# EPIDEMIOLOGY

## Therapeutic Efficacy of Chloroquine and Sulphapyrimethamine

### (i) In Uncomplicated falciparum Malaria (WHO Funded Project under RBM-TSN/DRP)

A standardized protocol has been developed by WHO to assess the therapeutic efficacy of antimalarial drugs against clinically manifested infection with *P. falciparum* in individuals of various age groups. The therapeutic efficacy protocol is based on clinical and parasitological responses of the patients and it has the purpose of determining the practical efficacy of the drug regimen in study areas with the ultimate objective of ascertaining its continued usefulness or the necessity for replacing it in the routine treatment.

Present study has been conducted at seven sites— Kathiatali and Simonabasti of District Nowgong, Assam; Sonapur and Boko of District Kamrup, Assam; Keonjhar Town, Padmapur and Basudepur of District Keonjhar, Orissa. In order to reduce the patient recruitment time, health centre close to well-defined community was identified to conduct the activities at peak malaria season by selecting local pockets and organizing mobile clinics. Microscopically confirmed cases of *P. falciparum* were enrolled according to the inclusion and exclusion criteria. Treatment with recommended drug was given under supervision and the test schedule to follow-up the patients at various intervals for 28 days was maintained. Assessment of efficacy of both chloroquine (CQ) and sulphapyrimethamine (SP) was conducted at five sites each.

## HIGHLIGHTS

- Therapeutic efficacy studies of chloroquine in falciparum malaria have shown up to 30% treatment failure of CQ in northeast states and 3% in Orissa
- Therapeutic efficacy studies of chloroquine in uncomplicated vivax malaria in Navi Mumbai, Gautam Budh Nagar and Chennai City showed no treatment failure of CQ
- Evaluation of *Pf* diagnostic kit— ICT Binax showed 100% sensitivity and specificity in detection of *P. falciparum*
- Malaria trend, spatio-temporal dynamics and epidemic cycle in Mewat region of Haryana were worked out using GIS
- Situation analysis of malaria in Gadchiroli district, Maharashtra emphasized the need of health education and community involvement for malaria control
- Impact of climate on malaria was studied in Tumkur (Karnataka) and Bikaner (Rajasthan)
- Analysis of correlationship between malaria cases and meteorological indicators indicated that there is positive as well as negative relationship with meteorological indicators in different areas
- A field trial site for malaria vaccine is being developed

In CQ study areas, wherever patients showed treatment failure to CQ were treated with second line drug— SP and they were then followed-up as per study protocol. It is observed that 30% cases showed treatment failure to CQ in District Nowgong (NK), where revised drug policy has already been introduced. In Kamrup district (KS and KB), treatment failure with CQ was less than 25%, which denotes the said regimen is still effective. Almost all the patients from Padmapur and Basudepur of District Keonjhar responded to CQ, treatment failure was noticed only in two patients (3%). Treatment with SP showed adequate clinical and parasitological responses in all the patients except one in KS, Kamrup.

**Table 1. Baseline characteristics in chloroquine treated patients**

| Parameters | Navi Mumbai | G.B. Nagar |
|---|---|---|
| Drug dose (over 3 days) | 25 mg/kg | 25 mg/kg |
| No. of cases | 85 | 48 |
| Males/Females | 68/17 | 31/17 |
| Age (range) | 1–65yrs | 3–59 yrs |
| % patients with fever (Day 0) | 55 | 45.8 |
| H/o use of antimalarials | None | None |
| *Adverse effects* | | |
| Vomiting | 8.2% | — |
| Giddiness | 2.3% | — |
| Purities | — | 6.9% |
| Parasitaemia (range) | — | 280–10,960 ?l |
| *Fever clearance time* | | |
| 24 h | 87% | 95% |
| 48 h | 100% | 95% |
| 72 h | 100% | 100% |
| *Parasite clearance time* | | |
| 48 h | 98.8% | 100% |
| 72 h | 100% | — |

### (ii) In Uncomplicated vivax Malaria

**Navi Mumbai:** Study was conducted in collaboration with Navi-Mumbai Municipal Corporation at six urban health posts—CBD, Karave, Nerul, Sanpada, Koparkhirne and Airoli. Up to December 2002, 85 patients of *P. vivax* malaria were enrolled and 28 day follow-up was conducted. The baseline characteristics of patients are listed in Table 1. All patients responded to treatment with chloroquine.

**Gautam Budh Nagar (Uttar Pradesh):** The district has seasonal transmission of malaria and is epidemic prone. A total of 48 (31 males + 17 females) patients of vivax malaria in the age range of 3–59 years were enrolled from PHC Dadri, Distt. Gautam Budh Nagar. There was no treatment failure up to Day 28 in this group of patients (Table 1).

**Chennai City (Tamil Nadu):** The study was undertaken in Sowcarpet, which is situated in the northern coastal belt of Chennai. The area is hyper endemic for malaria and the transmission is perennial. Study was conducted from the Central Malaria Laboratory of the Chennai Corporation located at Basin Water Works street (Div. 38) in Sowcarpet area. All fever cases reporting at the clinic were screened for malaria positivity by the Chennai Corporation staff. Patients with vivax malaria were sent to the MRC clinic established in the same building for enrollment in the study. Up to December 2002, 143 (130 males + 13 females) cases were enrolled of which 136 (95.1%) cases were adults. No recrudescence was observed in any patient in 28 day period. The parasite clearance time was 2 days in 87.8% patients, 3 days in 8.7% and >3 days in 3.5% (Table 2).

