Development of a Field Site for Malaria Vaccine Trial

A study has been initiated to understand the epidemiology of malaria in Sundergarh, Orissa with an objective to develop a field site for vaccine trial. There are 13 study villages with a total population of 4,221 under Gurundia and Birkera PHCs of Sundergarh district, out of which eight villages with a population of 2,058 are located in deep forests and five villages with a total population of 2,163 are located in a plain area (Fig. 7). The study villages are predominantly inhabited by ethnic tribals — Oram, Munda, Khadia, etc.

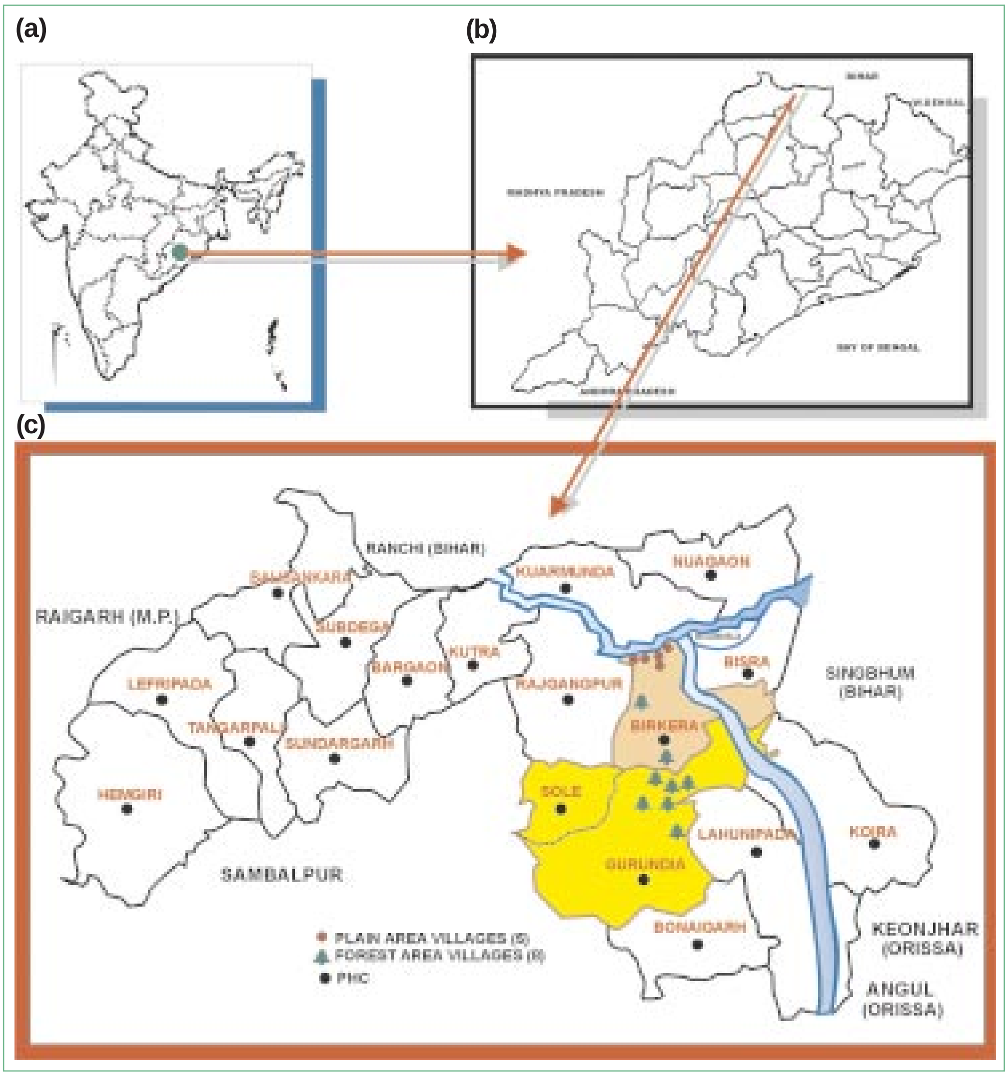

Fig. 7: **Study area (a) India map showing location of Orissa state; (b) Orissa state showing location of District Sundergarh; and (c) District Sundergarh showing villages in two PHCs—Gurundia and Birkera**

