Development of Field Sites for Malaria Vaccine Trial

A study has been initiated to understand the epidemiology of malaria in Sundargarh, Orissa and Jabalpur, Madhya Pradesh with an objective to develop field sites for vaccine trial.

Site I: Sundargarh, Orissa

Sundargarh district is located in the Garhjat hills of eastern Deccan plateau between 21°35'N and 22°35'N latitudes, and between 83°32'E and 85°22'E

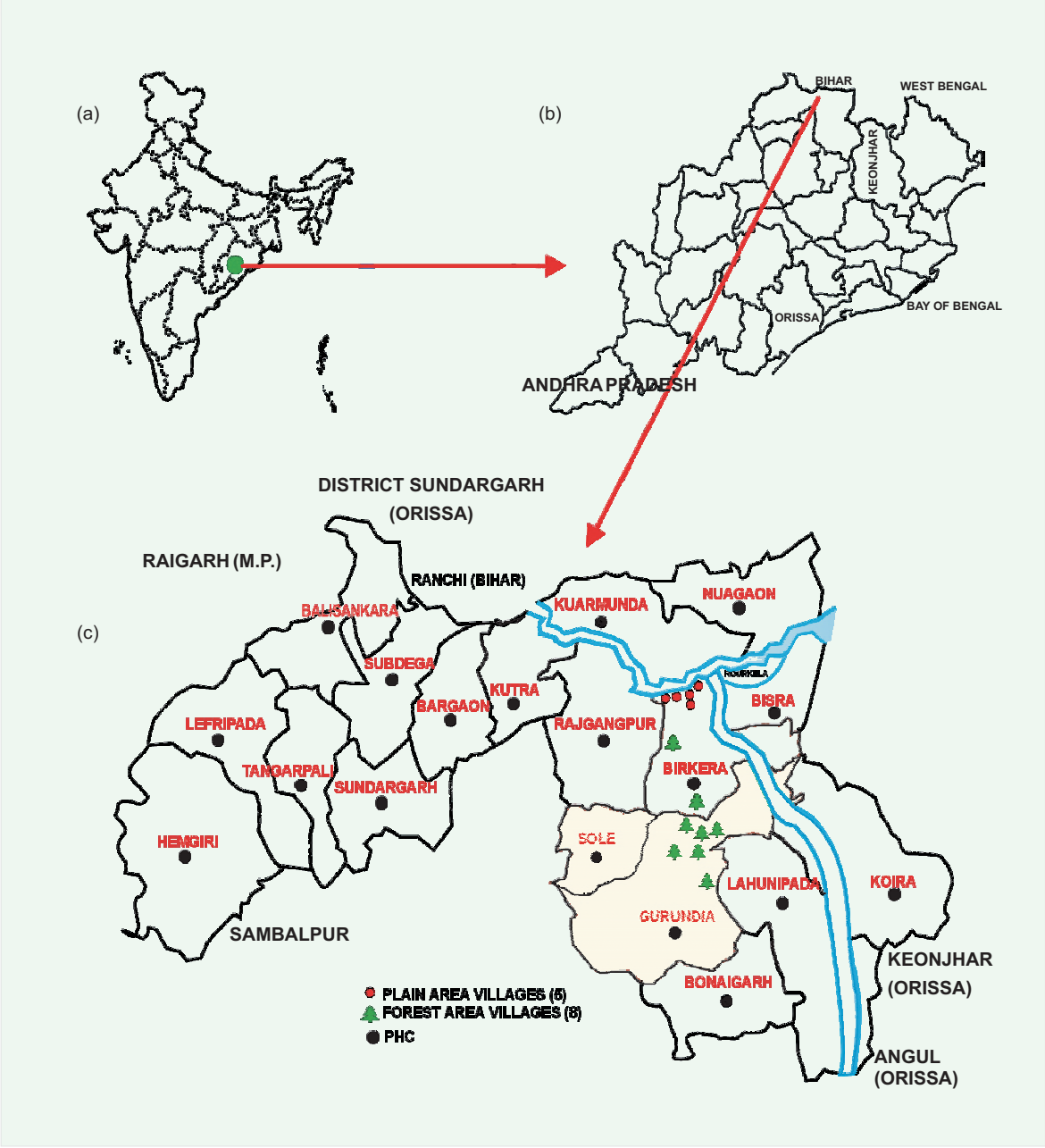


Fig. 21: Study area (a) India map showing location of Orissa state; (b) Orissa state showing location of District Sundargarh; and (c) District Sundargarh showing villages in two PHCs—Gurundia and Birkera

longitudes, at an altitude in the range of 200 to 900 m above the sea level. Topographically, the area presents ideal ecological conditions for malaria transmission with undulating uplands intersected by forested hills, rocky streams, and paddy fields. The area is characterized by a tropical humid climate and receives rainfall between June and September from the 'south-west monsoon' and in December and January from the 'northeast monsoon'. Average annual rainfall ranges between 160 and 200 cm and mean annual temperature ranges between 22 and 27°C. The weather seasons are: hot dry summer from April to mid-June, monsoon from mid-June to September, autumn from October to November, winter from December to January, and spring from February to March. The maximum temperature during summer rises to 40–45°C and the minimum temperature during winter falls to 5–10°C. A total of 51% of the area is covered with forests and is inhabited predominantly by tribals, that constitute 62% of the total population. The area is rich in mineral resources and industrialization based on these resources has lead to the development of new settlements, deforestation and ecological changes resulting in changes in malaria transmission patterns.

A study has been initiated to understand the epidemiology of malaria in Sundargarh, Orissa with an objective to develop a field site for vaccine trial. Initially, a study was conducted in 13 villages with a total population of 4221 under Gurundia and Birkera PHCs of Sundargarh district, out of which eight villages with a population of 2058 are located in deep forests and five villages with a total population of 2,163 are located in plain area (Fig. 21). The study villages are predominantly inhabited by ethnic tribals— Oram, Munda, Khadia, etc. Later, the study was extended to 35 villages (23 forested and 12 plain) with a total population 15,847.

Epidemiology of Malaria

Malaria is persistent throughout the year in both the areas but peak transmission was observed during post monsoon months–September, October and November. The proportion of *P. falciparum, P. vivax* and *P. malariae* species in the forest area was 85, 14 and 1 respectively, whereas it was 75, 25 and nil respectively in the plain area. A malaria episode was defined as a case where an individual had an axillary temperature more than 37.5°C and asexual forms of *P. falciparum*, *P. vivax* or *P. malariae* were detectable in thick blood smears. A second episode of fever occurring within 28 days of first episode was considered as recrudescence and treated as a single episode. A total of 3993 and 379 malaria cases were reported from forest and plain area villages respectively during the study period from January 2001 to December 2007. Over the 7-year study period an average of 65% of malaria cases were detected through active surveillance each year from the study area. The remaining malaria cases were detected through passive surveillance. The month-wise incidence of malaria in forest and plain areas during the study period is shown in Fig. 22. In the forest area, malaria transmission is perennial and fresh *P. falciparum* malaria cases are reported throughout the year. A similar pattern is seen in the plain area but the incidence is markedly low as compared to the forest area. The average number of *P. falciparum* cases/1000 population/year in the forest area was 225.4, whereas it was 17 in the plain area.

In the forest area, the average malaria incidence rate of *P. falciparum* (only first episode per individual per year) was ranging between 9.1 and 18% over different years and the highest incidence rate of 63.9% was recorded in the 1–5 years age group and it was inversely proportional to increasing age, whereas in the plain area, the incidence rate was

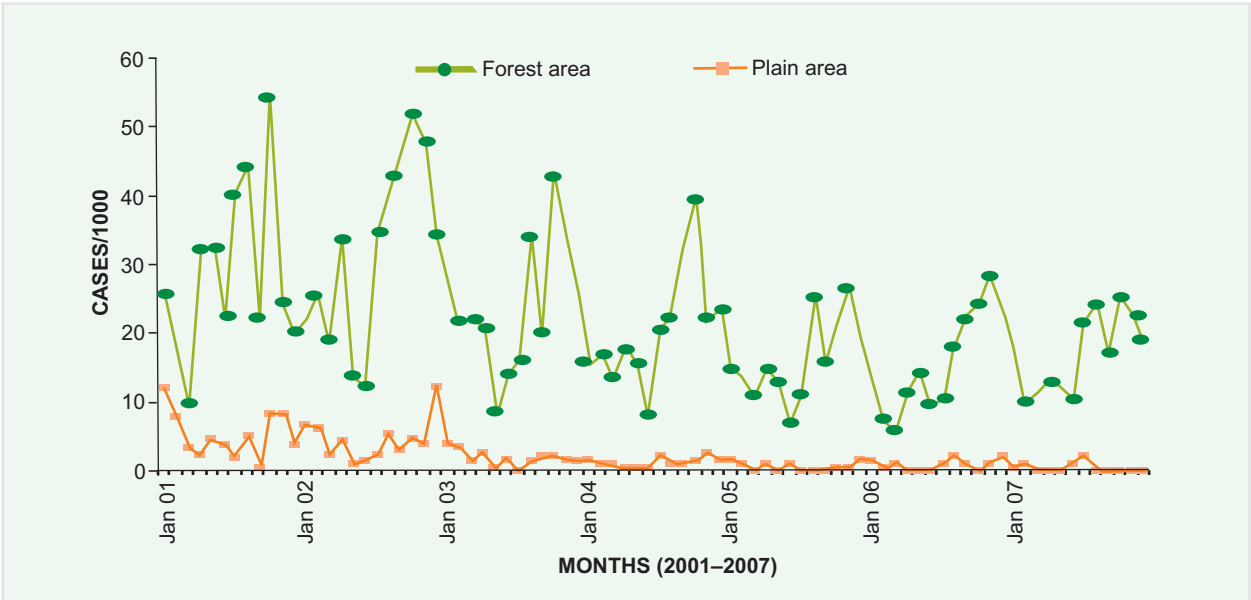


Fig. 22: Month-wise malaria incidence in the forest and plain area in Sundargarh district (2001–07)

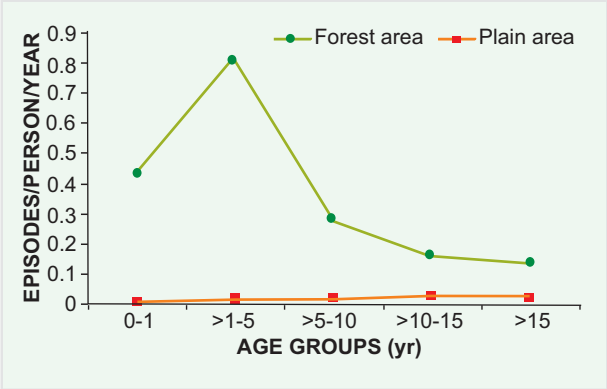


Fig. 23: Malaria attack rate due to *P. falciparum* in different age groups in the forest and plain area study villages during 2001–07

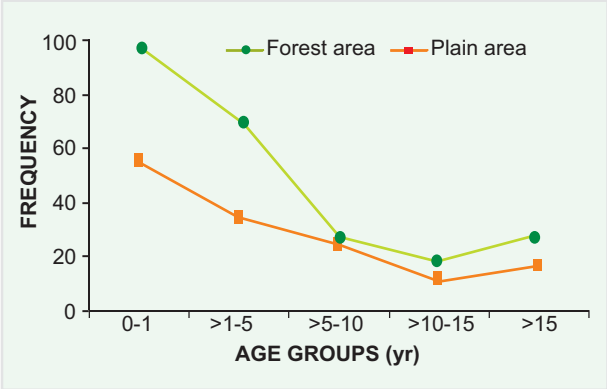


Fig. 25: Frequency of severe and moderate anaemia (< 70–100g Hb/l) in different age groups in the forest and plain area study villages recorded through cross-sectional malaria prevalence surveys

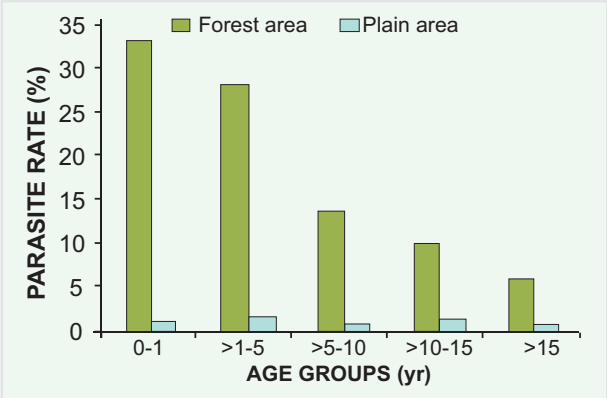


Fig. 24: Parasite rate in different age groups in the forest and plain areas in Sundargarh district as recorded through cross-sectional prevalence surveys during 2001 to 2006

low and ranged between 0.16 and 1%; and all the age groups were equally affected. The malaria attack rate due to *P. falciparum* infection (number of episodes per person per year) was determined in different age groups in both the study areas based on the weekly surveillance data collected over seven years from January 2001 to December 2007. The average attack rate in the forest area for all age groups over the 7-year period was 0.23 episodes/person/year respectively, whereas the average attack rate in the plain area for all age groups was 0.016. The difference in *P. falciparum* malaria attack rate between forest and plain areas was significant ($p<0.01$). In the forest area, the *P. falciparum* malaria attack rate varies with age (Fig. 23). The attack rate was found to be highest in 1 to 5 year old children during all the seven years of study. There was a gradual decline in the attack rate with increase in age. In the plain area, there was no correlation of attack rate with increase in age (Fig. 23).

Cross-sectional surveys were carried out each year in March, June and November representing intermediate, low and high malaria transmission seasons respectively. The average annual parasite rate in the forest and plain area was 12 and 1.3 respectively. The highest parasite rate in the forest area during these surveys was found in 1–5 years age group with a gradual decline as the age progresses, whereas in the plain area parasite rate was low and all the age groups were equally affected (Fig. 24). Out of total parasite positive cases found in the forest area during cross-sectional surveys, about 40% of the cases were asymptomatic and 9% were found gametocyte carriers. The average spleen rate in children and adults in the forest area was 82 and 20.5% respectively, whereas in the plain area it was 15.0 and 0.70% respectively. Estimation of haemoglobin in the study population showed that about 33% of the population in the forest area had moderate to severe anaemia, whereas in the plain area 20% population have anaemia of varying degree. About 18% of the population in both the areas was found to be G-6-PD deficient and 6% population in the forest area was found with sickle-cell trait (Table 9).

