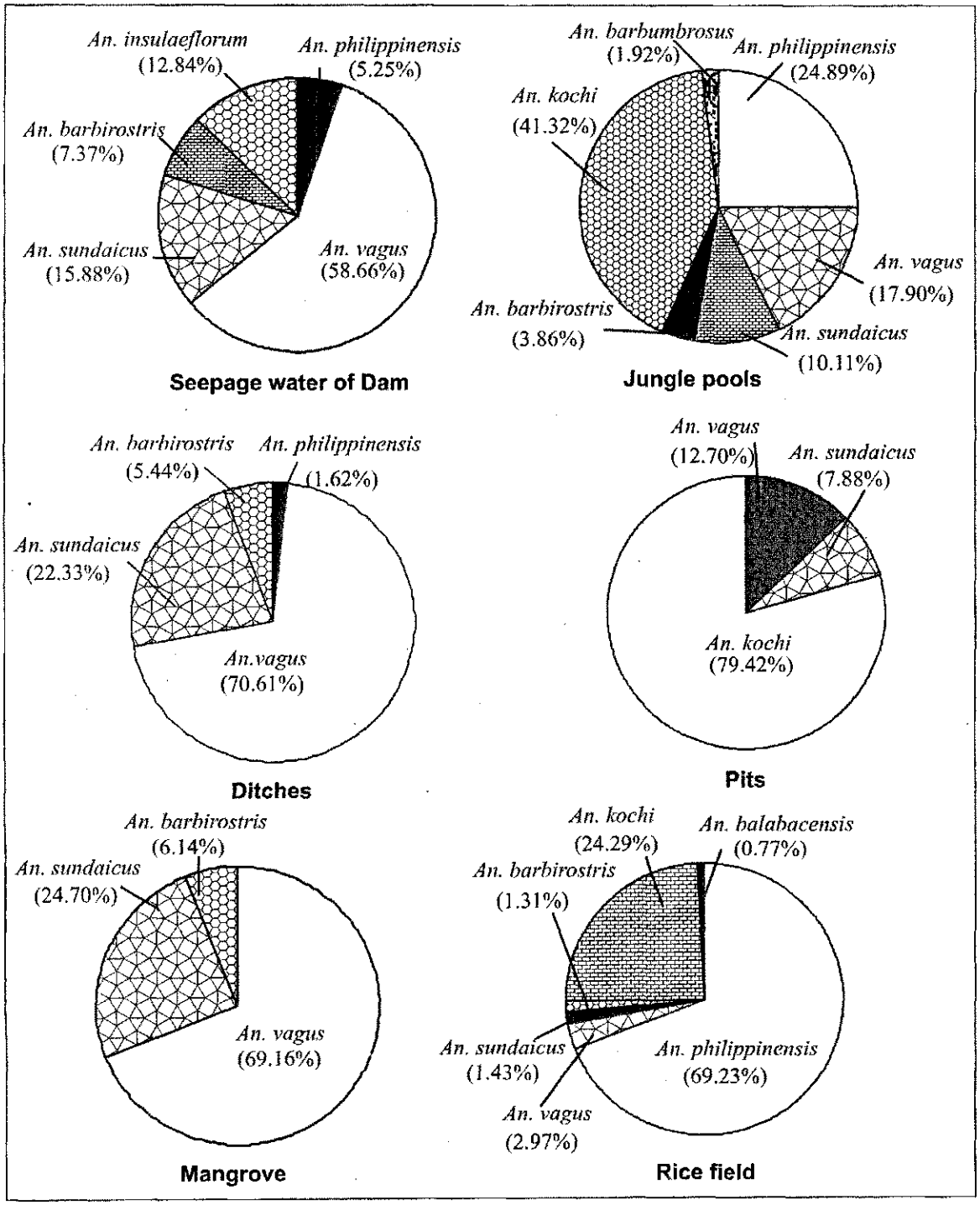


Fig. 3: Habitat-wise composition of anophelines (*contd...*)



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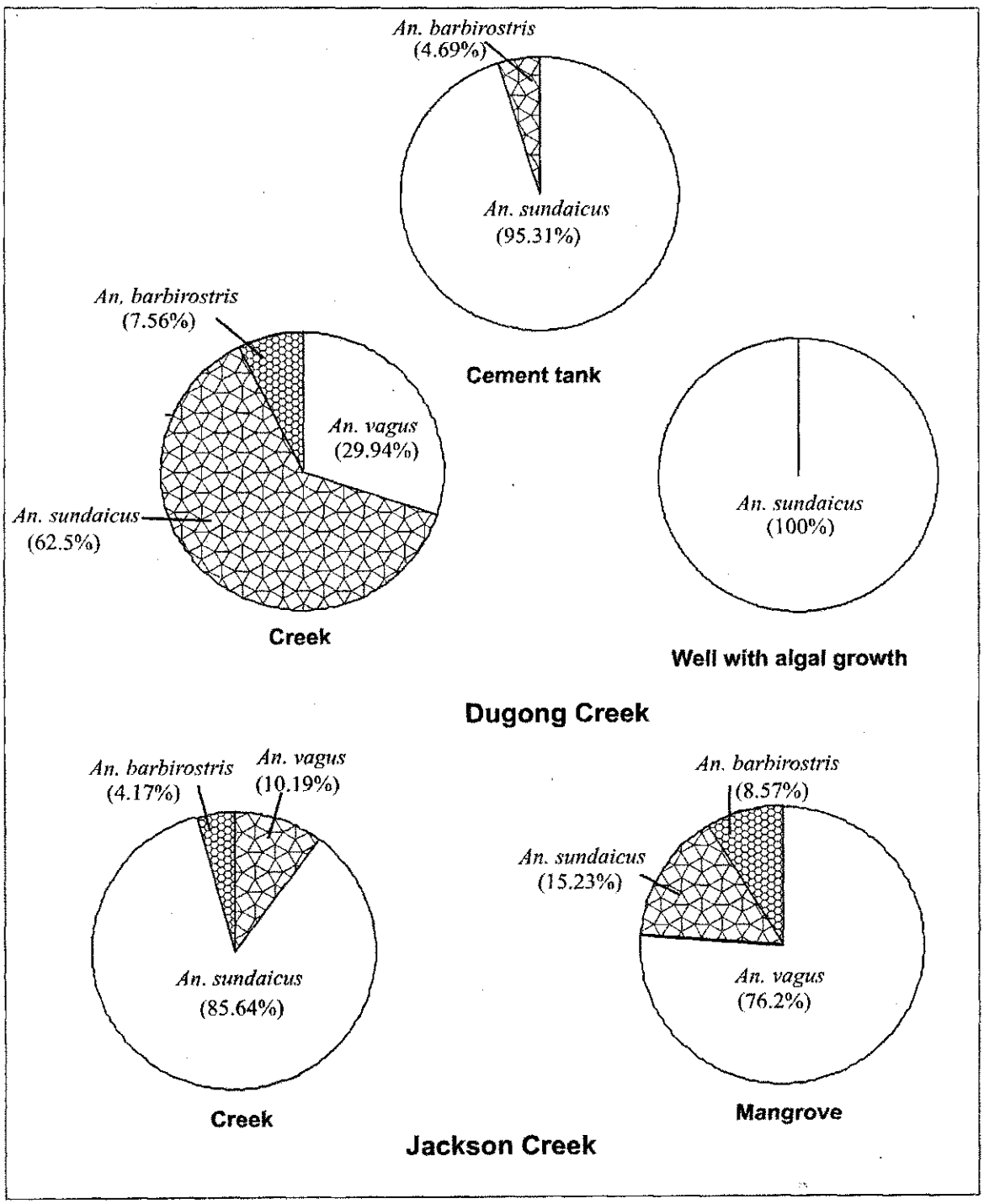

