Vector Biology and Control

1.1 Studies on *Anopheles* species Complex

1.1.1 *Anopheles culicifacies* Complex

Bionomics and distribution pattern

Analysis of *Anopheles culicifacies* populations from malaria-affected Gaurella and Pendra blocks of District Bilaspur (Chhattisgarh) revealed prevalence of species B and C with predominance of the latter. *An. culicifacies* C appeared to be the prime vector of malaria as it was found sympatric with *An. fluviatilis* T, a poor/non-vector in Khargone, Harda and Khandwa districts of Madhya Pradesh state. *An. culicifacies* samples collected during peak transmission season were analysed for sibling species composition and blood meal source. Only established vector species were encountered constituting 76.5% *An. culicifacies* and 23.5% *An. fluviatilis*. Both species were found predominantly zoophagic. Similarly, *An. culicifacies* examined from villages selected for malaria vaccine trial in and around Jabalpur (Madhya Pradesh) revealed the prevalence of species B, C and D with predominance of species C (62%). The established vector species (C and D) together constituted 90% of the total *An. culicifacies* population, while the prevalence of non-vector species was low (~10%) suggesting high malariogenic potential of the study area. In District Jalpaiguri (West Bengal), a few samples analysed from PHC Alipurdwar area showed the prevalence of *An. culicifacies* species B and C in almost equal proportion.

1.1.2 *Anopheles fluviatilis* Complex

Distribution, bionomics and biology of sibling species

In district Jalpaiguri of West Bengal, a malaria endemic district, *An. fluviatilis* was found sympatric with *An. minimus* in villages under PHCs Alipurdwar and Kalchini. Identification of the collected specimens to sibling species, using cytological technique and allele-specific PCR assay, revealed presence of *An. fluviatilis* species T. Blood meal source analysis using counter current immuno-electrophoresis showed species T to be totally zoophagic suggesting that the species might be playing a secondary/negligible role in malaria transmission in the district. Similarly, in Gaurella and Pendra blocks of District Bilaspur (Chhattisgarh) that witnessed spurt in malaria cases during October–November 2006, cytogenetic characterisation of *An. fluviatilis* population revealed the prevalence of species T which was polymorphic for q^1 inversion. In study villages, this species was found resting predominantly in cattlesheds and was totally zoophagic which indicates its limited role in malaria transmission in District Bilaspur.

1.1.3 *Anopheles minimus* Complex

Anopheles minimus collected from Districts Sonapur and Dibrugarh of Assam and District Jalpaiguri of West Bengal were characterised for Internal Transcribed Spacer 2 (ITS2) and 28S rDNA (D2-D3 domain) and based on these sequences, all were identified as species A (*An. minimus s.s.*). Molecular characterisation of morphologically identified *An. fluviatilis* collected from District Sonapur (Assam) revealed that their ITS2 and 28S rDNA are homologous to sympatric species *An. minimus s.s.* and are different than all reported members of the Fluviatilis Complex. Based on these findings, it was inferred that morphologically identified *An. fluviatilis* from Assam are, in fact, morphological variant (hypermelanic form) of *An. minimus s.s.* Cytogenetic characterisation of the *An. minimus s.s.* samples revealed striking resemblance in their banding

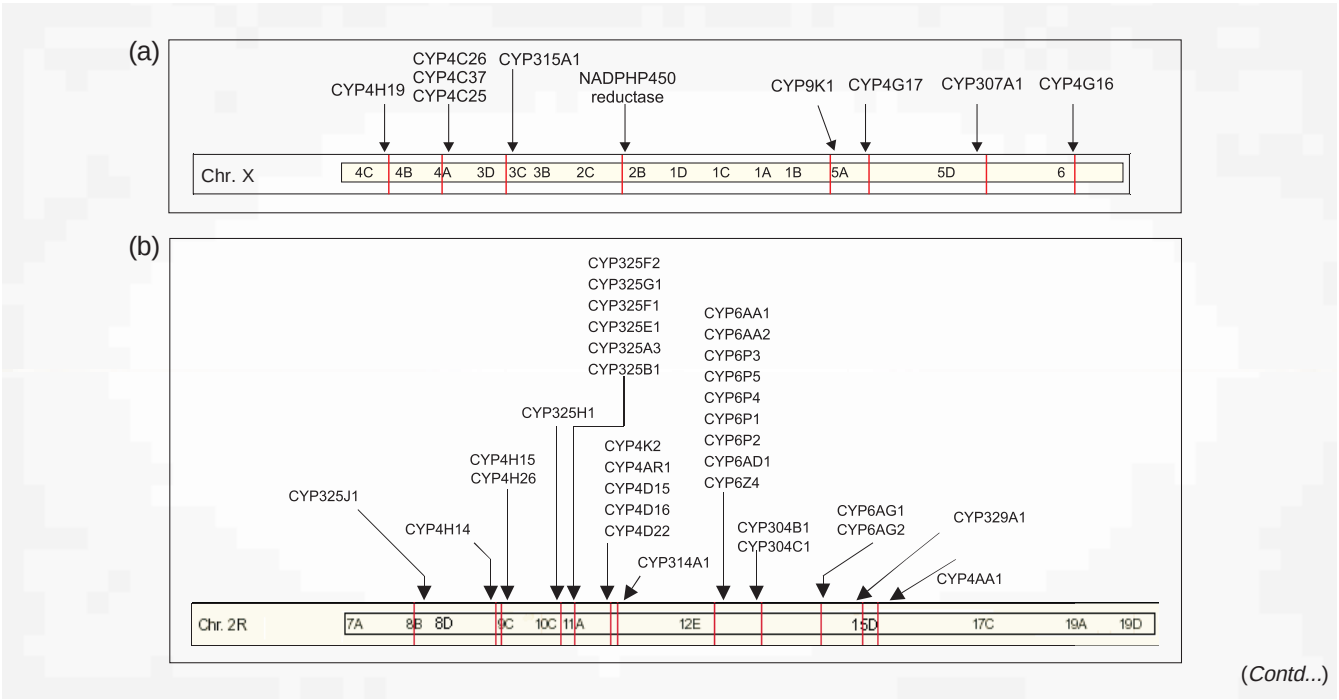
pattern of polytene chromosomes with that of *An. flvuiatilis* species U. However, molecular characterisation of these two species based on 28S (D2-D3) and ITS2 ribosomal DNA and cytochrome oxidase II (mtDNA) sequences revealed that these two species are genetically distant. A detailed photomap of polytene chromosomes complement of *An. minimus* is under preparation. *An. minimus* species A prevalent in hilly forested areas in districts of Assam and West Bengal was found resting predominantly in human dwellings. Blood meal source analysis revealed species A to be highly anthropophagic with anthropophilic index (AI) > 90%. These observations strongly suggest that *An. minimus* species A is playing a major role in malaria transmission in the study areas of the districts surveyed.

1.2 Vector Genomics

1.2.1 Genomic characterisation of cytochrome P450 genes in *Anopheles gambiae*

Origin and spread of insecticide resistance is an important example of natural selection, and the factors governing the maintenance of resistance-associated mutations are both of academic and of applied importance. The primary causes of insecticide resistance are: (i) alterations in the target sites; and (ii) increase in the rate of insecticide metabolism. Metabolic-based resistance mechanisms are important in conferring insecticide resistance and biochemical analyses have identified three enzyme families responsible in insecticide metabolism, the cytochrome P450s (P450s), GSTs (glutathione-S-transferases) and COE (carboxylesterases).

Cytochrome P450 (CYP) is an important and diverse superfamily of hydrophobic, haeme—containing enzymes involved in the metabolism of numerous endogenous and exogenous compounds. In the present work, genes of this superfamily were identified by fine scale scanning of all the four major chromosomal arms of the African malaria vector, *An. gambiae*, since the whole genome sequence of this species of *Anopheles* is available at the public domain. Scanning of the X, 2R, 2L, 3R and 3L chromosomes for CYP genes using the ENSEMBL (www.ensembl.org) genome browser database (release 43, February 2007) revealed a total of 83 CYP genes, clustered in different subfamilies found across the whole genome (Figs. 1a–e). Interestingly, as revealed from the figures, clustering of genes are seen at several places related to genes from a particular subfamily. Of particular importance,



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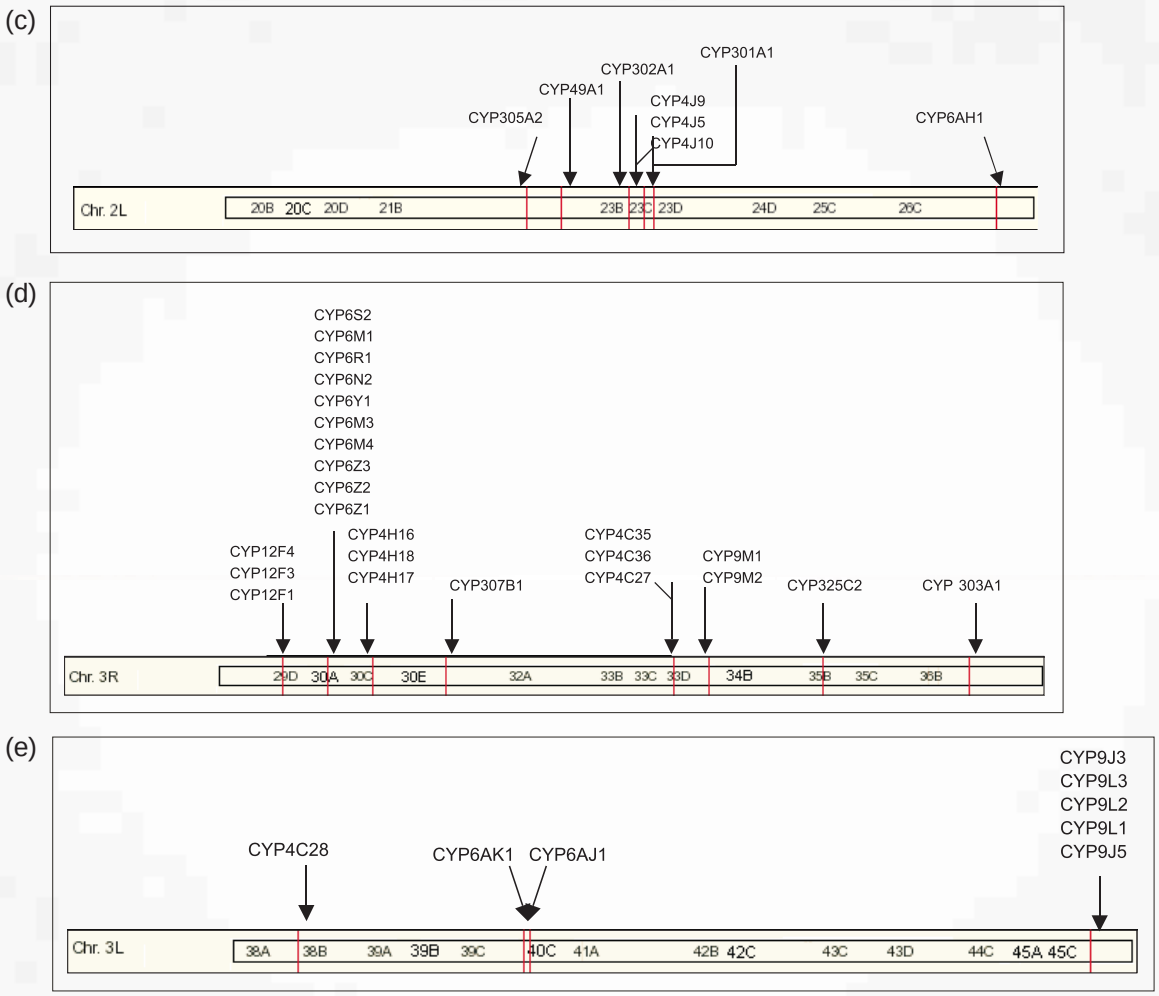


Fig. 1: **Schematic diagram showing the location of cytochrome P450 genes in (a) X- chromosome; (b) chromosome 2R; (c) chromosome 2L; (d) chromosome 3R; and (e) chromosome 3L of *An. gambiae***

two subfamilies, CYP6 and CYP4 are found to be present as clusters in whole genome, but CYP9 subfamily clusters are present only in chromosome 3L. In chromosome 3 most of the CYP genes are present at the telomere and proximal ends. In chromosome 2 the CYP genes are present at only the proximal ends. In contrast, most of the CYP genes are centrally located in the telocentric X-chromosome. The distribution of total CYP genes in each chromosomal arm is shown in Fig. 2 and the schematic diagram showing organisation of each CYP gene along with the sizes are shown in Fig. 3.

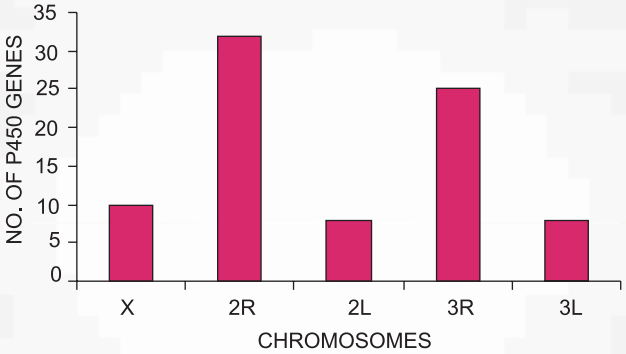


Fig. 2: **Distribution of cytochrome P450 genes in chromosomes of *An. gambiae***

Fig. 3: Schematic diagram showing the organisation of all 82 cytochrome P450 genes in different chromosomes of *An. gambiae*. Each solid rectangle represents a gene. Arrow marks show the directions of transcription. Green coloured rectangles denote forward and yellow coloured denote reverse transcription. Size (in nucleotide base pairs) of each gene is shown inside the rectangles (genes)

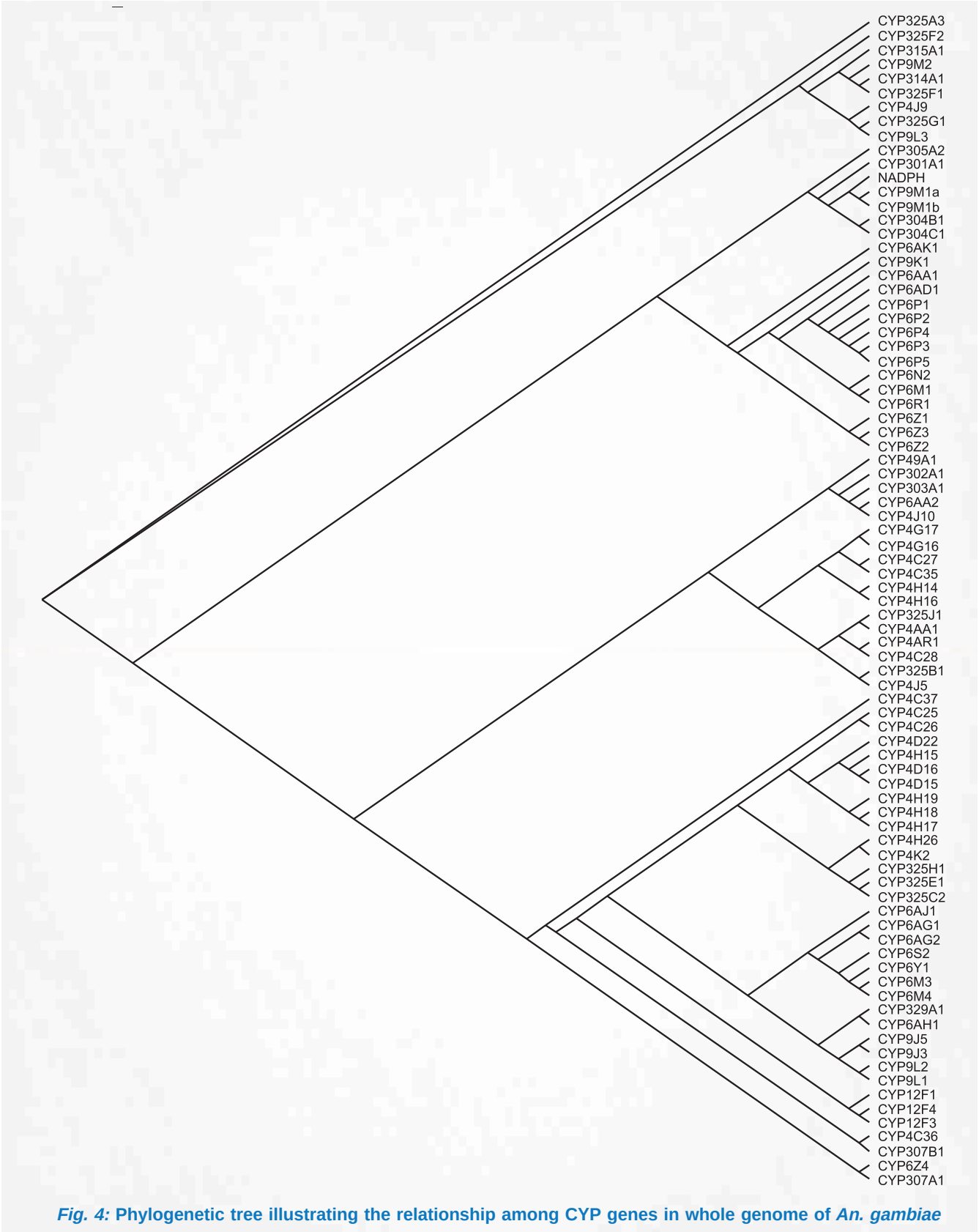


Fig. 4: **Phylogenetic tree illustrating the relationship among CYP genes in whole genome of *An. gambiae***

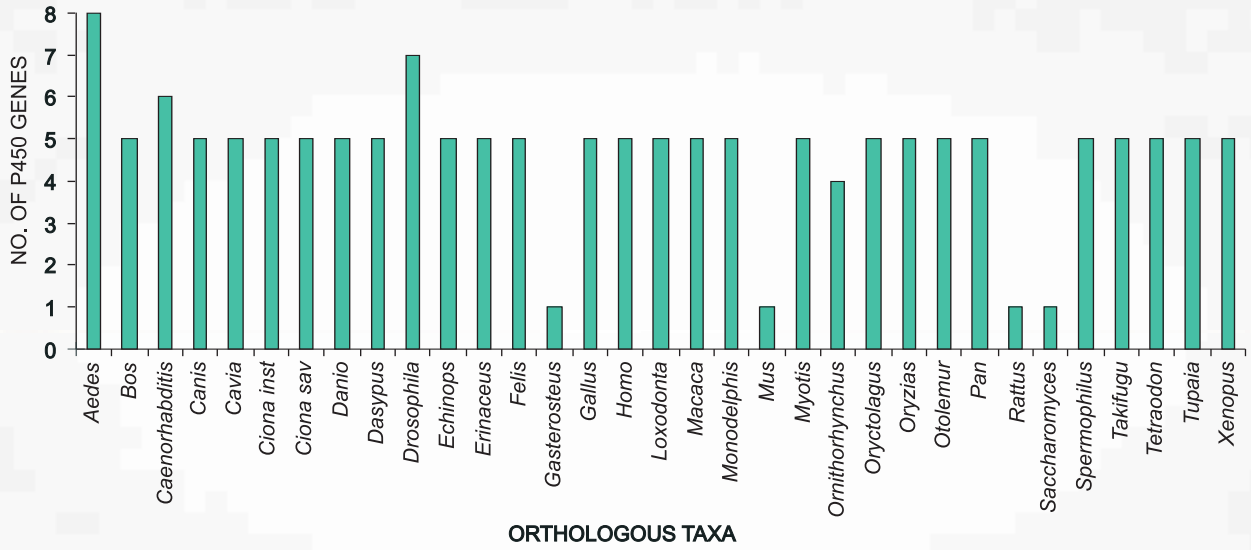


Fig. 5: **Distribution of CYP genes orthologous to *An. gambiae* in different taxa**

The nucleotide sequences of all the 83 CYP genes of *An. gambiae* were downloaded and multiple sequence alignment was successfully performed following ClustalX algorithm. The alignment is followed by construction of phylogenetic trees with the help of different softwares and bioinformatics tools. The trees show the evolutionary relationship among the CYP genes (Fig. 4). Interestingly, the CYP325A3 gene seems to behave like an out-group sequence and thus could have been very much similar to the ancestral sequence of the whole CYP gene families (Fig. 4). The secondary structures of all the genes are also predicted to know about the presence of helix, coils and extended loops.

In order to find if these genes have homologous sequences across taxa, we made a BLAST search in ENSEMBLE (release 44, April 2007) which revealed that *Aedes*, *Drosophila* and *Caenorhabditis* are genetically close at the CYP genes of *An. gambiae*. Nine CYP genes of *An. gambiae* were found to be conserved in >20 taxa and remaining were homologous in <10 taxa (Fig. 5). Interestingly, genes that are homologous to high number of taxa were found to be present in chromosome-X, followed by the right and left arms of the third chromosome.

The above evolutionary bioinformatic studies of cytochrome P450 genes in *An. gambiae* provide greater insights into genomic characterisation of insecticide resistance genes in the genus *Anopheles*. This information would be of tremendous importance to study the vectors of local importance in India such as *An. culicifacies*, *An. fluviatilis*, *An. minimus*, etc. Since the centrally and proximally located genes are expected to show high recombination, genetic variations in these genes are expected to me more. Thus, a comparison of genetic diversity among genes located in different regions of the genome in the above species of importance to India could be achieved through population genetic studies, which would ultimately lead to vector control strategy.

1.3 Vector control

1.3.1 Phase-I evaluation of chlorfenapyr (pyrrole insecticide) against susceptible and resistant strains of mosquito species

Chlorfenapyr, a pyrrole group pro-insecticide, acts by inhibiting the reaction of conversion of mitochondrial ADP to ATP. The observed diagnostic time of exposure and recovery period was determined.

An. culicifacies species A, species C, *An. stephensi* and *Cx. quinquefasciatus* were exposed to different concentrations (0.25–5%) of the insecticide for 15 to 180 min with recovery periods of 24, 48 and 72 h. Exposure of 120 min and recovery period of 48 h was needed to determine the susceptibility. The study is in progress.

1.3.2 Bioefficacy of "Advanced Odomos" repellent cream against vector mosquitoes with particular reference to *Anopheles stephensi*, malaria vector and *Aedes aegypti*, vector of dengue and DHF

This study was carried out to evaluate the bioefficacy of "Advanced Odomos" repellent cream against vector mosquitoes with particular reference to *An. stephensi* and *Ae. aegypti* under laboratory conditions and to determine average protection time against target mosquito species. DEET cream was used as positive control. Laboratory evaluation of Odomos repellent cream showed slightly higher protection time against *An. stephensi* than *Ae. aegypti* but the difference was not significant. The efficacy of Odomos cream in repelling the mosquitoes was comparable with 12% DEET cream. Exposure of mosquitoes up to 4 h on human skin (hand) applied with Advanced Odomos @ 1–10 mg/m^2 in a cage, produced 51.6–100% protection against *An. stephensi* and 55.1– 96.5% protection against *Ae. aegypti*, whereas exposure to DEET cream applied at the same dosages, i.e. 1–10 mg/m^2, produced 57.3–100% protection against the bites of *An. stephensi* and 59.9– 97.2% protection against *Ae. aegypti* (Tables 1–2). Application of Odomos cream on human skin at 10 mg/cm^2 gave 100% protection up to 4 h against *An. stephensi* but 100% protection was obtained at 12 mg/cm^2 in the case of *Ae. aegypti*.

Field trials of Advanced Odomos vs. DEET cream against malaria vectors were carried out in Pechera village in Loni PHC, District Ghaziabad, while field trials against *Ae. aegypti* were carried out during day-time in Railway Colony, Badarpur, Delhi. Results of field trials of Advanced Odomos vs DEET cream during whole night bait collections against different species of mosquito showed that percent repellency with Odomos cream applied at 4–10 mg/cm^2 ranged between 80.5 and 100% against *An. culicifacies*; and 70.5 and 100% against *An. stephensi*. Average protection time of 5.7–11 h against *An. culicifacies*, and 5–11 h against *An. stephensi* was observed. The percent repellency with DEET cream applied at the same dosages varied between 72 and 100% against *An. culicifacies*, 76.4 and 100% against *An. stephensi* and average protection time of 6–11 h against *An. culicifacies*, and 6.5–11 h against *An. stephensi* was observed (Table 3). The results revealed no considerable difference in the percent repellency of Odomos and DEET cream against the two malaria vector species tested.

TABLE 1

Laboratory evaluation of Advanced Odomos and DEET creams against *An. stephensi* and *Ae. aegypti* in cage bioassays

Dose (mg/cm^2)	% protection			
	An. stephensi		*Ae. aegypti*	
	Odomos	DEET	Odomos	DEET
1	33.7 (0)	45.0 (0)	32.5 (0)	39.7 (0)
2	62.7 (1)	69.2 (1)	56.2 (0)	72.5 (1)
4	77.0 (1)	81.8 (3)	72.0 (1)	85.5 (2)
8	100.0 (>4)	100.0 (>4)	97.9 (3)	98.0 (4)

Repellent cream was applied on nylon nets covered on Petri dish containing soaked cotton in glucose; Figures in parentheses are percent protection in hours.

TABLE 2

Laboratory evaluation of the repellent action of Advanced Odomos and DEET creams against *An. stephensi* and *Ae. aegypti* in cage bioassays (human exposure test)

Dose (mg/cm^2)	% protection			
	An. stephensi		*Ae. aegypti*	
	Odomos	DEET	Odomos	DEET
1	51.6 (1)	57.3 (1)	55.1 (0)	59.9 (0)
2	56.0 (1)	61.2 (1)	59.3 (1)	62.7 (1)
4	84.7 (1)	88.0 (2)	76.1 (0)	86.9 (0)
8	95.5 (2)	97.0 (2)	87.5 (2)	90.2 (2)
10	100.0 (4)	100.0 (4)	96.5 (4)	97.2 (3)
12	–	–	100.0 (4)	100.0 (4)

Figures in parentheses are protection time in hours.

TABLE 3

Field evaluation of Advanced Odomos cream against malaria vectors during whole night biting collection

Vector species	Repellent	Dose of repellent (mg/cm²)	No. of mosquitoes landed E/C	(%) Protection	Average protection (hours)
An. culicifacies	Odomos	4	7/36	80.5	5.7
		8	8/41	80.4	9.5
		10	0/37	100	11
	DEET	4	10/36	72.2	6
		8	6/41	85	9.1
		10	0/37	100	11
An. stephensi	Odomos	4	10/34	70.5	5
		8	18/100	82.5	8.1
		10	0/51	100	11
	DEET	4	8/34	76.4	6.5
		8	9/100	91	8.8
		10	0/51	100	11

Data of eight replicates; E—Experimental (Advanced Odomos cream); C—Control (Cream without any repellent ingredient).

The results of the field trials against *Ae. aegypti* mosquitoes showed 36.9 to 92.5% protection (repellency effect) at 4–10 mg/cm² application rate of Odomos cream and 39.1 to 96.2% protection at 4–10 mg/cm² of DEET cream (Table 4). Odomos cream considerably reduced the *Ae. aegypti* biting at 8 and 10 mg/cm² doses, as it resulted in 4 to 6 h protection at 8 and10 mg/cm². DEET cream produced 100% protection up to 5 and 6 h at the same doses. At lower dose of 4 mg/cm² Odomos cream failed to show any repellent effect against *Ae. aegypti* biting, whereas DEET cream gave repellency effect for one hour.

TABLE 4

Field evaluation of Advanced Odomos and DEET creams against *Ae. aegypti* during day time biting collection

Repellent	Dose of repellent (mg/cm²)	No. of mosquitoes biting E/C	(%) Protection	Average protection (hours)
Odomos	4	29/46	36.9	0.7
	8	7/64	89.0	4.7
	10	4/54	92.5	6.2
DEET	4	28/46	39.1	1.2
	8	5/64	92.1	5.2
	10	2/54	96.2	6.75

Data of four replicates; E—Experimental (Repellent cream); C—Control (Cream without any repellent ingredient).

All the volunteers participated in the study did not report any adverse effects such as itching, irritation, pungent smell, and other aberrations of the skin. Odomos cream applied on exposed body parts with 10 mg/cm² concentrations provided 100% protection from *An. culicifacies* for 11 h. Similarly, the protection time from the bites of *An. stephensi* for the same dose was 11 h. The present study also found that the Advanced Odomos cream when used in similar dosage on volunteers during day-time showed complete protection for 6 h against *Ae. aegypti*.

In conclusion, the efficacy of Advanced Odomos cream is comparable to the known repellent cream DEET for protection against the bites of malaria and dengue vectors.

1.3.3 Evaluation of ZeroFly®, an insecticide incorporated plastic sheeting against mosquitoes with particular reference to malaria vectors

This study on the efficacy of ZeroFly® plastic sheeting (incorporated with deltamethrin @ 265 mg a.i./m²) against malaria vectors, sponsored by M/s. Vestergaard Frandsen India Pvt. Ltd., New Delhi, was initiated in the month of July/August 2006 in labourer camps in Delhi and Noida and in the month of December in RAC police camp in Delhi.

Fig. 6: **Temporary shelter in a labourer camp made with ZeroFly**

In all these localities ZeroFly plastic sheets and untreated plastic sheets were fixed in tents (Fig. 6) at a distance of at least 1 km (Control). Entomological and epidemiological parameters were monitored at fortnightly intervals. The results showed considerable reduction in the indoor resting density of vector and non-vector insects in the labourer camps provided with ZeroFly plastic sheeting, as compared with the control plastic sheetings. In a labourer camp at a construction site in Noida, the indoor resting man hour density (MHD) of anophelines was in the range of 0–1.5 in the experimental area as compared to 1.5–232 in the control area. Similarly, in JJ cluster inhabiting agricultural labour in the Yamuna River belt area near Madanpur Khader in Delhi, the indoor resting MHD of anophelines was in the range of 0–5 in the experimental area as compared to 2–248 in the control area (Fig. 7). Slide positivity rate (SPR) in the construction site of labourer camp in Noida was 0–5 in the experimental area as compared to 10–33.3 in the control area. Similarly, in the JJ cluster in Delhi the SPR in experimental and control areas was 0 and 10–22.2. In RAC police camp preliminary collections revealed the reduction of culicine density in the experimental tents as compared to the control tents. In addition, evaluation of ZeroFly was also being undertaken in tribal hamlets in Orissa. The study is in progress.

1.3.4 Follow-up study on the efficacy of Olyset®Nets against malaria vectors and on malaria transmission in District Gautam Budh Nagar (Uttar Pradesh)

Follow-up studies were initiated in three villages to study the long-lasting efficacy of Olyset nets. Olyset nets and untreated nets were distributed in August 2004 in Khandera and Beel Akbarpur villages, respectively and Anandpur village was kept as control where nets were not used. Fortnightly monitoring of the MHD of mosquitoes and surveillance of malaria incidence was carried out. Results revealed a remarkable difference in the indoor resting density of mosquitoes particularly the major malaria vector *An. culicifacies* in the Olyset-

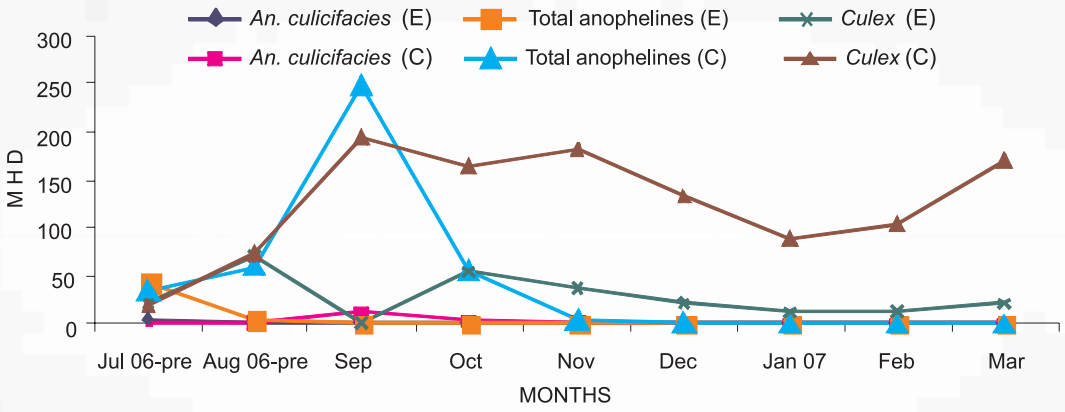


Fig. 7: **Indoor resting density of mosquitoes in experimental (ZeroFly) and control (Untreated plastic sheets) structures in a labourer camp in Delhi**

net village when compared to the untreated net and no net villages. The mean MHD of this species was 6.9 in human dwellings with Olyset net as compared to 19.2 and 21.9 in untreated net and no net villages respectively. Epidemiological results from the three villages revealed considerable difference in the prevalence of malaria. Cases/000 was nil in village with Olyset net as compared with 3.8 and 15 in plain net and no net villages respectively. Similarly, Pf/000 in the three villages was 0, 0.5 and 5.5 respectively. Results revealed that use of Olyset nets resulted in complete interruption in malaria transmission and also reduced mosquito nuisance. The study is still in progress.

