Vol. 37, Nos. 3–4
September–December 2000
CODEN : IJMAA9
ISSN : 0367–8326

Indian Journal of MALARIOLOGY

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

Indian Journal of Malariology
Vol. 37 (September–December 2000)

INDIAN JOURNAL OF MALARIOLOGY

Volume 37
Numbers 3–4
September–December 2000

CONTENTS

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.

Indian Journal of Malariology
Vol. 37, September–December 2000, pp. 53–60.

Occupational Malaria and Health Risk among Select Occupational Health Care Employee Groups in an Urban Hospital at Tirupati, A.P.

M. RAJASEKHAR[a] and N.V. NANDAKUMAR[a]

Epidemiological studies on occupational environments are very meagre in the developing countries like India. For this reason an attempt was made in the present investigation to see the occupational risk of malaria among health care workers of Sri Venkateswara Ramnarayan Ruia Government General Hospital (SVRRGGH), Tirupati, Andhra Pradesh. The cohort studies revealed association between exposure to occupational (hospital) environment and malaria among hospital staff. Retrospective cohort studies were made. 1,454 subjects namely hospital staff exposed to occupational environment included civil assistant surgeons and physicians, the students of medicine, the students of nursing, permanent nursing staff and the class IV employees (class IV included janitorials, male nursing orderlies, female nursing orderlies, attenders, laundry workers and the rest). Retrospective epidemiological studies were carried out for four years on the cohorts and the hospital staff. The data showed statistically significant relative risk and attributable risk for malaria. Physicians and civil assistant surgeons showed no incidence of malaria. The relative risk for malaria in the class IV employees was 1.27, 0, 5.8 and 2.9 for the years 1995–98. The students of nursing showed 4.2, 2.42. 3.3 and 0 relative risk for malaria, whereas the students of medicine showed 2, 2, 2 and 1.6 for the years 1995 to 1998. The attributable risk was ranged from 21.76–82.70, 58.75–76.17, 50–80 for the class IV employees, the students of nursing and the students of medicine retrospectively. These results provide an evidence for an association between occupational environment and malaria for the hospital staff and is more prevalent among certain groups of the hospital staff.

Keywords: Malaria, Nursing, Occupational environment, Students of medicine

[a]Division of Environmental Biology, Department of Zoology, S.V. University, Tirupati–517 502, India.

INTRODUCTION

After the initial success, India is now unable to get the upper hand in malaria control. Over the past decade or so the number of malaria cases have remained unchanged with the addition of increase in falciparum and vivax malaria. Thus the issue today is that malaria has now entered the new ecological niches.[1] Chloroquine resistant strains were reported in Kenya, Somalia, Sudan and interior Ethiopia.[2] Malaria occurrence is associated with rainfall and season.[3,4] The new paradigm of malaria consists of new ecotypes such as irrigation malaria, urban malaria, development project malaria, migration and boarder malaria.[1] The ecotype, namely occupational malaria has not been given much attention and investigations on this direction are inadequate. Occupational malaria may be defined as malaria due to causes and conditions attributable to a particular working environment and not due to stimuli encountered outside the work place. There is a great deal of research addressing many aspects of current and emerging infectious diseases like malaria ranging from anecdotal works to case studies of epidemics to detailed models of vector physiology. However, research into the epidemiological aspects of occupational malaria on health care workers is very meagre in India and also first of its kind in the Rayalaseema province of Andhra Pradesh.

MATERIALS AND METHODS

Epidemiological studies on occupationally exposed health care workers for malaria were

Table 1. Categories of hospital employees in Sri Venkateswara Ramnarayan Ruia Government General Hospital, Tirupati, A.P.

Designation of the employee	No. of employees
Civil assistant surgeons and physicians	49
Nurses	147
Lab. technicians	10
Lab. attenders	9
Pharmacists	12
Administration work employees	32
Clerical grade employees	76
Class IV employees	
Sweepers (Scavengers)	23
MNO's* + FNO's**	80
All round attenders	15
Laundry workers	7
Cooks	11
Other group	
Thoties***	31
Stretcher bearers	14
Barber	1
Cleaner	1
Watchmen	15
Watermen	7
Gatemen (security)	1
Gardeners	3
Painter	1
Junior and record assistants	49
Medical college students	500
Nursing school students (Total of six schools)	360

*Male nursing orderlies; **Female nursing orderlies; ***Help in transporting dead or diseased.

compared to occupationally not exposed group. Retrospective cohort studies were made for four years from 1995–1998 on 1,454 subjects namely the hospital staff of SVRRGGH, Tirupati, (A.P.). The hospital staff include civil assistant surgeons and physicians, the students of medicine, the students of nursing and the class IV employees (class IV includes janitorials, male nursing orderlies, female nursing orderlies, attenders, laundry workers, etc. (Table 1). The studies were based on the morbidity and mortality data of health care workers taken from the registered health cards (in patients) of above Government General Hospital. Employees of Sri Venkateswara University and the students of degree who are not exposed to occupational environment and registered their morbidity in SVU Health Centre were taken as the cohort control and these were compiled as described by Cody *et al.*[5] Occupationally exposed health care workers are the permanent employees of SVRRGGH and whenever they get ill health they undergo treatment in the same hospital and hence, the employees were considered as experimental group. The control group, who are not exposed to the occupational environment are the permanent employees and the students of Sri Venkateswara University and they will undergo treatment for their ill health in the University Health Centre. If the subject has one time admission or number of admissions within two months for a disease, it is taken as one time admission only. A patient with chronic disease visiting several times in a year is also taken as one time admission. The morbidity data from the registered health cards could be compiled from 1995 to 1998 only. The health cards for the year 1999 are not yet sorted out and stored in the record room, therefore, the data has been compiled up to 1998 only. The hospital campus has open gutters and drainages which are partly covered and are the potential breeding places for mosquitoes. The mosquitoes are also seen under surface of lush green leaves, scrubs and bushes. These moquitoes enter into the hospital as windows and doors are not provided with mosquito proof nylon wire meshes (Table 2). Statistical applications include incidence of malaria, relative risk and attributable risk as recommended by Beaglehole *et al.*[6], and Park and Park.[7]

The formulae used for incidence of disease, relative risk and attributable risk are as follows:

$$\text{Incidence} = \frac{\text{No. of people who get a disease in a specified period}}{\text{Population at risk}} \times 100$$

$$\text{Relative risk} = \frac{\text{Incidence of disease among exposed}}{\text{Incidence of disease among non-exposed}}$$

$$\text{Attributable risk} = \frac{(\text{Incidence of disease among exposed}) - (\text{Incidence of disease among non-exposed})}{\text{Incidence of disease among exposed}} \times 100$$

The incidence of malaria among the occupational and the non-occupational groups has been compared using paired *t*-test for its statistical significance over a period of four years. Software "Data analysis park of M.S. Excel" had been used to compute paired *t*-test.

Table 2. Potential factors associated with malaria at Sri Venkateswara Ramnarayan Ruia Government General Hospital, Tirupati, A.P.

Potential factors	Physicians and Surgeons	Students of nursing	Students of medicine	Class IV employees
Using mosquito repellents in their work enviroment	80%	5%	50%	Nil
Sojourn time in personal rooms	>80%	Nil	>50%	Nil
Per cent of wearing thick uppercoat/fully covered dress	95%	60%	90%	70%
Using preventive antimalarial drugs	50%	Nil	Nil	Nil
Mean working hours per day in occupational environment	8	7	6	8
Mean working hours per night in occupational environment in shift system	3	4	2	4
Using mosquito proof wires or nylon wire meshes or curtains for rooms	Nil	Nil	Nil	Nil

Table 3. Disease incidence among occupationally exposed health care workers belonging to Sri Venkateswara Ramnarayan Ruia Government General Hospital, Tirupati, A.P.

Health care workers	Incidence of malaria among occupationally exposed (experimental) group (cases/100/year)				Population at risk	Incidence of malaria among control group (cases/100/year)				Population at risk
	1995	1996	1997	1998		1995	1996	1997	1998	
Civil assistant surgeons and physicians	0	0	0	0	49	–	–	–	–	
Students of nursing	2.77	0.8	1.1	0	360	0.66	0.33	0.33	0	300
Students of medicine	0.4	0.4	0.8	1.0	500	0.2	0.2	0.4	0.6	500
Class IV employees	2.94	0	4.41	2.20	136	1.53	0	0.76	0.76	130

RESULTS

A strong relationship was observed for increased risks associated with the occupational exposure and the occurrence of malaria. Among the four categories of health care workers, the civil assistant surgeons and physicians showed no incidence of malaria for all the four years from 1995 to 1998. The incidence of malaria was high among the experimental group (Table 3). The highest incidence was observed among the class IV employees and the students of nursing. The incidence among the students of nursing was 2.77, 0.8, 1.1 and 0 cases/100/year for the years 1995 to 1998 respectively. The incidence among the class IV employees was 2.94, 0, 4.41, and 2.20 cases/100/year for the years 1995 to 1998 respectively, whereas the students of medicine showed 0.4, 0.4, 0.8 and 1.0 cases/100/year (Table 3). The relative risk also showed the same pattern among the four designated health care worker groups. The relative risk and the attributable risk was difficult to calculate for this group because of absence of controls and non occurrence of malaria in this group during the study period. The estimated relative risk among the students of nursing was 4.2, 2.42, 3.3 and 0 for the years 1995 to 1998 respectively. The relative risk among the class-IV employees was 1.27, 0, 5.8 and 2.9 for the years 1995 to 1998 respectively, whereas the students of medicine showed 2, 2, 2, 1.6 (Table 4). The relative risk of one indicates no association; greater than one suggests positive association between occupational exposure and malaria and less than one indicates reduction in the incidence rate in exposed individuals when compared to the unexposed.[8] The attributable risk also showed similar pattern among the select occupational groups. The students of nursing showed 76.17, 58.75, 70 per cent for the years 1995 to 1998 respectively. The attributable

Table 4. Risk for malaria among health care workers of select employee groups of Sri Venkateswara Ramnarayan Ruia Government General Hospital, Tirupati, A.P.

Health care workers	Relative risk				Attributable risk			
	1995	1996	1997	1998	1995	1996	1997	1998
Civil assistant surgeons and physicians	0	0	0	0	0	0	0	0
Students of nursing	4.2	2.42	3.3	0	76.17	58.75	70	0
Students of medicine	2	2	2	1.6	50	50	50	80
Class IV employees	1.27	0	5.8	2.9	21.76	0	82.7	65.4

risk among the class IV employees was 21.76, 0, 82.7 and 65.4 per cent for the years 1995 to 1998 respectively, whereas, 50, 50, 50 and 80 per cent was observed among the students of medicine for the years 1995 to 1998. The attributable risk indicates, to what extent the disease can be attributed to the exposure.

DISCUSSION

The results provide an evidence for an association between the occupational environment and malaria for the health care workers and is more prevalent among certain categories. The study reveals that the mean night exposure time and extent of dress cover (Table 2) are the possible influencing factors for occupational malaria besides other potential factors.

Except the civil assistant surgeons, physicians and the students of medicine, the other health care categories, namely the students of nursing and the class IV employee groups are the most afflicted and showed higher incidence. Accordingly the relative risk and attributable risk were also high among the students of nursing and the class IV employees than the other categories, namely the civil assistant surgeons, physicians and the students of medicine. It is suggested that the civil assistant surgeons, physicians and the students of medicine have medical knowledge on occurrence, source, precautionary measures, drugs to be used for malaria, whereas the students of nursing were yet to be trained or the lower literacy and lack of aptitude among the class IV employees might be responsible for the occurrence of malaria. The paired *t*-test values are significant for the medical students and insiginificant for the nursing students and the class IV employees. The insignificance of the above two groups can be attributed to high variance in the incidence of malaria (Table 5). These are several ambient environmental factors which are also responsible for the occurrence of higher incidence of malaria among the students of nursing and the class IV employees (Table 2). For example an observation on the prophylactic measures, so-

Table 5. Paired *t*-test values for malaria among the occupational and the non-occupational groups for a period of four years

Categories of employee	Mean		Variance		*t*-value	p-value
	Experimental group	Control group	Experimental group	Control group		
Students of nursing	1.17	0.33	1.36	0.07	1.85	0.08
Students of medicine	0.65	0.35	0.09	0.04	5.196	0.007*
Class IV employees	2.3875	0.9575	3.378	0.18	1.594	0.105

*Significant at 0.01 level.

journ in personal rooms, dress cover, prophylactic and antimalarial drug use revealed that physicians and surgeons are better equipped than the other categories of health care workers (Table 1). It is suggested that the class IV employees and the students of nursing have to be educated and appropriate measures have to be taken to reduce the incidence of malaria among these groups. The hospital environment is another possible potential factor.[9] There are no meshes for the windows and doors to prevent the entry of mosquitoes. There are no prophylactic measures such as general fumigation or use of domestic mosquito repellents (electrical gadgets) in the general wards where the class IV employees and the sojourn of students of nursing is more. The risk factors and the incidence have to be further reduced initiating appropriate prophylactic measures for the health of both the patients and the health care workers.

We have found that the hospital environment is the potential threat for occupational malaria. However, in many of the government general hospitals in developing countries like India, non-adherence to safe work place standards[10, 11] and biohazardous waste transportation standards[12] are still continuing. Hence, we suggest studies in developing countries — such studies should include collection of morbidity and mortality data, hygiene-related practices in hospitals, mosquito bite frequencies among select groups, biohazardous waste regulation, waste handling and waste transportation and the rest. Such information will help to define the situations in which occupational malaria may possibly be controlled. We hope that these important results would help in healthy hospital occupational environment.

ACKNOWLEDGEMENTS

We thank the Chief Superintendent of SVRRGGH, Tirupati and also Medical record technicians for their cooperation in the collection of morbidity data from health cards. We also thank the staff of S.V.University Health Centre, Tirupati, for giving the morbidity data for control groups.

REFERENCES

1. Sharma, V.P. (1996). Malaria and its control in India. *Global Meet on Parasitic Diseases: Abstracts and Scientific Programme* (Malaria Research Centre, Delhi): 2.
2. Tulu, A.N. (1993). Malaria. *The Ecology of Health and Disease in Ethiopia* (Boulder Westview Press): 341–352.
3. Corradetti, A.(1938). Ricerche epidemiologiche sulla malaria nella regione uollo-jeggiu durautela stergione della piogge (Epidemiologic research on malaria in the uollo–Juggiu region during rainy season). *Revista di Malariologia*, **2:** 101–110.
4. Corradetti, A. (1940). L'epidemiologia della malaria regione uollo–Jeggiu (Africa Orientale Italiola). *Rivista di Malariologia*, **19:** 39–64.
5. Cody, R.P., C.P. Weisel, G. Birnhaum and P.J. Lioy (1992). The effect of ozone associated with summer time photochemical smog on the frequency of asthma visits to hospital emergency departments. *Environ. Res.*, **58:** 184–194.
6. Beaglehole, R., R. Bonita and T. Kjellstrom (1993). *Basic Epidemiology* (Published by Orient Longman, in association with, World Health Organisation, Geneva): 55–69.

7. Park, J.E. and K. Park (1991). Textbook of preventive and social medicine, XIII edn. (Banarsidas Bhannot Publishers, Jabalpur): 68.

8. Schlesselman, J.J. (1982). *Case Control Studies* (Oxford University Press, New York).

9. Anon. (1983). Occupational Safety and Health Care System. OSHA CPL 2-2 (U.S. Department of Labour, Washington D.C.): 36.

10. Anon. (1991). *Occupational Safety and Health Administration: Occupational Exposure to Blood Borne Pathogens;* Final Rule. Federal Register (U.S. Department of Labour, Washington D.C.) **56:** 64004–64182.

11. Anon. (1991). *Occcupational Safety and Health Administration.* Consultation Services for the Employer. OSHA CPL 2-2 (U.S. Department of Labour, Washington D.C.): 3047.

12. Anon. (1990). *Occuaptional Safety and Health Act of 1970.* Public Law 91-596, 91st Congress, December 29, 1970 as amended by Public Law (U.S. Department of Labour, Washington D.C.): 101–552.

Indian Journal of Malariology
Vol. 37, September–December 2000, pp. 61–67.

Prognostic Implication of Hypocalcemia and QTc Interval in Malaria

C.L. SONI[a], M.R. KUMHAR[a], B.K. GUPTA[a], V.B. SINGH[a], L. SRIMALI[a], K.C. NAYAK[a] and V.S. CHADDA[a]

Hundred confirmed cases of malaria were included in the present study to determine the clinical and prognostic implications of hypocalcemia and corrected QT interval (QTc) prolongation in malaria. Peripheral blood smear examination was done to determine the parasite species and the parasite load. Serum calcium level and QTc measurements in electrocardiogram were done for each patient. Fifty patients were of *P. falciparum* malaria (38 complicated and 12 uncomplicated), 40 of vivax malaria and 10 patients were having mixed (*P. falciparum* and *P. vivax*) infection. Hypocalcemia was found in 26 cases in which QTc was prolonged. Ten patients who had convulsions, all of them were having QTc prolongation and eight had hypocalcemia. A total number of eight patients had muscle spasm, of which six had QTc prolongation and four had hypocalcemia. There were 34 cases of cerebral malaria, of which 18 had hypocalcemia as well as QTc prolongation, 12 of them developed renal failure and 14 had high parasitaemia. Four patients died who had hypocalcemia and QTc prolongation due to hepatorenal syndrome. The mean parasite load, QTc interval and serum calcium were 2.69 ± 1.0, 0.468 ± 0.055 sec and 8.16 ± 0.86 mg/dl respectively in complicated falciparum malaria; 1.6 ± 0.55, 0.442 ± 0.043 sec and 8.72 ± 0.97 mg/dl in complicated mixed (*Pf*+*Pv*) infection. 1.33±0.52, 0.435 ± 0.035 sec and 9.77 ± 1.34 mg/dl in uncomplicated falciparum malaria and 1.35 ± 0.58, 0.403 ± 0.019 sec and 9.68 ± 0.99 mg/dl in vivax malaria. The difference was significant between complicated falciparum and mixed (*Pf*+*Pv*) infection when compared to uncomplicated falciparum and vivax malaria ($p < 0.05$).

Keywords: Calcium, Hypocalcemia, QTc prolongation

[a]Department of Medicine, S.P. Medical College, Bikaner–334 001, India.

INTRODUCTION

Malaria is known to exist as a killer disease of mankind for thousands of years. Today, at the door of the 21st century, there is a rapid resurgence of the disease in an explosive manner especially with emergence of drug-resistant virulent forms of *Plasmodium falciparum.*[1] It is posing to be a big threat to countries of southeast Asia specially the Indian subcontinent.[2] Severe malaria may lead to various metabolic abnormalities including metabolic acidosis, hypoglycemia, low plasma concentration of glucose level or sodium, bicarbonate, calcium, phosphate and albumin, together with the elevation in lactate, blood urea, creatine, urate, muscle and liver enzymes, and conjugated and unconjugated bilirubin. Although there is mention about hypocalcemia in malaria but there is no information on its prevalence and clinical implication.[3] There are also reports of hypocalcemia in bacteremic patients[4] and intensive care unit patients.[5] Hypocalcemia is known to produce QTc prolongation in electrocardiogram[6], which may lead to ventricular arrhythmias and sudden death.