In the forest area, anaemia was absent in 26.7% of the persons screened, while 40.3% had mild anaemia and 33% had moderate to severe anaemia. The distribution of moderate to severe anaemia in

Table 9. Prevalence of G-6-PD deficiency in the forest and plain areas

Area	Population	No. tested	Normal	Deficient (%)	Malaria (*Pf*)	
					Normal	deficient (%)
Forest	2204	1133	926	207 (18.3)	301 (18.3)	92 (44.4)
Plain	2341	857	702	155 (18.1)	18 (2.6)	6 (3.9)

different age groups in the forest area is shown in Fig. 25. The highest prevalence of moderate to severe anaemia was found in 0–1 year old children (94.7%) followed by 1–5 year old children (68.2%). The frequency of incidence of moderate to severe anaemia decreased with age with incidence dropping to 16.9% in 10 to 15 year old children. In the plain area, 29.6% of the population screened had normal haemoglobin values, while 49.1% had mild anaemia, 20.8% had moderate anaemia and 0.5% had severe anaemia. The distribution of moderate to severe anaemia in different age groups in the plain area is shown in Fig. 25. The prevalence of moderate and severe anaemia in different age groups was similar to the forest area but the frequency of anaemia was significantly lower in comparison to the forest area ($p<0.05$).

Entomological Studies

During the study period, 15 anopheline species from the forest area and 13 species from the plain area were recorded and the percent composition of different *Anopheles* species in both the areas is shown in Table 10. Two primary vector species, *An. culicifacies* and *An. fluviatilis* were found in the forest area whereas the latter species was altogether absent in the plain area. *An. culicifacies* was most prevalent species and constituted 39.1 and 36.1% of all the total anopheline species in the forest and plain area respectively. *Anopheles fluviatilis* constituted 7.1% of all the anopheline species recorded from the forest area. *Anopheles annularis*, which is a secondary malaria vector in some parts of India but not incriminated as a vector in Sundargarh district was also found in good numbers and comprised 11.6 and 16.4% of the total species in the forest and plain areas respectively.

The month wise person-hour density (PHD) of *An. culicifacies* and *An. fluviatilis* in both the areas is shown in Fig. 26. The relative abundance of *An. culicifacies* was high throughout the year, although there were wide seasonal fluctuations in the density of *An. culicifacies* in both the areas. In the forest area, highest density of this vector species was observed between February and August and lowest during September to January. In the plain area, although the density fluctuations of this species were similar but peak was observed during January. The distribution pattern of this species in the forest and plain areas was almost similar and small density variations were insignificant. *Anopheles fluviatilis,* which was found only in the forest area, maintained low density throughout the year (range: 0.01 to 16.3). The highest prevalence of this species was recorded during post-monsoon months of September to December and lowest during hot dry months of May and June.

Mosquito blood meal analysis of the vector species revealed that the human blood index (HBI) of *An. culicifacies* and *An. fluviatilis* was 0.007 and 0.98 respectively, showing that *An. culicifacies* was

Table 10. Percent composition of anopheline species in forest and plain area of Sundargarh district as recorded through indoor resting collections from January 2001 to December 2007

S.No.	Species	Forest area (%)	Plain area (%)
1.	*An. culicifacies*	6737 (39.1)	7781 (36.1)
2.	*An. fluviatilis*	1222 (7.1)	0
3.	*An. annularis*	2001 (11.6)	3540 (16.4)
4.	*An. subpictus*	3414 (19.8)	4218 (19.6)
5.	*An. vagus*	2148 (12.4)	2649 (12.3)
6.	*An. pallidus*	940 (5.4)	1409 (6.5)
7.	*An. aconitus*	147 (0.8)	1323 (6.1)
8.	*An. nigerrimus*	277 (1.6)	348 (1.6)
9.	*An. barbirostris*	111 (0.6)	109 (0.5)
10.	*An. varuna*	32 (0.2)	5 (0.02)
11.	*An. splendidus*	178 (1.0)	112 (0.5)
12.	*An. tessellatus*	23 (0.1)	46 (0.2)
13.	*An. ramsayi*	5 (0.03)	2 (0.009)
14.	*An. jamesi*	2 (0.01)	1 (0.005)
15.	*An. jeyporiensis*	4 (0.02)	0
	Total	17241	21543

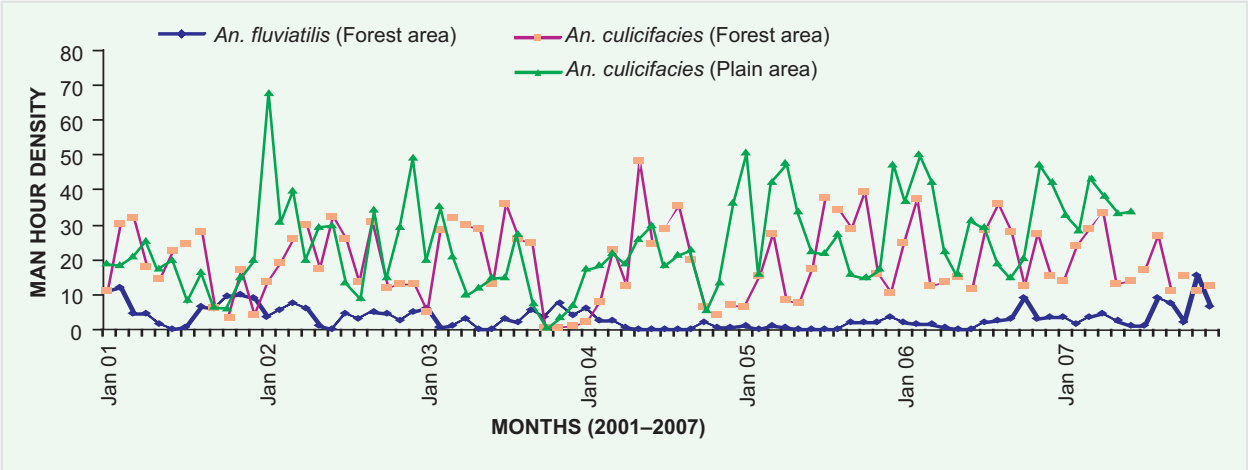


Fig. 26: Density of *An. culicifacies* and *An. fluviatilis* in the forest and plain areas of Sundargah district (2001–07)

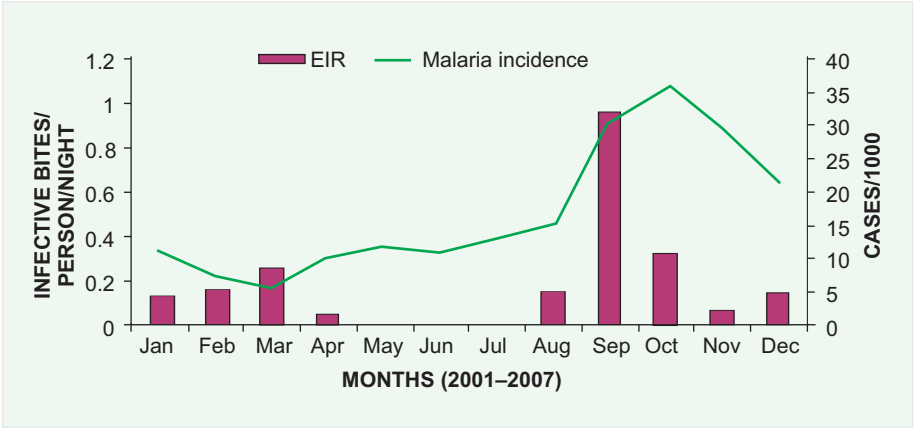


Fig. 27: Relationship of entomological inoculation rate (EIR) with malaria incidence (2002–06)

primarily a zoophagic species whereas *An. fluviatilis* is strongly anthropophagic. The results of blood meal analysis were also supported by direct observations on the biting activities of these species during whole night human bait collections. Transmission load in two areas during different transmission seasons was estimated by calculating the entomological inoculation rate (EIR). The EIR in the forest area varied significantly during different seasons of the year. The EIR during low, intermediate and high transmission seasons was 0.02, 0.085 and 0.35 infective bites per person per night respectively thereby showing high transmission load during most part of the year. In the plain area, EIR was 0.005, 0.005 and 0.014 infective bites per person per night respectively in low, intermediate and high transmission seasons. There was direct correlation of EIR with the malaria incidence in the study area (Fig. 27).

Genetic Diversity Studies

Plasmodium falciparum field isolates were collected in two ecosystems i.e. forest and plain with differential malaria transmission of District Sundargarh, Orissa. A total of 270 field isolates of *P. falciparum* were collected on filter paper strips and of which 230 were from villages of forest and 40 from villages of plain ecotypes. These isolates were collected at different transmission seasons and the genotypes of the parasite population were determined by amplification of polymorphic regions of two *P. falciparum* antigen genes: *MSP-1* (block 2) and *MSP-2* (block 3) by family specific nested polymerase chain reaction (PCR).

Study revealed highly polymorphic nature of both markers namely *MSP-1* and *MSP-2* in study isolates of two ecotypes, however, allelic diversity observed was slightly higher in the forest ecotype compared to the plain ecotype. *MSP-1* was represented by three reported families, namely K1, MAD20 and RO33 and two families, namely FC27 and 3D7 of *MSP-2*. It is revealed that composition of *MSP-1* and *MSP-2* families was same among the isolates of two ecotypes though variations in the proportional prevalence of families were observed.

A good number of isolates showed multiple infections of *MSP-1* and *MSP-2* judged on the basis of presence of more than one family or presence of more than one PCR fragment (allele) of the family in the same individual. Proportion of isolates with multiclone was significantly higher in the forest ecotype with high malaria transmission ($p<0.002$ for *MSP-1* and $p<0.026$ for *MSP-2*) and vice versa for single clone isolates in plain ecotype ($p<0.003$ for *MSP-2*) with low malaria transmission (Fig. 28).

Among 270 samples at *MSP-1* locus, 8 alleles of the K1 family ranging from 125 to 300 bp, 8 alleles of MAD20 ranged from 110 to 270 bp and monomorphic RO33 family having single allele of 160 bp were observed. For *MSP-2* locus, 12 alleles of FC27 ranged from 200 to 600 bp and 13 alleles of 3D7 ranging from 370–660 bp were observed. Fig. 29 shows the agarose gel electrophoretogram showing allelic variations in families of *MSP-1* and *MSP-2*.

Presence of all the allelic families of *MSP-1* and *MSP-2* among the isolates of both the ecotypes suggests for the same population in both the ecotypes. Absence of any significant difference in the prevalence of *MSP-1* and *MSP-2* families among isolates of forest and plain ecotypes could be due to the close proximity of both the ecotypes (located within a distance of 20–30 km) and population

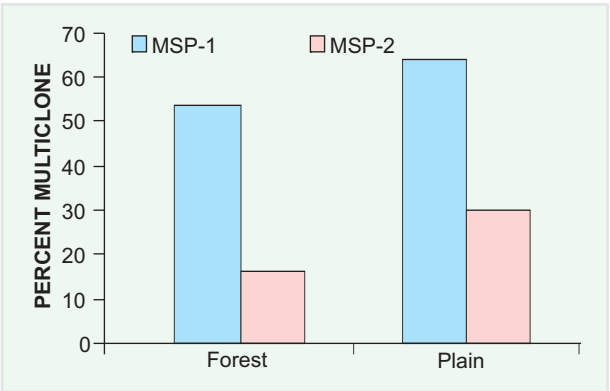


Fig. 28: Proportion distribution of multiclone isolates in forest and plain ecotypes of Sundargarh, Orissa

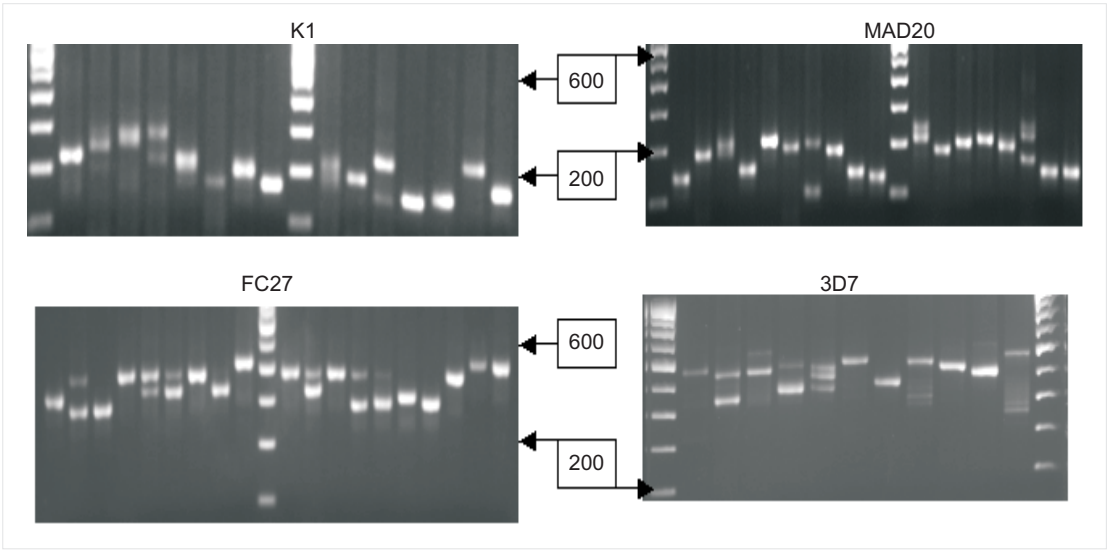


Fig. 29: Allelic polymorphism observed among *Plasmodium falciparum* field isolates of District Sundargarh, Orissa

movement between the two ecotypes thus supporting a common pool of parasites in both the ecotypes.

A high level of genetic diversity was present in isolates of both forest and plain ecotypes of District Sundargarh, Orissa. A significantly higher proportion of multiclone infections of different genotypes in isolates of forest ecotype, compared to plain ecotype may be attributed to the differential vector potential of the species (*An. fluvitilis*) prevalent in the area which is reflected in the malaria transmission pattern also, thus, supporting positive association of multiclone infection with high malaria transmission. Of both the marker systems, *MSP-2* has shown generally a higher level of polymorphism, revealed higher proportion of multiple genotype infections as well as higher multiplicity of infection (MOI) in both the transmission seasons as well as both ecotypes. MOI has shown an increase from lower to higher transmission season.