1.3.5 Evaluation of Bacticide

Results of the simulated trial with Bacticide tablets in plastic containers revealed 100% mortality of *Ae. aegypti* larvae in small containers of 10 and 50 litres but in bigger containers of 100 litres of water treated with one tablet, there was only 90% mortality in *Ae. aegypti* larvae and the remaining larvae got pupated and emerged as adult mosquitoes. The tablet was more effective in smaller containers of ~10 to 50 litres as it produced 100% reduction in the density of *Ae. aegypti* after 2 to 3 days and this impact persisted up to 2 weeks of observation. However, in tanks of ~100 litres or more, the tablets did not result in 100% reduction in the density of III and IV instar larvae.

1.3.6 Evaluation of mosquito larvicide Temephos for use in polluted water

This study was initiated in the month of January 2007 to evaluate the efficacy and persistence of the larvicide Temephos 50% EC in different types of polluted water at different application rates (25, 50,100 and 200g a.i./ha doses) in three different urban areas in India. The larvicide was applied with the help of a Knapsack sprayer at the rate of 20 ml/m^2 after making required dilutions for different doses. The impact of larvicide was assessed by monitoring the immature density before and after application of larvicide at different time intervals on Day 1, 2, 3, 7, 10, 14 and then at weekly intervals and determining the percent reduction based on untreated control. The study is in progress.

1.3.7 Studies on the adulticidal activity of *Fusarium pallideroseum* and *Aspergyllus nidulans* isolated from adult *Culex quinquefasciatus* in Delhi

Studies were carried out to isolate, identify and determine natural entomopathogenic fungi from field collected moribund adult *Cx. quinquefasciatus*, and their mosquitocidal activity against adult *Cx. quinquefasciatus*. Two fungal species *Aspergyllus nidulans* var *acristatus* (ATCC-6327.04) and *Fusarium pallideroseum* (ATCC-6324.06) were isolated. Adult bioassays were carried out using spore impregnated papers essentially following WHO susceptibility test method. *F. pallideroseum* was found to be more effective compared to *A. nidulans* var *acristatus*. About 90% mortality was observed after 4 h exposure to *F. pallideroseum* spore impregnated paper at a concentration of 1.11x 10^{10} spores/m^2.

1.3.8 Induction of chymoelastase (Pr1) of *Metarhizium anisopliae* and its role in causing mortality in mosquito larvae

Three isolates of insect pathogenic fungus, *Metarhizium anisopliae* produced extracellular cuticle-degrading enzymes chymoelastase (Pr1) and trypsin like protease (Pr2) in variable amounts. Induction of both Pr1 and Pr2 was directly proportional to the incubation time of different carbon and nitrogen sources and maximum inducer was from mosquito cuticle. The induction of Pr1 was found to be higher in the *M. anisopliae* 892 strain than in the strains 3210 and 4102 [Strains obtained from MTCC]. The larvae of *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti* were susceptible to different strains of *M. anisopliae* in the following order: strain 892>3210>4102. *Cx. quinquefasciatus* was the most susceptible. The cuticle of *Cx. quinquefasciatus* has induced maximum Pr1 than the rest of the mosquito cuticles. A direct relation between the quantum of induction of Pr1 and mortality was observed. Among the cuticles of four *Anopheles* spp added basal medium, highest induction of Pr1 was observed in *An. culicifacies* species C and lowest with *An. fluviatilis*. Pr2 induction of *M. anisopliae* did not correlate with the mortality of mosquitoes.

1.3.9 Micropropagation of an antimalarial plant (*Spilanthes acmella* L.) and investigation for larvicidal efficacy

Micropropagation has been achieved for a promising larvicidal asteraceous taxon *Spilanthes acmella* L. using seedling leaf explants and reared on a variety of growth regulators. Bioassays were conducted with crude extract in hexane from different plant parts of *in vitro* regenerated plants such as flower head, leaf, stem and roots, and the toxicity was in the order of flower>leaf>stem, and root was most effective against *An. stephensi* and *Cx. quinquefasciatus*. The LC_{50} with root extract was 2.71 ppm (*An. stephensi*) and 1.19 ppm (*Cx. quinquefasciatus*). The work for chemical characterisation of the compound responsible for lethality is in progress.

1.3.10 Bioefficacy of herbal extract from flower heads of *Spilanthes acmella* L.

Hexane extracts of flower head of three species of the Genus *Spilanthes: S. acmella* L. var *oleracea* Clark, *S. calva* and *S. paniculata* Wall. ex DC were tested for their efficacy against *An. stephensi*, *An. culicifacies* and *Cx. quinquefasciatus* at different concentrations in the range of 1.5625–50 ppm. The LC_{50} and LC_{90} for different species against *An. stephensi* is 4.57 and 7.83 ppm (*S. acmella* L. var *oleracea*), 5.1 and 8.46 ppm (*S. calva* L.) and 5.09 and 13.09 ppm (*S. paniculata* Wall. ex DC), respectively. This is the first report on the larvicidal activity of *S. calva* and *S. oleracea*.

1.4 Insecticide resistance

1.4.1 Insecticide resistance studies in Surat (Gujarat)

Field studies were carried out in the villages in Ukalda PHC area, District Surat in November 2006. Studies carried out in November 2005 and in the present study, *An. culicifacies s.l.* populations have indicated complete reversal of pyrethroid resistance reported in 2002. In these villages insecticide spray was withdrawn about 5–6 years back. Mosquitoes were exposed to the designated WHO diagnostic doses of different insecticides. This species was found resistant to DDT and malathion, while it was susceptible to other organophosphates (fenitrothion 100%), carbamates (propoxur 96%; bendiocarb 99%) and pyrethroids (deltamethrin 99%; lambdacyhalothrin 97%; cyfluthrin 97% and permethrin 96%). There was no variation in the susceptibility of this species in studies carried out in 2005 and 2006 except in DDT and malathion. As observed in earlier studies in 2005, *An. culicifacies* had shown narrow spectrum of resistance and the synergistic studies have confirmed involvement of malathion carboxylesterase (MCE) for conferring malathion resistance.

1.4.2 Study on the susceptibility of *Culex* larvae to Fenthion in urban localities of Delhi

The efficacy of Fenthion against *Cx. quinquefasciatus* larvae collected from urban areas of Delhi from June 2006 to January 2007 was studied in the laboratory conditions using WHO discriminatory dose. The field evaluation of Fenthion was carried out in waste water collections and in ditches of four localities of Delhi—Harsh Vihar (east zone), Ranaji Enclave (west zone), Kadi Vihar (north zone) and Roop Nagar (south zone). Pre-treatment baseline larval/pupal densities were recorded in each habitat using standard larval dippers. In drains, 10 dips were taken at every 5-metre distance for measurement density of *Culex* immatures. Similarly, 5 dips were taken from each of the pits/ditches.

National Vector Borne Disease Control Programme (NVBDCP) recommends mixing 5 cc of Fenthion 100% EC formulation with 10 litres of clean water for spraying in mosquito breeding habitats using a Knapsack sprayer @ 20 cc/m^2. After pre-treatment recording of larval density, spraying was done in experimental habitats while the unsprayed habitats were used as control for comparison. Four replicates of experimental and 2 replicates of control habitats were selected. The larval densities were recorded in all the Fenthion-treated and control habitats on Day 0 (pre-treatment) and thereafter on Days 1, 3, 5 and 7 post-treatment. Second and third applications of Fenthion were made at weekly intervals and larval density was monitored. The

reduction in III/IV instar larvae was calculated using Mulla's formula.

To evaluate susceptibility/resistance of *Cx. quinquefasciatus* larvae to Fenthion, III/IV instar larvae were collected from trial habitats and brought to the laboratory. Such larvae were exposed to Fenthion at the diagnostic concentration of 0.125 ppm (as well as two serially lower concentrations of 0.05 ppm and 0.025 ppm) in the laboratory. One ml of the Fenthion stock solution supplied by NIMR HQs was added in 249 ml of the water in beakers. Insecticide was added just below the surface of the water and stirred. At least three replicates of 25 larvae each for test and concurrent controls with two replicates of 25 larvae were run. After 24 h of exposure, mortality was determined scoring the dead and moribund larvae. Larvae, which pupated, were discarded and counted for calculation of mortality. Corrected percent mortality was calculated using Abbott's formula when the larval mortality in control was between 5 and 20%.

Field evaluation

The results indicate that spray by Fenthion resulted in reduction of larval density up to a maximum of 72.2% on Day 1 in waste water collections. Reduction was achieved only up to third day of spray. In ditches also, the reduction in larval density was observed up to third day after spray and the maximum percent reduction was 90.6 on Day 1. Thereafter there was no reduction in larval density and on the fifth day the density rather increased and percent reduction values were in negative. Even second and third rounds of spray did not result in improvement in percent reduction after third day. In all the four sites in Delhi, the larval density in experimental habitats on Day 21 was more than that of on Day 0.

Laboratory evaluation

The results of laboratory evaluation on susceptibility of *Culex* larvae to different concentrations of Fenthion are given in Table 5. It was found that there was 89.5% mortality at 0.125 ppm, 53.4% at 0.05 ppm and 40.9% at 0.025 ppm.

It can be concluded that the spraying of Fenthion at the NVBDCP recommended dose produced a very low impact on larval densities in general. The effect lasted only up to the third day. The gradual rounds of spray resulted in further reduction in larval densities. On Day 21 of post-spray, the larval density was more than that of on Day 0, indicating no impact of larvicide. The results of the larval susceptibility tests on *Cx. quinquefasciatus* indicated that the corrected percent mortality in larvae at the dose of 0.05 ppm was 53.4% which revealed that *Cx. quinquefasciatus* had developed resistance to Fenthion. In Delhi, Fenthion is in operation for the last 20 years.

TABLE 5

Susceptibility of *Cx. quinquefasciatus* III and IV instar larvae to Fenthion under laboratory conditions in Delhi

Concentration (in ppm)	No. of replicates	No. of larvae exposed	Corrected % Mortality
0.125	4	100	89.5
0.05	4	100	53.4
0.025	4	100	40.9
Control (for each concentration)	2	50	6–14

1.5 Vector-parasite interactions

1.5.1 Studies on *Plasmodium*-refractory *An. culicifacies*

Differential expression of serine protease gene

Serine protease (acsp30) gene of *Plasmodium vivax*-refractory *An. culicifacies* was found to be *Plasmodium* inducible. Considerable differences were noticed between the sequences of the promotor region of refractory (R) and susceptible (S) strains, with ~1.5 fold higher promotor strength in the R strain. The increase in promoter activity in the R strain was attributed to sequence differences within the 400 bp region spanning –333 to –702 bp upstream of the start codon. To determine the effect of such differences on the binding of nuclear proteins, electrophoretic mobility shift assays (EMSAs) were performed. EMSA experiments with three different probes (400, 188 and 100 bp)

allowed us to determine the minimum upstream region that shows difference in binding of nuclear proteins and thus might be responsible for differential expression of acsp30 in the two strains. EMSAs using nuclear extracts from the R strain and R400/S400 probe revealed two complexes, a sharp slow migrating band, complex A and a faster moving diffused band, complex B (Fig. 8A, lane 3). Both the complexes were observed when R188 probe was incubated with nuclear extract from R strain (Fig. 9B, lane 3). Interestingly, formation of complex B was nearly abolished on R100 probe but complex A formation remained unaffected (Fig. 10B). These results clearly indicated that the 88 bases (–602 to –514 bp) missing in R100 probe were critical for the assembly of transcription factors forming complex B but the 100 bp upstream region (–702 to –602 bp) was important and sufficient for binding of nuclear proteins forming complex A. In general, the binding of nuclear proteins to probes derived from the S strain was less compared to that from the R strain, which further emphasised the importance of differences in upstream regions of acsp30 from both the strains. The greater intensity of the bands with the R probes showed that the formation of both the complexes was more on R probe than S; an approximately 25% increase in DNA binding activity of both the complexes was observed on R400 as compared to S400 probe. Importantly, when S188 was used as a probe, there was an approximately 50% reduction in the formation of complex B than on R188. This could be a consequence of an increase in sequence divergence (70%) in this region between the two strains. Specificity of interaction of nuclear proteins with various probes from R and S strains was evaluated by competition assays in the presence of corresponding specific cold probe. The binding of nuclear factors to 400 bp upstream sequence from R strain (R400) was highly specific as the formation of complexes A and B was reduced to 25% in the presence of 100-fold molar excess of unlabelled 400 bp cold probe in the EMSA binding reaction mixture (Fig. 8B). Competition experiments with sequentially shorter fragments from R and S strains also generated similar results showing the specificity of binding of nuclear factors to all the probes.

We also performed EMSA experiments using nuclear extract from both the strains to evaluate the presence of additional transcription factors in the R strain that could be absent in the S strain. Noticeably, the binding pattern of nuclear proteins from S strain to S188 and R188 probes (Fig. 9A) were different from that of nuclear proteins from R strain. A similar result was obtained with S100 and R100 probes (Fig. 10). When a nuclear extract from the S strain was used with R100 probe, the faster migrating band, complex B did not form. This is indicative of either a lack of the transcription binding factors in the S strain that form complex B or their low concentrations that prevent detection. The association of putative binding factors was quantified by converting intensity of signals to numerical values by using the Image Analysis Software, ImageQuant TL (Amersham Biosciences) and the results were presented as bars in Figs. 8, 9 and 10.

Analysis of the Phenoloxidase enzyme

Third and fourth instars male/female larvae of refractory strain were taken separately from same batch of mosquitoes in sodium phosphate buffer pH 6.8. Similarly, male and female pupae were also taken at 0 and 24 h of pupation, these were labelled as fresh and old pupae respectively. Prior to Phenoloxidase (PO) assay protein in the samples was estimated quantitatively using Bradford method. Quantitative PO assays were carried out using tyrosine as substrate. The end product was read at wavelength of 420 nm. Qualitative PO assays were carried out on 5% SDS-PAGE gels. Enzyme activity was visualised on gels using tyrosine for monophenoloxidase (MPO) and DOPA for diphenoloxidase (DPO).

Quantitative assay revealed higher enzyme activity in IV instar than III instar larvae with highly significant difference. Similarly, older pupae had higher PO expression than the freshly developed pupae. Enzyme activity was nearly 3–4 times higher in larvae than pupae. Females of both III and IV instar larvae had significantly higher PO activity than the males. However, the PO activity in freshly emerged female pupae was nearly equal to freshly emerged male pupae whereas in older female

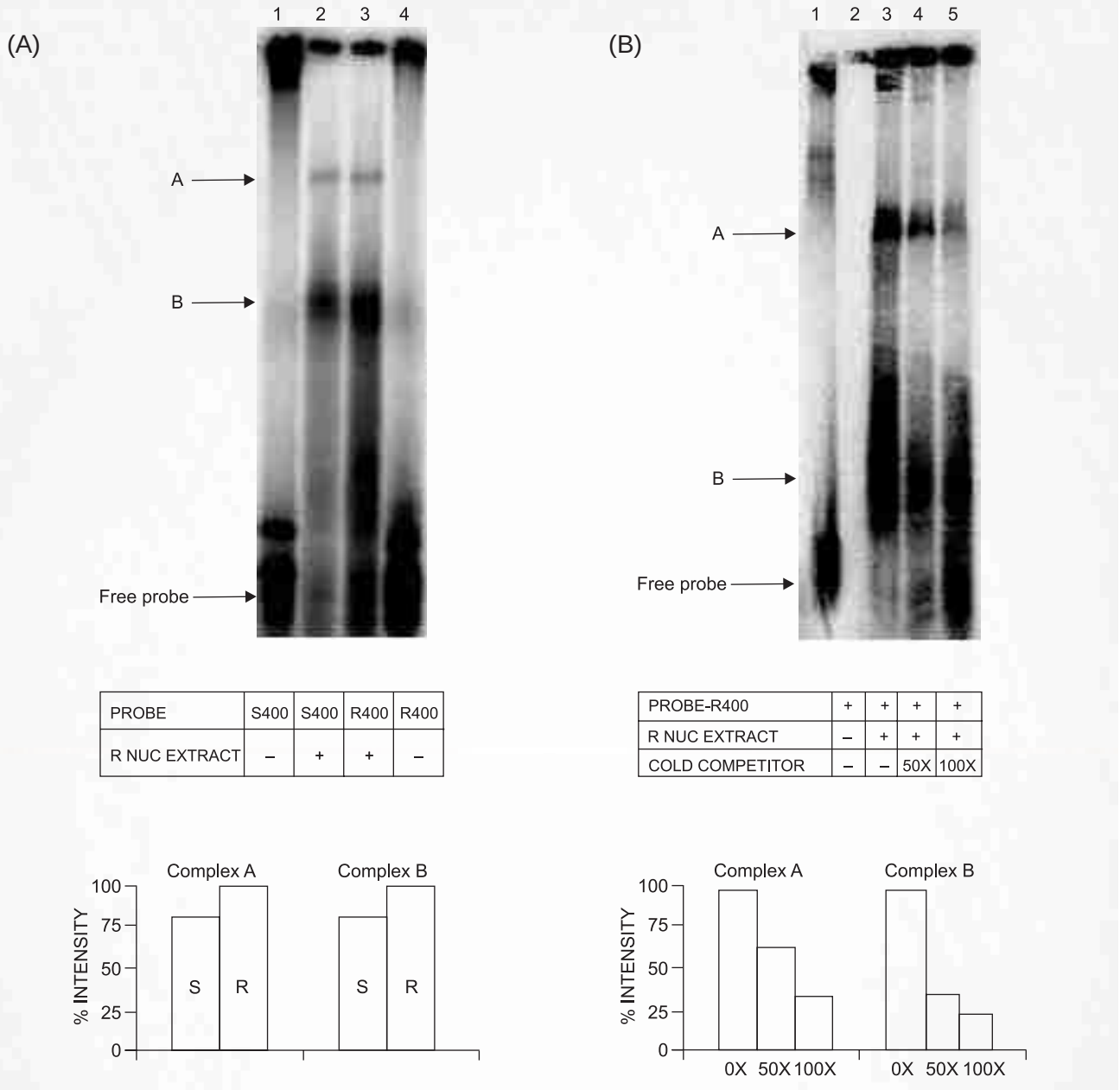


Fig. 8: **EMSAs with *An. culicifacies* refractory (R) strain nuclear extract and 400 bp probes from upstream sequences of acsp30 of S and R strains. (A) EMSA showing the binding pattern of nuclear proteins extracted from body tissue of five-day old adult female R strain mosquitoes when incubated with S400 (lane 2) and R400 (lane 3) probes at 37°C for 25 min. Free probes were run in lane 1 (S400) and lane 4 (R400); and (B) The radiolabelled probe R400 was incubated with refractory nuclear extracts without competitor (lane 3) and in the presence of unlabelled probe at 50-fold (lane 4) and 100-fold (lane 5) molar excess. Arrows indicate the migration of complex A (slow migrating) and B (fast migrating). The intensities of the signals were quantified with respect to PhosphoImager signals by the Image Analysis Software, ImageQuant TL (Amersham Biosciences) and represented as the percentage ratio of S400 to R400 signal intensities and as percentage ratio to the non-competed (R400) signal intensities**

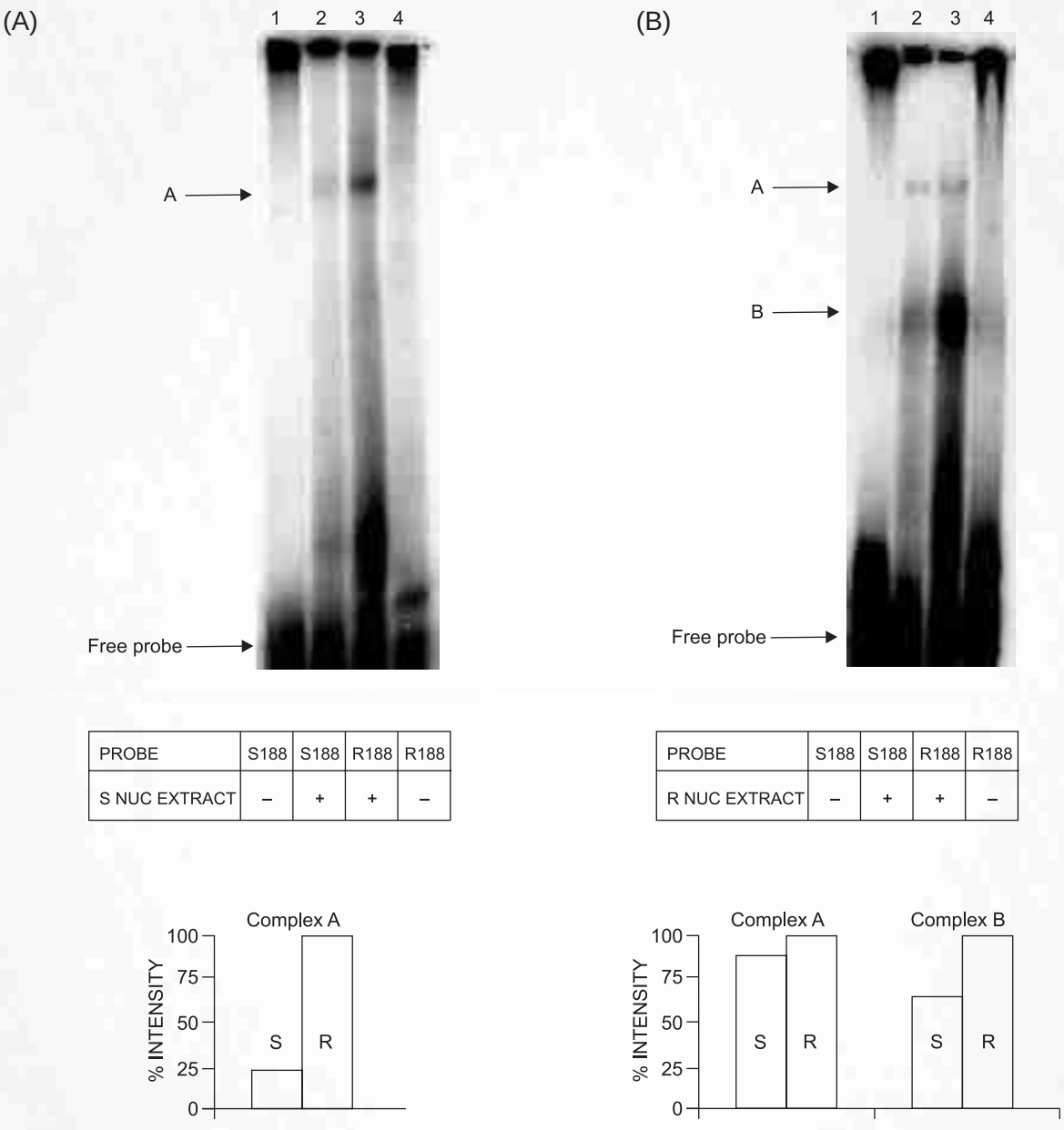


Fig. 9: **EMSAs using nuclear extracts and 188 bp probes from acsp30 upstream sequences. Nuclear proteins were extracted from body tissue of 5-day old adult female of S strain (A) and R strain (B) and incubated with S188 (lane 2) and R188 (lane 3) probes. Free probes were run in lane 1 (S188) and lane 4 (R188). Arrows indicate the migration of complex A (slow migrating) and complex B (fast migrating). The intensities of the signals were quantified with respect to PhospholImager signals by the Image Analysis Software, ImageQuant TL (Amersham Biosciences) and represented as the percentage ratio of S188 to R188 signal intensities**

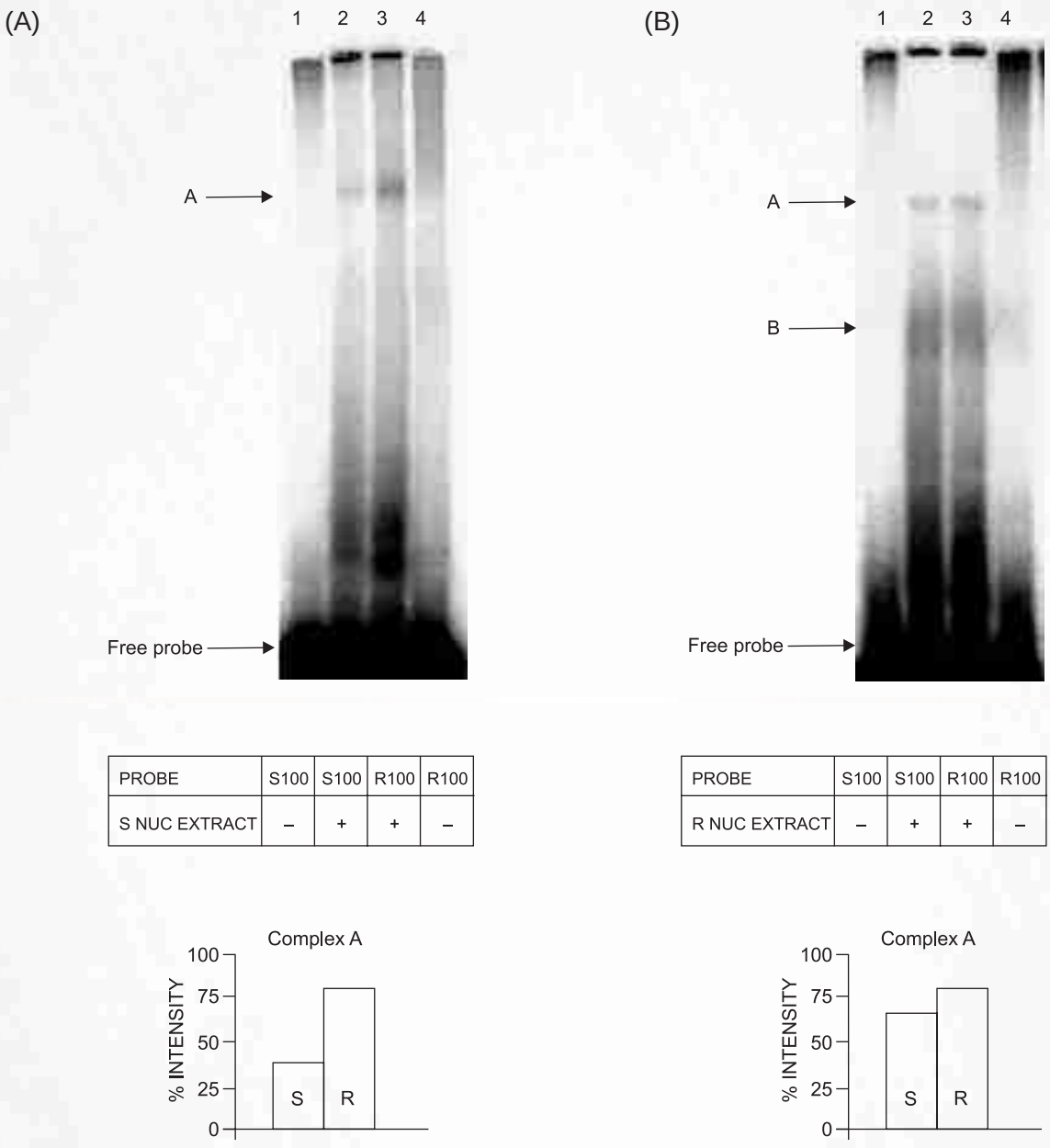


Fig. 10: **EMSAs using nuclear extracts and 100 bp probes from acsp30 upstream sequences. Nuclear proteins were extracted from body tissue of 5-day old adult female of S strain (A) and R strain (B) and were incubated with S100 (lane 2) and R100 (lane 3) probes. Free probes were run in lane 1 (S100) and lane 4 (R100). Arrows indicate the migration of complex A (slow migrating) and complex B (fast migrating). The intensities of the signals were quantified with respect to PhosphoImager signals by the Image Analysis Software, ImageQuant TL (Amersham Biosciences) and represented as the percentage ratio of S100 to R100 signal intensities**

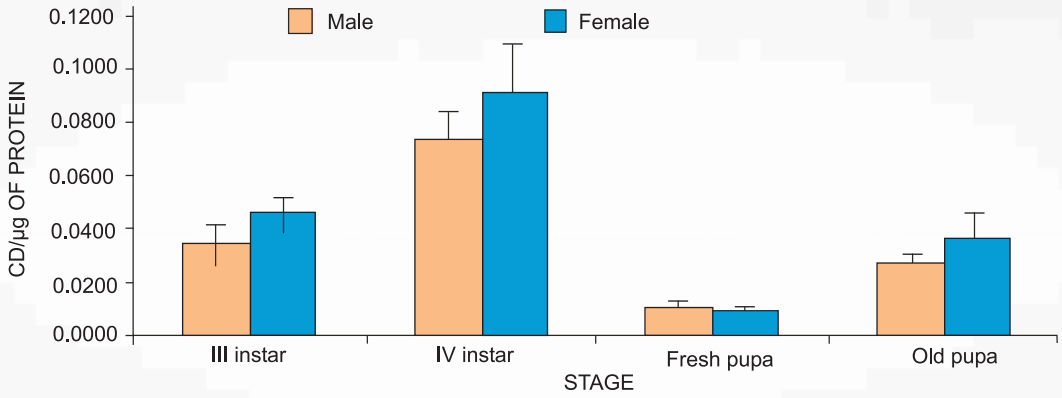


Fig. 11: **Comparative Phenoloxidase activity in developmental stages of refractory *An. culicifacies***

pupae it was insignificantly higher than in the corresponding male (Fig. 11).

Zymogram showed two clear bands of MPO and DPO in both the sexes of III instar larvae. Similarly, IV instar male larvae also had two clear bands, while IV instar female larvae showed only one comparatively darker and broader band (Fig. 12). Fresh male and female pupae showed single band while older pupae had two bands (Fig. 13). Activity of MPO is found slightly higher than the DPO on SDS-PAGE.

1.5.2 Biochemical and molecular characterisation of Nitric oxide synthase in *An. culicifacies*: relevance for refractory mechanism

Earlier we reported the specific activities of *An. culicifacies* Nitric Oxide Synthase (AcNOS) in lysates of non-blood fed, uninfected or *P. falciparum*-infected mosquitoes at 6 and 9 days post-blood meal activity with or without NOS inhibitor L-NAME and amplified the NOS gene in *An. culicifacies* species A and species B and established NOS as an informative marker.

