

**INDIAN JOURNAL OF MALARIOLOGY**

**Volume 33**

**Number 3**

**September 1996**

**MALARIA RESEARCH CENTRE**

Indian Council of Medical Research

22-Sham Nath Marg

Delhi-110 054

## INDIAN J. MALARIOL.

Quarterly

© Malaria Research Centre 1996

Year of Revival: 1981

### SUBSCRIPTION RATE

Annual	India	Rs. 75.00*
	Other countries (including airmail postage)	US \$ 20.00

\*25% discount would be admissible to individual-subscribers on annual basis.

Subscription may be made by a **Demand Draft** drawn in favour of  
"Director, Malaria Research Centre, Delhi" payable at Delhi  
and sent to the *Editor, Indian Journal of Malariology,*  
*Malaria Research Centre, 20-Madhuwan, Delhi-110 092.*

---

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

# INDIAN JOURNAL OF MALARIOLOGY

*Chairperson*  
Dr. G.V. Satyavati

*Editor-in-Chief*  
Dr. V.P. Sharma

*Consultant Editors*  
Mr. N.L. Kalra  
Dr. M.K.K. Pillai

*Editor*  
Dr. Aruna Srivastava

## EDITORIAL BOARD

Dr. S.S. Agarwal  
Director  
Sanjay Gandhi Postgraduate  
Institute of Medical Sciences  
Lucknow-226 001.

Dr. V.P. Kamboj  
Director  
Central Drug Research Institute  
Lucknow-226 001.

Dr. R.C. Mahajan  
Prof. and Head  
Department of Parasitology  
Postgraduate Institute of Medical  
Education and Research  
Chandigarh-160 012.

Dr. K.N. Mehrotra  
Prof. of Eminence (Retd.)  
Department of Entomology  
Indian Agricultural Research  
Institute  
New Delhi-110 012.

Prof. Kamini Mendis  
Department of Parasitology  
Faculty of Medicine  
University of Colombo  
Colombo 8, Sri Lanka.

Dr. Ramesh Kumar  
Prof. and Head  
Department of Microbiology  
All India Institute of Medical  
Sciences  
New Delhi-110 029.

---

*Assistant Editor*  
Seema Vedantam

*Associate Editor*  
Dr. B.N. Nagpal

*DTP Operator*  
Kamini Verma

*Publication Assistant*  
Jitender Kumar

*Production*  
D.S. Sontiyal  
Arti Sharma

*Artist*  
Tarun Malhotra

*Photographer*  
Gulshan Malhotra

## INDIAN JOURNAL OF MALARIOLOGY

---

### CONTENTS

Volume 33  
Number 3  
September 1996

---

- Study on the Feasibility of Delineating Mosquitogenic Conditions in and around Delhi using Indian Remote Sensing Satellite Data 107

*V.P. Sharma, R.C. Dhiman, M.A. Ansari, B.N. Nagpal, Aruna Srivastava, P. Manavalan, S. Adiga, K. Radhakrishnan and M.G. Chandrasekhar*

- A Study of Sensitivity of *P. falciparum* to Chloroquine in a Rural Area of Bharuch District, Gujarat 126

*SEWA-Rural Research Team*

- Blood Lipid Changes in Repeated Infections of vivax Malaria 131

*K. Sumitha, K. Ravichandiran and R. Selvam*

- Preliminary Evaluation of Safety Aspects of Neem Oil in Kerosene Lamp 139

*Neena Valecha, M.A. Ansari, S. Prabhu and R.K. Razdan*

- Application of Peptide ELISA in Tribal Malaria of Madhya Pradesh 144

*Arati Roy, Sukla Biswas and Neeru Singh*

### Short Notes

- Seasonal Prevalence of Common Anophelines in Sagar Island 154

*Sagartirtha Sarkar and Mihir K. Pramanik*

A Case of *Plasmodium malariae* Infection in the Dooars Region  
of West Bengal, India

159

*S. Das, P. Malakar, G.K. Saha, B. Dasgupta and A.K. Hati*

---

*Note:* The editor assumes no responsibility for the statements and opinions expressed by the contributors.

## **Study on the Feasibility of Delineating Mosquitogenic Conditions in and around Delhi using Indian Remote Sensing Satellite Data**

V.P. SHARMA, R.C. DHIMAN<sup>a</sup>, M.A. ANSARI, B.N. NAGPAL, ARUNA SRIVASTAVA, P. MANAVALAN<sup>b</sup>, S. ADIGA<sup>b</sup>, K. RADHAKRISHNAN<sup>b</sup> and M.G. CHANDRASEKHAR<sup>b</sup>

A feasibility study to identify mosquitogenic conditions in six study sites in and around Delhi (Bhalaswa lake, Nazafgarh drain, Seelampur lake, Sanjay lake, Okhla barrage and Hindon barrage) using Indian Remote Sensing Satellites was carried out. The water bodies with marshy areas, vegetation and human settlements were considered as environmental variables responsible for mosquitogenic conditions. Multidate IRS 1A and B, LISS-II satellite data were collected and analysed on Vax 11/780 computers. False colour composite (FCC) images were generated and land cover assessed using supervised classification based on ground truth training sets. Ground truth validation of satellite data was done on satellite pass dates. Concurrent monitoring of larval and adult mosquito density was performed by selecting sub-sites in each study site. The results indicate that mosquitogenic conditions can be identified (with limitation of resolution, i.e. 36.5 m) using FCC images and these images can be used as base maps of study sites. Characterization of study sites based on land cover was done from the view point of mosquitogenic conditions. Spatial changes in mosquito density vis-a-vis changes in environmental variables revealed positive correlation with water bodies and vegetation in some study sites.

**Keywords:** Delhi, Malaria, Mosquitogenic conditions, Remote sensing

---

Malaria Research Centre, 20-Madhuvan, Delhi-110 092, India.

<sup>a</sup>Malaria Research Centre, 2-Nanak Enclave, Delhi-110 009, India.

<sup>b</sup>Regional Remote Sensing Service Centre, ISRO, Bangalore-560 070, India.

## INTRODUCTION

Although malaria has been endemic in Delhi for many centuries, recent prolific urban sprawl has created new and expanding habitats for a range of mosquito vectors. The sources of mosquito production in Delhi are the Yamuna River, choked open drains, nallahs with defective gradients, improper disposal of domestic water, over head water tanks, coolers, cisterns and agricultural fields. Immature mosquitoes breed in water and emerging adults require shelter and host blood meal for survival and propagation. These factors are variable. Prior to mosquito vector control, precise knowledge of their breeding habitats and other factors responsible for their survival and propagation is required.

Conventional ground surveys are often time consuming, labour intensive and expensive. With the advent of remote sensing technology through satellites, it has become possible to monitor land-use features on earth's surface over various time interval. In this study we evaluated the feasibility of using satellite remote sensing technology to delineate mosquitogenic conditions and to determine if the correlation between varying land-use features and mosquito density can be determined with satellite imageries only.

## MATERIALS AND METHODS

### Study area

Six study sites representing different types of ecosystems were selected in

and around Delhi. The sites were Bhalaswa lake and Nazafgarh drain (west zone, Delhi), Sanjay lake and Seelampur lake (Shahdara zone, Delhi), Okhla barrage and Hindon barrage (District Ghaziabad, Uttar Pradesh). Each study site represents a unique ecosystem encountered in urban and peri-urban situation. An area of 3 km radius (27 sq km) was selected around a major water body in each study site (Fig. 1).

### Satellite data

The data from Indian Remote Sensing Satellite IRS 1A and B (LISS-II) were used in the study. The IRS satellite has a revisit period of 22 days. Taking into account data quality and cloud cover, a maximum of six out of a possible eight data collection dates (9.7.91, 5.10.91, 27.10.91, 7.11.91, 12.1.92, 25.2.92, 20.4.92 and 1.5.92) could be used for any individual study site. The digital data was obtained in the form of computer compatible tapes (CCT) with a spatial resolution of 36.25 m and in 4 spectral bands (3 visible and one near infra-red), virtually identical to corresponding Landsat TM 1-4 bands. The data were corrected geometrically and radiometrically for georeferencing of images and for base map preparation.

The satellite data in respect of Bhalaswa lake and Nazafgarh drain were of IRS LISS-II A2 scenes of 29-47 while the remaining sites were covered by IRS LISS-II B2 scenes of 29-47. The digital image processing was carried out using VIPS-32 image processing

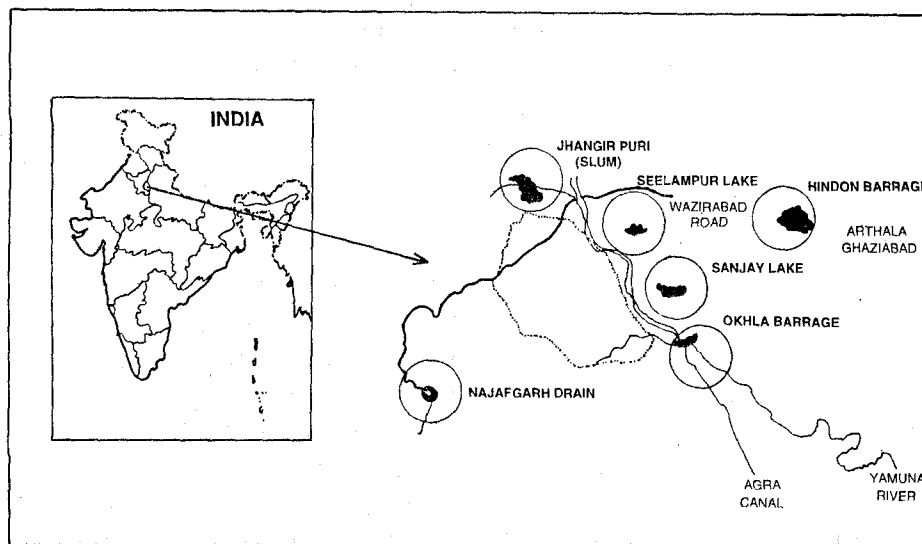


Fig. 1: Location map of the study site in Delhi.

software package installed on Vax 11/780 computer at the Regional Remote Sensing Service Centre (RRSSC), Bangalore. Initially, the satellite data were geometrically rectified with respect to SOI toposheets on 1:50,000 scale using a second order polynomial transformation model with the necessary ground control points. For georeferencing of images and for base map preparation data for other dates and study sites were then rectified with respect to aforesaid rectified scene using similar procedure. Masks for study sites were created by registering the circular boundaries defined on the toposheets with a radius equivalent to 3 km from the central point of the major water body (with a total of 27 sq km area). The masked image data for different dates corresponding to the study sites were then subjected to sun elevation angle correction so as to re-

duce the variation in spectral response of a given non-changing category due to different sun illumination conditions in different seasons.

After correction, the enhanced false colour composite (FCC) images were generated for each site. Supervised classification adopting maximum likelihood algorithm was employed. Care was taken to define homogenous and evenly distributed training samples all over the scene. Training sets of unchanging category such as forest/plantation were more or less kept at same location for different dates data for a given site to minimise the differential classification results. The other categories included water, marshy areas, weeds, barren areas, agriculture/crops and scrubs. Output maps depicting the spatial distribution of above categories in each study site as on differ-



ent dates were generated. The statistics of all land-use features were obtained so as to quantify the land-use categories. Depending upon the varying land cover categories, study sites were described on the basis of their ecological characteristics.

### **Ground data**

The study was carried out in 1991 and 1992. Ground data in respect of larval and adult mosquito density in six study sites were collected on the dates of satellite pass over Delhi. Further sub-sites around major water body were selected for each study site for sampling of larval density and collection of adult mosquitoes from human settlements. The sampling of adult mosquitoes was done from 0600 to 0900 hrs from cattlesheds and human dwellings situated within the area in each sub-site. Larval samplings were made from the major water bodies visible in satellite imagery and the surrounding area by taking dips. Ground truth data in respect of land-use features was also collected on every satellite pass.

### **Correlation of mosquito population with spatial changes in land-use/land cover variables**

The changes in mosquito (larval and adult) densities were analysed with season. Seasonal trend analysis was carried out by observing the temporal changes (increase/decrease) in adult mosquito/larval population density

with the corresponding changes in the area of environmental parameters (water/marshy/weed/vegetation cover). Correlation coefficients were determined using six pairs of variables obtained for each study site. A high correlation coefficient indicates the strong influence of the given environmental parameter on the mosquito/larval population densities during monsoon, winter and summer months. Consistency and integrity of data were checked and any abnormal values were excluded from the analysis. The change in mosquito population was analysed with the change in three main variables, namely, water, vegetation and settlements. As water bodies and marshy areas are potential breeding sites, the larval densities were correlated with them. As vegetation and human settlements support the thriving of adult mosquitoes by providing food and shelter, the adult densities were correlated with spatial changes in vegetation cover and changes in human settlement areas. However, the interactive effects of water, vegetation and settlements together may have significant effect on larval and adult density variation, were not considered.

### **RESULTS**

It is observed that all six sites chosen for the study support only two genera, namely, *Culex* and *Anopheles* for which measured densities of immatures and adults are summarized in Table 1. Density of *Culex* mosquitoes was more

**Table 1. Summary of relative density of immatures and adults at different sites**

Date	Av. larvae (No./Dip]		Total density	Av. adult (MHD)		Total density
	Culex	Anopheles		Culex	Anopheles	
<i>Bhalaswa lake</i>						
05.10.91	1.71	2.96	4.67	18.75	3.70	22.45
27.10.91	14.14	0.25	14.39	24.05	3.15	27.20
12.01.92	2.27	0.0	2.27	42.0	0.75	42.75
25.02.92	1.10	0.0	1.10	22.0	0.5	22.50
20.04.92	3.53	0.0	3.53	65.3	0.2	65.5
01.05.92	4.53	0.0	4.53	164.8	1.4	166.2
<i>Nazafgarh drain</i>						
05.10.91	10.44	0.0	10.44	21.0	0.0	21.0
27.10.91	9.37	0.17	9.54	10.4	1.4	11.8
12.01.92	7.53	1.67	9.20	43.6	0.7	44.3
25.02.92	27.0	0.0	27.0	42.0	0.0	42.0
20.04.92	34.9	0.0	34.9	38.1	0.0	38.1
01.05.92	59.81	0.0	59.81	34.0	0.1	34.1
<i>Sanjay lake</i>						
09.07.91	1.16	0.0	1.16	61.70	0.0	61.70
05.10.91	3.03	0.0	3.03	166.4	0.0	166.4
27.10.91	0.94	0.0	0.94	278.0	0.0	278.0
07.11.91	3.45	0.0	3.45	270.0	0.0	270.0
12.01.92	1.22	0.0	1.22	46.4	0.0	46.4
25.02.92	0.41	0.0	0.41	76.4	0.0	76.4
20.04.92	1.83	0.0	1.83	158.5	0.0	158.5

contd...

**Table 1. (contd.)**

Date	Av. larvae (No./Dip)		Total density	Av. adult (MHD)		Total density
	Culex	Anopheles		Culex	Anopheles	
<i>Seelampur lake</i>						
09.07.91	0.0	0.0	0.0	67.7	0.0	67.7
05.10.91	0.0	0.0	0.0	176.0	0.0	176.0
27.10.91	2.64	0.0	2.64	198.0	0.0	198.0
07.11.91	1.09	0.0	1.09	189.0	0.0	189.0
12.01.92	0.0	0.0	0.0	126.0	0.0	126.0
25.02.92	0.0	0.0	0.0	65.5	0.0	65.5
20.04.92	0.0	0.0	0.0	69.5	0.0	69.5
<i>Okhla barrage</i>						
09.07.91	22.6	8.4	31.0	11.8	4.4	16.2
05.10.91	28.0	47.8	75.8	33.6	57.6	91.2
27.10.91	313.2	28.4	341.6	251.0	27.8	278.8
07.11.91	231.6	20.0	251.6	119.4	18.6	138.0
12.01.92	19.0	35.4	54.4	30.0	45.6	75.6
25.02.92	38.0	19.8	57.8	107.6	8.8	116.4
20.04.92	165.0	10.0	175.0	309.0	5.2	314.2
<i>Hindon barrage</i>						
09.07.91	30.5	1.3	31.8	43.0	0.7	43.7
05.10.91	92.44	8.94	101.38	103.8	13.0	116.8
27.10.91	84.88	6.07	90.95	202.6	12.8	215.4
07.11.91	50.1	3.02	53.12	268.4	19.2	287.6
12.01.92	82.84	0.40	83.24	238.4	17.0	255.4
25.02.92	14.60	0.40	15.0	250.8	8.6	259.4
20.04.92	23.94	2.9	26.84	305.0	8.6	313.6

than *Anopheles* in all the study sites. The maximum density of anopheline larvae (47.8 per dip) was at Okhla barrage. The man hour density of adult anopheline mosquitoes was also highest at Hindon barrage and Okhla barrage. At Seelampur and Sanjay lakes no anopheline larvae or adults were encountered. In contrast to anopheline densities, *Culex* densities at Sanjay lake and Seelampur lake were high, although larval densities were disproportionately low in comparison to adult densities. This probably indicates that the most important breeding sites for *Culex* were too small to be represented in the present larval sampling strategy. Analyses of temporal fluctuations in mosquito densities indicate that adult mosquito densities are generally highest in winter season, and lowest in July.