**Table 2. Analysis of parasite count in patients**

| Day | No. of cases | Mean parasites/?l | SD | Minimum | Maximum |
|---|---|---|---|---|---|
| Day 0 | 115 | 7237.7 | 6499.03 | 352 | 39680 |
| Day 2 | 115 | 32.14 | 163.46 | 0 | 1360 |
| Day 3 | 115 | 13.2 | 18.0 | 0 | 120 |
| Day 7,14, 21 and 28 | 115 | Nil | Nil | Nil | Nil |

## Evaluation of Diagnostic Kit— ICT Binax

In continuation of earlier studies evaluation of ICT binax diagnostic kit based on detection of HRP-2 was done. The results are shown in Table 3. The study showed that ICT binax kit is highly sensitive and specific for the detection of *P. falciparum.*

**Table 3. Results of ICT Binax diagnosis**

| Microscopic diagnosis | No. tested | Test kit result | |
|---|---|---|---|
| | | +ve | –ve |
| *P. falciparum* | 77 | 77 | 0 |
| *P. vivax* | 40 | 0 | 40 |
| –ve | 32 | 0 | 32 |
| Total | 149 | 77 | 72 |

## Association of Leptospirosis in Patients of Severe falciparum Malaria

Severe and complicated falciparum malaria presents with many complications like cerebral malaria, jaundice, acute renal failure, etc. Among these, acute renal failure occurs in < 1% cases but the mortality from these cases are reported up to 45%. Data from Ispat General Hospital, Rourkela shows nearly two fold increase in the number of patients with severe complications like acute renal failure and jaundice over a period of five years. Leptospirosis is an acute anthropozoonotic infection prevalent worldwide and is emerging as an important public health problem in India. The clinical picture of leptospirosis mimicks severe and complicated malaria especially that of acute renal failure and jaundice. The recent increase of acute renal failure and jaundice among malaria patients at Rourkela may be due to leptospirosis alone or in combination with malaria. Hence, a collaborative study was undertaken to rule out the presence and/or association of leptospirosis among these patients. A detailed clinical, hematological and biochemical examination was done in these patients. There were 13 severe malaria patients (microscopy/ICT +ve 8; microscopy/ICT –ve 5) and 8 uncomplicated malaria (microscopy/ICT +ve). Serum samples were tested for leptospirosis by Leptospira IgM specific Agglutination Test and Lepto Tek Dri Dot Test at NICD, Delhi. None of these were positive by serology for leptospirosis. The study will be continued with larger sample size representing all age groups.

## Spatio-temporal Dynamics of Malaria in Mewat

### Malaria Trend and Epidemic Cycle

In Mewat, API during 1991-2001 revealed that from 1991–1993 there was a declining trend and the API reached below 2, later during 1994 and 1995 API increased to ~ 5. In 1996, the API was around 33, statistically more than average ±2 S.D.) and was declared as an epidemic year. Thereafter a decline was observed and the API in 1998 reached below 2, subsequently by 2001 it was < 0.5 (API < 2 is considered as very low risk area and need not ot be covered by indoor residual spraying as per NAMP norms) (Fig. 1). Statistically malaria incidence in the

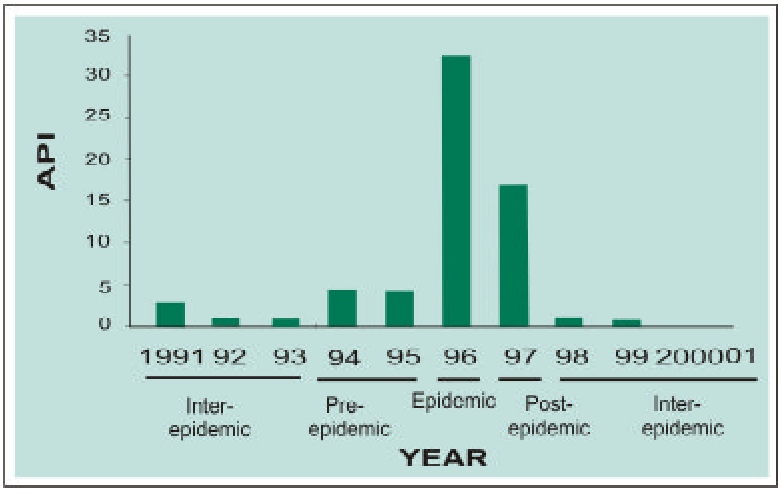


*Fig. 1:* Annual parasite incidence in Mewat from 1991 to 2001 showing different epidemic phases