Fig. 12: **MPO & DPO activities in IV and III instar larvae on SDS-PAGE**

Fig. 13: **MPO & DPO activities in freshly emerged and older pupae on SDS-PAGE (3LM: III instar male larvae; 4LM: IV instar male larvae; 3LF: III instar female larvae; 4LF: IV instar female larvae; FPM: Fresh male pupae; OPM: Older male pupae; FPF: Fresh female pupae; OPF: Older female pupae)**

In continuation with our earlier studies, we have identified and characterised expression of AcNOS, which is highly homologous to characterised NOS genes and was detected in the midgut soon after invasion of the midgut by *P. vivax* at the beginning of blood feeding. Increased levels of mRNAs (encoding iNOS) were observed 9–14 days after ingestion of an infected blood meal using RT-PCR analyses (Fig. 14). *An. culicifacies* NOS (AcNOS) expression was studied by using semi-quantitative RT-PCR. Total RNA was isolated from 30–40 each non-blood fed, blood fed uninfected, and *P. vivax*-infected mosquitoes at 6, 9, 14, and 18 days pBM. A strong induction occurred in infected mosquitoes at 7 days and 9–14 days. Semiquantitative RT-PCR experiments established that AcNOS is parasite inducible as shown by significant increase in transcript levels (9–14 days) after feeding parasite-infected blood to mosquitoes (Fig. 14).

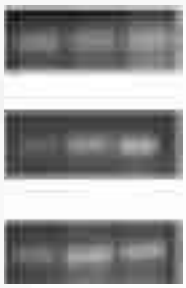


Fig. 14: **Agarose gel showing semi-quantitative RT-PCR assayed transcriptional induction of AcNOS in parasite induced midgets from *An. culicifacies* species A and species B (U–Unfed; F–Fed uninfected; FI–Fed infected; N–Normal; I–Infected) 7 and 14 days. All cDNA template were normalised for equal yield of ribosomal protein S7 RT-PCR product**

Parasite Biology

2.1 Molecular Genetics of *Plasmodium falciparum* and *P. vivax*

2.1.1 Sequence diversity of *Plasmodium vivax* MSP-3α in Indian field isolates

A study was undertaken to know the sequence variations existing among the Indian field isolates having three length variants of 1.2, 1.4 and 1.8 kb as revealed from PCR-RFLP. It was observed that Indian isolates have 90 to 100% sequence similarity among themselves and average identity of Indian isolates with isolates of other regions was in the range of 80 to 99% (Fig. 1). Highest level of identity was observed in 1.2 kb variant, while identity observed in 1.8 kb variant was the lowest. Existence of common allelic composition in different parts of the globe and segregation of Indian isolates with isolates of different regions suggests that Indian isolates have global allelic representation (Fig. 2).

2.1.2 Comparison of genetic diversity of *Plasmodium vivax* among Indian, southeast Asian and other regions

Plasmodium vivax apical membrane antigen-1 (*PvAMA-1*) is a potential asexual stage vaccine candidate and highly polymorphic in nature. DNA sequence variation in the *PvAMA-1* gene from 41 *P. vivax* isolates from India were examined and compared with previous reported studies of *PvAMA-1* from Papua New Guinea, Thailand, Sri Lanka, Indonesia, Africa, India, China, Solomon Islands and Philippines. In total, 86 haplotypes, 25 polymorphic sites and 31 mutations were identified. The rate of non-synonymous substitutions was very high compared to the rate of synonymous substitutions indicating that *PvAMA-1* is under positive selection pressure. Twenty out of 83 haplotypes showed sharing among different population and remaining 63 haplotypes were clustered among different populations (Fig. 3).

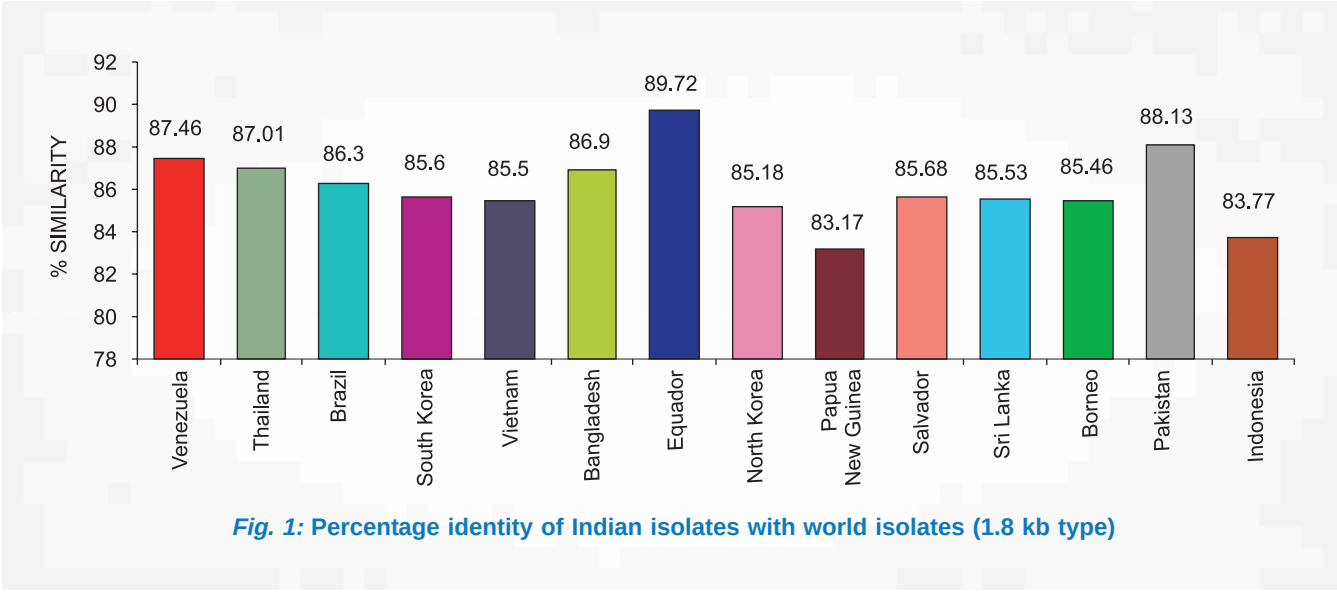


Fig. 1: **Percentage identity of Indian isolates with world isolates (1.8 kb type)**

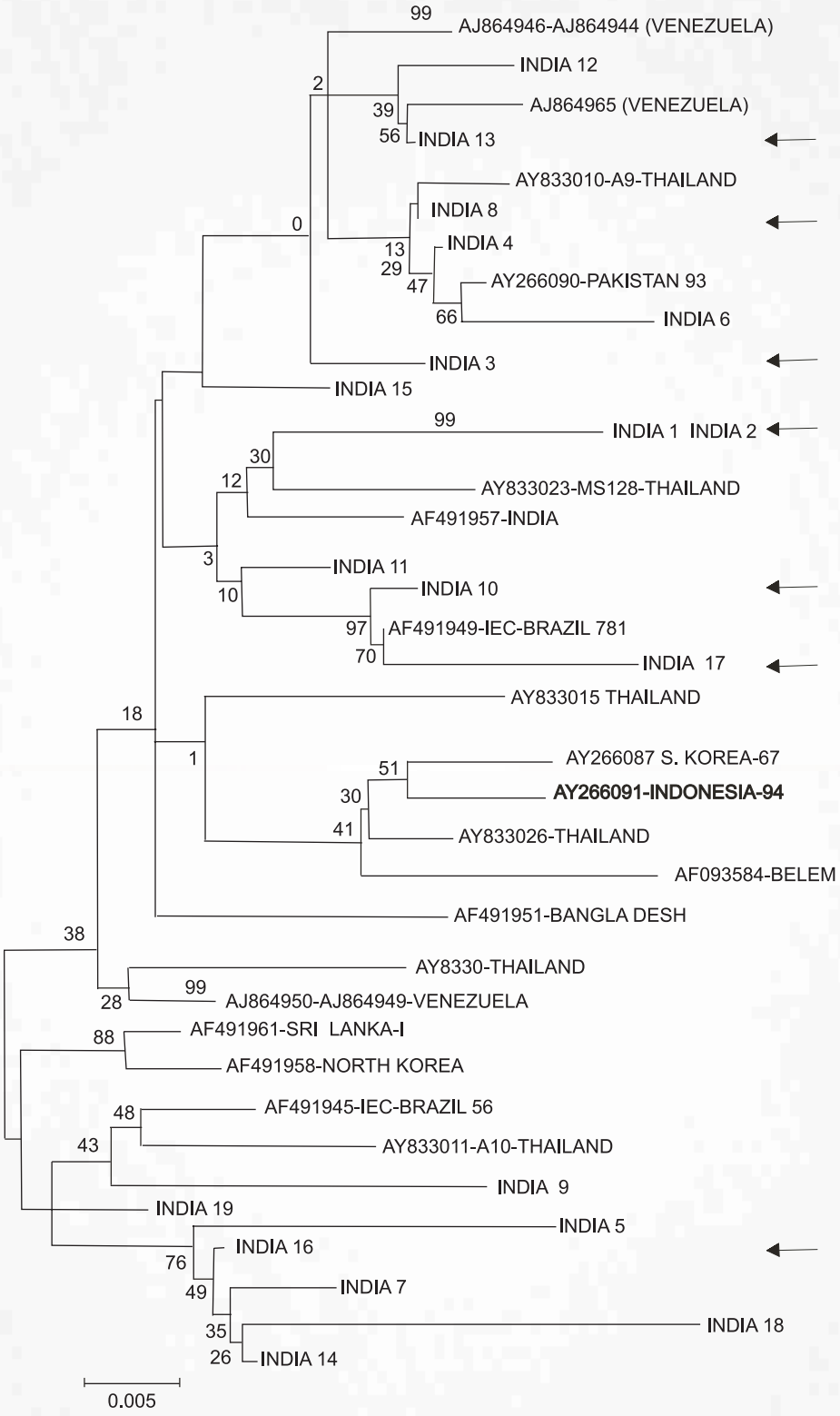


Fig. 2: **Phylogenetic analysis of Indian and other global isolates using MSP-3α sequences**

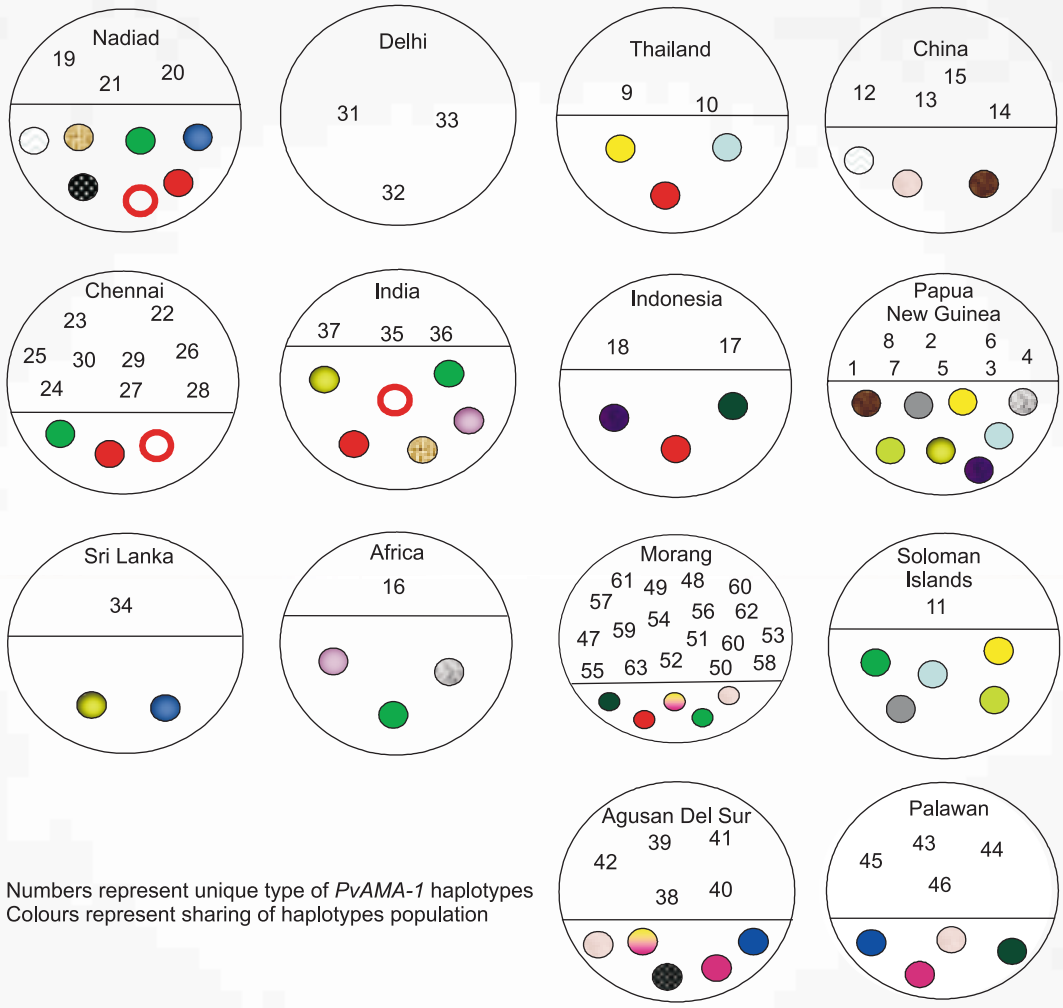


Fig. 3: **Distribution of *PvAMA-1* haplotypes among Indian and other southeast Asian regions**

2.1.3 MSP-3α gene of *Plasmodium vivax*

DNA amplification and sequence analysis of MSP-3α genes of *P. vivax* positive patient isolates from different regions of India like Delhi, Patna, Chennai, and Daltonganj were conducted. PCR/RFLP protocol-based on PvMSP-3α genes has been standardised to demonstrate its utility in analysis of *P. vivax* diversity (Figs. 4 & 5).

2.1.4 Glutathione S-transferase in both *Plasmodium vivax* and *Plasmodium falciparum*

Glutathione S-transferase (GST), an intracellular antioxidant with complex biological functions and well-established metabolic regulator, when decreased is associated with number of disease states including malaria. GST estimation has been investigated in Indian population infected with malaria. Clinical isolates were analysed to understand the role of GST as biochemical marker in vivax patients as well as to compare GST activity in adults with non-complicated vivax malaria with healthy controls. In continuation to biochemical work on GST, PCR protocol-based on GSTP1, GSTM1 and GSTT1 genes has been standardised.

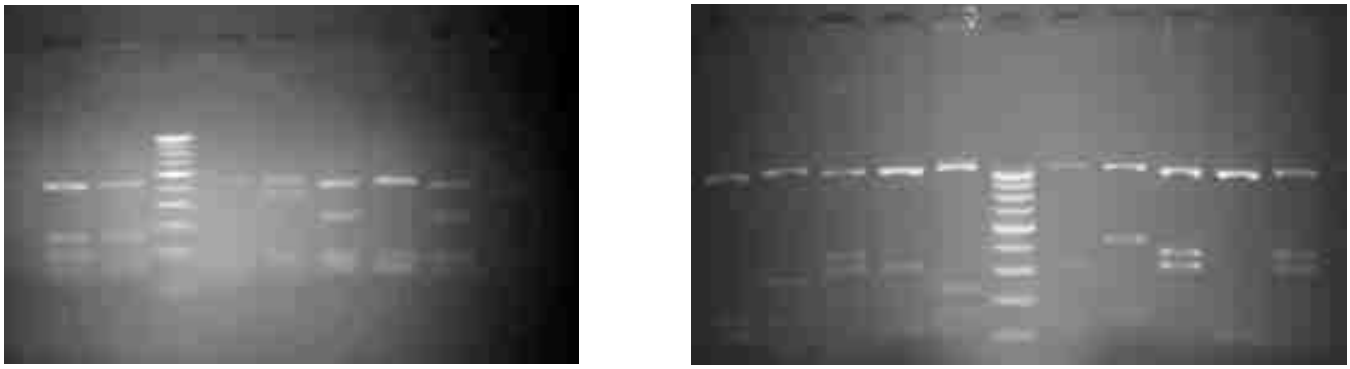


Fig. 4: **Gel electrophoretograms showing size variations of MSP-3α by employing PCR/RFLP among *P. vivax* isolates**

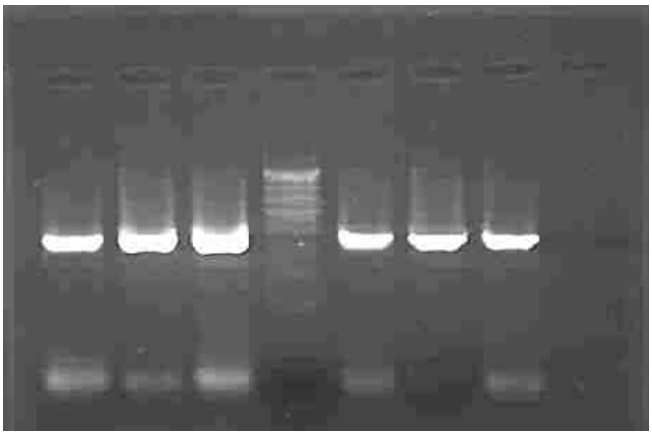


Fig. 5: **Agarose gel electrophoretogram showing 433 bp of GSTP1 allele among *P. vivax* isolates**

2.1.5 Molecular genotyping of clinical resistance in *Plasmodium falciparum*

Samples collected from *P. falciparum* patients on Day 0 and the day of recrudescence during therapeutic efficacy studies of various antimalarials conducted at different parts of the country were analysed for molecular genotyping using highly polymorphic surface protein markers as well as drug resistance related markers. Results revealed high proportion of recrudescent infections with the same genotype that of Day 0 among isolates of Gujarat, Tamil Nadu, Orissa and Assam in case of CQ efficacy studies thus suggesting for resistance. Analysis of mutations in *Pfcrt* and *Pfmdr1* genes revealed prevalence of threonine (T) at codon76 of *Pfcrt* and asparagines (N) at codon86 of *Pfmdr* gene among the isolates of different areas. The study further revealed SVMNT as the predominant haplotype, however, in areas of high malaria endemicity, haplotype diversity observed was much higher (Fig. 6). High prevalence of mutant alleles and haplotypes among the isolates of different

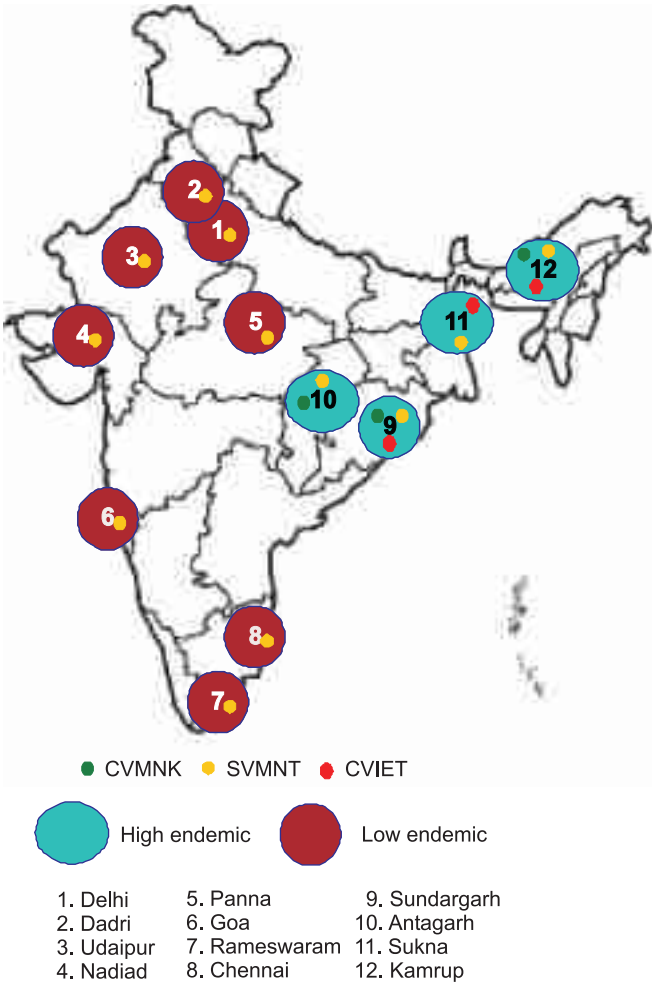


Fig. 6: **Distribution pattern of *Pfcrt* haplotypes in India**

regions definitely reflect presence of CQ resistant strain in the country.

2.1.6 MSP-1 and MSP-2 in genotypes of *Plasmodium falciparum*

The data of *P. falciparum* positive blood samples collected from different villages of Sundargarh, Orissa have been analysed using polymorphic markers and vaccine candidate antigens such as the merozoite surface proteins MSP-1 and MSP-2 after extracting DNA from the samples and performing PCR. Multiplicity of infection and clonality (samples having single and multiple clones) at three different transmission levels—low, moderate and high with respect to different age groups and two ecosystems were calculated (Figs. 7 and 8).

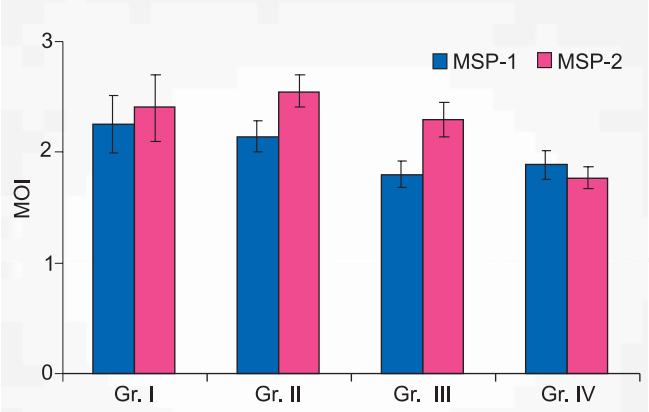


Fig. 7: **MSP-1 and MSP-2 polymorphism in different age groups**

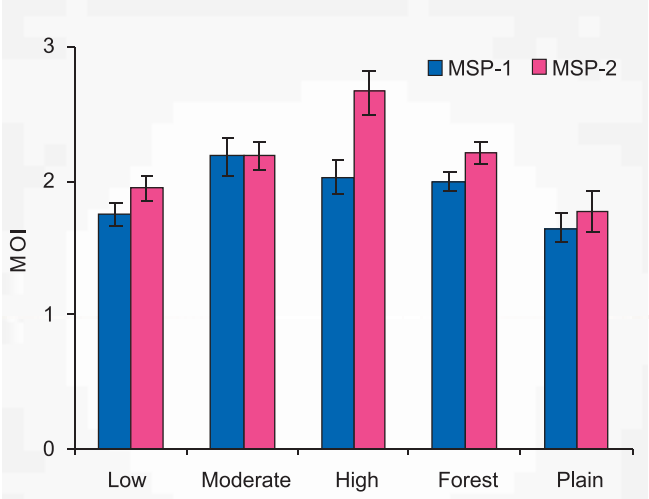


Fig. 8: **MSP-1 and MSP-2 polymorphism in forest and plain areas**

2.1.7 Genetic diversity studies of *Plasmodium falciparum*: SNP analysis in putatively neutral loci

In order to infer haplotype diversity with SNP, it is essential to use field isolates having infection with single parasite type. Therefore, about 400 field isolates collected from nine different study sites were analysed with three highly polymorphic surface protein markers to identify clonality in them. A total of nine PCR assays were carried out for each isolate to identify single clone infection isolates. Fig. 9 shows the distribution of single and multiclone isolates in different study sites and these were used for further analysis by selected sets of primers to

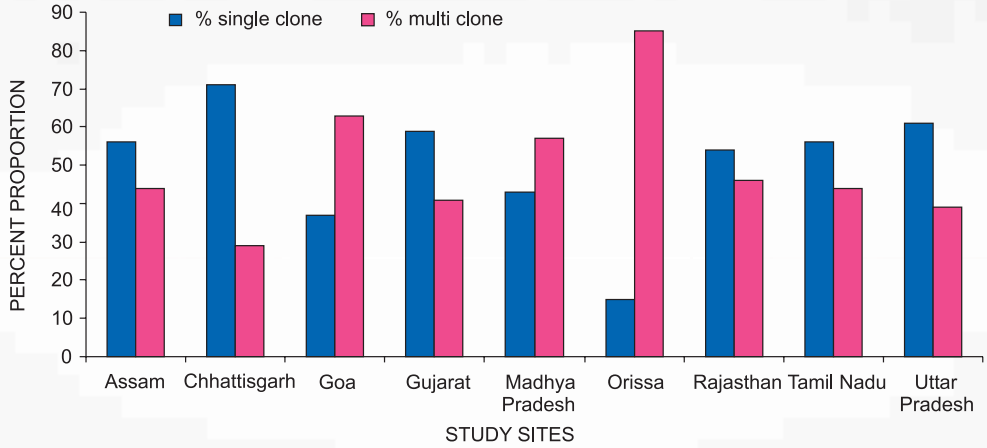


Fig. 9: **Distribution of single and multiclonal isolates in different study sites**

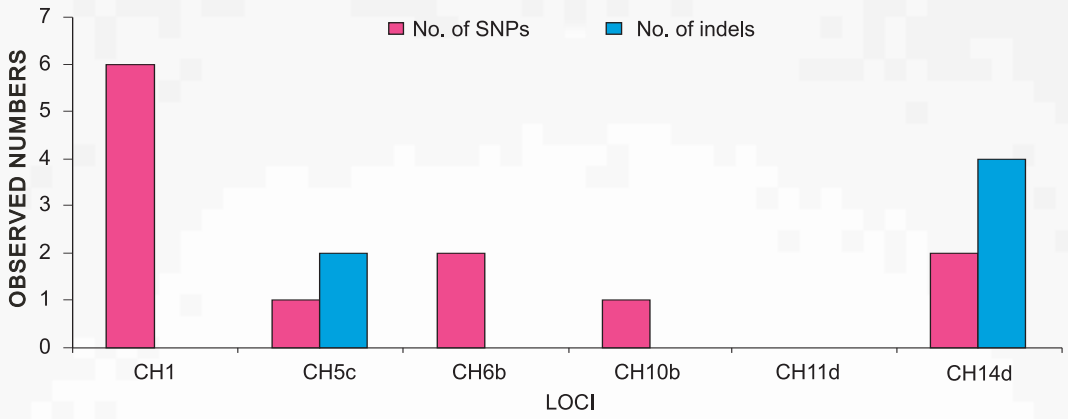


Fig. 10: **Distribution of SNPs and indels (insertion/deletion) observed at neutral loci studied**

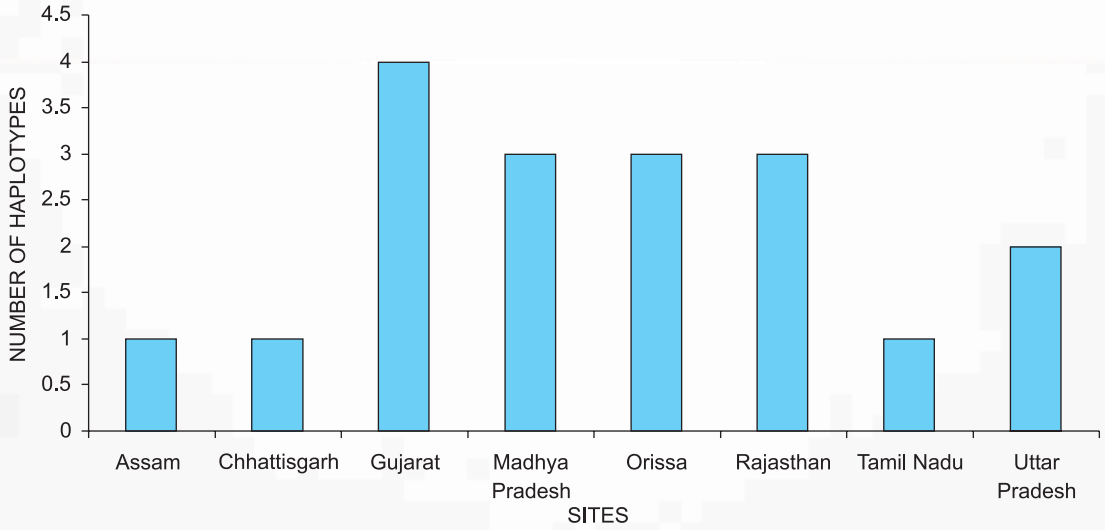


Fig. 11: **Distribution of CH1 haplotypes in different study sites**

amplify intronic regions. PCR amplified products were gel purified and sequenced.

Analysis of sequence data revealed five polymorphic loci (Fig. 10). In addition, a number of indels (insertion/deletions) were also observed and this was in conformity with published data of *P. falciparum.* Analysis of data using population genetic softwares, DnaSP and Arlequine revealed that CH1 locus had six different haplotypes and six polymorphic sites. Isolates of different study sites had shown varied degree of heterozygosity (Fig. 11) but *F* statistics calculated for different populations was non-significant. The phylogenetic tree constructed, based on sequence data, 3D7 and respective *P. reichenowi* sequences using MEGA 3.1 with 1000 boot strap replicates has not shown much divergence between the populations. Analysis of other loci is in progress.

2.1.8 Relatively simple genotype of *Plasmodium falciparum* isolates from northeastern states as determined by Anchored Primer Amplification of DNA (APAD)

Twenty isolates have been studied for genetic

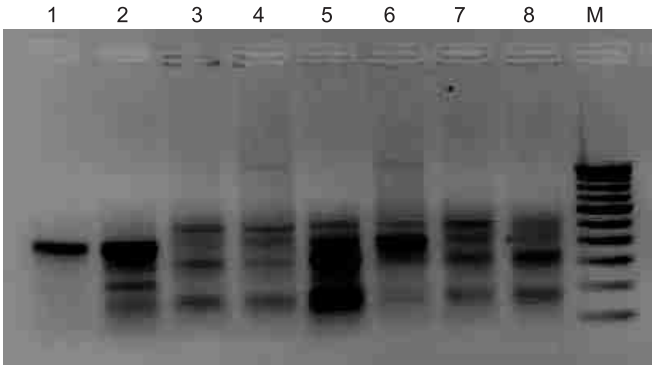


Fig. 12: **Anchored primer amplification of gDNA of *P. falciparum* isolates with primer $(A)_{14}$CATGCC M: Marker, 100 bp DNA ladder; Lane 1:(CQ - 11); 2:(CQ-16); 3:(CQ-19); 4:(CQ-24); 5:(C-4); 6: (C-9); 7:(C-25); 8:(C-26)**

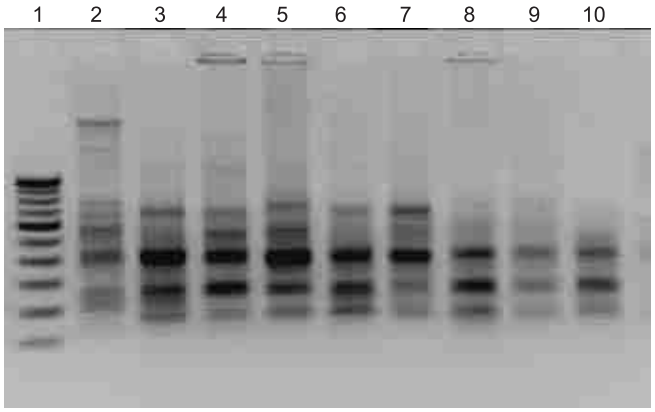


Fig. 13: **Anchored primer amplification O of gDNA of *P. falciparum* isolates with primer $(A)_{14}$GGTTCC M: Marker, 100 bp DNA ladder; Lane 1:(CQ-11); 2: (CQ-16); 3:(CQ-19); 4:(CQ-24); 5:(C-4); 6:(C-9); 7:(C-25); 8:(C-26); 9: (C-29); 10:(C-32)**

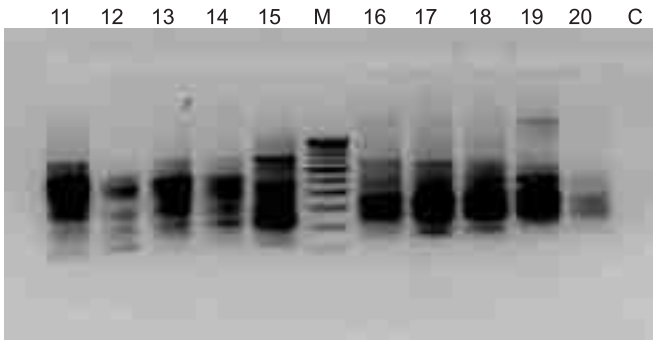


Fig. 14: **Anchored primer amplification of gDNA of *P. falciparum* isolates with primers $(A)_{15}$GTGTA M: Marker,100 bp DNA ladder; Lane 11:(ACT-3); 12:(ACT-4); 13:(ACT-5); 14:(ACT-6); 15:(ACT-7); 16:(ACT-8); 17:(ACT-11); 18:(ACT-12); 19: (ACT-13); 20:(ACT-14); C : Control**

diversity by APAD. Variable multiple bands were obtained, depending on the type and number of nucleotides attached to the 3' end of the oligo-nucleotide primer (Figs.12–14). The appearance of the polymorphic bands also depend upon which anchored primers and DNA templates were used. Thus, by using various combinations of anchored nucleotides, numerous and unique patterns of bands were obtained. By using this method, isolates from the same region could be categorised into groups using the same primer and different primers as well. These results could be used for detecting variations between closely related parasites. Some primers gave multiple and discrete bands with all the isolates and showed difference in the patterns of bands with the same primer (Figs. 12–14). Difference in the patterns of band with the same primer in different isolates revealed the presence of diversity among the isolates. This method can be very useful for identifying polymorphism that can distinguish closely related parasite isolates. This method is very useful for rapid parasite typing because large number of loci in the genome can be checked quickly. The method also provides a potentially powerful tool for developing hundreds of genetic markers from different parasite species.