Fig. 3: Habitat-wise composition of anophelines

Nagar and Vivekanandpur village of Little Andaman were included with other collection methods and are given in Table 2. In Hutbay forest area, 103 *An. vagus*, 43 *An. sundaicus*, 10 *An. kochi*, 8 *An. philippinensis* and one *An. barbirostris* were collected from human bait (indoor). Twenty-six *Culex* specimens were also collected. From animal bait (Bovine, outdoor), 398 *An. vagus*, 291 *An. philippinensis*, 231 *An. sundaicus*, 82 *An. kochi* and 23 *An. barbirostris* were collected. A total of 1104 specimens belonging to the genus *Anopheles*, *Aedes*, *Culex* and *Mansonia* were collected from animal bait. In Kichard basti, 39 *An. vagus* and 17 *An. sundaicus* were collected from human bait (indoor). From animal bait (Bovine, outdoor), 182 *An. philippinensis*, 141 *An. vagus*, 84 *An. kochi*, 9 *An. sundaicus* and 2 *An. barbirostris* were collected. About 446 specimens belonging to the genus *Anopheles*, *Aedes* and *Culex* were collected from animal bait. In Netaji Nagar, 87 *An. vagus*, 27 *An. sundaicus*, 4 *An. kochi* and 3 *An. philippinensis* were collected from human bait (indoor). From animal bait (Bovine, outdoor), 262 *An. vagus*, 175 *An. philippinensis*, 124 *An. sundaicus*, 78 *An. kochi* and 16 *An. barbirostris* were collected. A total of 699 specimens belonging to the genus *Anopheles*, *Aedes*, *Culex* and *Mansonia* were collected from animal bait. In Vivekanandpur village, 139 *An. vagus*, 42 *An. sundaicus*, 7 *An. philippinensis* and 5 *An. kochi* were collected from human bait (indoor). From animal bait (Bovine, outdoor), 240 *An. vagus*, 193 *An. philippinensis*, 101 *An. sundaicus*, 78 *An. kochi* and 20 *An. barbirostris* were collected. A total of 672 specimens belonging to the genus *Anopheles*, *Aedes*, *Culex* and *Mansonia* were collected from animal bait.

Five Anopheline species were collected from all night bait collections made in Little Andaman. A total of 1409 specimens of *An. vagus* were collected. About 501 specimens were collected from Hutbay forest area followed by Vivekanandapur (379 specimens), Netaji Nagar (349 specimens) and Kichard basti (180 specimens). The maximum number was found biting between 1830 and 0030 hours.

Only 859 specimens of *An. philippinensis* were collected. About 299 specimens were collected from Hutbay forest area followed by Vivekanandapur (200 specimens), Kichard basti (182 specimens) and Netaji Nagar (178 specimens). The maximum number of biting was found from 1730–2230 hours. The majority of this species were collected from animal bait.

A total of 594 specimens of *An. sundaicus* were collected and of these 274 specimens were collected from Hutbay forest area followed by Netaji Nagar (151 specimens), Vivekanandapur (143 specimens) and Kichard basti (26 specimens). The maximum number were found biting during 1730–0230 hours. During daytime no species was found either from the cattleshed or from the house.

A total of 342 specimens of *An. kochi* were collected. About 93 specimens were collected from Hutbay forest area followed by Kichard basti (84 specimens), Vivekanandpur (83 specimens) and Netaji Nagar (82 specimens). The maximum number of specimens were collected from animal bait during 1730–2350 hours.