Therefore, this study was undertaken to determine the incidence of hypocalcemia in different types of malaria and resulted change in the QTc interval in electrocardiogram of the patient with malaria, clinical and prognostic implications of calcium level and QTc interval in the cases of malaria.

MATERIALS AND METHODS

The present study was carried out on 100 consecutive adult cases of malaria admitted in the Department of Medicine, S.P. Medical College and Associated Group of Hospitals, Bikaner. Malaria was diagnosed by the positive blood smear for asexual phase of the parasite.[7] All routine investigations were done in each case including liver function tests, renal function tests, serum electrolyte, serum calcium level and electrocardiogram with special emphasis on peripheral blood film examination. The density of parasite/μl of blood or the average number of parasites per oil immersion field in thin smear was determined by the grading system.[7]

Grade 1+ = 1–10 parasites per 100 fields;
2+ = 1–10 parasites per 10 fields;
3+ = 1–10 parasites per field; and
4+ = >10 parasites per field.

o-Cresolphthalein complexion without deproteinization method was used[8] to estimate the serum calcium level. Serum calcium level was corrected with the simultaneous concentration of serum albumin (normal range of total serum calcium is 8.4–10.4 mg/dl). Blood samples were collected without using a tourniquet. Those who had low calcium levels were given eight hourly intravenous 10 per cent calcium gluconate, and serial calcium estimation was carried out till the value returned to normal.

Baseline electrocardiogram was also recorded in each patient and QT interval was measured manually (by experienced single doctor who was blinded) from first deflection of QRS complex to point of T-wave offset, defined as return of the T-wave to baseline if U-wave is present then offset is defined as the nadir be-

tween the T-wave and U-wave.[9] QT was corrected (QTc) by Bazette's[10] formula ($QTc = QT/\sqrt{RR}$). Quinine was used to treat complicated and chloroquine resistant malaria patients, whereas chloroquine was used to treat the uncomplicated malaria patients.[11] Quinine doses were reduced by half to quarter in patients with renal failure, hepatic failure or prolonged QTc since the conventional doses produced cardiotoxicity.[11]

Malaria case was graded as severe or complicated when presented with one or more features including cerebral malaria, severe haemolytic anaemia, renal failure, pulmonary oedema, hypoglycemia, disseminated intravascular coagulation, acidosis, malarial haemoglobinuria, severe hepatitis, algid malaria and heavy parasitaemia.[12]

RESULTS

In this study, the age of the patients ranged between 14 and 82 years and the most vulnerable group of patients were in the range of 21–30 years with 72 males and 28 females. Mostly the patients had high grade continuous fever or fever with daily peaks. A total of twenty-four per cent of cases had chills and rigor, 56 per cent had nausea and vomiting, headache in 52 per cent, altered sensorium in 34 per cent, generalised bodyache in 24 per cent, pain abdomen in 24 per cent, loose motion in 16 per cent, jaundice in 12 per cent, convulsions in 10 per cent, muscle cramps in eight per cent and oliguria in four per cent cases were observed. On clinical examination splenomegaly was present in 76 per cent, hepatomegaly in 36 per cent, anaemia in 44 per cent and jaundice in 24 per cent cases. Laboratory examination revealed anaemia in 90 per cent cases and most of them were moderately anaemic and of normocytic and normochromic type (80 per cent). Some cases had macrocytic (eight per cent) and microcytic hypochromic anaemia (12 per cent). The most common finding during the study was elevated serum bilirubin (in 56 per cent of cases), out of them six patients had serum bilirubin between 18–23 mg/dl. Renal failure was seen in 12 per cent cases with serum creatinine > 2 mg/dl. Peripheral blood smears were positive for *P. falciparum* in 50 per cent, *P. vivax* in 40 per cent and for mixed (*Pf* + *Pv*) infection in 10 per cent . Total 48 cases were presented as severe malaria (38 *P. falciparum* and 10 mixed infection).

Out of 60 cases of *P. falciparum* and mixed infection, 26 had hypocalcemia, whereas none of *P. vivax* case had hypocalcemia (Table 1). Twenty-four out of 48 complicated cases (22 *P. falciparum* and two mixed infection) had hypocalcemia against two out of 52 uncomplicated cases ($p < 0.01$). A total 38 cases had QTc prolongation, 32 of them were related to complicated falciparum malaria and mixed (*Pf* + *Pv*) infection. Out of 60 cases of *P. falciparum* malaria and mixed (*Pf* + *Pv*) infection 36 had QTc prolongation, whereas only two cases out of 40 *P. vivax* malaria had QTc prolongation. All cases with hypocalcemia had prolonged QTc interval. Table 2 shows the mean calcium level and the average parasitic load in different types of malaria. There was a

Table 1. Parasitic load, serum calcium and QTc interval in various types of malaria

Type of malaria	Parasitic load			Serum calcium (mg%)			QTc interval (sec)		
	1+, 2+	3 +	4 +	≤ 8.4	8.5–10.4	>10.4	<0.36	0.36–0.43	>0.43
Complicated falciparum	18	6	14	22	16	–	–	12	26
Complicated mixed infection (*Pf*+*Pv*)	10	–	–	2	8	–	–	4	6
Uncomplicated falciparum	10	2	–	2	6	4	–	8	4
Vivax	38	2	–	–	32	8	2	36	2

Table 2. Comparison between complicated malaria and other types of malaria in regard to parasitic load, serum calcium level and QTc interval

Type of malaria	No. of cases	Parasitic load Mean ± SD	QTc interval Mean ± SD (sec)	Serum calcium Mean ± SD (mg%)
Complicated falciparum	38	2.69 ± 1.00	0.468 ± 0.055	8.16 ± 0.86
Complicated mixed infection (*Pf*+*Pv*)	10	1.60 ± 0.55	0.442 ± 0.043	8.72 ± 0.97
Uncomplicated falciparum	12	1.33 ± 0.52	0.435 ± 0.035	9.77 ± 1.34
Vivax	40	1.35 ± 0.58	0.403 ± 0.019	9.68 ± 0.99

good correlation between parasitic load and degree of hypocalcemia ($r = 0.630$; $p < 0.001$).

In our study, four patients died due to hepatorenal failure, as they all had hypocalcemia and prolonged QTc interval. Patients presented with convulsions (10 cases) and muscle cramps (eight cases) also had hypocalcemia and QTc prolongation (8, 4 and 10, 6 cases respectively). Out of 34 cases of cerebral malaria, 18 cases had hypocalcemia as well as QTc prolongation. All the patients with heavy parasitaemia (14 cases) and renal failure (12 cases) had hypocalcemia and QTc prolongation.

The difference in the mean parasitic load, serum calcium and QTc interval was significant between complicated falciparum and uncomplicated falciparum malaria ($t = 3.79, 2.91, 2.45$ and $p < 0.13, 0.50, 0.58$ respectively) as well as complicated falciparum and vivax malaria ($t = 4.17, 6.48, 4.76$ and $p < 0.001, < 0.0001,$

< 0.0001 respectively) whereas no significant difference was observed between the complicated falciparum and mixed (*Pf*+ *Pv*) infection ($p > 0.05$).

In this study no correlation ($p > 0.05$) was found with serum calcium and blood sugar level and no case was presented with hypoglycemia. All patients who had hypocalcemia were treated with intravenous calcium gluconate and normal return of serum calcium was coincided with the parasite clearance and clinical recovery of the patients.

DISCUSSION

Calcium is an essential element which is required for many biological processes including neuronal conduction, synaptic transmission, hormone secretion, mitotic division, cardiac automaticity and excitation, contraction and coupling in muscle. Calcium is also a major intracellular messenger which is essential for cellular processes that require movement of ion and protein, and is required by many enzymes for regular activity.[13] Deficiency of this important ion is common in the patients who are critically ill.[5,13] Hypocalcemia in such patients may be because of poor sun light exposure, poor dietary intake and deficiency of vitamin D. The disease of pancreas, liver, biliary system or intestine may also cause malabsorption and deficiency of vitamin D leading to hypocalcemia. The disease of kidney or liver can disrupt the calcium homeostasis by impairing vitamin D activation leading to hypocalcemia.

Desai *et al.*[5] observed a strong association between sepsis and hypocalcemia. They found that mortality of hypocalcemic patients (44 per cent) were significantly greater ($p < 0.05$) than mortality of the normocalcemic patients (17 per cent). There are reports of hypocalcemia in bacteremic patients as observed by Aderka *et al.*[4] They found that hypocalcemic patient had a significantly higher maximal temperature than normocalcemic. These findings suggest that hypocalcemia is a very common abnormality in acutely ill patients and is associated with a poor prognosis.

We found that hypocalcemia was present in 26 per cent cases of complicated falciparum malaria in whom QTc interval was prolonged (>0.43 sec) and these patients had high parasitaemia. This finding is consistent with study done by Prabha *et al.*[14]

We also observed convulsions in 10 cases, of which eight had low serum calcium level and muscle cramps in eight cases, of which four had hypocalcemia. The convulsions may be due to hypocalcemia or severe malaria itself (blood sugar and sodium levels were normal). These clinical features are related to neuronal irritability due to hypocalcemia.[14] In spite of low calcium levels, tetany was observed rarely probably because all of these patients were on quinine which is known to suppress tetany.[15]

The patients who had hypocalcemia were given intravenous calcium gluconate and they recovered as the serum calcium level and QTc interval returned to normal due to parasite clearance

and clinical improvement. Only four deaths occurred during the study period and these patients had acute hepatorenal syndrome. All of them had hypocalcemia and prolonged QTc.

The difference in relation to the mean parasitic load, the mean serum calcium level and the mean QTc interval was highly significant between complicated falciparum as well as mixed (*Pf*+ *Pv*) infection compared to uncomplicated falciparum and vivax malaria ($p < 0.001$).

In this study, the mechanism by which hypocalcemia occurred in severe falciparum malaria could not be ascertained. Possibly the general causes of hypocalcemia of severe illness like poor intake of calcium diet, prolonged parenteral infusion without calcium, alkalosis, associated renal, hepatic, parathyroid and pancreatic disfunction could have contributed[4,5] and hypocalcemia per se may lead QTc prolongation.[16]

Our study indicates that though hypocalcemia is usually asymptomatic, it is a significant prognostic biochemical marker and it should be monitored and treated (if present) to reduce the morbidity and mortality in malaria. Daily determination of QTc in ECG should be done in all cases of severe malaria specially in those with hypocalcemia, as prolonged QTc may lead to serious ventricular arrhythmias.

ACKNOWLEDGEMENTS

The authors are grateful to Prof. S.C. Lodha, Principal and Controller, S.P. Medical College, Bikaner for his assistance and kind permission to conduct the study. We are also grateful to Prof. C.R. Vyas, Head, Department of Biochemistry and Prof. V.B. Kalra, Head, Department of Pathology who helped us considerably in this work.

REFERENCES

1. Chodankar, C.N. and K.P. Deodhar (1995). Malaria still a master killer. *J. Assoc. Physic. India*, **43:** 261–262.
2. WHO (1990). Division control of tropical disease. Severe and complicated malaria. *Trans. R. Soc. Trop. Med. Hyg.*, **84**(suppl 2): 1–65.
3. White, N.J. and J.G. Breman (1998). Malaria and other diseases caused by red cell parasites. *Harrison's Principles of Internal Medicine,* XIV edn. Eds. A.S. Fauci, E. Braunwald, K.J. Isselbacher, J.D. Wilson, J.B. Martin, D.L. Kasper, S.C. Hauslen and D.L. Zougo (McGraw Hill, New York): 1180–1189.
4. Aderka, D., D. Schwartz, M. Dan and Y. Levo (1987). Bacteremic hypocalcemia : A comparison between the calcium levels of bacteremic and non-bacteremic patients with infection. *Arch. Intern. Med.*, **147:** 232–236.
5. Desai, T.K., R.W. Carlson and M.A. Geheb (1988). Prevalence and clinical complication in acutely ill patients in a medical intensive care setting. *American J. Med.*, **84:** 209–214.
6. Goldman, M.J. (1979). Hypocalcemia. *Principle of Clinical Electro Cardiography,* X edn. (Lange Medical Publications, Singapore): 297–298.
7. WHO (1991). *Basic Malaria Microscopy*. Pt I: *Learner's Guide* (World Health Organisation, Geneva): 68–89.
8. Gindler, E.N. and J.D. King (1972). *o*-Cresolphthalein complexion without deproteinization

method of calcium estimation. *American J. Clin. Pathol.*, **58:** 376–379.

9. Statters, D.J., M. Malik, D.T. Ward and A.J. Camm (1994). QT dispersion problems of methodology and clinical significance. *J. Cardio. Electrophysiol.*, **5:** 672–685.

10. Bazette, H.C. (1920). An analysis of the time relation of electrocardiogram. *Heart*, **7:** 353–370.

11. White, N.J. (1996). The treatment of malaria. *New England J. Med.*, **12**; **335** (11): 800–806.

12. Gilles, H.M. (1991). Severe and complicated malaria. *Management of Severe and Complicated Malaria,* II edn. Eds. D.A. Warrell, M.E. Molyneux and P.F. Beales (World Health Organization, Geneva): 5–9.

13. Zaloga, G.P. and B. Chernow (1986). Hypocalcemia in critical illness. *J. Amer. Med. Assoc.*, **256**(14): 1924–1929.

14. Prabha, M.R.A., P. Pereira, N. Chowta and B.M. Hegde (1998). Clinical implication of hypocalcemia in malaria. *Indian J. Med. Res.*, **108:** 62–65.

15. Webster, L.T. Jr. (1990). Drugs used in chemotherapy of protozoal infections: Malaria. *Goodman and Gilman's the Pharmacological Basis of Therapeutics,* VIII edn. Eds. A.G. Gilman, T.W. Rall, A.I. Nies and P. Taylor (McGraw Hill, New York): 978–998.

16. Rumancik, W.M., J.K. Denlinger and M.L. Namrwold (1978). The QT interval and serum ionized calcium. *J. Amer. Med. Assoc.*, **240:** 366–368.

Indian Journal of Malariology
Vol. 37, September–December 2000, pp. 68–73.

Splenomegaly in School Children in a Remote Tribal Area of Dhule District, Maharashtra

K. GHOSH[a], M.B. MUKHERJEE[a], R.R. SURVE[a], U. SHANKARKUMAR[a], S.L. KATE[b], S.B. NAGTILAK[c], R.B COLAH[a], A.A. TAMANKAR[a] and D. MOHANTY[a]

Sickle-cell gene is known to protect against *P. falciparum* infection and provides a selective survival advantage in those areas where *P. falciparum* infection is endemic. This protection is not absolute and many other factors, inherited and acquired also contribute to the immunity against *P. falciparum* infection. We investigated incidence of splenomegaly and typical history of malaria in the past two years in apparently healthy school children in a tribal area in Dhule district of Maharashtra to see whether the incidence of malaria (splenomegaly and typical history) was different in children having sickle-cell trait to that of those who did not have this trait. A total of 480 school children were clinically examined for splenomegaly and history of typical malarial fever and/or blood slide positivity for malaria in the past two years. About 9.55 per cent of normal population had either splenomegaly or convincing history of malarial infection in the past two years which is not statistically different from the sickle-cell trait patients having evidence of past malaria (8.79 per cent; $p > 0.05$).

Keywords: Healthy school children, Malaria, Sickle-cell, Splenomegaly

INTRODUCTION

Dhadgaon is one of the remote areas in Dhule district of Maharashtra state where malaria is endemic. "Bhils" and "Pawras" are the two major endogamous tribal groups with a higher prevalence of sickle-cell gene and have been living in this area for the centuries. Several stud-

[a]Institute of Immunohaematology (ICMR), 13th Floor, KEM Hospital Campus, Parel, Mumbai–400 012, India.
[b]Department of Genetics, B.J. Medical College, Pune–411 001, India.
[c]Department of Biochemistry, A.C.P. Medical College, Jawahar Medical Foundation, Dhule–424 001 India.

ies have already established that sickle-cell trait protects against severe *P. falciparum*[1] as well as milder *P. falciparum*[2] infection.

We studied 480 apparently healthy school children aged between 12 and 18 years from a school with a view to find out splenomegaly and convincing history of malarial infection in this population over the past two years. As sickle-cell trait itself does not produce splenomegaly, the incidence of splenomegaly and history of malarial infection together will provide some measures of prevalence of malarial infection in this population. RBC indices and haemoglobin analysis for sickle-cell trait were carried out in this population with a view to find out whether malarial infection is significantly less in the students having sickle-cell traits compared to those who did not have it.

MATERIALS AND METHODS

Health Supervisor of Dhadgaon region was approached for various malaria related incidences of that area for 1996. A total of 480 students in the age group of 12 to 18 years from the school was clinically examined for splenomegaly, anemia and fever. A clinical proforma was filled-up with special emphasis on fever with chill and rigor in the past two years and whether the episode was checked by peripheral blood smear examination and the fever responded to antimalarial treatment.

As most of the students were in nineth and tenth standards, communication and understanding was proper and easy. The spleen was examined by one of us (KG) using standard technique.[3] If the spleen was not palpable by this technique then the patient was put on his/her right side with left hip and knee flexed at right angle and left hand kept flexed over patient's head. Then lower left rib cage was supported with the left palm and the spleen was palpated with tips of right index and middle fingers with patient in deep breathing. The enlargement was measured in centimeters from the left costal margin.

Five ml of blood was collected from these students from the antecubital vein and was anticoagulated with dipotassium EDTA. RBC indices on all samples were done in an automated cell counter (ERMA-PC 6, Japan). The haemoglobin analysis was carried out in Variant Haemoglobin Testing System (Bio-Rad, Inc. Ohio, USA) using β-thalassemia short programme. This is an automated system which uses microcolumn ion exchange chromatography as the principle of separation of different haemoglobin variants. Sickle-cell haemoglobin was additionally tested by solubility test and cellulose acetate electrophoresis with Tris-EDTA borate buffer at pH 8.9[4] (Fig. 1).