As proposed in the project, data on all above parameters would be generated in the second study site that has already been selected in Meghalaya state, bordering Bangladesh. Epidemiological data of the selected site, CHC Dalu (Indo-Bangla border) of West Garo Hill district of Meghalaya has shown high *P. falciparum* prevalence with API of 22.4 in 2006 and deaths were also reported.

2.1.9 Molecular analysis of aspartic protease gene in *Plasmodium vivax*

Parasite proteases are essential for parasite survival. Proteases help parasite in host cell invasion, nutrition, growth and the processing of precursor proteins. Proteases are believed to be the promising targets for antimalarial chemotherapy as plasmepsins have been characterised in *P. falciparum*. Aspartic proteases have now been characterised in *P. vivax* (Sharma *et al. J Biochem* 2005; *138:* 71–8). As an extension of our previous work, we have characterised aspartic protease

gene in *P. vivax.* Nucleotide sequences encoding the plasmepsins, PvPM4 and PvPM5 of *P. vivax* were retrieved from the Blast searches of available malaria genome sequences database (The Institute for Genomic Research through the website at http://www.tigr.org) using the nucleotide sequences of *P. falciparum* plasmepsins as query sequences. PCR was performed with ~50 ng of *P. vivax* genomic DNA by using specific oligo-nucleotide primers for each gene.

PvPM4
(5'ATGGATATAGCAGTGAAAGAACAAGACTACTCAAA-3' and 5'TTAATTCTTTGCGATGGCAAAACCGACACTCTC-3')

PvPM5
(5'-ATGGTCGGAGCGAGCTTGGGGCCCCCCGGT-3' and 5'-CTACGCATCCGCGGGCGCCTTGCCCTCGGAGG-3')

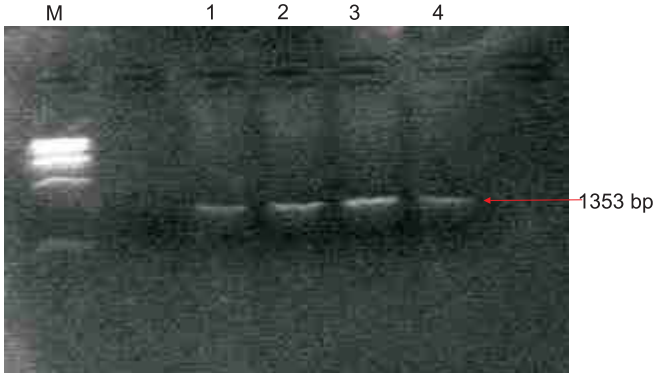


Fig. 15: **PCR amplification of aspartic protease gene using primer PvPM4 in *P. vivax***

P. vivax isolates from Chennai were amplified by PCR using specific primers for PvPM4 and PvPM5 (Data not shown). Each PCR product appeared at the expected sizes of 1353 (Fig. 15) and 1611 bp. Characterisation at the biochemical and molecular level will be helpful for designing new drugs against *P. vivax* and *P. falciparum.*

2.2 Genome Informatics of *Plasmodium falciparum* and *P. vivax*

2.2.1 Bioinformatic study of *Plasmodium falciparum* genome

Distribution and relative position of introns in each gene in all the 14 chromosomes of *P. falciparum* have been studied. The study revealed that introns

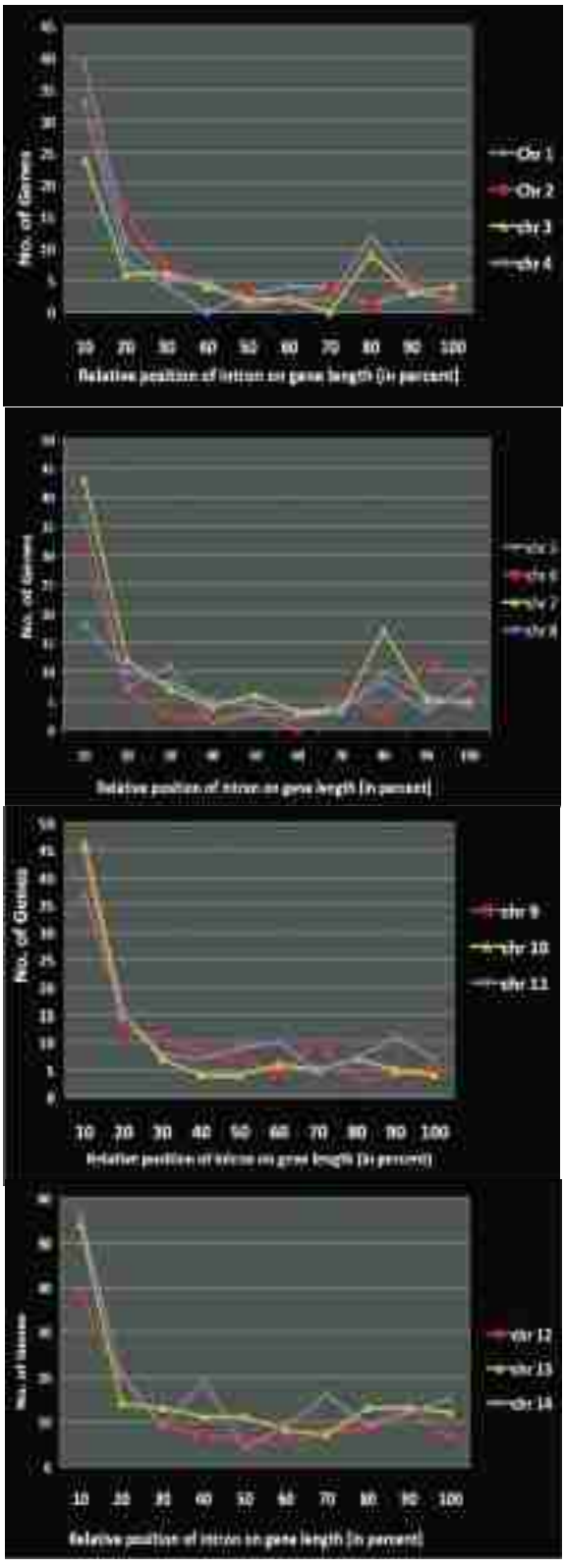


Fig. 16: **Distribution of introns on gene length in *P. falciparum* genes, having one intron per gene**

with length of 100–200 bp were very common in *P. falciparum* genome. Chromosome 13 has maximum number of introns with <400 bp and introns with > 400 bp were mostly present on the chromosomes 10 and 14. Introns in the *P. falciparum* genes were biased towards the 5' end of the genes. Majority of the first introns lies within the first 10% of the total gene length. The results were consistent in all the 14 chromosomes of the *P. falciparum* (Fig. 16). An intron database providing exclusive information about the *P. falciparum* introns with user friendly search and output options was developed. This data base can be assessed from http://www.plasmonimr.org/index_files page1473. htm.

2.2.2 Evolutionary perspective of chloroquine resistant malaria

Drug resistance in *P. falciparum* represents a major health problem in malaria endemic countries like India. *P. falciparum* chloroquine (CQ) resistance transporter (*Pfcrt*) gene has been shown to be primarily responsible in conferring resistance to CQ. In India, CQ resistance in field isolates was first reported from Assam in 1973 and its resurgence has recently been seen in several regions of India. Studies with microsatellite loci in 87 worldwide isolates of *P. falciparum* at the *Pfcrt* gene and flanking DNA sequences have found little variations among CQ resistant isolates but more among CQ sensitive isolates. The observations in India contradict those in global scenario observed for genetic diversity at the *Pfcrt* gene in *P. falciparum*. Indian populations of *P. falciparum* show high genetic variations at the *Pfcrt* gene which do not fit either selective sweep model or the assumptions of origin of drug resistance caused by drug pressure in India. Whereas the global isolates seem to have an almost fixed *Pfcrt* gene following the selective sweep model of evolution by natural selection (Fig. 17a), the *Pfcrt* gene in Indian isolates seems to be in the process of a massive 'genetic reconstruction' (Fig. 17b). Possession of high genetic diversity in Indian isolates is frightening. This would provide a platform where new (beneficial) mutations followed by new associations among different mutations might arise in the population and help a parasite to evade new combination drugs that are now in use in the field. Thus, combining results from recent studies mean that the prospects of testing and using new antimalarial drugs and/or vaccine in India are discouraging. Thus knowledge of the detailed population genetic structure of the parasite in India

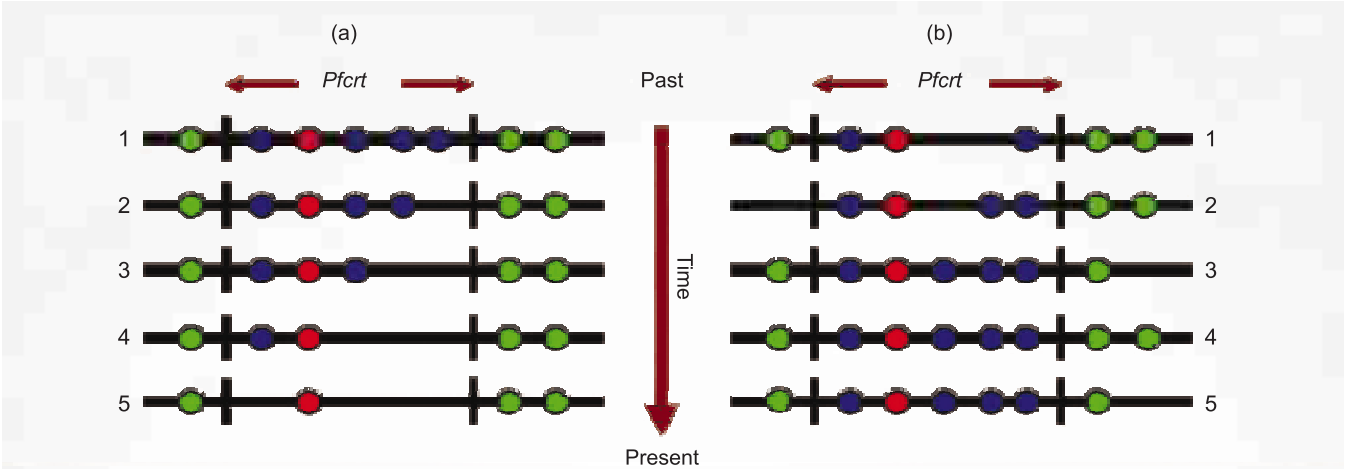


Fig. 17: **Evolutionary trajectory of *Pfcrt* gene in chloroquine resistant *P. falciparum* populations (a)—Over time and drug selection pressure, the positively selected mutation (*e.g.* K76T) is swept away by natural selection along with the linked neutral genetic variations (steps 1–5). However, the variations that are far from the selected mutation are unaffected by the selective sweep. Thus, neutral variations in the flanking regions of *Pfcrt* are maintained (step 5); and (b)— A completely opposite evolutionary trend, in which neutral mutations progressively accumulate over time (steps 1–5) inside the chloroquine resistance gene (*Pfcrt*), especially in and around the positively selected mutation (*e.g.* K76T) (step 5)**

is needed before the field trial of a new drug or vaccine is initiated.

2.2.3 *In silico* genetic characterisation of *Plasmodium falciparum* chromosome 7

The fact that malaria still an uncontrolled disease, is reflected by the genetic organisation of the parasite genome. Efforts to curb malaria should begin with proper understanding of the mechanism by which the parasites evade human immune system and evolve resistance to different antimalarial drugs. We have initiated such a study and present herewith the results from the *in silico* understanding of a seventh chromosomal region of the malaria parasite, *P. falciparum* encompassing

Fig. 18: **The ~270 kilobase specified region of *Plasmodium falciparum* chromosome 7**

the antigenic *var* genes (coding *Pfemp1*) and the drug-resistant gene *Pfcrt* located at a specified region of the chromosome 7 (Fig. 18). We found 60 genes of various functions and lengths (Fig. 19), majority (61.67%) are performing unknown

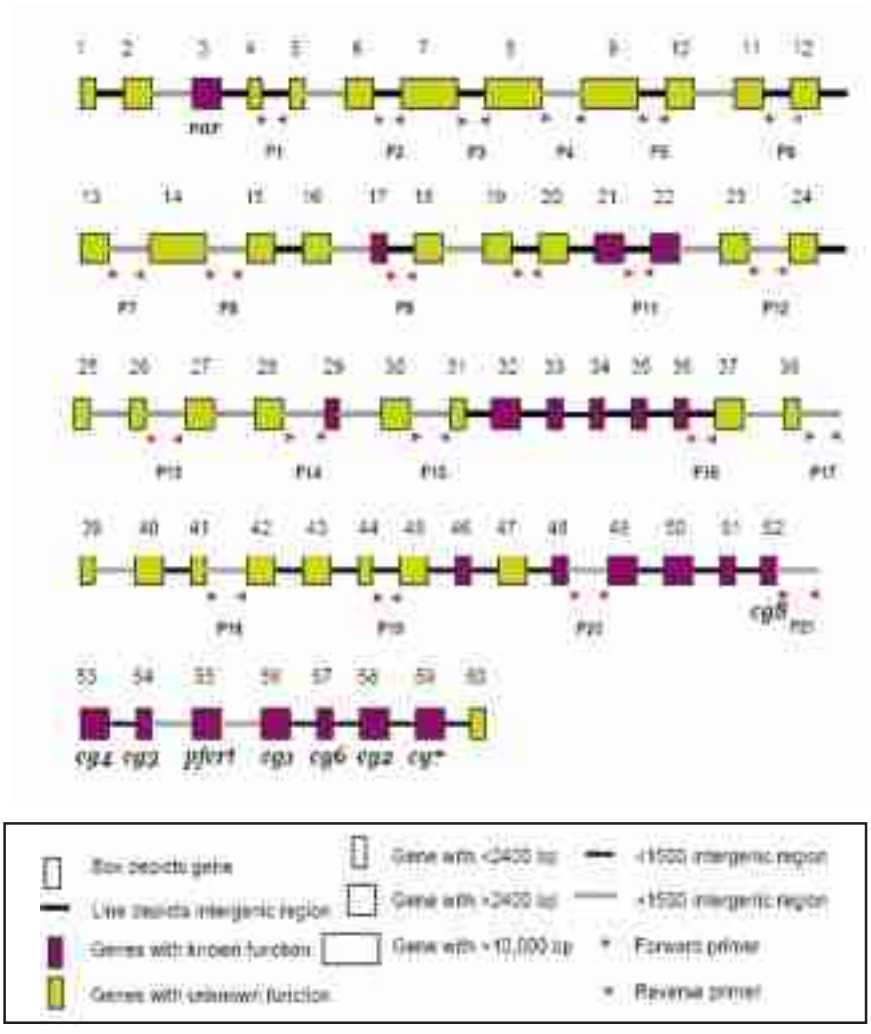


Fig. 19: **Fine details of the specified region showing the genes, intergenic regions and locations of the primers designed for amplification of different regions**

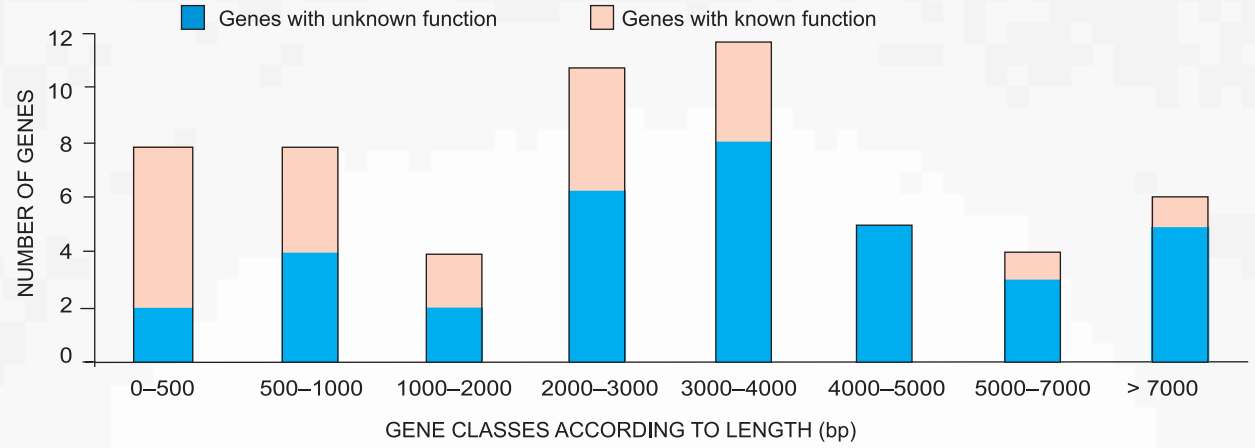


Fig. 20: **Distribution of genes with known and unknown functions in the specified region**

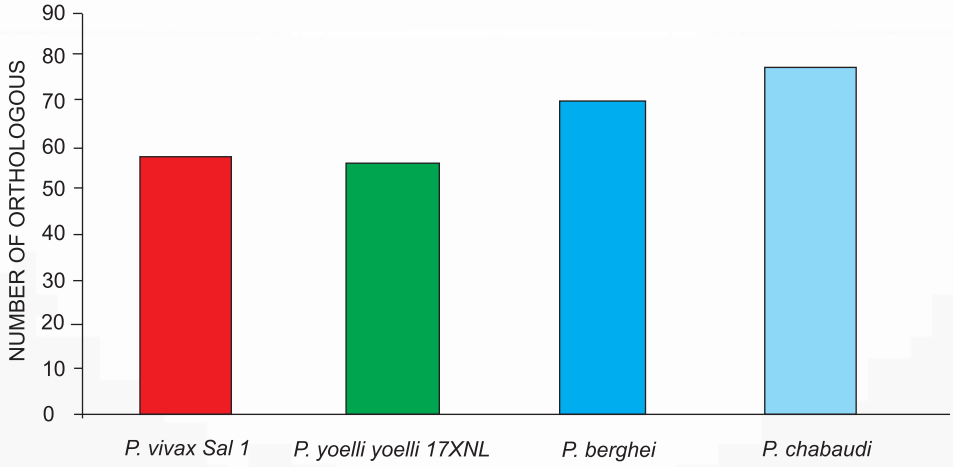


Fig. 21: **Number of genes in the specified region that are orthologous to other species of the genus *Plasmodium***

functions (Fig. 20). Almost all genes have orthologs in other four species of *Plasmodium*, of which *P. chabaudi* seems to be the closest to *P. falciparum* (Fig. 21). However, only two genes were found to have paralogs. Interestingly, the drug-resistant gene, *Pfcrt* was found to be surrounded by seven genes coding for several CG proteins out of which six were reported to be responsible for providing drug resistance to *P. vivax*. The intergenic regions, in this specified region were generally bigger in size, majority (73%) of them were found to be of > 500 nucleotide base pair length. We also designed primers for amplification of 21 non-coding DNA fragments in the whole region for estimating genetic diversity and inferring the evolutionary history of this region of *P. falciparum* genome.

The study, for the first time, provides fine-scale genetic insights into a chromosomal region of the malaria parasite genome of high importance. The study, in addition to characterisation of different genes, their homology pattern, intron-exon compositions and pattern of intergenic regions, also reveal a very interesting phenomenon that the gene conferring chloroquine-resistance to the malaria parasite, *P. falciparum* is flanked by seven CG-protein coding genes otherwise known to be responsible for drug resistance in *P. vivax*. The study would help

furthering malaria research by understanding the pattern of genetic diversity in this specified region and help inferring evolutionary mysteries of both drug resistance and high antigenic variations.

2.2.4 Localisation and characterisation of the antigenic *var* genes in *Plasmodium falciparum* genome

Recent research has shown that around 10% of the 3-Mb *P. falciparum* genome is committed to the expression and generation of diversity of the virulence genes and these genes are randomly distributed throughout the genome. Three families of variant virulence genes have been characterised in *P. falciparum*; the *var* gene encoding *P. falciparum* erythrocyte membrane protein 1 (*Pfemp1*); the repetitive interspersed family (*rif*) of gene; and the sub-telomeric variant open reading frame (*stevor*) gene. The *var* and *rif* gene families are considered to be responsible for the key virulence factors and the functions of *stevor* gene are not yet well understood. Parasite protein *Pfemp1* adherences to certain disease receptors are more commonly associated with certain disease outcomes, such as cerebral and placental malaria. *P. falciparum* infections are persistent, and this chronicity is promoted by antigenic variation at the infected red blood cell surface. Further, expression of *var* genes from a chromosomal domain known for frequent rearrangements has important implication for the mechanism of *var* gene switching and the generation of novel antigenic and adhesive phenotypes.

To understand mechanisms by which the parasite is able to do so, it is important, at the first hand, to localise the position of each of the *var* genes in the genome of *P. falciparum*. Also, by mapping chromosomal locations of *var* genes expressed in *P. falciparum* isolate 3D7, we can identify the *var* gene map within sub-telomeric and centrally placed locations. In the present study, we utilised the *P. falciparum* genome web database to localise each of the *var* genes in all the 14 chromosomes of *P. falciparum* genome. The schematic representation of the results is shown in (Figs. 22 a & b). As shown in the figure, the studies revealed the presence of 104 *var* genes in all the 14 chromosomes. Majority of *var* genes were located at the sub-telomeric regions of the chromosome, the region, generally with high recombination rate. Another small set of *var* genes was found to be arranged in chromosome internal clusters in some of the chromosomes (Figs. 22 a & b). Phylogenetic analysis revealed the ancestry and high evolutionary resemblance among the various *var* genes within the chromosome and also across chromosomes, in the whole genome (Fig. 23).

2.2.5 Genomic characterisation of *vir* multigene family in *Plasmodium vivax*

The estimated global burden due to *P. vivax* is approximately 70 to 80 million cases annually with about 80–90% cases in the middleeast, Asia and western pacific, 10–15% in central and south America, and 10–20% in sub-Saharan Africa. It is now known that the virulence, pathogenicity and successful invasion of the parasites into the host have genetic basis, thus, characterisation of specific genes in the genome should be first step to understand the whole mechanisms. To this respect, the *vir* multigene family is an important virulence determining gene family in *P. vivax.* One of the most striking features of this gene family is the antigenic property and enormous amount of genetic variation that helps the parasite in successfully evading the host immunity and thus, plays a major role in malaria pathogenicity. The almost completed *P. vivax* whole genome sequence available at public domain, provides opportunities to characterise different genes and family of genes. The present study was carried out to characterise the entire *vir* gene family in detail with a genome outlook and establish evolutionary relationships among different gene families. There are altogether 32 genes reported across the entire gene family. Using the PlasmoDB (www.plasmodb.org) web database, data on five (A, B, C, D and E) *vir* gene subfamilies were retrieved. Each of the subfamilies except the subfamily B has about 400–570 different gene sequences. These sequences are nothing but the multiple copies of all 32 genes. Subfamily B has 192 sequences in the database. We divided each subfamily into 32 groups according to the gene similarities and aligned all the genes within each group. We found several conserved domains within

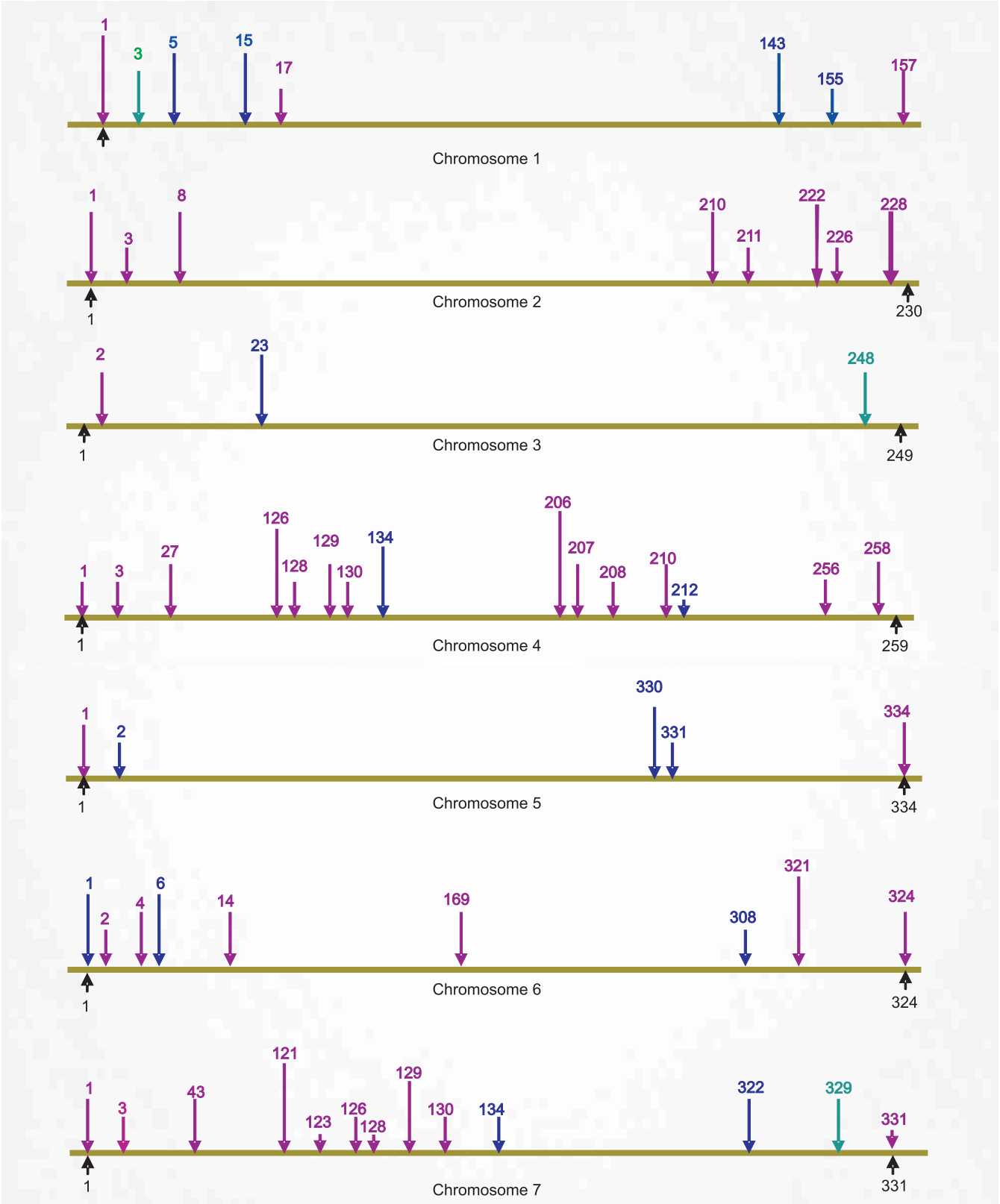


Fig. 22a: **Schematic map of *P. falciparum* chromosomes showing the location of *var* genes (chromosomes 1–7)**

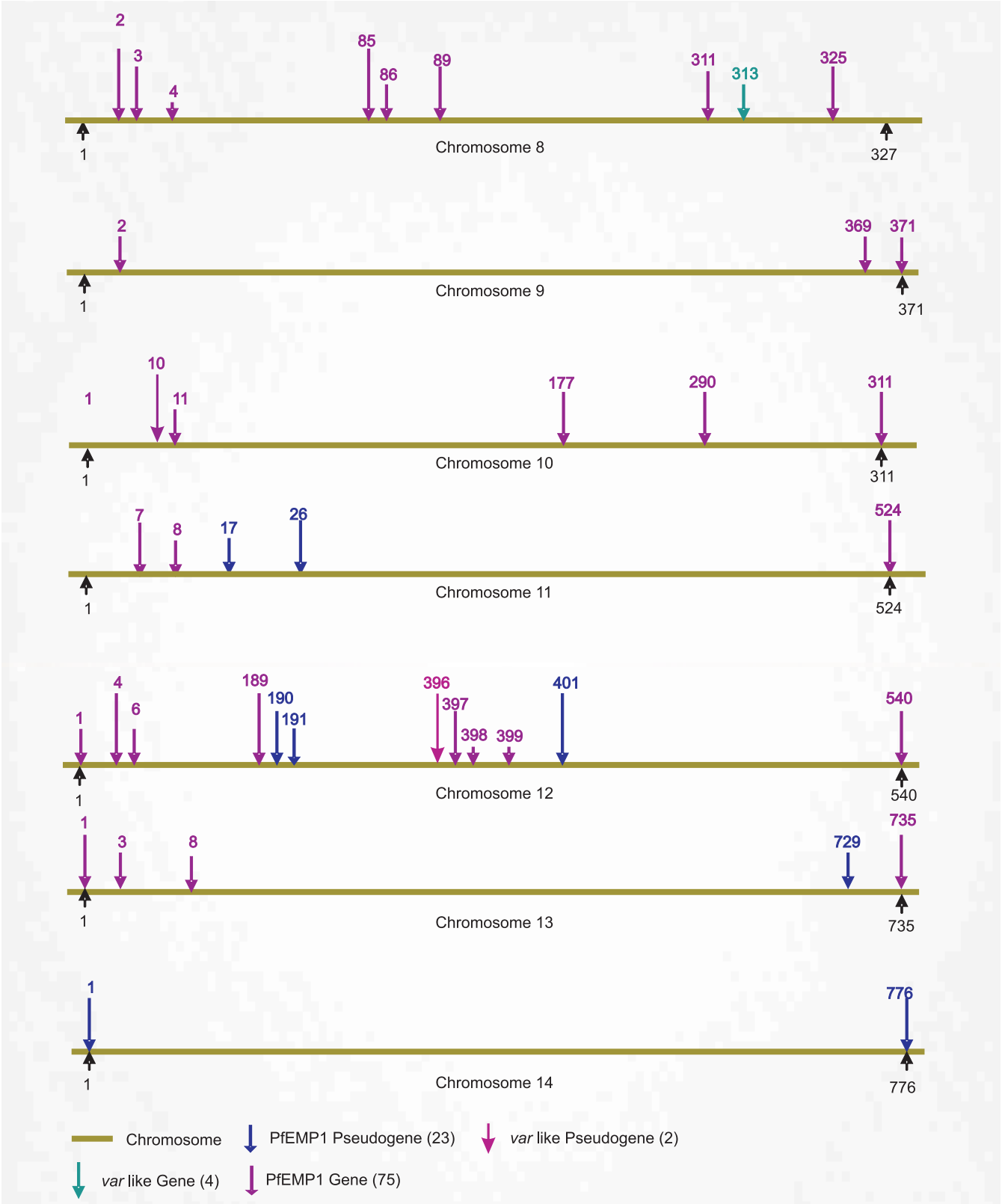


Fig. 22b: **Schematic map of *P. falciparum* chromosomes showing the location of *var* genes (chromosomes 8–14)**

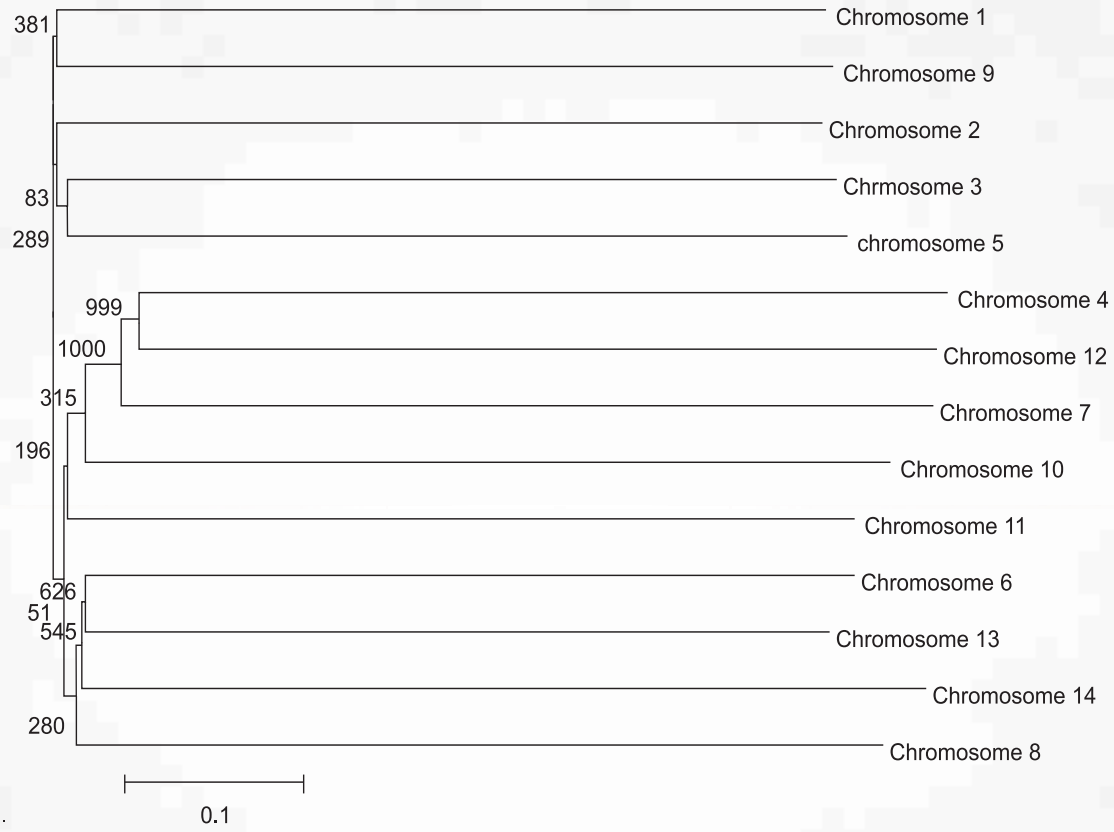


Fig. 23: **Phylogenetic relationships among the *var* genes across the *P. falciparum* genome. Consensus sequences were generated for each chromosome using the computer program "Bioedit". The numbers show the strength of the internal node through generation of bootstrapped trees**

each group, in certain cases, this extends even up to 80%. Fig. 24 shows the contribution of each gene subfamily into each of the 32 divided groups. It was evident from the figure that some of the genes show high number of copies and few show very less number in different groups.