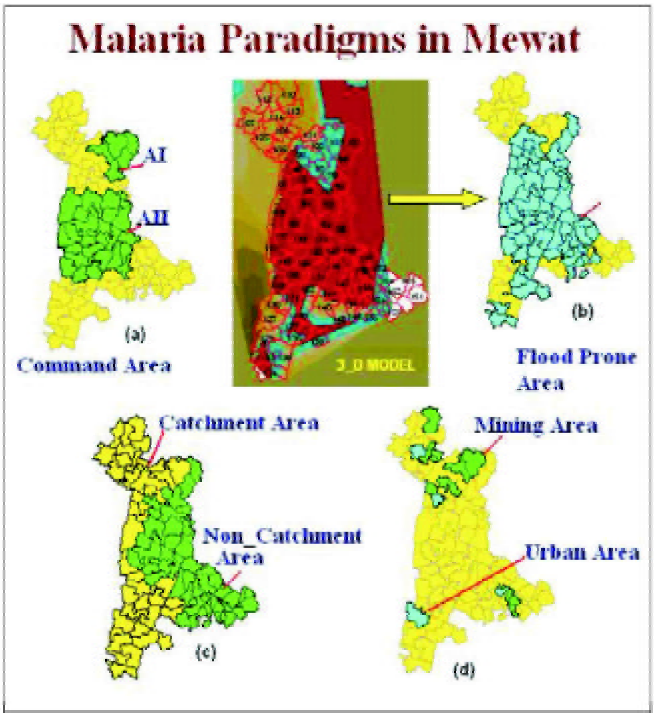


*Fig. 2:* (a) Two command areas AI and AII in Mewat; (b) Flood prone areas; (c) Catchment and non-catchment areas; and (d) Urban and mining areas

years 1993 and 1998 were found similar. Thus the entire cycle, 1991 to 2001, a span of 11 years was classified into different epidemic phases, such as inter-epidemic (1991–93), pre-epidemic (1994–95), epidemic (1996), post-epidemic (1997) and once again as the inter-epidemic phase (1998–2001). A post-epidemic investigation in 1996, revealed that *P. vivax* was the prominent species of malaria but *P. falciparum* was also prevalent in some pockets.

## Identification of Paradigms

Using GIS, based on geographic reconnaissance, ecological and socioeconomic profile initially five malaria paradigms—irrigation command, catchment, mining, urban and flood prone areas were identified (Fig. 2). Section-wise map of the area was overlaid on thematic maps to delineate the sections falling in 5 malaria paradigms. Each paradigm exhibited its own eco-epidemiological characteristics and potential for maintaining malaria transmission of varying intensity.

## Malaria Receptivity by Paradigm

It may be seen from the Fig. 3 that during 1991 overall API in all the paradigms was around 5, then declined to around 2 by 1993, again increased during 1994 and 95 representing pre-epidemic phase when it touched around 7 API in all paradigms. Epidemic phase started in 1996, 1997 was the post-epidemic year and 1998–1999 once again represent the inter-epidemic period. During 1996 different paradigms responded differently, maximum amplification occurred in urban/semi urban paradigms with API about 45. This was followed by flood prone, command area A-II and noncatchment paradigms which exhibited the same amount of amplification and the API reached around 40. Mining paradigms showed about 20. The lowest malaria incidence was observed in command area AI (API about 10). By 1998 malaria incidence reached below 2 in all the paradigms. Further decline continued to reach API below 1 by 1999 subsequently in 2000 and 2001 API reached below 0.5 in all paradigms.

Spatio-temporal distribution map of malaria for the years 1991 to 2001 depicted spatial spread of various epidemic phases (Fig. 4). The sections other than < 2 API were extracted for the years 1993 and 1998 and overlaid on paradigm maps to study eco-epidemiological profile of malaria during inter-epidemic phase. It revealed that API in 1993 and 1998, the years of similar malaria situation in the last two inter-epidemic periods, flood prone area, irrigation

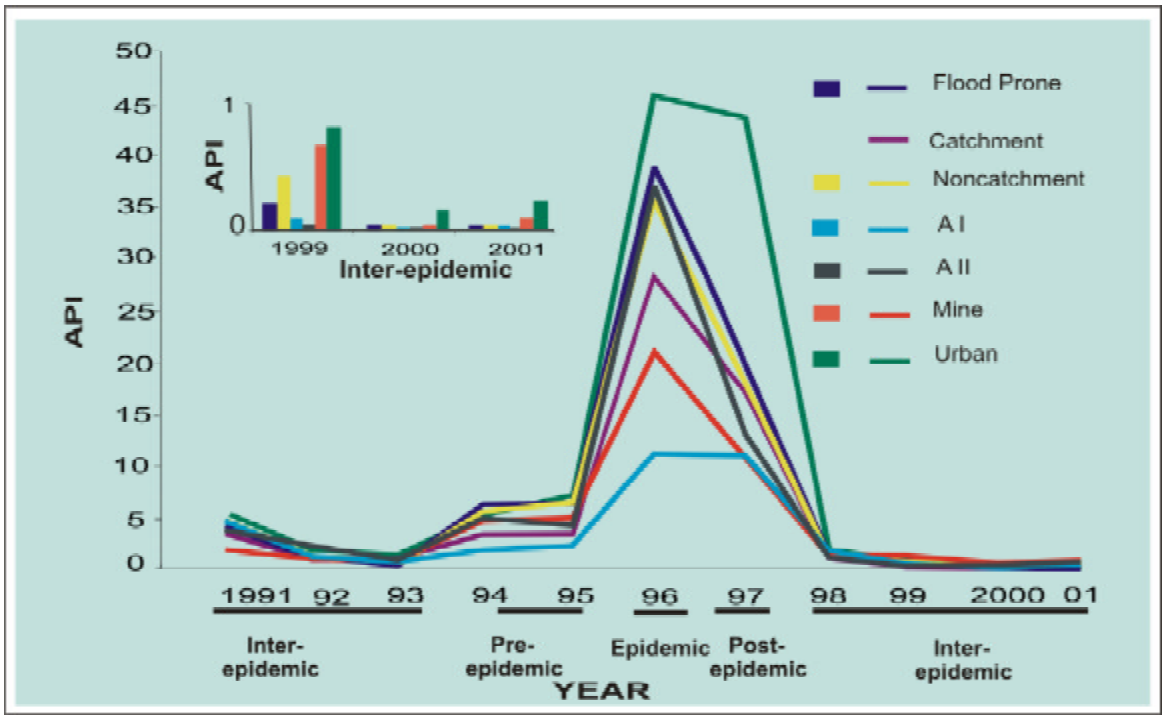


*Fig. 3:* Paradigm-wise different epidemic phases in Mewat (1991–2001).