About 62 specimens of *An. barbirostris* were collected. Out of these 24 specimens were col-

Table 3. Per cent composition of different anopheline species in different breeding habitats

Breeding sites	Species emerged								
	An.philippinensis	*An. vagus*	*An.sundaicus*	*An.barbirostris*	*An.insulaeflorum*	*An. kochi*	*An.barbumbrosus*	*An. roperi*	*An.balabacensis*
Creek	0	975 (65.65)	510 (34.35)	0	0	0	0	0	0
Marshy area	0	713 (68.36)	218 (20.90)	58 (5.27)	34 (3.26)	0	0	0	20 (1.91)
Stream	1190 (89.13)	48 (3.60)	32 (2.40)	47 (3.52)	18 (1.35)	0	0	0	0
Shaded stream in deep jungle	1478 (90.50)	52 (3.19)	12 (0.73)	19 (1.17)	57 (3.49)	0	7 (0.43)	8 (0.49)	0
Forest nallah (with growth of vegetation)	1025 (81.34)	87 (6.91)	47 (3.73)	52 (4.13)	49 (3.89)	0	0	0	0
Pond	11 (0.82)	729 (54.69)	564 (42.31)	29 (2.18)	0	0	0	0	0
Seepage water of dam	172 (5.25)	1981 (58.66)	519 (15.88)	241 (7.37)	420 (12.84)	0	0	0	0
Mangrove	0	529 (69.16)	189 (24.70)	47 (6.14)	0	0	0	0	0
Pits	0	229 (12.70)	142 (7.88)	0	0	1432 (79.42)	0	0	0
Ditches	37 (1.62)	1619 (70.61)	512 (22.33)	125 (5.44)	0	0	0	0	0
Jungle Pools	271 (24.89)	195 (17.90)	110 (10.11)	42 (3.86)	0	450 (41.32)	21 (1.92)	0	0
Rice Fields	630 (69.23)	27 (2.97)	13 (1.43)	12 (1.31)	0	221 (24.29)	0	0	7 (0.77)
Dugong creek									
Creek	0	392 (29.94)	818 (62.50)	99 (7.56)	0	0	0	0	0
Well with algal growth	0	0	415 (100.00)	0	0	0	0	0	0
Jackson Creek									
Creek	0	22 (10.19)	185 (85.64)	9 (4.17)	0	0	0	0	0
Mangrove	0	240 (76.20)	48 (15.23)	27 (8.57)	0	0	0	0	0
Total	4814	7775	4334	807	578	2103	28	8	27

Figures in parentheses indicate percentages.

lected from Hutbay forest area followed by Vivekanandapur (20 specimens), Netaji Nagar (16 specimens) and Kichard basti (2 specimens). The maximum number of specimens were collected from animal bait during 1730-0430 hours. Only one specimen was collected from human bait during the all night collection.

This is the first record of the mosquito fauna of Little Andaman Island. The present study also highlights the breeding status of different *Anopheles* species with reference to *An. sundaicus* the malaria vector of Andaman and Nicobar Islands. This will be of immense importance in formulating strategy for the antilarval operation such as being tried under alternative approach through bioenvironmental control of malaria at Car Nicobar Islands.

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Study of Malaria in a Village of Lower Myanmar

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Malaria endemicity in lower Myanmar has been studied to identify the causes for the prevalence of malaria in Yeasitkan village of lower Myanmar. Vector mosquitoes were collected by mosquito net in cattlesheds and in human dwellings (indoor and outdoor) by biting and catching procedure for the identification of species, insecticide susceptibility test and sporozoites detection. Larvae of mosquitoes were also collected in and around the village for vector identification and for breeding sources. Malaria infection in humans was examined by blood examination and blood antibody detection by ELISA method. Results showed that malaria infection was 43.2% in children under 10 years of age and *An. dirus* and *An. minimus* were found as main vectors. Total parasite positive rate was found to be 41.28% and in this 78.87% were *P. falciparum* infections and remaining 18.31% were of *P. vivax*. Spleen positive rate has been found very high in children between 2 and 9 years (52.94%). Study indicates that villages near to dam areas are more prone to malaria infection.

Keywords: Incidence rate, Malaria, Mosquitoes, Plasmodium, Village

INTRODUCTION

Malaria is a common infectious disease of tropical countries and a major public health problem of Southeast Asia.[1–3] In Myanmar *An. dirus* and *An. minimus* have been found as main vectors and their prevalence depend upon the distribution of forest.[4–7] However, other suspected vectors are *An. sundaicus, An. annularis, An. culicifacies, An. aconitus, An. maculatus* sp, *An. philippinensis* and *An. vagus*.[8–10] Insecticide sensitivity studies in Anophelines in Oktwin Township of Bago Division and Mudon Township of Mon state revealed that *An. dirus, An. minimus, An. culicifacies, An. maculatus* and *An. annularis* were found to be sensitive to Icon, deltamethrin 0.025% and permethrin.[11]

Coosemans *et al.*[12] found high rate of malaria in less irrigated area of the Rosizi valley of Burundi, but Gaddal *et al.*[13] found that irrigated

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areas have more outbreak of infections compared to that of less irrigated areas in Sudan. Epidemiological studies of Tigray region of Sudan showed high incidence of malaria, schistosomiasis and intestinal parasites in children in villages close to dam area.[14–15] Similar results also observed in studies of children of northern Ethiopia.[16] Present survey is planned to investigate the role of vector, parasite, risk area and malaria infection in human population in Yeasitkan village of lower Myanmar.

MATERIALS AND METHODS

Study area

Yeasitkan village of Taikkyi Township in lower Myanmar is situated in the southern part of the Bago mountain range and about 120 m above sea level. Half of the total area is covered by dense forest and very close to Ghophyu Dam. There is one Primary Health Centre and one water-sterilizing pond (200 sq ft). Plant supplies sterilized water to Yangon city by pipeline. Most of the villagers are employed in dam work and water sterilization plant and remaining village folks earn their livelihood by cutting and selling wood, bamboo and making charcoal from the forest area. The study was conducted in December 1999.

Meteorological data

Area gets highest rainfall during the month of July (±390 mm) and relative humidity (RH) has been found more than 80%. In December rains occur rarely and RH remains between 52 and 56% and approximate environmental temperature ranges from 16–31°C.

Experimental protocol

Details of human subjects and clinical findings are shown in Table 1.