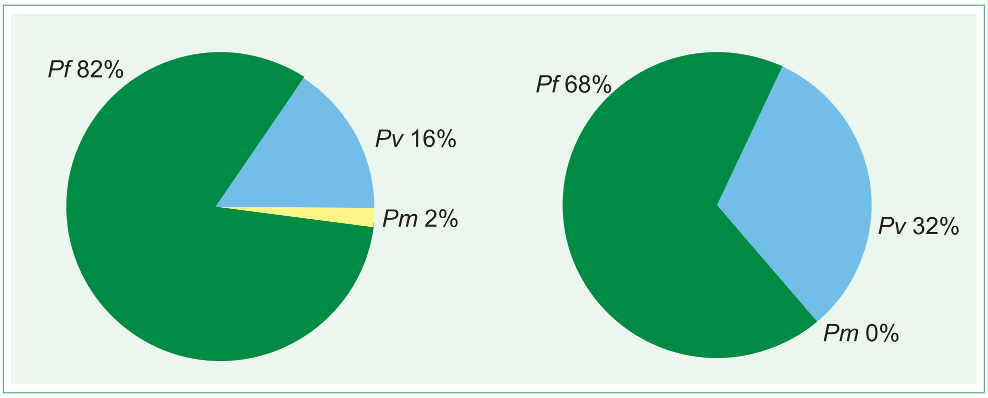

Fig. 8: **Proportion of different *Plasmodium* species in the study area**

Parasitological Surveys

Out of the total malaria cases in the forest area, the prevalence of *P. falciparum*, *P. vivax and P. malariae* accounted for 82, 16 and 2 per cent respectively and in the plain area, the prevalence of *Pf* and *Pv* were 68 and 32 per cent respectively (Fig. 8).

The longitudinal and cross-sectional parasitological surveys were conducted in all the study villages. The SPR, SfR, *Pf* per cent and annual parasite incidence (API) respectively in the forest villages were 38.1, 30.8, 80.9 and 323.1, whereas in the plain area villages were 20.7, 13.4, 64.5 and 43. The malaria incidence was more in the younger age groups up to 15 years and the highest incidence was in the 0–5 years age group in the forest area while in the plain area no malaria case was found in the 0–5 years age group and the malaria cases were evenly distributed among the age groups of 5–10, 10–15 and >15 years (Fig. 9).

The spleen rate in children living in the forest area villages was above 75 per cent throughout the year and in adults it was above 40%, whereas in the plain area the spleen rate in children and adults ranged from 40–82 and 9–14% respectively.

Entomological Surveys

An. culicifacies was widely prevalent in both the areas, whereas *An. fluviatilis* was exclusively found in the forest area villages (Fig. 10). All night mosquito landing collections on human baits showed

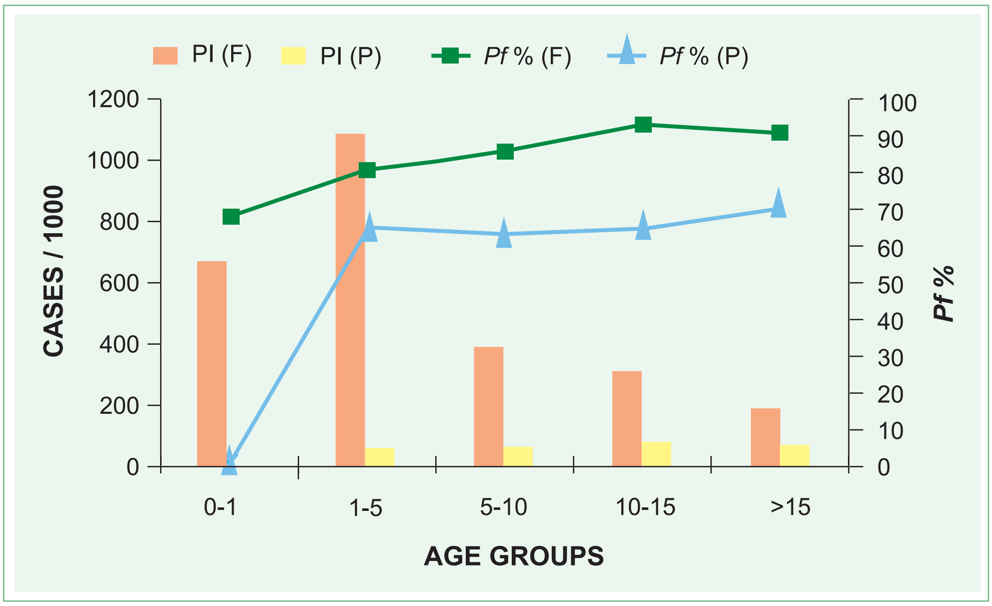

Fig. 9: **Age-wise distribution of malaria cases and *P. falciparum* proportion in forest and plain area village (Data generated through weekly surveillance) PI (F) Parasite incidence in forest area; PI (P) Parasite incidence in plain area**