The analysis of land-use/land cover details in the study sites indicate varying composition depicting unique environment (Table 2). The characterization of each study site based on land-use/land cover analysis through remote sensing has been done (Table 3). Bhalaswa lake site consists of big and small water bodies, marshy areas (15-20%), vegetation and scrub (30-40%) and human settlements (40%). The Nazafgarh drain site is characterized by the presence of a choked drain having highly polluted water full of water hyacinth. During the study period significant construction activities were taking place in the area, as well as an ongoing attempt to clean the drain.

At this site water and marshy areas account only 1-5%, while vegetation and barren/scrubs are 65-70% with 30% human settlements.

Along the Yamuna River belt on western side, agricultural fields are located. Sanjay lake is characterized by dense human settlements (27-40%). The water of lake is mainly sewage water from surrounding residential areas. Water and weeds occupy about 3-8%, while vegetation and barren/scrub 45-55% of the area. The Seelampur lake site is dominated by densely populated area. The water in lake is free from weeds and is moderately polluted. Agricultural land along with some farm ponds are seen in the southern side of the site. At this site the extent of water bodies is about 3-6%, vegetation, barren/scrub 35-40% and settlements 60%.

The Okhla barrage site is located along the Yamuna belt. The Agra canal is running across this site. A barrage constructed across the Yamuna River causes the impoundment of water resulting in formation of a large water body. There are weeds like water hyacinth, azolla and algae and they stagnate during lean river flow. This site exhibits a rural environment dominated by natural vegetation, water logged and low-lying areas. Water, weed and marshy area account for 30-35%, while vegetation, barren/scrub areas 45-50% and settlements approximately 15%. The Hindon barrage site is located along the Hindon River

**Table 2. Details of land-use categories derived from IRS LISS-II data****(a) Bhalaswa lake**

Land-use category	Areas (sq km) of land-use categories on different dates					
	05.10.91	27.10.91	12.01.92	25.02.92	20.04.92	01.05.92
Urban	5.34 (19.27)	5.02 (18.12)	6.00 (21.6)	5.54 (20.0)	4.46 (16.8)	5.41 (19.5)
Rural	5.16 (18.62)	4.48 (16.17)	3.60 (12.99)	4.32 (15.5)	7.81 (28.19)	5.47 (19.7)
Barren	3.32 (11.98)	3.68 (13.28)	4.62 (16.6)	3.69 (13.32)	2.15 (7.76)	1.79 (6.46)
Water	0.81 (2.92)	0.63 (2.29)	0.41 (1.48)	0.44 (1.58)	0.60 (2.16)	0.33 (1.19)
Marsh	4.78 (17.25)	4.47 (17.94)	4.20 (15.16)	3.73 (13.46)	4.75 (17.14)	3.64 (13.14)
Plantation and agriculture	5.79 (20.9)	6.97 (25.15)	5.47 (19.74)	6.86 (24.75)	5.46 (19.7)	8.16 (29.45)
Unclassified	2.58 (9.31)	2.50 (9.02)	3.46 (12.49)	3.18 (11.48)	2.34 (8.44)	2.79 (10.07)

**(b) Nazafgarh drain**

Land-use category	Areas (sq km) of land-use categories on different dates					
	09.07.91	05.10.91	27.10.91	12.01.92	25.02.92	01.05.92
Water	0.53 (1.89)	1.11 (3.97)	0.18 (0.64)	0.27 (0.96)	0.48 (1.72)	0.54 (1.93)
Marsh	0.83 (2.97)	0.07 (0.25)	0.58 (2.07)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Settlement	6.64 (23.79)	8.89 (31.86)	10.62 (38.06)	7.98 (28.6)	7.74 (27.74)	5.15 (18.45)
Barren	9.31 (33.36)	5.39 (19.31)	1.74 (6.23)	9.23 (33.08)	8.13 (29.13)	9.49 (34.01)

*contd...*

**Table 2. (contd.)****(b) Nazafgarh drain**

Land-use category	Areas (sq km) of land-use categories on different dates					
	09.07.91	05.10.91	27.10.91	12.01.92	25.02.92	01.05.92
Dense scrub	0.00 (0.00)	2.72 (9.74)	2.52 (9.03)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Crop/other vegetation	3.03 (10.86)	3.76 (13.47)	2.66 (9.53)	3.85 (13.79)	6.00 (21.5)	2.95 (10.57)
Plantation	6.51 (23.33)	5.56 (19.22)	8.39 (30.07)	6.32 (22.65)	4.67 (16.73)	7.84 (28.10)
Unclassified	1.03 (3.69)	0.38 (1.36)	1.21 (4.33)	0.25 (0.89)	0.87 (3.11)	1.92 (6.88)

**(c) Sanjay lake**

Land-use category	Areas (sq km) of land-use categories on different dates						
	09.07.91	05.10.91	27.10.91	7.11.91	12.01.92	25.02.92	20.04.92
Water	0.86 (3.12)	0.910 (3.29)	0.383 (1.38)	0.974 (3.52)	0.504 (1.82)	1.033 (3.73)	0.453 (1.63)
Weed	0.316 (1.14)	0.985 (7.17)	0.616 (2.22)	0.764 (2.76)	0.260 (0.94)	0.157 (0.57)	0.595 (2.15)
Settlement	11.414 (41.21)	8.778 (31.68)	10.32 (36.22)	7.571 (27.33)	10.120 (36.53)	9.788 (35.34)	11.086 (40.02)
Barren	3.646 (13.16)	3.216 (11.61)	5.625 (20.31)	7.588 (27.39)	5.671 (20.47)	8.090 (29.21)	5.635 (20.34)
Agriculture	1.514 (5.46)	4.192 (15.13)	3.389 (12.24)	4.326 (15.62)	1.881 (6.80)	2.308 (8.33)	3.326 (12.01)
Agriculture fallow	1.467 (5.30)	4.704 (16.98)	4.462 (16.11)	4.093 (14.78)	7.287 (26.31)	2.558 (9.23)	4.425 (15.98)
Plantation	5.756 (20.78)	1.618 (5.84)	1.671 (6.03)	1.616 (5.83)	1.048 (3.78)	1.922 (6.94)	1.994 (7.19)

contd...

**Table 2. (contd.)****(d) Seelampur lake**

Land-use category	Areas (sq km) of land-use categories on different dates						
	09.07.91	05.10.91	27.10.91	7.11.91	12.01.92	25.02.92	24.04.92
Water	0.74 (2.64)	1.35 (4.82)	1.58 (5.64)	0.9 (3.21)	1.12 (4.13)	1.8 (6.42)	1.16 (4.14)
Settlement	18.07 (64.50)	17.40 (62.14)	17.20 (61.40)	19.30 (68.92)	14.20 (52.70)	16.60 (59.20)	17.70 (63.20)
Barren	6.85 (24.46)	7.20 (25.71)	6.22 (22.21)	5.60 (20.25)	6.93 (25.50)	5.69 (20.31)	6.33 (22.52)
Plantation	0.0 0.0	0.0 0.0	0.83 (2.96)	0.32 (1.14)	3.4 (12.54)	0.89 (3.17)	1.26 (4.50)
Agriculture	2.37 (8.46)	2.01 (7.17)	2.17 (3.57)	1.74 (6.21)	1.4 (5.16)	2.91 (10.3)	1.57 (5.60)

**(e) Okhla barrage**

Land-use category	Areas (sq km) of land-use categories on different dates						
	09.07.91	05.10.91	27.10.91	7.11.91	12.01.92	25.02.92	20.04.92
Water	4.47 (16.1)	6.56 (23.64)	2.92 (10.51)	3.29 (11.85)	2.37 (8.56)	2.15 (7.75)	2.63 (9.48)
Marsh	1.12 (4.05)	1.22 (4.40)	2.11 (7.62)	5.81 (20.94)	3.5 (12.22)	3.39 (12.22)	1.3 (4.68)
Settlement	4.40 (16.18)	9.24 (33.2)	8.25 (29.7)	6.22 (22.42)	5.17 (18.6)	4.11 (14.82)	3.48 (12.53)
Barren	2.40 (8.66)	1.32 (4.78)	0.88 (3.17)	1.65 (5.94)	5.30 (19.1)	5.64 (20.32)	4.19 (15.11)
Plantation	3.97 (14.32)	2.32 (8.36)	3.3 (11.89)	1.28 (4.64)	3.18 (11.47)	2.28 (8.2)	3.06 (11.02)
Agriculture	5.6 (20.29)	4.55 (16.3)	5.21 (18.7)	6.17 (22.2)	6.17 (22.23)	4.67 (16.8)	9.29 (33.47)

contd...

**Table 2. (contd.)****(e) Okhla barrage**

Land-use category	Areas (sq km) of land-use categories on different dates						
	09.07.91	05.10.91	27.10.91	7.11.91	12.01.92	25.02.92	20.04.92
Weeds	3.8 (13.77)	1.7 (6.36)	3.5 (12.6)	1.58 (5.71)	0.88 (3.17)	3.19 (1.49)	2.73 (9.84)
Unclassified	1.8 (6.62)	0.75 (2.47)	1.55 (5.6)	1.73 (6.26)	1.08 (3.89)	2.3 (8.29)	1.11 (4.03)

**(f) Hindon barrage**

Land-use category	Areas (sq km) of land-use categories on different dates						
	09.07.91	05.10.91	27.10.91	7.11.91	12.01.92	25.02.92	20.04.92
Water	0.52 (1.86)	0.86 (3.05)	1.88 (3.84)	1.12 (3.97)	1.2 (4.26)	0.92 (2.24)	0.74 (2.63)
Marsh	2.64 (9.30)	4.43 (15.69)	2.93 (10.40)	0.2 (0.73)	1.08 (3.83)	1.75 (6.22)	1.01 (3.60)
Settlement	8.96 (31.75)	7.94 (28.15)	10.93 (38.71)	9.36 (33.16)	12.01 (42.5)	9.84 (34.89)	9.88 (35.01)
Barren	6.86 (24.3)	3.47 (12.29)	2.83 (10.04)	6.44 (22.94)	0.74 (02.65)	3.00 (10.68)	10.27 (36.4)
Plantation	2.87 (10.16)	2.09 (7.43)	4.26 (15.01)	5.48 (19.42)	4.84 (17.17)	2.16 (7.65)	1.48 (5.21)
Agriculture	3.72 (13.19)	8.24 (29.21)	5.71 (20.19)	4.92 (17.44)	7.6 (26.92)	9.02 (31.96)	3.93 (13.92)
Weeds	0.0 0.0	0.0 0.0	0.01 (0.038)	0.1 (0.4)	0.28 (1.02)	0.86 (3.07)	0.2 (0.94)
Unclassified	2.63 (9.03)	1.17 (4.15)	0.4 (1.64)	0.53 (1.84)	0.41 (1.46)	0.63 (2.25)	0.61 (2.16)

Figures in parentheses indicate percentage of total area.



Table 3. Characterization of study sites with regard to land cover and mosquito-genic conditions

Site	Land cover	Nature and condition	Mosquito-genic condition (Av. density)				
			Larvae		Adult		Total
			Culex	Anopheles	Total Culex	Anopheles	
Bhalaswa lake	Water, weed and marsh (15-20%)	Bhalaswa lake less polluted, other water bodies are marshy and full of weeds	4.55	0.55	5.09	1.62	57.77
	Vegetation and barren/scrub (30-40%)	Agriculture and barren					
	Settlement (40%)	Rural and sparse					
Nazafgarh drain	Water, weed and marsh (1-5%)	Drain highly polluted					
	Vegetation and barren/scrub (65-70%)	Agriculture and plantation	24.84	0.31	25.15	0.37	31.89
	Settlement (30%)	Rural and sparse					
Sanjay lake	Water, weed and marsh (3-8%)	Moderately polluted	-	-	1.72	-	151.06
	Vegetation and barren/scrub (45-55%)	Open, barren areas dominant					
	Settlement (27-40%)	Dense					

contd...

Table 3. (contd.)

Site	Land cover	Nature and condition	Mosquitogenic condition (Av. density)					
			Larvae			Adult		
			Culex	Anopheles	Total	Culex	Anopheles	
Seelampur lake	Water, weed and marsh (36%)	Moderately polluted, less weed, less marshy areas	-	-	0.53	-	-	127.39
	Vegetation and barren/scrub (35-40%)	More barren areas						
	Settlement (60%)	Dense						
	Water, weed and marsh (30-35%)	Moderately polluted lowlying areas with water logging	118.23	24.26	142.49	123.20	24.0	1147.20
Okhla barrage	Vegetation and barren/scrub (45-50%)	Agriculture fields						
	Settlement (15%)	Rural and sparse						
	Water, weed and marsh (5-15%)	Moderately polluted, presence of weeds, marshy areas	54.12	3.30	57.42	201.71	11.41	213.11
	Vegetation and barren/scrub (50-60%)	Agriculture fields						
Hindon barrage	Settlement (35%)	Dense						

which flows only during monsoon months. The water stagnation around the barrage causes weed growth. Agricultural fields are dominating at this site and are located in the southern side. However, settlements are located in the eastern and western sides. Together, water and marsh constitute about 5-15% of the area, vegetation, barren/scrub 50-60% and settlements 35%.

### **Correlation of site characteristics with mosquito density**

Spatial extent of various land-use categories derived from IRS LISS-II data for different dates of the study are given in Table 2 (a-f). The trends of spatial changes of various land-use categories were identified for different seasons. The trend analysis was performed by grouping land-use categories with possible spectral overlaps, namely, water, weed and marshy areas; plantations, agricultural crops and other vegetation. The results were analysed for different seasons and presented in Table 4. During monsoon (July-October), there was increasing trend of water and/or marshy weed areas except for Okhla barrage site. Parallel increasing trend of larval population was observed in this season thus indicating the positive correlation. There were also positive correlation during the winter months except at Seelampur lake and Hindon barrage. However in summer with the decrease in water and/or marshy weed areas, the larval

density increased, indicating negative correlation.

During monsoon months the total area under cultivation, plantations and other vegetation increased at two sites (Bhalaswa lake and Hindon barrage), but decreased in the other four. However, with the exception of the Nazafgarh drain site, an increasing trend of adult mosquitoes was seen, resulting in positive correlations in three areas (Bhalaswa lake, Hindon barrage and Nazafgarh drain) and negative correlations in the rest. The situation in winter and summer was consistent with that described above.

### **DISCUSSION**

The overall results of this study reveal that identification of water bodies, human settlements and vegetation which contribute as mosquitogenic environment is possible with remote sensing technique. Spatial resolution of IRS LISS-II data restricts the identification/delineation of area of smaller water bodies. However, features of linear pattern like drains, canals, road network are discernible. With the help of false colour composite image, base maps of the study area can be prepared (Figs. 2 and 3). Supervised classification helped in quantification of land-use features and their correlation with mosquito density.

A study elsewhere points out that a minimum water area equivalent to

**Table 4. Land-use trend and its correlation with mosquito density trend**

Site	Category		Season		
			Monsoon (Jul-Oct)	Winter (Nov-Jan)	Summer (Mar-Jun)
Bhalaswa lake	(i) Water and marsh	(a) Trend	Increase	Decrease	Decrease
		(b) Correlation with larval density	Positive	Positive	Negative
	(ii) Plantation and agriculture	(a) Trend	Increase	Decrease	Increase
		(b) Correlation with adult density	Positive	Negative	Positive
Nazafgarh drain	(i) Water and marsh	(a) Trend	Decrease	Decrease	Decrease
		(b) Correlation with larval density	Positive	Positive	Negative
	(ii) Plantation, agriculture, other vegetation and scrub	(a) Trend	Decrease	Decrease	Increase
		(b) Correlation with adult density	Positive	Negative	Negative
Sanjay lake	(i) Water and marsh	(a) Trend	Increase	Decrease	Decrease
		(b) Correlation with larval density	Positive	Positive	Negative
	(ii) Plantation and agriculture	(a) Trend	Decrease	Decrease	Increase
		(b) Correlation with adult density	Negative	Positive	Positive
Seelampur lake	(i) Water	(a) Trend	Increase	Increase	Decrease
		(b) Correlation with larval density	Positive	Negative	Negative
	(ii) Plantation and agriculture	(a) Trend	Decrease	Increase	Decrease
		(b) Correlation with adult density	Negative	Negative	Negative

contd...