Entomological survey: Four corners of the village area were used for mosquito collection and identification of vectors. Human bite mosquito samples were collected by WHO sucking tube and in cattle by a big mosquito net (330 x 330 x 180 cm). Collections of mosquitoes were done for three days and three nights. Volunteers for mosquito collection were given mefloquine prophylaxis regimen for a week as a prophylactic measure. Larvae of mosquito were collected within 3 km radius of the village for three days and samples of larvae from different sites were brought to DMR laboratory for mosquito identification. Larvae were reared and after attaining the IV instar stage of development they are separated in individual vials for identifica-

Table 1. Details of human subjects and clinical findings in Yeasitkan village

Total village population	Male	Female	Total +ve fever	Clinical symptoms		
				Fever	Spleen	Fever+Spleen
253	116 (45.85%)	137 (54.15%)	172 (67.98%)	96 (37.94%)	30 (11.86%)	46 (18.18%)

tion. Samples collected from human, cattle and larvae of mosquitoes were identified for Anopheline species. Insecticide susceptibility tests were performed by WHO insecticide kit and insecticide exposed mosquitoes had been tested for the presence of sporozoite in salivary gland by immunological assay.

Mosquito species identification: Mosquito species were identified by morphological methods.[17–23]

Sporozoite detection by ELISA method: Enzyme-linked immunosorbent assay (ELISA) for circumsporozoite protein was done by the method of Wirtz *et al.*[23]

Human survey

Clinical examination: About 253 village subjects were clinically examined for fever and enlarged spleen. Spleen examination was done according to Hackett's Index[24] and 172 subjects have been found clinically positive for fever and spleen enlargement (clinical malaria) and were used in the present study.

Blood examination: Blood samples were collected on 3 mm Wattman filter paper for ELISA study by finger prick method. Blood smears (thick and thin) have been stained in 10% Giemsa stain and slides were examined for the presence of malaria parasite. Parasites were counted by the WHO method.

RESULTS

Mosquito identification study: A total of 1382 Anopheles mosquitoes were collected and only 13 species were found in this area (Table 2). Only one *An. minimus* and one *An. maculatus* have been found positive in ELISA studies for *P. falciparum* in salivary gland circumsporozoite protein assay. Other potential and suspected vectors for transmission of malaria were also found—*An. annularis* and *An. aconitus* in the study.

Larval survey: Table 2 shows the mosquitoes emerged from larval collections from breeding site. *An. jamesii* larvae were found in maximum number followed by *An. maculatus, An. aconitus, An. annularis* and *An. vagus*. Few *An. dirus* and *An. minimus* were also found.

Insecticide susceptibility tests: *An. jamesii, An. minimus, An. maculatus, An. tessellatus, An. aconitus, An. splendidus, An. kochi, An. varuna, An. hyrcanus* and *An. barbirostris* were found highly susceptible to DDT (4%), deltamethrin (0.025%), permethrin (0.025%) and malathion (5%). But *An. annularis* and *An. vagus* were found resistant to DDT (4%).

Human blood study: Table 3 shows the results of microscopic examination of blood in 172 human subjects and 71 cases of malaria. It was found that children between 5 and 9 years age group were highly affected by this disease (53.03%). Also age-specific parasite rate and cumulative gametocyte positive rate for *P. falciparum* are shown in Table 3.

Malaria ELISA immunological assay: Results of ELISA immunoassay of blood samples of 172 human subjects showed that 51.16%

Table 2. Mean values of mosquito biting in human and animals (3 days) and sporozoite positive mosquito rate by ELISA

Anopheline species	No. of mosquitoes and sporozoite +ve rate					HBR/ night
	Human bite (indoor + outdoor)	Sporozoite +ve mosquito (ELISA)	Cattle bite	Total mosquitoes and sporozoite +ve rate	Sporozoite +ve rate (ELISA)	
An. jamesii	75		588	663		6.25
An. minimus	10	1	18	28	1 (3.57)	0.83
An. maculatus	44	1	140	184	1 (0.54)	3.67
An. tessellatus	13		21	34		1.08
An. aconitus	17		36	53		1.41
An. annularis	25		67	92		2.08
An. splendidus	12		30	42		1.00
An. kawari	10		22	32		0.83
An. kochi	11		26	37		0.92
An. vagus	23		59	82		1.91
An. barbirostris	13		42	55		1.03
An. hyrcanus	12		27	39		1.00
An. varuna	13		28	41		1.08
Total	278	2	1104	1382	2 (0.15)	23.16

(88/172) subjects were positive for malaria antibody (O.D. 0.93 ± 0.07).

Table 3. Parasite positive rate in humans by microscopic examination in Yeasitkan village

Age group	% +ve (Subjects)	*P. falciparum* gametocyte +ve rate	% +ve (Spleen +ve rate)
0–1 month	42.85	–	1 (14.29)
12–23 months	22.22	–	3 (33.33)
2–4 yrs	30.55	–	19 (52.78)
5–9 yrs	53.03	1 (1.32)	35 (53.03)
10–14 yrs	36.84	–	6 (31.58)
>15 yrs	37.14	1 (2.86)	12 (34.29)

DISCUSSION

The results of the present study revealed that malaria infection was very high in under 9 years children and the infant parasite rate was found to be 42.85%. The results of the present study are in conformity with the earlier studies carried out by Myint Lwin *et al.*,[25] Myo Paing *et al.*[10] and Tun Lin *et al.*[26] who have also observed high rate of malaria prevalence in infants and children between 2 and 9 years of age groups in Thabyewa village, western and eastern parts of Pago Yoma in Myanmar. Our finding showed that *P. falciparum* prevalence in village population is 78.87 %; *P. vivax* is 18.31% and mixed infection is 2.82%. Studies of Myo Paing