We have conducted phylogenetic studies by constructing neighbour-joining trees among all the five subfamilies of the *vir* gene family and the results are shown in Fig. 25. The analysis showed a close relationship between the subfamily A and B (Fig. 25). Since, genes in the *vir* gene family were responsible for the antigenic properties of *P. vivax* which also help the parasite in virulence and pathogenicity the evolutionary bioinformatics studies presented here would be helpful in furthering research in this area.

2.3 Parasite characterisation and Immunology

2.3.1 Characterisation of the *Plasmodium falciparum* strains prevalent in northeastern states

As per study protocol, malaria prevalence studies were started in Dhubri and Nalbari districts to recruit patients for therapeutic efficacy studies.

District Dhubri

As per discussions with state/district health officials, Chappar and Gazarikandi PHCs were screened for prevalence of *P. falciparum* malaria. During 12–24 July 2006, 411 subjects of Chappar Tea Estate were screened for blood smears and only 8 (1.9%) were positive for malaria. Of these, 6 (75%) were

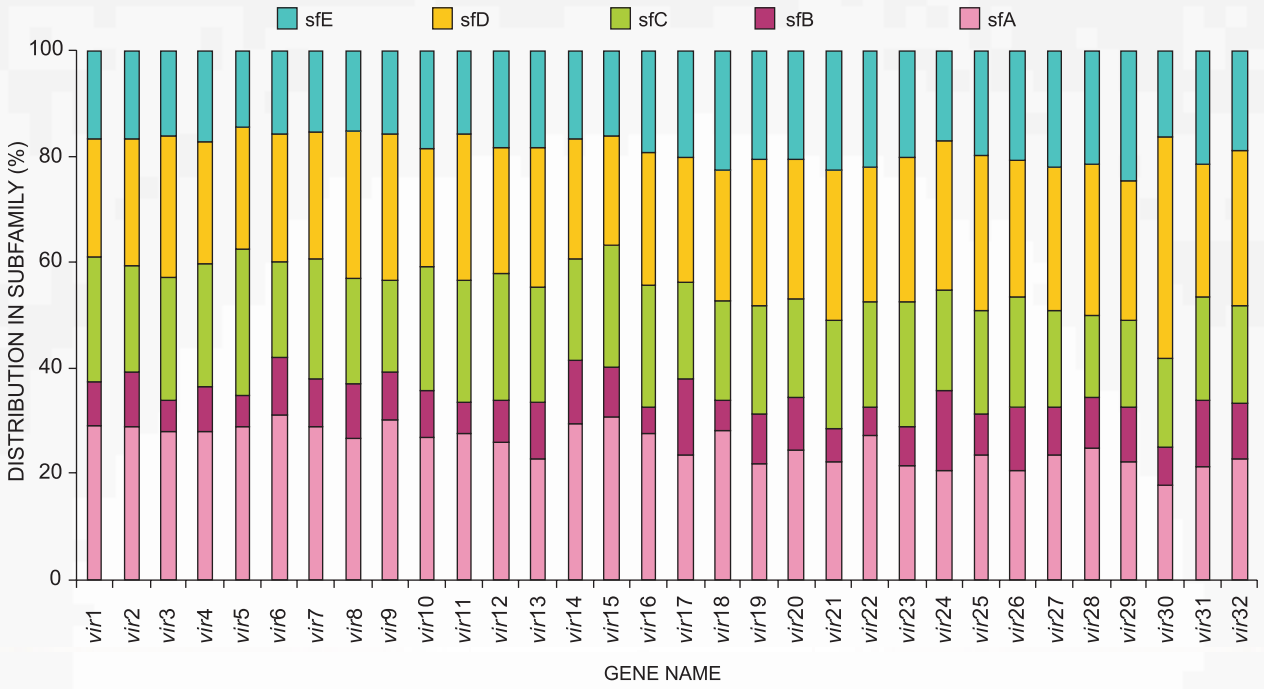


Fig. 24: **Distribution of different *vir* gene subfamilies across 32 different groups**

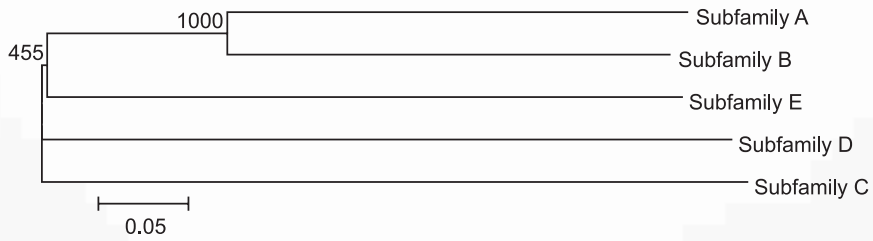


Fig. 25: **Phylogram showing the relationship among different subfamilies of *vir* gene. The numbers indicate the strength of the internal nodes, established through generation of bootstrapped trees. A high number (1000) separating the subfamily A and B from C, D and E are highly statistically significant**

P. falciparum, and the remaining were *P. vivax* cases. Due to low prevalence of malaria at the given time, the team was shifted to Gazarikandi PHC located along Indo-Bhutan border in consultation with the District Malaria Officer.

Active fever surveys were conducted in villages of Sadullabari, Kakrapara and Mankachar for detection of malaria cases. Of 456 blood-smears examined, 8 (1.7%) were positive for malaria. Most were *P. falciparum* (88%) cases. Among a total of 14 cases that were positive for *P. falciparum*, only one subject met the inclusion criteria. The site has to be abandoned due to low malaria prevalence for the period of study and work started in District Nalbari.

Therapeutic efficacy of drugs used by the State Health Authorities was ascertained for treatment of uncomplicated *P. falciparum* malaria in endemic villages located along Indo-Bhutan border in Tamulpur PHC of Nalbari district of

Fig. 26: Map of the Northeastern region of India showing Assam, Arunachal Pradesh, Meghalaya, Manipur, Mizoram, Nagaland and Tripura states and the international borders with neighbouring countries. The location of Tamulpur (Nalbari distrcit), Gazarikandi (Dhubri district), and the proposed site in Tura, Dula CHC (Meghalaya) are indicated

Assam (Fig. 26). For enrolment of study subjects, in addition to active surveillance in high-risk villages, a clinic was established in Menoka Tea Estate, Kumarikata. During the study period from August–October 2006, all those reporting fever were screened for malaria parasites in their peripheral blood smear (Table 1).

Chloroquine efficacy

Based on inclusion criteria, 33 subjects were selected for treatment with standard regimen of chloroquine (CQ), out of these, 30 completed the 28-day follow-up investigations, 8 (27%), 14 (46%) and 8 (27%) subjects were observed to be ETF, LCF and ACPR respectively.

TABLE 1

The prevalence of malaria among the febrile villagers in Kumarikata, Tamulpur PHC, Nalbari district, Assam*

Study village	No. of blood-smears examined	No. positive for malaria	No. positive for *P. falciparum*
Angarkata	220	95 (43)	47 (49)
Pani Tanki	36	10 (28)	7 (70)
Vinoypur	14	2 (14)	2 (100)
Palasbari	22	7 (32)	1 (14)
Menoka Tea Estate	626	168 (27)	103 (61)
Total	918	282 (31)	160 (57)

*Study period: August–October 2006; Figures in parentheses indicate percentage.

ACT (SP+Artesunate) efficacy

Using this combination therapy, 53 subjects that met the inclusion criteria were included. Of these, 47 (89%) were treatment successes (ACPR) and 6 (11%) were LCF owing to recrudescence on Day 21 (4 cases) and Day 28 (2 cases). All drug failure cases were referred for curative therapy under hospital care. Table 2 shows the baseline characteristics of patients in Tamulpur PHC of Nalbari district. Table 3 presents summary of treatment response among the patients in Tamulpur PHC of District Nalbari.

TABLE 2

Baseline characteristics of patients in Tamulpur, District Nalbari, Assam

Drug: Chloroquine	Dose 25 mg/kg over 3 days
No. of cases	30
Male/Female	18/12
Age (Range)	6–45 yr
Parasitaemia (Range)	1280–63, 520 µl
Drug: AS + SP	AS: Dose 4 mg/kg/day × 3 SP: 25mg/kg single dose
No. of cases	53
Male/Female	30/23
Age (Range)	2–52 yr
Parasitaemia (Range)	1040–99, 280 µl

TABLE 3

Summary of classification of treatment response in Tamulpur, District Nalbari, Assam

	Chloroquine		SP + ACT	
	No.	Prevalence	No.	Prevalence
ETF	9	0.31	0	0
LCF	12	0.414	6	0.113
LPF	0	0	0	0
ACPR	8	0.276	47	0.887
Total analysis	29	–	53	–
Withdrawals	0	–	0	–
Lost to follow-up	1	0.033	0	0
Total	30	–	53	–

Genotyping studies

Genotyping of recrudescence infection observed in CQ and ACT drug therapy was carried out using three surface protein markers, MSP-1, MSP-2 and GLURP. Nested PCR assays were carried out. Results revealed same genotype in three out of five paired samples (zero day and on the day of recrudescence) tested for LTF of chloroquine efficacy, suggesting of recrudescence. Similarly, in ACT efficacy trial, four LTF cases tested showed different genotypes thus suggesting for new infection.

Drug-resistant markers

Samples of clinical isolates collected from individual *P. falciparum* malaria patients participated in the therapeutic efficacy study were analysed by PCR for mutations in *Pfcrt* and *DHFR* genes. In *Pfcrt* analysis, 13.34% demonstrated wild type K76, 33.33% showed mutant type T76, while 53.33% had mixture of both K76 and T76.

Point mutations in *DHFR* codons 16, 51, 59, 108 and 164 have also been analysed. In the study group, 80% demonstrated wild type A16, 3.33% showed mutant type V16 and 16.67% had mixture of both A16 and V16. At codon 51, 70% demonstrated wild type N51, 6.67% showed mutant type I51 and 23.33% showed mixture of both N51 and I51. Codon 59 demonstrated 46.67% wild type C59, 30% showed mutant type R59, while 23.33% had mixture of both C59 and R59. Number of wild type variants in codon 108 are 16.67% (S108); mutant type of N108 found in 60% and 23.33% showed mixed type S108N. Mutant type Thr108 has not been detected among these isolates. Codon 164 demonstrated 76.67% wild type I164, 10% showed mutant type L164, while 13.33% showed mixture of both I164 and L164.

Blood samples collected before and at the time of recurrence of asexual parasitaemia were also analysed. Ten patients who did not respond to the drugs showed selection of resistant subpopulation. All of them had mutant type T76 in case of CQ. In DHFR, presence of double (1), triple (5) and quadruple (4) mutant types have been observed as a result of treatment failure by selecting resistant parasite population (Table 4).

TABLE 4

Variants of *Pfcrt* and DHFR present in *P. falciparum* in samples collected before (D_0) and after treatment (R)

Pairs	Code No.	Sample on D_0 / R	*Pfcrt* K76T	DHFR				
				A16*V*	N51*I*	C59*R*	S108*N/T*	I164*L*
1	CQ-2	D_0	K+*T*	A+*V*	N	C+*R*	S+*N*	I
	ACT-5	R-D_{21}	T	*V*	N	*R*	*N*	I
2	C-1	D_0	*T*	A	*I*	R	N	I
	C-1	R-D_{14}	*T*	A	*I*	*R*	*N*	I
3	C-3	D_0	K+*T*	A	N	C+*R*	S+*N*	*L*
	C-3	R-D_7	*T*	A+*V*	N	C+*R*	*N*	I
4	C-9	D_0	K+*T*	A+*V*	N	C+*R*	S+*N*	I+L
	ACT-11	R-D_{21}	*T*	*V*	N	C+*R*	*N*	I+*L*
5	C-27	D_0	K+*T*	*V*	N	C+*R*	S+*N*	I
	ACT-27	R-D_3	*T*	A	N	*R*	*N*	I+*L*
6	C-33	D_0	K+*T*	A+*V*	N	C+*R*	*N*	I+*L*
	ACT-33	R-D_{21}	*T*	A+*V*	N	C+*R*	S+*N*	I+*L*
7	ACT-18	D_0	*T*	A	*I*	*R*	*N*	I+*L*
	ACT-18	R-D_{21}	*T*	A	N+*I*	*R*	*N*	*L*
8	ACT-20	D_0	*T*	*V*	N	*R*	*N*	I
	ACT-20	R-D_{21}	*T*	*V*	N	*R*	*N*	*L*
9	ACT-23	D_0	*T*	A	*I*	*R*	*N*	*L*
	ACT-23	R-D_{23}	*T*	A	N	*R*	*N*	I
10	ACT-28	D_0	*T*	A	N	*R*	*N*	I
	ACT-28	R-D_{21}	*T*	A	*I*	*R*	*N*	I

Mutant genotypes are indicated in italics.

2.3.2 Naturally acquired humoral immune responses to defined *Plasmodium falciparum* erythrocytic stage antigens in a population of eastern India

In malaria endemic areas, acquired protective immunity to *Plasmodium* is achieved in adults as a cumulative effect after repeated exposure. The humoral response to various antigens of parasite is thought to be one of the important mechanisms mediating this immune state. Human sera of various age groups living in malaria endemic regions, naturally exposed to seasonal or perennial transmission, have been used as probes in defining *P. falciparum* antigens that may be involved in the induction of immunity. Several antigens of *P. falciparum* erythrocytic merozoite stage were under investigation for their inclusion in a subunit vaccine; among them merozoite surface proteins 1 and 2 (MSP-1 and MSP-2), the apical membrane antigen-1 (AMA-1), rhoptry associated protein-1 (RAP-1) and erythrocyte binding antigen-175 (EBA-175) showed high immunogenicity eliciting partial or total protection in monkey malaria. By conducting epidemiologic studies, sera of the inhabitants living in malaria endemic areas showed marked immunoglobulin-G reactivities to these antigens.

Individuals of two subpopulations of town and forest areas were studied for their parasitologic and immunologic profiles (Fig. 27). Sera of three different age-matched groups were screened by ELISA to measure antigen-specific IgG for characterising antibody responses to the B-cell epitopic sequences of *P. falciparum* MSP-1, AMA-1, RAP-1, EBA-175

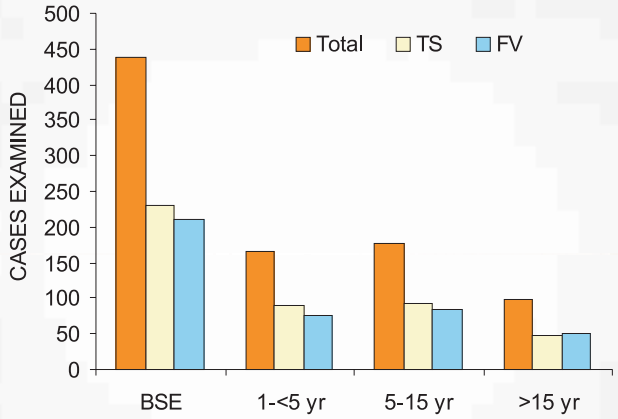


Fig. 27: **Screening of fever cases of different age groups**

and *P. falciparum* infected erythrocyte lysate (PfC) (Fig. 28). In addition, the sera with high antibody titres were also assessed in *P. falciparum* culture in the presence of peripheral blood monocytes by antibody dependent cellular inhibition (ADCI) assay to determine the cytophilicity of circulating antibodies. The results demonstrated that humoral responses to the erythrocytic stage antigens were acquired in an age-dependent manner in these two populations during natural course of infection, and those sera containing antigen-specific antibodies mediated in parasite phagocytosis.

2.3.3 Differential recognition of antigens expressed on the surface of *Plasmodium falciparum*-infected erythrocytes by sera collected from villages of northern India

The natural isolates of *P. falciparum* mainly consist of heterogeneous population of parasites. The antigenic diversity found to be common among various *P. falciparum* strains. The antigens expressed on the surface of *P. falciparum*-infected erythrocytes recognised differentially by the sera of children and adults even from the same location.

P. falciparum-infected blood from a group of young children was collected before giving antimalarial treatment. Blood was collected from the same subjects during convalescent period. Adults of the same area with a past history of repeated malarial infections were also included as immune subjects. Sera from adults of the same area were also tested for antigenic diversity associated with the *P. falciparum* parasites isolated from children. The test parameters included were *in vitro* parasite growth inhibition, erythrocytic stage-specific antibodies, agglutination of parasites with various sera, direct antiglobulin test, *in vitro* rosette formation and identification of proteins on western blot with sera of children and adults.

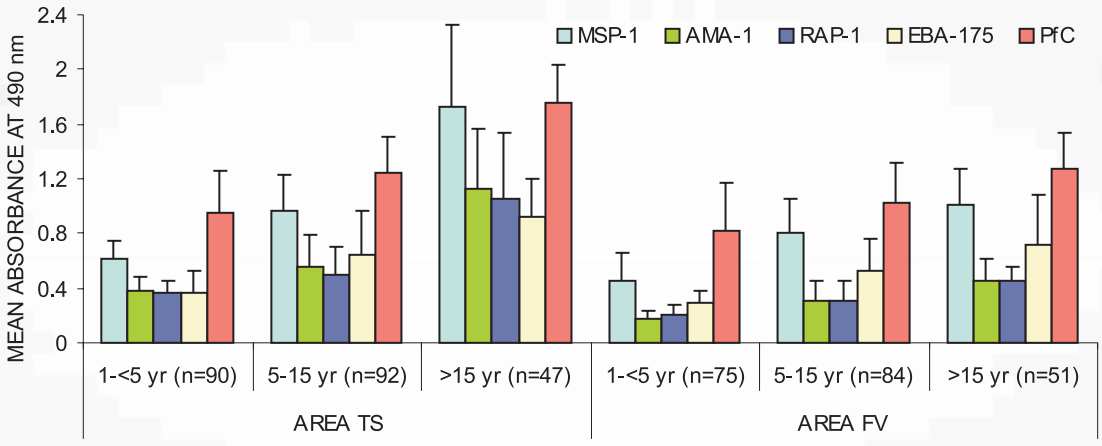


Fig. 28: **Antibody profile of subpopulations of town and forest areas**

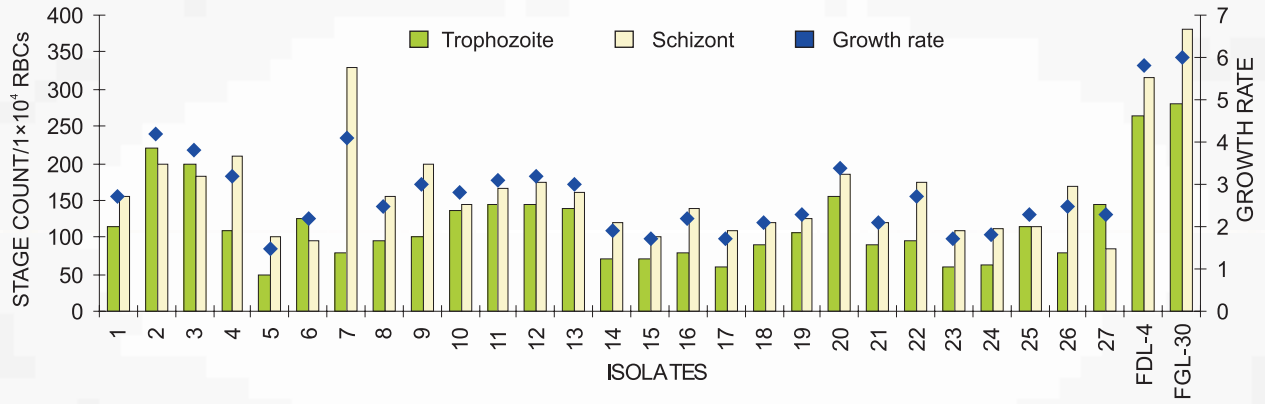


***Fig. 29: P. falciparum* stages and growth rate in culture after 96 hours**

Sera positive for circulating antibodies showed *in vitro* growth inhibitory activity in *P. falciparum* and there was a trend in higher activity with increased age. Rosette formation was observed in some of the isolates and they got disrupted in the presence of autologous sera of children during convalescent stage. Growth inhibitory activity was noticed as a result of agglutination of parasites with autologous convalescent sera. Some of the children developed anaemia at the time of recovery from malaria. The erythrocytes showed agglutination in direct Coombs test. On western blot, sera of these children recognised less number of proteins in comparison to adult immune sera (Fig. 29).

From this study, it was observed that children during acute phase of infection were lacking humoral immunity to combat the infection, but there was a progressive accumulation of antigen-specific antibodies during convalescent period. Variability in response to different isolates and the differential characteristics showed serological diversity in *P. falciparum* within small area experienced with seasonal transmission of malaria.

2.3.4 Immunochromatographic test for determining antimalarial antibody in patients' blood

This study was undertaken to develop an immuno-chromatographic test by dot-immuno binding assay and to compare the results with Plate- ELISA. Blood samples were collected from 92 subjects of age ranging from 5–20 yr, who belong to Nanoo village of Loni PHC, Ghaziabad district after obtaining informed consent. Almost all of them had previous history of malaria. Indirect ELISA was done to estimate antimalarial antibodies in sera samples. Of the 92 sera, 34 had high, 51 had moderate and 7 had low levels of *P. falciparum* erythrocyte stage antigen-specific antibodies. Sera of the children demonstrated low or moderate and sera of adults demonstrated moderate or high antibody profiles. The ELISA results of 92 individuals were compared with that of healthy individuals (negative sera) and malaria immune subjects (positive sera). The results of Dot-blot assay on nitrocellulose membrane were comparable with Plate-ELISA results. Sera containing low titre showed very faint appearance of coloured dots, whereas reactions of moderate and high titre sera showed prominent coloured dots of various intensities when blots were read freshly after completion of reaction.

High level of antibodies determined both by Plate-ELISA and Microdot ELISA indicate past experience of malaria in these individuals. The area where the study subjects reside comes under the influence of seasonal malaria transmission. Since the results of two assays were comparable, they could be equally useful for estimation of antibodies in

patient's serum. The merits of plate or conventional ELISA remain in the measurement of the reactions outcome by an ELISA reader, whereas dot-blot assay results are only readable visually. Therefore, in this case the test outcome is subjective to decide the highest or high or moderate reactions. But antibody positive serum sample always gives positive reaction as a coloured dot, which is easily readable. Moreover, the requirement of antigens, sera, conjugate and other reagents are minimal. The total time requirement for completion of dot-immuno binding assay is shorter than conventional Plate-ELISA. The assay was found to be easy to perform and rapid for producing results.

2.3.5 Source of mosquito blood meal and determination of antimalarial antibodies in ingested blood meal

A total of 576 fed mosquitoes were caught from different areas of Delhi and Ghaziabad. Elution of blood meal was done in 100 µl PBS in 96-well microtitre plates. The eluates were tested by Microdot ELISA to determine the source of blood meal. Of the 576 eluates, 369 were found positive for human blood, whereas 207 blood eluates were cattle positive. Overall human blood meal positivity was 64.1% in these batches of samples. In the study, only 60 mosquitoes (10.4%) were anophelines out of 576, rest were non-vector species. Indirect ELISA tool using *P. falciparum* erythrocytic antigen was applied in testing the eluates positive for human blood meal for antimalarial antibodies. Seropositivity was determined for 325 samples. In some areas almost all the eluates were detected antimalarial antibody negative, but in some pockets samples were found positive for antibody. Overall seropositivity was detected in 78 blood eluates (24%) out of 325.

2.3.6 Allelic variation and immunogenicity of synthetic peptides of T-helper cell epitopic regions of circumsporozoite protein of *Plasmodium falciparum* isolates from India: relevance for vaccine development

T-helper cell epitopes (Th-2R and Th-3R) of circumsporozoite protein of *P. falciparum* show variation which is a serious impediment for development of T-cell epitope-based vaccine against sporozoite. However, if the variants are restricted and could be categorised into groups then the prototype variants from the group could be included into a subunit polyvalent vaccine against sporozoites. We studied altogether 283 isolates of *P. falciparum* from different geographical regions of India. The variants were found to be restricted, could be categorised into groups. The variants were also found to be regionally unbiased in the sense that similar types of variants were found in different geographical regions of India. Studies on the immunogenicity of synthetic peptides of different allelic variants of T-helper cell epitopes found in these studies revealed that combinations of peptides were more immunogenic than individual peptides or peptides of Th-2R and Th-3R from a single group. Therefore, prototype variants from the groups could be used in a subunit polyvalent vaccine against sporozoites.

❑

Epidemiology and Clinical Studies

3.1 Remote Sensing and Geographic Information System

3.1.1 Regional level mapping of malaria vectors using RS and GIS in northeastern states in India to develop strategic plan for malaria control

IRS-P6 LISS-III sensor data of two districts of Assam namely, Nagaon and Sonitpur were procured from National Remote Sensing Agency (NRSA), Hyderabad. Landsat TM of 19 December 1999, 28 November 2000 and 15 November 2001 covering two districts were downloaded from EROS Data Centre USGS website for the purpose of registration. IRS-P LISS-III images were geo-referenced with the help of Landsat TM data. Base layer for Nagaon district created, showing district boundary, road, rail and major drainage network. Preliminary visual interpretation of water bodies, wetlands, built-up areas, forest classes have been done for Nagaon district. It is noticed that Sonitpur district is covered by three IRS-P6 LISS-III scenes, the present Sonitpur district is carved out of the erstwhile Darrang district, hence new approved boundary was obtained and digitised. Besides this, blockwise malaria data have been procured and put on the GIS platform along with other census information. The work is in progress.

3.1.2 Micro level mapping of malaria vectors using GIS in bordering districts of Assam and Arunachal Pradesh in India to assist malaria control

Twelve satellite scenes of IRS-1D LISS-III were acquired from two districts of Assam namely, Kamrup and Sonitpur. Raw images were radiometrically corrected using dark pixel removal technique and noise removal. Well-identified ground control points were taken to rectify the satellite images to provide latitude and longitude information using raster-based geometric corrections, the satellite images were geo-coded using geo-referenced Landsat TM data. Sub-pixel image-to-image accuracy was achieved through repeated attempts. Different images were joined together to get one single false colour composite (FCC). Histogram matching was performed to correct the radiometric difference of the mosaic images. Area of interest extracted from mosaiced FCC scene is shown in (Fig. 1). Digital image processing of satellite data was done through different algorithms and mathematical indices for unsupervised classification and the images were classified into 250 cluster classes. These clusters were grouped under

Fig. 1: **Mosaiced false colour composite**

different classes using visual interpretation of images. AOI generation of various classes for land cover classification namely, river, inland water bodies, tea gardens, vegetation, habitation, open land, etc. was done to get more accurate classified image. Normalised difference vegetation index (NDVI) technique was applied to further classify vegetation into different categories namely, dense forest, open forest, scrub land, grass, water bodies

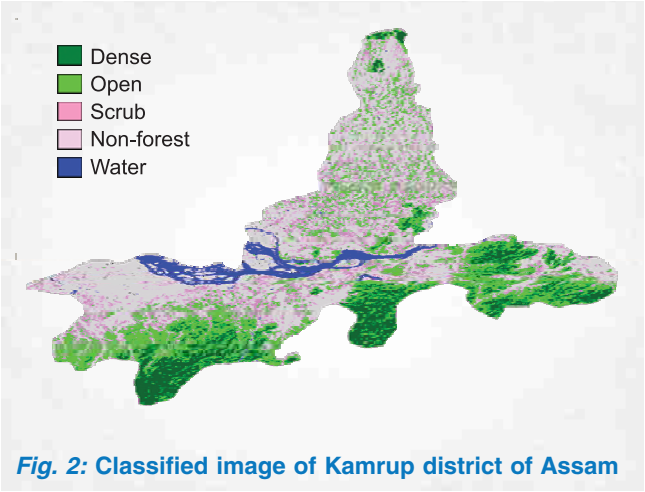


Fig. 2: **Classified image of Kamrup district of Assam**

Fig. 3: **GIS layer's overlaid on satellite image**

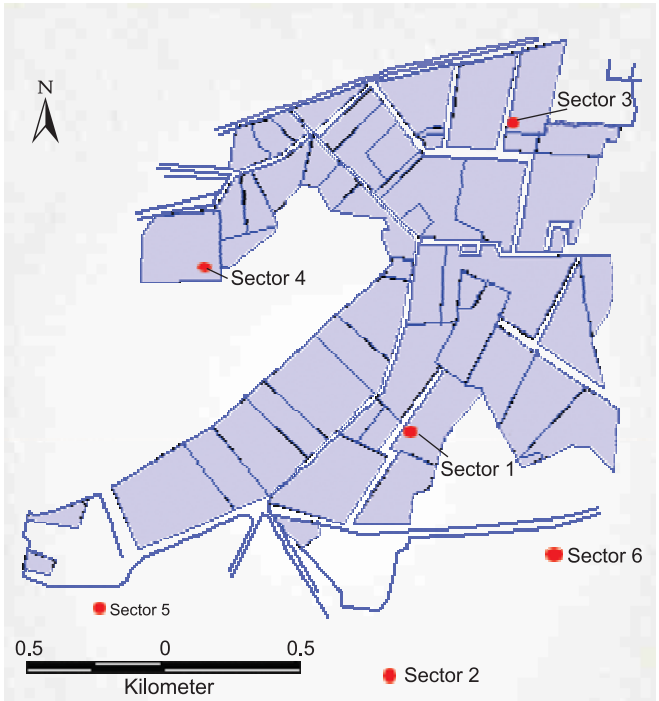


Fig. 4: **Showing different sectors of Jodhpur Cantonment area**

and non-forested areas (Fig. 2). Satellite images were again geometrically rectified with the shape file of the district coverage to get an exact overlay of GIS layers on the images (Fig. 3).