RESULTS

Out of 480 students examined, 315 were boys and 165 were girls. None had any complaints and none was suffering from febrile illness in the past two weeks. About 12 (2.5 per cent) were suffering from either β-thalassemia trait or homozygous sickle-cell anemia and they were excluded from the present analysis. The mean haemoglobin in our study population was

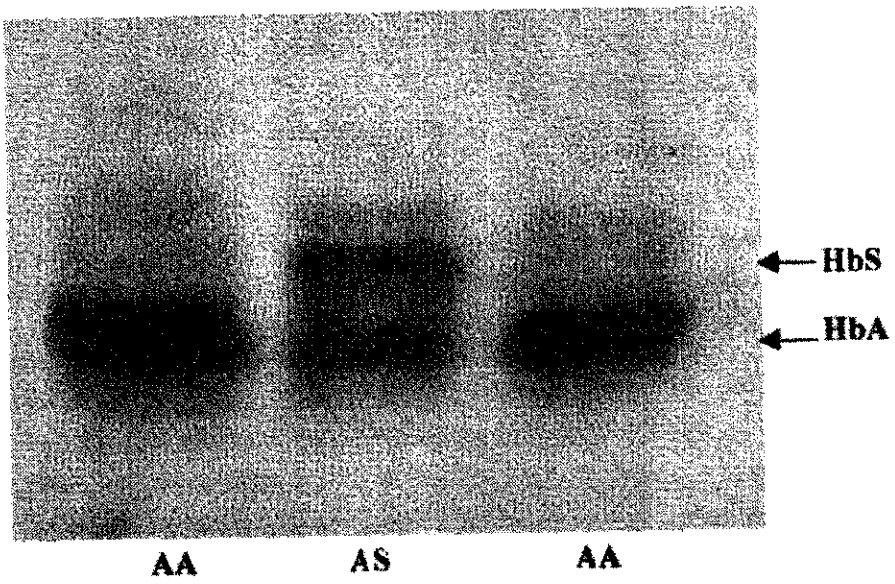

Fig. 1: Hb electrophoresis on cellulose acetate membrane at pH 8.9

11.94 ± 1.60 g/dl and 40 students (9.7 per cent) had haemoglobin less than 10 g/dl (16 boys and 24 girls). A total of 376 students (80.5 per cent) had mean carpuscular volume (MCV) < 75 fl.

Table 1 shows the spleen rate and malaria occurrence in the school children studied. Out of 377 students who did not have sickle-cell traits, spleen (2–5 cm) was variably palpable in 25 students (6.63 per cent). All these students gave typical history of malaria during the last two years. Additionally, 11 students from this group gave history of typical malarial attack in the last two years and they responded antimalarial drugs, but they did not have palpable spleen. Hence, 36 students (9.55 per cent) from 377 non sickle-cell trait group had some evidence of malaria.

Ninety-one students from the whole group were positive for sickle-cell trait. Six patients (6.59 per cent) from this group had variable (2–6 cm) splenomegaly and additional two from this group gave typical history of malaria without palpable spleen. Hence eight students (8.79 per cent) had some evidence of malaria in the past. All the patients with splenomegaly in this group gave history of malaria. The difference in the incidence of malaria in these two groups of students were not statistically significant ($p > 0.05$) for both. The total incidence of splenomegaly in this population turns to be 31/468 (6.62 per cent).

The annual parasite incidence[5] (which is calculated as confirmed cases during one year out of the population under surveillance x 1000) was found to be 20.36 and the slide positive

Table 1. Spleen rate and occurrence of malaria in sickle-cell trait and normal healthy school children in Dhule

Status	No. of cases	Spleen		Malaria	
		Palpable	Not palpable	Present	Absent
Normal	377	25 (6.63)	352 (93.37)	36 (9.55)	341 (90.45)
Sickle-cell trait	91	6 (6.59)	85 (93.71)	8 (8.79)	83 (91.21)
Total	468	31 (6.62)	437 (93.38)	44 (9.40)	424 (90.60)

Figures in parentheses indicate percentage.

rate was 30 per cent where as slide falciparum rate was 17 per cent (17 slides out of 100 slides) in the patients with fever was positive for *P. falciparum*. All indices showed the area to be endemic for malaria with *P. falciparum* as the predominating species.

DISCUSSION

The spleen rate has been used as an indicator for the prevalence of malaria in the population surveys.[5] We studied a relatively healthy school student population and the sickle-cell trait in this tribal population was quite high. About 91/468 (19.4 per cent) students in our study had sickle-cell trait. As several studies have proved that sickle-cell traits provide some measure of protection against *P. falciparum* malaria, we hoped to see significantly less malaria related indices or history of malaria in our sickle-cell trait population. However, the present study showed no differences in the spleen rate or in the history of malaria in the students with and without sickle-cell traits. Similar observations have been made by Hersan *et al.*,[6] where they found that the prevalence rates of *P. falciparum* was similar in normal and sickle-cell individuals. Though, the parasite densities increased less rapidly in sickle-cell trait than in normal children.

In most studies sickle-cell gene was found to provide a measure of protection against serious *P. falciparum* infection[1] and whether this protection is operative in milder infection is debatable.[2] It has been postulated that the malaria parasite metabolized HbS less easily than HbA[7] and the difference in the immune response between sicklers and non-sicklers might account for the relative resistance of the sicklers.[8] Miller *et al.*[9] suggested that parasitization of red cells in the sickle-cell trait brought about selective sickling of parasitized cells and hence their more effective removal, while in other study, Luzzatto *et al.*[10] demonstrated increased sickling of parasitized cells on *in vitro* incubation. The parasite was thought to bring about its own destruction by identifying its host cell to the splenic reticulo-endothelial tissue. This proposed mechanism depended on significant sickling at physiological oxygen tension in the sickle-cell trait, which seemed unlikely although it is now recognized that in falciparum malaria, parasitized cells are

sequestered deep within the reticuloendothelial system and exposed to low oxygen tensions permitting potassium loss and low pH, factors known to inhibit parasite growth *in vitro*.[11,12]

By design most of our patients had milder form of *P. falciparum* infection, hence the present study does not analyse the fact that sickle-cell gene protects against severe *P. falciparum* infection. Unlike Africa, HbS has not been shown to have definite correlation with malaria in India.[13] Demonstration of similar proportion of patients with *P. falciparum* malaria in both the groups and demonstration of similar proportion of splenomegaly suggest that *P. falciparum* infection was mainly responsible for splenomegaly. This suggestion is also strengthened by the fact that splenomegaly was not found in the patients with sickle-cell trait who did not have malaria. It may be worthwhile to note that not a single patient died of *P. falciparum* infection in Dhadgaon area in the study year.

Several genetic factors like ABO blood groups,G-6-PD deficiency, TNF allelles, NSO_2 genes and HLA antigens, influence the severity of disease due to infection with *P. falciparum*.[14,15] When all or some of these factors are operative in an endogamous population then the protection offered by sickle-cell gene may become marginal as other equally important factors remain operative.

Iron deficiency anemia as an acquired condition also offer some protection against malarial and other infections.[16-19] In the population studied though β-thalassemia was excluded but MCV < 75 fl was present in 80.5 per cent of cases. It is certain that many of these patients were iron deficient and this acquired deficiency could as well be operative in protection against malaria apart from acquired immunity to this condition. The spleen rate (6.62 per cent) in the population as a whole was higher when compared to 2.86 per cent in college freshmen in USA where malaria is non existent and mostly imported.[20]

ACKNOWLEDGEMENTS

This study was a part of the sickle-cell anemia screening project funded by Indian Council of Medical Research, New Delhi. The authors gratefully acknowledge the help of Dr. Niti Mohanty, Director, Jigyansu Tribal Research Centre, New Delhi and Jawahar Medical Foundation, Dhule. Thanks are also due to Mr. C.T. Kulkarni at IIH for excellent typing of the manuscript.

REFERENCES

1. Sears, A.D. (1976). The morbidity of sickle-cell trait : A review of the literature. *American J. Med.*, **64:** 1021–1036.
2. Allison, A.C. (1954). Protection afforded by sickle-cell trait against subtertian malarial infection. *British Med. J.*, **1:** 290–294.
3. Anon. (1983). The alimentary and genito urinary system. *Clinical Examination*. Ed. John Mcleod (Churchill Livingstone, Edinbergh): 206.
4. Dacie, J.V. and S.M. Lewis (1975). *Practical Hematology*, V edn. (Churchill Livingstone, London): 227.
5. Park, J.E. and K. Park (1989). Malaria. *Text Book of Preventive and Social Medicine*. XIIth edn.

(Banarsidas Bharat Publishers, Jabalpur, India): 182–192.

6. Hersan, Le, J.Y., I. Personne, P. Personne, N. Fievet, B. Dubois, M. Beyeme, C. Boudin, M. Cot and P. Deloron (1999). Longitudinal study of *Plasmodium falciparum* infection and immune responses in infants with or without sickle-cell trait. *Intl. J. Epidemiol.*, **28:** 793–798.

7. Mackey, J.P. and F. Vivarelli (1954). Sickle-cell anemia. *British Med. J.*, **1:** 276.

8. Edozien, J.C., A.E. Boyo and D.C. Morley (1960). The relationship of serum gamma-globulin concentration to malaria and sickling. *J. Clin. Pathol.*, **13:** 118–123.

9. Miller, M.J., J.V. Neel and F.B. Livingstone (1956). Distribution of parasites in the red cells of sickle-cell trait carriers infected with *Plasmodium falciparum*. *Trans. R. Soc. Trop. Med. Hyg.*, **50:** 294–296.

10. Luzzatto, L., E.S. Nwachuku–Jarrett and S. Reddy (1970). Increased sickling of parasitised erythrocytes as mechanism of resistance against malaria in the sickle-cell trait. *Lancet*, **1:** 319–321.

11. Pasvol, G., D.J. Weatherall and R.J.M. Wilson (1978). Cellular mechanism for the protective effect of hemoglobin-S against *P. falciparum* malaria. *Nature*, **274:** 701–703.

12. Friedman, M.J., E.F. Roth, R.L. Nagel and W. Trager (1979). *Plasmodium falciparum*: Physiological interactions with the human sickle-cell. *Expl. Parasitol.*, **47:** 73–80.

13. Sharma, A. (1983). Hemoglobinopathies in India. *Peoples of India : Some Genetical Aspects.* (Indian Council of Medical Research, New Delhi): 31–49.

14. Hill, A.V.S., G.E.M. Allsop, D. Kwiakowski, N.M. Anstey, P. Twumasi, P.A. Rowe, S. Bennett, D. Brewster, A.J. McMichael and B.M. Greenwood (1991). Common West African HLA antigens are associated with protection from severe malaria. *Nature*, **352** : 595–600.

15. Migot-Nabias, F., L.E. Mombo, A.J. Luty, B. Dubois, R. Nabias, C. Bisseye, P. Millet, C.Y. Lu and P. Deloron (2000). Human genetic factors related to susceptibility to mild malaria in Gabon. *Genes Immun.*, **1:** 435–441.

16. Anon. (1974). Editorial. *Lancet*, **2** : 372.

17. Brock, J.H. (1984). Iron in infection, immunity, inflammation and neoplasia. *Iron Metabolism in Health and Disease*. Eds. J.H. Brock, J.W. Halliday, M.J. Pippard and L.W. Powel (W.B. Saunders, London): 353–389.

18. Murray, M. J., A.B. Murray, N.J. Murray and M.B. Murray (1975). Refeeding malaria and hyperferrimia. *Lancet*, **1:** 653–654.

19. Murray, M.J., A.B. Murray, M.B. Murray and N.J. Murray (1978). The adverse effect of iron repletion on the course of certain infections. *British Med. J.*, **2:** 1113–1115.

20. McIntyre, O.R. and F.G. (Jr.) Ebaugh (1967). Palpable spleen in college freshman. *Ann. Int. Med.*, **66:** 301–306.

Indian Journal of Malariology
Vol. 37, Septemebr–December 2000, pp. 74–81.

Mosquito Fauna and Malaria Vectors in Jairampur, District Changlang, Arunachal Pradesh

ANIL PRAKASH[a], D.R. BHATTACHARYYA[a], P.K. MOHAPATRA[a] and J. MAHANTA[a]

In an entomological study, covering three biotopes like village, panikheti (wet rice cultivated land) and urban agglomeration, a total of 35 species of mosquitoes in eight genera were recorded in Jairampur area of Arunachal Pradesh. Two mosquito species — *Orthopodomyia anopheloides* and *Tripteroides indicus* were recorded for the first time in Arunachal Pradesh. *Anopheles dirus* and *An. minimus*, the two well-known malaria vectors were encountered in the study along with several potential vector species like *An. annularis, An. culicifacies, An. maculatus* group and *An. philippinensis/nivipes*. The diversity of mosquito species as well as the man-vector contact was maximum in the panikheti and minimum in the village biotope.

Keywords: Biotopes, Malaria vectors, Man-mosquito contact, Mosquito fauna

INTRODUCTION

Jairampur sub-division in Changlang district of Arunachal Pradesh is endemic for malaria with high degree of chloroquine resistance to *Plasmodium falciparum*.[1,2] A mosquito survey carried out during 1979 in Jairampur[3] (in erstwhile Tirap district) reported a total of 28 species of mosquitoes including 13 anopheline species. Later, during the course of malaria transmission studies, between 1986 and 1990, in Jairampur area, anopheline species varying

[a]Regional Medical Research Centre, N.E. Region (ICMR), Post Box No. 105, Dibrugarh–786 001, India.

from 11 to 16 were recorded and *An. dirus* was incriminated as vector.[4-6] However, these studies are now nearly a decade old and moreover, ecological changes like deforestation, expansion of agriculture, developmental activities, population increase and the rest may have brought some changes in abundance of mosquito species, malaria vectors, its ecological succession and ultimately the malaria epidemiology. In view of this, an entomological study was undertaken in Jairampur area during 1997 to investigate the composition of various mosquito species with particular reference to malaria vectors. The study covered three different biotopes in the same area to find out the local variations in the abundance of mosquito species and man-vector contact.

MATERIALS AND METHODS

Study site

The study was conducted at three locations representing different socio-ecological settings (biotopes) existing in Jairampur area. Location-I (village Kobin) was a typical Tangsa tribal village situated on a small hillock and surrounded by the broken forest on two sides. Location-II was a flat, low-lying wet paddy cultivated area, generally referred as 'panikheti', inhabited by the immigrant Nepalese labourers having houses interspersed with paddy fields. Location-III (colony Premnagar) was a congested locality/urban agglomeration in Jairampur town. All the three study sites were located at the bank of the Namchik river and were close by (~ 0.5 km) to the forest fringe. The distance between location-I and location-II was < one km and between location-I and location-III about five km.

Mosquito collections

Four entomological surveys, in the months of April, June, September and December 1997 were carried out in all the three locations. Adult mosquitoes were collected from the human dwellings (in all the locations) and cattlesheds (in location-II) with the help of battery operated CDC miniature light-traps from dusk-to-dawn. A total of 10 trap-night collections (Location-I: four; Location-II: four; and Location-III: two) from the human dwellings and five trap-night collections from the cattlesheds were made during the study. The man-mosquito contact was estimated by carrying out whole night human landing collections by self baiting technique in the verandah of the human dwellings for 15 nights (Location-I: seven; Location-II and III: four each). Indoor resting mosquitoes were collected with the help of suction tube and flashlights from the human dwellings in the morning between 0500 and 0700 hrs (in Location-II only). A minimum 120 min indoor resting collections from 8 to 10 houses during each survey was carried out. Mosquito catching stations, in all the locations, for different types of collection remained unchanged throughout the study. The adult mosquitoes collected by various methods were carried to the camp laboratory and identified using the standard keys.[7-10] Suitable specimens of the known and potential malaria vectors were dissected to detect gut

and gland infection. In addition, mosquito immatures were collected from different breeding habitats. The collected larvae were link reared in plastic photo vials till emergence for proper identification.

RESULTS

A total of 35 species of mosquitoes in eight genera were collected during the study (Table 1). In light-trap catches, the diversity (19 spp) as well as density (29.9/trap night) of mosquitoes was highest in the location-II, whereas the man-mosquito contact was maximum in the location-III with a mean landing rate of 163.5 mosquitoes/person/night, 85 per cent of it contributed by *Cx. quinquefasciatus* alone (Table 2). Compared to this, the mean landing rate was though less in the loation-II (48.3 mosquitoes/person/night), yet the number of mosquito species was the highest (16 spp) there. Further, mosquito density was minimum in the location-I both in light-trap (two mosqutioes/trap-night, six spp) as well as human landing collections (5.3 mosquitoes/person/night, 10 spp).

In indoor resting catches, only three species— *An. culicifacies, An. vagus* and *Cx. quinquefasciatus* were collected from the human dwellings (Table 1). Of these, *An. culicifacies* (per man hour density, PMHD 4.5) was captured only during June; *An. vagus* was collected during June (PMHD 13.3) and September (PMHD 4); and *Cx. quinquefasciatus* was caught in April (PMHD 1), September (PMHD 4) and December (PMHD 3).

The number of mosquito breeding habitats were limited in the village biotope which included a couple of small fish rearing ponds, pit wells, tree holes, bamboo stumps and a stream. In addition to these sites, paddy fields, ditches and seepages were other breeding sources in the panikheti biotope; whereas drains, ground pools and pits were the main peridomestic mosquito breeding sites in the urban agglomeration. Of the 20 species of mosquitoes that emerged from larval collections, eight species — *Ae. annandalei, Ae. albopictus, An. nivipes, Cx. fuscanus, Cx. mimulus, Or. anopheloides, Tx. splendens* and *Tp. indicus* were collected exclusively as immatures whereas the remaining 12 species were also collected as adults (Table 1).

A total of six species of malaria vectors in India — *An. annularis, An. culicifacies, An. dirus, An. maculatus* group, *An. minimus* and *An. philippinensis/nivipes* were encountered during the study. While the first two species were collected only in the panikheti biotope, *An. minimus* was present in the village as well as in the panikheti biotope while *An. dirus, An. maculatus* group and *An. philippinensis/nivipes* were found in all the three biotopes. The human landing density of all vector species together was highest in the panikheti (14.3/person/night) followed by urban agglomeration (4/person/night) and village (2.5/person/night) (Table 2).

Suitable specimens of *An. culicifacies* (n = 20), *An. dirus* (n = 14), *An. maculatus* group (n = 45), *An. minimus* (n = 10) and

Table 1. Mosquitoes collected in Jairampur area

Species	Collection method			
	Light-trap	Human landing	Indoor resting	Immature collection
Aedes albopictus	–	–	–	C
Ae. annandalei	–	–	–	C
Ae. vexans	–	C	–	–
Anopheles annularis	C	C	–	–
An. barbirostris	C	–	–	C
An. culicifacies	C	–	C	–
An. dirus	C	C	–	–
An. hyrcanus group	C	–	–	C
An. karwari	C	–	–	–
An. kochi	C	–	–	C
An. maculatus group	C	C	–	C
An. minimus	C	C	–	–
An. nivipes	–	–	–	C
An. philippinensis	C	C	–	C
An. pseudowillmori	–	C	–	–
An. tessellatus	C	–	–	–
An. vagus	C	C	C	C
Armigeres durhami	–	C	–	–
Ar. flavus	–	C	–	–
Culex bitaeniorhynchus	C	C	–	C
Cx. fuscanus	–	–	–	C
Cx. fuscocephala	C	C	–	C
Cx. gelidus	–	C	–	–
Cx. mimulus	–	–	–	C
Cx. pseudovishnui	–	C	–	C
Cx. quinquefasciatus	C	C	C	C
Cx. tritaeniorhynchus	–	C	–	–
Cx. vishnui	C	C	–	C
Cx. whitmorei	C	–	–	–
Mansonia annulifera	C	–	–	–
Ma. dives	C	C	–	–
Ma. uniformis	–	C	–	–
*Orthopodomyia anopheloides**	–	–	–	C
Toxorhynchites splendens	–	–	–	C
*Tripteroides indicus**	–	–	–	C

*First report from Arunachal Pradesh; (C) — Collected; (–) Not collected.