Table 4. Detection of Anopheline sp. emerged from larvae

S. No.	Name of area	Type of mosquito sp.	Density/Dip				
			1st	2nd	3rd	4th	p-value
1.	Small pools on the side of dam	*An. jamesii*	5	6	4	3.66	2
		An. vagus	1	2	3	1	0.66
		An. annularis	3	2	1.66	2.33	0.33
2.	Rock pools under dense forest	*An. dirus*	1	1	1	2	0.33
3.	Bamboo stumps in deep forest	*An. dirus*	–	1	2	1	0.33
		Ae. subalbopictus	2	3	3	5	2
		Armigeres annissus	2	2	3	2	1
4.	Small jungle streams	*An. minimus*	–	1	1.33	1	0.33
		An. maculatus	1	3	3	2.33	1.33

et al.[22] and Tun Lin *et al.*[26] reported that 80% of malaria caused by *P. falciparum* in Thabyewa village Bago mountain range in all seasons. Also study in Yepyu Township, Dewae district, Taninthayi division done by Htay Aung *et al.*[27] and Kowthaung Township Taninthayi division[28], Kazuma village Magwe division[29] showed high incidence of *P. falciparum* infection. However, *P. vivax* infection rate has been found more in other parts of Myanmar—Thaindi village.[30] Total parasite density index (PDI) in village Yeasitkan has been found to be 5.03, which is very high.

This study in Yeasitkan village of Taikkyi Township showed that *An. minimus* is a causative vector for transmission of malaria in humans, however, studies of other workers found that *An. dirus* and *An. minimus* are main vectors for transmission of malaria and may be due to the presence of water wells, small rock pools, small jungle, stems and bamboo stumps, etc.[9, 31, 32] (Table 4).

Our ELISA immunoassay result of mosquito salivary gland study found only one *An. minimus* and one *An. maculatus* positive for *P. falciparum* sporozoite. We have measured man and cattle biting ratio for *An. minimus* and found to be 1 : 2. The study suggests that high rate of malaria may be due to the presence of a big dam near to Yeasitkan village and dam area creating conducive environment for breeding of mosquitoes. It is also helping growth of large numbers of shrubs and plants in and around dam area which is causing the increase in population of mosquitoes and may be responsible for higher incidence of malaria in Yeasitkan village.

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SHORT NOTES

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Clinical Analysis of Malaria Cases Treated at MGM Hospital, Navi Mumbai

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Keywords: Epidemiology, Malaria, Malaria surveillance, Navi Mumbai, *P. falciparum*, *P. vivax*

Malaria is a serious problem in India and abroad.[1,2] Overall 2.6 to 2.7 million cases are reported annually in middle south Asia, out of which 80 per cent of cases occur in India.[3] The recent trend of increasing mortality related to malaria is due to rising incidence of falciparum malaria and drug resistance. *Plasmodium falciparum* infection may be frequently accompanied by renal, hepatic and/or cerebral involvement and complications.

Navi Mumbai (New Bombay) is the area close to Mumbai having a population about 7.22 lakh, out of this 40 per cent population reside in slum areas. It is a fast developing city and large number of construction activities are in process. There are about 136 quarries in this area and large number of migratory labourers are congregated in and around these projects. The land is marshy and low-lying and results in the stagnation of water which is conducive for mosquito breeding. Mosquitogenic potential and incidence of malaria in this area are comparatively higher than the other areas, with peak malaria transmission between July and November every year. Till date there have been no documented reports of malaria situation in this region. Hence, present work was undertaken to study epidemiological and clinical characteristics of laboratory confirmed cases of malaria diagnosed in Mahatma

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Gandhi Mission's Medical College and Hospital at CBD-Belapur for five years (January 1997–December 2001). This hospital is centrally located in Navi Mumbai and receives patients from all over including nearby rural and urban areas.

Patients were clinically examined by the physician and suspected patients were referred for blood smear examination. Thick and thin blood smears were stained by Giemsa stain and examined for malaria parasites for the identification of species. All blood smear positive malaria cases were included in the study. Detailed case report containing clinical and laboratory information was recorded for every patient. This includes name, address, age, sex, date, time, place of onset of illness, laboratory results, details of species, previous history of malaria, clinical complications and drugs used for the treatment. Record of clinical complications of the infection was maintained carefully.

Diagnosis of cerebral malaria was done by well-experienced physician as per the WHO guidelines.[4] Following laboratory investigations were done as supportive majors — cerebrospinal fluid (CSF) for cerebral involvement; ABG for metabolic acidosis; liver function tests (LFTs)— SGOT (serum glutamate-oxaloacetate transminase), SGPT (serum glutamate-pyruvate transaminase) and serum bilirubin (patients suffering from chronic liver disease and hepatitis were excluded); renal function tests–serum creatinine and blood urea for renal complications. For the diagnosis of multi system organ failure (MSOF) involvement of more than two systems/organs were considered. In addition to hepatic and renal involvement disseminated intravascular coagulation (DIC), acute respiratory distress syndrome (ARDS) and vascular collapse were also observed in these patients.

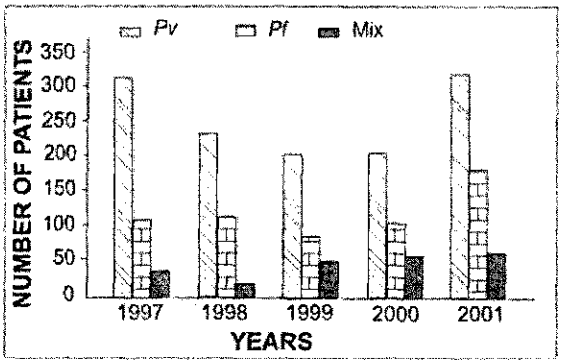

Fig.1: Distribution of *Pv, Pf* and mixed infections (1997–2001)