**Table 4. (contd.)**

Site	Category		Season		
			Monsoon (Jul-Oct)	Winter (Nov-Jan)	Summer (Mar-Jun)
Okhla barrage	(i) Water, marsh and weed	(a) Trend	Decrease	Decrease	Decrease
		(b) Correlation with larval density	Negative	Positive	Negative
	(ii) Plantation and agriculture	(a) Trend	Decrease	Increase	Increase
		(b) Correlation with adult density	Negative	Negative	Positive
Hindon barrage	(i) Water, marsh and weed	(a) Trend	Increase	Increase	Decrease
		(b) Correlation with larval density	Positive	Negative	Negative
	(ii) Plantation and agriculture	(a) Trend	Increase	Increase	Decrease
		(b) Correlation with adult density	Positive	Positive	Negative

## BHALASWA LAKE

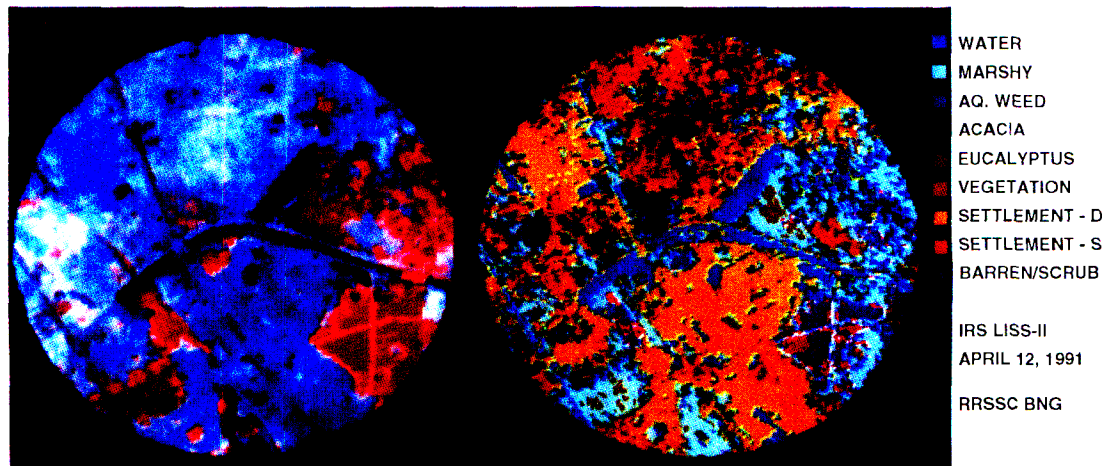


Fig. 2: Multispectral IRS LISS-II data (FCC and classified images) of Bhalaswa lake

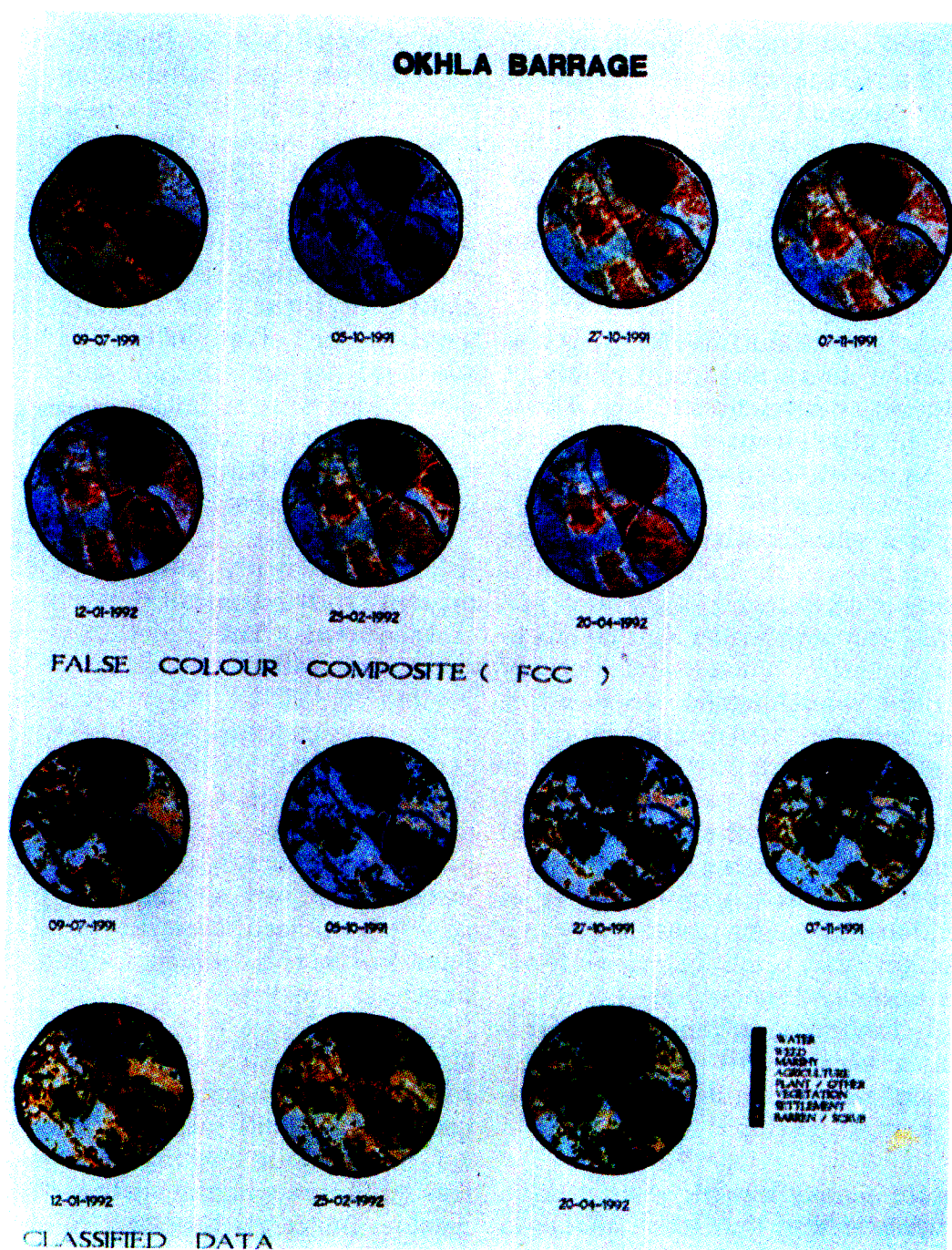


Fig. 3: Multidate IRS LISS-II data (FCC and classified images) of Okhla barrage

4 pixels is needed for definite identification and a minimum equivalent to 9 pixels for reliable estimation of aerial spread. As such the smaller water bodies such as ponds, pools, water stagnations even in hoof marks of animals, the potential breeding habitats of *Anopheles* mosquitoes, can not be identified remotely.

Quantification of land-use features has been found as an important ability of satellite data derived information which helped in characterization of ecosystems. As the atmospheric correction of satellite data was not carried out, multi-spectral classification of multirate data might result in non-identical mapping of unchanged categories. For example, the area under settlement is not expected to change considerably within one year duration. However, it is observed that due to spectral overlap between barren and settlement classes (the extent of overlap influenced by different atmospheric conditions) unequal settlement area estimates were obtained. Similarly, varying degree of spectral mixing is also possible between plantation and other crops and between water and marshy areas. Keeping this limitation in mind, the correlation analysis was performed by grouping land-use categories with possible spectral overlaps.

Mosquito density was highest at Okhla site, while it was lowest at Bhalaswa lake and Nazafgarh drain. It can be

explained due to presence of maximum area of water bodies, highest vegetation cover and least urbanization. *Anopheles* mosquitoes are known to prefer fresh water, while *Culex* breeds in polluted water. Thus, the availability of fresh water bodies at Okhla and Hindon barrage sites is likely to explain why *Anopheles* densities were highest at these sites. On the other hand, the lack of anophelines at Sanjay lake and Seelampur lake can be explained due to dense urbanization, low vegetation cover and highly polluted water bodies leading to the production of only *Culex* mosquitoes.

All the six study sites selected in present study represent different types of ecosystems. The correlation of water bodies with larval density is not positive for all the study sites. Similarly, the vegetation also had variable correlation with adult mosquito density. It indicates that environmental factors such as type and density of human settlements, distance from the water bodies and specification of vegetation and local weather conditions have interactive influencing effect on mosquito densities.

Mosquito densities are known to peak in the post-monsoon season, when the quality of available satellite data is poorest, due to cloud coverage. Because of this limitation valuable information regarding mosquito breeding cannot be gathered remotely during the monsoon

period, and monthly seasonal fluctuations in land-use features cannot be derived with reliability.

Species level vegetation classification can further provide specific information on the association with particular mosquito species<sup>1</sup>. It can be concluded that using multitemporal IRS LISS-II data, mosquito-generating potential of an area arising due to specific environmental composition of water, weed, vegetation, settlement etc. can be assessed for macro-stratification and for prioritising the areas for mosquito control programmes. Remote sensing technology is rapid and accurate at macro-level for identification of different larval habitats of mosquitoes<sup>1-3</sup> and enables identification of villages with high risk malaria transmission<sup>4</sup>. In addition to mosquitoes, Remote sensing technology has proved useful as a tool for surveillance of various arthropod vectors<sup>5</sup>.

In future, Geographic Information Systems and the availability of more frequent and higher resolution of IRS 1C data may further improve our understanding of the epidemiology of vectors and vector-borne diseases.

#### ACKNOWLEDGEMENTS

The authors express their sincere thanks to the staff of Malaria Research Centre, Delhi and Regional Remote Sensing Service Centre, Bangalore for

help in extensive field work and analysis of data. Thanks are also due to Dr. M. Bouma, London School of Hygiene and Tropical Medicine, London for critically evaluating the manuscript and offering valuable suggestions.

#### REFERENCES

1. Hayes, Richard O., Eugene L. Maxwell, Carl J. Mitchell and Thomas L. Woodruff (1985). Detection, identification and classification of mosquito larval habitats using remote sensing scanners in earth-orbiting satellites. *Bull. WHO*, **63**(2): 361-374.
2. Wagner, V.E., R. Hill-Rowley, S.A. Narlock, and H.D. Newson (1979). Remote sensing: A rapid and accurate method of data acquisition for a newly formed mosquito control district. *Mosq. News*, **39**: 283-287.
3. Wood, B.L., L.R. Beck, R.K. Washino, K. Hibbard and J.S. Salute (1992). Estimating high mosquito producing rice-fields using spectral and spatial data. *Internatl. J. Remote Sensing*, **13**: 2813-2826.
4. Beck, Louisa R., Mario H. Rodriguez, Sheri R. Dister, America D. Rodriguez, Eliska Reimankova, Armando Ullova, Rosa A. Meza, Donald R. Roberts, Jack F. Paris, Michael A. Spanner, Robert K. Washino, Carl Hacker and Llewellyn J. Legters, (1994). Remote sensing as a landscape epidemiologic tool to identify villages at high risk for malaria transmission. *American J. Trop. Med. Hyg.*, **51**(3): 271-280.
5. Washino, Robert K. and Byron L. Wood (1994). Application of remote sensing to arthropod vector surveillance and control. *American J. Trop. Med. Hyg.*, **50**(6) (Suppl): 134-144.



## **A Study of Sensitivity of *P. falciparum* to Chloroquine in a Rural Area of Bharuch District, Gujarat**

SEWA-RURAL RESEARCH TEAM\*

A study was undertaken to determine the level of chloroquine sensitivity in *P. falciparum* strains prevalent in a rural, tribal-belt of Bharuch district, Gujarat during 1992-93. Of the 32 cases for which the 7-day follow-up was completed, only in one RII level of resistance was noted, with the mean parasite clearance time in the remaining cases being 1.87 days. Thus, chloroquine continues to be effective in treatment on falciparum malaria in this area.

**Keywords:** Chloroquine-sensitivity, Gujarat, Malaria, *P. falciparum*

### **INTRODUCTION**

After initiating a countrywide malaria eradication campaign under NMEP, encouraging results were achieved in the control of disease. Thereafter, in the late sixties and seventies, outbreaks appeared in many parts of the country including Gujarat. Bharuch district also showed an increase in the inci-

dence, especially of *P. falciparum* infection. Resistance of *P. falciparum* to drugs has been one of the causes attributed to such alarming increase in this type of infection. The paper describes a study that attempted to measure the level of sensitivity to chloroquine among *P. falciparum* strains in a rural, tribal area of Bharuch district.

---

\*SEWA-Rural (Society for Education, Welfare and Action-Rural) Research Team, is a voluntary organisation working for health and development in a rural tribal-belt of western India since 1980. The main contributors to the study were Dr. Ashwin Shah, Mr. A.S. Nair and Dr. Pankaj Shah, Jhagadia-393 110, India.

In India, drug resistant strains of *P. falciparum* were first reported from north eastern states<sup>1,2</sup>. The *P. falciparum* monitoring teams are observing drug resistance in falciparum malaria throughout the country. In Gujarat, the proportion of *P. falciparum* cases has increased from less than 5% in 1980 to more than 34% of total malaria cases in 1988<sup>3</sup>. Results of studies in Gujarat have shown increasing trends in resistance, both, in its level and proportion since 1984 and RI and RII resistance have been reported in some districts of Gujarat including Bharuch district<sup>3</sup>.

The study was set in the project area of SEWA-Rural, a voluntary service organisation working in Bharuch district, Gujarat. The project area constitutes a Primary Health Centre fully managed by the organisation, where all activities of national health programmes are carried out, including malaria control, through a cadre of workers similar to that of a typical PHC. A study of the incidence in Bharuch district and SEWA-Rural pro-

ject area over the last few years (Table 1) indicated that the incidence of falciparum malaria was much higher in the project area as compared to remaining areas of the district. There are two possible reasons, topographically the project area is better irrigated than other areas, and there is a sizeable number of people going to nearby industrial areas which attract a large number of migrant labourers from distant places. The likelihood of importing resistant strains is thus obvious.

Hence, a study was undertaken to determine the level of drug resistance in *P. falciparum* strain prevalent in SEWA-Rural project area and to suggest any change in the drugs used, if need be. The study was conducted over a period of 14 months during 1992-93.

#### MATERIALS AND METHODS

The study was conducted along with other routine work of the field staff of the PHC project area. Blood smears were

**Table 1. Comparison of malaria indices for the SEWA-Rural project area with the remaining rural areas of Bharuch district for 1989-91**

Indices	1989		1990		1991	
	SR	District	SR	District	SR	District
ABER	19.6	122.2	15.1	24.6	23.9	21.4
API	13.1	25.0	9.3	28.3	15.6	21.7
SPR	6.9	11.4	6.2	11.5	6.5	10.1
SfR	3.8	2.2	3.8	2.3	4.5	2.1

SR — SEWA-Rural project area.

collected by special mass survey and intensive surveillance of *P. falciparum*-predominant villages that were selected based on epidemiological situation of the past three years.

The collected blood smears were examined on the very day or the next day, after staining with Giemsa stain. Those *P. falciparum* cases that showed ring forms alone, or both ring and gametocytes, were taken up for the study, and results were conveyed to the respective field staff. A urine test for presence of chloroquine was done initially by the Dill and Glazko method<sup>4</sup>, and patients with a positive test were not included for study. A total dose of 25 mg/kg body weight, in three divided doses was administered to each selected patient as per WHO standard field test procedures (7-days test)<sup>5</sup>. All tablets of chloroquine were administered in presence of field staff. A urine test for the presence of chloroquine was repeated on Day 1 to confirm absorption of chloroquine. Follow-up blood smears in duplicate were collected on Days 1, 2, 3 and 7 and examined for parasite both asexual forms and gametocytes. Parasite count was done on blood smears collected on all the above days. The count per cu mm of blood was calculated on the basis of 8000 WBC per cu mm after counting the number of parasites and WBC in 100 oil immersion fields. Those cases which showed less than 1000 asexual parasites on Day 0 were omitted while analyzing the results.