Table 2. Mosquito abundance and man-mosquito contact in different biotopes in Jairampur

Species	Mean density/trap-night				Mean landing rate/person/night		
	L–I (n = 4)	L–II (n = 9)	L–III (n = 2)	Total (%)	L–I (n = 7)	L–II (n = 4)	L–III (n = 4)
Aedes vexans	–	–	–	–	0.1	0.0	0.0
Anopheles annularis	0.0	0.3	0.0	3 (0.9)	0.0	0.3	0.0
An. barbirostris	0.0	0.1	0.0	1 (0.3)	–	–	–
An. culicifacies	0.0	0.1	0.0	1 (0.3)	–	–	–
An. dirus	0.0	0.1	0.0	1 (0.3)	0.3	0.0	3.0
An. hyrcanus group	0.0	2.8	0.0	25 (8.2)	–	–	–
An. karwari	0.0	1.9	0.0	17 (5.6)	–	–	–
An. kochi	0.0	0.1	0.0	1 (0.3)	–	–	–
An. maculatus group	0.3	1.7	0.0	17 (5.6)	0.4	5.0	0.5
An. minimus	0.0	0.6	0.0	5 (1.6)	0.3	1.0	0.0
An. philippinensis/nivipes	0.3	2.6	0.0	24 (7.9)	1.5	8.8	0.5
An. pseudowillmori	–	–	–	–	0.0	1.3	0.0
An. tessellatus	0.0	0.1	0.0	1 (0.3)	–	–	–
An. vagus	0.5	0.8	0.0	9 (2.9)	0.1	1.3	0.3
Armigeres durhami	–	–	–	–	0.0	0.0	16.8
Ar. flavus	–	–	–	–	0.0	0.3	0.3
Culex bitaeniorhynchus	0.0	0.4	0.0	4 (1.3)	0.1	0.5	0.0
Cx. fuscocephala	0.5	2.6	0.0	25 (8.2)	0.0	7.0	0.0
Cx. gelidus	–	–	–	–	0.0	0.5	1.0
Cx. pseudovishnui	–	–	–	–	0.4	5.0	1.0
Cx. quinquefasciatus	0.3	0.1	13.0	28 (9.2)	1.3	15.0	139.0
Cx. tritaeniorhynchus	–	–	–	–	0.0	0.3	0.0
Cx. vishnui	–	–	–	–	0.0	0.5	0.5
Cx. vishnui group*	0.3	11.2	0.0	102 (33.5)	–	–	–
Cx. whitmorei	0.0	1.9	0.0	17 (5.6)	–	–	–
Mansonia annulifera	0.0	0.1	0.0	1 (0.3)	–	–	–
Ma. dives	0.0	2.6	0.0	23 (7.5)	0.7	1.5	0.8
Ma. uniformis	–	–	–	–	0.0	0.3	0.0
Total	2.0	29.9	13.0	305 (100)	5.3	48.3	163.5

*Light-trap catches of *Cx. vishnui, Cx. pseudovishnui* and *Cx. tritaeniorhynchus* clubbed together due to uncertain identification of the individual species; L–I — Location I (Village); L–II — Location II (Panikheti); L–III — Location III (Urban agglomeration); (–) Not collected.

An. philippinensis/nivipes (n = 44) collected during the study were dissected for gut and gland infection, but none was found positive.

DISCUSSION

Nagpal and Sharma[11] recorded 40 species of mosquitoes in six genera from Arunachal Pradesh. Jairampur area was, however, not covered in their survey. Malhotra *et al.*[3] reported 28 mosquito species belonging to eight genera during 1979 from Jairampur. Compared to this, we collected mosquitoes of 35 species in eight genera from the same area including *Orthopodomyia anopheloides* and *Tripteroides indicus*, which were recorded for the first time in Arunachal Pradesh (Table 1). *Or. anopheloides*, as noted by Barraud,[8] is commonly available in the western and eastern Himalayas, in Assam along with Brahamputra valley, hills of south Assam while, *Tp. indicus* is distributed in Assam, Meghalaya and Darjeeling.

An. minimus was earlier not reported from Jairampur area[3-6] as well as some other parts of Tirap district of Arunachal Pradesh.[3,12] However, it was incriminated from Namsang circle of Tirap district.[13] Collection of *An. minimus* in good numbers in the present study indicates that *An. minimus* is now well established, though having patchy distribution, in Tirap and Changlang districts of Arunachal Pradesh and playing a role in the malaria transmission along with *An. dirus*, another incriminated vector in District Tirap.[4,12]

Both, *An. philippinensis* and *An. nivipes* were identified in larval collections using diagnostic larval and pupal characters.[9] Both the species bred together in paddy fields, seepage water and ditches. Thus, present study confirms the earlier report, which was based on wing characters, of presence of *An. nivipes* along with *An. philippinensis* in northeast India.[11] Recently, unequivocal cytotaxonomic evidence for the presence of *An. nivipes* in northeast India including Arunachal Pradesh was provided by Subbarao *et al.*[14] *An. philippinensis*, once incriminated from Byrnihat area of Meghalaya,[15] is considered as a potential vector in Changlang district of Arunachal Pradesh.[6] Further studies are required to work out the distribution and vectorial status of *An. philippinensis* and *An. nivipes* in the northeastern region.

The high densities of *An. maculatus* group mosqutioes in our study are in accordance with earlier reports.[3,6] Of the eight biological species identified, so far, in *An. maculatus* group/complex from southeast Asia, four species — sp B (*An. maculatus* s.s), sp C (*An. dravidicus*), sp H (*An. willmorei*) and sp I (*An. pseudowillmori*) are reported in India.[16] Following the key of Rattanarithikul and Panthusiri,[17] a few specimens of *An. pseudowillmori* were morphologically identified in our collections and all uncertain specimen of this complex were shown as *An. maculatus* group. The occurrence of *An. maculatus* and *An. willmorei* (as *An. maculatus* var *willmorei*) is already on record from Arunachal Pradesh.[3,11] *An. maculatus* group of mos-

quitoes are important malaria vectors in Malaysia, Myanmar, Thailand and Nepal and were incriminated, in 1940s from Assam and Meghalaya.[18] However, its present vectorial status in northeast India is unclear and as such the distribution of species of *An. maculatus* group and its role in the malaria transmission needs critical assessment.

The study noted variations in mosquito abundance, species distribution and diversity in the three biotopes within the same geographic zone. Though the overall man-mosquito contact was high in urban agglomeration followed by panikheti and village biotope, the man-vector contact (in turn broadly the risk of contracting malaria) was maximum in the panikheti followed by the urban agglomeration and the village area in Jairampur area. These local variations might be the function of availability of species-specific mosqutio breeding habitats and alternate hosts in different biotopes. Jambulingam *et al.*[19] attributed the variations in vector abundance and malaria transmission intensity between the villages, within one geographical zone of Orissa, to the availability of extent and type of mosquito breeding places. Similarly, Greenwood[20] considered the small area variations in exposure to mosquito bites as the most important factor in explaining the differences in malaria risk factors in a given geographical area.

This study brings out the mosquito fauna of a well-known malairous area besides highlighting the local variations of mosquito prevalence and man-mosquito contact within a geographical area. It would be worthwhile to investigate the likely variations in malaria endemicity, epidemiological and antimalarial drug resistance pattern in different biotopes of Jairampur area in view of its importance in evaluating and optimizing the malaria control operations.

ACKNOWLEDGEMENTS

We are grateful to the Commanding Officer of 28, Assam Rifles for providing accommodation during the course of the study. Technical help provided by Mr. A.C. Rabha and Mr. Dipak Dutta is appreciated.

REFERENCES

1. WHO (1986). Areas where resistance of *P. falciparum* to chloroquine is reported. *The Clinical Management of Acute Malaria*. WHO Regional Publications, Southeast Asia Ser. No. 9: 65–68.
2. Satyanarayana, S., S.K. Sharma, P.K. Chelleng, P. Dutta, L.P. Dutta and R.N.S. Yadav (1991). Chloroquine resistant *P. falciparum* malaria in Arunachal Pradesh. *Indian J. Malariol.*, **28:** 137–140.
3. Malhotra, P.R., P.K. Sarkar, N.G. Das, S. Hazarika and V.M. John (1987). Mosquito survey in Tirap and Subansiri districts of Arunachal Pradesh. *Indian J. Malariol.*, **24:** 151–158.
4. Dutta, P., D.R. Bhattacharyya, C.K. Sarma and L.P. Dutta (1989). The importance of *Anopheles dirus* (*An. balabacensis*) as a vector of malaria in northeast India. *Indian J. Malariol.*, **26:** 95–101.
5. Dutta, P., D.R. Bhattacharyya, C.K. Sarma and L.P. Dutta (1992). Anopheline fauna of parts of Tirap district, Arunachal Pradesh with reference

to malaria transmission. *Indian J. Med. Res.*, **95(A):** 245–249.

6. Dutta, P., V. Dev and D.R. Bhattacharyya (1993). Anopheline fauna and malaria incidence in Changlang district (Arunachal Pradesh). *Indian J. Malariol.*, **30:** 135–143.
7. Christophers, S.R. (1933). *The Fauna of British India inlcuding Ceylon and Burma*, v. IV. Family: Culicidae,Tribe : Anophelinae (Taylor and Francis, London): 1-371.
8. Barraud, P.J. (1934). *The Fauna of British India inlcuding Ceylon and Burma*, v. IV. Family: Culicidae,Tribe : Megarhinini and Culicinae (Taylor and Francis, London): 1–452.
9. Reid, J.A. (1968). Anopheline mosquitoes of Malaya and Borneo. *Stud. Inst. Med. Res. Malaya*, **31:** 1–520.
10. Das, B.P., R. Rajagopal and J. Akiyama (1990). Pictorial key to the species of Indian anopheline mosquitoes. *Zoology*, **2**(3): 131–162.
11. Nagpal, B.N. and V.P. Sharma (1987). Survey of mosquito fauna of northeastern region of India. *Indian J. Malariol.*, **24:** 143–149.
12. Sen, A.K., V.M. John, K.S. Krishnan and R. Rajagopal (1973). Studies on malaria transmission in Tirap district, Arunachal Pradesh (NEFA). *J. Com. Dis.*, **5:** 98–110.
13. Dutta, P. and B.D. Baruah (1987). Incrimination of *Anopheles minimus* Theobald as a vector of malaria in Arunachal Pradesh. *Indian J. Malariol.*, **24:** 159–162.
14. Subbarao, S.K., K. Vasantha Kumar, N. Nanda, B.N. Nagpal, V. Dev and V.P. Sharma (2000). Cytotaxonomic evidence for the presence of *Anopheles nivipes* in India. *J. Amer. Mosq. Contr. Assoc.*, **16:** 71–74.
15. Rajagopal, R. (1976). Studies on persistent transmission of malaria in Byrnihat, Meghalaya. *J. Com. Dis.*, **8:** 235–245.
16. Subbarao, S.K. (1998). *Anopheles* species complexes in southeast Asia. *WHO/SEARO Tech. Pub. Ser. No.* **18:** 1–82.
17. Rattanarithikul, R. and P. Panthusiri (1994). Illustrated keys to the medically important mosquitoes of Thailand. *Southeast Asian J. Trop. Med. Pub Hlth.*, **25** (suppl. 1): 1–66.
18. Rao, T. Ramachandra (1984). *The Anophelines of India.* Rev. edn. (Malaria Research Centre, Indian Council of Medical Research, Delhi): 1–518.
19. Jambulingam, P., S.S.S. Mohapatra, P. Govardhini, L.K. Das, A. Manoharan, S.P. Pani and P.K. Das (1991). Microlevel epidemiological variations in malaria and its implications on control strategy. *Indian J. Med. Res.*, **93**(A): 371–378.
20. Greenwood, B.N. (1989). The microepidemiology of malaria and its importance to malaria control. *Trans. R. Soc. Trop. Med. Hyg.*, **83** (suppl): 25–29.

Indian Journal of Malariology
Vol. 37, September–December 2000, pp. 82–87.

Estimating Parasite Density in Patients Suffering from Falciparum Malaria in an Endemic Area in Kolkata

A.K. HATI[a], B. MONDAL[b] P. CHAUDHURI[c], S. PURKAYASTHA[c], H. MUKHERJEE[a] and A.K. MUKHOPADHYAY[d]

Parasite density of one hundred patients suffering from falciparum malaria in an endemic area in Kolkata was determined using three different methods. In the first of these, parasite density per microlitre of blood in a patient was determined using parasite count adjusted by average WBC count (i.e. 8000/µl) observed in microscopic fields of the thick film. In the remaining two methods, only raw (i.e. unadjusted) parasite counts in microscopic fields of the same slide were used. A statistical analysis was carried out in detail to compare these methods based on raw and adjusted parasite counts and also to find out a suitable method which can be used in practice. Estimating the density of parasites is of primary importance in determining the severity of infection. Furthermore, parasite density can help in identifying short-treatment and long-treatment failure vis-a-vis detection of development of resistance in *P. falciparum* against the drug used. This article reports some findings that indicate the existence of a potentially dangerous situation in the study area.

Keywords: Malaria, Parasite density, RBC and WBC counts

INTRODUCTION

Estimation of parasite density in the patients infected with falciparum malaria is required not only to determine the severity and prognosis of the disease but also to detect the early treatment failure as well as late treatment failure. It is also useful in detecting the development of re-

[a]Department of Medical Entomology, Calcutta School of Tropical Medicine, Kolkata–700 073, India.
[b]ESI Hospital, Kolkata–700 054, India.
[c]Indian Statistical Institute, 203, B.T. Road, Kolkata–700 035, India.
[d]Regional Office of Health and Family Welfare, 48-A, Hindustan Park, Kolkata–700 029, India.

sistance against the drug used. There is dearth of literarture on parasite density in Indian patients suffering from falciparum malaria since its resurgence. Malaria is now endemic in big cities posing a serious threat to public health. A recent study on comparative evaluation of methods of malaria parasite density estimation in blood samples from patients and laboratory animals was carried out by Dubey *et al.*[1] will be discussed in more detail in the concluding section.

This communication analyses data on parasite density of altogether one hundred patients suffering from falciparum malaria in an endemic area in Kolkata. Three different methods, which are standardised and well-known in practice, have been used simultaneously for each patient and evaluated statistically to find out the most suitable method that can be conveniently used in practice. Moreover, the range of prasitaemia and the average density of parasites in the endemic area were also evaluated.

MATERIALS AND METHODS

The study area consisted of about three sq km situated at the northwestern part of Kolkata. A total of 100 patients suffering from falciparum malaria were considered for the study reported in this paper. From each patient a thick film was drawn on the glass slide, stained with Giemsa and examined under oil immersion at a magnification of 1000. The number of parasitized red cells (containing only the rings) per 200 white blood cells was counted. To count the parasites, three methods were used. In the first method, assuming the white blood cell count to be 8000/μl of blood, the number of parasites thus counted in each positive slide was multiplied by 40 to get the number of parasites/μl of blood applying the formula of Wernsdorfer and McGregor.[2]

$$\text{Adjusted count} = \frac{\text{No. of parasites}}{\text{No. of leucocytes}} \times 8000$$

$$= \text{No.of parasites/}\mu\text{l of blood}$$

In the second method,[3] the number of parasites was counted in each microscopic field and each case was graded as having high density for 20 or more parasites per field, as having medium density for 2–19 parasites per field and as having low density for one or less parasite per field. For each positive slide on an average 10 fields were examined. This is termed as the raw count-I. In the third method,[4] the cases were classified according to the parasite density as follows:

+ : 1–10 rings per 100 fields; ++ : 11–100 rings per 100 fields; +++ : < 10 rings per field; and ++++ : > 10 rings per field.

This is termed as the raw count-II.

Statistical analysis of data

One of our main objectives is to find out how the raw parasite counts and adjusted parasite counts are correlated for different patients. We have plotted the logarithms of raw and adjusted parasite counts for all hundred falciparum ma-

laria patients in the present study in a scattergram (Fig. 1). The strong linear pattern, which is clearly visible in the scattergram, suggests a very high linear correlation between the logarithms of the two counts and the actual value of the correlation in this case is 0.86.

On careful examination of the data, it was observed that most of the patients with adjusted count 3000 or less, were classied in the "low" category according to raw count-I and in the + or the ++ category according to raw count-II. The cases for which the adjusted count was between 3000 and 20,000, were classified mostly as "medium" according to raw count-I and as +++ according to raw count-II. The cases with adjusted count more than 20,000 were classified mostly as "high" according to raw count-I and as ++++ according to raw count-II. This motivated us to introduce a new classification based on adjusted count as follows.

The cases with adjusted count 3000 or less may be classified as mild, those with adjusted count between 3000 and 20,000 may be classified as moderate and those with adjusted count above 20,000 may be classified as high. In the next section we report some results obtained from statistical analysis of the cross classified data when each case was classified by different methods.

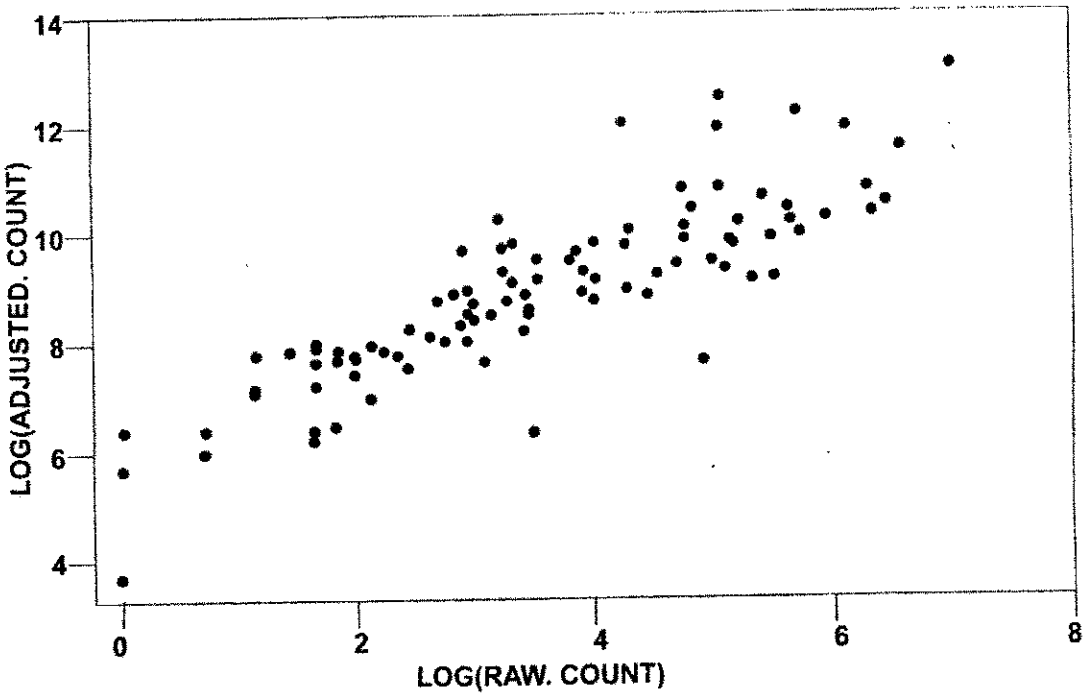

Fig. 1: Scattergram for logarithms of adjusted and raw counts

RESULTS

According to the first method, i.e. adjusted count, the infected cases were observed to be distributed as 33 per cent "mild" cases, 42 per cent "moderate" cases and 25 per cent "high" cases. According to the second method, i.e. raw count-I, 33 per cent of the cases were classified as "low", 53 per cent as "medium" and 14 per cent as "high" (Table 1). In the case of the third method (raw count-II), there were five per cent cases in + category, 23 per cent cases in ++ category, 42 per cent cases in the +++ category and 30 per cent cases in the ++++ category (Table 2).