Malaria was clinically suspected in 9504 patients out of these 2132 (22.43%) patients were reported blood smear positive. Distribution of malaria cases during the entire study period is presented in Fig.1. Incidence of *P. vivax* infection was 60.27 per cent (1285/2132). Incidence of *P. falciparum,* infection was 28.61 per cent (610/2132) and for mixed infection due to *P. vivax* and *P. falciparum,* was 11.12 per cent (237/2132). *P. malariae* and *P. ovalae* infections were not reported. Malaria prevalence is increasing from 1997–2001 with the increase in falciparum malaria. Maximum number of cases was seen in the year 2001. Malaria cases were seen throughout the year in all age groups, but prevalence was high in 21–30 years age group followed by 31–40 and 11–20 age groups (Fig. 2). Malaria prevalence was more predominant in males than females. *P. vivax* cases were recorded throughout the year but *P.*

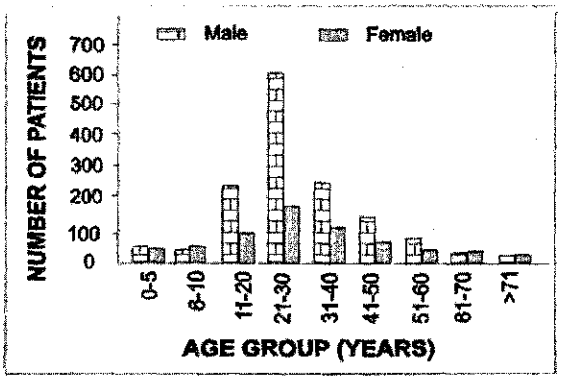

Fig. 2: Age-group and sex-wise distribution of malaria

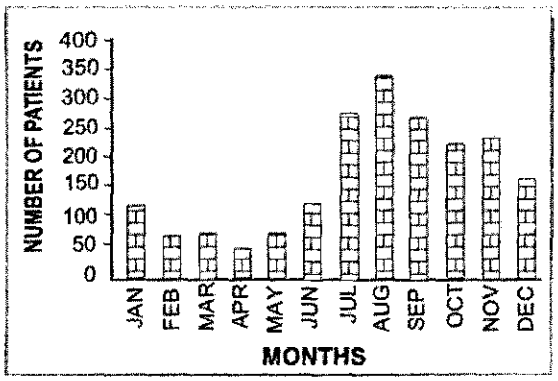

Fig. 3: Malaria incidence in different months

falciparum malaria cases were observed to be increased from June to November — after rainy season (Fig. 3).

Out of 2132 patients 944 had developed complicated malaria and were hospitalised. Among these, 48.73 per cent (460/944) had *P. falciparum* infection, 32.10 per cent (303/944) had mixed infection due to *P. falciparum* and *P. vivax* and the remaining were *P. vivax* infections (181/944). Record of these 763 patients admitted due to falciparum malaria and mixed infection was maintained and analysed carefully and it was observed that 17.56 per cent (134/763) patients had anaemia (Hb $\leq$ 5%), 10.22 per cent (78/763) had hepatic complications, 3.2 per cent (25/763) had renal changes, 0.92 per cent (7/763) had cerebral malaria and 1.44 per cent (11/763) had multi-organ system failure with 0.4 per cent (3/763) deaths. All these patients had high grade fever with coma and generalised seizures, hypotension, and multi-organ failure, hypokalemia, metabolic acidosis and haemolytic anaemia with very high parasitic index. Out of these cases three deaths were reported.The results of various biochemical parameters are presented in the Table1. It is clear from the results that elevation of

Table 1. Details of laboratory investigations in complicated malaria patients admitted at MGM Hospital, Navi Mumbai

Parameters	Mean ± S.D.			Method of estimation
	Pv (n = 181)	*Pf* (n = 460)	*Pv* ± *Pf* (n = 303)	
Haemoglobin (12–16 g/dl)	12.4 ± 2.2	9.6 ± 3.5	10.5 ± 3.5	Cyanohaemoglobin
S. bilirubin (0–2 mg/dl)	1.6 ± 1.3	3.6 ± 3.8	3.7 ± 3	Kinetic RA-50
SGOT (0–40 IU/dl)	51.6 ± 74	219 ± 427	64 ± 176	Kinetic RA-50
SGPT (0–40 IU/dl)	63.6 ± 92	363 ± 543	104 ± 237	Kinetic RA-50
S. creatinine (0.8–2 mg/dl)	11 ± 1.3	2.9 ± 2	1.5 ± 1.4	Picrate RA-50
Blood urea nitrogen (4–21 mg/dl)	8.2 ± 2.3	29.1 ± 42.9	17.8 ± 21	UV-RA-50

Figures in parentheses indicate normal range observed in healthy adults.

S. bilirubin, SGOT, SGPT, blood urea, S. creatinine is observed to be high in the *P. falciparum* patients followed by mixed and *P. vivax* infections.

A total of 10.22 per cent (78/763) patients were studied in detail for hepatic complications. It was observed that serum bilirubin, SGOT and SGPT were significantly higher in *P. falciparum* than other patients. After chemotherapy the liver function tests returned to normal within two weeks.

The results of the present study in Navi Mumbai for the last five years indicates the fact that the prevalence of malaria is high in this region and malaria cases were reported in every month and a good number of cases were reported during rainy season—July to November which indicates seasonal transmission. The high incidence of malaria in this region may be due to the prevailing conditions especially in rainy season such as lower temperature in winter (20° to 30°C) which is favourable to the parasite growth; humidity more than 60 per cent which is favourable for the longevity and activity of mosquitoes; man-made water habitats during construction activities; water accumulation in low-lying areas; small water collections during rains; poor drainage facilities, etc. are conducive for mosquito breeding in this area. Hence in the rainy season and post rainy season high malaria incidence is reported. Further, the persistence of parasite load, poor surveillance, lack of sense of hygine in the labour population congregated in nearby areas and slums contributes to continuous malaria transmission in this locality. The results of the study are in conformity to the Indian scenario where malaria transmission is high during July to November months[5] in most parts of India and in all age groups.[6]