Pregnant women, infants, seriously ill patients and non cooperative patients were omitted from the study. Duplicate blood smears were sent to the laboratory of the Regional Director, Rural Health and Family Welfare, Ahmedabad, and the results were thus cross-checked and confirmed.

## RESULTS

A total of 2416 blood smears were collected from 18 villages during the period of the study. Out of these, 86 (3.4%) malaria positive cases were detected, of which 61 (70.9%) showed asexual forms alone or along with gametocytes while 5 (5.8%) showed only gametocytes of *P. falciparum*. *P. vivax* cases were 20 (23.3%).

Out of a total of 66 *P. falciparum* cases, 61 cases showing asexual forms were taken up for the study. However, only 32 (52.5%) cases could be given full treatment and followed up to 7 days. The remaining 29 (47.5%) were not included in the study as they showed very low parasitaemia (<1000 cu mm) or were non-cooperative or were taking treatment from other sources. Out of 32 cases 9 (28.1%) showed only asexual stages and the remaining 23 (71.9%) both asexual stages and gametocytes on Day 0. Again, 9 (28.1%) showed complete clearance of asexual parasites on Day 1, a further 18 (56.3%) cases on Day 2, three (9.4%) more cases on Day 3, while 1 (3.1%) case did not show clear-

**Table 2. Day-wise progress in the clearance of parasites from the blood**

Age group (yrs)	n (32)	Day 0		Day 1		Day 2		Day 3		Day 7	
		n	MPC	n	MPC	n	MPC	n	MPC	n	MPC
0 - 15	14	14	4988	9	5032	2	507	2	131	1	677
16 - 30	14	14	4390	11	617	2	32	0		0	
> 30	4	4	4026	3	2830	1	232	0		0	

MPC — Mean parasite count; n — Number of smear positive subjects on that day.

ance of parasites till Day 4 and showed higher parasitaemia on Day 7 (Table 2). The mean parasite clearance time of 31 cases was 1.87 days. The patient showing resistance was treated with an alternative regimen.

## DISCUSSION

Out of 32 cases studied, only one case (3.12%) has shown RII resistance and 31 (96.88%) cases showed sensitivity or RI resistance. It was not possible to distinguish between the latter two, since the extended test (28 days follow-up) could not be carried out. The seven day test carried out in a neighboring district, Surat (Gujarat) among 97 cases reported, *P. falciparum* strain was found sensitive to chloroquine<sup>6</sup>. The *P. falciparum* monitoring team of Government of India carried out tests in a neighboring PHC of Bharuch district in 1987 and reported RI level of resistance to chloroquine in 48%, RII in 16% and normal sensitivity in 36%<sup>3</sup>. The result of repeat tests in same PHC carried out in 1990 showed only 7.1% RII resistance while rest of the infections were sensitive to chloroquine (personal communication).

It can be inferred that chloroquine is still effective against asexual stages of *P. falciparum* strain prevalent in this area as only 5 (19.35%) cases out of 31 cases have shown clearance time more than the mean clearance time of 1.87 days and 31 (96.88%) cases have shown sensitivity to chloroquine in the study. However, it is essential to carry out a 7 day study as well as a 28 day (extended period) study of a sufficient number of cases in the next transmission season to find out any increase in mean parasite clearance time and also presence of RI level of resistance.

Since, the study indicates that chloroquine is still effective against asexual stages of *P. falciparum* strains prevalent at the study area, no change in drug regime is required at present. However, it is essential to liquidate detected RII foci.

## ACKNOWLEDGEMENTS

The research team is thankful to the Regional Director and staff of Rural Health and Family Welfare Department, Ahmedabad, for training the laboratory

technician, cross-checking of blood smears, and helping with literature search. The team is also thankful to Technical Officer, Research and Technical Intelligence, Malaria Action Programme, WHO, for technical guidance. The team is also grateful to Desai Laboratories, Surat, the Librarians at the National Medical Library (Medlars) and to the Chief District Health Officer, Bharuch District, Bharuch for help in various stages of this study.

#### REFERENCES

1. Sehgal, P.N., M.I.D. Sharma, S.L. Sharma and S. Gogoi (1973). Resistance to chloroquine in falciparum malaria in Assam state, India. *J. Com. Dis.*, **5**: 177-180.
2. Das, S., R.G. Roy and S. Pattanayak (1979). The note of chloroquine-resistance tests on *P. falciparum* in Nagaland. *Indian J. Med. Res.*, **70** (Suppl.): 30-33.
3. Karmakar, P., S.C. Dutt, M.V.V.L. Narasimham and R.C. Sharma (1990). Status of *Plasmodium falciparum* resistance to chloroquine in Gujarat, Rajasthan and Maharashtra states of India. *Indian J. Malariol.*, **27**: 101-109.
4. Lelijveld, J. and H. Kortmann (1970). The eosin colour test of Dill and Glazko: A simple field test to detect chloroquine in urine. *Bull. WHO*, **42**: 477-479.
5. WHO (1981). Chemotherapy of malaria. Procedures for assessing the response of malaria parasites to drugs *in vivo*. 2nd Edn, WHO Monograph Ser. No. **27**: 205-210.
6. De, C.M., S.C. Dutta, B. Chowdhury, R.G. Roy and S. Pattanayak (1979). Results of chloroquine sensitivity test in *P. falciparum* in some districts of Gujarat and Maharashtra states. *Indian J. Med. Res.*, **70** (Suppl.): 23-26.

## Blood Lipid Changes in Repeated Infections of vivax Malaria

K. SUMITHA, K. RAVICHANDIRAN and R. SELVAM

Alterations in the levels of plasma and erythrocyte membrane lipids in fresh and repeated *P. vivax* malarial patients were studied. A significant fall in plasma cholesterol and phospholipids was observed in repeated malaria. The decrease was highly significant when the number of attacks were more than five ( $p < 0.0001$ ). A significant increase in plasma triglycerides and non-esterified fatty acids were observed when the number of attacks was between 4-5 ( $p < 0.0001$ ). Erythrocyte membrane cholesterol and phospholipids were increased in repeated malaria. The increase in erythrocyte membrane cholesterol and phospholipids, was significant in more than five attacks ( $p < 0.0001$ ). The activities of LCAT and LPL were decreased significantly in repeated malaria, when the number of attacks were between 4-5 ( $p < 0.0001$ ). It is suggested that repeated malarial attack alters the lipid metabolism and the changes are marked with increase in the number of malarial attacks.

**Keywords:** Lipids, *Plasmodium vivax*; Repeated malaria attacks

### INTRODUCTION

Malaria remains one the the most prevalent and devastating diseases, in spite of the various efforts taken to control it. Nearly 40% of the world's population is threatened by it<sup>1</sup>. Annu-

ally, about 280 million malaria cases are reported with 1-2 million deaths<sup>2</sup>. India's public health system is being undermined by malarial infection<sup>3</sup> and in Tamil Nadu, the number of malaria cases reported is steadily increasing, from 1988 onwards<sup>4</sup>.

It is well-known that vivax malaria causes serious problems because of its frequently recurring nature, either due to reinfection or due to the dormant forms of the parasite in the host. Studies on the lipids in vivax malaria have revealed a decrease in serum cholesterol and total lipids<sup>5</sup>. Cholesterol rises during chill and decreases during afebrile periods<sup>6</sup>. Studies on the lipid levels of vivax malaria in relation to parasitaemia and chemotherapy are extensive<sup>5,6</sup>, but, no detailed investigation has been undertaken to study the effect of repeated vivax malarial infections on the lipid status of the individual. This investigation thus presents the lipid status in fresh and repeated infections of *P. vivax* malaria.

#### MATERIALS AND METHODS

Blood samples were collected and examined for malarial parasites from patients (13-38 yrs) attending the Central Malaria Clinic of the Corporation of Madras (Division 55), at Elephant Gate. Informations on the patient's repeated malarial attacks within a year and symptoms of present attack were obtained by oral admission of the patients as well as from the Corporation's Malaria Clinic. At the time of blood sampling, only patients with vivax malaria were considered for this study. The malarial patients reported general symptoms of malaria, like sudden onset of fever, usually in the late afternoons or at nights. Profuse sweating periods were also reported. Headache and general body pain was noticed in

all cases with nausea and vomiting in most. They also reported irritation with watering of eyes. Venous blood from these patients was collected in the morning before breakfast, in tubes containing anticoagulant, EDTA, before the commencement of antimalarial treatment. Blood samples from healthy, age and sex-matched volunteers served as controls. Considering the number of malarial attacks suffered at the time of blood sampling the subjects were subdivided into the following groups:

'F' group consisting of blood samples from 15 fresh malarial patients.

'F<sub>1</sub>' group consisting of 20 patients suffering from 2-3 repeated attacks within 3 months from first attack.

'F<sub>2</sub>' group consisting of 15 patients suffering from 4-5 repeated attacks within 5-6 months.

'F<sub>3</sub>' group consisting of 15 patients suffering from more than 5 repeated attacks of malaria in a year.

'C' group consisted of 20 patients age- and sex-matched control subjects.

Plasma was separated from the blood by centrifugation at 2000 rpm for 10 min and used for analysis of lipids. Total cholesterol was estimated by the method of Parekh and Jung<sup>7</sup>, triglycerides by the method of Rice<sup>8</sup>, phospholipids by Rouser *et al.*<sup>9</sup> and NEFA by the method of Hron and Menahan<sup>10</sup>. Plasma LCAT and LPL were assayed

by the methods of Hilz *et al.*<sup>11</sup> and Schmidt<sup>12</sup> respectively.

**RBC membrane isolation:** Erythrocyte membrane lipids were extracted by the method of Folch *et al.*<sup>13</sup>, with a slight modification. Plasma was separated from whole blood and the erythrocyte membrane pellet was washed 3-4 times in 0.89% saline to remove the buffy coat. Then, one part of RBC membranes was added to one part of methanol and kept aside for one hour. Then, two parts of chloroform was added and the lipids were extracted overnight. The lipid extract was washed with 0.4 ml of 0.1 M/L potassium chloride, after centrifugation. The chloroform layer was filtered and dried. Total lipids were estimated on dry weight basis. The dried lipid extract was made upto a known volume in chloroform : methanol mixture (2:1) and lipid constituents were estimated.

**Statistical analysis:** The data was analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keul's multiple comparison test. Differences between groups were considered significant at  $p < 0.05$ .

## RESULTS

The changes observed in plasma lipids in C, F, F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> groups are given in Table 1. Plasma cholesterol and phospholipid levels were significantly decreased in patients with primary and repeated malarial attack ( $p < 0.0001$ ).

The decrease was significant, when repeated attacks were compared with fresh malaria ( $p < 0.05$ ). The C/P ratio was found to decrease with increase in the number of attacks.

Plasma triglyceride level was found to increase during malaria ( $p < 0.0001$ ). The increase was significant when the numbers of attacks were increased ( $p < 0.05$ ). The C/T ratio was found to decrease with increase in number of attacks.

The NEFA level was found to increase significantly in malarial infection ( $p < 0.0001$ ), but, this level decreased in repeated malaria, even though the level was higher than that of controls ( $p < 0.05$ ).

Table 2, shows the activities of LCAT and LPL, the lipid metabolizing enzymes in the plasma of malarial patients and healthy controls. Activity of LCAT enzyme in plasma was found to decrease in malarial attacks ( $p < 0.0001$ ). Activity of LPL enzyme in plasma also decreased during malaria ( $p < 0.0036$ ). The decrease in activities of LCAT and LPL in plasma was also significant as the number of malarial attacks increased ( $p < 0.05$ ).

The changes observed in erythrocyte membrane cholesterol and phospholipids, of malaria patients and controls are given in Table 3. Membrane cholesterol and phospholipid levels increased during malarial infection



Table 1. Plasma lipids in control and *P. vivax*-infected malarial patients

Parameter	Control (C) (n = 20)	Fresh malaria (F) (n = 15)	Repeated malaria		F-ratio
			F <sub>1</sub> (n = 20)	F <sub>2</sub> (n = 15)	
Total cholesterol (mg/dl of plasma)	235.68±26.31	211.31±19.49 <sup>a</sup>	154.89±7.83 <sup>ab</sup>	120.18±8.44 <sup>abc</sup>	109.67±9.13 <sup>abc</sup> p<0.0001
Phospholipids (mg/dl of plasma)	218.77±15.77	191.75±23.87 <sup>a</sup>	136.76±21.78 <sup>ab</sup>	121.17±11.53 <sup>abc</sup>	117.41±11.26 <sup>abc</sup> p<0.0001
Triglycerides (mg/dl of plasma)	126.33±13.86	138.39±15.40 <sup>a</sup>	147.76±16.23 <sup>a</sup>	223.99±19.6 <sup>abc</sup>	219.13±15.86 <sup>abc</sup> p<0.0001
Free fatty acids (mg/dl of plasma)	6.50±1.27	14.98±2.55 <sup>a</sup>	13.58±2.07 <sup>a</sup>	12.14±3.20 <sup>ab</sup>	10.09±3.23 <sup>abcd</sup> p<0.0001
C/P ratio	1.08	1.10	1.13	0.99	0.93
C/T ratio	1.87	1.53	1.05	0.54	0.50

Values are expressed as mean ± standard deviation; Pairs of groups significantly different at p<0.05 level, by Newman-Keul test, are indicated by: a — Compared to control (C), b — Compared to fresh malaria (F), c — Compared to repeated malaria (F<sub>1</sub>), d — Compared to repeated malaria (F<sub>2</sub>).

**Table 2. Activities of LCAT and LPL in the plasma of control and *P. vivax*-infected malarial patients**

Parameter	Control (C)	Fresh malaria (F)	Repeated malaria			F-ratio
			F <sub>1</sub>	F <sub>2</sub>	F <sub>3</sub>	
LCAT (nmol CE/ml plasma/h)	44.51±7.37 (n=20)	38.35±7.74 <sup>a</sup> (n=15)	32.02±5.41 <sup>ab</sup> (n=20)	25.16±7.55 <sup>abc</sup> (n=15)	27.64±4.63 <sup>abc</sup> (n=15)	p<0.0001
LPL (µmol ffa/ml plasma/h)	7.06±0.52 (n=10)	6.24±0.61 (n=8)	6.12±0.69 (n=12)	5.47±0.62 <sup>abc</sup> (n=8)	5.25±0.34 <sup>abc</sup> (n=6)	p<0.0036

Values are expressed as mean ± standard deviation; Pairs of groups significantly different at p<0.05 level, by Newman-Keul test, are indicated by: a — Compared to control (C), b — Compared to fresh malaria (F), c — Compared to repeated malaria (F<sub>1</sub>).

**Table 3. Erythrocyte membrane cholesterol and phospholipid content of control and *P. vivax*-infected malarial patients**

Parameter	Control (C) (n = 20)	Fresh malaria (n = 15)	Repeated malaria			F-ratio
			F <sub>1</sub> (n = 20)	F <sub>2</sub> (n = 15)	F <sub>3</sub> (n = 15)	
Cholesterol (mg/ml of PCV)	1.45±0.27	1.85±0.36 <sup>a</sup>	2.03±0.30 <sup>a</sup>	1.80±0.29 <sup>a</sup>	1.78±0.39 <sup>a</sup>	p<0.0001
Phospholipids (mg/ml of PCV)	1.92±0.59	2.45±0.51 <sup>a</sup>	2.95±0.56 <sup>ab</sup>	3.25±0.54 <sup>ab</sup>	3.26±0.49 <sup>ab</sup>	p<0.0001
C/P ratio	0.76	0.76	0.69	0.55	0.55	

Values are expressed as mean ± standard deviation; Pairs of groups significantly different at p<0.05 level, by Newman-Keul test, are indicated by: a — Compared to control (C), b — Compared to fresh malaria (F).

( $p < 0.0001$ ). The increase in membrane lipids was significant when fresh cases were compared with repeated attacks ( $p < 0.05$ ). The C/P ratio decreased with increase in number of attacks.

## DISCUSSION

Haematologic indices were observed to decrease in the fresh and repeated malaria attack patients, in this study suggesting anaemia. A variety of hematologic abnormalities have been reported in malaria<sup>14</sup>. Malaria is accompanied by thrombocytopenia, granulocytopenia and by anaemia, the degree of which is influenced by the infecting species, immune status and duration of parasitaemia<sup>14-16</sup>.