command area AII and noncatchment area retained as active pockets of malaria transmission. Amplification started during 1994–1995 (pre-epidemic phase) and engulfed all paradigms by 1996 (epidemic year). Therefore, there is a need for quantitative assessment through field surveys in these paradigms to explain above phenomenon of residual malaria and to identify 'epidemic risk factors' to prevent future epidemics.

## Serological Profile following Malaria Outbreak in Mewat Region of Haryana

Mewat region of Gurgaon district in Haryana experienced heavy rain and inundation in 1996, followed by a severe malaria outbreak. High incidence of *P. falciparum* infection and deaths were reported though control measures were taken at a large-scale to combat the epidemic situation of the area by government and local agencies. Assessment of the efficacy of intervention measures to control malaria transmission, however, received little attention.

In addition to this nothing was known about the antimalarial immune status and parasitological exposure level of the population. In an attempt to understand

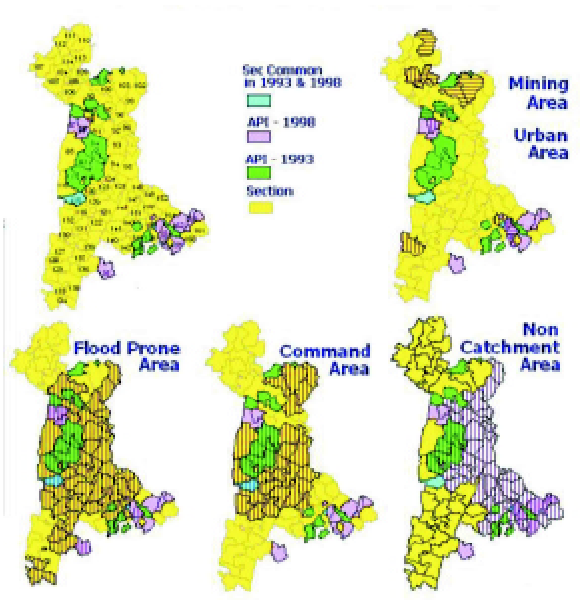


*Fig. 4:* GIS identifies malaria active sections during inter-epidemic period and their related paradigms. It revealed that active pockets confined to three paradigms—flood prone, non-catchment and command area AII.

**Table 4. Anti AR1 and anti *Pf* antibody (IgG) levels in the finger prick blood samples collected from residents of CHCs Nuh and Firozepur Jhirka in the aftermath of malaria outbreak (1997 and 2001)**

| Year | Village | Total no. of blood samples | Mean±SD ELISA $OD_{490}$ | | % Mean seropositivity | |
|---|---|---|---|---|---|---|
| | | | AR1 | *Pf* | AR1 | *Pf* |
| 1997 | FN | 211 | 0.96±0.15 | 0.90±0.09 | 98.8 | 98.2 |
| | SL | 195 | 0.66±0.25 | 0.74±0.28 | 82.0 | 82.2 |
| | ML | 261 | 0.79±0.20 | 0.95±0.16 | 96.0 | 95.6 |
| | KH | 179 | 0.83±0.17 | 0.80±0.15 | 96.4 | 95.8 |
| 2001 | FN | 47 | 0.65±0.24 | 0.56±0.22 | 69.8 | 68.9 |
| | SL | 83 | 1.01±0.23 | 0.93±0.22 | 98.8 | 98.6 |
| | ML | 70 | 0.95±0.26 | 0.75±0.19 | 96.4 | 94.8 |
| | KH | 48 | 0.77±0.19 | 0.68±0.18 | 89.6 | 85.5 |
| | FN (School children) | 45 | 0.436±0.064 | 0.53±0.07 | 80.0 | 17.0 |

FN—Firozepur Namak; SL—Salmbha; ML—Malabh; KH—Khedla.

the epidemic situation and subsequent consequences, cross-sectional surveys were conducted during February–March 1997 and April 2001 to study the antimalaria immune status of the residents of four villages of community health centres (CHCs) Nuh and Firozepur Jhirka, using ELISA as a tool and parasitological exposure level by microscopic blood slide examination.

Seroepidemiological observation in the aftermath of outbreak showed high titers of IgG antibody directed against AR1 synthetic peptide (EENVEHDA–C) and *P. falciparum* crude antigens. Parasitological results—slide positivity rate (SPR), slide falciparum rate (SfR) and per cent *Pf* were observed to be low during these surveys. A negative relationship was noticed between the levels of anti AR1, *Pf* antibody and parasitological results in the residents (Table 4).

Seroepidemiological studies could be used to evaluate the immune status of the population, transmission pattern and assessment of efficacy of intervention measures. The data could also be utilized for effective surveillance of malaria.