3.1.3 Application of GIS to map distribution of malaria vectors and to develop disease surveillance system in Jodhpur Cantonment area

The study was carried out in the cantonment area in Jodhpur district. This area has diversified population movements from all over India. Statewise contribution of the imported cases from different states, depicts highest contribution from Rajasthan followed by Madhya Pradesh and Uttar Pradesh states.

The area is divided into six sectors (Fig. 4). Indoor and outdoor density of adult mosquitoes was recorded from various GPS registered localities using manual aspirators (per man hour density) and CDC light-traps. Locations of major breeding sites and larval density of mosquitoes from different water bodies were recorded. Mosquitoes collected from each sector were identified into different species. The meteorological data (January 2004– December 2005), number of malaria cases among troops and family members reported in Army Hospital (July 2004 to March 2005), details of hygiene and chemicals used for mosquito control programme were analysed.

Malaria cases from the Army Cantonment Hospital for the past two years were taken into consideration in July 2004 and September 2005 showed highest number of malaria cases in respective years. It also shows the correlation between malaria cases and meteorological data. Relative humidity (RH) parameter was more correlated than any other parameters. When RH was high, malaria cases were more and with low RH, malaria cases were less.

The study on consumption of hygiene chemicals during 2004 and 2005 revealed highest consumption of Baytex 1000 Fenthion mainly during the months of July to August in both the years

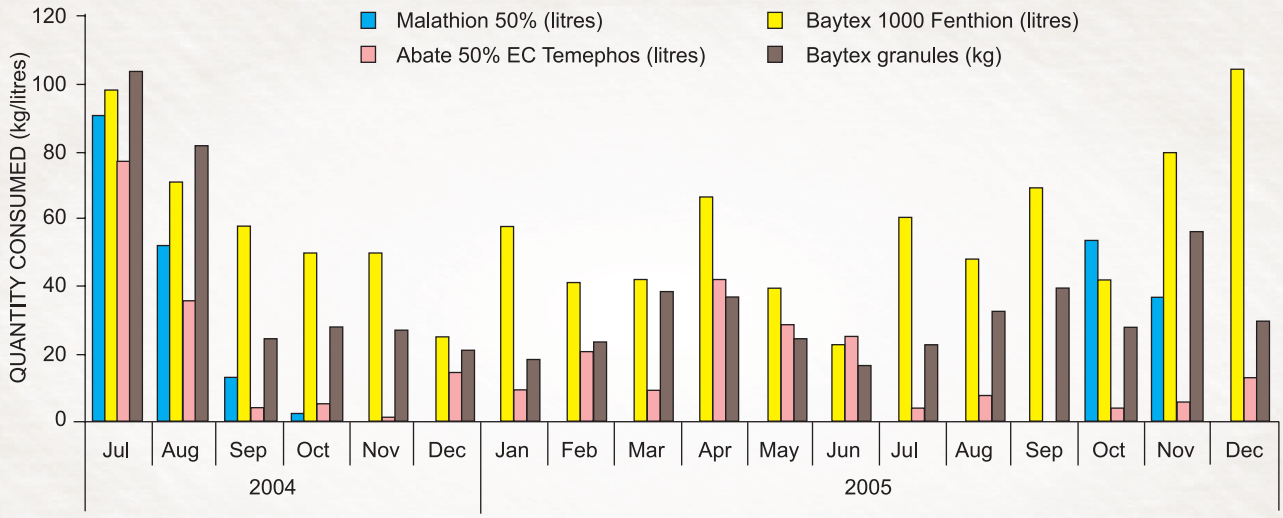


Fig. 5: **Consumption pattern of hygiene chemicals in Cantonment area, Jodhpur**

followed by Abate 50% EC Temephos. Malathion was stopped in between. Baytex granules have also been used in large quantity (Fig. 5). Baygon/ Propoxur was used in less quantity in the year 2004 but in 2005 it was used extensively, especially during July–September. Consumption of Odomos was found high in July and August in 2004 whereas in 2005 high consumption was seen during February– April.

Entomological findings

Indoor and outdoor entomological surveys were conducted in all the six sectors of the Cantonment area in the months of October 2004 and November 2005. Two anopheline mosquito species were collected from Sectors 1, 3 and 6 (Civilian area) during indoor collection. In outdoor collections, two specimens were collected from Sector 3 only.

Indoor and outdoor *Culex* mosquitoes were collected from all the sectors in both the surveys. High density of *Culex* was found indoors as compared to outdoors. Highest indoor density was found in Sector 3 followed by Sector 4, whereas outdoor density in October 2004 was highest in Sector 1 followed by Sector 3. In November 2005 *Culex* density was very low.

Larval density of *Anopheles* and *Culex* mosquitoes was estimated. High larval density of anophelines was observed in the year 2004 than 2005. Highest anopheline larval density was recorded in Sector 1 followed by Sector 6 (Civilian area) in October 2004 whereas in November 2005 highest larval density was recorded in Civilian area followed by Sector 3. *Culex* larval density was reported as high as 452 in Sector 5 followed by 115 in Sector 4 during November 2005. In October 2004 highest larval density was found in Sector 2. *Culex* larval density was higher in 2005 as compared to 2004 collection.

3.1.4 Implementation of GIS research in National Vector Borne Disease Control Programme

GIS-based information system for decision support of kala-azar control in Bihar

For the first time in India, an attempt has been made to design kala-azar control strategy at national level utilising GIS platform for Bihar state. It aimed at identifying high risk pockets and also the risk factors for the decision support of prompt and cost-effective control of kala-azar. There are 38 districts in Bihar, where 31 are endemic for kala-azar and nine are severely affected namely, Gopalgunj, Muzaffarpur, Saharsa, Saran, Vaishali, Araria, East Champaran and Madhepura. Despite implementation of various control strategies, the status of morbidity and mortality due to kala-azar in several districts

remained the same. For GIS platform geo-referenced digital maps of villages/tehsils/districts were used. A three tier database was constructed—districtwise, tehsilwise and village-wise. Attribute data such as villagewise population, schedule caste/schedule tribe population, medical facilities, primary health centres, etc. and data on kala-azar incidence/death for six years—from 2001 to 2006, were attached to the villages on the maps and were used for the analysis for decision support in formulation of control strategies. An example of Gopalgunj is given below.

Gopalgunj

Gopalgunj has about 8.3 thousand to 2.9 lakh population in its 14 tehsils. In the years 2001 to 2003 the kala-azar cases were 6, 19 and 52 respectively, confined to only Baikunthpur Tehsil/PHC of Gopalgunj district. This problem started from east of Gopalgunj and gradually built-up cases in

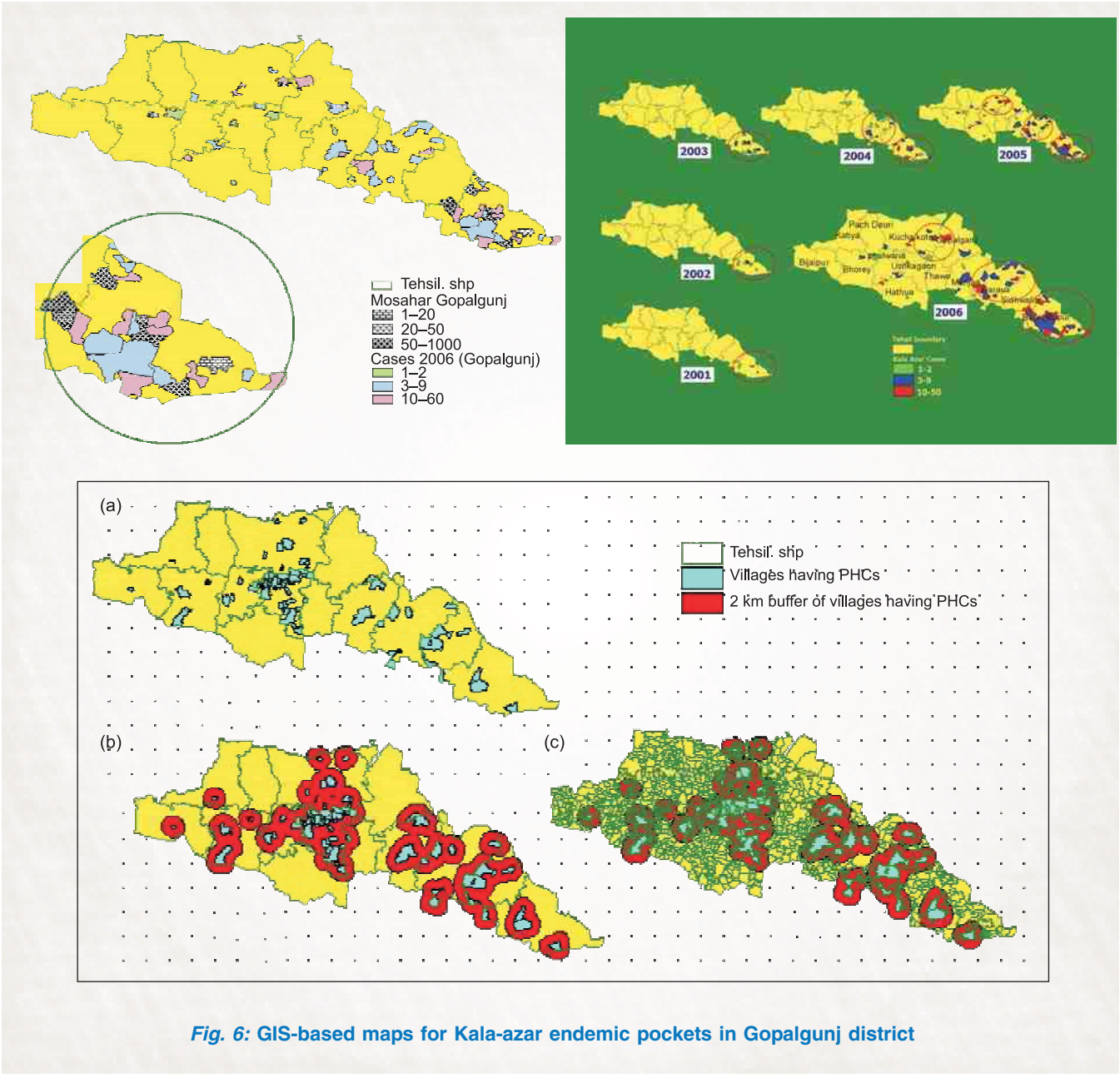


Fig. 6: **GIS-based maps for Kala-azar endemic pockets in Gopalgunj district**

western tehsils with time and in 2006, there was widespread incidence of kala-azar and the disease engulfed 50% of the tehsils and 23 villages having >10 cases. Overall, 8 tehsils of Gopalgunj namely, Baikunthpur Barauli, Gopalgunj, Hathua, Kuchaikot, Bhorey, Uchkagaon and Manjha have reported kala-azar cases from 2001 to 2006 and kala-azar control may be intensified in these villages.

Overlaying yearwise kala-azar cases over Musahar population, a tribe in Bihar, reveals a strong correlation of kala-azar cases with Musahar population. Fig. 6 shows such a correlation of Musahar population with kala-azar cases of 2006, the year of highest incidence. Villages where Primary Health Centres located in different tehsils of Gopalgunj were mapped and a buffer zone was created at 2 km and village boundaries were overlaid to show the accessibility for patients to closest PHC, and the areas where there is a need to establish new PHCs. Similar studies have been conducted for other kala-azar affected districts in Bihar and West Bengal.

GIS-based dengue information system for Delhi

In India, 21 states have reported dengue cases in 2006. A total of 10,935 cases and 171 deaths were reported from all over the country (provisional). The case fatality rate was 1.6%. Out of total cases, 31% were reported from Delhi and adjoining areas. Delhi also reported maximum number of deaths among all the states. A GIS-based Dengue Surveillance System was developed for monitoring and control of dengue in Delhi.

Delhi consists of about 139 million population spread over three localities namely, Municipal Corporation of Delhi (MCD), New Delhi Municipal Committee (NDMC) and Cantonment area. In MCD, there are 12 zones and 133 wards. NDMC consists of one zone and nine wards whereas in Cantonment area, there is only one ward in one zone. Digital map up to the level of street was used to create the GIS database. For all the three areas wardwise number of households, population, literacy rate, scheduled caste population, etc. as per 2001 census were attached. Streetwise reported dengue cases were mapped to identify clusters requiring intense attention for the control of disease (Fig. 7). A routine sample survey for breeding sites supporting breeding of dengue vector is carried out by the National Institute of Malaria Research. These data were overlaid to identify breeding source contributing more for proliferation of dengue vectors, to undertake situation-specific control measures. Based on GIS mapping, formulation of focused control strategy for dengue is in progress.

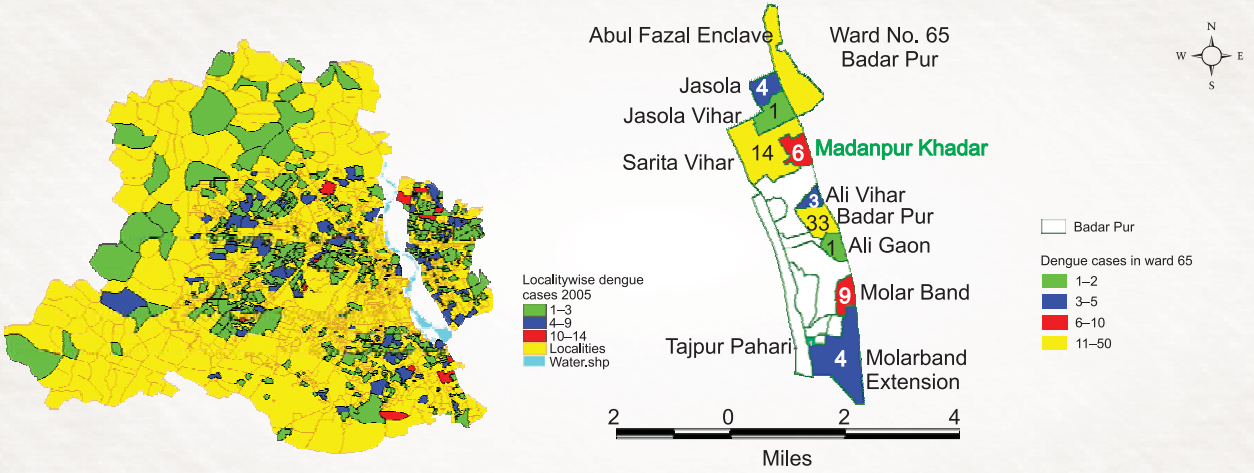


Fig. 7: **GIS map showing localitywise dengue cases in Delhi**

3.1.5 GIS-based analysis of current malaria scenario in India

In India, about 1.47 malaria cases were reported in the year 2006 with 46% *Pf* cases and 1263 deaths. In collaboration with National Vector Borne Disease Control Programme on API basis districts were categorised in four strata namely, having API <1.33, 1.34–5 and >5 and on *Pf* percent basis districts having < or >30%. GIS mapping of current malaria situation was carried out for decision support of formulating control strategy. GIS mapping revealed that in 2006, out of 208 districts 91 districts showed >5 API and 97 districts showed >30% *Pf* cases. Malaria incidence was correlated with drug resistance status.

In northeastern states, 9 and 20 districts showed 2–5 and >5 API respectively. In all, 24 districts had >30% *Pf* cases. In West Bengal and Jharkhand 9 districts showed API 2–5 and 13 showed >5 API. Also nine districts showed >30% *Pf* (Fig. 8). In Bihar no district was found to be having >5 API only one district had >30% *Pf*. The malaria in Orissa and Chhattisgarh is more with five districts in 2–5 API range and 19 in >5 API and overall 35 districts in 30% *Pf* zone. Similarly, for each state high risk districts were geographically identified to intensify the control activities in a focused way.

Districts falling on interstate borders with high malaria or *Pf*% were identified and it was proposed that same intervention should be followed in bordering districts to avoid increase in malaria cases due to infiltration of the population. Similar analysis has been done at block level for Madhya Pradesh (Fig. 8).

3.1.6 RS and GIS in mapping the malaria receptivity of Indira Sagar and Omkareshwar Dam project areas

The work on mapping of the receptivity of Indira Sagar and Omkareshwar Dam project areas is in progress. Digital map of villages of District Dhar was prepared, attached with attribute and malaria data. Trend analysis of epidemiological data from

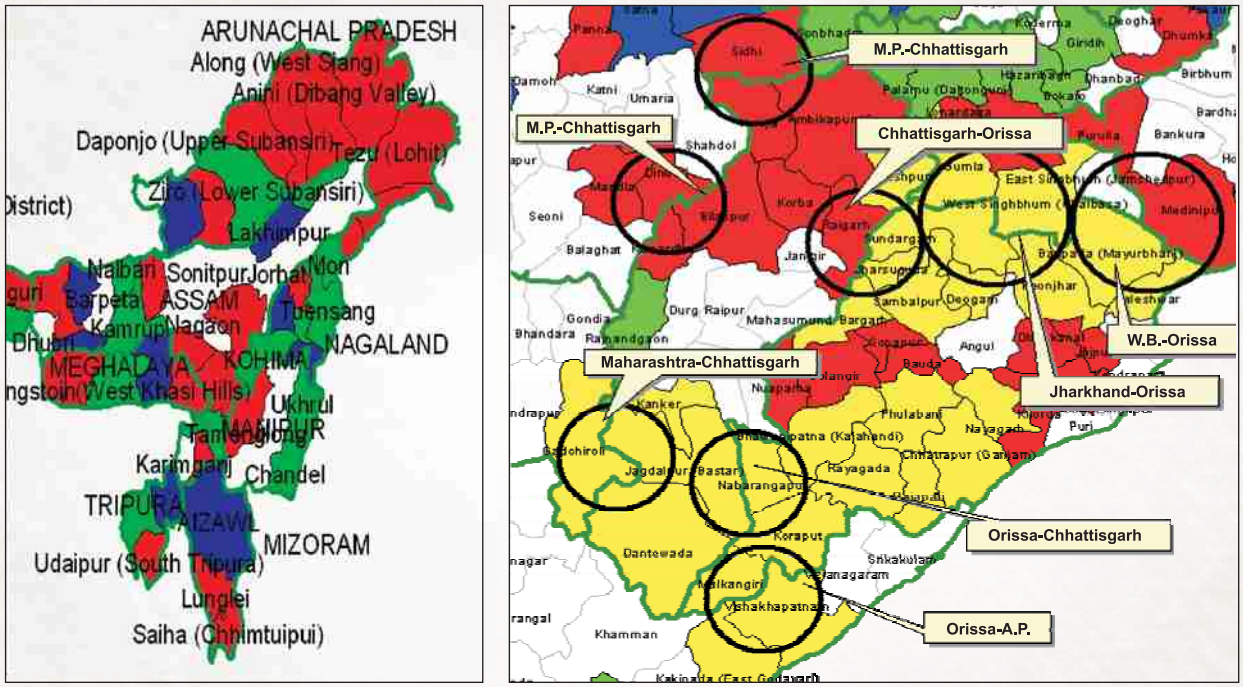


Fig. 8: **GIS-based maps for malaria incidence classification**

2002–05 has been done. The data on various entomological and parasitological parameters were being collected through periodic surveys regularly and are put in GIS-based frame work to view the impact of the construction of dams in space and time.

After completing each survey, meetings were held with the Vice-Chairman, NVDA and state authorities and survey highlights and actions required for developing mitigating measures—engineering, epidemiological and entomological to control the vector borne diseases were suggested and a review was also taken for the implementation of the previous recommendations.

3.1.7 Identification of epidemiological risk factors of malaria for development of strategic action plan for malaria control in problematic districts in Karnataka

In connection with our collaborative project on "Identification of epidemiological risk factors of malaria for development of strategic action plan for malaria control in problematic districts in Karnataka", a field visit was undertaken in Upper Krishna Project area of Raichur, Gulbarga, Bijapur and Bhagalkot districts during November (peak transmission month) 2006. Based on villagewise data of past three years, villages from highest and lowest malaria endemicity were selected for detailed survey. Altogether, 27 villages were surveyed for types of breeding habitats prevalent, man hour density (MHD) of adult vectors and fever cases. Malariogenic conditions around each village were also mapped.

Of the 25 villages surveyed under high malaria endemicity category, almost all had conducive malariogenic conditions. The problems were exacerbated by the vicinity of irrigation channel or seepage and the borrow pits in the vicinity of households and scattered households with poor economic conditions. The scattered breeding habitats and the hutments over vast area in jungle made the surveillance and intervention task bit difficult. The MHD of *An. culicifacies* was up to a maximum of seven even in the month of November. The villages, which were away from irrigation canal/ seepage water, etc. and were established enough were found to be of low endemicity.

Field visit was also undertaken in the month of April (low peak) 2007 in selected 10 villages. Highest MHD was 0.4 as compared to 7 in the month of November 2006. Data were also generated on socioeconomic attributes of 10 villages through questionnaires (293). Ground truth data in selected PHCs of Chitradurga and Tumkur districts were also collected. Satellite data of IRSP6 LISS IV for November 2006 were analysed and false colour composite (FCC) images at village-level were generated. The study is in progress to cover vast areas along the reservoir from Narayanpur to Almatti.

3.1.8 Identification of malaria risk factors in different ecosystems of Assam using remote sensing

Studies were initiated in Defence Research Laboratory, Tezpur sponsored project in Assam to identify ecological and environmental risk factors of malaria using satellite remote sensing at village-level for early warning of malaria outbreaks.

Most districts of the northeastern states are malaria endemic and many pockets are vulnerable to focal outbreaks. Epidemiological data of malaria for all the districts of Assam were procured from Govt. of Assam. Based on the incidence of malaria, Kamrup and Sonitpur districts were selected for detailed investigation. One PHC with highest malaria and the other with least malaria were selected from each district. Three to five villages from each category of PHCs were selected for detailed entomological, parasitological and ecological data generation.

Monthly meteorological data containing rainfall, temperature and relative humidity were collected from Indian Meteorological Department, Guwahati (Assam) in respect of Tezpur, Kamrup, Dhubri, Lakhimpur and Mohanbari (Dibrugarh) for the years 2003–05. The transmission windows of malaria were identified based on seasonal occurrence of cases and minimum temperature and RH required for ensuing transmission of *P. falciparum* malaria.

Field visits to the selected areas were made in November 2006. Data on entomological, parasitological and ecological aspects were generated.

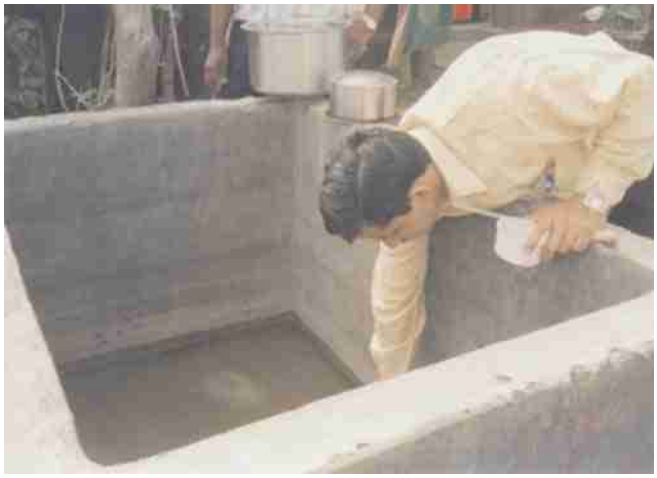
The findings are as follows: (i) Rivers, ponds, drains, ditches/pits and canals were the main breeding habitats in the area surveyed. Maximum larval density was found in ponds while minimum in drains; (ii) Results of adult mosquito collection revealed that seven species of anophelines were collected from Kamrup and Sonitpur districts. In Kamrup district, the highest MHD of *An. minimus*, the major vector of malaria in northeast was nine in high risk village. In Sonitpur district also, the highest density (5) of *An. minimus* was found in high risk village; (iii) In Sonapur PHC (high risk area under Kamrup district), malaria positivity was found in all the four villages surveyed and SPR ranged from 15.3 to 42.1 with overall SPR as 34.87%. *P. falciparum* percentage was 64.7. In Upperhali PHC (low risk area under Kamrup district), of the two villages surveyed, malaria case was detected in only one and SPR was 4.2%; (iv) In Northjamaguri PHC (high risk area of Sonitpur district), of seven villages surveyed, malaria cases were found in four villages. SPR ranged from 20 to 100 with overall SPR as 52%. *Pf* percentage was 84.61. In Behguri PHC (low risk area), of the two villages surveyed, malaria cases were found in only one and SPR was 30.3%; (v) In Kamrup district, the highest peak of *P. falciparum* reaches in June/July while the lowest peak is in December to February. During January the temperature remains < 18°C. Based on minimum requited temperature (T) and RH, the transmission window (TW) is supposed to remain open for 11 months. In Sonitpur district, the incidence is lower than Kamrup district and the fluctuation of cases is similar to Kamrup district and transmission windows (TWs) are open for 10–11 months; and (vi) Retrospective analysis of *P. falciparum* and meteorological parameters to find out the suitable indicators responsible for malaria outbreaks is underway. Analysis of IRS 1D LISS III data did not reveal much landscape features at village-level to identify the ecological factors responsible for malaria endemicity. The work is in progress.

3.2 Health Impact Assessment

3.2.1 Health impact assessment of Indira Sagar Dam and resettlement and rehabilitation colonies in SSP Reservoir impoundment areas in Narmada Valley in Madhya Pradesh

During 2004–06, nine surveys (4 in pre-monsoon, 3 in post-monsoon and 2 in monsoon season) were carried out in seven districts—Khandwa, Badwani, Khargaon, Devash, Dhar, Harda and Jhabua of Indira Sagar, Omkeshwar and Sardar Sarovar Project areas. Mosquitogenic conditions created due to dam construction—seepage of the reservoir, pits and pools of down streams, new canals, pools created, curing tanks, etc. have been identified. Surrounding to these, a total of 33 villages, 18 rehabilitation and resettlement centres and 3 command area villages under seven districts

Mosquito larval collection at Dam site



Larval collection in storage tanks

have been surveyed for entomological and epidemiological data for all the vector borne diseases—malaria, dengue, JE and filariasis.

It is worth to mention that before the construction of dam, no malaria cases were recorded from these surveyed villages. In 2006 till September, 137 cases of chikungunya and 112 cases of typhoid were also recorded by NHDC Hospital, Narmada Nagar, Khandwa. Samples were also collected for dengue and JE but none was found positive.

Man hour density/per room density of malaria vectors *An. culicifacies* and *An. stephensi*, filaria vector *Cx. quinquefasciatus*, JE vector *Cx. vishnui* and dengue vector *Ae. aegypti* were calculated in all the three seasons. Impact of dam construction was observed in nine villages as the vector density was reported high in all the three seasons. To establish the transmission, other entomological parameters—biting habit, parity rate, gonotropic cycle, sporozoite rate, human blood index and presence of sibling species were also carried out. Breeding sites created due to dam construction were surveyed for larval breeding and species-specific breeding sites were identified for all the disease vectors. The susceptibility test for *An. culicifacies* was also carried out in all the three seasons and *An. culicifacies* was found resistant to DDT and susceptible to synthetic pyrethroids.

3.3 Epidemiology of Urban Malaria

3.3.1 Studies on the epidemiology of urban malaria in mega, medium and small cities of India

Delhi

A total of 22 localities were selected to carry out the entomological and epidemiological studies in consultation with officials of the Municipal Corporation of Delhi (Health Department). These 22 localities represent high, middle and poor income groups and also the commercial, recreation, industrial and transport areas. From November 2005 to June 2006, a total of 704 active slides were collected from these 22 localities. Out of these 704 slides, 20 were found positive for *P. vivax* and none was found positive for *P. falciparum*. Maximum number of cases were recorded from Okhla (Industrial area) and Trilokpuri (residential low income group area).

The morbidity and mortality data of malaria were also collected from about 25 agencies such as Government/private hospitals; private practitioners, clinics and nursing homes; cremation ground and diagnostic centres—malaria clinics, private and pathological laboratories.

Adult mosquito collection

Weekly hand catch collections were carried out in all the 22 localities from November 2005 to June 2006. Eight species of anopheline namely

Active fever surveillance in study population

Adult mosquito collection in rooms

An. stephensi, An. culicifacies, An. subpictus, An. annularis, An. aconitus, An. pulcherrimus, An. nigerrimus and *An. vagus* were collected from indoor resting places such as human dwellings, cattlesheds and mixed dwellings. Of these eight species, *An. stephensi* and *An. culicifacies* are well-known vectors of malaria. *An. culicifacies* was collected from Yamnua River belt—Sonia Vihar and peripheral areas of the city like Najafgarh and Mahipalpur. *An. stephensi* was collected from all the localities surveyed. Besides the anophelines, *Culex*, *Aedes, Mansonia* and *Armigeres* species were also collected. *Cx. quinquefasciatus* was the predominant species among all the mosquitoes collected during night as well as day-time collections. *Ae. aegypti* were also collected from the human dwellings. The total catch collection was also carried out in all the localities on monthly basis.

Identification of mosquito species

In bait collections (night collection), two *An. stephensi* were collected from Rohini area and three from Okhla industrial area. Three *An. culicifacies* were collected from Sonia Vihar. The biting time of both the species was recorded between 2200 and 2400 hrs.

Mosquito larval collection

Larval collection was carried out in all the 22 localities from 12 different breeding sites like overhead tanks, ground tanks, domestic containers, coolers, drains, ponds, pits, dump tyres, septic tanks, mud pots, fountain pools, riverbed pools, store drums, etc. The breeding of malaria vector species— *An. stephensi* was recorded from OHTs, ground tanks, domestic containers, fountains, pits and stone pools whereas the breeding of *An. culicifacies* was recorded from riverbed pools and pits only. The breeding of *Ae. aegypti* was recorded from drums, tyres, coolers, mud pots exclusively while in OHTs, ground tanks, domestic containers the breeding was also recorded in association with *An. stephensi*. Breeding of *Cx. quinquefasciatus* was recorded from almost all the breeding sites. The breeding of *An. stephensi* and *Ae. aegypti* in OHTs was recorded from November 2005 to May 2006 and maximum breeding was recorded during the months of November 2005 and March 2006, whereas ground tanks supported the breeding of both the species from November 2005 to June 2006 and the maximum breeding was recorded in the months of June and November. Maximum breeding of *An. culicifacies* was recorded from riverbed pools in the month of February and June. Some of the important breeding sites supporting the breeding of vector species are given below.