Sequence Diversity

The sequence diversity in three malaria vaccine candidates, namely MSP-1_{19} (C- terminal 19 kDa fragment of MSP-1), EBA 175-RII and TRAP was determined in *P. falciparum* isolates collected from forested villages. Sequencing of 16 field isolates for MSP-1 has shown polymorphism only at 5-amino acid positions. Out of which four were reported earlier by other workers. Sequencing of TRAP N-terminal region in field isolates showed polymorphism at 25 sites, and three were reported for the first time. Sequencing of EBA-F2 region in 16 field isolates has shown polymorphism at 19-amino acid positions. Only five of these polymorphisms were reported between different strains. The study further revealed that a few selected amino acids are targeted for change. This selection may be to maintain non-synonymous polymorphism in EBA region II, thus, not affecting the functional aspects. Significant levels of cross-reactive antibodies are generated against different PfMSP-1_{19} allelic forms in a *P. falciparum* infected natural human population. Studies further indicated that antibodies elicited by the vaccine candidate, region F2 of EBA-175 (Camp strain), can block the binding of variant F2 regions observed among the field isolates with similar efficiency suggesting that the binding site within F2 may be conserved. Observation that anti-F2 (Camp) sera blocks binding of diverse variants with comparable efficiency provides support for the development of recombinant F2 as a blood stage vaccine for *P. falciparum* malaria.

Immunological Profile

Finger-prick blood samples were collected from different age groups by repeated cross-sectional surveys at two sites each of forest and plain areas during low and high transmission seasons. Indirect ELISA was done to measure the antibody levels against *P. falciparum* MSP-1_{19}, EBA-175 and TRAP antigens in 222 (110 from forest and 112 from plain areas) and 248 (138 from forest and 110 from plain areas) blood samples collected during low and high transmission seasons, respectively.

In the forest area, *P. falciparum* infection was detected in 12.7% (14/110) persons during low transmission phase, whereas in plain area among 112, only 3 (2.7%) were found positive. During high transmission, *P. falciparum* positivity was detected in 28.2% (39/138) in the forest area and 8.2% (9/110) in the plain area. It was observed that overall IgG profiles against MSP-1_{19}, EBA-175 and TRAP were higher in study subjects of forest area than plain in both low and high transmission seasons (Figs. 30–33). The age-dependent increase of specific antibody levels was noticed in individuals of two areas in both the seasons. The mean ELISA O.D. was significantly lower in children <5 years compared to adults ($p<0.001$). Proportion of high responders was more in adults than in children ($p<0.01$). However,

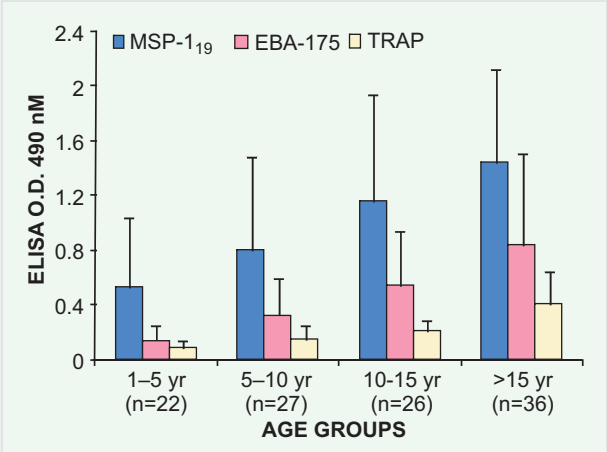


Fig. 30: Prevalence of MSP-1, EBA-175 and TRAP in different age groups in low transmission forest area in Sundargarh district, Orissa

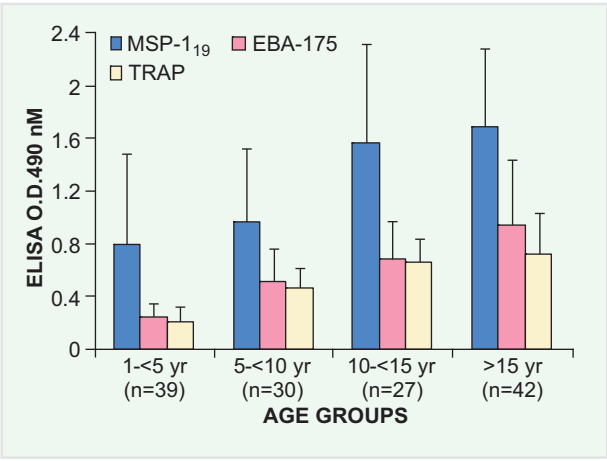


Fig. 31: Prevalence of MSP-1, EBA-175 and TRAP in different age groups in high transmission forest area in Sundargarh district, Orissa

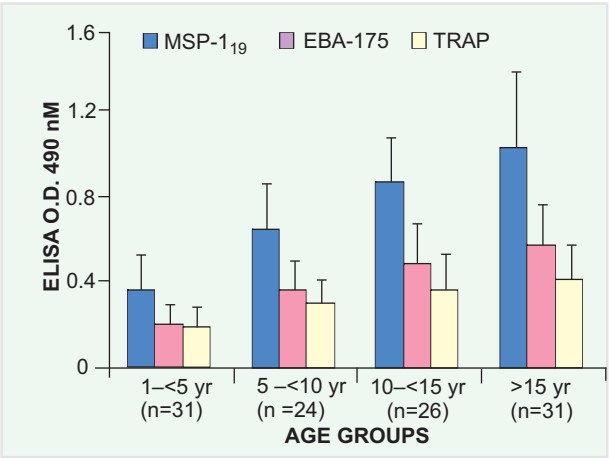


Fig. 32: Prevalence of MSP-1, EBA-175 and TRAP in different age groups in low transmission plain area in Sundargarh district, Orissa

acquisition of antibodies during the time of high transmission phase was more compared to low transmission (Fig. 34). A total of 127 and 68 blood samples, collected from the forest and plain areas during mass survey were tested for their IgG subtypes. Age-wise increase in IgG level has been observed in individuals of both forest and plain areas.

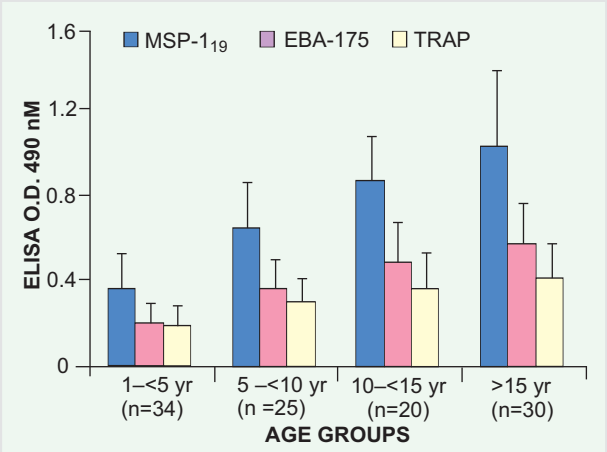


Fig. 33: Prevalence of MSP-1, EBA-175 and TRAP in different age groups in high transmission plain area in Sundargarh district, Orissa

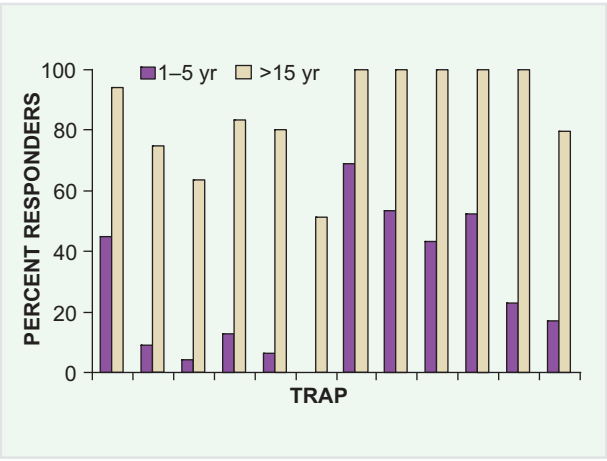


Fig. 34: The responder frequency of children and adults for TRAP in District Sundargarh, Orissa

The IgG1 and IgG2 were the predominant subclass responses to all three antigens (Figs. 35–37).

In another set of repeated cross-sectional surveys at four sites each of forest and plain areas, individuals were categorized on the basis of fever and/or presence or absence of *P. falciparum* infection. In afebrile (healthy) and febrile/non-malarial subjects, IgG levels against all three antigens were higher than febrile/*Pf*+ patients. However, antigen specific IgM was higher in this group compared to afebrile/healthy and non-malarial fever cases.

Overall IgG profiles against MSP-1$_{19}$, EBA-175 and TRAP were higher in study subjects of the forest area than plain in both low and high transmission seasons. The age-dependent increase of specific antibody levels was noticed in individuals of two areas in both seasons. Boosting in antibody production has been observed against these molecules by natural infections.

On the basis of existing epidemiological data as well as immune status of the study population, the children in the age group of 1–5 years are eligible for vaccine trial in the forest area. The required number of target children for vaccine trial will be sufficiently met out of the existing study population.

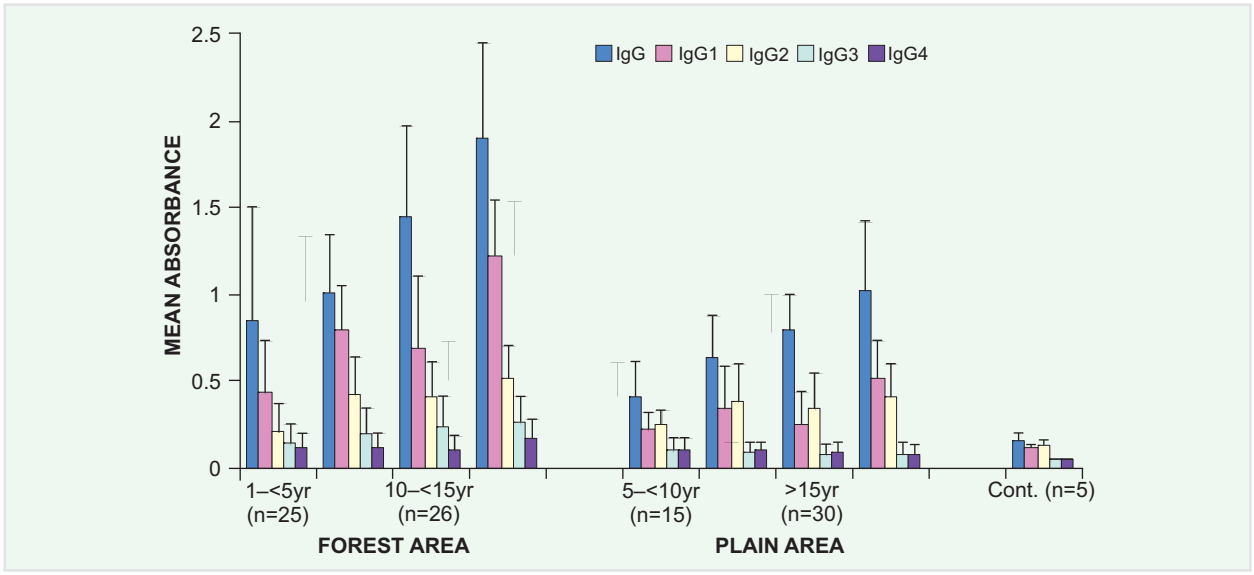


Fig. 35: IgG subtypes profile in different age groups against MSP-1_{19} in Sundargarh district, Orissa

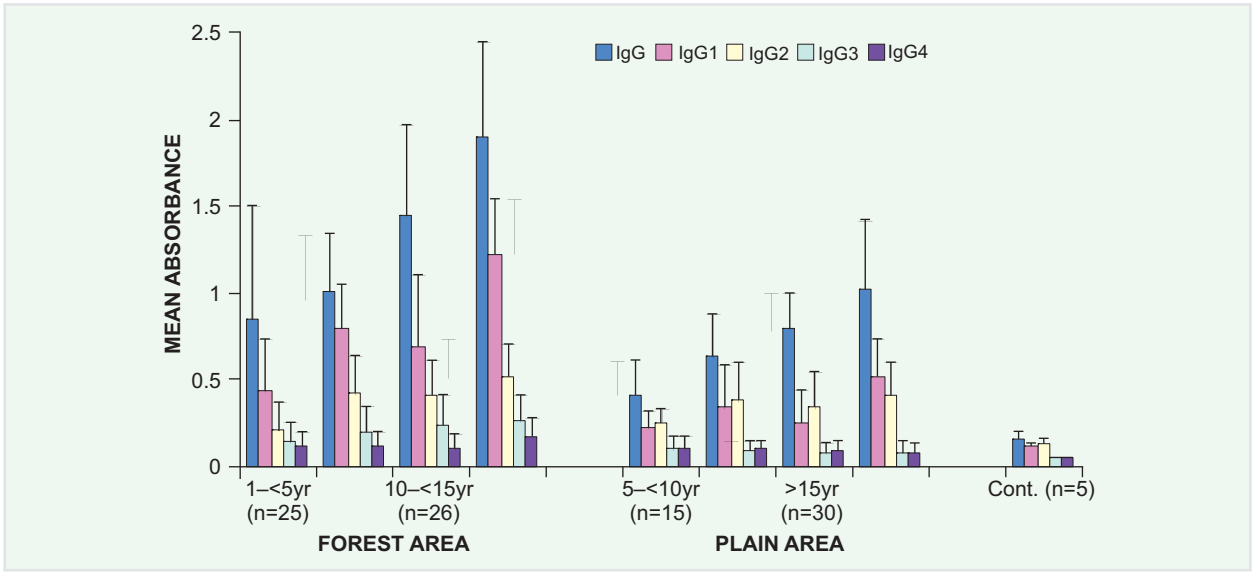


Fig. 36: IgG subtypes profile in different age groups against EBA-175 in Sundargarh district, Orissa

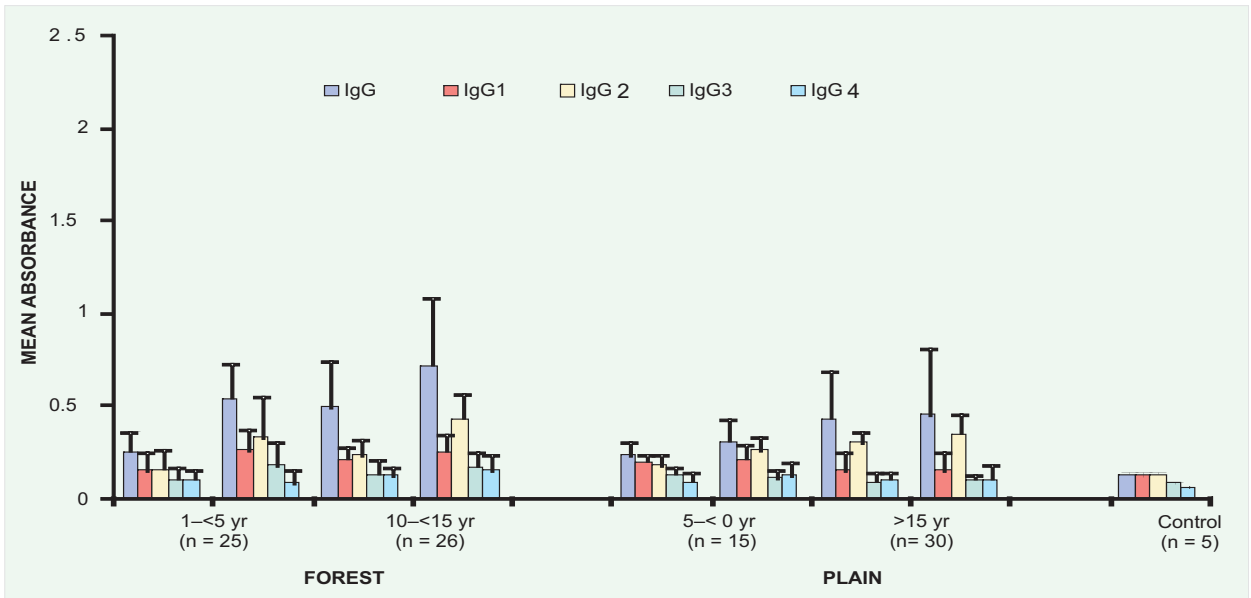


Fig. 37: IgG subtypes profile in different age groups against TRAP in Sundargarh district, Orissa

GIS Database

Village boundaries of plain area villages, namely Balupatra, Chikatmati, Sarala, Mahaliapalli and Mallikpalli were digitized showing landscape features such as highways, village roads, walk ways, rivers, canals, branch canals, water bodies, houses, schools, shops, clubs, churches, industries, open space, rice-fields, *etc*.