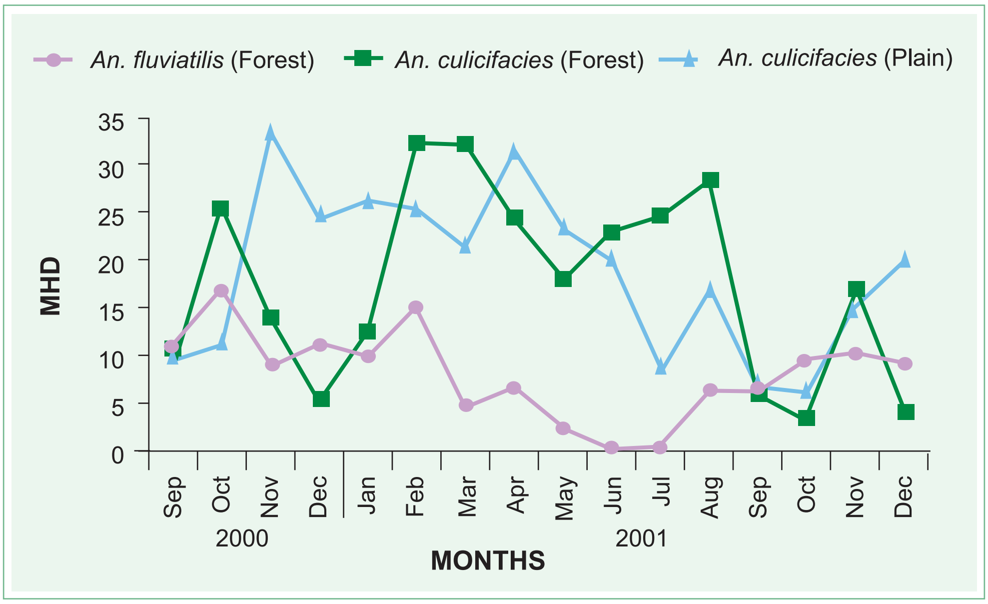

Fig. 10: **Indoor resting density of *An. culicifacies* and *An. fluviatilis***

An. fluviatilis to have preference to bite humans and the man landing rate in the forest area was 13.5 bites per person per night while the man landing rate of *An. culicifacies* in the forest and plain areas was 0.3 and 0.5 bites per person per night respectively. The sporozoite rate of *An. culicifacies* and *An. fluviatilis* was found to be 0.70 and 2.82 respectively and entomological inoculation rate (EIR) for these species was calculated as 0.002 and 0.38 infective bites per person per night in the forest area villages, whereas it was nil in the plain area.

Multiplicity of Infection

Multiplicity of infection is an important marker to get information about the intensity of malaria transmission as well as development of host-immune responses. Field collected blood spots from *P. falciparum* positive patients from forested villages were genotyped using MSP-1 (block 2) and MSP-2 (central variable region) by PCR assay. Primers used were gene-specific in primary PCR and family-specific in the nested PCR condition. Multiplicity of infection among isolates ranged from 1.1 to 3.28. Number of alleles observed were 22 in MSP-1 and 24 in MSP-2. A high proportion of isolates (65–100%) had multiple infectivity with different genotypes of MSP-1 and MSP-2.

Sequence Diversity

The sequence diversity in three malaria vaccine candidates namely MSP-1_{19} (C- terminal 19kD fragment of MSP-1), EBA 175-RII and TRAP was determined in *P. falciparum* isolates collected from forested villages. Primers were designed covering part of block 16 and entire block 17 of MSP-1 complete N-terminal portion of TRAP and region F2 of EBA-175. Sequencing of 10 field isolates for MSP-1 has shown polymorphism only at 5- amino acid positions. Out of which four were reported earlier by other workers. Rest of the sequence was conserved in all the 10 field isolates. 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. 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.

Immunological Profile

Immunological studies were carried out to study antibody profiles for three antigens (MSP-1, EBA-175 and TRAP). The antibody levels were higher in individuals from forest areas than those residing in the plain areas. However, age-wise increase in antibody level was observed both in forest and plain areas.

GIS Database

Village boundaries of plain area villages 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. 11).

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 (Fig. 11).
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. Per cent 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.

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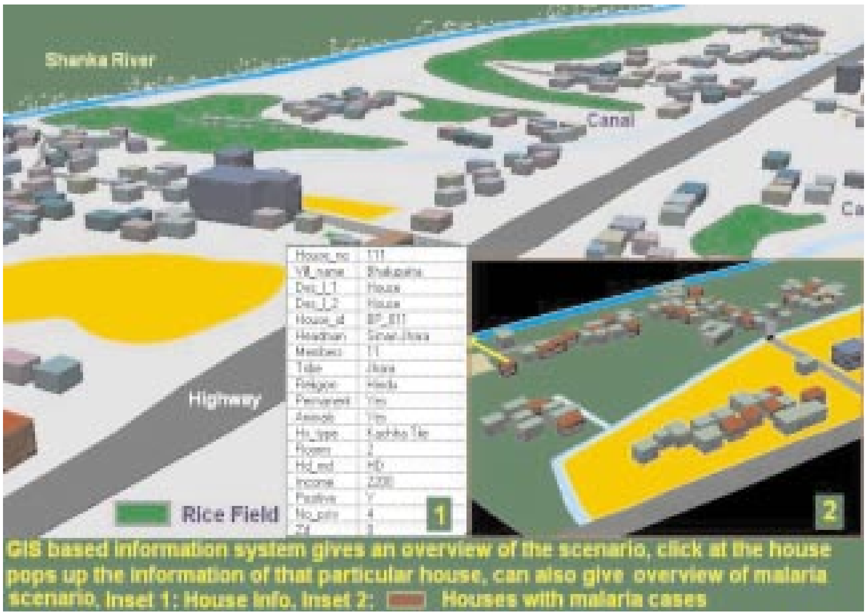

GIS based information system gives an overview of the scenario, click at the house pops up the information of that particular house, can also give overview of malaria scenario, Inset 1: House Info, Inset 2: 🟥 Houses with malaria cases

Fig.11 : **GIS-based information system of a study village in Sundergarh district**