## Seroepidemiological Assessment of Resurgence of Malaria in Haldwani Area, after withdrawal of the Bioenvironmental Control Programme

Cross-sectional seroepidemiological studies were undertaken in Haldwani area including plain and forested areas in pre and post-bioenvironmental control programme (implemented in 1986) to assess the actual malaria situation in the area and the impact of control programme. Bioenvironmental control programme was withdrawn (in 1990's) as malaria situation was improved and decline in malaria incidence was reported. Before the implementation of bioenvironmental control programme, sero-reactivity against AR1 antigen (a nonapeptide [EENVEHDA(C)] representing an epitopes in the 3 carboxy terminal immunodominant repeat region of the ring infected erythrocyte surface antigen (RESA/*Pf* 155) of *P. facipuram* was observed to be 0.695±0.150 and 0.628±0.105 in children population, but after the implementation, seroreactivity decreased considerably. The AP1 and ELISA O.D. recorded in children population in 1991 in nontransmission and transmission periods were 0.244±0.089 and 0.134±0.064

respectively in the years 1992 and 1993, it remained low (0.145± 0.058 and 0.269±0.09) indicating improvement in malaria situation. Antimalarial antibody levels measured in 368 small children from the plain area in 1997 showed moderate levels of anti AR1 IgG antibody. High levels of anti AR1 IgG antibody were observed in adult population in the forested area in 1997. Gradual rise in malaria incidence was noticed in Haldwani area as 456 active fever cases with 11.2 SPR and 1.1 SfR in 1998 and 625 fever cases with 18.9 SPR and 9.9 SfR in 1999 were reported, supporting our observation and confirms the resurgence of malaria (Tables 5). Using linear regression analysis of ELISA O.D. values and known annual parasite index (API) of a given area a formula was developed to calculate equivalent transmission index (ETI=270.55/ELISA O.D.±7.40) from which the level of endemicity could be estimated. Five villages from plain areas were considered for estimating malaria status through serology. Each village showed moderate anti AR1 ELISA O.D. which indicates a moderate malaria transmission. On comparison of seropositivity and equivalent transmission index, the values confirms the same.

**Table 5. Impact of bioenvironmental control programme on the seroreactivity against AR1 *P. falciparum* antigen and malariogenic condition of Haldwani region (1989-2000)**

| Year | Month | Mode of transmission | Total no. of children population | AR1 ELISA O.D. | API | SPR | ETI |
|---|---|---|---|---|---|---|---|
| 1989 | Oct | Peak | 208 | 0.695 ± 0.150 | 52.2 | 20.4 | 196.8 |
| 1990 | Jan–Feb | Non | 684 | 0.628 ± 0.105 | 42.3 | 20.3 | 177 |
| 1991 | Jan–Feb | Non | 161 | 0.24 ± 0.089 | 17.4 | 17.5 | 72.3 |
| 1991 | Nov | Peak | 104 | 0.134 ± 0.064 | 5.2 | 5.8 | 43.6 |
| 1992 | Sep/Oct | Peak | 104 | 0.145 ± 0.058 | 4.2 | 7.5 | 46.6 |
| 1993 | Jan | Non | 146 | 0.269 ± 0.090 | 6 | 6.8 | 88.4 |
| 1997 | Feb–Mar | Non | 368 | 0.42 ± 0.069 | – | – | 118.31 |
| 2000 | Feb–Mar | Non | 84 | 0.73 ± 0.15 | – | – | 205.13 |

Whereas in two forested villages where implementation of control programme is impossible due to lack of accessibility, malaria incidence was very high. Annual parasite index collected from MRC field stations were 231.2 and 162.99 for HP and JL villages. Accordingly, mean AR1 ELISA O.D., percentage seropositivity and equivalent transmission index, all the values are very high which indicates the correlation of parasitological and serological data (Table 6).

**Table 6. Village-wise malaria status in plain and forested area of Haldwani during February–March 1997 in all age groups after the withdrawal of bioenvironmental control programme**

| Area | Villages | No. examined | Mean AR1 O.D. ± S.D. | % Seropositivity | ETI |
|---|---|---|---|---|---|
| Plain | AR | 91 | 0.43 ± 0.08 | 55.9 | 118.41 |
| | SNBC | 19 | 0.42 ± 0.09 | 51.0 | 115.90 |
| | NVC | 171 | 0.40 ± 0.14 | 56.5 | 113.03 |
| | RP | 58 | 0.35 ± 0.09 | 36.2 | 98.73 |
| | JN | 29 | 0.54 ± 0.15 | 51.0 | 145.48 |
| Forest | HP | 231.2 | 0.85 ± 0.22 | 89.10 | 221.73 |
| | HP2 | 23.12 | 0.92 ± 0.13 | 96.00 | 238.95 |
| | JL | 162.99 | 0.69 ± 1.80 | 86.86 | 182.62 |

Cut of value for AR1 = 0.35 ELISA O.D.

Blood samples from three villages were collected from nonmalarial in-

**Table 7. Anti AR1 IgG antibody titer in adult population of Haldwani (Plain area) during March 2000 (Nontransmission season)**

| Area | No. examined | AR1 ELISA O.D. | ETI |
|---|---|---|---|
| Daval Chaur | 33 | 0.67±0.18 | 188.66 |
| Haripur Motia | 28 | 0.78±0.13 | 218.43 |
| Kishan Pur | 23 | 0.75±0.14 | 210.31 |

Cut off value for AR1 = 0.35 ELISA O.D.

dividuals. Each village showed moderate to high transmission as per anti ARI ELISA O.D. and equivalent transmission index. From our hypothesis, this information indicates that villagers experienced malaria in the previous year of sample collection (Table 7). In conclusion, seroepidemiological information can estimate the malaria status of a population much better compared to other classical methods.