The three methods were subjected to detailed statistical evaluation. There was very high degree of association between the "high-medium-low" classification based on the raw counts (the sencond method) and the "high-moderate-mild" classification based on the adjusted counts (the first method). Kendall's correlation,[5] between the two classification procedures based on concordant and discordant pairs was 0.982 as computed from the data presented in Table 1. If the raw count classification happened to be "low", there was about 90 per cent chance that the adjusted count classification would be "mild", and similarly if the raw count classification happened to be "high", there was about 93 per cent chance that the adjusted count classification would be "high". When the raw count classification was "medium", the chances for the adjusted count classification to be "moderate" and "high" were 72 and 23 per cent respectively.

Table 1. Classification of parasitaemia in *P. falciparum* infected patients according to adjusted count and raw count-I

Adjusted count	Raw count-I			
	Low	Medium	High	Total
Mild	30	3	0	33
Moderate	3	38	1	42
High	0	12	13	25
Total	33	53	14	100

Table 2. Classification of parasitaemia in *P. falciparum* infected patients according to adjusted count and raw count-II

Adjusted count	Raw count-II			
	+/++	+++	++++	Total
Mild	28	4	1	33
Moderate	0	35	7	42
High	0	3	22	25
Total	28	42	30	100

Since there were only five of the 100 cases in the + category, we have tabulated the cross classification data in Table 2 after merging the + and the ++ categories. The Kendall's correlation[5] computed using concordant and discordant pairs based on this table for the two classification procedures (one based on raw counts and the other on adjusted counts) was 0.96 which was quite high indicating a strong association between the data obtained from two

procedures. When a case in + or ++ category was taken, the corresponding classification based on adjusted count was always "mild". Similarly a case falling in +++ category would likely be classified as "moderate" based on adjusted count with a chance of about 83 per cent and the chance for a case in ++++ category to be classified as "high" according to adjusted count is about 73 per cent.

DISCUSSION

Our analysis indicates that the correlation between the classification based on adjusted counts and the classification based on raw counts is extremely high, and so one can use only the raw counts and avoid complexities involved in obtaining the adjusted counts. Since obtaining adjusted counts involves getting WBC counts and observing more fields in positive slides than what is required in the case of raw counts, doing the analysis using only raw counts results in a more convenient and less expensive procedure. This would also reduce the time to examine a slide.

Severity of the disease has some relation with the density of parasites. This is also useful to detect the development of resistance and select treatment modalities. One of the criteria of severe malaria in a patient is haematocrit < 15 per cent or haemoglobin < 5 g/dl in the presence of parasitaemia with parasite density > 10,000/µl. Patients having hyperparasitaemia with parasite density > 100,000/µl have the risk of increased mortality, and when parasite density is > 500,000/µl, high mortality is expected.[6] According to these parameters, 43 per cent of the patients examined possess the risk of attack with severe malaria in the sense that the parasite density in them is > 10,000/µl. In a study in the same endemic area, 0.63 per cent of the patients infected with falciparum malaria has haemoglobin < 5 g/dl.[7] However, it should be noted that in endemic areas, such type of high parasitaemia may be well tolerated by semi-immune population.

It was observed that seven per cent of the patients in the present study possess parasitaemia with parasite density > 100,000/µl of blood in whom severe manifestations may occur. In no patient, however, parasite density was observed to exceed 500,000/µl. The observed range in 100 patients was 40/µl to 4,96,000/µl, and the mean parasite density was 27,462/µl.

Counting of density of parasites in the blood can take a significant role to detect the early treatment failure, late treatment failure and treatment success.[8] It can thereby help in identifying the resistant cases and selecting treatment modalities. When parasite density on Day 3 is more than 25 per cent of the density of Day 0, early treatment failure is apprehended. Again, subjects whose fever has initially been cleared (i.e. temperature < 37.5°C), and who become parasite negative or parasite positive on Day 3 (i.e. parasite density becomes ≤ 25 per cent of the density on Day 0) but who show fever or parasitaemia on any day from Day 4 to Day 14 are regarded as cases of late treatment failure. Treatment success means afebrile subjects who are parasite negative on Day 3 and thereafter

(early recovery) or subjects with or without fever and parasite positive on Day 3 (i.e. parasite density $\leq$ to 25 per cent of the density on Day 0) and parasite negative thereafter (late recovery).

Studies on density of parasitaemia in falciparum patients in this hypoendemic region indicate the existence of a potentially dangerous situation, having the risk of life, specially when falciparum malaria is increasing gradually from 10 per cent in 1980 to 36–38 per cent in recent years in India.[9,10]

We conclude by drawing the reader's attention to a similar work by Dubey *et al.*[1] This work also considers several methods of evaluating parasite density and their statistical comparison. However, the number of patients suffering from falciparum malaria considered by them is only 12 as opposed to 100 in our study. Also, their modes of comparison are quite different from ours. This is primarily because one of the methods used by them was assumed to give the correct measure of parasite density and the other methods were tested against this method, which was taken like a standard, using statistical *t*-test for comparison of parasite counts obtained by different methods. On the other hand, we have focussed on the degree of correlation between parasite counts obtained by three standard procedures used in practice.

REFERENCES

1. Dubey, M.L., C. Weingken, N.K. Ganguly and R.C. Mahajan (1999). Comparative evaluation of methods of malaria parasite density determination in blood samples from patients and experimental animals. *Indian J. Med. Res.*, **109:** 20–27.
2. Wernsdorfer, W.H. and I. McGregor (1988). *Malaria: Principles and Practice of Malariology*, Chapter 5 — The microscopic diagnosis of malaria by *G.T. Shute* (Churchill Livingstone, London): 781.
3. WHO (1980). *Manual of Basic Techniques for a Health Laboratory* (World Health Organisation, Geneva): 487.
4. Bruce-Chwatt, L.J. (1980). *Essential Malariology*. (William Heineman Medical Books Ltd., London): 86.
5. Kendall, M.G. (1938). A new measure of rank correlation. *Biometrika*, **30:** 81–93.
6. Cook, G. (1996). *Manson's Tropical Diseases*. Chapter 61: Malaria, XX edn. Ed. N.J. White (ELBS with W.B. Saunders, London): 1111.
7. Hati, A.K., B. Mandal, N.K. Mitra, A.K. Mukhopadhyay and H. Mukherjee (1998). Malaria in Calcutta : Recent character. *Sci. Cul.*, **64:** 150–156.
8. Ejor, M.N., T. Tun, S. Aung and K. Sein (1999). Response of falciparum malaria to different anti-malarials in Myanmar. *Bull. WHO*, **77:** 244–249.
9. Hati, A.K. (1993-94). *Assessment Related to Abatement of Some Vector-Borne Diseases in India*. Presidential Address, 81st Session of Indian Science Congress (Section of Medical and Veterinary Sciences, Jaipur): 24.
10. NMEP (1988). *Country Scenario of Malaria and its Control in India* (Directorate of National Malaria Eradication Programme, Delhi): 98.

Indian Journal of Malariology
Vol. 37, September–December 2000, pp. 88–96.

Entomological and Epidemiological Studies on Malaria in Rajmahal Range, Bihar

N.G. DAS[a], M. BHUYAN[a] and S.C. DAS[a]

The epidemiological investigations carried out in six tribal villages in Rajmahal range hill in south Bihar revealed average slide positivity rate (SPR) 25.1 ranging between 9.8 and 37.8 per cent. *Plasmodium falciparum* was the dominant parasite which accounted 64.2 per cent of the total infections. Results of mass blood survey indicated the presence of high percentage of asymptomatic carriers of malaria parasites in the local populace. In an entomological survey, 25 species of mosquitoes under five genera were collected in 20 trap nights. The average density of mosquitoes recorded was 230.6 per trap night. *Anopheles* mosquitoes alone accounted 72.8 per cent of the total collection whereas malaria vectors *Anopheles maculatus*, *An. minimus*, *An. philippinensis*, *An. varuna* and *An. annularis* accounted 32.8 per cent of the total anophelines collected. Both anopheline and culicine mosquitoes were found susceptible to DDT (4 per cent) and malathion (5 per cent) in 30 min exposure. Dissection of malaria vectors *An. minimus*, *An. maculatus* and *An. philippinensis* revealed very high percentage of parity rate (77.8 per cent) which gives a strong indication about their vectorial status in the transmission of malaria. Poor socio-economic condition, lack of sense of hygiene worsen the situation in the presence of asymptomatic carriers.

Keywords : Asymptomatic carrier, Malaria vector, *Plasmodium falciparum*, Tribals

INTRODUCTION

Malaria is one of the oldest and endemic disease in Bihar, causing serious health hazards mainly in the rural areas, where 90 per cent of the state population lives in scattered villages. Geographical location, tropical climate and poor socio-economic conditions make it an excellent abode for occurrence and persistent transmission of malaria.[1] Rajmahal range of hills in Santhal Pargana district of south

[a]Defence Research Laboratory, Post Bag No. 2, Tezpur–784 001, India.

Bihar is one of such areas endemic for malaria recording number of deaths every year. *An. culicifacies*, *An. fluviatilis* and *An. stephensi* have been reported as the vectors of malaria in rural areas, foothills and urban cities in Bihar.[2] High incidence of malaria has been recorded by Medical Inspection (MI) room of the Cantonment during 1994–97. The slide positivity rate ranged between 11.2 and 20.5 per cent with *P. falciparum* constituting more than 76.5 per cent of the total positive cases except in 1997, when it was only 50 per cent. Indoor residual spray was regularly carried out in the Cantonment, but no records of spray were available in the adjoining villages (as per record of the MI room of Air Force Station, Singhorsi).

In view of the above, epidemiological and entomological studies were carried out in Rajmahal ranges in hills of south Bihar during October 1998 to investigate the persistence of malaria transmission and vectors responsible for it, and the susceptibility status of vectors to insecticides.

Topography

Rajmahal range of hills with the highest peak of 1860 ft is located in southeast Bihar, covering a large area of Santhal Pargana district bordering West Bengal. The climate is wet tropical and the rainfall is moderate to heavy with the intervals of sunshine. The range is covered with tall trees and dense undergrowth interspersed with perennial streams and nallahs forming innumerable water pockets and some marshy lands. The Gamani lake is the main water reservoir of the Cantonment with grassy margins and huge marshy land all around located in the vicinity. It is also the perennial source of anopheline breeding. Small sylvan villages (hamlets) with thick vegetation mainly inhabited by the Santhals and Adivasis are located by the side of perennial streams/nallahs.

Socio-economic condition of villagers is poor and the people solely depend on paddy and jhoom cultivation. Forested terrain, tall trees, dense undergrowth and perennial streams maintained moderate climate throughout the year which is congenial for rapid multiplication and longevity of malaria vectors.

MATERIALS AND METHODS

Epidemiology

Mass blood survey was carried out in six affected villages—Balami, Mudgaon, Tatpara, Mamamore, Chabi and Singhorsi. Blood smears (both thick and thin) were prepared, stained with 1:10 Giemsa diluted in pH 7.2 buffer and examined under the microscope. Epidemiological parameters such as parasite species, SPR, *Pf* per cent, age and sexwise distribution of malaria cases were analysed.

Entomology

Adult mosquitoes were collected from human dwellings, cattlesheds and goat cabins with the aid of six volt battery operated CDC miniature light-traps (Communicable Disease Centre,

USA) from dusk-to-dawn (1800-0500 hours). Traps were hung in the middle of the room at a height of about two metre from the ground level. Smoking and other illuminations were prevented during the operation of the traps. Hand catch collection of adult mosquitoes with the help of aspirator tube between 1800 and 2100 hours was also made. Collected mosquitoes were identified as per standard keys of Christophers,[3] Barraud,[4] Puri,[5] and Wattal and Kalra.[6] Mosquitoes of hand catch collection were utilised to determine the susceptibility status of malaria vectors against DDT (4 per cent) and malathion (5 per cent) as per standard WHO procedure.[7] Alive anophelines were dissected to determine their physiological age and sporozoite infection of salivary glands.[8]

RESULTS AND DISCUSSION

The examination of blood smears revealed an average slide positivity rate (SPR) of 25.1. The highest incidence (37.8 per cent) was recorded in Singhorsi followed by Balami (30.8 per cent), Mudgaon (29.4 per cent), Chabi (19.4 per cent), Mamamore (16.8 per cent) and the lowest (9.8 per cent) in Tatpara village which is located in the vicinity of Airforce Medical Inspection (MI) room. *P. falciparum*, the dominant species accounted 64.8 per cent of the total infections ranging between 40 and 100 per cent in the study villages (Table 1). High percentage of *P. falciparum* has also been reported from northeastern region of India.[9–11]

The presence of high percentage of asymptomatic carriers (25.1 per cent) by detecting malaria parasite from the population that have no symptoms during mass blood survey in the area act as reservoirs in transmitting the disease to the healthy persons through vector mosquitoes as the locals are not in a habit of using mosquito nets or any other personal pro-

Table 1. Results of mass blood survey in Rajmahal Range, Bihar

Village	BSC/E	(+) ve	*Pf*	SPR	SfR	*Pf* %
Balami	107	33	21	30.8	19.6	63.6
Mudgaon	51	15	6	29.4	11.8	40.0
Tatpara	41	4	4	9.8	9.8	100
Mamamore	95	16	10	16.8	10.5	62.5
Chabi	67	13	8	19.4	11.9	61.5
Singhorsi	74	28	21	37.8	28.4	75.0
Total	435	109	70	25.1	16.1	64.2

BSC/E — Blood smears collected/examined.

tection measures, thus become more vulnerable for contacting malaria. However, detected positive cases were given radical treatment as per NAMP schedule to prevent further transmission of the disease. Repeated infections and non-clearance of parasites from the blood due to under-dosage of antimalarial drugs might be the cause of development of immunity and asymptomatic carriers in the community. Population below 20 years (SPR 32.7) suffer more than the population above 21 years (SPR 15.3) of age (Table 2). This is in conformity with the earlier observations made by Dutta and Rajvir[12] in north Indian districts and Das *et al*[11] in Assam. Analysis of data revealed a little difference in the incidence of malaria among the male and female population indicating the SPR 26.1 and 23.8 per cent respectively (Table 3), which confirmed the earlier observations made by Dutta and Bhattacharyya[13] in Tirap district of Arunachal Pradesh and Das *et al.*[11] in Nalbari district of Assam.

Table 2. Incidence of malaria among different age groups

Age group (yrs)	BSC/E	*Pf*	*Pv*	SPR	*Pf* %
< 5	40	6	4	25.0	60.0
6–10	90	19	12	34.4	61.3
11–15	61	16	7	37.7	69.6
16–20	54	10	6	29.6	62.5
> 21	190	19	10	15.3	65.5
Total	435	70	39	25.1	64.2

BSC/E — Blood smears collected/examined.

Table 3. Incidence of malaria among different sex

Sex	BSC/E	*Pf*	*Pv*	SPR	*Pf* %
Male	234	37	24	26.1	60.7
Female	201	33	15	23.8	68.8
Total	435	70	39	25.1	64.2

BSC/E — Blood smears collected/examined.

In entomological surveys, a total of 4,616 mosquitoes comprising 25 species under five genera—*Anopheles* (15 spp), *Culex* (5 spp), *Aedes* (2 spp), *Armigeres* (1 sp) and *Mansonia* (2 spp) were collected in 20 trap nights (Table 4). Habitats of collection were human dwellings, cattlesheds and goat cabins with per trap night density of 106.8, 305.6 and 164.4 respectively. *Anopheles* mosquitoes accounted to 72.8 per cent of the total collection. Highest density (229.9 per trap night) of anophelines was observed in the cattlesheds followed by the goat cabins (112) and human dwellings (67.5) (Table 5). Major malaria vectors encountered were *Anopheles maculatus* (642), *An. minimus* (306), *An. philippinensis* (87), *An. varuna* (56) and *An. annularis* (12) which formed 32.8 per cent of the total anopheline collection. Highest catch of malaria vectors were made from the cattlesheds followed by the goat cabins and the lowest in human dwellings which indicates the zoophilic nature of the vector species (Table 5). Among the culicines, *Culex vishnui* group (540), was the predominant species followed by *Cx. malayi* (266), *Mansonia uniformis* (108) and *Cx. gelidus* (107).

Table 4. Mosquitoes collected by CDC light-trap in Rajmahal range

Mosquito species	Balami	Gamani	Mudgaon	Mamamore	Chabi
Anopheles aconitus	–	11 (5.5)	–	–	7 (3.5)
An. annularis	5 (0.4)	–	3 (0.8)	5 (1.7)	–
An. barbirostris	15 (1.7)	21 (10.5)	15 (3.8)	21 (7.0)	6 (3.0)
An. crawfordi	69 (7.7)	45 (22.5)	35 (8.8)	49 (16.3)	11 (5.5)
An. jamesi	15 (1.7)	40 (20.0)	39 (9.8)	23 (7.7)	34 (17.0)
An. jeyporiensis	108 (12.0)	71 (35.5)	93 (23.3)	70 (23.3)	59 (29.5)
An. maculatus	159 (17.7)	115 (57.5)	136 (34.0)	113 (37.7)	119 (59.5)
An. maculatus var. *willmorei*	24 (2.7)	–	3 (0.8)	27 (9.0)	–
An. minimus	94 (10.4)	181 (90.5)	5 (1.3)	4 (1.3)	22 (11.0)
An. philippinensis	47 (5.2)	19 (9.5)	5 (1.3)	10 (3.3)	6 (3.0)
An. ramsayi	29 (3.2)	53 (26.5)	10 (2.5)	11 (3.7)	9 (4.5)
An. splendidus	169 (18.8)	226 (113.0)	160 (40.0)	146 (48.7)	80 (40.0)
An. theobaldi	66 (7.3)	59 (29.5)	106 (26.5)	79 (26.3)	73 (36.5)
An. vagus	27 (3.0)	2 (1.0)	5 (1.3)	17 (3.7)	18 (9.0)
An. varuna	36 (4.0)	4 (2.0)	14 (3.5)	2 (0.7)	–
Cx. bitaeniorhynchus	9 (1.0)	24 (12.0)	20 (5.0)	11 (3.7)	8 (4.0)
Cx. gelidus	16 (1.8)	33 (16.5)	10 (2.5)	38 (12.7)	10 (5.0)
Cx. malayi	67 (7.4)	52 (26.0)	46 (11.5)	73 (24.3)	28 (14.0)
Cx.quinquefasciatus	15 (1.7)	–	13 (3.3)	10 (3.3)	2 (1.0)
Cx.vishnui group	160 (17.8)	85 (42.5)	108 (27.0)	126 (42.0)	61 (30.5)
Armigeres subalbatus	23 (2.6)	11 (5.5)	16 (4.0)	27 (9.0)	9 (4.5)
Aedes albopictus	3 (0.3)	7 (3.5)	3 (0.8)	3 (1.0)	–
Ae. pseudotaeniatus	–	3 (1.5)	–	2 (0.7)	–
Mansonia annulifera	17 (1.9)	–	3 (0.8)	–	7 (3.5)
Ma. uniformis	43 (4.8)	19 (9.5)	14 (3.5)	9 (3.0)	23 (11.5)
Total	1215	1081	852	876	592

Figures in parentheses indicate per trap night density of mosquitoes.