Data Architecture

A three-tier GIS database has been generated. First level— village-wise data, which include census information and malaria data; second—house-wise data, where data of individual house pertaining to house number, number of rooms in the house, type of house—kuchcha or pucca/human dwelling or mixed dwelling, name of the headman, number of persons in the house, their names, age, sex, religion, tribe, income, *etc*, number of animals in the house and malaria history (houses have been depicted by square blocks on the village boundary); and third level—personal level data, namely name, age, sex, marital status, education level, occupation, malaria history up to four malaria episodes of each person in the village have been included. Persons in the houses have been shown by dots. Number of dots in a house (shown by boxes) show number of persons (Fig. 38).

Out of five plain area villages house-wise data of three villages have been obtained and put in the GIS database. Forest villages data are being processed for developing GIS data base.

Functionality of the System

1. Information of any village/house or person can be retrieved at the click of the mouse within village boundary/house/dots respectively on the map.
2. Using zoom-in facility one can blow up houses and can see number of persons, by assigning different colour to positive and negative cases both for houses or persons, one can see the house-wise malaria spread or in houses how many persons are sick to evaluate the disease scenario.
3. Malaria epidemiology can be studied both in space and time where change in malaria situation in any village can be correlated to any specific breeding site or the activity in that area to take situation-specific control measure.
4. Percent composition of any parameter can easily be mapped to review the situation. For example, if one needs to know the per cent parasite composition— *Pv* and *Pf*, instantly situation of the entire area/houses can be known.

Depending upon the requirement, database can be tailor-made and so is the analysis algorithm to achieve the desired result.

Site 2: Jabalpur, Madhya Pradesh

This study was initiated in June 2005 with the objective to develop a well characterized site, where the epidemiology of the disease, immune response to malarial antigens, diversity of parasite genes and vector characteristics are well understood. The study has four arms which are as under:

(a) Epidemiology
(b) Immunoepidemiology
(c) Molecular epidemiology and
(d) Entomology

Epidemiologic Study

The main objectives of the study were based on: (i) to measure the rate of morbidity and mortality in all age groups in a selected population particularly infants, children and pregnant women; (ii) to

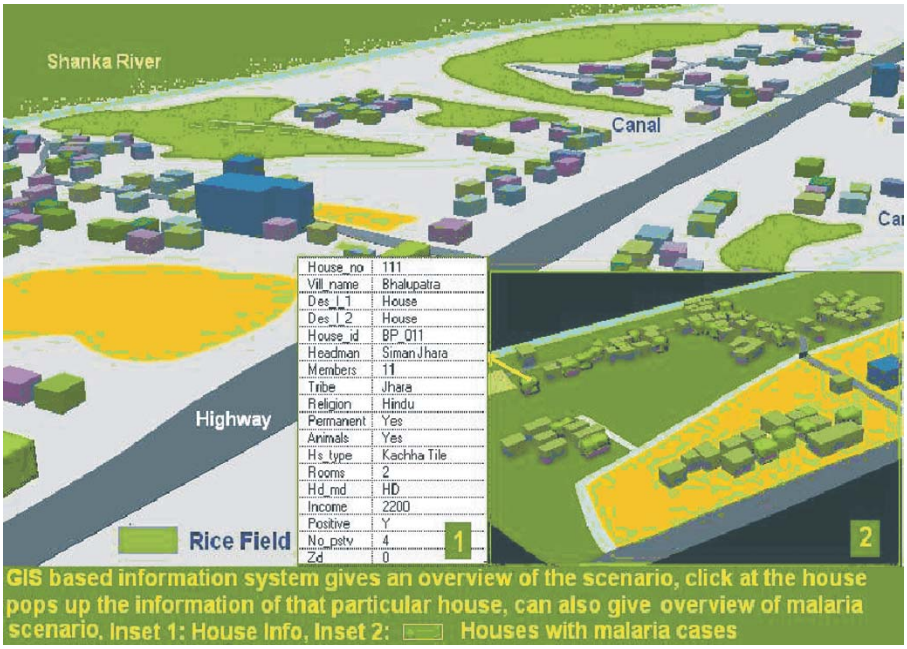


Fig. 38: GIS-based information system of a study village in Sundargarh district, Orissa

Fig. 39: Study villages in Jabalpur (M.P.)

determine the frequency and level of parasitaemia, onset of fever due to *P. vivax* and *P. falciparum* in different age groups; (iii) to examine the relationship between placental malaria and infant low birth weight and mortality.

This study was started in November 2005 in the Bargi PHC of District Jabalpur (Fig. 39). In the cohort, nine villages (2414 persons) were on the bank of Bargi dam reservoir, 17 in forested (8831 persons) and 36 (21,455 persons) in the plain area (Fig. 40). The cohort was established by conducting baseline census in the study area to generate a real picture of different settings.

Baseline census of study villages consisted of collection of demographic, socio-economic information of all the individuals in the study area. A household is defined as a group of people living under one roof and sharing the same cooking spot/pot. At registration an identity number is allocated to each household which is painted on top of the front entrance door frame of the household. Thereafter every cooking pot sharing unit is denoted by the unique identifier number as a single household (Fig. 41). Quality assurance was maintained by conducting re-interview of 5% randomly selected households. Locally designed software in ADO.NET is used for data entry. The system is developed based on the MS SQL 2000 along with data collection and entry guidelines of Household Registration System Software, Population Council (Flowchart 1).

Baseline population of the study area is 32,700 residing in 5813 households of 62 villages. Average family size is 5.6 per household. This population is

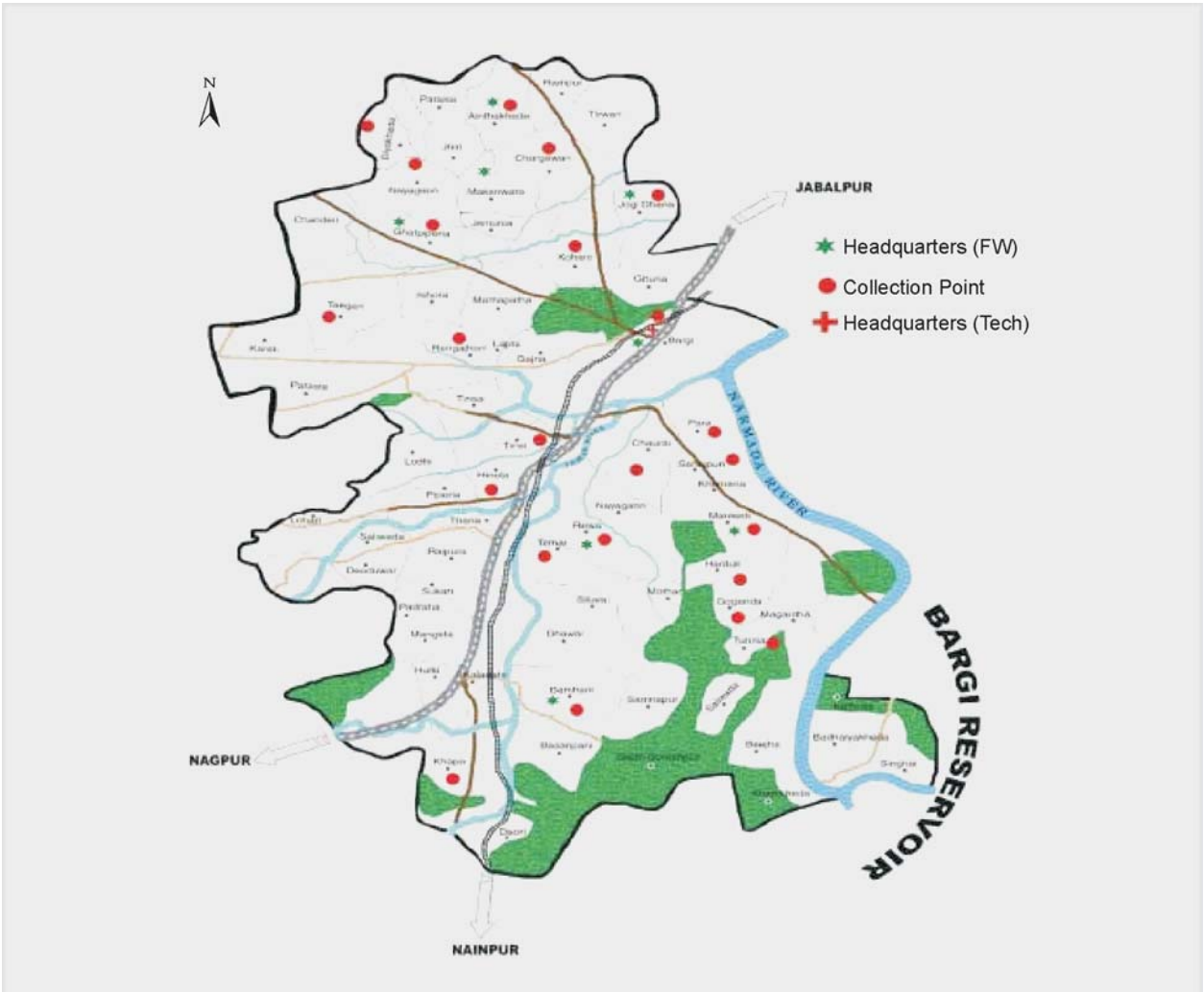


Fig. 40: Map of Bargi PHC showing study villages

Flowchart 1: Demographic database management

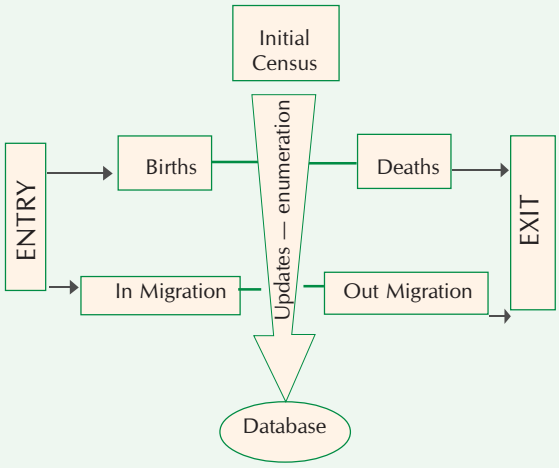


Table 11. Baseline census at a glance

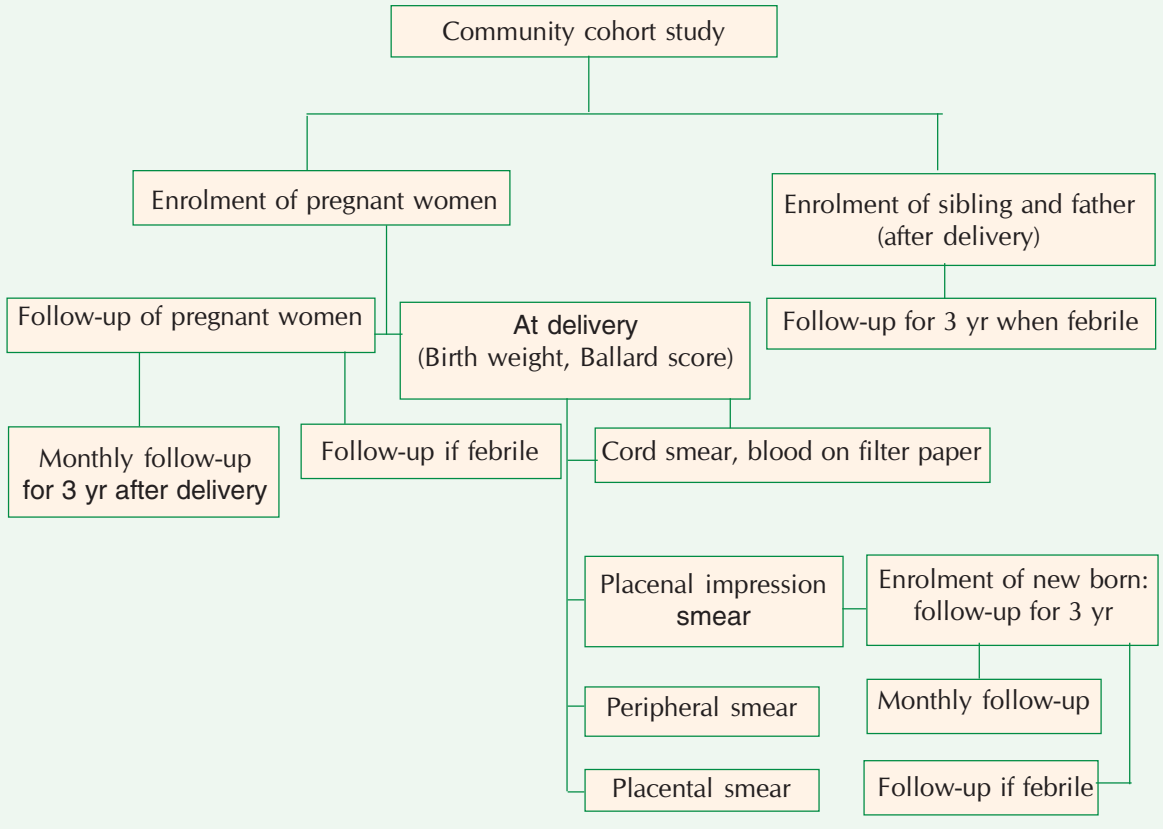
Age interval	Population	Male	Female
0–1	1357	701	656
>1–4	2636	1341	1295
>4–8	3600	1778	1822
>8–14	4691	2396	2295
>14–18	2332	1320	1012
>18	18084	9155	8929

mainly ethnic 'Gond' tribes (54.4%). Age-wise distribution of the total population under different age groups is given in Table 11. Sex ratio is 959 female/ 1000 male populations.