## Delineation of Breeding Habitats and Landscape Features Suitable For *An. culicifacies* Abundance using Satellite Remote Sensing (ICMR Task Force Project)

The study was continued in three selected PHCs of Tumkur district. Entomological surveys including positivity of breeding habitats, emergence of adult species and man hour density of anopheline vectors (indoors) were undertaken along with ecological changes in January and June 2002. Parasitological surveys for point prevalence were also carried out.

Satellite data (IRS 1C/D LISS III and PAN) products were procured for the dates of entomological surveys. False colour composite/hybrid colour composite images were generated from merged products of LISS III and PAN. Supervised classification was done and statistics of land use features like water bodies, barren area, rocks with vegetation, barren rocks, etc. were generated in respect of 27 villages for May 2001. Based on data of January 2002, FCCs images were generated and statistics of land use features were also generated in respect of six villages of Tovinkere PHC. Findings are given below:

(i) Tanks, streams, ponds, marshy areas and irrigation wells were found as breeding habitats of *An. culicifacies* in the decreasing order.

(ii) May/June month was found critical in differentiating entomological and ecological parameters supporting *An. culicifacies* populations.

(iii) Analysis of satellite data revealed that tanks, streams, ponds, marshy areas were detectable by LISS III and PAN merged products. Irrigation wells whose positivity for *An. culicifacies* was insignificant, were not detectable.

(iv) The difference in land use features in villages of high and low malaria categories indicated that in villages of high malaria incidence presence of water in water bodies (0.36–35.78%), more vegetation cover (24.9–85.72%), less barren area and scrub (0–12.9%), less barren rocks (0.13–9.68%) as compared to 1.44–3.2% water bodies, 15.07–34.19% vegetation cover, 8.57–30.21% barren area and scrub, and 0.35–20.33% barren rocks in least malarious area (Byalya). The presence of streams and tanks in the vicinity of human settlements were found more productive for breeding of *An. culicifacies.*

(v) The village-wise analysis of satellite image revealed that remote sensing may be used for ecological change detection at village level and for stratification of high/low malarious areas.

## Impact of Climate Change on Malaria in India

As a part of Ministry of Environment and Forests, Government of India, preparation of India's Initial National Communication (NATCOM) to the UNFCCC on vulnerability assessment and adaptive measures due to climate change, a study was undertaken on the impact of climate change on malaria in India.

Based on monthly fluctuations in malaria cases in the year 2000 (NAMP data), regions vulnerable to climate change were identified. Keeping in view the minimum requirement of temperature and relative humidity (RH) for development of *P. vivax* and *P. falciparum* parasites, transmission windows (TWs) of malaria in different cities (representing concerned state) were determined. Based on the areas vulnerable to climate change were further identified.

The projected rise in temperature (T) due to climate change (1.5, 2.4 and 3.8°C by the year 2020, 2050 and 2080 respectively) and precipitation (2 $\pm$ 1% by 2020, 3 $\pm$ 1% in 2050 and 7 $\pm$ 3% by 2080) were added in monthly mean temperature and RH of baseline year 2000. The TWs were determined based on the TWs temperature alone as well as in combination of T and RH.

When we look at the projected temperature in the years 2020, the TW is likely to increase by one month in northeastern states, Rajasthan, Uttar Pradesh and Gujarat while by two months in J&K, Himachal Pradesh and Madhya Pradesh. The TW is likely to remain unaffected in Andhra Pradesh, Chhattisgarh, Haryana, Punjab, Karnataka, Kerala, Maharashtra, Orissa, Tamil Nadu, Uttaranchal and West Bengal. Since malaria transmission dynamics is complex, affected by rainfall pattern, agricultural practices, socio-economic conditions and the intervention measures undertaken, projection of malaria based on temperature alone may not hold true. If intervention measures being practiced in India are implemented effectively, the whole scenario of malaria may change (drastic reduction), let temperature or rainfall be suitable.

Longitudinal case studies were undertaken in Tumkur (Karnataka) and Bikaner (Rajasthan) districts to find out correlation of climatic factors—temperature, RH and rainfall with malaria to find out suitable adaptive measures. Surveys on socio-economic conditions prevalent in the areas were also made to assess the impact of climate change on socioeconomic conditions and thereby on malaria. The results of the case studies in Tumkur revealed that temperature fluctuation during different years is not much but the rainfall pattern at the threshold of TW helps in providing suitable RH for effective transmission of malaria. In Bikaner district, the rainfall at the threshold of TW for at least two consecutive months is important in causing increase in malaria cases. The overall analysis of correlationship between malaria cases and meteorological indicators indicate that there is positive as well as negative relationship with meteorological parameters in different areas.