Table 5. Density of mosquitoes in different habitats, Rajmahal range, Bihar

Mosquito species	Human dwelling Total (4 TN)	Cattleshed Total (11 TN)	Goat cabin Total (5 TN)
Anopheles aconitus	–	18 (1.63)	–
An. annularis	3 (0.8)	8 (0.7)	1 (0.2)
An. barbirostris	7 (1.8)	66 (6.0)	5 (1.0)
An. crawfordi	19 (4.8)	155 (14.0)	35 (7.0)
An. jamesi	7 (1.75)	125 (11.3)	19 (3.8)
An. jeyporiensis	54 (13.5)	279 (24.5)	68 (13.6)
An. maculatus	49 (12.25)	466 (42.36)	127 (25.4)
An. maculatus var. *willmorei*	3 (0.8)	31 (2.8)	20 (4.0)
An. minimus	16 (4.0)	247 (22.5)	43 (8.6)
An. philippinensis	8 (2.0)	62 (5.6)	17 (3.4)
An. ramsayi	9 (2.3)	82 (75.0)	21 (4.2)
An. splendidus	46 (11.5)	618 (56.2)	117 (23.4)
An. theobaldi	34 (8.5)	294 (26.7)	55 (11.0)
An. vagus	4 (1.0)	55 (5.0)	10 (2.0)
An. varuna	11 (2.8)	23 (2.1)	22 (4.4)
Culex bitaeniorhynchus	5 (1.3)	50 (4.5)	7 (1.4)
Cx. gelidus	6 (1.5)	84 (7.6)	17 (3.4)
Cx. malayi	36 (9.0)	182 (16.5)	48 (9.6)
Cx. quinquefasciatus	12 (3.0)	20 (1.8)	8 (1.6)
Cx. vishnui group	67 (16.8)	346 (31.5)	127 (25.4)
Armigeres subalbatus	12 (3.0)	54 (4.9)	20 (4.0)
Aedes albopictus (Skuse)	–	15 (1.4)	1 (0.2)
Ae. pseudotaeniatus	–	5 (0.5)	–
Mansonia annulifera	3 (0.8)	17 (1.5)	7 (1.4)
Ma. uniformis	16 (4.0)	65 (5.9)	27 (5.4)
Total	426 (106.7)	3367 (306.0)	822 (164.5)

Figures in parentheses indicate per trap night; TN — Trap night.

Table 6. Susceptibility status of mosquitoes to insecticides

Mosquito species	DDT (4%) (Exposure time 30 min)			Malathion (5%) (Exposure time 30 min)			Control mortality (%)
	Exposed	Dead	Alive	Exposed	Dead	Alive	
An. annularis	8	8	0	5	5	0	0
An. crawfordi	41	41	0	34	34	0	0
An. jamesi	33	33	0	28	28	0	10.5
An. jeyporiensis	46	46	0	41	41	0	10
An. maculatus	93	93	0	83	83	0	9.1
An. minimus	30	30	0	26	26	0	0
An. philippinensis	17	17	0	16	16	0	0
An. splendidus	56	56	0	52	52	0	0
An. theobaldi	40	40	0	31	31	0	0
An. vagus	24	24	0	20	20	0	0
An. varuna	20	20	0	18	18	0	0
Cx. malayi	19	19	0	10	10	0	0
Cx. vishnui group	34	34	0	21	21	0	0

Banerjee and Sinha[2] reported *An. fluviatilis* as a principal vector in foothills of Bihar while *An. culicifacies* and *An. stephensi* play the same role in rural and urban areas. *An. fluviatilis* and *An. culicifacies* could not be recorded during the survey. However, *An. minimus*, *An. maculatus* and *An. philippinensis* which are the known vectors of malaria in other states of India,[10,14,15] the role of these species in the transmission of malaria in Rajmahal hills can not be ruled out. The average man hour density of *An. annularis*, *An. maculatus*, *An. minimus* and *An. philippinensis* in hand catch collection was 18. *An. annularis* may not be a major vector of malaria but it has an importance as a local vector in several localities.[16]

Both anopheline and culicine mosquitoes were found susceptible to DDT (4 per cent) and malathion (5 per cent) in 30 min exposure (Table 6). This indicates the usefulness of indoor residual spray of insecticides in controlling mosquitoes in the valley.

Table 7. Parity status of vector mosquitoes in Rajmahal range, Bihar

Mosquito species	Nuli-parous	Parous	% parous	Salivary gland infection
An. minimus	15	48	76.2	–
An. maculatus	75	270	78.3	–
An. philippinensis	6	15	71.4	Not done
Total	96	333	77.6	

Dissection of malaria vectors revealed high parity rate (77.6 per cent) ranging between 71.4 and 78.3 per cent (Table 7) which gives a strong indication about their vectorial status in the transmission of the disease.[12]

The water reservoir with grassy margins, marshy land, overflow of streams, nallahs and springs are the major sources of mosquito breeding. Water lodged paddy fields can also be seen in the downhill. High parity rate of vectors, the poor socio-economic condition, the lack of awareness about hygiene, the poor sanitation and the prevalence of malaria in the locals might be the prime causes of the perennial transmission of malaria in Rajmahal range, Bihar.

As the vectors are susceptible to insecticides, indoor residual spray, active surveillance, malaria awareness camp and timely antimalarial measures can certainly improve the situation in this area.

ACKNOWLEDGEMENTS

The authors thank Dr. M.P. Chacharkar, Director, Defence Research Laboratory, Tezpur for valuable suggestions and continuous encouragement in this investigation.

REFERENCES

1. Sinha, V.P. (1971). Observations on the progress of National Malaria Eradication Programme–Bihar and its impact on the green revolution in villages. *Patna J. Med.*, **45:** 297–302.
2. Banerjee, B.D. and V.P. Sinha (1977). Alert: Malaia is back. *Patna J. Med.*, **51**(9) : 228–232.
3. Christophers, S.R. (1933). *The Fauna of British India including Ceylon and Burma*, v. 4 (Taylor and Francis, London).
4. Barraud, P.J. (1934). *The Fauna of British India including Ceylon and Burma*, v. 5 (Taylor and Francis, London).
5. Puri, I.M. (1955). Synaptic table for the identification of the anopheline mosquitoes of India, iv edn. *Hlth. Bull.* No. **10**.
6. Wattal, B.L. and N.L. Kalra (1961). Region-wise pictorial keys to the female Indian Anopheles. *Bull. Natl. Soc. Ind. Mal. Mosq. Dis.*, **9:** 85–138.
7. WHO (1981). Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides. Diagnostic test. WHO/VBC/81. 803 (Mimeographed document).
8. Detinova, T.S. (1962). *Age grouping Methods in Diptera of Medical Importance* (World Health Organization, Geneva): 74–76.
9. Jana-Kara, B.R., W.A. Jihullah, B. Sahi, Vas Dev, C.F. Curtis and V.P. Sharma (1995). Deltamethrin impregnated bednets against *Anopheles minimus* transmitted malaria in Assam, India. *J. Trop. Med. Hyg.*, **98:** 73–83.
10. Prakash, A., P.K. Mohapatra and V.K. Srivastava (1996). Vector incrimination in Tamulpur Primary Health Centre, district Nalbari, lower Assam during

malaria outbreak 1995. *Indian J. Med. Res.* **103**(2): 146–149.

11. Das, N.G., I. Baruah, S. Kamal, P.K. Sarkar, S.C. Das and K. Santhanam (1997). An epidemiological and entomological investigation on malaria outbreak at Tamulpurr PHC, Assam. *Indian J. Malariol.*, **34:** 164–170.

12. Dutta, P.K. and B. Rajvir (1991). An epidemiological study in North Indian district. *J. Com. Dis.*, **23**(1): 29–33.

13. Dutta, P. and D.R. Bhattacharyya (1990). Malaria survey in some parts of Namsang Circle of Tirap district, Arunachal Pradesh. *J. Com. Dis.*, **22:** 92–97.

14. Das, S.C. and I. Baruah (1985). Incrimination of *Anopheles minimus*, Theobald and *Anopheles balabacensis balabacensis* Baisas (*An. dirus*) as malaria vectors in Mizoram. *Indian J. Med. Res.*, **22:** 53–55.

15. Rajagopal, R. (1976). Studies on persistence transmission of malaria in Meghalaya. *J. Com. Dis.*, **8**(4): 235–245.

16. Rao, T. Ramachandra (1984). *The Anophelines of India.* Rev. edn. (Malaria Research Centre, Indian Council of Medical Research, Delhi): 383–391.

SHORT NOTES

Indian Journal of Malariology
Vol. 37, September–December 2000, pp. 97–102.

Preliminary Observations on Man-mosquito Contact in Soraipung Village of District Dibrugarh, Assam (India)

ANIL PRAKASH[a], D.R. BHATTACHARYYA[a], P.K. MOHAPATRA[a] and J. MAHANTA[a]

Keywords: Ecosystem, JE vectors, Malaria vectors, Man-mosquito contact, Biting rhythm

A longitudinal study on man-mosquito contact was conducted during 1995–96 in a remote forest-fringed village—Soraipung under Tengakhat Primary Health Centre (PHC) of District Dibrugarh, Assam. The sociological and meteorological conditions of the study area have already been described elsewhere.[1] Dusk-to-dawn man landing mosquito collections on a local volunteer relaxing on a cot were carried out inside a village hut situated at a distance of 15 m from the forest-fringe at monthly interval from August 1995 to August 1996 by two pairs of collectors working in two shifts of six hours each, using suction tube and flashlights. The collection hut, remained unchanged throughout the study. Hour-wise collections of mosquitoes were kept separately and identified in the camp laboratory next morning using the standard keys. The frequency of man-mosquito contact, expressed as mosquito landing rate (MLR) was calculated as Williams' geometric mean.[2] Data of only those months which recorded high mosquito densities were utilised for computing the biting rhythm of common vector species encountered during the study.

A total of 1941 mosquitoes belonging to four genera and 18 species were collected during 13

[a]Regional Medical Research Centre, N.E. Region (ICMR), Dibrugarh–786 001, India.

Table 1. Indoor man landing mosquitoes in Soraipung village, District Dibrugarh, Assam

Species	Number of landing mosquitoes/person/night													Total	Mosquito landing rate*
	1995					1996									
	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug		
Anopheles dirus	36	52	60	9	0	0	0	0	19	30	40	119	23	388	9.4
Culex bitaeniorhynchus	1	1	0	0	0	0	0	0	2	1	0	3	1	9	0.92
Cx. fuscocephala	2	0	0	0	0	0	0	0	0	0	0	7	0	9	0.69
Cx. gelidus	26	323	0	0	0	0	0	0	5	13	18	75	15	475	4.37
Cx. pseudovishnui	13	67	1	1	0	0	1	6	126	54	112	203	134	718	11.99
Cx. quinquefasciatus	1	0	10	1	0	0	0	1	0	0	1	0	2	16	1.0
Cx. tritaeniorhynchus	0	9	2	0	0	0	0	1	0	0	0	0	12	24	0.98
Cx. vishnui	15	30	7	2	0	0	0	0	13	5	14	51	10	147	4.51
Cx. whitmorei	0	28	0	0	0	0	0	2	0	0	2	0	1	33	0.91
Mansonia dives	1	20	23	0	0	0	0	0	0	7	22	0	9	82	2.01
Ma. uniformis	0	20	1	0	0	0	0	0	0	0	0	2	9	30	1.02
Others**	1	1	1	1	0	0	0	0	2	1	1	0	0	8	0.94
Total	96	551	105	14	0	0	1	10	167	111	210	460	216	1941	42.9

*Williams' geometric mean following n + 0.5 transformation: **Include 7 species : One specimen each of *Aedes aegypti* (May), *Ae. nigrostriatus* (June), *An. hyrcanus* group (Oct), *An. kochi* (Aug), *An. philippinensis* (Nov), *An. vagus* (Sep) and two specimens of *Ma. indiana* (Apr).

nights of collection with the mean mosquito landing rate of 42.9 per person per night (Table 1). Species diversity and density of man landing mosquitoes was maximum in the month of September accounting for 28.4 per cent of the total collection in 10 species.

Anopheles dirus was the predominant anopheline species (99 per cent) collected. This species is a major vector of forest malaria in many southeast asian countries[3] including India (N.E. Region).[1] The landing rate of this species was high from May to October with the overall mean landing rate of 9.4 per person per night. Biting rhythm of *An. dirus,* as found in the present investigation has been described elsewhere.[4] Briefly, the biting was minimal (7.1 per cent) in the I quarter (1800–2100 hours), maximum (37.5 per cent) in the II quarter (2100–2400 hours), high (35.6 per cent) in the III quarter (2400–0300 hours) and moderate (19.8 per cent) in the IV quarter (0300–0600 hours) of the night.

Among JE vectors, the proportions of *Culex pseudovishnui*, *Cx. vishnui* and *Cx. gelidus*, the three predominant *Culex* spp were 37, 7.6 and 24.5 per cent respectively. Their mean landing rates were 11.99, 4.51 and 4.37 per person per night respectively during the study. Maximum number of these mosquitoes were collected between April and September. Members of *Cx. vishnui* sub group of mosquitoes are the known vectors of Japanese encephalitis virus (JEV). In addition, JEV has also been isolated in different parts of India from *Cx. gelidus, Cx. fuscocephala, Cx. bitaeniorhynchus, Cx. quinquefasciatus* and *Cx. whitmorei*.[5] All these species were collected in low to moderate numbers in the present study. Indoor landing of *Cx. pseudovishnui* was noted throughout the night with a peak immediately after dusk. Subsequently, landing decreased gradually in the night (Fig. 1). Maximum landing (51.6 per cent) of this mosquito took place in the I quarter of the night, which was significantly higher ($p < 0.05$) than the biting occurred in III and IV quarters and at par with II quarter landing. In contrast to this, the peak indoor biting of *Cx. pseudovishnui* was found between 0130 and 0330 hours in a JE affected plain village of Assam.[6] Variable peak biting times of this mosquito were also reported from south India,[7] Pakistan[8] and Philippines.[9] This behavioural difference might perhaps be due to the involvement of different populations of *Cx. pseudovishnui* in different places and biotypes.

Landing activity of *Cx. vishnui* also started early and continued throughout the night with gradual decline after 2300 hours (Fig. 1). The landing was minimal and significantly less ($p < 0.01$) in the IV quarter of the night while intense and almost equal amount of landing took place in I and II quarters of the night totalling to 84 per cent together. Further, 2000–2100 and 2200–2300 hours were the peak landing hours of this species accounting for 23.3 and 21.8 per cent of the total landing respectively. Similar pattern of nocturnal biting activity of *Cx. vishnui* was found in other studies[6,10] from Assam. However, in North Arcot district of Tamil Nadu, a rounded unimodel biting pattern of this species with a peak at 2300 hours was reported.[7]

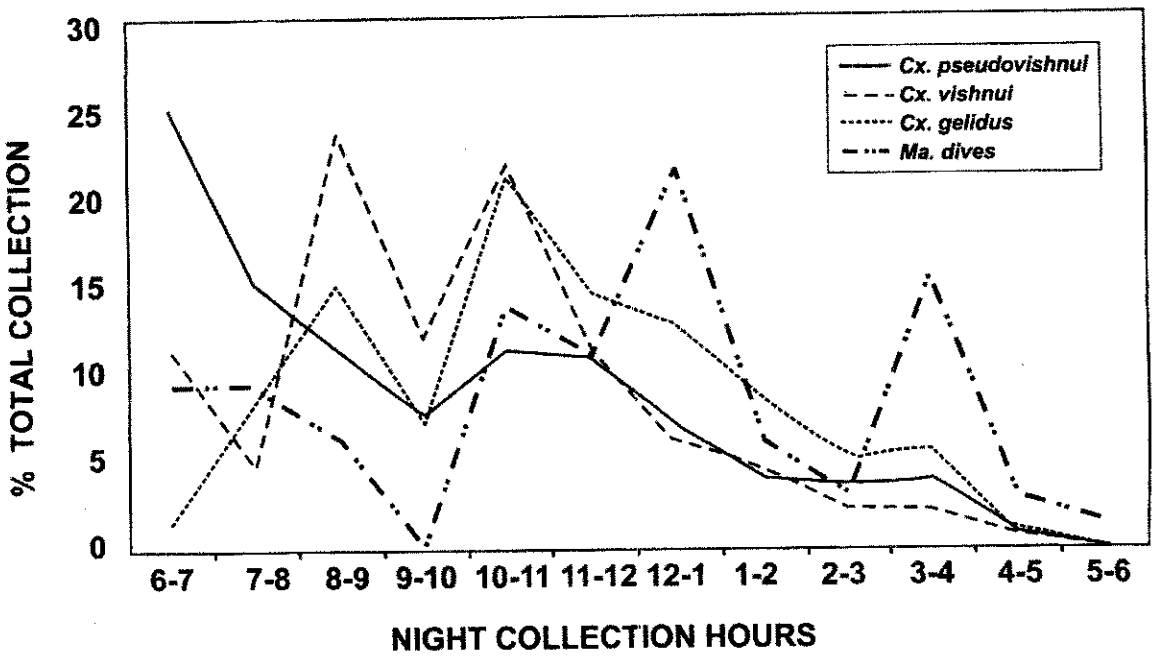

Fig. 1: Indoor biting rhythm of potential JE vector mosquitoes

The landing of *Cx. gelidus* started subduedly on a low note at the dusk hours. It picked up gradually and attained the peak during 2200–2300 hours before slopping down (Fig. 1). The maximum (38.8 per cent) and minimum (6.0 per cent) landing occurred in II and IV quarters of the night with no significant difference ($p > 0.05$) in biting in different quarters.