Results of clinical examinations revealed that complications such as ARDS, cerebral malaria, DIC, ARF, multisystem organ failure, hepatic complications are reported in *P. falciparum* and mixed infections and the duration of recovery after chemotherapy is high. Only few vivax infections showed complications and they recovered very shortly after chemotherapy. Hyperbilirubinemia may be due to increased conversion of haemoglobin into bile pigments, intravascular haemolysis of parasitised red blood cells, hepatic dysfunction due to septicemia. Heavy parasitemia, hyperpyrexia and volume depletion may be responsible for acute renal failure.[7–9] Elevation in transaminases may be mainly due to impaired permeability arising due to the destruction of infected red blood cells, hepatic cells and parenchymal cells in the liver due to liver dysfunction and damage caused by the parasites.[10–11]

It can be concluded from the present study that malaria situation in Navi-Mumbai is alarming and needs immediate attention for malaria control in this region. Appropriate vector control measures are indicated in this region to control mosquito breeding and proliferation.

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Malaria Vectors of Southern Rajasthan, India

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Keywords: *An. culicifacies, An. fluviatilis, An. stephensi*, Southern Rajasthan

Rajasthan lies between 23°3' and 30°12' north latitude and 64°5' and 78°17' east longitude. It has an area of 3,56,700 sq km with eastwest diagonal of about 836 km and northsouth diagonal of 836 km. The region is typical arid and semi arid consisting of dry sand desert areas interspersed with fertile plains and plateaus as well as forest clad hills. The Aravallis form the main hill range rising as high as 1219 m above the sea level. Extreme climate is very characteristic features of Rajasthan. Winter extends from December to February with mean minimum temperature varying from 5 to 10°C, whereas summer extends from April to June with May as the hottest month. Summer is quite intense and temperature up to 45°C is a common feature. Rainy season extends from July to September. Western dry area receives annual rainfall as low as 100 mm whereas hilly and plateau areas on an average recieve around 750 mm rainfall (Table 1). These are innumerable perennial and monsoon streams and lakes in the undulating landscape of hilly and plateau areas. On the basis of physiography and climate, Rajasthan can be divided into four natural divisions—(i) East Rajasthan plains; (ii) West Rajasthan desert plains; (iii) Rajasthan hilly regions; and (iv) Rajasthan plateau.

Rajasthan has been experiencing local and regional epidemics of malaria with considerable patches of hyperendemicity, while western desert areas are prone to epidemics, the eastern plains show variable endemicity. The hilly and plateau regions because of absence of extreme climatic conditions show stable malaria. Investigations in the past have revealed *An. culicifacies* associated with epidemics in western desert region and *An. stephensi* as a vector of urban and rural areas. *An. fluviatilis* and *An. culicifacies* are the vectors in the hilly tracts and plateau regions.[1–5]

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Table 1. Average climatological data of southern districts of Rajasthan (1995–2001)

Month	Temperature (°C)	Rainfall (mm)	Relative humidity (%)
Jan	25.8	0	79
Feb	25.4	0	55
Mar	31.5	0	46
Apr	38	0.8	40
May	38.9	52.4	63
Jun	39	99	66
Jul	30.9	172.8	83
Aug	30.2	168	84
Sep	28.1	125	84
Oct	35.4	30	67
Nov	33.8	0	72
Dec	28	0	64

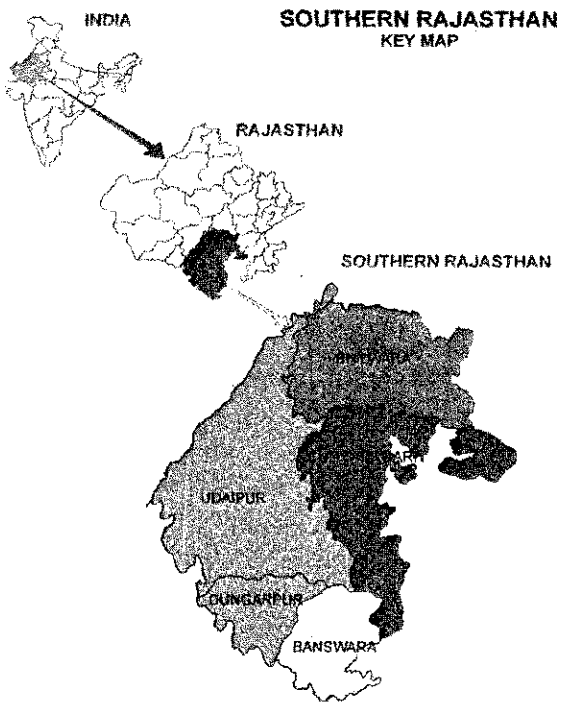

Fig. 1: Map of southern districts of Rajasthan

Over the successive five year development plans, Rajasthan topography has undergone radical changes. Introduction of Indira Gandhi Canal with an irrigation potential of 1200 million hectares has transformed the western desert region into that of greenery. Similarly, eastern plains and plateau regions have introduced many irrigation schemes and industrial projects respectively, producing in the process a very high breeding potential of vector mosquitoes and that of transmission of malaria. It was in this context that present longitudinal study was planned in southern Rajasthan (Plateau region) to determine the prevalence of malaria vectors in the altered scenario.

Mosquitoes sampling was undertaken in different villages of Banswara, Chittorgarh, Dungarpur, Rajsamand and Udaipur districts of southern Rajasthan (Fig. 1) from January 1995 to December 2001 following WHO standard entomological techniques. Mosquitoes were identified under the stereoscopic microscope using the keys of Das *et al.*[6] and Nagpal and Sharma.[7] The prevalence of malaria vectors was determined in terms of per man hour (PMH) density. Climatological data during the period of studies were collected from meteorological department of C.T.A.E., Udaipur.