## SITUATION ANALYSIS

**District Ghaziabad (U.P.):** Study was carried out in two PHCs Garh Mukteshwar (high risk of malaria) and Dasna (low risk of malaria) in Ghaziabad district (U.P.) from 7 to 17 October

**Table 8. Epidemiological indices in PHC, Garh Mukteshwar, Distt. Ghaziabad (U.P.)**

| Village | Population | TBS | SPR | SfR | Cases/000 | *Pf*/000 |
|---|---|---|---|---|---|---|
| Nanu Pura | 5505 | 7 | 0 | 0 | 0 | 0 |
| Bhagwantpur | 392 | 3 | 33.3 | 33.3 | 2.5 | 2.5 |
| Jireena | 3415 | 8 | 12.5 | 0 | 0.29 | 0 |
| Salar Pur | 2741 | 5 | 0 | 0 | 0 | 0 |
| Sharifabad | 790 | 2 | 0 | 0 | 0 | 0 |

2002. Parasitological survey was carried out in both the PHCs for few days and was compared with the data collected by DMO, Ghaziabad In PHC, Garh Mukteshwar the slide positivity rate (SPR) ranged from 0 – 33.3 and SfR also ranged from 0–33.3 (Table 8). In PHC, Dasna no case was reported. Malaria cases recorded by the District Malaria Officer in five villages of each PHC based on active case detection at an interval of 15 days were nil in these villages. Entomological observation revealed that *An. culicifacies* was predominant species and per man hour densities ranged between 7 and 27 in Garh Mukteshwar PHC and in Dasna PHC *An. culicifacies* man hour density was in between 7 and 14 during October. Susceptibility test was carried out as per WHO procedure and found that *An. culicifacies* was resistant to DDT in both the PHCs (Table 9).

## District Gadchiroli (Maharashtra)

Maharashtra government has been able to reduce malaria in most of the districts but the problem in Gadchiroli district was still persisting in spite of implementation of the main tools of intervention—indoor residual spray (IRS) by deltamethrin and fever radical treatment (FRT) to the best possible. An investigation was made to find out the reasons of persistence of malaria in Gadchiroli district during November 2002.

Annual blood examination rate was found to be ranging from 49 to 94 indicating that the health workers were reaching to community efficiently. *Pf* % during 1997 to 2001 ranged from 51 to 66%. The slide positivity rate ranged from 1 to 2.72. The coverage by two rounds of IRS (synthetic pyrethroids) was around 90% and for better compliance to radical treatment,

**Table 9. Result of insecticide susceptibility test in PHC, Garh Mukteshwar, Distt. Ghaziabad (U.P.)**

| Insecticide | Species | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *An. culicifacies* | | | | | *Cx. quinquefasciatus* | | | | |
| | Doses (%) | No. exposed | Exposed period (hr) | % knock-down | Corrected mortality | Doses (%) | No. exposed | Exposed period (h) | % knock-down | Corrected mortality |
| Deltamethrin | 0.02 | 15 x 2 | 1 | 100 | 100* | 0.02 | 25 x 2 | 1 | 44 | 100* |
| DDT | 4 | 15 x 2 | 1 | 1 | 0 | 4 | 25 x 2 | 1 | — | 0 |
| Malathion | 5 | 15 x 2 | 1 | 100 | 100 | 5 | 25 x 2 | 1 | 38 | 100 |

*100% knock-down was obtained within 15–25 min of exposure.

blister pack was introduced containing 4 tablets of chloroquine of 600 mg each and 4 tablets of 45 mg primaquine.

Detailed parasitological investigations revealed that 25% of positive cases detected during the survey were having gametocyte stage of *P. falciparum* parasite. It indicates that compliance to FRT was not satisfactory. Entomological findings revealed that the main vector, *An. culicifacies* was resting indoor and was 100% sensitive to the sprayed insecticide—deltamehthrin. *An. fluviatilis* was encountered rarely. Results of cone bioassay indicated that on the wall surface sprayed before 10 days, mortality of *An. culicifacies* was around 68%, while the mortality was only 13% on the surface sprayed before one month. It indicates that the quality of spray was not satisfactory and required close surpervision. It was learnt that just after second round of spray in October, the inhabitants got their houses smeared/white washed.

A deep insight into the reasons of persistence of malaria revealed that the intervention measures were being affected by the social factors like locked houses, not allowing spray in all the rooms and smearing of houses by mud/white wash. FRT was affected by the approach of inhabitants to seek the help of local quacks rather health services and not taking full course of antimalarials. The overall findings emphasize the importance of health education to community and their involvement in malaria control for achieving best results.

## Development of a Field Site for Malaria Vaccine Trial (A Collaborative Project with International Centre for Genetic Engineering and Biotechnology, New Delhi – Funded by Department of Biotechnology, Govt. of India)

Studies were continued in hyperendemic and low endemic areas of Sundargarh district, Orissa for preparation of a site for malaria vaccine trial. There are 13 study villages with a total population of 4221 under Gurundia and Birkera PHCs of Sundargarh district out of which, 8 villages with a population of 2058 are located in deep forest close to the streams, under the influence of *An. fluviatilis* and have persistent malaria transmission. The remaining five villages with a total population of 2163 are located in a plain area close to a perennial river where *An. culicifacies* is the main vector and malaria transmission is low and seasonal. The study villages are predominantly inhabited by ethnic tribals—Oram, Munda, Khadia, etc. The geographical reconnaissance and mapping of the study villages were completed and a computer based epidemiological as well as GIS database has been developed.