A detailed GPS survey has been carried out in east Delhi for the purpose of geo-referencing. Eighty-eight GPS waypoints located in different parts of eastern Delhi, mainly road intersections, have been randomly selected as registration point. Map has been digitised in ArcGIS 8.1 and the selected locations have been identified in the map for registration. To register map existing latitude and longitude has replaced in real world coordinates. A base map showing locations of different colonies

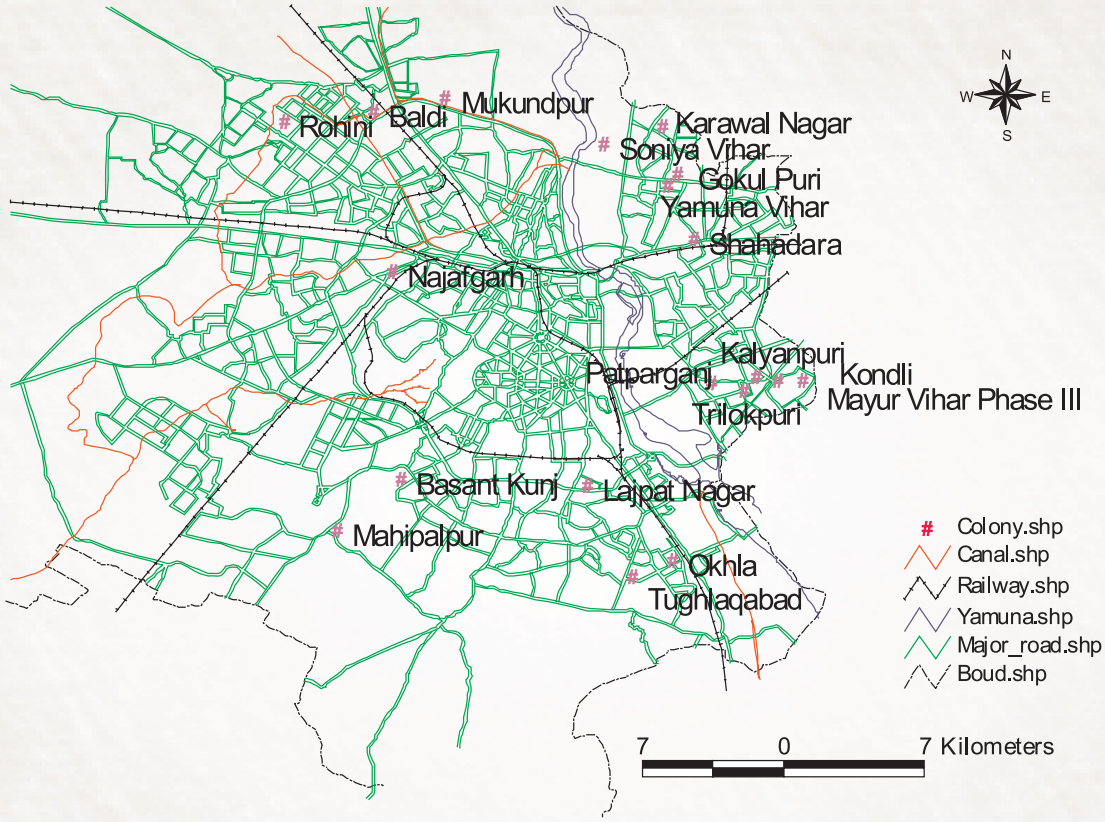


Fig. 9: **Map showing different colonies of Delhi under survey (GIS analysis)**

of Delhi under survey has been prepared for GIS analysis (Fig. 9).

Ajmer (Rajasthan)

Epidemiological survey

A total of 825 blood slides were collected in surveys carried out during November 2005, January and June 2006 from inhabitants of 20 localities representing high, middle and poor income groups and also from inhabitants residing in commercial, recreation, industrial and transport areas. Out of these, seven slides were found positive for *P. vivax* and one for *P. falciparum*.

Entomological survey

Adult collection

Hand catch collections were carried out in all the 20 localities during November 2005, January and June 2006. A total of five species of anophelines—*An. stephensi*, *An. culicifacies*, *An. subpictus*, *An. annularis* and *An. pulcherrimus* were collected from indoor resting places (human dwellings, cattlesheds

Mosquito collection on cattle bait

Domestic mosquito breeding habitats

and mixed dwellings). Out of these five species, *An. stephensi* was the most dominant species and collected in all the three surveys carried out, whereas *An. culicifacies* was collected in the months of November 2005 and June 2006 only. *An. stephensi* was collected from all the 20 localities surveyed, whereas *An. culicifacies* was collected from nine localities.

Larval collection

Larval collection was carried out in all the 20 localities from 15 different breeding sites like OHTs (cement and sintex), ground tanks, drains, drain pits, coolers, matkas, side pits, tankas, mud pots, fountains in marriage homes, fountain pools, tanks at ice cream factory, drum wells, etc. *An. stephensi* was found breeding in OHTs, ground tanks, matkas and fountains, whereas *An. culicifacies* was found breeding in fountain pools and tankas. *Ae. aegypti* was found exclusively in coolers, mud pots and drums, but in association with *An. stephensi* in OHTs, ground tanks and in matkas. The maximum breeding of vector species was recorded in ground tanks 63% during November 2005, 27.5% during January and 56.4% during June 2006 followed by OHTs. It is noteworthy to mention that *Ae. aegypti* has not been recorded for more than one decade.

Larval collection in water storage tanks

Vishakhapatnam (Andhra Pradesh)

Vishakhapatnam district is one of the northeastern districts of Andhra Pradesh situated within the geographic coordinates of 17° north latitude and 83° east longitude. The district is bounded by

Active surveillance in study villages

Domestic mosquito breeding habitats

Vizianagaram district on the north, East Godavari district in the south, Koraput of Orissa in the west and Bay of Bengal in the east. The total population of the district is around 3.28 million. Rural population of the district is approximately 60%. The scheduled castes and scheduled tribes population in the district is 7.8 and 14.3%, respectively. The field work in Vishakhapatnam has been initiated recently and analysis of data is in progress.

3.4 Clinical Trials

Assessment of therapeutic efficacy of antimalarial drugs against uncomplicated *P. falciparum* malaria

Antimalarial drug resistance is a major obstacle in the fight against malaria. A systematic surveillance system with periodic updating is essential for containment of drug resistance. The treatment policies can be updated only if the information on extent of the problem is known from various parts of the country. Since India is a vast country with several eco-epidemiological subtypes of malaria, it is necessary to evaluate efficacy of first and second line antimalarial drugs at several sites so as to devise alternative strategies for treatment as per need. India is also contemplating a partial change in its drug policy including a consideration for use of combination therapy in drug resistance areas. Therefore, while the studies on the occurrence of resistance to first line drugs should be continued, there is a need to evaluate efficacy of these proposed new regimens. Thus, in view of urgent need to update and rationalise treatment policies, which can be done only after updating the information on drug efficacy and safety with existing

drugs used in the country, these studies were initiated with the following objectives: (a) assessment of therapeutic efficacy of chloroquine (CQ) or sulphadoxine-pyrimethamine (SP) or combination therapy in uncomplicated *P. falciparum* malaria in endemic districts of Orissa, Jharkhand and Indo-Bhutan border districts in Assam; and (b) to validate the *in vivo* drug resistance data using molecular markers and therapeutic concentration of drugs.

Assam

District Udalguri was selected as a study site. Fifty-three patients were enrolled, out of which 51 completed the study. Efficacy of combination therapy (AS+SP) was evaluated. The regimen was found to be very effective with high cure rates, adequate clinical and parasitological response (ACPR 94.1%), rapid parasite clearance time and was well tolerated.

Orissa

The study has been completed in Keonjhar district. ACT (AS+SP) was found to be highly effective at this site also with cure rates of 98.5%. The results were similar to those observed in Assam. The town was already under the second line drug SP treatment. However, in adjoining PHC, Banspal, where chloroquine was the first line drug, the cure rates with CQ were only 21%.

Jharkhand

Tathaitangar PHC, Jaldega sub-centre in District Simdega and Angara PHC in District Ranchi were selected for the study. Orientation workshop was held at District Simdega on 7 July 2006 just before initiation of the study. Therapeutic efficacy of chloroquine was studied and results are discussed.

The studies were conduced according to WHO protocol for therapeutic efficacy. The procedural steps in brief are as follows: (i) Enrolment of subjects using pre-determined criteria by active/ passive surveillance; (ii) Collection of peripheral smears and samples for molecular biology; (iii) Follow-up for 28 days for clinical and parasitological cure; (iv) Rescue medication for treatment failures;

Investigations meeting with state health officials

(v) Survival analysis of data using specifically designed software; and (vi) Genotyping of blood spots on Day 0 and Day of recrudescence by MSP-1, MSP-2 and GLURP—Nested PCR with family-specific assay.

District Simdega

Fifty-one cases completed the follow-up and although total failure rates with CQ (25 mg/kg body weight over 3 days) were 34.2%, the early failures were low (7.8%) (Tables 1 a & b). Since the failure was much above the cut-off level for change of policy, the drug policy has been revised by the national programme.

District Ranchi

In Angara PHC, 68 patients were enrolled out of which 63 completed the study. High failure rates were observed with CQ (25 mg/kg over 3 days) at this site also, leading to change in drug policy (Tables 2 a & b).

TABLE 1a

Baseline characteristics of patients in Tathaitangar PHC, District Simdega

Drug: CQ		Dose: 25 mg/kg × 3 days
No. of cases enrolled	:	51
Male/Female	:	(19/32)
Age (Range)	:	0.5 months–40 yr
Parasitaemia/µl on D0 (Range)	:	1000–99, 440

TABLE 1b

Therapeutic response to chloroquine

Classification	No. of patients	Prevalence
ETF	4	0.078
LCF	10	0.196
LPF	4	0.078
ACPR	33	0.647
Total analysis	51	
With	0	
Loss	0	0
Total	51	

TABLE 2a

Baseline characteristics of patients in Angara PHC, District Ranchi

Drug: CQ		Dose: 25 mg/kg × 3 days
No. of cases enrolled	:	68
Male/Female	:	(37/31)
Age (Range)	:	2.5–50 yr
Parasitaemia/µl on D0 (Range)	:	1000–82,400

TABLE 2b

Therapeutic response

Classification	No. of patients	Prevalence
ETF	2	0.032
LCF	12	0.190
LPF	4	0.063
ACPR	45	0.714
Total analysis	63	
With	1	
Loss	4	0.074
Total	68	

3.4.2 A Phase II, double-blind, parallel-group, randomised, dose-ranging study assessing the antimalarial activity and safety of RBx 11160 administered for 7 days in patients with acute uncomplicated *P. falciparum* malaria

RBx 11160 (Arterolane) a new peroxide, is a synthetic trioxolane that is easy to synthesise, inexpensive, achiral and orally rapidly acting with high antimalarial activity. It is a potential new antimalarial agent with demonstrable activity in pre clinical models and a substantial safety margin between an effective dose for malaria and the toxic dose. Mechanism of action of the drug: reductive activation by haeme, released as a result of haemoglobin digestion; irreversible redox reaction produces carbon-centred free radicals, leading to alkylation of haeme and proteins (enzymes) and one enzyme is the sarcoplasmic endoplasmic reticulum ATPase PfATP6.

Studies carried out indicate that RBx 11160 is safe and does not produce any clinically significant effect on behavioural parameters and cardiovascular systems. The present study was designed to assess the clinical safety and efficacy of three dose levels of RBx 11160 (50, 100 or 200 mg), administered for 7 days in patients with acute uncomplicated *P. falciparum* malaria. The primary objective was to compare three (50, 100 and 200 mg) RBx 11160 dose levels administered orally for seven consecutive days on time to 90% parasite clearance (PC90) in patients with acute uncomplicated *P. falciparum* malaria and to identify the most appropriate dose of RBx 11160 for further investigation. The trial was conducted according to good clinical practices (GCP) guidelines.

Male or female patients aged 13 to 65 years, with no clinical evidence of severe malnutrition and presence of acute uncomplicated falciparum malaria with asexual parasitaemia between 1000 and 100,000 asexual parasites/µl blood were included. Patients with mixed infection, severe malaria, antimalarial treatment during two weeks prior to screening, history of hypersensitivity or allergic reactions to artemisinin, electrocardiogram

Monitoring and auditing according to GCP-ICH guidelines at IGH Hospital, Rourkela

(ECG) abnormalities with clinical significance, lactating or pregnant woman, evidence of other clinically significant diseases were not included.

The multicentric study has been completed on 80 patients in Rourkela field unit (Table 3) in collaboration with Ispat General Hospital, Orissa. All the three doses resulted in parasite clearance in all the patients by Day 7 and drug was well tolerated. The mean parasite clearance time for all dose ranges was 37 h. About 94% (74/79) patients cleared parasites by 72 h. High recrudescence was observed during 28 day follow-up. Further studies are planned in combination with long acting antimalarial. RBx 11160 (Arterolane) is an effective synthetic alternative to Artemisinin and further studies in combination with long acting partner drug are important. The study was conducted in two parts as shown below.

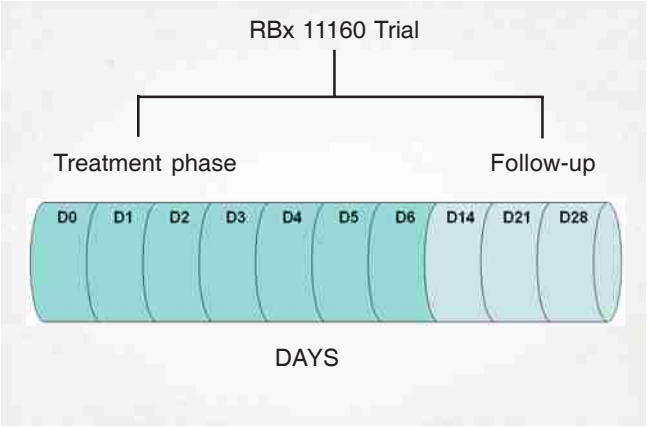


TABLE 3

Final global enrolment status

Study centre	No. of patients enrolled
Bangkok, Thailand	90
Rourkela, India	80
Bagamoyo, Tanzania	43
Kivunge, Zanzibar	17
Total	230

Drug storage

3.4.3 A Phase III, randomised, non-inferiority trial, to assess the efficacy and safety of Dihydroartemisinin + Piperaquine (DHA + PPQ, Artekin) in comparison with Artesunate + Mefloquine (AS + MQ) in patients affected by acute, uncomplicated *P. falciparum* malaria

Artekin™ was developed in China and is registered in China and Cambodia. It has been evaluated

extensively in clinical trials in Thailand, Vietnam, Cambodia and China. Efficacy has been high and tolerability was uniformly excellent in all the trials in these multidrug-resistant areas. Artekin™ is a second generation Artemisinin-based combination therapy (ACT) with similar efficacy to that of Coartem (Artemether + Lumefantrine) or Artesunate + Mefloquine but with a simpler dosing scheme that will aid better compliance. Moreover, its good safety profile and affordable cost make it ideal for resource constrained countries. Most of the clinical trials evaluating Artekin™ have been conducted without the sponsorship of a pharmaceutical company. In addition, the formulation of DHA+PPQ used in some trials through compliant with Chinese GMPs, was not compliant with the GMP standards laid by the European or US Health Authorities. For the later reasons Sigma-Tau has used the information/data generated from previous trials to support the design of a new phase III development strategy using Artekin product according to GMP and European regulatory standards as Euartekin. DHA – PPQ is highly effective and tolerated by all the age groups. DHA is active metabolite of Artemisinin. Piperaquine related to CQ and has similar mechanism of action and is long acting. The dose regimen includes 3 day therapy (2.1 mg/kg DHA & 16.8 mg/kg piperaquine). Reported side-effects are vomiting, dizziness and nausea. The primary objective of the study was to measure the Day 63, PCR corrected cure rates of Artekin and AS+MQ and demonstrate that the cure rate of Artekin is non-inferior to that of AS+MQ (non-inferiority margin = 5%).

The study was conducted at three sites—Goa, in collaboration with Goa Medical College & Hospital; Mangalore, in collaboration with Wenlock District Hospital, Mangalore; and Guwahati, in collaboration with Down Town Hospital. Investigator meetings were held before starting the study**.** This was a phase III, randomised, open label, two arms study to include 1050 patients (700 DHA+PPQ; 350 AS+MQ) at all the sites in India and other countries. In order to ensure concealment of treatment allocation and avoid other biases the randomisation was under blind conditions and treatment allocation was concealed until the final recruitment of the

Investigators meeting at Goa

Rajiv Gandhi Memorial Medical College and Hospital, Goa

patients. The primary endpoint was the PCR-corrected ACPR at D63. Patients classified as failures by clinical and parasitological criteria were considered ACPR if the PCR analysis showed a new infection rather than a recrudescence.

Males and females aged >18 years having microscopically confirmed, monoinfection of *P. falciparum* (asexual forms parasitaemia >1000/µl <100,000/µl or mixed infection), history of fever or presence of fever (temperature at >37.5°C) were included. Known hypersensitivity to the study drugs, severe malaria, presence of intercurrent illness, pregnant or lactating women were excluded. A total of 154 cases of *P. falciparum* were enrolled at all the sites

TABLE 4

Enrolment status at study sites in India

Characteristics	Guwahati	Mangalore	Goa
Number enrolled	69	57	28
Screen failures	1	3	0
No. finally enrolled	68	54	28
No. of patients completed the study	65	30	27
No. of SAE	0	0	0
Parasite clearance time	1 to 3 days	1 to 3 days	1 to 3 days
Adverse reaction	3	3	Nil
Recrudescence	Nil	3	Nil

(Table 4). Artekin is an effective and well-tolerated combination regimen.

3.4.4 Multicentre, open-label, randomised clinical trial of efficacy and tolerability of the fixed-dose artesunate/amodiaquine (AS/AQ) combination therapy and amodiaquine (AQ) monotherapy for treatment of uncom plicated falciparum malaria in India

At present, only one ACT (Artemether Lumefantrine–Coartem®) is available as a fixed dose drug in which both compounds are co-administered. Co-packaging of the combination partner drugs in a blister pack is highly recommended in order to make them user-friendly, and to increase adherence to the complete therapy. DND*i* in association with UNICEF/UNDP/World Bank/WHO (TDR) is developing a new fixed-dose combination of Artesunate and Amodiaquine that will allow a simple treatment of just three days, with a single daily administration of two tablets.

Since WHO has decreed that artemisinin-based combinations to be used in malaria endemic countries, the testing of AS/AQ is relevant for informing drug policy makers. Comparing AS/AQ with chloroquine, the current first line drug, would not be an optimal choice because chloroquine will have to be replaced in future. The present study with AS/AQ and AQ will allow gathering of data on amodiaquine for the first time in India.

The primary objective of the study was to measure

Community Welfare Society Hospital, Rourkela

Investigators meeting at Ranchi

the clinical and parasitological efficacy of the fixed-dose Artesunate/Amodiaquine combination therapy among children and adult patients suffering from uncomplicated falciparum malaria, by determining the proportion of patients having negative peripheral smear for malaria without relapse before 28 days (cure rate). The study has been initiated at Ranchi and Rourkela in collaboration with Community Welfare Society Hospital, Rourkela. Investigator meeting was held at Ranchi.

Inclusion criteria were: (i) children and adults from 6 months to 60 years of age; both gender; for children (aged 6 months), body weight 5 kg; (ii) presenting with uncomplicated falciparum malaria; (iii) axillary temperature >37.5°C (99.5°F); (iv) positive *P. falciparum* parasitaemia (1000–100,000 asexual parasites/µl); and (v) written informed consent (participant or parent/guardian). The exclusion criteria were: (i) any other concomitant condition that could explain the fever episode (upper respiratory tract infection or ENT infection for example); (ii) any concomitant infection by *P. vivax*; (iii) any chronic ailment; (iv) pregnancy; (v) Hb < 7 g/dl; (vi) ALT/AST > 2.5 ULN; and (vii) S.

TABLE 5
Enrolment status at study sites in India

Parameters	Ranchi		Rourkela	
	A (AS+AQ)	B (AQ)	A (AS+AQ)	B (AQ)
No. of patients enrolled	10	4	14	8
No. of patients finally enrolled	10	4	14	8
No. of patients completed the study	6	4	9	4
No. of SAE	0	0	1	0
Parasite clearance time	1–2 days	1–8 days*	1–3 days	1–2 days
Adverse reaction	0	0	0	0
Recrudescence	0	0	0	1

* One patient

creatinine >1.2 ULN. Thirty-six cases of *P. falciparum* were enrolled till date at both the sites (Table 5).

3.5 Monitoring and Evaluation of Disease Control Programme

3.5.1 Monitoring and supervision of spray operation in kala-azar affected Districts of Bihar state

In Bihar state, two visits in three districts namely Madhuvani, Darbhanga and Arwal from 19 to 28 May and from 18 to 26 June 2006 and two visits in District Madhepura from 21 to 28 February and from 17 to 22 March 2007 were made to supervise the DDT (50%) spray operation activities. In the above three districts, spray operations were ongoing in two PHCs in each district and four villages in each PHC. In district Madhuvani, the spray was started from 8 May 2006, in Darbhanga from 24 May 2006, but no spray was started in District Arwal during May 2006. The spray operations were affected due to non-payment of wages to spray teams. The spray reports revealed that in the above two districts advance planning of spray operations was made but no advance information regarding spray operations to the villagers was given. Recruitment of spray workers and preparations for spray suspension was found satisfactory. Out of 35 stirrup pumps checked, 4 pumps were found defective. Nozzle discharge rate was found satisfactory in rest of the pumps. Training/knowledge of spray workers was not satisfactory. Refusal varied from 5 to 15% due to untimely instructions to the villagers. Spray coverage in houses ranged from 45 to 85% and in rooms it varied from 65 to 92%. It was suggested that DMO should organise meetings with villagers to develop awareness and willingness towards spray operations. IEC programme should be made effective to check refusal rate and the staff deputed to check and supervise spray operations should visit the village for full coverage of spray.

During 2007, two visits in District Madhepura were made to supervise spray operations in all the PHCs. Two villages in each PHC were checked and the proforma of checklist for monitoring and supervision of IRS activities for kala-azar control was duly filled up. The observations recorded were that funds were not released for payment of wages to the spray workers at district level, refusals in Musahar and high communities were recorded, percent coverage of spray in the houses varied from 76.2 to 95.5% and in rooms it ranged from 74.3 to 91.9% which revealed high refusal rate by the community. It was suggested that bed rooms should not be spared for DDT spray at any cost, Medical Officer/Incharge of the concerned PHC should invariably check spray squad, monitoring and supervisory team framed by the district officials should check the spray activities in the villages to achieve cent percent coverage.

3.5.2 Assessment of malaria treatment practices in public and private health sectors

The early diagnosis and prompt treatment of cases of malaria is a key component of the global malaria

control strategy and the National Vector Borne Disease Control Programme in India executes this through the primary health care system. A large proportion of suspected cases of malaria are being treated outside the public health system. Antimalarial drugs are available over the counters and they are used injudiciously and sometimes in inappropriate doses. Exact information on the malaria treatment practices in private sector is lacking. The knowledge about the compliance with prescribed drug policy at different levels across the public health facilities is also not available. The study was planned at Delhi, Gujarat and Orissa to ascertain the allopathic treatment practices of malaria through a questionnaire survey among public and private health sectors at various levels of health system in the urban and rural areas.

The objectives of the study were: (i) to evaluate allopathic treatment practices for malaria in public and private health sectors; (ii) to assess and compare the awareness for National Drug Policy in public (teaching & non-teaching sectors) versus private sectors; and (iii) to assess the proportion of clinicians using rational treatment for the disease.

Questionnaire for the study has been designed. Meetings with social scientists and statisticians were held for finalising the questionnaire, and for estimation of sample size. A pilot study was carried

TABLE 6

General information and response of training

	Type of practice (%)	
	Government (n = 115)	Private (n = 70)
Awareness about malaria training course for doctors		
Yes	64	46.4
No	36	53.6
Report of malaria cases to concerned authorities		
Yes	43.2	27.9
No	56.8	72.1

TABLE 7

Diagnosis of malaria patients

	Type of practice (%)	
	Government (n = 115)	Private (n = 70)
Availability of diagnostic facility		
Yes	93	79.7
No	7.1	20.3
Treatment according to report of peripheral smear		
Yes	70.2	75.0
No	29.8	25.0
Awareness of RDT		
Yes	67.5	61.8

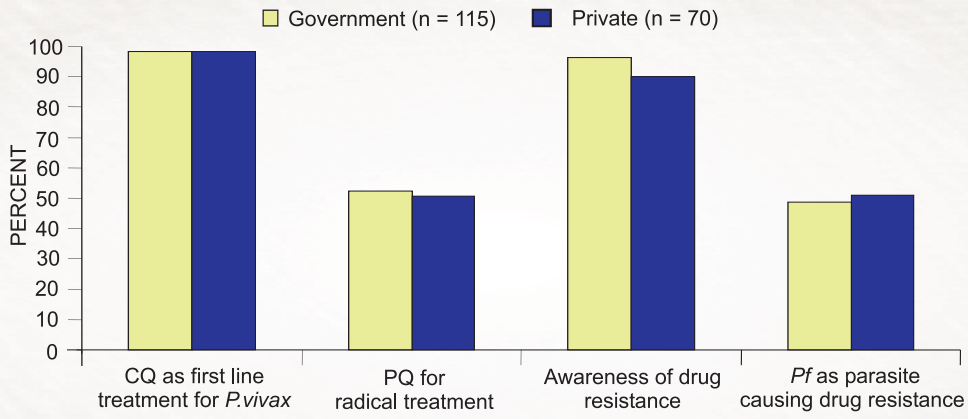


Fig. 10: **Awareness of treatment and drug assistance**

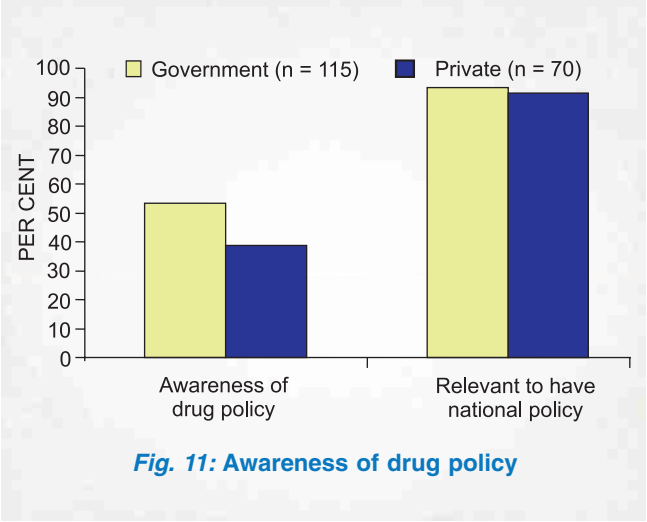


Fig. 11: **Awareness of drug policy**

out on 100 practitioners and based on the findings a sample size of 500 practitioners from each study site has been calculated. Questionnaires were completed from a total of six hundred fifty respondents. Of these, 200 were from Delhi, 400 from Gujarat and 50 were from Orissa.

The results of analysis available from 200 respondents of Delhi are as follows: 64% of the public health sector is aware about the malaria course conducted for training doctors (Table 6). Only 43% of the public health sector and 27% of private sector doctors report malaria cases to the concerned authorities (Table 6). A total of 93% of the public sector reported about the availability of diagnostic facility but only 70% treat according to the report of peripheral smear (Table 7). About 68 and 62% respectively from public and private health sectors are aware of the rapid diagnostic tests (Table 7). Except for three respondents, all the others reported chloroquine as the first line treatment for *P. vivax* (Fig.10). Use of primaquine for radical treatment was reported only by 50% from public and private health sectors (Fig.10). About 94% of the respondents are aware about the drug resistance but only 50% reported *Pf* as the parasite causing drug resistance (Fig.10). Awareness about drug policy for malaria was reported only by 53 and 39% respectively from public and private health sectors though 93 and 91% respectively felt that it is relevant to have national drug policy (Fig.11).

3.5.3 Studies on drug use practice and pre-packaged blister pack drugs

Blister packs for the radical treatment of adult patients (15 yr and above) who constitute nearly 60% of all the cases of malaria have been introduced for the first time in the national programme to improve acceptance of antimalarial drugs and compliance of the full course of radical treatment. The advantage of these blister packs is that less number of tablets has to be consumed for each dose. However, exact information on malaria treatment practices in public sector and compliance with prescribed drug policy at different levels across the public health facilities/sector is lacking in Jharkhand state. It is proposed to evaluate drug use practice and use of pre-packaged blister pack drugs through a combined review and observational study at various levels of health infrastructure.

The objectives of the study were: (i) to evaluate drug use practices at various levels of health infrastructure with emphasis on districts with change in drug policy in Jharkhand state; (ii) to study the knowledge and skills of paramedical personnel in the use of blister packs (including ACT) and its acceptance by paramedical personnel and in the community; and (iii) to study the compliance for blister pack, its impact on malaria epidemiology and the serious adverse events if any with the usage of blister packs.

The study is a questionnaire-based survey. The survey will involve primary health centres with first and second line of treatment, district health facilities and registered medical practitioners. The study will be done in two districts of Jharkhand state which are highly endemic for malaria. The primary health centres with first and second line of treatment and district health facilities will be selected from the study sites. The questionnaires will be completed by personal interaction with DMOs, SPOs, MO PHCs and other clinicians. Special emphasis will be given to knowledge of drug policy, drug resistance and usage of blister packs including ACT.

A workshop was held at NIMR involving experts from Jharkhand and Delhi states before initiation of the study. Field visit was undertaken to initiate

the study. Discussions were held with the District Malaria Officer, Simdega, Jharkhand for the proposed work. Data was collected on number of PHCs in Simdega district, supply of blister pack drugs, etc. The pilot study has been initiated in the Simdega district.

3.5.4 Institutional assessment of the NVBDCP Directorate and the assessment of the capacity building of the states

Successful implementation of the National Vector Borne Disease Control Programme requires enhanced capacities and capabilities both at the central level as well as in disease endemic states. Assessment is required of the current capacity of the Directorate of NVBDCP and its counterparts in the states and districts for improving programme performance to reduce the burden of malaria and other vector borne diseases.

An assessment of the functioning of 17 selected states including two municipal corporations was undertaken by the National Institute of Malaria Research (NIMR) during the period from November 2005 to November 2006. Of these, the states and municipal corporations assessed during September to November 2006 are Gujarat, Andhra Pradesh, Karnataka, Bihar, West Bengal, Rajasthan and the municipal corporations of Lucknow and Navi Mumbai. Institutional assessment of the Directorate of NVBDCP and four states namely Karnataka, Bihar, West Bengal and Rajasthan was assigned to the Indian Institute of Health Management Research (IIHMR), Jaipur.

The institutional assessment included overall functioning of the NVBDCP Directorate and state health organisations, role and functional integration of state and district level societies, review of ability of decentralised health system and capacity of district level organisations to prepare microaction plan and to manage financial resources. Assessment was also made of the linkages of health system with NGOs and private sectors/faith-based organisations, and the requirements for motivation, training and monitoring. Outcome of this exercise

Workshop in progress

is likely to benefit the NVBDCP in improving its overall performance taking advantages of the health sector reforms as well as intersectoral cooperation and community support at various levels.