Pregnant women were identified and enrolled in the study with or without fever after obtaining written informed consent. At the time of enrolment, data concerning reproductive history indicators were collected. Each woman was followed every month during her pregnancy. Both current and recent clinical

Fig. 41: Numbering system for identification (Location ID) of houses

Flowchart 2. Community cohort study



signs and symptoms associated with malaria and antimalarial treatment was recorded. Thick and thin film prepared and finger-prick blood samples were collected for measurement of hemoglobin (Hb), immunological and molecular biological study during follow-up (Flowchart 2).

In all 23 sample collection points were established to collect placenta samples at the time of delivery with the help of traditional birth attendant and to keep them at our sample collection points. A cold chain was also established in these sample collection points. Delivery questionnaire was filled to collect information about the clinical sign of malaria and any other complications during delivery. Maternal peripheral blood smear, birth weight, gestational age and birth outcome was recorded.

Mother-infant pairs were followed-up every month after delivery. Questionnaire was filled during follow-up to obtain information on each child's health and axillary body temperature. Simultaneously, father and siblings were also enrolled after delivery and followed-up every month for clinical signs and as symptoms associated with malaria and antimalarial treatment. Blood samples collected from study subjects were used in immunologic and molecular biologic studies. G-6-PD and sickle-cell trait were examined in all enrolled subjects. In addition, fever surveys were also carried out weekly to measure the rate of morbidity and mortality in all age groups in the cohort area.

Hospital-based cross-sectional study was carried out at NSCB Medical College, Jabalpur and Civil Hospital, Maihar. Patients were screened for malaria parasite and malaria positive patients were enrolled in the study after obtaining written informed consent. All fever cases were given presumptive treatment and all subjects found positive for malaria were treated as per guidelines of NVBDCP within 24 h.

In all 1100 pregnant women (PW) were enrolled in the community cohort (Fig. 42). At the time of enrolment only 14.5% PW were having fever or history of fever and 37 PW were found positive for malaria (16 *Pv*, 21 *Pf*) of which only 14 were symptomatic and remaining 23 asymptomatic. During

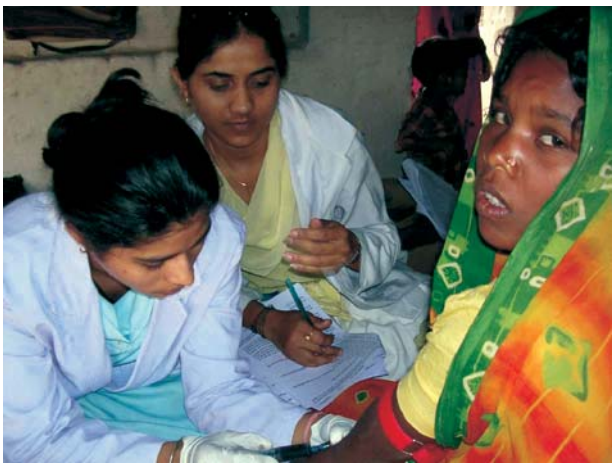


Fig. 42: Enrolment of pregnant women

the follow-up a total of 36 PW were found infected for malaria (17 *Pv*, 19 *Pf*) of which 37% were febrile (Table 12) while 38% infected PW having repeated episode of malaria during their pregnancy and post-pregnancy (Table 13). More than 90% PW were followed successfully till delivery while the overall follow-up rate among the PW was 68%. Mean haemoglobin of these PW were 10.04 ± 1.71 g% (4.7–16.6). Mild anaemia (9.86 ± 0.55 g%) was recorded in 52% of the subjects, moderate anaemia (8.30 ± 0.52 g%) in 23% and severe anemia in 3% (6.38 ± 0.66). Sickle-cell heterozygote and G-6-PD deficiency were found in 11.5 and 2% of women respectively in all the enrolled subjects.

A total of 678 infants were enrolled and followed subsequently every month. At the time of enrolment 8.7% were having fever or history of fever and 6.4% during subsequent follow-up. Two infants were found positive for malaria (1 *Pf*, 1 *Pv*) during enrolment and 5 (3 *Pf*, 2 *Pv*) during subsequent follow-up (Table 12) and all of them were symptomatic. Also 217 fathers and 113 siblings were enrolled and followed on monthly basis. Out of those 8.3% fathers and 6.1% siblings were found symptomatic and one from each group was positive for malaria during the enrolment (Table 12). Details of malaria episodes among enrolled pregnant women are shown in Table 13.

Table 12. Malaria infection in the study subject at enrolment and during follow-up

Study subjects	Enrolled (E)/Follow-up (F)	Malaria +ve	No. of parasite (Parasite density/ml)	
			Pv	*Pf*
Pregnant women (n = 1100)	E	37	16 (1800/ml)	21 (1080/ml)
	F	36	17 (2400/ml)	19 (3280/ml)
New born (n = 678)	E	2	1 (160/ml)	1 (1040/ml)
	F	5	2 (3360/ml)	3 (1080/ml)
Father (n = 217)	E	1	1 (3020/ml)	0
	F	0	0	0
Sibling (n = 113)	E	1	1 (160/ml)	0
	F	0	0	0

Table 13. Malarial episode among pregnant women

No. of pregnant women	Repeated episodes
8	2
4	3
1	4 (first 3 *Pf* and 4th one is *Pv*)
1	5 (first 3 *Pf* and 4th & 5th one is *Pv*)
23	1

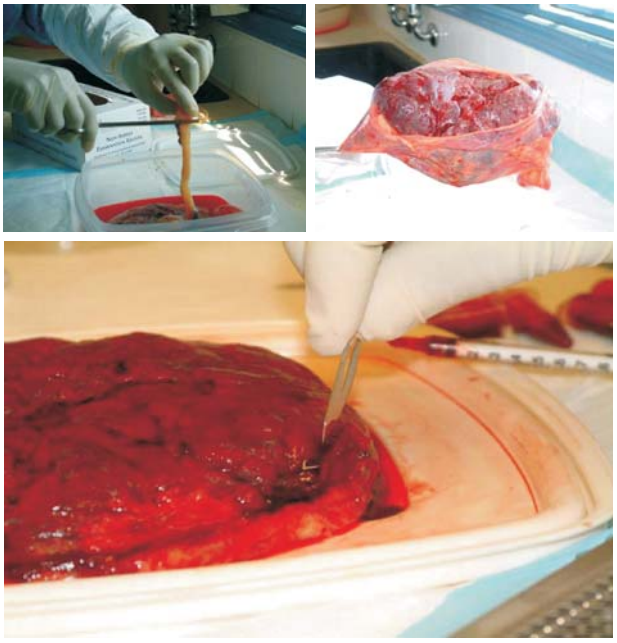


Fig. 43: Processing of placenta

Of the 1100 enrolled pregnant women, 735 deliveries took place. Out of 735 deliveries, 554 could be attended and 3.8% women had fever at the time of delivery. Peripheral smears of nine mothers were positive for malaria; 394 placentas were collected and processed successfully (Fig. 43). Still birth and neonatal death were recorded in 1.8 and 1.5% subjects respectively. Abortion was recorded in 26 (3.53%)

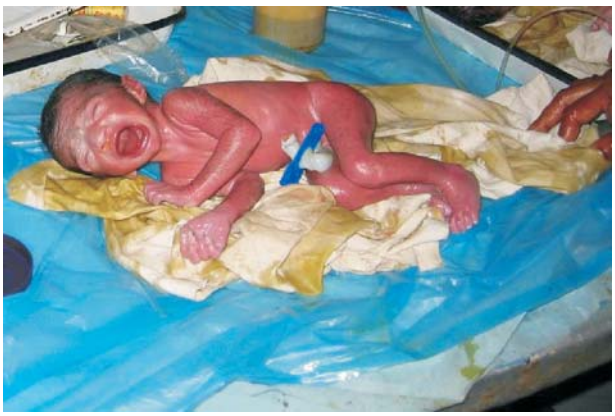


Fig. 44: New born baby (Low birth weight)

subjects. Only one maternal death was found. Low birth weight was found in 38% (2.14 ± 0.47 kg) cases (Fig. 44). The details of placental malaria are shown in Table 14.

Active fever survey was conducted in the cohort area. Both *P. vivax* and *P. falciparum* were prevalent in the study area and as the transmission season progressed, there was an increasing trend in *Pf* ratio from 28 to 83% (Fig. 45). Overall SPR was 14 and *Pf*% was 64%. The age group >4–14 years found to be highly susceptible for malaria (Fig. 46) as compared to other age groups combined (OR = 2.34;

Table 14. Placental investigation for malaria parasite

Smears (n = 394)	Positive (no. of cases)	Species (Parasite density)	
		Pf	*Pv*
Mother peripheral smears	9	5 (496/µl)	4 (4200/µl)
Cord blood smears	4	1 (200/µl)	3 (220/µl)
Placental smears	6	2 (12940/µl)	4 (547/µl)
Tissue impression smears	6	2 (12980/µl)	4 (1400/µl)

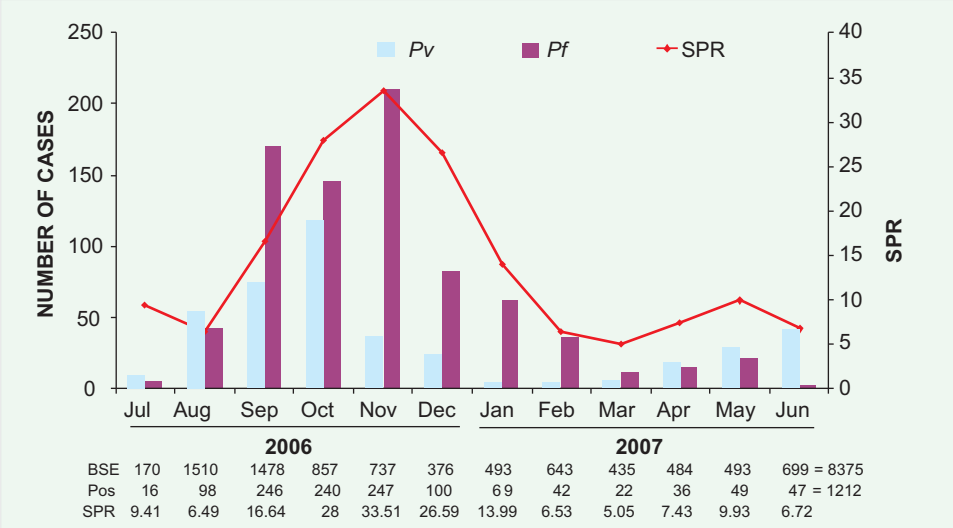


Fig. 45: Malaria prevalence in cohort area

Table 15. Hospital-based cross-sectional study

Case distribution (n = 75 in each category)	Samples collected
Severe malaria anaemia (Hb <5 g/dl)	31 (3.94 ± 0.67)
Moderate malaria anaemia (Hb ≥5 & ≤7.9 g/dl)	65 (6.65 ± 0.79)
Mild malaria anaemia (Hb ≥8 & ≤10.9 g/dl)	75 (9.37 ± 0.78)
Cerebral malaria	50
Uncomplicated malaria	74 (12.8 ± 1.56)
Acute renal failure	14

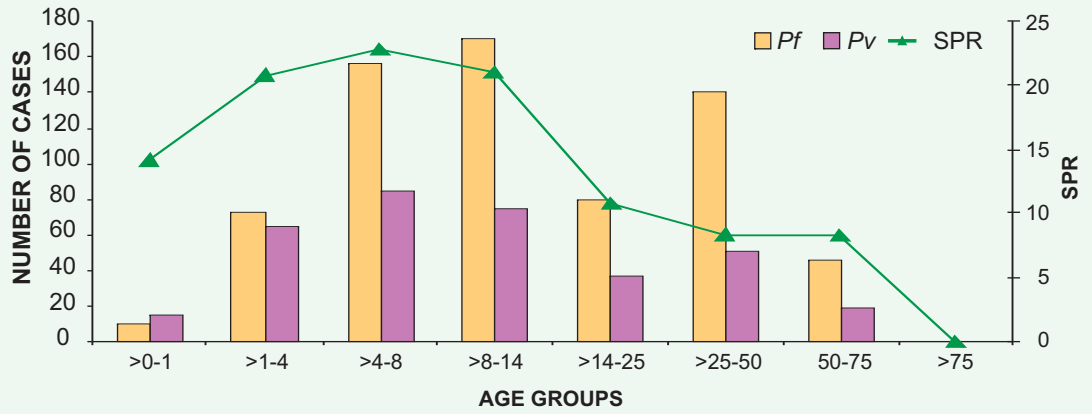
Fig. 47: Malaria prevalence in cohort area (Site-wise)

Table 16. Hospital-based cross-sectional study in pregnant women

Case distribution (n = 106 in each category)	Samples collected
Pregnant women with *Plasmodium falciparum* infection	53
Pregnant women with *Plasmodium vivax* infection	6
Healthy controls	36

95% CI 2.04–2.69). Further analysis revealed that the highest malaria prevalence was recorded in the villages surrounded by the dam reservoir (20.5%) as compared to the forested villages (15.4%) [OR-0.70; 95% CI-0.57–0.86] and plain villages (11.5%) [OR-0.50; 95% CI-0.43–0.60] as shown in Fig. 47. Similarly, the prevalence of *P. falciparum* was also found to be relatively higher in the villages surrounded by the dam reservoir (14.4%) than the forested villages (9.4%) [OR-0.62; 95% CI-0.48–0.78] and the plain villages (7.1%) [OR-0.45; 95% CI-0.37–0.55].