In this study, it was observed that plasma cholesterol and phospholipids decreased severely in patients with repeated malarial attacks. Though it has been shown that cholesterol level is restored to its pre-infection level by the end of four weeks<sup>17</sup>, this study infers that frequent reinfection depletes cholesterol severely. This may be due to increased utilization of cholesterol by the parasites. A similar decrease in cholesterol level has been observed in malaria, irrespective of the infecting species<sup>18,19</sup>. Malarial parasites, like the mature mammalian RBCs, are incapable of lipid biosynthesis *de novo*<sup>20</sup>, so, the parasites developing within the host erythrocytes use the membrane lipids, which are dynamically exchanged with serum lipids. The cholesterol and lipoprotein levels have been

reported to reflect parasitic activity, returning to normal on recovery<sup>21</sup>. In contrast to plasma cholesterol and phospholipids, the erythrocyte membrane cholesterol and phospholipids increases in malaria. This could be attributed to the asexual development of the parasite within the mature mammalian erythrocytes with increase in size of the plasmodia and parasitophorous vascular membranes<sup>22</sup>.

Increase in plasma triglycerides and NEFA, observed in the study, can be attributed to increased mobilization of NEFA and triglycerides from adipose tissues<sup>23</sup> due to the hyperactivity of the sympathetic nervous system, which usually occurs during infection<sup>24</sup>. An 8-fold increase in plasma FFA with concentration of unsaturated FFA being higher than that of saturated FFA has been reported<sup>25</sup>. Post-hepatic damage due to centrilobular necrosis of liver parenchymal cells in malaria<sup>26</sup>, also causes a decrease in LPL activity, leading to hyper-triglyceridemia<sup>27</sup>. Hyper-triglyceridemia becomes very much evident in repeated attacks, as noticed in the study.

Post-hepatic damage<sup>27</sup>, parasite induced endo-toxins<sup>28</sup> or increase in release of TNF<sup>27,29</sup> may result in decrease in LPL activity. The observed decrease in LCAT activity may be due to hepatic dysfunction and to circulating inhibitors released into the blood stream from the parasitized erythrocytes, at each end of the schizogonic cycle<sup>30,31</sup>. Another element may be the

decrease in apo A-1, which is a known LCAT enzyme-activator, as evidenced in falciparum malaria<sup>31</sup>.

The present study shows that the change in the lipid status in vivax malaria becomes severe as the number of attacks increases.

#### ACKNOWLEDGEMENTS

We wish to express our gratitude to Mr. M.P. Kannabiran, Health Officer and Mr. B. Dhanraj, Senior Entomologist, Corporation of Madras, for their encouragement and providing samples throughout the period of the work. We thank Dr. P. Venkatesan, Institute of Thoracic Medicine, for his help in the statistical analysis of the data.

#### REFERENCES

1. Anon. (1993). Global malaria control. *Bull. WHO*, **71**: 281-284.
2. Thathy, V., D.W. Seversom and B.M. Christensen (1994). Reinterpretation of genetics of susceptibility of *Aedes aegypti* to *P. falciparum*. *J. Parasitol.*, **80**(5): 705-712.
3. Nandan, G. (1994). Malaria tests India's public health system (news). *British Med. J.*, **309**: 1183-1184.
4. Anon. (1992). World malaria situation in 1990. *Bull. WHO*, **70**: 801-807.
5. Seshadri, C., B. Ramakrishna Shetty, N. Gowri, S. Venkataraghavan and M.V. Chari (1981). Serum cholesterol and total lipids in *Plasmodium vivax* malaria—A preliminary study. *Indian J. Med. Res.*, **74**: 513-516.
6. Martin, D. Young (1970). *A Manual of Tropical Medicine*. 4th ed. (Oxford and IBH Publishing Co., Calcutta): 346-348.
7. Parekh, A.C. and D.H. Jung (1970). Cholesterol determination with ferric chloride-uranyl acetate and sulfuric acid ferrous sulfate reagents. *Annals. Chem.*, **42**: 1423-1427.
8. Rice, E.W. (1970). Triglycerides in serum. In *Standard Method of Clinical Chemistry*. Eds. P. Roderick and MacDonald (Academic Press, New York), **6**: 215-222.
9. Rouser, G., S. Fleisher and A. Yamanaoro (1970). Two dimensional TLC separation of lipids and determination of phospholipids by phosphorous analysis of spots. *Lipids*, **5**: 495-496.
10. Hron, W.T. and L.A. Menahan (1981). A sensitive method for the determination of free fatty acids in plasma. *J. Lipid Res.*, **22**: 377-381.
11. Hilz, T., J. Stermmetz and G. Siest (1983). Plasma LCAT, reference values and effects of Xenobiotics. *Clin. Chem. Acta*, **133**: 85-96.
12. Schmidt, A. (1974). Measurement of LPL and hepatic TGL and hepatic TGL in human post-heparin plasma. *Methods in Enzymol.*, **72**: 325-337.
13. Folch, J., M. Loes and S.G.H. Stanely (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **266**: 497-509.
14. Wyler, D.J. (1982). Malaria: Host-pathogen biology. *Rev. Inf. Dis.*, **4**: 785-797.
15. Looareesuwan, S., D.A. Warrell, N.J. White, P. Chantaranich, M.J. Warrell, S. Chontaratherkitti, S. Changswak, L. Chongaman Kongcheep and L. Kanchanaraya (1983). Retinal haemorrhage, a

- common sign of prognostic significance in cerebral malaria. *Amer. J. Trop. Med. Hyg.*, **32**: 911-915.
16. McGregor, I.A. (1987). The significance of parasitic infections in terms of clinical disease: A personal view. *Parasitology*, **94**: 159-178.
  17. Mohanty, S., S.K. Mishra, B.S. Das, S.K. Satpathy, D. Mohanty, T.K. Patnaik and T.K. Bose (1992). Altered plasma lipid pattern in falciparum malaria. *Ann. Trop. Med. Parasitol.*, **86**: 601-606.
  18. Ebisawa, I., H. Ohara and K. Tanabe (1990). Biochemical abnormalities in malaria. *Japanese J. Trop. Med. Hyg.*, **18**(3): 247-253.
  19. Angus, M.G.N., K.A. Fletcher and B.G. Maegraith (1971). Studies on the lipids of *P. knowlesi*-infected rhesus monkeys I. Changes in serum lipids. *Ann. Trop. Med. Parasitol.*, **65**: 135-154.
  20. Rock, R.C. (1971). Incorporation of  $^{14}\text{C}$ -labeled non-lipid precursors into lipid of *P. knowlesi* in vitro. *Biochem. Physiol.*, **40**(B): (a) 657-669; (b) 893-906.
  21. Nolte, K.H., P. Cremer, A. Kuhlencord, W. Bommer and D. Seidel (1988). Changed lipid metabolism parameters in malarial patients — A diagnostic criterion? *Mitteilungen-der-Osterreichischen-Gesellschaft-fur-Tropenmedizin-und-Parasitologie*, **10**: 223-233.
  22. Vial, H.J., M.L. Ancelin, J.R. Philippot and M.J. Thuet (1990). Biosynthesis and dynamics of lipids in Plasmodium-infected mature mammalian erythrocytes. *Blood Cells*, **16**: 531-555.
  23. Nilsson-Ehle, I. and P. Nilsson-Ehle (1990). Changes in plasma lipoproteins in acute malaria. *J. In. Med.*, **227**(3): 151-155.
  24. Skirrow, H.B., T. Chonsaphajai Sidhi and B.G. Maegraith (1964). The circulation in malaria II. Portal argiogeaphy in monkeys injected with *P. knowlesi* and in shock, following manipulation of the gut. *Ann. Trop. Med. Parasitol.*, **58**: 502-510.
  25. Roth, E.F., S. Schulman, J. Varderberg and J.A. Olson (1986). Pathways for reduction of oxidised glutathione in *P. falciparum*-infected erythrocytes. *Blood*, **67**: 827-830.
  26. Gray, Allan and G. Discombe (1966). *Clinical Pathology* (Blackwell Scientific Publications, Oxford).
  27. Beutler, B. and A. Cerami (1987) Cachectin: More than a tumour necrosis factor. *New England J. Med.*, **316**: 379-385.
  28. Kaufmann, R.L., C.F. Matson and W.R. Beisel (1987). Hypertriglyceridemia produced by endotoxin: Role of impaired Tg disposal mechanism. *J. Inf. Dis.*, **133**: 548-555.
  29. Grau, G.E., T.E. Taylor, M.E. Molyneux, J.J. Wirima, P. Vassalli, M. Hommel and P.H. Lambert (1989). TNF and disease severity in children with falciparum malaria. *New England J. Med.*, **320**: 1586-1591.
  30. Maurois, P., M. Pessah, I. Briche and L.G. Aleinder (1985). Alteration of LCAT activity during *P. chabaudi* rodent malaria. *Biochimie*, **67**: 227-239.
  31. Djoumessi, S. (1989). Serum lipids and lipoproteins during malaria infection. *Pathol. Biol.*, **37**: 909-911.

## Preliminary Evaluation of Safety Aspects of Neem Oil in Kerosene Lamp

NEENA VALECHA, M.A. ANSARI<sup>a</sup>, S. PRABHU<sup>b</sup> and R.K. RAZDAN<sup>a</sup>

Kerosene lamps containing one per cent neem oil were used for mosquito repellent action in a village near Delhi. The safety aspects of this personal protection method developed by Malaria Research Centre were evaluated by animal studies and clinical examination of population before and after exposure. Single application of neem oil (1%) did not produce skin irritation in rabbits and adverse effect on guinea pigs after exposure to aerosol. Clinical examination of 156 adults and 110 children did not reveal any major adverse effects after one year of exposure to 1% neem oil.

**Keywords:** Neem oil, Repellent, Safety

### INTRODUCTION

Vector-borne diseases like malaria, filaria, dengue, japanese encephalitis etc. are important causes of morbidity in tropical and subtropical countries including India. Mosquito nuisance is unbearable in many parts of the country. Residual spraying of insecticides in rural areas and antilarval measures in

urban areas have not produced any dent in evergrowing mosquito nuisance. In addition, they also contribute to the environment pollution<sup>1</sup>. Personal protection measures are, therefore, used as supplementary measures to avoid mosquito bites.

Neem (*Azadirachta indica*), a native of Indian subcontinent, has remarkable

---

Malaria Research Centre (ICMR), 22-Sham Nath Marg, Delhi-110 054, India.

<sup>a</sup>Malaria Research Centre (ICMR), 20-Madhuban, Delhi-110 092, India.

<sup>b</sup>Maulana Azad Medical College, Bahadur Shah Zafar Marg, New Delhi-110 002, India.

insecticidal properties<sup>2,3</sup>. The repellent action of neem products on mosquitoes particularly anophelines and sandflies, the vectors of malaria and kala-azar, have also been demonstrated<sup>4,5</sup>. Repellent action of neem oil against *An. culicifacies* was also evident when neem oil was added to kerosene lamps to give 1% concentration of neem oil in kerosene. These lamps were lighted from dusk-to-dawn in rural areas<sup>6</sup>.

However, the impact of neem in kerosene lamp on inhabitants has not been evaluated. Therefore, experiments were conducted to generate data on some safety aspects of the method during the field trials. In addition, dermal and inhalational toxicity in rabbits and guinea pigs was determined. Results of this study are presented in the paper.

## MATERIALS AND METHODS

### Study area

Field trial was carried out in Beel Akbarpur village, Dadri PHC, District Ghaziabad (U.P.) situated about 40 kms southeast of Delhi. Neem oil was added to kerosene to obtain 1% concentration. Lamps containing this mixture were lighted in all human dwellings from dusk-to-dawn (1800-0600 hrs) during June 1992 to May 1993. The average size of room was 10 x 10 x 10 ft and average number of occupants was four in each room. Atleast one window of each room was kept open. Neem oil was not used in kerosene lamp in Anandpur village of same PHC and taken as control.

## Animal studies

### Primary skin irritation test on rabbits:

The study was conducted in three groups (2 male + 2 female) of rabbits. They were housed singly in climatized room with regular lighting conditions and allowed food and water *ad libitum*. Hair were removed from both sides of back (approx. 4 sq cm area) one day before experiment, 0.5 ml of test compound and/or vehicle was applied to prepared site and secured with impervious sheet of aluminium foil and firmly held in place with adhesive tape. Visual assessment of skin changes (erythema and oedema) was made as per classification of Draize<sup>7</sup>. The animals were under observation for a period of 7 days for development of any adverse symptoms.

**Acute inhalation toxicity:** Fasted guinea pigs of either sex weighing from 250-350 g were confined in a gas chamber of about 4 litre capacity<sup>8</sup>. Six animals (3 male + 3 female) were exposed to vehicle, i.e. kerosene and 6 to kerosene in 1% neem oil. The aerosol was lead to the chamber through an opening (2 cm diam) with the help of nebuliser and compressed air (a second opening allows excess to escape). The animals were observed for adverse effect. The pre-convulsion time was determined and compared with positive control (0.2% histamine diphosphate aerosol).

### Clinical study

Studies were carried out in villages of Anandpur and Beel Akbarpur situated 40 km in east of Delhi. Ordinary kero-

**Table 1. Results of primary skin irritation in rabbits**

Treatment	Dose	Skin irritation		Signs of toxicity (upto 7 days)
		(24 h)	(72 h)	
Control	-	Erythma	Nil	Nil
		Oedema	Nil	
Kerosene	0.5 ml	Erythma	Nil	Nil
		Oedema	Nil	
Kerosene + Neem oil (1%)	0.5 ml	Erythma	Nil	Nil
		Oedema	Nil	

Each group comprised 2 male and 2 female animals and 0.5 ml of test compounds were applied over 4 sq cm area.

sene lamps made of tin with a regulator to adjust the wick were used. The wick was adjusted by field workers and kept constant throughout the study. Neem oil lamp was used in Beel Akbarpur village (Distt. Ghaziabad) and Anandpur village served as control, where only kerosene lamps were used. Approximately 25% of population (occupants of every 4th house) of each village was examined clinically before starting the experiment and thereafter at 3, 6 and 12 months.

General physical examination with cardiovascular and central nervous systems were studied clinically. Blood samples were collected for estimation of haemogram, liver and renal function.

## RESULTS AND DISCUSSION

A single application of neem oil (1%) in kerosene on the skin of male and female

rabbits at tested dose level did not indicate symptoms of primary skin irritation. There were no gross pathological changes and delayed adverse effects. (Table 1).

The pre-convulsion time in vehicle and experimental group of guinea pigs was more than 6-10 min. Three animals in neem oil group and two in vehicle group had fine tremors after 3 min of exposure. There was no mortality in either group. The animals exposed to histamine and pre-convulsion time of less than 3 min ( $n = 3$ ).

In the clinical study, 156 adults (83 M + 73 F) and 110 children in experimental village and 180 adults (99 M + 81 F) and 125 children in control village were selected for periodic observation. Approximately 25% population in both the groups was suffering from skin diseases (mainly scabies) and was treated ac-



**Table 2. Effect of kerosene lamp with or without 1% neem oil on renal and liver functions (n = 6)**

Parameters	Control		Experimental	
	Before 3 months	After 3 months	Before 3 months	After 3 months
B. urea (mg/dl)	26.32±4.34	25.8±4.77	24.92±4.32	23.86±5.8
S. creatinine (mg/dl)	1.09±0.24	1.05±0.09	0.81±0.18	0.84±0.1
SGPT (U/L)	29.30±11.1	22.3±7.36	31.16±5.51	31.47±5.14
SGOT (U/L)	36.80±21.13	37.1±8.25	28.21±3.83	28.42±2.94

cordingly. Four adults in experimental and one in control villages were hypertensive. There was no aggravation of symptoms/signs of pre-existing diseases during the study period. Mild respiratory irritation was experienced by two subjects each in control and experimental group thereby showing that addition of neem oil to routinely used kerosene lamp did not increase side effects. However, occasional vomiting was complained by 0.01% (n = 3) subjects in experimental village. No other subjective symptoms or phenomenon was complained by any individual in either group. All vital parameters remained within normal limits in both the groups, indicating that physiological status of liver and kidney was not affected (Table 2).

Thus the results of the study indicate that addition of 1% neem oil in kerosene lamp which is used regularly in these villages does not produce any adverse effects except vomiting in 0.01% cases. However, long-term effects need further assessment.

#### ACKNOWLEDGEMENTS

The authors are grateful to Dr. V.P. Sharma, Director, Malaria Research Centre, Delhi for his constant guidance and support. Thanks are also due to Mr. A.R. Kotnala and Mr. Keshari Prasad for technical assistance. The sincere work carried out by Mr. Bhopal Singh is also acknowledged.