Examination of anopheline mosquitoes confirmed the presence of three vector species of *Anopheles—An. culicifacies, An. stephensi* and *An. fluviatilis* in this region. Analysis of the

Table 2. Average prevalence of malaria vectors in southern districts of Rajasthan (1995–2001)

District	*An. culicifacies* (PMH)	*An. stephensi* (PMH)	*An. fluviatilis* (PMH)
Udaipur	2–134	0–3	0–15
Banswara	8–200	0–3	0–12
Dungarpur	5–165	0–2	0–14
Chittorgarh	1–80	0–1	0–13
Rajsamand	1–35	0–2	0–5

results (Table 2) revealed that among the three species only *An. culicifacies* was found dominant and other two species—*An. stephensi* and *An. fluviatilis* were sparsely found in different villages of southern Rajasthan. Observations for the years 1995–2001 (Tables 3, 4 and 5) indicated that the vector density started to increase in March to May and highest density was observed in June to September, thereafter it gradually decreased in the months of October and November. Almost similar trend was observed during the period of study (1995 –2001).

Prevalence of mosquito species depends on the availability of natural breeding water in an ecosystem. Hilly and plateau areas have many slow moving streams and stagnant water bodies, which provide facilities for breeding of *An. fluviatilis* and *An. culicifacies* respectively. Since, *An. culicifacies* being monsoon associated species, exploits transient water collections and build up high density. This is reflected in Table 3. During monsoon the species density crosses 100 PMH mark. *An. fluviatilis*

Table 3. Density (PMH) of *An. culicifacies* in southern districts of Rajasthan (1995–2001)

Month	1995	1996	1997	1998	1999	2000	2001
Jan	3	1	1	2	2	2	2
Feb	5	4	1	5	4	4.5	4
Mar	20	22.3	12	18	13	16.8	13
Apr	58	55	32	50	38	42.8	30
May	69	64	45.5	55	43	51	40
Jun	130	134	126	133	128.8	116	130.2
Jul	148.9	140	132.6	144.7	134	133	136.8
Aug	148	129	131	130.3	85.6	122.7	132.6
Sep	132	100.6	130	134	84.3	90	85
Oct	89	80	67	82	71	60	68.2
Nov	45	39	37.7	48	42	45	39.3
Dec	1	1	1	3.5	2	2	3

Table 4. Density (PMH) of *An. fluviatilis* in southern districts of Rajasthan (1995–2001)

Month	1995	1996	1997	1998	1999	2000	2001
Jan	0	0	0	0	0	0	0
Feb	2	0	1	0	4	0	3.5
Mar	3	4	4	4	3	4	4.3
Apr	3.4	6.5	5.8	6	7	8.5	8.6
May	7	10.2	9.9	6	9	8.8	9.8
Jun	12	15	13.5	10	11.7	12.3	13
Jul	14.8	14	13.6	12	13.2	14.2	15
Aug	13.2	12.2	13.6	13	11	13	14.2
Sep	12	13	13	15	12	10	11.2
Oct	9.7	8.8	9	10	9	6	9.5
Nov	5	6	4	3	1	3	4
Dec	0	0	0	0	0	0	0

Table 5. Density (PMH) of *An. stephensi* in southern districts of Rajasthan (1995–2001)

Month	1995	1996	1997	1998	1999	2000	2001
Jan	0	0	0	0	0	0	0
Feb	0	0	0	0	0	0	0
Mar	1	1	0	0	1	0	1
Apr	1	2	1	1	1	1	1
May	2	2	2	1	1	2	2
Jun	3	3	2	3	2	2	3
Jul	3	3	3	3	3	3	3
Aug	2	2	3	3	3	2	3
Sep	2	2	2	3	2	2	2
Oct	1	1	1	2	1	2	1
Nov	0	0	0	0	0	0	0
Dec	0	0	0	0	0	0	0

generally more predominant during post-monsoon period (Table 4) when torrents get stabilised. *An. fluviatilis* has been detected positive for sporozoites of malaria parasite in Udaipur and it is apparent that both *An. culicifacies* and *An. fluviatilis* maintain relay transmission to give rise to endemic malaria in southern Gujarat. *An. stephensi* is a domestic species and the density is directly correlated with water storage practices in the region. Since the region receive higher rainfall than western region, water storage practices are limited and this accounts for low prevalence of *An. stephensi* (Table 5). In view of this, *An. stephensi* role may be restricted to highly urbanised towns in the region.

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molar	M	intraperitoneal	ip
milli molar (mole/litre)	*mM*	*intravenous*	*iv*
micromolar (mole/litre)	µM	intramuscular	im
mole (quantity of substance)	mol	subcutaneous	sc
normal (normality)	*N*	oral	po
metre	m	ampere	A
centimetre	cm	milli Ampere	mA
square centimetre	cm^2	watt	W
millimetre	mm	Angstrom	A
micrometre	µm	volume ratio (volume per volume)	vol/vol
nanometre	nm	volume	vol
picometre	pm	weight	wt
hectare	ha	mg/100 ml	mg/dl
foot or feet	ft	weight per volume	wt/vol
cubic centimetre	cc	weight ratio (weight per weight)	wt/wt
litre	L	Confidence Interval (Statistics)	CI
millilitre	ml	chi square	χ^2
microlitre	µl	significant at 1% level	**
gallon	gal	significant at 5 % level	*
gram	g	standard deviation	σ
milligram	mg	standard error	$S_{\bar{x}}$
kilogram	kg	probability	*P*
figure	Fig.	ultra low volume	ULV
hour(s)	h	active ingredient	AI
minute(s)	min	emulsifiable concentrate	EC
second(s)	sec	lethal dose-50	LD_{50}
weekes	wk	diameter	diam
mean	$\bar{x}$	revolutions per minute	rpm
year(s)	yr	counts per minute	cpm
number	no.	relative humidity	RH
number (statistical)	*n*	sensu stricto	s.s
ortho	*0*	sensui lato	s.l.
meta	*m*	curie	Ci
para	*p*	gravity	g

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