Mansoniodes mosquitoes are the recognised vectors of malayan filariasis in India.[2,11] Recently, these have also been implicited in the transmission of JE in view of isolation of JEV from *Mansonia annulifera* in Assam;[12] and *Ma. uniformis* and *Ma. indiana* from Kerala.[13] In the present study three species—*Ma. dives, Ma. uniformis* and *Ma. indiana* were collected. Of these, *Ma. dives* was the predominant species with the landing rate of 2.01 per person per night. Landing of *Ma. dives* occurred throughout the night arythmically with pronounced landing between 2400 and 0100 hours and again between 0300 and 0400 hours (Fig. 1). The extent of landing was almost equal ($p > 0.05$) in all four quarters of the night. Sizable indoor man landing activity of *Ma. dives* in upper Assam was also reported earlier.[6]

Ecological conditions of an area largely influence the vector density and man-vector contact which in turn determines the epidemiology and intensity of disease transmission.[14] Forest-fringed areas in northeastern region are basically ecotone zones, where due to the availability of

large number of breeding habitats and suitable micro-climate throughout the year, generally high mosquito densities and species diversity with higher survival rate are encountered. It makes forest-fringed areas relatively more receptive to the mosquito-borne diseases in comparison to deep forest or plain non-forested areas. High prevalence of *An. dirus* transmitted *P. falciparum* malaria from the present study area has been reported.[1] Further, Dibrugarh district is highly endemic for JE, reporting large number of human JE cases and deaths regularly since 1978.[15] Present study recorded high degree of man to JE vectors contact. This factor coupled with the availability of pigs in the village and the adjoining forest area, makes this area further vulnerable to JE transmission which needs regular monitoring.

ACKNOWLEDGEMENTS

We are thankful to Messrs A.C. Rabha, P. Doloi, Raju Sonowal and Dipak Dutta for their technical assistance during the study.

REFERENCES

1. Prakash, Anil, D.R. Bhattacharyya, P.K. Mohapatra and J. Mahanta (1997). Seasonal prevalence of *Anopheles dirus* and malaria transmission in a forest-fringed village of Assam, India. *Indian J. Malariol.*, **34** : 117–125.
2. Pradeep, Kumar, N., S. Sabesan and K.N. Panicker (1989). Biting rhythm of the vectors of malayan filariasis, *Mansonia annulifera*, *Ma. uniformis* and *Ma. indiana* in Shertalla (Kerala state), India. *Indian J. Med. Res.*, **89:** 52–55.
3. Rosenberg, R. and N.P. Maheswari (1982). Forest malaria in Bangladesh II. Transmission by *Anopheles dirus*. *American J. Trop. Med. Hyg.*, **31:** 183–191.
4. Prakash, Anil, D.R. Bhattacharyya, P.K. Mohapatra and J. Mahanta (1997). Studies on bionomics of *Anopheles dirus* Peyton and Harrison, 1979 (Diptera : Culicidae) in Assam, India. *Proceedings of the Second Symposium on Vectors and Vector-Borne Diseases* (National Academy of Vector-Borne Diseases, Bhubaneswar): 236–246.
5. Reuben, R., H.N. Kaul and R.S. Soman (1988). Mosquitoes of arboviral importance in India. *Mosq. Borne Dis. Bull.*, **5:** 48–54.
6. Khan, S.A., K. Narain, P. Dutta, R. Handique, V.K. Srivastava and J. Mahanta (1997). Biting behaviour and biting rhythm of potential Japanese encephalitis vectors in Assam. *J. Com. Dis.*, **29:** 109–120.
7. Reuben, R. (1971). Studies on the mosquitoes of North Arcot district, Madras state, India. Pt 2. Biting cycles and behaviour on human and bovine baits at two villages. *J. Med. Entomol.*, **8:** 127–134.
8. Reisen, W.K. and M. Aslamkhan (1978). Biting rhythms of some Pakistan mosquitoes (Diptera : Culicidae). *Bull. Entomol. Res.*, **68:** 313–330.
9. Schultz, G.W. and C.G. Hayes (1993). Ecology of mosquitoes (Diptera : Culicidae) at a site endemic with Japanese encephalitis on Luzon, Republic of the Philippines. *Southeast Asian J. Trop. Med. Pub. Hlth.*, **24:** 157–164.
10. Bhattacharyya, D.R., P. Dutta, S.A. Khan, P. Doloi and B.K. Goswami (1995). Biting cycles of some potential vector mosquitoes of Japanese encephalitis in Assam, India. *Southeast Asian J. Trop. Med. Pub. Hlth.*, **26:** 177–179.
11. Manson Bahr, P.E.C. and F.I.C. Apted (1982). Filariasis. *Manson's Tropical Diseases*, 18th edn. (The ELBS and Bailliere Tindal, London): 148–180.
12. Chakraborty, S.K., A.K. Chakraborty, K.K. Mukerjee, A.C. Mitra, A.K. Hati and M.S. Chakraborty (1981). Isolation of Japanese en-

cephalitis (JE) virus from *Mansonia annulifera* species of mosquitoes in Assam. *Bull. Cal. Sch. Trop. Med.*, **129:** 3–4.

13. Dhanda, V., V. Thenmozhi, N.P. Kumar, J. Hiriyan, N. Arunachalam, A. Ilango and A. Gajanana (1997). Virus isolation from wild caught mosquitoes during a JE outbreak in Kerala in 1996. *Indian J. Med. Res.*, **106:** 4–6.

14. Rao, T. Ramachandra (1984). *The Anophelines of India*. Rev. edn. (Malaria Research Centre, ICMR, Delhi).

15. Ahmed, F.U. (1992). Epidemiology of Japanese encephalitis in Assam. *Proceedings of the Workshop on Japanese encephalitis Diagnosis and Management* (Assam Medical College, Dibrugarh): 7–12.

Indian Journal of Malariology
Vol. 37, September–December 2000, pp. 103–105.

Control of *Aedes* Breeding using Bactoculicide and Neem Oil Combination in Evaporation Coolers

R.K. SINGH[a] and S.P. SINGH[a]

Keywords: Bactoculicide, Dengue, Mosquito, Neem oil, Vector control

Dengue has become a serious problem in our country. Forty-two outbreaks of dengue till date in different parts of the country have been reported.[1-4] *Aedes aegypti*, the well-known vector of dengue fever in urban areas breeds in domestic water containers, water storage tanks, earthen pots, desert coolers, used/unused wells, curing tanks, old tyre dumps, discarded drums, over head water tanks, tree holes and the rest.[5-8] For the control of *Aedes* breeding in evaporation coolers, a trial of neem oil[9] and bactoculicide[10] have been carried out so as to get instant mortality by bactoculicide and continued prolonged mortality by neem oil. In this study, a simple method using balls made of fine wheat flour, neem oil (*Azadirachta indica*) and bactoculicide (*Bacillus thuringiensis* var *israelensis* H-14, *Bti*) was used.

Forty ml of neem oil (Unjha Pharmacy, Jhansi) and 20 g of bactoculicide with 5–8 per cent endotoxin (Biotech International Ltd., Delhi) were mixed with two kg fine wheat flour along with 1060 ml distilled water. From this formulation, 40 balls of equal weight were made, each ball containing 50 g weight, 0.5 g *Bti* and one ml neem oil. The prepared balls were kept for complete drying in shade for about a week. These balls were introduced in evaporation coolers with heavy breeding of *Aedes aegypti*. Results of the impact as well as residual effect of formulation balls on mosquito control are reported in this paper.

R.K. Puram, Sector 1, west block in South Delhi was selected for the field trials. This colony has 250 evaporation coolers. A preliminary survey

[a]Malaria Research Centre (ICMR), 2-Nanak Enclave, Delhi–110 09, India.

of mosquito breeding in evaporation coolers was carried out from 12–26 July 1994 and the results showed that out of 250 coolers surveyed, mosquito breeding was found in 200 coolers.

Forty evaporation coolers with equal dimension (70 x 70 x 88 cm) and (15 cm) depth of water were selected for this study, of which 20 were treated with one ball formulation (containing neem oil and *Bti*) and 20 were held as untreated control. Before the introduction of balls, the average larval density of *Aedes aegypti* was measured in each of the experimental and control coolers by taking five dips using a dipper of 500 ml capacity.[9] In each dip, number of larvae and pupae were counted for the estimation of average density per dip. The impact of balls on the larval density was observed and recorded. Observations were made from 29 July to 15 September 1994 regularly, at an interval of two to five days. A proper care was taken not to cause mortality of larvae during handling. All the larvae found in dips were reintroduced into the same cooler after counting and the data were recorded. The per cent reduction in the density of larvae was calculated using Mulla's formula[10] as given below:

$$\text{Per cent reduction} = 100 - \frac{C_1}{T_1} \times \frac{T_2}{C_2} \times 100$$

Where, C_1 = No. of larvae in control before treatment; C_2 = No. of larvae in control after

Table 1. Impact of balls made by fine wheat flour, neem oil and bactoculicide on the control of mosquito breeding in evaporation coolers in R.K. Puram, New Delhi

Date of survey	Day	Control		Experimental		% reduction	
		Larvae	Pupae	Larvae	Pupae	Larvae	Pupae
Pre-treatment							
29.7.94	0	19.8	2.1	19.0	2.1	0.0	0.0
Post-treatment							
1.8.94	3	17.9	1.6	4.7	0.3	72.6	81.3
4.8.94	6	18.6	0.8	2.3	0.3	87.1	62.5
8.8.94	10	15.4	0.8	1.6	0.1	89.2	87.5
11.8.94	13	11.9	0.7	1.4	0.0	87.7	100
16.8.94	18	12.9	0.6	0.6	0.0	51.5	100
22.8.94	24	8.4	0.9	0.6	0.0	92.6	100
25.8.94	28	5.0	0.3	1.0	0.0	79.2	100
30.8.94	33	7.9	0.2	1.4	0.0	81.5	100
05.9.94	39	4.8	0.2	0.9	0.0	80.4	100
08.9.94	42	2.6	0.2	0.8	0.0	67.9	100
11.9.94	46	1.0	0.1	1.1	0.0	0.0	100
15.9.94	49	1.4	0.1	1.4	0.0	0.0	100

treatment; T_1 = No. of larvae in experimental (before treatment); and T_2 = No. of larvae in experimental (after treatment).

The average density of larvae before treatment (Day 0) was found to be 19 per dip in experimental coolers and 19.8 in control. The average pupal density was 2.1 per dip in both control and experimental coolers in July 1994. The results revealed that the application of neem oil and bactoculicide balls reduced both the larval and pupal densities compared to that of in control (untreated) coolers. In coolers treated with the formulation, larval and pupal densities per dip were reduced from 19 and 2.1 to 1.4 and nil respectively up to Day 49 (Table 1). The observations showed that coolers treated with the formulation resulted in 90 per cent reduction in the larval densities from Day 4 onwards till Day 49.

In India, evaporation coolers support heavy breeding of *Aedes aegypti* mosquitoes and one such method would be sufficient to control *Aedes aegypti* breeding from June to September.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. V.P. Shrama, former Director, Malaria Research Centre for providing the facilities and to Mrs. Sarita Kumar, Dy. Director, Medical Entomology Division, NICD, Delhi, for her valuable suggestions and encouragement. The authors are also thankful to the staff Mr. Hari Om, Mr. Madan Pal, Mr. Hari Chand, Mr. Dharam Veer and Mr. Veer Pal for their help in the field.

REFERENCES

1. Ilkal, M.A., V. Dhanda, M.M. Hassan, Mangala Mavale, P.V.M. Mahadev, P.S. Shetty, S.N. Guttikar and K. Banerjee (1991). Entomological investigations during outbreaks of dengue fever in certain villages in Maharashtra state. *Indian J. Med. Res.*, **93:** 174–178.
2. Morman, G.A., Theodre and A. Joseph (1991). An insular outbreak of dengue fever in a rural south Indian village. *J. Com. Dis.*, **23:** 185–190.
3. Kabra, S.K., I.C. Verma, N.K. Arora, Y. Jain and V. Kalra (1992). Dengue haemorrhagic fever in children in Delhi. *Bull. WHO*, **70:** 105–108.
4. Uperty, H.C., P.K. Srivastava, B.N. Nagpal and V.P. Sharma (1983). Mosquito breeding survey in urban Delhi. *Indian J. Malariol.*, **20:** 79–82.
5. Chandrahas, R.K. and V.P. Sharma (1987). Small-scale field trials with polystyrene beads for the control of mosquito breeding. *Indian J. Malariol.*, **24:** 175–180.
6. Shetty, P.S. and G. Geevarghese (1977). Tree hole breeding of *Ae. aegypti* in Poona city — A short note. *Indian J. Med. Res.*, **66:** 172–174.
7. Nagpal, B.N. and Aruna Srivastava (1993). Experience in the use of expanded polystyrene beads to control mosquito breeding in over head water tanks in Delhi. *Community Participation in Malaria Control*, edited by V.P. Sharma. (Malaria Research Centre, Delhi): 243–246.
8. Nagpal, B.N., Aruna Srivastava and V.P. Sharma (1995). Control of mosquito breeding using wood scrapings treated with neem oil. *Indian J. Malariol.*, **32:** 64–69.
9. Batra, C.P., P.K. Mittal and T. Adak (2000). Control of *Ae. aegypti* breeding in desert coolers and tyres using *Bacillus thuringiensis israelensis* formulation. *J. Amer. Mosq. Contr. Assoc.*, **16**(4): 321–323.
10. Mulla, M.S. (1971). Control of chironomid midges in recreational lake. *J. Econ. Entomol.*, **64:** 300–307.

Indian Journal of Malariology
Vol. 37, September–December 2000, pp. 106–110.

An Outbreak of Malaria in a Village in Faridabad District, Haryana

K.B. SINGH[a], K. ANAND[a], T. KRISHNA[a], S. KANT[a], T. RAY[a] and S.K. KAPOOR[a]

Keywords: Epidemic, Falciparum, Faridabad, Malaria

Malaria continues to be a public health problem in India, though, after the implementation of Malaria Action Programme by the Government of India in 1995, there has been declining trend in the incidence of malaria in the country.[1] But sporadic outbreaks still do occur in some parts of the country.[2-4] In Gurgaon district of Haryana, an outbreak of malaria was reported in 1996. Three community blocks namely, Nuh, Ferozepur Jhirka and Punehana of Mewat area reported a total of 16,270 cases of malaria out of which almost 50 per cent were due to *P. falciparum*.

In the months of Septemebr and October 1998, four children from Mohabbatabad village who came to Civil Hospital, Ballabgarh were found positive for *Pf* on peripheral blood smear examination. Civil Hospital, Ballabgarh is a part of the Comprehensive Rural Health Services Project being run by the All India Institute of Medical Sciences (AIIMS), New Delhi. It is a sixty-bed hospital which draws patients from Ballabgarh and Palwal blocks of District Faridabad. The information was sent to the PHC Dhauj under which this village falls, so that the necessary control measures could be taken. Based on this information on *Pf* cases from a single village, an investigation was done in the village Mohabbatabad in Faridabad district of Haryana where an outbreak of malaria occurred in 1998.

Mohabbatabad village is situated at the foothill of the Aravali ranges about 15 km away from the hospital and the accessibility to this area is quite difficult, as there is no adequate transport facilities. Three faculty members along with four health workers and one laboratory technician visited the village for the initial survey on 28 November. All fever cases were examined and blood smears were prepared. As there were many cases of fever in the community which could not be seen on that day, it was decided to

[a]Comprehensive Rural Health Service Project (All India Institute of Medical Sciences), Ballabhgarh–121 004, India.

visit a week later. An ecological investigation into the probable causes of the epidemic was also done.

In next visit on 5 December 1998, three teams equipped with necessary equipment and anti-malarials for presumptive treatment reached the village. Each team comprised of a doctor, a laboratory technician and a health worker. Three places in different parts of the village were selected for easy accessibility from any part of the village. All patients who had fever during the fifteen days prior to the visit were clinically examined. Peripheral smears were taken by finger-prick method from all the symptomatic cases and presumptive treatment was given. The smears were taken to the laboratory at Civil Hospital, Ballabgarh, where they were stained by JSB and examined as per the NAMP guidelines.

Weekly follow-up visits were made to the village for radical treatment of cases diagnosed at the last visit and searching for fresh cases. This active surveillance continued till the outbreak was controlled as defined by nil or one cases at the time of visit. All patients found to be positive for malaria were followed-up by weekly smears. Chloroquine (10 mg/kg body wt) was given as presumptive treatment on the first contact. On the second contact chloroquine (15 mg/kg/ body wt) was given to be taken over two days. Primaquine was given in doses recommended under the programme for all positive cases.

On the first preliminary visit on 28 November 1998, a total of 79 smears were made out of which, 28 slides were positive for malaria with a slide (falciparum) positivity rate of 35.4 per cent. Following this, a more extensive investigation was done. The population of the village was estimated to be about 3200 based on approximate sizes of each mohalla, the number of voters in the village. The number of smears made on each visit and the results are shown in Table 1.

Table 1. Results of malaria outbreak investigation in village Mohabbatabad

Date	No. of smears examined	*Pf*	*Pv*	Total	SPR	SfR
28.11.98	79	28	0	28	35.4	35.4
05.12.98	275	13	16	29	10.5	5.8
12.12.98	7	3	0	3	42.8	42.8
19.12.98	14	1	1	2	14.2	7.1
26.12.98	5	0	0	0	0.0	0.0
02.01.99	5	2	0	2	40.0	40.0
09.01.99	2	2	0	2	100	100
16.01.99	5	1	0	1	20.0	20.0
Total	392	50	17	67	17.1	12.7

On the second visit, 275 slides were made from new fever cases and 29 new cases of malaria were detected. On subsequent weekly visits the number of fever cases and malaria cases declined significantly. The investigation was called off on 16 January 1999 after two months of active surveillance as only sporadic cases were being detected.

During the second visit, the concerned health workers from PHC Dhauj were present. They had been unaware of the sudden increase in fever cases. They had come to know only through our information which was sent to their PHC . The village Sarpanch (village head) or other villagers did not take the initiative to inform either the health workers posted for their village or the Medical Officer at the PHC.

Thus, during the investigation a total of 392 slides were made in the village and a total of 67 cases of malaria were detected giving 20.9 cases per 1000 population. There were 50 cases of *Pf* and 17 cases of *Pv* malaria. There was no difference in the slide positivity rate among the two genders (Table 2).

Heavy rainfall during the monsoon season (October to November) has resulted in stagnation of water in the village as the village has poor drainage system. This facilitated mosquito breeding which might have contributed in malaria transmission.

The very high incidence rate of malaria in the village during the study period clearly demonstrated that there was an epidemic of malaria during this period. There was a preponderance of *Pf* cases. However, all cases responded to chloroquine. It was also seen that a good proportion of cases had only gametocytes in the blood. This increases the risk of malaria transmission. Early detection and prompt treatment with chloroquine and primaquine resulted in break in further transmission of malaria and duration of the epidemic.