#### REFERENCES

1. Jakson, R.J. (1983). Pesticides as a public health concern in California. *West. J. Med.*, **139**: 363-364.
2. Schmutterer, H. (1990). Properties and potential of natural pesticides from neem tree, *Azadirachta indica*. *Annual Rev. Entomol.*, **35**: 271-297.
3. Sharma, V.P. (1993). Malaria control in neem research and development. In *Neem Research and Development*. Eds. N.S. Randhawa and B.S. Parmar (Publication No. 3, Society of Pesticide Science, India): 235-241.
4. Sharma, V.P., M.A. Ansari and R.K. Razdan (1993). Mosquito repellent action of Neem (*Azadirachta indica*) oil. *J. Mosq. Contr. Assoc.*, **9**(3): 359-360.

5. Sharma, V.P. and R.C. Dhiman (1993). Neem oil as a sandfly (Diptera: Psychodidae) repellent. *J. Mosq. Contr. Assoc.*, **9**(3): 364-366.
6. Sharma, V.P. and M.A. Ansari (1994). Personal protection from mosquitoes (Diptera: Culicidae) by burning neem oil in Kerosene. *J. Med. Entomol.*, **31**(3): 505-507.
7. Draize, J.M. (1959). Dermal toxicity in appraisal of the safety of chemicals in food, drugs and cosmetics (Association of food and Drug Officials of the United States of America).
8. Seigmund, O.H., H.R. Granger and A.M. Lands (1947). The bronchodilator action of compounds structurally related to epinephrine. *J. Pharmacol.*, **90**: 254.

## Application of Peptide ELISA in Tribal Malaria of Madhya Pradesh

ARATI ROY, SUKLA BISWAS and NEERU SINGH<sup>a</sup>

A recently developed peptide ELISA method was used for monitoring the efficacy of malaria control programme in the tribal areas of Madhya Pradesh. Both crude *Pf* antigen and synthetic nonapeptide were used in ELISA for seroepidemiological studies. Both antigen responded equally well but the synthetic peptide had advantages of purity, defined characteristic and easy availability. Population of Mandla protected by vector control measures showed lower antibody titre and lower percentage positivity compared to the unprotected population of Jabalpur. A 0-5 yrs sentinel population from Haldwani almost seronegative has been taken as control.

**Keywords:** ELISA, Seroepidemiology, Tribal malaria

### INTRODUCTION

Malaria is a major public health problem in tribal areas of India and contributes a major portion of total malaria cases in India. 7.8% of tribal population of seven states in the country accounts for almost 40% malaria cases and 60% *P. falciparum* cases<sup>1</sup>. Madhya Pradesh, having largest tribal

population contributes 13.27% of total malaria cases and 16.67% of *Pf* cases. Malaria Research Centre has initiated bioenvironmental control of malaria<sup>2</sup>, an integrated approach for vector control which has yielded excellent result in Mandla<sup>3</sup>. However, any control measure requires an efficient epidemiological surveillance and monitoring method. Importance of serology

---

Malaria Research Centre, 22, Sham Nath Marg, Delhi-110 054, India.

<sup>a</sup>Malaria Research Centre (Field Station), Medical College Building, Jabalpur-482 003, India.

for epidemiological surveillance is well recognised<sup>4-6</sup>. Extensive studies has been carried out on seroepidemiology of malaria in India<sup>7,8</sup>. Among various methods, ELISA has been successfully applied for serodiagnosis of malaria<sup>9-11</sup>. In the present report we try to revalidate the peptide ELISA developed in our laboratory using samples from Mandla villages. Bioenvironmental control programme has been operative from 1986 onwards in Mandla. An adjoining area to Jabalpur<sup>12</sup> which is unprotected has been included for comparison (Fig. 1).

## MATERIALS AND METHODS

### Study area

Bizadandi block of Mandla district covers an area of 471 sq km with a resident population of about 50,000. The main geographical features are hilly and forest terrain with extensive network of streams and nallahs. The rainy season is from June to September with maximum precipitation in July and August. The villages are scattered remotely and become inaccessible due to flood and difficult terrain during

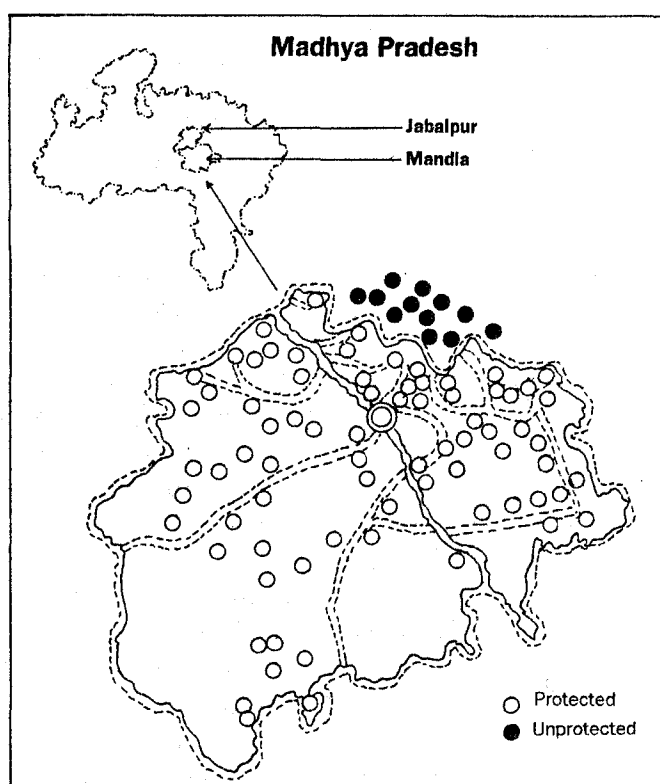


Fig. 1: Location of protected and unprotected villages of Mandla and Jabalpur (Madhya Pradesh)

rains. Ecological condition favours prolonged transmission of malaria accentuated by back and forth movement of population and labour migration. The area has preponderance of *P. falciparum* and presence of two vectors species namely, *An. culicifacies* and *An. fluviatilis* that are resistant to DDT. Presence of chloroquine resistant *P. falciparum* has also been detected in these areas.

Malaria control in most areas of Mandla district is difficult due to inaccessibility. Therefore, personal protection measures such as insecticide impregnated bednets has been adopted in some of the villages. Deltamethrin impregnated bednets of various fibres and mesh sizes were tested in some villages. The results are encouraging and there are clear indications of its useful role in reducing man-vector contact.

In Mandla, Malaria transmission is perennial and there are two distinct peaks of malaria, one is of *P. vivax* followed by *P. falciparum*.

#### **Control area**

Haldwani, where malaria control programme had been implemented for last five years and API became zero was considered as a negative control area. Children of age group 0-5 yrs were considered for the study.

**Study population and measure:** Eighty per cent of the population in

the study area belongs to scheduled tribes (Gond) with poor socio-economic conditions (low literacy rate and poverty). A group of 21 villages surrounding Bijadandi block were taken as the control where bioenvironmental control measures are operational. The houses in experimental areas have attached cattlesheds and are made of bamboo with mud plaster on both sides. Villages are separated by a distance of 2-10 kms. Most houses are found in clusters of 4 to 5 and proper boundaries of villages are not defined. Proper approach roads to majority of villages are absent. only *kuccha* lanes connect villages through forest. People are ignorant of modern medical facilities and do not avail them from PHCs and mostly depend on herbal treatment and witchcraft. As a result most cases of malaria are neither noticed nor treated.

**Sample collection:** A total of 721 samples were collected from 8 villages. Every third house was selected for sample collection. Fifty  $\mu$ l blood through finger prick was collected from normal individuals of all age groups on Whatman paper no. 3 in April 1990 and were stored at  $-20^{\circ}\text{C}$  till used.

**Antigen:** RI antigen is a nonapeptide (EENVEHDA-Cys) and is custom synthesised commercially from M/s. Cambridge Research Biochemicals, U.K. It is octapeptide epitope from the 3'-repeat region of ring infected erythrocyte surface antigen (RESA)<sup>13</sup> with a

Cys at the C'-terminal end and subsequently, for ELISA as published elsewhere<sup>7,9</sup>.

**Pf:** Soluble extract of trophozoite schizont enriched antigen fraction was obtained through percoll gradient and subsequently standardised for ELISA as reported earlier<sup>14</sup>. Each blood sample on filter paper was eluted with 1 ml PBS at +4°C overnight to give 1:40 dilution which was made to react with RI and 1:1024 dilution was used for *Pf*.

**Enzyme-linked immunosorbent assay:** 50 ng/well (RI) and 2 µg/well (*Pf*) antigen were coated in fast binding (Costar, USA) ELISA plate and kept overnight at +4°C followed by 1 h at 37°C. The plates were washed with PBST (0.02% Tween) three times and blocked with 200 µl/well 1% BSA for 1 h at 37°C. Appropriate dilutions of eluate was added at 50 µl/well and kept at 37°C for 1 h. After subsequent washings, 50 µl/well antihuman IgG-HRPo conjugate (Dakopatt) was added and kept at 37°C for 1 h followed by addition of orthophenylene diamine and plates were read at 490 nm in Biotek reader. Each ELISA test included standard positive and negative serum to make correction for plate-to-plate variation.

## RESULTS

Information regarding parasitology in infants and children of the two groups

under study are shown in Table 1. There are marked differences among slide falciparum rate and annual parasite index in protected (P) and unprotected (UP) villages. Less number of parasite positive cases have been reported from P villages. These observations may indicate that malaria transmission is lower in P villages than in UP village.

The effect of parasite load in P and UP groups have been evaluated by measuring the antibody level in individuals in a cross-sectional survey during non-transmission season. Data on samples from four P villages of Mandla, the control village of Haldwani which is non-endemic (Fig. 2) and four UP villages of Jabalpur are presented in Fig. 3. Overall level of antibody against RI and *Pf* (Fig. 2) in P group and control group are much less compared to UP group. In the negative control area 0-5 yrs age group became seronegative as expected. The serological profile of Jabalpur area adjoining Mandla district, shows high antibody response indicating high endemicity (Table 1).

In P villages antibody titres are lower in comparison to API values. In P and UP villages, mean ELISA OD values and percentage seropositivity have been compared using both the antigens (Tables 2 and 3). A low antibody titre and high API value were accompanied with high antibody titre and a higher percentage of seropositivity (99%) as shown in Table 3.

**Table 1. Epidemiological situation of malaria in Mandla and Jabalpur districts (1987-1989)**

Year	No. of villages	Population		Infants (0-1 yr)			Popula- tion	Children (2-6 yrs)			(Av. age 6.5 yrs)	
				API	ABER	SPR		API	ABER	SPR		
1987	80	981	P	23.45	73.19	3.20	8061	121.45	84.98	14.29	4.69	
	12	277	UP	223.44	54.51	41.22	1433	473.31	74.74	64.25	35.46	
1988	103	1724	P	20.66	35.15	4.62	11701	120.50	66.16	18.21	6.43	
	18	338	UP	349.11	87.28	40.00	1881	795.32	152.36	52.20	32.00	
1989	105	1601	P	16.24	26.48	5.61	15772	116.66	45.67	23.03	5.01	
	21	624	UP	129.87	51.60	23.08	4520	216.36	54.78	34.64	10.40	

P — Protected; UP — Unprotected; Mandla — P — Infant (Av.) 20.12 : P — Children (Av.) 119.54; Jabalpur — UP — Infant (Av.) 234.14 : UP — Children (Av.) 495.0.

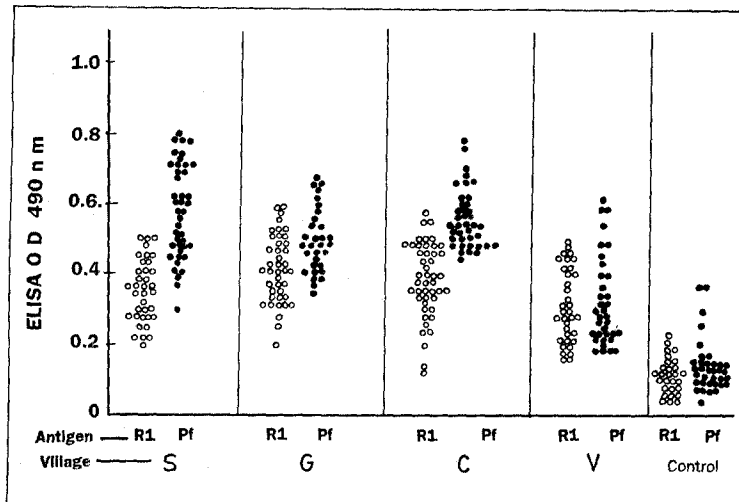


Fig. 2: Antibody reactivity against R1 and *Pf* antigen in four protected villages of Mandla with control (non-endemic) population

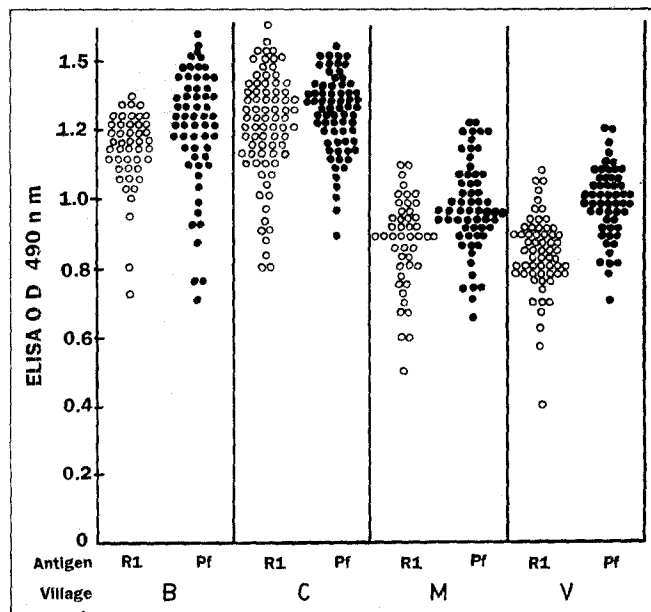


Fig. 3: Antibody reactivity against R1 and *Pf* antigen in four unprotected villages of Jabalpur



**Table 2. Parasitological and serological results of Mandla (study village) and Haldwani (control area)**

Village (1990)	API	Population examined for serology		RI		Pf	
		No.	% examined	Mean ELISA	% positivity	Mean ELISA	% positivity
Somnapur (S)	291	90	21.5	0.30	35	0.49	21
Ghota (G)	189	81	60.6	0.44	32	0.53	60
Chargaon (C)	100	69	38.2	0.42	55	0.56	38
Vijaypur (V)	81	118	37.3	0.32	33	0.54	37
Average	165.2	358	39.4	0.37	38	0.52	39
Haldwani (1991) (0-5 yrs) control area	0	40	20.0	0.14	7.5	0.15	7.5

**Table 3. Parasitological and serological results of Jabalpur study villages**

Village (1990)	API (1989)	No. examined	% population examined for serology	RI*		Pf*	
				Mean ELISA	% positivity	Mean ELISA	% positivity
Bilnagari (B)	210.64	120	25.5	1.10	100	1.15	100
Chargaonkala (E)	293.77	103	15.5	1.19	100	1.04	99.4
Majhgaon (M)	381.44	92	ND	1.27	100	0.948	99.2
Tarwani (T)	234.88	48	13.5	0.85	100	1.20	100

\*Cut-off value for RI is 0.35 O.D. and Pf 0.55 O.D., based on non-endemic Srinagar population; ND — Not determined.

## DISCUSSION

Antibodies to crude *Pf* antigens are regarded as an indicator of exposure and not of protective immunity<sup>15</sup>. Usefulness of ELISA methods as a tool for evaluation of malaria epidemiology such as transmission have been investigated by several workers<sup>10,16-18</sup>. It has been shown that antibody profile of the population reflects its malaria experience. The major limitation of this assay however, is the supply of standardised reagents, particularly defined antigen<sup>10</sup>. Here we are reporting the possibility of monitoring malaria transmission through seroepidemiological studies using RI antigen which has several advantages compared to *Pf* antigen. In synthetic peptide, a high degree of purity in antigen, consistency in test system, uniformity and specificity in results can be maintained<sup>7,9</sup>. Moreover, its production in large-scale is possible. The quantity of RI required is much less per sample than *Pf* crude antigen as Cystine at the C' facilitates efficient binding on the plate<sup>7</sup>. We demonstrated that RI-ELISA is a desirable method with specific antigen for determining status of malaria transmission in an endemic area<sup>9</sup>. This report summarizes the epidemiological and immunological findings of endemic *P. falciparum* and *P. vivax* malaria transmission among Gond tribes of Mandla and Jabalpur. The study shows that in a defined population, the antibody titre to both *Pf* and RI antigens are comparable. The interesting finding is

that antibodies to both the antigens indicate that the trend in each group of population is correlated with transmission status of the areas. An early diagnosis and prompt treatment reduced both the antibody titre and low percentage positivity in Mandla, reflecting low-level of incidence of malaria (Fig. 2, Table 2). A high antibody titre and a high percentage positivity in Jabalpur with both the antigens, indicate a high transmission which is well corroborated with high API values (Fig. 3, Table 3).