**Entomology and Parasitology:** Longitudinal and cross-sectional parasitological and entomological surveys were conducted in all the study villages. The weekly surveillance was conducted in all the study villages through village volunteer workers. The SPR, SFR, Pf per cent and annual parasite index (API) in the forest villages were 41.8, 35.1, 84.0 and 397.5 respectively, whereas in the plain area villages these were 17.1, 12.1, 70.8 and 55.5 respectively. The malaria incidence was more in the younger age groups up to 15 years and the highest incidence was in the 0–5 age groups in the forest area but in the plain area, malaria cases were evenly distributed in all the age groups.

During the year, cross-sectional mass blood surveys were conducted in all the study villages during March, June and November covering all the transmission seasons— low, moderate and high respectively. The parasite rate in the forest and plain area during March, June and November was 11.4. 1.1; 9.8 and 0.6, 20.2, 1.84 respectively. Malaria was more prevalent in the younger age groups as compared to adults. Out of the total malaria cases in forest area, the prevalence of *P. falciparum*, *P. vivax* and *P. malariae* accounted for 82, 16 and 2 per cent respectively but in the plain area, the prevalence of *Pf* and *Pv* were 68 and 32 per cent respectively. The spleen rate in children living in forest area villages was above 80 per cent throughout the year and also in adults it was above 30 per cent, whereas, in plain area the spleen rate in children and adults was ranging from 20–35 and 2–12 respectively. A study on the therapeutic response of chloroquine in the study population is in progress.

Entomological surveys were carried out in two indicator villages each in forest and plain areas. During the year, 14 anopheline species from forest area and 11 species from plain area were recorded. *An. culicifacies* was widely prevalent in both the areas whereas *An. fluviatilis* was totally absent in the plain area. The man hour density (MHD) of *An. culicifacies* in the forest and plain area was in the range of 3.3–35.0 and 8.0–26.0 respectively. The MHD of *An. fluviatilis* in the forest area ranged from 0.2–15.3. Results of all night mosquito landing collections on human baits showed that *An. fluviatilis* prefer to bite humans and the man biting rate in forest area during low, moderate and high transmission seasons was 0.62, 6.5 and 16.5 bites per person per night respectively, whereas it was nil in the plain area. The average man biting rate of *An. culicifacies* in the forest and plain area was 0.3 and 0.5 bites per person per night respectively. The sporozoite rate in the forest area during low, moderate and high transmission seasons was 0, 1.32 and 2.1 respectively and it was nil in the plain area. The entomological inoculation rate (EIR) in the forest area during low, moderate and high malaria transmission seasons was 0, 0.085 and 0.35 infective bites per person per night respectively whereas, it was nil in the plain area. The study population is being increased to 15,000 for which new villages have been identified and baseline data collection as well as census operation is in progress.

**Host Immune Responses:** Study proposed on host immune responses was to determine the antibody level to vaccine candidate *P. falciparum* antigens, namely MSP-$1_{19}$, EBA175 and TRAP developed in the course of natural infection in different age groups in the study population.

Finger prick blood samples were collected by cross-sectional survey from individuals belonging to forest and plain areas. Indirect ELISA was done to assess the antibody level against three recombinant peptides. From the results, it was observed that overall IgG antibody profile with three antigens were higher in individuals of forest areas than those in plain areas. There was an age-wise increase in IgG level in both areas. Antigen specific IgM profile in study group was low and almost similar in two areas. Anti-TRAP antibody level found moderately high in adults of both forest and plain areas. IgG1 and IgG2 were the predominant subclass responses to all three antigens. Proportion of high responders to MSP-$1_{19}$ and EBA175 was comparable in children and adults. A group of sera from older age group showed elevated level of IgG3 to MSP-$1_{19}$ and EBA175. There was an association between high IgG/IgG1 antibody to MSP-$1_{19}$ and EBA175 responses and lower prevalence of *P. falciparum* parasitaemia.

A longitudinal cohort study of parasite episodes, reinfection and antibody level against *P. falciparum* antigens of interest has been proposed.

**Multiplicity of Infection:** Field collected blood spots from *P. falciparum* positive patients were genotyped using PCR method. Primers of MSP-1 (block-2) and MSP-2 (central variable region) were used for PCR assay. Primary reaction primers were gene specific and nested PCR primers were family specific. About 105 samples were analyzed for two years during different transmission seasons. Multiplicity of infection ranged from 1.1 to 3.28 in low and high transmission seasons. Number of alleles observed was 22 in MSP-1 and 24 in MSP-2. A high proportion of isolates (>60%) had multiplicity of infection greater than 1.

**Sequence Diversity:** The sequence diversity in three malaria vaccine candidates namely MSP-1 (C-terminal 19kDa), EBA175 region II, and TRAP was determined in *P. falciparum* isolates. Primers were designed covering part of block-16 and entire block-17 of MSP-1. Sequencing of 16 field isolates has shown polymorphisms only at few amino acid positions. Eight alleles were observed in Indian field isolates, out of which three are unreported till now (from any other country study).

Primers were designed for TRAP N-terminal and C-terminal regions. Sequencing of 10 field isolates for TRAP N-terminal region showed polymorphisms at 25 amino acid positions, and three were reported for the first time. Important motifs like RGD, IQQ and the motif in thrombospondin related proteins were conserved in all the field isolates. Sequencing of 10 field isolates for TRAP C-terminal region showed variable PNP repeats in different isolates.

Sequencing of EBA175 F2 region of 16 field isolates has shown polymorphisms at 19 amino acid positions. Only five of these were reported between different strains. Only selected amino acid residues were targeted for mutations.