Table 2. Age and gender distribution of malaria cases

Parameter	No. examined	(+)ve	p-value
Age group (yr)			
<1	2	0 (0)	
1–4	35	5 (14.3)	
5–14	212	39 (18.4)	<0.000
≥15	143	23 (16.0)	
Sex			
Male	238	42 (17.6)	0.80
Female	154	25 (16.2)	

Figures in parentheses indicate percentage.

The epidemic occurred in a village which is adjacent to the region which reported a major epidemic in 1996. However, despite that we found that neither the surveillance system was working adequately nor the villagers were aware of the problem. Thus, this epidemic raises the issues related to the role of various agencies in malaria control. The three important elements in any disease surviellance are community mem-

bers, primary level health workers and hospitals.

Community has to own up its responsibility for its own health. For its own benefit, it has to adopt community-based approaches for malaria control like preventing water collection, informing higher authorities in case of outbreaks. In addition, every individual should adopt personal protective measures and seek appropriate treatment in case of fever. However, in practice for various socio-culture reasons, community members fail to take up the onus upon themselves. This was clearly demonstrated in Mohabbatabad, where despite such high morbidity, nothing was done for improving the drainage system. Despite informing repeatedly, the villagers linked malaria to dirty drinking water and not to collect water at different parts of the village. This also switched the responsibility for the outbreak from them to the government which is responsible for providing drinking water.

If the active malaria surveillance is carried out by the field workers as envisaged under the programme, then the workers should be able to recognise an epidemic at an early stage, thus avoiding morbidity and mortality . As per the malaria action programme, if an epidemic with *P. falciparum* predominance is seen with deaths of microscopically confirmed cases, then it indicates that (i) both rounds of inseciticidal spray was either not given or coverage was extremely poor or (ii) case detection and drug distribution were not done for at least four to five months.[5] In District Faridabad, only focal insecticidal spraying is being carried out. This is restricted to the villages where a case of malaria has been reported. The failure of active surveillance, however, needs investigation. The data from PHC Dhauj show that the active surveillance was above one per cent during the months of July to October. This dropped to 0.3 per cent in November and December during the study year. As per reports, 21 cases of *Pf* and no case of *P. vivax* malaria was reported for the last six months of 1998 in the whole PHC of Dhauj.

Hospital-based passive surveillance is the next higher level where a vigilant system can pick up an outbreak early. This could either be a primary or secondary level hospital. The outbreak was not identified at the concerned PHC as the villagers do not go to the PHC, mainly on account of inaccessibility. The secondary level hospital at Ballabgarh is in a town where people visit for work or for their marketing . This opportunity is used for availing medical services as well. Hospital-based surveillance is a less effective system as the cases may distribute themselves over different private and government hospitals. This may result in non-identification or late identification of an epidemic, as in reality there is little sharing of information between hospitals even within the government system. Also, there is need for a person with a public health background at block level who can be alerted on such occasions.

Though, this paper pertains to an outbreak of malaria, the issues raised here are relevant to outbreak of any communicable disease. De-

layed recognition of an epidemic and its control measures result in high morbidity and mortality. If future epidemics are to be prevented, then there is a need to strengthen the surveillance mechanism as well as to improve communtiy participation.

REFERENCES

1. Anon. (1999). *Annual Report 1989-99* (Minsitry of Health and Family Welfare, Government of India, New Delhi): 149–150.
2. Srivastava, H.C., R. Kant and M.R. Bhat (1995). Epidemiological observation on malaria in villages under Buhari PHC, Surat, Gujarat. *Indian J. Malariol.*, **32:** 140–152.
3. Shukla, R.P., A.C. Pandey and A. Mathur (1995). Investigation of malaria outbreak in Rajasthan. *Indian J. Malariol.*, **32:** 119–128.
4. Dev, V. (1996). Malaria survey in Tarajulie Tea Estate and adjoining hamlets in Sonitpur District, Assam. *Indian J. Malariol.*, **33:** 21–29.
5. Anon. (1995). *Operational Manual for Malaria Action Programme (MAP)* (National Malaria Eradication Programme, Directorate General of Health Services, Ministry of Health and Family Welfare, New Delhi): 102.

INDIAN JOURNAL OF MALARIOLOGY

Instructions to authors

Editorial Policy

The *Indian Journal of Malariology* is devoted to the publication of original research papers which contribute significantly to any field of malariology. Papers of routine and repetitive nature dealing with gross observations may not be included. Articles will be published at the Editor's discretion in the order accepted. The date of acceptance will be the date on which the copy is accepted in final form for publication. The authors should also submit names of three experts in the field of research on which the paper has been submitted. If there is no expert in India, experts from outside the country may be suggested. Manuscripts in triplicate along with the undertaking form duly filled by the author(s) should be submitted to:

The Managing Editor
Indian Journal of Malariology
20 Madhuvan
Delhi–110 092, India.

Classes of Items Published

In addition to full papers, the journal publishes a short note. Review articles are also invited. Book reviews may also be published at the end of the journal.

Format

The matter should be arranged in the following order: Title, Name(s) of the author(s) with address of the institute/university (as footnotes and indicated serially in superscript), Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements and References. Authors should provide keywords and a short title to be used as a running title of not more than five words.

Preparation of Copy

Manuscript should be typewritten in English on one side of the paper leaving a $1^1/_2$ inch left-hand margin. The entire matter should be typed in double space including references, tables and captions. The abstract, tables, references and legends for illustrations should be typed on separate sheets of paper. Pages are to be numbered consecutively.

Tables should be placed singly on sheets of paper, along with relevant headings and footnotes. Table width should not be more than 80 characters (including column space), and should be self-explanatory and referred to in the text. Tables should be numbered in arabic numerals (e.g. 1, 2); avoid roman numerals (e.g. I, II). Do not use any horizontal or vertical lines in the body of the table.

Footnotes to the text should be avoided as far as possible; parenthetical insertions are preferable.

Manuscript in $3^1/_2$" floppies in MS word or PageMaker is appreciated.

Illustrations should be sent in triplicate. All illustrations including figures, photographs, graphs and maps should be numbered consecutively in the order in which they appear in the text. Captions and legends should be typed separately and must not appear on the face of illustrations. Authors should identify each illustration on the reverse side with the author(s) name(s), figure number and abbreviated captions. Line drawings should be clear, and letters and numerals should be planned for legibility after reduction. Labelling should be neat and accurate. Photographs should be sharp, glossy, black and white prints, preferably mounted and covered with a transparent overlay for protection. The photographs and art work should be in size: 7.5 x 8.5 cm for quarter page; 15.5 x 8.5 cm for half a page and 15.0 x 19.0 cm for a full page.

Data for tables, graphs, etc. should be carefully verified. All statistics, percentages and other calculations should be checked thoroughly before submission of a paper. Once a paper is accepted for publication, data in it would be treated as final.

Nomenclature: Abbreviations of scientific names of insects should be omitted in the abstract and title, but should be included at the first instance in the body of the text.

Numbers less than one should have a zero set before the decimal point, e.g. 0.1.

Measurements should follow the International System (SI) of units. Kindly see WHO publication *The SI for the Health Professional*, WHO, Geneva, 1977. Use of the 24-hour time system (e.g. 0830 hrs, not 8:30 A.M.) is preferable.

References should include only published references and papers in press. References to literature cited should be numbered consecutively and placed at the end of the manuscript. In the text, they should be indicated above the line as a superscript number. As far as possible, mentioning names of author(s) under references should be avoided in the text. For references to a paper accepted for publication, the words "in press" should appear after the title of the periodical. Citations of unpublished work should be incorporated in the text itself (e.g. R.G. Roy, unpublished data; or S. Pattanayak, personal communication). If the reference is to an article published without any authorship in a periodical, in place of the author's name the word "Anonymous" (Anon.) should be used. Titles of periodicals cited in the references are to be abbreviated as in the *World List of Scientific Periodicals*.

The following style is accepted for this journal:

Research Paper

Sharma, V.P. (1976). Elimination of aziridine residues from chemosterilised mosquitoes. *Nature*, **261**: 135.

Book/Monograph

Rao, T. Ramachandra (1981). *The Anophelines of India* (WQ, Judge Press, Bangalore).

Landau, I. and Y. Boulard (1978). *Rodent Malaria*, eds. R. Killick-Kendrick and W. Peters (Academic Press Inc., London): 53–84.

Paper presented at Symposium/Conference

Subbarao, S.K. (1981). *Cytoplasmic incompatibility in mosquitoes*. Paper presented at the international symposium on recent developments in the genetics of insect disease vectors. Bellagio, Italy, 20–24 April.

Authors are requested to verify the spellings, punctuation, titles and dates of all references. The address of the publisher should be given for books. References are attributable to authors, not to editors in the case of compilations or contributory texts, e.g.

Killick-Kendrick, R. and W. Peters (1978). Ed. *Rodent Malaria.* (Academic Press Inc., London): 406. **(Incorrect)**.

Landau, I. and Y. Boulard (1978). *Rodent Malaria,* eds. R. Killick-Kendrick and W. Peters (Academic Press Inc., London): 53–84. **(Correct).**

Providing correct and complete references is the sole responsibility of the author(s).

Short notes should be prepared in a manner similar to the research papers and should contain a title, Name(s) of the author(s) with the Address of institute/university as footnotes, Acknowledgements and References.

Proofs

Page proofs of the articles will be sent to the authors for correction. Corrected proofs must be returned promptly to the editor or else the article may not be printed in the stated issue, or may be printed as it stands. Only minimal changes, i.e. those that do not substantially alter the page make-up are permissible at the proof stage and only in exceptional cases. Alterations which are disallowed by the Editor shall be deleted or charged to author.

From 1994 onwards, the reprint service has been discontinued. All senior first authors will be provided with a copy of the journal free of cost containing their paper.

Check-list

1. Manuscript to be checked as per the format of IJM.
2. Three copies of the manuscript in double space with floppy and a covering letter.
3. Short title of the research paper (max. five words)
4. Keywords.
5. Undertaking by the author(s).
6. Names of at least three experts on the subject of the paper submitted for publication.
7. Set of figures with legends and captions in triplicate on a separate sheet.

ANNOUNCEMENT

MALARIA PARASITE BANK

A Centralized National Facility for the Advancement of Research on Malaria

Malaria Research Centre, 2 Nanak Enclave, Delhi-110 009

Malaria Parasite Bank is a National resource at Malaria Research Centre. The bank is involved in the collection and maintenance of species of human, rodent, avian and simian malaria parasites. The human malaria parasite isolates are collected during epidemics and from rural, urban and tribal endemic areas from different geographical regions of India. A few of these isolates are characterized for the chloroquine sensitivity status and variations in merozoite surface proteins 1 and 2 (MSP 1 and 2). Sera/plasma collected from blood of humans and animals infected with malaria parasites are also available.

The bank is actively involved in imparting training to students and researchers on *in vitro* cultivation, preservation and handling of parasites, *in vitro* chloroquine sensitivity testing and screening of herbal products for their antimalarial properties. This centralized facility is being utilized by various **research organizations, universities** and **medical colleges** for various parasitological, pharmacological, immunobiological, biochemical and molecular biological studies.

The biological materials supplied to research organizations (except those having collaborative research projects with Malaria Parasite Bank) will be charged as per rates shown in Annexure-I. The funds generated will be utilized for expansion and maintenance of the parasite bank.

For further information please contact:

Dr. Sarala K. Subbarao
Director
Malaria Research Centre
22-Sham Nath Marg
Delhi-110 054

Tel. (O): 011-3946150, 3981690, 3981905;
(R): 011-7246177, 7141791;

Fax: 011-3946150, 7234234;

e-mail: sks@ndf.vsnl.net.in

Dr. C.R. Pillai
Assistant Director
Parasitology & Parasite Bank
Malaria Research Centre 2, Nanak Enclave
Delhi-110 009

Tel. (O): 011-7123079, 7234234;
(R): 011-7410045;

Fax: 011-7234234, 3946150;

e-mail: cr_pillai@hotmail.com

Annexure-I

Biological Materials	Rate
P. falciparum adapted and characterized for chloroquine sensitivity	Rs. 1500/1 ml vial (cryopreserved or running culture)
P. falciparum characterized for erythrocyte invasion phenotype and cytoadherence properties	Rs. 1500/1 ml vial (cryopreserved or running culture)
P. falciparum cultivated and adapted *in vitro*	Rs. 1000/1 ml vial (cryopreserved or running culture)
P. falciparum cultivated *in vitro* (Short-term cultivation/non-adapted)	Rs. 1000/1 ml vial (cryopreserved or running culture)
P. falciparum (original stock)	Rs. 1000/1 ml vial (cryopreserved)
P. vivax (original stock)	Rs. 1000/1 ml vial (cryopreserved)
P. falciparum culture supernatant (spent media)	Rs. 500/250 ml (frozen)
Serum/Plasma from *P. falciparum, P. vivax* infected blood	Rs. 250/1 ml vial (cryopreserved)
Non-human plasmodia	Rs. 500/1 ml vial (cryopreserved)
Sera/plasma from non-human Plasmodia infected animal	Rs. 100/1 ml vial (cryopreserved)

Note: The rates exclude the packing and transportation charges. The parasites will be supplied on first come first serve basis.

The cheque/draft payable at Delhi only should be drawn in favour of the **Director, Malaria Research Centre, Delhi A/C Malaria Parasite Bank.**

MALARIA RESEARCH CENTRE

PRICED PUBLICATIONS

Indian Journal of Malariology

Volume 18 Nos. 1-2 (1981) Volume 19 Nos. 1-2 (1982) Volume 20 Nos. 1-2 (1983) Volume 21 Nos. 1-2 (1984) Volume 22 Nos. 1-2 (1985) Volume 23 Nos. 1-2 (1986)	India Rs. 30.00 Foreign US$10.00
Volume 24 Nos. 1-2 (1987) Volume 25 Nos. 1-2 (1988) Volume 26 Nos. 1-4 (1989) Volume 27 Nos. 1-4 (1990) Volume 28 Nos. 1-4 (1991) Volume 29 Nos. 1-4 (1992) Volume 30 Nos. 1-4 (1993) Volume 31 Nos. 1-4 (1994) Volume 32 Nos. 1-4 (1995) Volume 33 Nos. 1-4 (1996) Volume 34 Nos. 1-4 (1997)	India Rs. 75.00 Foreign US$20.00
Volume 35 Nos. 1-4 (1998) Volume 36 Nos. 1-4 (1999) Volume 37 Nos. 1-4 (2000) Volume 38 Nos. 1-4 (2001)	India Rs. 150.00 Foreign US$40.00

The Managing Editor
Indian Journal of Malariology
Malaria Research Centre (ICMR)
20 Madhuvan
Delhi–110092, India

Sir,

I enclose herewith a bank draft No(s) ..... for $/Rs. ..... (in favour of the **"Director, Malaria Research Centre, Delhi"** towards subscription for the *Indian Journal of Malariology* for the year(s) .....(two/four Nos.) The journals should be mailed to me/my client at the following address:

.....
.....
.....
.....

INDIAN JOURNAL OF MALARIOLOGY

Editorial Acknowledgements

The editor gratefully acknowledges the help and support rendered by the following scientists who have kindly reviewed the papers for the year 2000 issues of the *Indian Journal of Malariology.*

Dr. K. Balaraman, Vector Control Research Centre, Pondicherry; **Dr. V.S. Chauhan**, International Centre for Genetic Engineering and Biotechnology, New Delhi; **Dr. Neeraj Dhingra**, National Anti Malaria Programme, Delhi; **Dr. V.K. Dua**, Malaria Research Centre, Hardwar; **Dr. C.M. Gupta**, Central Drug Research Institute, Lucknow; **Shri N.L. Kalra,** Malaria Research Centre, Delhi; **Dr. M. Kalyanasundaram,** Vector Control Research Centre, Pondicherry; **Dr. D.K. Kochar**, C-54, Sadul Ganj, Bikaner; **Dr. Ramesh Kumar**, B-601, Rishi Apartments, Alaknanda, New Delhi; **Dr. Shiv Lal,** Directorate General of Health Services, Ministry of Health and Family Welfare, New Delhi; **Dr. R.C. Mahajan**, Post Graduate Institute of Medical Education and Research, Chandigarh; **Dr. S.P. Misra,** Malaria Control and Research Project, Surat; **Dr. P.K. Mittal**, Malaria Research Centre, Delhi; **Dr. S. Pattanayak,** B-91, Swasthya Vihar, Delhi; **Dr. M.K.K. Pillai**, 37-Saakshara Apartments, Paschim Vihar, New Delhi; **Dr. P.K. Rajagopalan,** 142, Ist Seaward Road, Valmike Nagar, Chennai; **Dr. R. Reuben,** 52, Rashmi Apartments, D'Monte Road, Bandra, Mumbai; **Dr. H.N. Saiyed**, National Institute of Occupational Health, Ahmedabad; **Dr. S.K. Sharma,** National Institute of Communicable Diseases, Delhi; **Dr. V.P. Sharma,** World Health Organisation, New Delhi; **Dr. Y.D. Sharma**, All India Institute of Medical Sciences, New Delhi; **Dr. Neeru Singh**, Malaria Research Centre, Jabalpur; **Dr. R.P. Singh**, Indian Agricultural Research Institute, New Delhi; **Dr. Neena Valecha**, Malaria Research Centre, Delhi; **Dr. R.S. Yadav**, Malaria Research Centre, Nadiad.

INDIAN JOURNAL OF MALARIOLOGY

Author Index

INDIAN JOURNAL OF MALARIOLOGY

Volume 37 **Numbers 3–4**

September–December 2000

MALARIA RESEARCH CENTRE
Indian Council of Medical Research
22 Sham Nath Marg
Delhi–110054

INDIAN J. MALARIOL.

Quarterly

Year of Revival: 1981

SUBSCRIPTION RATE

Annual	India	Rs. 150.00*
	Other countries (including air mail postage)	US$40.00

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

Subscription may be made by a **demand draft** or **cheque** drawn in favour of the **"Director, Malaria Research Centre, Delhi"**, payable at Delhi and sent to the Managing Editor, *Indian Journal of Malariology*, Malaria Research Centre, 20 Madhuvan, Delhi-110 092.

The *Indian Journal of Malariology* is indexed by *BIOSIS, Drugs and Pharmaceuticals Current Indian Titles, EMBASE/Excerpta Medica, Index Medicus, Indian Science Abstracts, Review of Applied Entomology, Protozoological Abstracts, Quarterly Bibliography of Major Tropical Diseases* and it is selectively abstracted by the *Tropical Diseases Bulletin*. This journal is also accessible on the CAB Computer Database, ExtraMed CD-ROM, SourceOne UnCover and MEDLINE.

R.N. 2346/57

Published by Dr. Sarala K. Subbarao, Director, Malaria Research Centre(ICMR), 22 Sham Nath Marg, Delhi-110 054 and Printed at Chandu Press, D-97, Shakarpur, Delhi-110 092