Results of the present study indicate the difference between seropositivity in P and UP population. Similar study was done by Voller *et al.*<sup>10</sup> Since seropositivity is dependent on malaria experience (recent/past) this obviously would give correct information about endemicity<sup>19,20</sup> of the disease than slide positivity rate. In disappearing malaria repeat cross sectional seroepidemiological studies by IFA using *Pf* antigen showed a steady decline in the sentinel population (1-5 yrs)<sup>21</sup>. Similar observations were made using ELISA in Haldwani population (unpublished data). Some percentage of infections are definitely missed when parasitaemia is too low or the patient had received antimalarial treatment resulting in a low SPR. Results show that ELISA using crude *Pf* antigen or synthetic peptide are equally suitable to indicate the endemicity of malaria in a given community and this can be useful in monitoring its control.

## ACKNOWLEDGEMENTS

Authors are grateful to Dr. V.P. Sharma, Director, Malaria Research Centre, for guidance during the study. Thanks are also due to Prof. K.B. Roy, Jawaharlal Nehru University, for valuable suggestions in preparation of the manuscript and Mr. Dixit and Mr. Rajput for collecting samples from Jabalpur field station. The technical help of immunology group, MRC, is highly appreciated.

## REFERENCES

1. Malaria control in tribal and non-tribal rural areas with special emphasis on disease management, prevention and emergencies (1993). In *National Task Force on Revised Strategy for Control of Malaria* (Final Report). Government of India/WHO: 13-43.
2. Sharma, V.P. (1987). Community-based malaria control in India. *Parasitol. Today*, **3**(7): 222-225.
3. Singh, Neeru, V.P. Sharma, A.K. Mishra and O.P. Singh (1989). Bioenvironmental control of malaria in a tribal area of Mandla district, Madhya Pradesh, India. *Indian J. Malariol.*, **26**: 103-120.
4. Huang, T.Y., R. Hezi, F.X. Zheng, A.H. Jaing, D.X. Xiao, A.W. Yie, B.Y. Cheng and H. Yu (1989). Study of a seroepidemiological methods of evaluation of malaria during the advanced stage of malaria control. *WHO/Mal.*, **89**: 1053.
5. Molineaux, L. and G. Gramiccia (1980). *The Garki Project: Research on the Epidemiology and Control of Malaria in the Sudan Savanna of West Africa* (WHO, Geneva).
6. Ray, Krishna, H.B. Upreti, A.K. Mukherjee, H.C. Sharma, R.N. Yadav, C.A. Sivaraman and R.N. Basu (1988). Seroepidemiological tools vis-a-vis conventional malariometric indices—A study in Orissa state, India. *J. Com. Dis.*, **20**(3): 232-246.
7. Roy, Arati, V.P. Sharma and V.S. Chauhan (1994). The use of peptide ELISA in determining malaria endemicity. *J. Immunol. Methods*, **167**: 139-143.
8. Anon. (1989). *Seroepidemiology of Human Malaria: A Multicentric Study*. Ed. V.P. Sharma (Malaria Research Centre, Delhi).
9. Roy, Arati, Sukla Biswas, L. Kabilan and V.P. Sharma (1995). Application of simple peptide ELISA for stratification of malaria endemicity. *Indian J. Malariol.*, **32**: 164-173.
10. Voller, A., R. Kornille, A.J. Brogger, A. Storey and L. Molineaux (1990). A longitudinal study of *Plasmodium falciparum* malaria in the west African Savanna using the ELISA technique. *Bull. WHO*, **33**(3): 429-453.
11. Quaky, I.A. (1980). The development and validation of an enzyme linked immunosorbent assay for malaria. *Trop. Parasitol.*, **31**: 325-333.
12. Singh, Neeru and V.P. Sharma (1989). Persistent malaria transmission in Kundam block, District Jabalpur (M.P.). *Indian J. Malariol.*, **26**: 1-7.
13. Perlmann, H., P. Perlmann, K. Berzils, B. Wahlin, M. Troy-Blomberg, I. Hagstedt, B. Anderson, E. Hog, Peterson and A. Bjorkmann (1989). Dissection of the human antibody response to malaria antigen Pfl55/RESA into epitope specific components. *Immunol. Rev.*, **112**: 115-132.
14. Biswas, S., Q.B. Saxena, A. Roy and V.P. Sharma (1989). Isolation of different erythrocytic stages of *Plasmodium falciparum* and synchronization in culture. *Indian J. Malariol.*, **25**: 7-10.

15. Chizzolini, Carlo, E. Delaportate, H.H. Kaufmann, J.P. Akue, A.B. Verdini, A. Fessi and G. Delgudice (1989). Age-related prevalence of antibody response against three different defined *Plasmodium falciparum* antigens in children from the Haut-Ogoone province in Gabon. *Trans. R. Soc. Trop. Med. Hyg.*, **83**: 147-151.
16. Deloron, Phillipe and M. Cot (1990). Antibodies to the ring-infected erythrocyte surface antigen and the circumsporozoite protein of *P. falciparum* in a rural community from Burkina Faso. *Trans. R. Soc. Trop. Med. Hyg.*, **84**: 191-195.
17. Peterson, E., B. Hogg, N.T. Marbiah, H. Perlmann, M. Willcox, E. Dolopate, A.P. Hanson, A. Bjorkmann and P. Perlmann (1990). A longitudinal study of antibodies to and the *P. falciparum* antigen Pf 155/RESA and immunity to malaria infection in adult Liberians. *Trans. R. Soc. Trop. Med. Hyg.*, **84**: 339-345.
18. Sanchez, E., H.A. Perez and C. Martinex (1990). Malaria in the Amazon. Prevalence of *P. falciparum* antibodies Amerindians inhabiting the Venezuelan Amazon. *Ann. Trop. Med. Parasitol.*, **84**(4): 307-312.
19. Doi, H., Syafei and A. Ishii (1990). Detection of malaria endemicity in community villages in north Sumatra, Indonesia by enzyme-linked immunosorbent assay. *Ann. Trop. Med. Parasitol.*, **84**: 301-305.
20. Gordon, D.M., D.R. Davis, M. Lee, C. Lambros, B.A. Harrison, R. Samuel, G.H. Campbell, M. Jegthesan, K. Selvarajan and G.E. Lewis Jr. (1991). Significance of circumsporozoite specific antibody in the natural transmission of *Plasmodium falciparum*, *Plasmodium vivax* and *Plasmodium malariae* in an aboriginal population of central peninsular Malaysia. *Amer. J. Trop. Med. Hyg.*, **45**(1): 49-56.
21. Srinivasa, H., H.G.V. Rao and P. Bhat (1988). *In vitro* cultured *P. falciparum* in younger age group - A sentinel population for assessment of malaria transmission. In *Seroepidemiology of Human Malaria : A Multicentric Study*. Ed. V.P. Sharma (Malaria Research Centre, Delhi): 153-160.

## SHORT NOTES

Indian Journal of Malariology  
Vol. 33, September 1996, pp. 154-158.

### Seasonal Prevalence of Common Anophelines in Sagar Island

SAGARTIRTHA SARKAR and MIHIR K. PRAMANIK

**Keywords:** Anophelines, Sagar Island, Seasonal prevalence

A detailed survey during 1991-92, on the distribution of anophelines and species predominance in relation to seasonal and climatic changes was carried out in Sagar Island. It is a large and well-populated estuarine Island of the river Hugli during 1991-92. Marked seasonal variations were recorded for all the adult anophelines.

Prevalence of anophelines in houses and cattlesheds has been reported earlier<sup>1</sup>, although faunastic survey of the mosquito populations of deltaic Ben-

gal<sup>2</sup> and that of the Sagar Island<sup>3</sup> has been done only recently.

Adult anopheline mosquitoes were collected by sucking tubes from definite cattlesheds of north, middle and south regions of Sagar Island. Collections were made from 0500 to 0700 hrs at every fifteen days interval in each season. At least two months were considered for every seasonal collections and were made four times by a person in each season. Temperature, relative humidity and pH of the water were also recorded. Adult mosquitoes were

brought to the laboratory for identification by using the key of Christophers<sup>4</sup> and Das *et al.*<sup>5</sup> Identifications were cross-checked at Malaria Research Centre, Delhi.

Collections were carried out throughout the year during 1991-92 in sixteen villages covering north, middle and south Sagar. Data shows (Table 1) all the four species of anopheline mosquitoes, namely *An. barbirostris*, *An. culicifacies*, *An. subpictus* and *An. annularis* with the population density, reaching its peak during the post-monsoon season followed by the winter density (Fig. 1). A very low anopheline density was a characteristic of monsoon and summer, though adaptation with summer climate seems to be much more prominent in these specimens except species *An. annularis*.

This indicates that all the four species reacted in same way during the four seasons and undoubtedly post-monsoon was the most favourable period, followed by winter, summer and rains. The results showed that one of the species, namely *An. annularis*, 183 (4.94%) specimens and 120 (3.24%) specimen in post-monsoon and winter respectively, fails to appear during the summer and rainy seasons. *An. barbirostris* was the most predominant species in the post-monsoon (46.4%) and winter (8.91%); whereas, it was *An. culicifacies* (3.16%) in summer. *An. culicifacies*, the established malaria vector is dominant in post-monsoon (12.52%) though its density was about one fourth that of

*An. barbirostris* during the same time. This suggests that *An. culicifacies* is not a dominant species except in summer (Table 1, Fig. 1).

The results on the seasonality of the ecoclimatic factors are shown in Table 1. Data indicates that certain ecoclimatic factors like temperature (25-27°C), relative humidity (85-88%) and pH of water (8.3-8.5) are pertinent in relation to the prevalence of these four species in post-monsoon season. But even slight alterations in these ecoclimatic parameters adversely affect the mosquito populations (Fig. 2).

Sen<sup>6,7</sup> reported the succession of anopheline species in rice fields in relation to the rice culture practices and growth of rice plants in lower Bengal. In coastal Orissa, Covell and Singh<sup>8</sup> and Senior-White *et al.*<sup>9</sup> found a high prevalence of *An. aconitus* and *An. annularis* in November-December and July-December which infact corresponds to the period of rice harvest. The results of the present study agree with their works. The rice fields and ponds, which are the two major sources of mosquito breeding, appear to regulate the seasonal prevalence of anophelines<sup>10</sup>. The study of the seasonal variations of anophelines in Sagar Island has revealed the presence of two potent malaria vectors namely, *An. culicifacies* and *An. annularis* in high densities. This study also indicates the knowledge of the specificity of seasonal prevalence of anophelines would be helpful in understanding the

**Table 1. Seasonal prevalence of common anopheline mosquito population along with seasonality of ecoclimate in Sagar Island during 1991-92**

Seasons	Temperature (°C)				Relative humidity (%)				pH								
	N		S		N		S		N		S						
	M	Av.	M	Av.	M	Av.	M	Av.	M	Av.	M	Av.					
Summer (Apr-May)	32	33	34	32.5	75	78	74	76	8.9	9.0	9.1	9.0					
Monsoon (Jul-Aug)	29	30	28	29	98	96	95	96	7.7	7.5	7.7	7.6					
Post-monsoon (Oct-Nov)	26	27	25	26	86	88	85	86	8.4	8.3	8.5	8.4					
Winter (Jan-Feb)	19	18	17	18	82	83	80	82	8.8	8.9	8.9	8.9					
Anopheline species													Season total				
An. annularis				An. barbirostris				An. culicifacies				An. subpictus					
N	M	S	T	N	M	S	T	N	M	S	T	N		M	S	T	
Summer (Apr-May)	0	0	0	0	7	14	11	32	32	41	44	117	55	34	17	106	255 (6.89)
Monsoon (Jul-Aug)	0	0	0	0	5	11	14	30	7	9	34	50	8	16	74	98	178 (4.81)
Post-monsoon (Oct-Nov)	59	64	60	183	863	185	668	1716	161	83	219	463	53	27	115	195	2557 (69.14)
Winter (Jan-Feb)	14	38	68	120	95	105	103	303	33	37	45	115	47	56	67	170	708 (19.14)
Group total	303				2081				745				569				3698
N — North; M — Middle; S — South; T — Total; Figures in parentheses indicate percentage.																	

N — North; M — Middle; S — South; T — Total; Figures in parentheses indicate percentage.

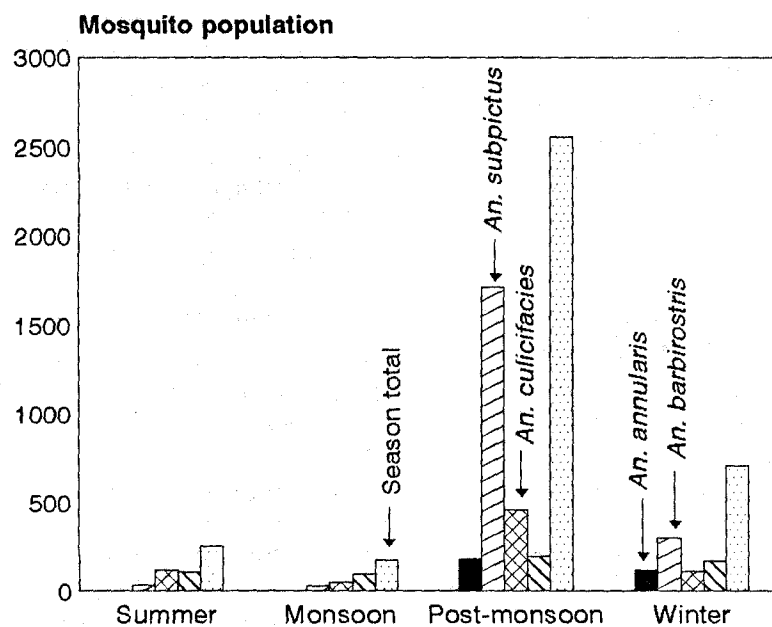


Fig. 1: Seasonal prevalence of common anopheline population in Sagar Island

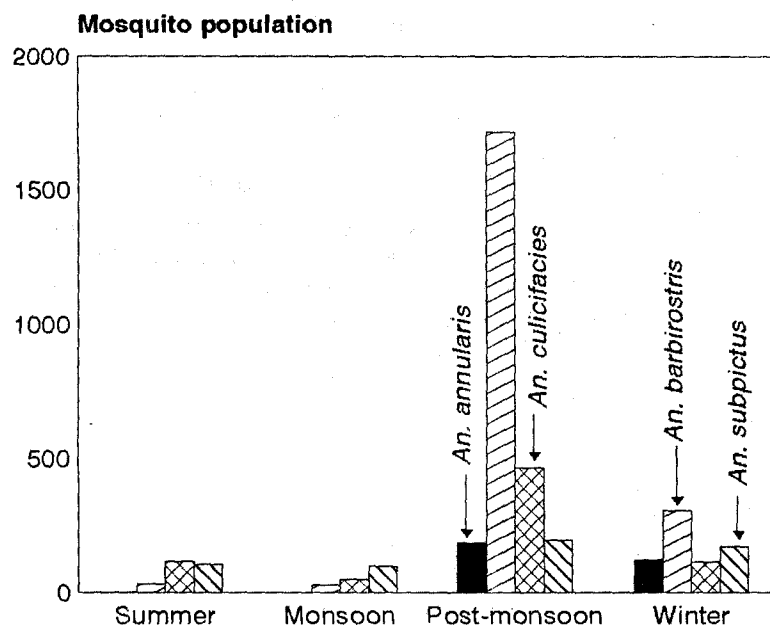


Fig. 2: Prevalence of anophelines against ecoclimates of Sagar Island



nature of competition that a vector faces in a certain niche at a particular time of the year. This would be helpful in planning precautionary measures for vector control.

#### ACKNOWLEDGEMENTS

We are thankful to Dr. B.N. Nagpal, Malaria Research Centre, Delhi for help; advice and cross-checking the specimens of anopheline. We are also thankful to the Head of the Department of Zoology, and to Prof. A Chowdhury, Department of Marine Science, Calcutta University for providing help at Sagar Island to carry out the survey. We thankfully acknowledge the help of Prof. Asish Duttgupta, Genetics Laboratory, Department of Zoology, Calcutta University for providing laboratory facilities and guidance.