In the hospital-based cross-sectional surveys, the patients were also enrolled following the protocol and blood samples were collected for immunological and molecular biology study with particular reference to establish correlation with clinical severity of the disease and serum cytokine levels e.g. TNF-α, IFN-γ, IL-4, IL-10, IL-12 and IP-10. Enrolled patients were summarized in Tables 15 & 16. Samples from hospital-based cross-sectional study of the PW cases allowed us to generate data on immunological parameters, such as cellular and humoral immune responses profile of women with *P. falciparum* and *P. vivax* infections. In addition, all fever cases coming to Civil Hospital Maihar, were also screened for malaria (Fig. 48). Age group >4–14 years were highly susceptible for malaria as compared to other age groups combined (OR = 1.79; 95% CI-1.54–2.09) as shown in Fig 49.



BSE	176	658	1052	1166	1080	2289	783	34 = 7238
Pos	25	137	240	245	117	192	65	0 = 1021
SPR	14.20	20.82	22.81	21.01	10.83	8.39	8.30	0

Age group 4–14 is highly suceptible to malaria; OR = 2.34 (2.04 – 2.69); RR = 2.05 (1.83 – 2.29); χ^2 = 158.96; P<0.001

Fig. 46: Malaria prevalence by age groups in cohort area

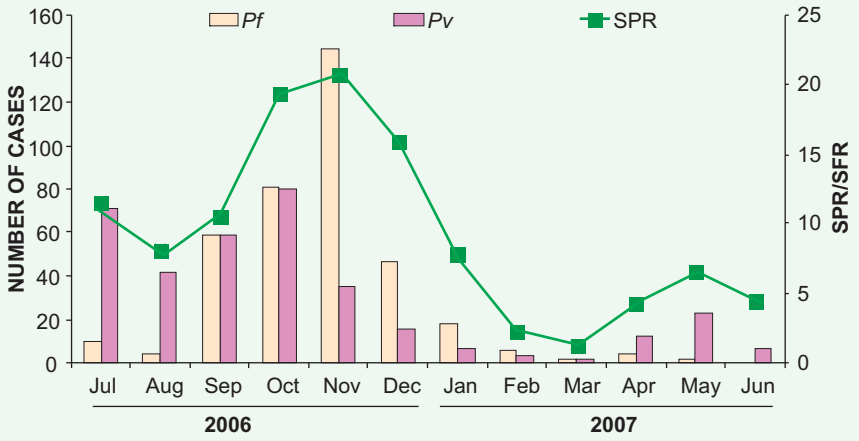


Fig. 48: Malaria prevalence in Maihar Civil Hospital, Satna

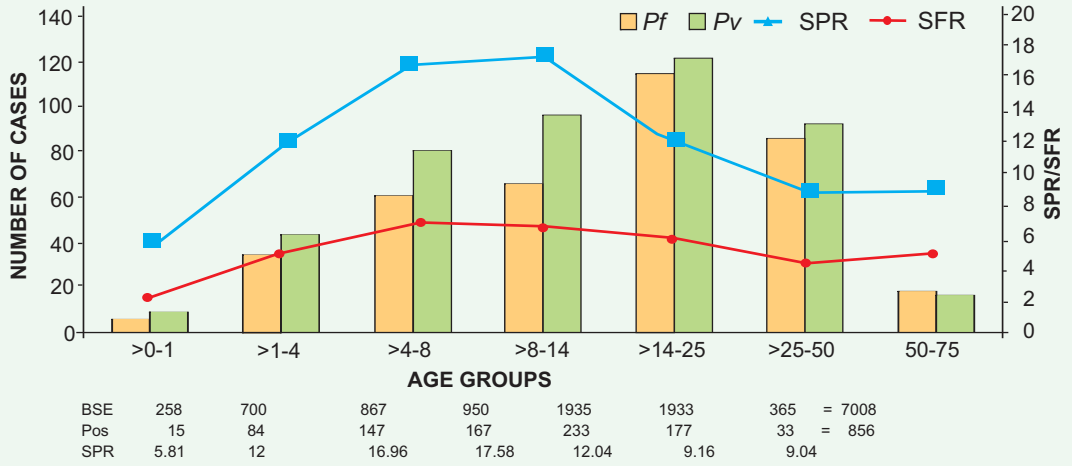


Fig. 49: Malaria prevalence by age groups

Immunoepidemiology

Naturally acquired immune responses to stage-specific *P. falciparum* and *P. vivax* antigens in a population of central India

The study has been initiated with an aim to characterize immune responses to stage-specific *P. falciparum* and *P. vivax* antigens in children and adults naturally exposed to malaria; to study the development and maintenance of immune responses in different age groups with emphasis of infants, their older siblings and mothers, including identification of epitopes that correlate with protection; to determine the role of stage-specific antigens in the development and maintenance of natural immunity to malaria; to evaluate the immune mechanisms, those are involved in pathogenesis of malaria, especially anaemia, cerebral malaria and placental malaria. Study was conducted in three populations. They are: (i) Infants, children and adults from the community; (ii) Pregnant women from the community; and (iii) Hospitalized patients with severe malaria. Peripheral blood, placental and cord blood at delivery were taken for determining antibodies against species and stage-specific antigens by enzyme immunoassay. The antibody levels were quantified using known antimalarial antibody positive controls and this allowed us to estimate antibody levels in O.D. values. Sera from non-endemic healthy subjects were taken as negative control (Figs. 50–53). Blood samples collected from a group of 149 malaria parasites positive (79 *P. falciparum* and 70 *P. vivax*) patients were tested for the determination of antibodies to species and stage-specific peptides. Proportionate sizes of malaria negative subjects (n = 50) were also taken for comparison. Seroprevalence was more in parasite positive cases. Differences in antibody responses between parasite positive and negative groups were categorized as high and low responders. Most of the parasite positive individuals showed high antibody responses to all the peptides, whereas the malaria negative individuals were mostly low responders (Figs. 54 and 55). The responders' frequency of *Pf* and *Pv* positive patients with malaria negative subjects was compared and results found significant between two groups. The level of