#### REFERENCES

1. Sen, P. (1937). The relative prevalence of anophelines in houses and cattlesheds in deltaic Bengal. *Rec. Mal. Surv. Ind.*, **7**: 147-153.
2. Bhattacharya, S., I. Sen and N. Tandon (1992). Preliminary observation on the distribution of anopheline species in deltaic zone of West Bengal. In *Proceedings of Zoological Society of Calcutta*, **45** (Suppl. A): 331-334.
3. Pramanik, M. and S. Sarkar (1993). Mosquitoes of Sagar Island. *Indian J. Malariol.*, **30**: 193-199.
4. Christophers, S.R. (1933). *The Fauna of British India including Ceylon and Burma*, v. 4 (Taylor and Francis, London): 1-360.
5. Das, B.P., R. Rajagopal and J. Akiyama (1990). Pictorial key to Indian anopheline mosquitoes. *J. Pure Appl. Zool.*, **2**(3): 131-162.
6. Sen, P. (1935). *Anopheles* breeding in relation to rice cultivation in lower Bengal. *Rec. Mal. Surv. Ind.*, **1**: 97-108.
7. Sen, P. (1948). *Anopheles* breeding in the rice-fields of lower Bengal: its relation with the cultural practices and with the growth of the rice fields. *Indian J. Malariol.*, **2**: 221-238.
8. Covell, G. and Pritam Singh (1942). Malaria in coastal belt of Orissa. *J. Mal. Inst. Ind.*, **4**: 457-488.
9. Senior-White, R., A.K. Adhikari and V. Ramkrishna (1943). On malaria transmission on the Orissa coastal plain. *J. Mal. Inst. Ind.*, **5**: 159-189.
10. Nagpal, B.N. (1986). *A contribution to the knowledge of family Culicidae (Diptera) from Orissa*. Ph.D. Thesis (University of Berhampur, Orissa).

## **A Case of *Plasmodium malariae* Infection in the Dooars Region of West Bengal, India**

S. DAS, P. MALAKAR, G.K. SAHA, B. DASGUPTA and A.K. HATI<sup>a</sup>

**Keywords:** Dooars, *P. malariae*, Quartan malaria

Resurgence of malaria in different parts of Dooars region of West Bengal, India has posed a serious concern to the public health management programme. The situation has become acute in the foothills and in the terai region of eastern Himalayas.

Knowles *et al.*<sup>1</sup> reported from undivided Bengal the presence of all three species of malarial parasites namely, *P. vivax*, *P. falciparum* and *P. malariae* and the latter constituted about 10% of the total infections. Earlier, Hati and Mukhopadhyay<sup>2</sup> compiled the relevant data during the National Malaria Eradication Programme (1971-1978) and reported that *P. malariae* had become virtually extinct from the country except in some parts of Karnataka, where it

was present in a sizeable number in the population<sup>3</sup>. Experimentally, Roy<sup>4</sup> and Hati and Mukhopadhyay<sup>2</sup> were unable to infect the salivary glands of *An. stephensi* with *P. malariae*.

However, while routine examination of blood smears taken from the patients suffering from malaria, a case of typical infection by *P. malariae* with trophozoites showing characteristic band pattern was noticed. The schizonts showed twelve nuclei and bird's eye form of rings as well as gametocytes in the blood smear. The patient was a 23 years old college student hailing from Dooars area of northern West Bengal. The patient was treated with antimalarial drugs and blood smears taken subsequently proved negative for in-

---

P.G. Department of Zoology, Darjeeling Government College, Darjeeling-734 101, India.

<sup>a</sup>School of Tropical Medicine, Calcutta-700 073, India.

fection. This finding appears to be the first case of quartan malaria infection reported from this part of the country in recent years. The area from where the patient came is surrounded by thick jungle harbouring gibbons. According to Garnham<sup>5</sup> gibbons carry a malaria parasite similar to *P. malariae*. Whether the present case is one of the zoonotic infection or not is open to further investigation.

#### ACKNOWLEDGEMENTS

Financial assistance for the present study provided by the Department of Science & Technology, Government of West Bengal is gratefully acknowledged.

#### REFERENCES

1. Knowles, R., R. Senior-White and B.M. Dasgupta (1930). Studies in the parasitology of malaria. *Indian Med. Res. Memoirs*, **18**: 80-86.
2. Hati, A.K. and N.C. Mukhopadhyay (1980). Distribution of *P. falciparum* in West Bengal. *Trans. R. Soc. Trop. Med. Hyg.*, **74**: 420-421.
3. Roy, R.G., N.M. Madesayya, N.L. Sitarman and R.B. Ghosh (1976). *Plasmodium malariae* infection in Karnataka state (1963-1973). *J. Com. Dis.*, **8**: 81-82.
4. Roy, D.N. (1943). The role of *An. subpictus* Grassi as a carrier of malaria. *J. Mal. Inst. Ind.*, **5**: 117-121.
5. Garnham, P.C.C. (1966). *Malaria parasites and other haemosporidea* (Blackwell Scientific Publications, Oxford): 273-275.

# 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. Date of acceptance will be the date on which 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 author(s) should be submitted to:

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

### Classes of Items Published

In addition to full papers the Journal publishes 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 running title, of not more than five words.

### Preparation of Copy

Manuscript should be typewritten in English on one side of the paper leaving 1½ inch left-hand margin. The entire matter should be typed double space including references, tables and captions. 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.

Illustrations should be sent in triplicate. All illustrations including figures, pho-

**We accept manuscript on 3½" and 5¼" floppies in MS word.**

tographs, 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 author's name, fig. no. 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. Photographs should have allowance for reduction to 1/3 size. The approximate sizes of art work should be : 24 x 21 cm for quarter page, 45 x 24 cm for half page and 57 x 45 for 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.* Authors of scientific names of insects should be omitted in abstract and title, but should be included at 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 references is to an article published without any authorship in a periodical, in place of 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). In *Rodent Malaria*, edited by 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 spelling, 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). In *Rodent Malaria*, edited by 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.

*Short notes* should be prepared in a manner similar to the research papers and should contain Title, Name(s) of author(s) with 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 reprint service has been discontinued. All senior authors (first) 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 a covering letter.
3. Short title of the research paper (max. 5 words).
4. Keywords.
5. Undertaking by the author(s).
6. Names of at least three experts on the subject of paper submitted for publication.
7. Set of figures with legends and captions in triplicate on a separate sheet.

## ***Announcement***

We prefer submission of manuscripts on electronic media.

- Acceptable medium is 3 $\frac{1}{2}$ " or 5 $\frac{1}{4}$ " disk in MSDOS compatible format with file name, software/hardware used.
- The contents on the disk should exactly match with the manuscript and should be submitted with the hard copy (printed copy). The disk would be sent back in case of revision; the same should be returned to editor along with the revised copy of the manuscript. The file on the disk and printout should be identical. 'R' should be marked with red ink with the file name for revised manuscript.
- Package used for graphs should be mentioned.
- Floppies will be sent back to the authors after a final decision on the manuscript only on request.

— Editors

### **OTHER PUBLICATIONS OF MALARIA RESEARCH CENTRE**

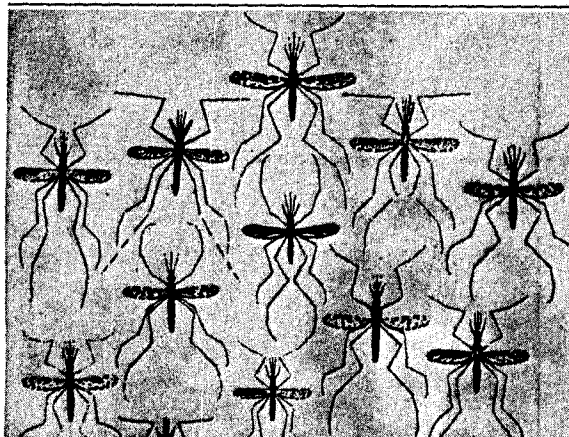
- (1) Proceedings of the ICMR/WHO Workshop on *Community Participation for Disease Vector Control* (1986) pp. 256  
*Edited by V.P. Sharma*
- (2) *Seroepidemiology of Human Malaria — A multicentric study* (1989), pp. 206  
*Edited by V.P. Sharma*
- (3) *Indigenous Larvivorous Fishes of India* (1991), pp. 66  
**A.G.K. Menon**
- (4) Proceedings of an Informal Consultative meeting WHO/MRC on *Forest Malaria in Southeast Asia* (1991), pp. 206  
*Editors V.P. Sharma and A.V. Kondrashin*
- (5) *Malaria Patrika* quaterly (Hindi) 1993 onwards.
- (6) *Community Participation in Malaria Control* (1993), pp. 295  
*Edited by V.P. Sharma*
- (7) *Larvivorous Fishes of Inland Ecosystem: Proceedings of the MRC-CICFRI Workshop* (1994), pp. 224  
*Editors V.P. Sharma and Apurba Ghosh*

# INDIAN ANOPHELINES

by

**B.N. NAGPAL • V.P. SHARMA**

## INDIAN ANOPHELINES



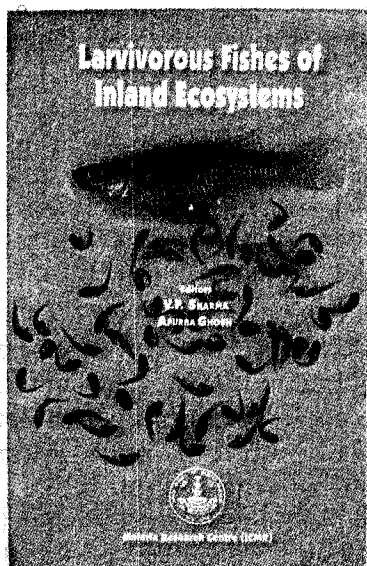
B.N. Nagpal • V.P. Sharma

**ISBN81-204-0929-9**  
**Size : Crown 4TO**  
**Price: Rs. 750/-**  
**pp. viii, 416 (Hardbound)**  
**1995**

Indian Anophelines is the first book of its kind on the fauna of anopheline mosquitoes from India. The book assumes special importance because of the deteriorating malaria situation in India, complicated by vector resistance to insecticides, ecological succession of mosquitoes, invasion of mosquitoes to new areas, as also their disappearance from certain areas. As a result mosquito fauna has undergone major changes and this precise knowledge at the local level in endemic regions is invariably lacking. Often the identification is made difficult due to variations in many appendages. For each anopheline species the book provides names, derivatives, type form availability, resting habits, breeding ecology, biting time, flight range, susceptibility to insecticides, relation to disease, reported distribution in India and the world, and results of vector incrimination studies.

© OXFORD & IBH PUBLISHING CO PVT. LTD.  
66, Janpath, New Delhi-110 001.





*An exhaustive volume which brings out the state of art and scope of larvivorous fishes in the management of vector-borne diseases.*

**Size: Royal 8 Vo**  
**pp. ix, 224 (Hardbound)**  
**1994**

*A complete review of the experience on community participation from various areas in the country in relation to vector/malaria control. A commendable document for future management of vector control through community participation.*

**Size: Crown 4To**  
**pp. vii, 295 (Hardbound)**  
**1993**

### *Community Participation in Malaria Control*



*Edited by*  
*Dr. V.P. Sharma*

## VIDEO FILMS PRODUCED BY MALARIA RESEARCH CENTRE

### DOCUMENTARIES

**Fighting Malaria** (English)

Master Tape No. 2001

**Malaria Control in Shahjahanpur**  
(English)

Master Tape No. 6003

**Malaria Control in Shahjahanpur**  
(Hindi)

Master Tape No. 6001

**Defeating the Invincible - Hardwar**  
(English)

Master Tape No. 6004

**A Seven Point Action Programme for  
Malaria Control in Madras** (English)

Master Tape No. 2010

**Tackling Malaria in Orissa** (English)

Master Tape No. 2011

**Insecticide Impregnated Bednets for  
Malaria Control** (Assamese)

Master Tape No. 2008

**Insecticide Impregnated Bednets for  
Malaria Control** (English)

Master Tape No. 2006

**Man Made Malaria** (English)

Master Tape No. 2002

**Sirf Ek Muskan** (Hindi)

Master Tape No. 2078

**Ek Anootha Prayog** (Hindi)

Master Tape No. 2003

**Insecticide Impregnated Bednets for  
Malaria Control** (Hindi)

Master Tape No. 2061

**Malaria Control in Madras** (English)

Master Tape No. 2153

**Man, Mines and Malaria** (English)

Master Tape No. 2018

**Mosquito Menace** (English)

Master Tape No. 6049

**A Seven Point Action Programme for  
Malaria Control in Madras** (Tamil)

Master Tape No. 2208

### SCIENTIFIC DISCUSSION

**Synthetic Malaria Vaccine: A Hope  
for Future** (English)

Master Tape No. 2121

**Malaria Vaccine: A Perspective**  
(English)

Master Tape No. 2204

**Malaria Vaccine : A State of Art**  
(English)

Master Tape No. 2122

**Malaria Vaccine : Status and Future  
Prospect** (English)

Master Tape No. 2211

**M-10, A New Environment Friendly Insecticide for Disease Vector Control** (English)

*Master Tape No. 2212*

**Global Malaria Control – An Approach Plan** (English)

*Master Tape No. 2275*

**Chelating Agent in Severe Malaria**

*Master Tape No. 2140*

### **TEACHING PROGRAMMES**

**Life cycle of Malaria Parasite**

(English)

*Master Tape No. 2247*

**The Microscope** (English)

*Master Tape No. 2240*

**How to Make a Blood Smear and Stain for Malaria Parasite** (English)

*Master Tape No. 6052*

**How to Treat Uncomplicated Malaria** (English)

*Master Tape No. 6045*

**Cerebral Malaria** (English)

*Master Tape No. 2200*

**Malaria in Pregnancy** (English)

*Master Tape No. 6060*

**Laboratory Diagnosis of Malaria** (English)

*Master Tape No. 6066*

### **HEALTH EDUCATION**

**Malaria – Bednets a TV Spot** (Hindi)

*Master Tape No. 2013*

**Malaria – Bednets a TV Spot** (English)

*Master Tape No. 2072*

**Malaria – Spread the Knowledge**

(English)

*Master Tape No. 2071*

**Malaria – Mukti Pavoo** (Hindi)

*Master Tape No. 2236*

**Malaria – Arivay Parappivoo** (Tamil)

*Master Tape No. 2214*

**Malaria – Gnanava Haradona**

(Kannada) *Master Tape No. 2261*

**Malaria – Overhead Tanks and Malaria Control – A TV Spot** (Tamil)

*Master Tape No. 2282*

---

Cost of each cassette is Rs. 100.00 + postal charges for 2 cassettes Rs 18.00; 3-4 — Rs. 24.00 and for 5 — Rs. 30/-.

These cassettes could be obtained by sending crossed **Demand Draft**, drawn in favour of “**Director, Malaria Research Centre, Delhi**”, and send to the Assistant Director, A.V.P. Unit, Malaria Research Centre, 2, Nanak Enclave, Delhi-110 009.

## UNDERTAKING BY AUTHORS

submitted for publication in the **Indian Journal of Malaria** :—

**Signature of Authors**

- |    |  |  |
|----|--|--|
| 1. |  |  |
| 2. |  |  |
| 3. |  |  |
| 4. |  |  |
| 5. |  |  |
| 6. |  |  |
| 7. |  |  |
| 8. |  |  |

1. All authors are required to sign independently in the form and in the sequence given above. A photocopy of this form may also be used.
2. No addition/deletion/ or any change in the sequence of the authorship will be permissible at a later stage, without valid reasons. If change is valid, then all the authors involved should attest to the change. The decision however, rests with the Editor.
3. If the authorship is contested at any stage, the article will be either returned or will not be processed for publication till the dispute is dissolved.

\* Please write the applicable statement below:

## 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)\*  
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)

Annual Subscription { India Rs. 75.00+  
Foreign US \$ 20.00  
+25% discount for individuals

\*Back issues are available at old rates, i.e. Rs. 30.00 or \$ 10.00

The Editor  
Indian Journal of Malariology  
Malaria Research Centre  
20-Madhuvan  
Delhi-110 092 (India)

Sir,

I enclose herewith a bank draft/postal order(s) No.(s) .....  
for \$/Rs. .... (in favour of the Director, Malaria Research Centre, Delhi) towards  
subscription/payment for **Indian Journal of Malariology** for the year(s) .....  
(2/4 Nos.). The journals should be mailed to me/my client at the following address:

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