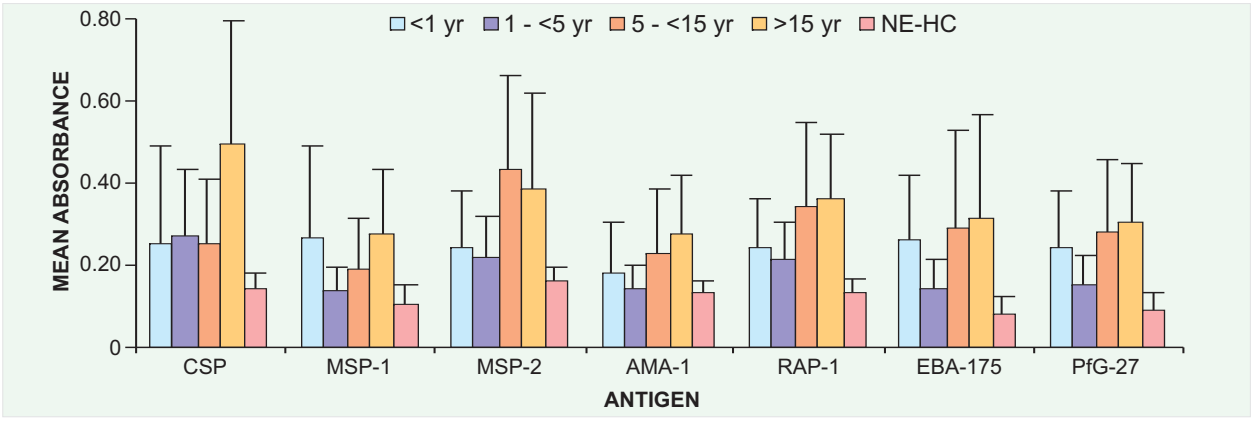


Fig. 50: Antibody profile with *P. falciparum* antigen

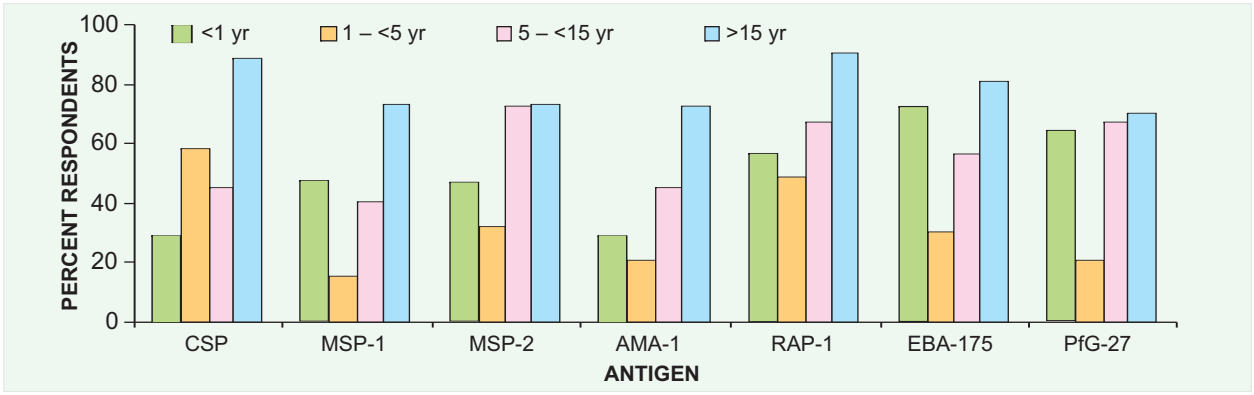


Fig. 51: Responder frequeny to *P. falciparum* antigen

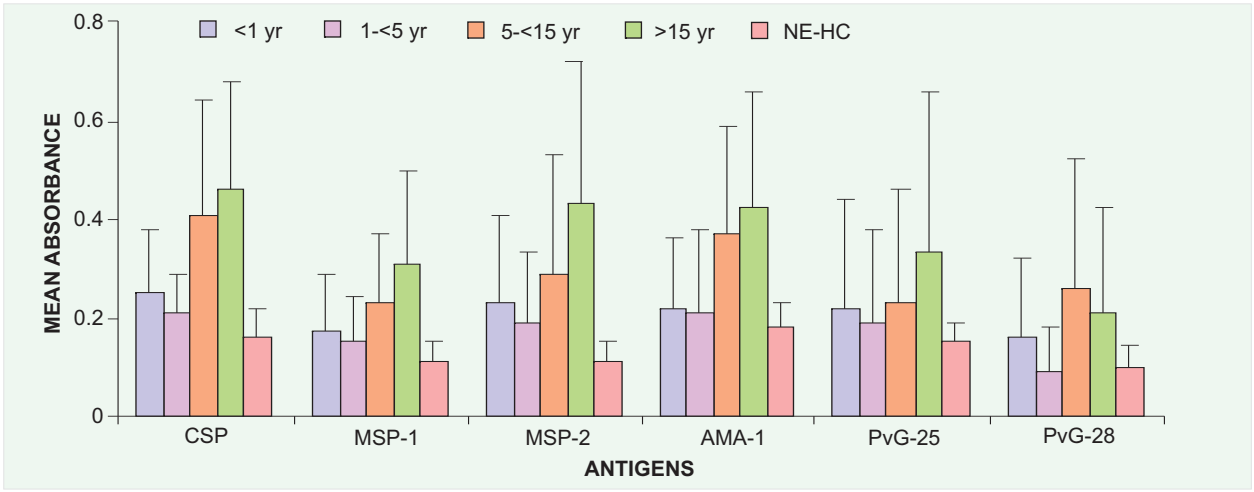


Fig. 52: Antibody profile with *P. vivax* antigens

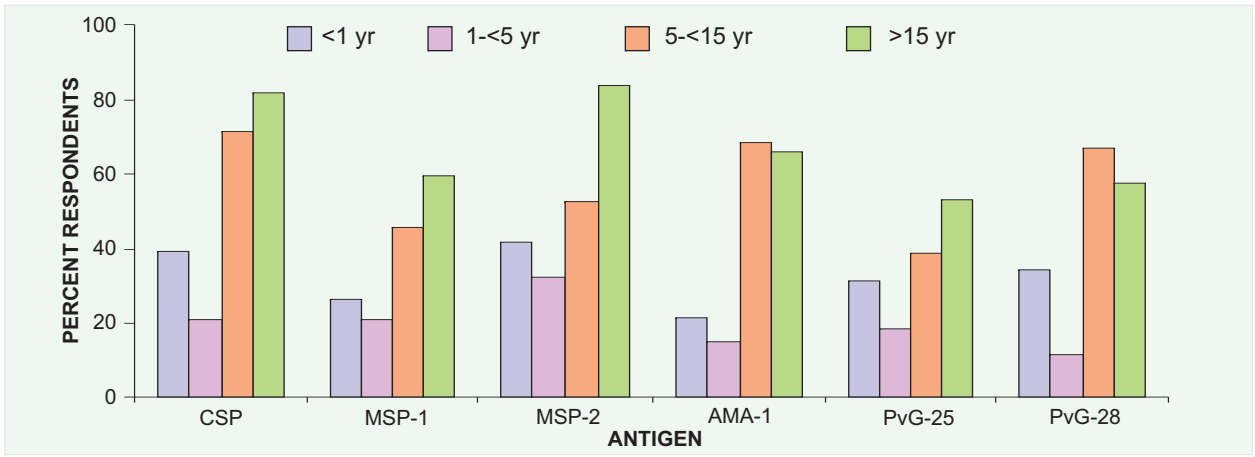


Fig. 53: Responder frequency to *P. vivax* antigens

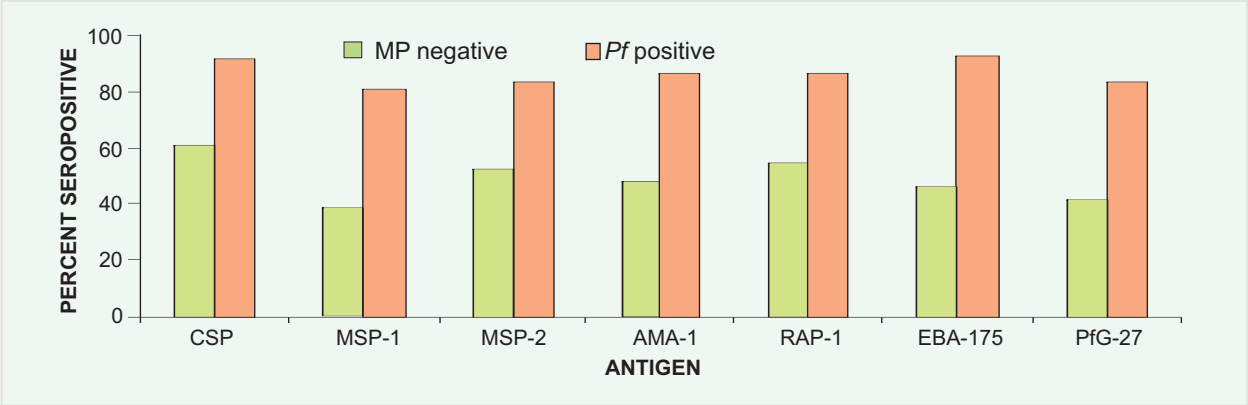


Fig. 54: Seroprevalence with *P. falciparum* antigen

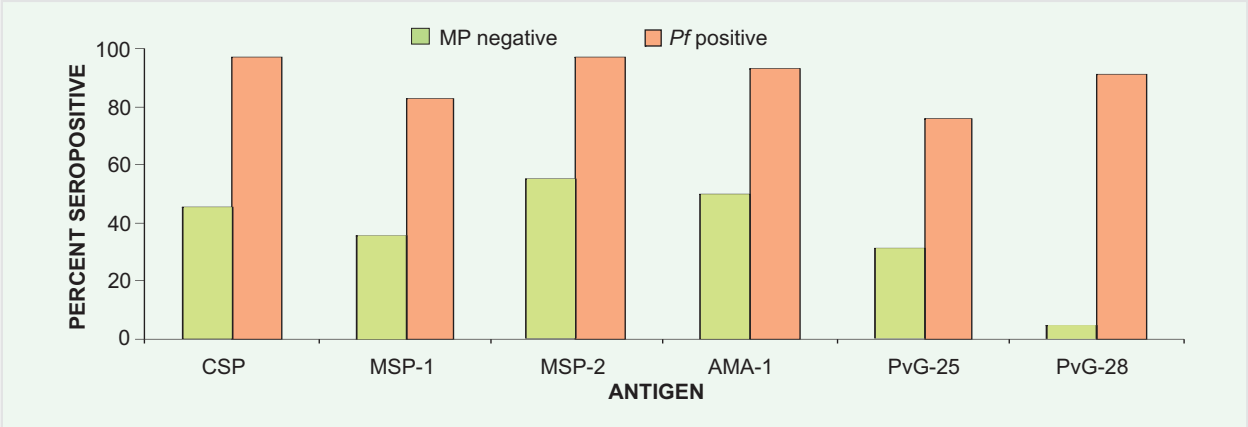


Fig. 55: Seroprevalence with *P. vivax* antigen

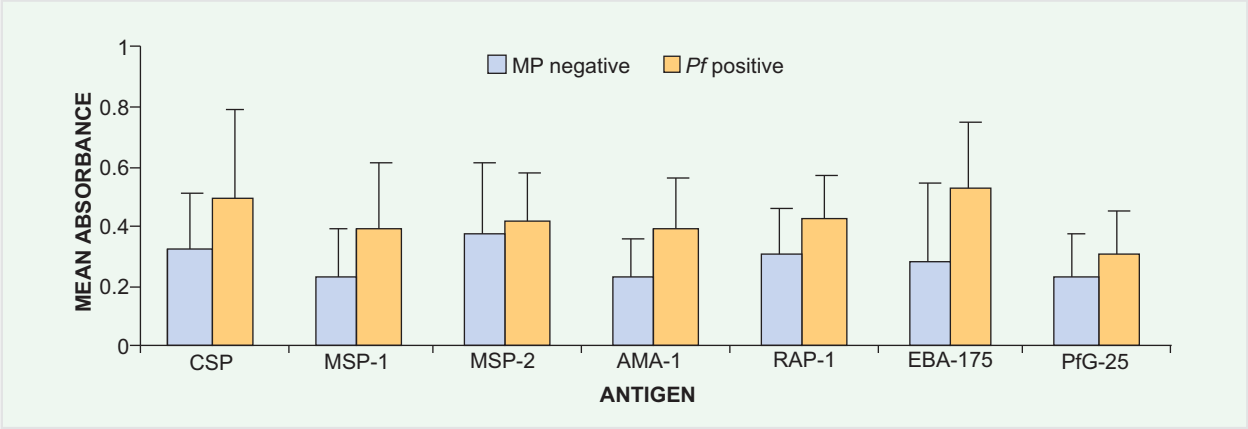


Fig. 56: Antibody profile of enrolled pregnant women with *P. falciparum*

antibodies appeared to be related to their exposures to the parasite during malaria transmission. Thus the results suggest that there might be natural boosting in antibody responses as a result of infection and that were measurable against these peptides.

Blood samples collected from enrolled pregnant women were checked (n = 600) for antibodies to species and stage-specific peptides. In this group also seroprevalence was more in parasite positive cases. Most of the parasite positive individuals (36 *P. falciparum* and 26 *P. vivax*) showed comparatively higher antibody response to all the peptides than the malaria negative individuals. The enrolled subjects were divided gravida-wise into three categories: primigravida (n = 180), secundigravida (n = 186) and multigravida (n = 234). Results of the antibody responses of three groups against individual peptides were compared. No significant differences have been observed between pregnant women of different gravidities (Figs. 56–59).

The infants' umbilical cord blood and mothers' placental blood were tested for antimalarial antibodies to both *Pf* and *Pv* antigens to determine the possible effects of maternal antimalarial IgG antibodies on protection against placental infection and infection in infants. The relationship between antimalarial antibodies in infants' cord blood (C) and mothers' placental blood (P) were drawn. Overall antimalarial IgG profile in mothers at the time of delivery was low and same response has been observed in respective

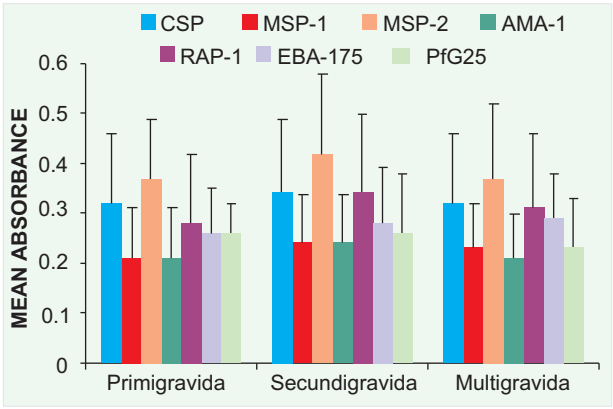


Fig. 57: Antibody profile with *P. falciparum* antigen

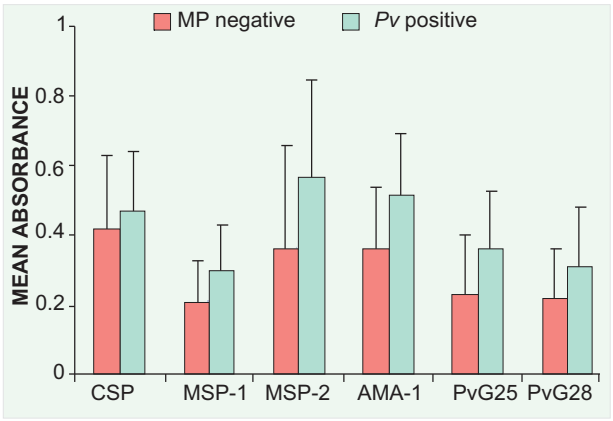


Fig. 58: Antibody profile of enrolled pregnant women with *P. vivax*

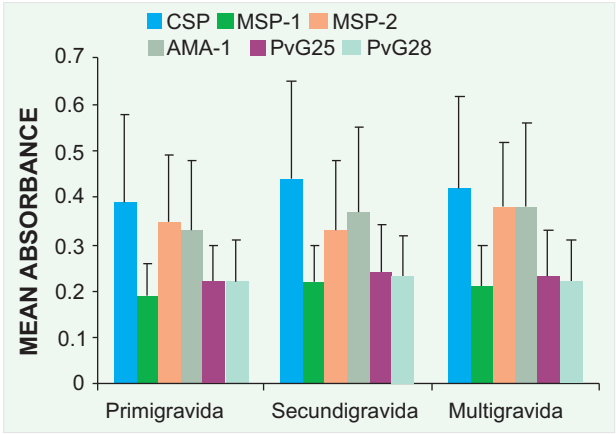


Fig. 59: Antibody profile with *P. vivax* antigen

infants. In these study cohorts, there was a trend for the higher antibody levels in maternal sample than cord against all *Pf* and *Pv* stage-specific peptides. We have also determined percent positive responders among different placental and cord blood samples. Among *Pf* peptides, *Pf*MSP-1 and *Pf*G-27 showed less seropositivity when compared to other peptides, whereas among *Pv* peptides *Pv*G-28 showed less seropositivity. Highest seropositivity was seen in *Pf* and *Pv*CSP.

Cellular response was determined in peripheral blood mononuclear cells by lymphocyte transformation test in the presence of *P. falciparum* and *P. vivax* antigens and cytokines (IL-4 and IFN-γ) level was estimated in activated T lymphocytes culture supernatant by sandwich ELISA. The peripheral blood mononuclear cells (PBMC) were isolated from venous blood of subjects who have past experience of malaria and the proliferative responses against *P. falciparum* and *P. vivax* antigens were determined in individual set of PBMCs. Only a subset of adults and older children above 14 year were participated in this study. In general, the study subjects responded to T-cell epitopes. The mean stimulation index (SI) was not significantly different among positive responders. The *in vitro* stimulation of T-cells from malaria-exposed donors results in the production of IL-4 and IFN-γ in concordance with the serum concentrations of antibodies specific for the antigens used for lymphocyte stimulation.

Peripheral blood from healthy cohort participants and hospital admitted *P. falciparum* malaria patients were collected in anticoagulant. Four categories of patients were identified: uncomplicated falciparum malaria (UFM), cerebral malaria (CM), severe malaria (SM) and severe malaria anaemia (SMA). Cytokines like IL-4, IL-10, IP-10, IFN-γ and TNF-α levels were estimated in plasma using commercially developed two-site ELISA assay kits. Our results revealed that IP-10, which is a proinflammatory chemokine, progressively increased with the disease severity. Its plasma levels were found to be significantly elevated during malaria illness compared to HC; with highest levels found among CM cases followed by SM, SMA and UFM and lowest levels among HC subjects. The levels of TNF-α and IFN-γ (both proinflammatory) were also increased with the severity of disease. In comparison to healthy controls, both were elevated among the severe cases of malaria. In our study, IL-10 was undetectable among the HC subjects but in patients it got up-regulated during the disease severity; in the SMA patients IL-10 level was low. Another anti-inflammatory cytokine IL-4 began rising in plasma during acute falciparum malaria and increased maximum among CM cases but in SM and SMA, IL-4 level was comparatively lower than CM. Cytokine levels were compared within groups and between groups and results were significant.

Further study needs to identify the antigen-specific antibody responses in the serum of pregnant women who do not suffer from placental malaria, which may suggest that these antibodies are important for protecting the mother against infection. Antibodies produced by adults (mothers) are correlated with protection. Therefore it is important to determine if infants mount antibody responses to species and stage-specific antigens and the association between infant's antibodies with protection. An important component of this study is to understand the role of immunologic factors in the pathogenesis of severe malaria. The current understanding of the immunology of severe malaria is mostly based on African studies. However, Indian populations face a different epidemiologic setting due

to differences in the malaria transmission and availability of both *P. falciparum* and *P. vivax*.

Molecular Epidemiology

Genomic DNA Extraction and Polymerase Chain reaction (PCR), Nucleotide Sequencing and Sequence Analysis

The genetic polymorphism in the vaccine candidate antigen genes (*MSP-1, MSP-2, MSP-3, TRAP, RAP-1, CSP, EBA-175* and *AMA-1*) and drug resistance genes (*pfcrt*, *pfdhfr* and *pfdhps*) were studied. Genomic DNA was extracted from *P. falciparum* infected blood. The aliquot of extracted DNA was used to amplify the vaccine candidate antigen genes and drug resistance genes using the respective gene-specific primers. Sequencing was performed using ABI Big Dye Terminator Ready Reaction Kit Version 3.1 on a 310 genetic analyzer (Applied Biosystems). Sequences were analyzed using BioEdit software and aligned by using GeneDoc (Table 17 and 18; Figs. 60 and 61).

P. falciparum MSP-1 gene was sequenced from 37 isolates. Majority (17 of 37) of the isolates had MAD20-type allele while 12 isolates showed K1-type MSP-1 alleles. Remaining eight isolates were showing RO33-type of alleles. It is noteworthy that MAD20 alleles further showed nine different variants (MI to MIX) among themselves whereas six variants (KI to KVI) were found of K1-type alleles. *MSP-2* gene was sequenced from 35 isolates. Majority (29 of 35) of the isolates showed FC27-type allele while, six isolates showed 3D7-type alleles. Eight variants of FC27-type (FI to FVIII) and three variants of 3D7-

Table 17. Details of the number of isolates sequenced for vaccine candidate antigens

Antigens	No. of isolates	Sequenced nucleotides (bp)	Results
MSP-1	85	555	K1–40%, MAD20–40% & RO33–20%
MSP-2	81	634	FC27–63% & 3D7–37%
MSP-3	32	550	K1–44%, FC27–34% & 3D7–22%
TRAP	67	757	G3–46% & (G8–G23)–54%
RAP-1	47	1133	GIII–36%, GII–23% & Rest (GI- GXI)–41%
EBA-175 (Region II)	31	1100	GIII–38%, GVIII–23% & Rest (GI–GX)–39%
CSP	30	1000 plus 450	Th2R GI–83% & Rest (GII–GV)–17% Th3R GI–87% & Rest (GII–GIV)–13%
AMA-1	90	540	GI–33%, GIII–20% & Rest (GII–GXI)–47%

Table 18. Details of the number of isolates sequenced for drug resistance loci

Drug loci	No. of Isolates	Sequenced nucleotides (bp)	Codons	Results
Pfdhps	96	653	436, 437, 540, 581 & 613	Wild–92% Single mutant–8%
Pfdhfr	93	542	16, 51, 59, 108 & 164	Wild–4% Single mutant–11% Double mutant–47% Triple mutant–38%
Pfcrt	78	582 plus 232	72–76 & 220	Wild–2% Single mutant–98%

Fig. 60: Electrophoretogram of merozoite surface protein-1

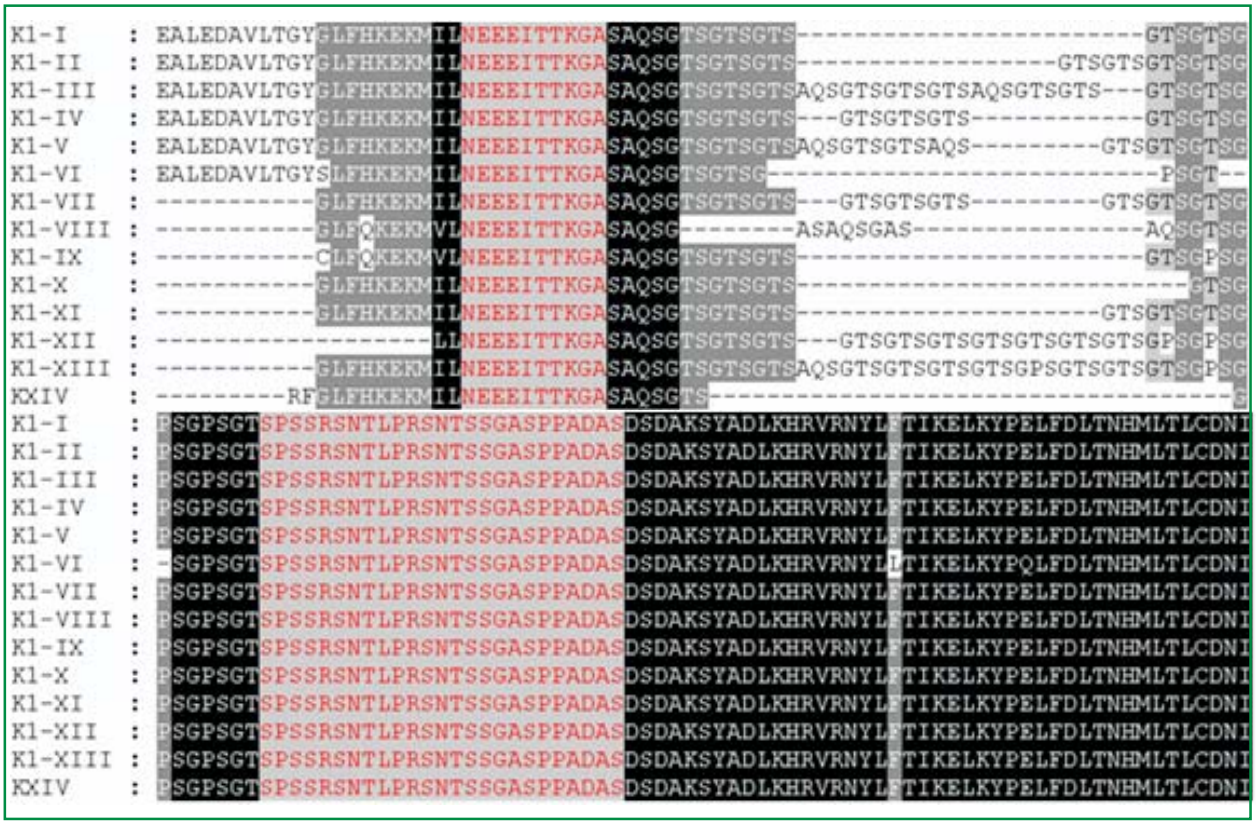


Fig. 61: Alignment of K1 like allele family (MSP)

type (3D7I to 3D7III) alleles were further observed. *MSP-3* gene was sequenced from 32 isolates. Majority (14 of 32) of the isolates had K1-type allele, while seven isolates showed 3D7-type MSP-3 alleles. Remaining 11 isolates were showing FC27-type alleles. Two variants each in K1 allele (K1-a and K1-b) and 3D7 alleles (3D7-a and 3D7-b) were found. TRAP gene was sequenced from 29 isolates. Seven allelic variants (GI to GVII) of the TRAP gene were observed. Majority (18 of 29) of the isolates belong to the GIII alleles. Five of the isolates showed GV alleles. Two isolates had GI alleles. The remaining four isolates had four different types of alleles GII, GIV, GVI and GVII. *AMA-1* gene was sequenced from 40 isolates. Those were classified into ten different alleles (GI to GX) according to their amino acid sequences. The predominant allele observed among the isolates was G1 allele.

Polymorphism in Drug Resistance Loci

Dhps gene of *P. falciparum* was sequenced from 35 isolates. Majority (32 of 35) of the isolates showed SAKAA (wild type) genotypes at the codons 436, 437, 540, 581 and 613 respectively. Two isolates had SGKAA (single mutant) at these codons while one isolate was having SA/EKAA genotype. *Dhfr* gene was sequenced in 36 isolates. Majority (25 of 36) of the isolates showed ANRNI (double mutant) genotypes at the codons 50, 51, 59, 108 and 164 respectively. Eight isolates had ANCNI (single

Setting up of experiment in molecular biology laboratory

Harvesting of labelled lymphocytes onto glass-fibre filters in Cell Harvester

Breeding site in forested villages

Breeding site in plain villages

mutant) at these codons while three isolates were having ANCSI (wild type) genotype. *Pfcrt* gene was sequenced in 35 isolates. All of the isolates showed SVMNTS genotype at the codons 72, 73, 74, 75, 76 and 220 respectively (Table 18).

Entomological study

Indoor Resting Mosquito Collections

Monthly collections of indoor resting anophelines were initiated from August 2006 in 10 study villages. Out of those, five are located in the plain area, three in the forest area and two near the dam site. Results of one year study revealed that the average per man hour density (MHD) of *Anopheles* mosquitoes was 42.59 (range 16 in June to 195.05 in August). Of which 66.8% were *An. culicifacies* (MHD 28.49, range 12.5 in May to 126.4 in August) (Fig. 62). The density of *An. fluviatilis* was recorded 0.48. Of which, the density in the forested villages was 0.97, which is significantly higher ($F = 9.23$; $p < 0.001$) as compared to that in villages located in the plain area and near the dam site (Fig. 63). *An. culicifacies* and total anophelines density was highest in the villages surrounded by dam reservoir and lowest in forested villages, although this difference is not significant statistically ($p > 0.05$).

Human Landing Collections

These collections were initiated from September 2006 by conducting all night collections in each month in indoor and outdoor sites of the study villages. Eleven months' results revealed that the average human landing of total *Anopheles* was 0.98 per man per night in indoors and 0.88 in outdoors. *An. culicifacies* was the most common species at both sites with 0.60 per man per night landing in indoors and 0.51 in outdoors (Fig. 64). *An. fluviatilis* landing was low (0.03 in indoors and 0.02 in outdoors). The

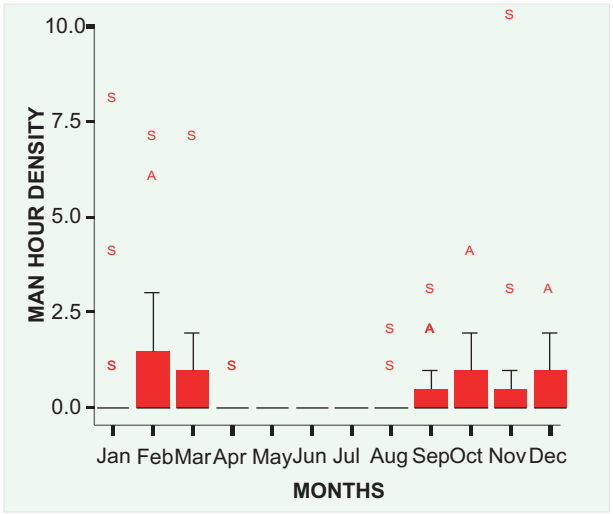


Fig. 63: Monthly man hour density of *An. fluviatilis*

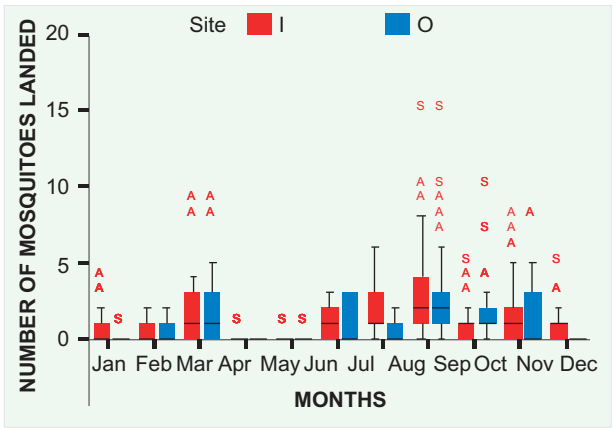


Fig. 62: Month-wise man hour density of *An. culicifacies*

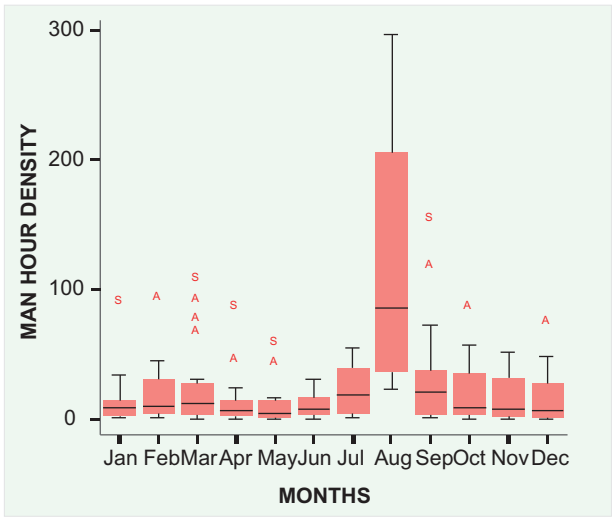


Fig. 64: Monthly human landing catches of *An. culicifacies*

Table 19. Results of vector incrimination by ELISA

Month of collection	No. of *An. culicifacies* tested	Results	No. of *An. fluviatilis* tested	Results
April 2007	135	1+ve (*Pf*) 2+ve (*Pv* mix of 247&210)	3	–ve
May	63	2 +ve (*Pv*247)	0	–ve
June	68	–ve	0	–ve
July	115	1+ve (*Pv*210)	1	–ve
August	210	–ve	8	–ve
September	215	1+ve (*Pv*247)	11	2+ve (*Pf*)
October	129	–ve	34	1+ve (*Pv*247)
November	117	–ve	34	–ve
December	83	–ve	22	–ve
January 2008	93	–ve	16	–ve
February	114	–ve	12	–ve
March	130	–ve	7	–ve
Total	1472	7 +ve (1*Pf*, 6*Pv*)	148	3+ve (2*Pf*, 1*Pv*)

landing rate of all anophelines at indoor sites was almost equal in villages located at plain, forest and dam sites, however, at outdoor sites it was higher in plain and dam sites as compared to forest villages. Further analysis revealed that significantly more *An. culicifacies* land between 1800 and 2200 hrs than after 2200 and 0600 hrs (t=4.73; p<0.0001).

Light Trap Collections

Light trap catches revealed that the average per trap per night catch of *Anopheles* mosquitoes was higher at outdoors (20.84) as compared to that at indoors (11.78). *Anopheles culicifacies* and *An. fluviatilis* were 4.44 and 0.84 at indoors and 6.18 and 1.28 at outdoors respectively. Area-wise results revealed that most of the anophelines were trapped in the plain villages. Further analysis revealed that significantly more *An. culicifacies* were trapped between 1800 to 2200 hrs than after 2200 hrs (t = 4.73; p<0.0001). The trend was similar for *An. fluviatilis* (t = 2.19; p<0.05). This would have direct implication for bednet as preventive measure.

Determination of Sporozoite Rate

The sporozoite determination by ELISA could be initiated in June 2007 after obtaining Monoclonals from CDC and standardization of the techniques at the field unit. The proportion of *An. culicifacies* and *An. fluviatilis* with positive salivary glands by ELISA is shown in Table 19. Monthly entomological surveillance carried out in 10 study villages revealed that average indoor resting density (per man hour) of anophelines during the year was 37.3 of which *An. culicifacies* was the dominant species (18.4) followed by *An. subpictus* (10.9), *An. annularis* (5.8) and *An. fluviatilis* (0.5). A total of 1472 *An. culicifacies* and 148 *An. fluviatilis*, collected from different localities were assayed during the year for sporozoite detection by ELISA technique of which seven *An. culicifacies* (1 *Pf* and 6 *Pv* strain) and three *An. fluviatilis* (2 *Pf* and 1 *Pv* strain) were found positive for the presence of sporozoites (Table 19).

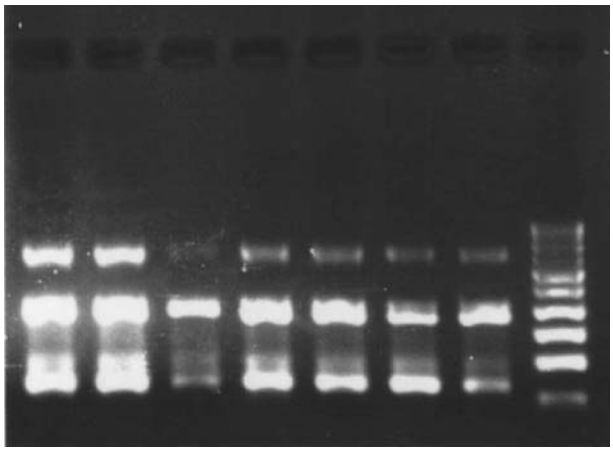


Fig. 65:Polymerase chain reaction assay for differentiation of members of *An. fluviatilis* complex

Sibling species determination

The *An. culicifacies* was further identified into the sibling species using cytotaxonomy and *An. fluviatilis* by using polymerase chain reaction (PCR) techniques at NIMR, Delhi. Results revealed that the majority of the *An. culicifacies* tested were of sibling species 'C' (66%). Species 'D' was detected in 24.6% and species 'B' in 9.1% of *An. culicifacies.* Area-wise results also showed the highest prevalence of sibling species 'C' in all the plain, forest and dam areas. All the 101 *An. fluviatilis* tested were of sibling species 'T' of which three were detected as sporozoite positive (Fig. 65).

As proposed in the project, data on all above parameters would be generated to fulfil the objectives in large number of samples. Once fully characterized, this site would be useful for testing of any tools available for the control and prevention of malaria, such as antimalarial vaccines and diagnostic reagents. The study is in progress. ❑