The investigation was conducted by holding deliberations at national level with the programme planners, senior programme managers, policy makers and NGO executives. In the states, assessment was made by examining available documents, reports, office orders and circulars issued by the ministries, health directorates of the states, NVBDCP Directorate, published research papers, reports and field records of various functionaries at the district and lower levels.

During the assessment it was recognised that the programme had achieved many commendable achievements. There are committed individuals who are waking in the face of formidable field conditions and numerous other practical constraints. The states and its institutions have capacity to

implement the proposed NVBDCP but certain gaps have been identified by critical review during assessment which can be removed by cautious efforts. Study has been completed and the detailed report has been submitted to the NVBDCP.

3.5.5 Monitoring of implementation of programme activities in high risk districts

The Directorate of NVBDCP has identified districts with high burden of malaria in 17 states—Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Chhattisgarh, Goa, Gujarat, Jharkhand, Karnataka, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Mizoram, Orissa, Rajasthan and West Bengal in the country for intensive monitoring for its effective control. Of these, monitoring of 21 districts in six states—Assam, Bihar, Goa, Karnataka, Madhya Pradesh and Orissa has been coordinated at NIMR, Delhi.

For monitoring each district, during each visit, two PHCs were selected and visited every month for a period of six months to evaluate parameters of programme activities. The various parameters evaluated are, study of epidemiological parameters for situational analysis such as trends of malaria incidence and deaths, identification of any high risk population and outbreaks, assessment of early diagnosis and prompt treatment, assessment of functioning of the PHC laboratory and record keeping, assessment of vector control methods undertaken and their impact, staff position and capacity building activities, status and functioning of district malaria control society, intersectoral co-ordination, availability of insecticides and antimalarials and IEC activities undertaken. Reports of the monitoring activities of these high risk visited districts have been prepared and submitted to the NVBDCP.

3.6 Vaccine Trial

3.6.1 Development of a site for malaria vaccine trials

This is a collaborative project with International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi and is being funded by the Department of Biotechnology, Govt. of India under Jai Vigyan Mission for vaccine development. The studies are being carried out to understand the epidemiology of malaria in Sundargarh district, Orissa that will facilitate the field trials for *P. falciparum* malaria vaccines through collection of clinical, entomological and molecular epidemiological/immunological indicators from the study site. The longitudinal and epidemiological studies were conducted in two sets of villages in the forest and plain areas characterised by hyper- and meso-endemic malaria situations, respectively. There are 35 study villages (Forest 23, Plain 12) with a total population of 15,847. These villages are grouped under Gurundia and Birkera primary health centres (PHCs) for the purpose of delivery of health services. Out of 35 study villages, 23 villages with a population of 8099 are situated in deep forests close to perennial streams and had persistent malaria transmission. The remaining 12 villages with a total population of 7748 are located in plain deforested areas with low levels of malaria transmission. All the twelve plain area villages come under Birkera PHC. Almost all (98%) of the residents of these 35 villages are tribals, predominantly of the Munda and Oram ethnic group. The average distance of all the study villages from the nearest PHC is 5–10 km. All the study villages are well-connected by roads and are located at a distance of 25 to 60 km from National Institute of Malaria Research field unit, Rourkela. The following longitudinal and cross-sectional studies were conducted as per protocol from the study area.

Detection of malaria cases was carried out through weekly active surveillance as well as passive surveillance in all the study villages of Phase I. The surveillance was carried out through village volunteers trained for this purpose. Data collected through weekly surveillance were subject to the analysis for malaria transmission pattern in two ecotypes, age distribution of malaria incidence and attack rate due to *P. falciparum*, proportion of *P. falciparum*/*P. vivax*/*P. malariae* and seasonal variations in the above parameters.

A longitudinal data on the above parameters from

the study site since August 2000 is available at NIMR field unit, Rourkela. 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 forest area was 89, 10 and 1 respectively, whereas it was 87.5, 12.5 and nil respectively in plain area. The malaria incidence rate of *P. falciparum* (only first episode per individual per year) during 2006–07 in forest and plain area was 18.7 and 0.5%, respectively. The highest incidence rate of 74% was recorded in the 1–5 yr age group and it was inversely proportional to increasing age, whereas in the plain area, no age-related correlation was found (Fig. 12). The average attack rate due to *P. falciparum* malaria in the total population was found to be 0.2 and 0.005 episodes per person per year in the forest and plain area, respectively. Three cross-sectional surveys were carried out during March, June and November representing intermediate, low and high malaria transmission seasons, respectively. About 40% of the study population was screened for malaria parasites. Blood samples were also collected for parasite genotyping and host immune response. Cross-sectional malaria prevalence data were analysed to obtain information on malaria prevalence in the study population, age distribution of parasite rate, spleen rate, asymptomatic cases, gametocyte carriers and seasonal variations in these parameters.

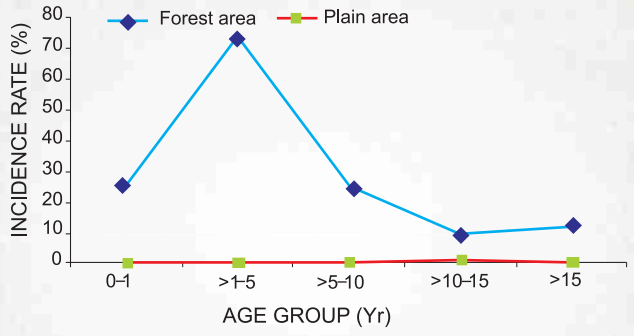


Fig. 12: **Malaria incidence rate in different age groups in forest and plain area of Sundargarh district during 2006–07**

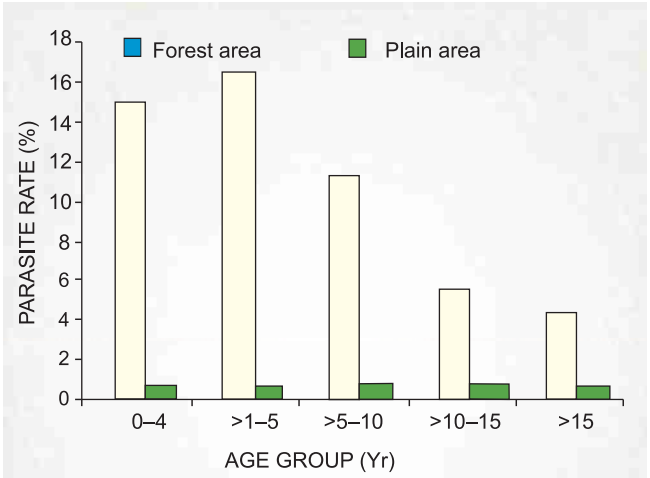


Fig.13: **Parasite rate in different age groups in forest and plain areas in Sundargarh district as recorded through cross-sectional prevalence surveys during 2006**

The average parasite rate in the forest and plain areas was 9.0 and 0.2, respectively. The highest parasite rate in the forest area during these surveys in 0–1 yr age group was found 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. 13). Out of total parasite positive cases found in the forest area during the cross-sectional surveys, 39% of the cases were asymptomatic and 6.4% were found to be gameto-

TABLE 8

Percent composition of anopheline species in the forest and plain areas of Sundargarh district during 2006–07

Species	Forest area		Plain area	
	Total No.	(%)	Total No.	(%)
An. culicifacies	1324	(39.2)	1913	(41.8)
An. fluviatilis	148	(4.4)	0	
An. annularis	317	(9.4)	540	(11.8)
An. subpictus	757	(22.4)	840	(18.3)
An. vagus	505	(15.0)	535	(11.8)
An. pallidus	187	(5.5)	308	(6.7)
An. aconitus	27	(0.8)	344	(7.5)
An. nigerrimus	32	(1.0)	48	(1.0)
An. splendidus	45	(1.3)	15	(0.3)
An. barbirostris	29	(0.9)	28	(0.6)
An. theobaldi	1	(0.03)	0	
An. tessellatus	1	(0.03)	4	(0.09)
An. ramsayi	1	(0.03)	2	(0.04)
Total	3374		4577	

cyte carriers. The average spleen rate in children and adults in the forest area was 52.7 and 12.8%, respectively, whereas in plain area it was 8.1 and 1.5%, respectively.

Entomological surveys were carried out in two indicator villages each in the forest and plain areas. Adult mosquito densities were monitored at monthly intervals by manual catching, using suction tube method. A total of 13 anopheline species from forest area and 11 species from plain area were recorded (Table 8). *An. culicifacies* was the most predominant species and accounted for 39 and 42% of the total anophelines in the forest and plain areas, respectively. *An. fluviatilis* was restricted to only forest area and its prevalence rate was 4.4%. The human blood index (HBI) of *An. culicifacies* and *An. fluviatilis* was 0.003 and 0.90, respectively thereby showing that the latter species is responsible for maximum transmission, whereas the former plays only complimentary role during spring and monsoon seasons. The entomological inoculation rate (EIR) in the forest area ranges between 0.02 and 0.61 infective bites per person per night during different seasons, thereby showing that transmission load in the forest ecotype is very high throughout the year, whereas it is low and seasonal in the plain area.

The existing epidemiological data from the study population will provide baseline information about the target population to be included in the field trial for malaria vaccines expected to be undertaken during 2008.

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Contribution of NIMR in Nobel Peace Prize, 2007 to IPCC

"The awarding of the Nobel peace prize to the Intergovernmental Panel on Climate Change (jointly with former US Vice-President Al Gore) is a remarkable testament to the dedication and commitment of the thousands of experts and participants who have produced the Panel's rigorous and comprehensive assessments of climate change research" (Press Release, IPCC October 12, Geneva).

"This is an honour that goes to all the scientists and authors who have contributed to the work of the IPCC, which alone has resulted in enormous prestige for this organization and the remarkable effectiveness of the message that it contains" – says Mr. Rajendra Pachauri, the Chairman of the IPCC.

"It is the most significant recognition that the IPCC has received for providing policymakers with objective and balanced information about the causes and impacts of climate change and possible response measures"– says Renate Christ, the Secretary of the IPCC.

"Hundreds of authors from all regions of the planet have devoted an incredible amount of time and labour to writing and reviewing the reports. None of them has been paid for their time".

Dr. R.C. Dhiman, Deputy Director (Senior Grade), scientist of National Institute of Malaria Research (Formerly, Malaria Research Centre) of Indian Council of Medical Research, Delhi also contributed in IPCC by serving as Reviewer in IPCC Working Group II, Fourth Assessment Report (Page 911 of Appendix III of Climate Change 2007: Impacts, Adaptation and Vulnerability).

4

Highlights of Research Activities under IDVC Project

Bangalore (Karnataka)

- Fish-based biocontrol of malaria vector *Anopheles culicifacies* — very effective, cheap and sustainable for malaria control in southern India, is being implemented.
- A change of mosquitogenic conditions was observed and an action plan has been prepared for mosquito control in Bangalore City.
- Establishment of therapeutic efficacy of chloroquine and its correlation with the molecular markers *Pfcrt* and *Pfmdr1* is underway.
- Situation analysis of malaria was carried out in three districts—Kolar, Raichur and Chitra-durga. Inadequate surveillance and untimely spray operations were responsible for rise in malaria. However, fish release show less malaria in these districts.
- Situation analysis of malaria was carried out in Andhra Pradesh. Inadequate surveillance and untimely spray operations were the major factors responsible for high proportion of *P. falciparum* malaria in the IMCP areas especially in the tribal areas.
- Indoor residual spray with Lambdacyhalothrin 10% CS formulation against *An. culicifacies* was found very effective up to 12 weeks.
- Training on entomological techniques was rendered to 21 entomologists in Karnataka.
- Larvivorous fish against *Aedes* larvae in cement tanks for control of dengue and chikungunya was found very effective.
- Completed randomised multicentric clinical trial of Artekin against *P. falciparum* malaria in Mangalore.
- Initiated evaluation of Alphacypermethin coated long-lasting insecticidal nets in Mangalore City against *An. stephensi*.

Chennai (Tamil Nadu)

- Studies on environmental, social and behavioural risk factors related to malaria transmission in Chennai were carried out and GR completed in experimental and control areas.
- Phase III trial of Lambdacyhalothrin 10% CS as IRS was undertaken in Hogenekkal and Nagasamudra PHCs of Dharmapuri district, Tamil Nadu. Trials in Rameswaram and Thangachimadam PHC, Ramanathapuram district were initiated and evaluation is in progress.
- Malaria clinic continued to offer early/prompt diagnosis and treatment.
- Other activities included technical support to various centres/institutes and collaborative research/scientific work. Health education and training programmes were undertaken.

Hardwar (Uttarakhand)

- Study on insecticidal properties of a plant code MRCHAR/04/04 was continued.
- Evaluation of bioefficacy and wash-resistance of K-O Tab 123, a formulation for long-lasting treatment of nets, against vector mosquitoes was done.
- Simultaneous determination of curcumin and piperine in plasma using high performance liquid chromatography was done.
- Study on accumulation of persistent organochlorine compounds in sub-Himalayan region of north India was continued.
- Field evaluation of neem oil-based Azadirachtin (0.15% EC) against the immatures of *Culex quinquefasciatus* and anopheline larvae was undertaken.
- Field evaluation of Pirimiphosmethyl (50% EC), an organophosphorous insecticide against the immatures of *Culex quinquefasciatus* and anophelines was done.

- Entomological and epidemiological investigations at Narora Atomic Power Station (NAPS), District Bullandshahar (Uttar Pradesh) were completed.
- Two patents were filed on: (i) A new composition for insect and pest control by V.K. Dua, M.F. Alam and A.P. Dash [Application No. 3234/DEL/2006 submitted final application]; and (ii) A new botanical formulation for mosquito control by V.K. Dua, A.C. Pandey and A.P. Dash [Application submitted for provisional registration].

Jabalpur (Madhya Pradesh)

- A study on cerebral malaria associated with neurological disorders in central India was continued.
- A project on rapid assessment of burden of malaria in pregnancy in Madhya Pradesh, India has been launched.
- Change has been done in drug policy in Bajag PHC of Dindori district, which contri-butes highest number of malaria cases in the state based on NIMR research findings.
- DDT was replaced by synthetic pyrethroids as *An. culicifacies* a major malaria vector was resistant to DDT.
- Panna and Satna districts were brought under Enhanced Malaria Control Programme and intensive intervention started as a result of our evaluation and monitoring.
- Workshops at national and state level were organised for medical officers and health staff.

Nadiad (Gujarat)

- Health impact assessment of Sardar Sarovar Project during pre-irrigation baseline period was completed in Phase I districts.
- For making evidence-based decision on the use of larvivorous fish in malaria control in semi-arid areas in north Gujarat, a randomised controlled trial of a native fish species, *Aphanius dispar* was conducted in District Kutch. Initial results are very encouraging.
- Technical support was given to Ahmedabad Municipal Corporation in surveillance, investigation and containment of chikungunya outbreak in Ahmedabad City.
- Completed field trial of a new insecticide formulation in collaboration with WHO Pesticide Evaluation Scheme; also evaluated two new larvicide formulations for urban malaria and dengue vector control in Ahmedabad.
- Provided technical support to the National Vector Borne Disease Control Programme in Gujarat state in terms of policies, annual programme planning, reviews of performance, training of health personnel and evaluation of malaria laboratory services.

Panaji (Goa)

- Study on estimation of malaria burden in Jharkhand, the 'Jharmal' Project was continued.
- Phase III, randomised, non-inferiority trial, to assess the efficacy and safety of Dihydro-artemisinin + Piperaquine (DHA + PPQ, Artekin) in comparison with Artesunate+ Mefloquine (AS +MQ) was continued at Goa Medical College.
- The project on community randomised evaluation of effectiveness of insecticide-treated nets for malaria control on construction workers in the urban slums of Goa was launched in October 2006.
- In the wake of chikungunya and dengue outbreaks in Goa, a survey on *Aedes* population dynamics and breeding habitat preferences in Goa was initiated.
- Evaluation of latex agglutination method for rapid diagnosis of malaria is underway.
- Monitoring of preparedness of Navi Mumbai Municipal Corporation (NMMC) for implementation of the World Bank supported project was done.
- Operational research on current practices of diagnosis and treatment of *P. falciparum* malaria in area with and without resistance to chloroquine in Govt. sector at primary, secondary and tertiary level and in private sectors, clinics, hospitals and general practi-tioners was undertaken in Goa.

Raipur (Chhattisgarh)

- Studies on the bionomics of mosquitoes,

particularly the vectors of malaria, dengue and filariasis in Raipur City were undertaken.

- Epidemiological investigation on malaria and therapeutic efficacy evaluation of chloroquine in Surguja district was carried out.
- Field evaluation of long-lasting insecticidal nets (LLINs) impregnated with Alphacypermethrin (Interceptor®) against anopheline vector mosquitoes was undertaken.
- Field evaluation of Pirimiphosmethyl (50% EC), an organophosphorous insecticide against the immatures of *Cx. quinquefasciatus* and anopheline was done.
- Investigation on outbreak of malaria in Basti PHC under Gaurella block of Bilaspur district was undertaken during 9–14 and 23–28 November 2006.
- Malaria clinic is serving as an early diagnosis and prompt treatment facility.
- Support is being provided to the State govt. by cross-checking of blood slides for malaria and filariasis.

Ranchi (Jharkhand)

- Cytogenetic study of *An. fluviatilis* revealed the presence of species T in Ranchi district.
- Insecticide susceptibility of malaria vector revealed that *An. culicifacies* and *An. annularis* are resistance to DDT (4%) in District Ranchi and *An. fluviatilis* developed partial resistance to DDT. Larvivorous fish hatcheries of *Gambusia affinis* and *Poecilia reticulata* were established for the first time in District Ranchi, Jharkhand state.
- Filariasis survey was carried out in the primitive Santhal tribes of Dumka district and Oram tribes of Ranchi district.
- *In vivo* 28 days studies to detect chloroquine resistance against *P. falciparum* malaria were carried out in PHC Jaldega of District Simdega and PHC Angara of Ranchi district. In PHC Jaldega, early treatment failure was seen in 4 cases (7.8%) and late treatment failure in 14 (27.2%) cases.
- Technical support was provided to the National Vector Borne Disease Control Programme in Jharkhand state for various activities such as malaria epidemic investigation and containment, malaria situation analysis, planning of antimalaria activities, dengue surveillance, establishment of larvivorous fish hatcheries, filariasis survey, cross-checking of slide, IEC, etc. Monitoring of programme implementation at PHC level with high malaria burden was undertaken in Jharkhand. Diagnostic and treatment facilities were provided to the patients reporting to malaria and filariasis clinic at the field unit.

Rourkela (Orissa)

- Studies on development of a site for malaria vaccine trials were continued in Sundargarh district.
- Evaluation of biolarvicide (Bti AS, VCRC B17 strain) against immatures of *Anopheles* and *Culex* species in Rourkela was undertaken.
- Phase III trial on the efficacy of long-lasting insecticidal nets (Olyset nets) was completed.
- Field evaluation of LLIN impregnated with Alphacypermethrin (Interceptor) was undertaken in Rourkela.
- Evaluation of ZeroFly—an insecticide incorporated plastic sheeting against mosquitoes was undertaken in Lathikata block of Sundargarh district.
- Phase III evaluation of high density polyethylene (HDPE) mosquito nets was undertaken.
- A phase II, double-blind, parallel-group, randomised, dose-ranging study assessing the antimalarial activity and safety of RBx 11160 administered for 7 days in patients with acute uncomplicated *P. falciparum* malaria was successfully completed.
- Study on multicentre, open-label randomised clinical trial of efficacy and tolerability of the fixed dose Artesunate/Amodiaquine (AS/AQ) combination therapy versus Amodiaquine (AQ) monotherapy for treatment of uncomplicated falciparum malaria in India was initiated.
- Monitoring of the Programme Implementation at PHC level in Sundargarh, Mayurbhanj, Bhadrak, Nayagarh and Bolangir districts of Orissa was done.
- In-depth review of National Vector Borne Disease Control Programme (Malaria) in

Sundargarh, Sambalpur and Nuapada districts of Orissa was undertaken.

Sonapur (Assam)

- Phase III evaluation of long-lasting insecticidal nets (Olyset nets) against *An. minimus* transmitted malaria in Assam was completed.
- Characterisation of *Plasmodium falciparum* strains prevalent in northeastern states was done.
- Field evaluation of long-lasting insecticidal nets (LLINs) impregnated with Alphacypermethrin (Interceptor) against anopheline vector mosquitoes in Assam was done.
- Phase III, randomised, non-inferiority trial, to assess the efficacy and safety of Dihydro-artemisinin + Piperaquine (DHA+PPQ, Artekin) in comparison with Artesunate + Mefloquine (AS+MQ) in patients affected by acute, uncomplicated *P. falciparum* malaria – multi-centric study in Asia was undertaken.
- In-depth review of NVBDCP for malaria in Darrang, Goalpara and Karbi Anglong districts of Assam was undertaken.
- Other activities included technical inputs to strengthen the malaria control activities specific to northeastern region—health education and capacity building measures, observance of antimalaria month, and mass propagation and distribution of larvivorous fishes (Guppy) in town areas, and malaria outbreak containment in affected districts of Assam.

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5

Research Support Facilities

5.1 Animal House Facility

Rabbits, pigeons, domestic fowls, laboratory mice, etc. are being maintained as per the guidelines issued by the concerned authorities. The animals are being procured from Indian Veterinary Research Institute, Rae Bareli, Uttar Pradesh. The animals were housed at 22, Sham Nath Marg, Delhi and were used as blood meal source to mosquitoes of different species and strains maintained at the Institute. Laboratory mice were used in screening the antimalarials, host-parasite interaction studies and maintenance of rodent plasmodia at the parasite bank. Sick animals were treated as and when found necessary and carcages were destroyed as per standard guidelines. Experiments on animals were performed with the approval of the Scientific Advisory Committee (SAC) and Institutional Animal Ethics Committee (IAEC) of the Institute.

5.2 Repository of Biological Material

5.2.1 Mosquito species

Different species of mosquitoes being maintained in the Insectary are listed below:

Mosquito species	Strain/Origin	Karyotype	Sibling species
Anopheles culicifacies	Burari	Sub	A
An. culicifacies	Dehra	Sub	A
An. culicifacies	Rameswaram	Sub	A
An. culicifacies	Rourkela	Sub	A
An. culicifacies	Haldwani (Refractory strain)	Acro	B
An. culicifacies	Ladpur	Acro	B
An. culicifacies	Jabalpur	Sub	C
An. culicifacies	Rourkela	Sub	C
An. fluviatilis	Rourkela		T
An. fluviatilis	Hardwar		T
An. fluviatilis	Haldwani		T
An. fluviatilis	Hardwar		U
An. stephensi	Delhi		
An. stephensi	Punjab		
An. stephensi	Haryana		
An. stephensi	Okhla, Delhi		
An. stephensi	Hardwar		
An. stephensi	Safiabad		
An. stephensi	Sonepat		
An. stephensi	Kutch		
Culex quinquefasciatus	BSSS (Sensitive to biocide)		
Aedes aegypti	Delhi		
Mutant Lines			
An. stephensi	Black larvae		
An. stephensi	Black larvae– White eye		
An. stephensi	Golden yellow		
Culex quinquefasciatus	Scarlet eye		
Cx. quinquefasciatus	Red eye		

5.2.2 Parasite species

Human Plasmodia

— Non-adapted cryopreserved isolates of *P. falciparum, P. vivax* and *P. malariae*
— Sera/plasma from infected patients

Plasmodium falciparum

— Adapted/Characterised isolates
— Different stages of the parasite from culture
— Merozoites (from culture supernatant)
— Ring (by synchronisation)
— Gametocytes (by Hypoxanthine treatment)
— Free parasites for antigen preparation (by Saponin lysis and ultrasonication)

Plasmodium vivax

— Sporozoites harvested from artificially fed mosquitoes

Cell lines

— Hepatoma cell line: Hep G2 A16 used in *in vitro* cultivation of pre-erythrocytic stage malaria parasites
— Myeloma cell line: SP2
— Hybridomas: 2A 10 (anti-*P. falciparum* sporozoite antibody secreting cells); 2 F2 1A7 (anti-*P. vivax* sporozoite antibody secreting cells)

Non-human Plasmodia

— Different species of avian, simian and rodent plasmodia
— Rodent plasmodia infected rats/mice
— Sera/plasma from respective vertebrate hosts

Non-human malaria parasites in the Parasite Bank

Simian malaria parasites

P. cynomolgi bastianelli (CDRI, Lucknow)
P. cynomolgi bastianelli (NICD, Delhi)
P. knowlesi (NICD, Delhi)
P. knowlesi (CDRI, Lucknow)
P. fragile (CDRI, Lucknow)

Avian malaria parasites

P. gallinaceum
P. relictum

Human malaria parasites in the Parasite Bank

Parasite species	Collection sites (States and Districts)	No. of isolates
P. falciparum	**Andhra Pradesh**	
	Visakhapatnam	12
	Assam	
	Sonapur	20
	Tezpur	6
	Nalbari	1
	Chhattisgarh	
	Jagdalpur	14
	Bilaspur	26
	Delhi	
	Delhi	192
	Gujarat	
	Anand	4
	Kheda	7
	Haryana	
	Gurgaon	25
	Karnataka	
	Mangalore	14
	Madhya Pradesh	
	Mandla/Jabalpur	14
	Meghalaya	
	Tura	18
	Orissa	
	Rayagada	29
	Sundargarh	42
	Rajasthan	
	Alwar	25
	Bharatpur	35
	Jaisalmer	38
	Tamil Nadu	
	Chennai	4
	Ramanathapuram	1
	Uttar Pradesh	
	Baharaich	22
	Gautam Budh Nagar	37
	Ghaziabad	17
	Allahabad	60
	West Bengal	
	Kolkata	18
	Midnapore	1
	Total	**682**
P. vivax	Karnataka	6
	Delhi, Uttar Pradesh, Orissa	53
	Tamil Nadu	9
	Total	**68**
P. malariae	Orissa	5

Rodent malaria parasites

P. berghei (CDRI, Lucknow)
P. berghei
P. berghei
P. berghei ANKA
P. berghei (*NK65*) (PGIMER, Chandigarh)
P. chabaudi (Paris)
P. yoelii nigeriensis (ICGEB, Delhi)
P. yoelii nigeriensis (CDRI, Lucknow)
P. yoelii nigeriensis (LSHTM, London)
P. yoelii yoelii (265 by) Paris

5.3 Library

The Institute has one of the best libraries in the country in the field of malaria having more than 6735 books, 4083 bound journals, 3573 reprints, 18 video cassettes, 27 audio cassettes, 20 microfilms, 19 theses and 106 national and international reports. About 52 journals (39 Foreign and 13 National) are being subscribed besides 8 journals which are received on complimentary and exchange basis. Two magazines and six newspapers are subscribed. About 201 new books had been added during this financial year.

The library renders its services not only to the scientists/research scholars of the Institute but also to various national and international universities and organisations. During the year modernisation process has been expedited and entry into LIBSYS software of about 6664 books and 2050 journals has been completed. Library has also been serving its users through DELNET. Other services such as information retrival, citation index, internet facility, inter library loan facility, reprographic services, etc. are also being provided to the users. J Gate and JCCC@ICMR & Proquest Medical Database are being provided to the NIMR scientists in Delhi and its IDVC field units located in different parts of India. In the process of modernisation application of barcode system is in progress. Library provides abstracts, references, CAS and SDI services, Medline CD search, etc. to its users.

Selection of books for the library

Inter-Institutional Collaboration

Collaborative projects were underatken with the following ICMR/Non-ICMR institutes and medical colleges of the country.

1. 'Development of site for Malaria Vaccine Trial at Sundargarh District, Orissa' in collaboration with International Centre for Genetic Engineering and Biotechnology, New Delhi and State Government of Orissa.
2. 'Developing Epitope-based Immunogen selecting different Stages of *Plasmodium vivax* using in-built Immunoadjuvants and Delivery in Microspheres' In collaboration with Department of Biochemistry, All India Institute of Medical Sciences, New Delhi.
3. 'Immunocapture-based Diagnostic Assay for the Detection of *Plasmodium falciparum* HRP-2 and LDH Antigen' in collaboration with Department of Biochemistry, All India Institute of Medical Sciences, New Delhi.
4. 'Complement Receptor 1 (CR1) and its Gene Polymorphisms in relation to the Pathophysiology and Susceptibility to Severe Malaria' in collaboration with Department of Biochemistry, All India Institute of Medical Sciences, New Delhi.
5. Studies on the Distribution of Members of *Anopheles dirus* Species Complex in North eartern states in collaboration with Defence Research Laboratory (DRDO), Tezpur, Assam.
6. 'Engineering Indian malaria vector *Anopheles culicifacies* mosquito genetically using transposable element' in collaboration with Maharshi Dayanand University, Rohtak, Haryana.
7. 'Identification of Epidemiological Risk Factors of Malaria for Development of Strategic Action Plan for Malaria Control in Problematic Districts in Karnataka'
8. 'Evaluation of Therapeutic Efficacy of Antimalarials' in collaboration with the State Governments of Assam, Goa, Gujarat, Madhya Pradesh, Tamil Nadu and Uttarakhand.
9. 'Clinical Drug Trials of Antimalarials' in collaboration with Medical Colleges of Guwahati, Jabalpur, Goa, Mangalore Hospital and Tea Estate, Assam.
10. 'Cerebral Malaria Associated Neurological Disorders in Central India' in collaboration with Medical College, Jabalpur, More House School of Medicine and Centre for Disease Control and Prevention, Atlanta, USA.
11. 'Primary Screening of Medical Plants from Northeastern States of India for their Antimalarial Activity' in collaboration with Defence Research and Development Organization, Tezpur, Assam.
12. 'Screening of Chloroquine Sensitivity Status of *P. falciparum* Parasites from Western Border Areas of India' in collaboration with Defence Research and Development Organization, Gwalior, Madhya Pradesh.
13. 'Molecular Characterisation of Nitric Oxide Synthase in *An. culicifacies*: Relevance for Refractory Mechanism' in collaboration with Institute for Cytology and Preventive Oncology, Noida, Uttar Pradesh.
14. 'Health Impact Assessment of Indira Sagar Dam and Resettlement Colonies in SSP Reservoir Impoundment Areas in Narmada Valley in Madhya Pradesh' in collaboration with National Institute of Virology, Pune, National Institute of Cholera and Enteric Diseases, Kolkata and Narmada Valley Corporation.
15. 'Characterisation of *P. falciparum* Strains Prevalent in Northeastern States' in collaboration with Regional Medical Research Centre (RMRC), Dibrugarh, Assam.

16. 'Screening of Antimalarial Activity of Synthetic Compounds in *P. falciparum* culture lines' in collaboration with Department of Chemistry, University of Delhi and Indian Institute of Chemical Technology, Hyderabad.
17. 'Larvicidal Activity of Extracts of different parts of a Tissue Cultured Plant (Code VA-1 of family Astereaceae)' in collaboration with University of Delhi, Delhi.
18. "Preparation of a Field Site for Malaria Vaccine Trial in and around Jabalpur" [ICMR National task force Project] funded by ICMR task force and CDC Atlanta.
19. "Transmission Dynamics of Malaria in Tribal Areas" in collaboration with RMRCT, Jabalpur and NIMR, Delhi under Tribal sub-plan", funded by ICMR.
20. "Assessing the Burden of Malaria in Pregnancy in India (Chhattisgarh)" in collaboration with Boston University School of Public Health (BUSPH) funded by ICMR and NIH, Washington.
21. "Rapid Assessment of Burden of Malaria in Pregnancy in Madhya Pradesh, India" in collaboration with CDC Atlanta, USA, Liverpool School of Medicine, UK, funded by USAID, New Delhi.
22. "Assessing the Burden of Malaria in Pregnancy in East India (Jharkhand)" in collaboration with Boston University School of Public Health (BUSPH), funded by USAID, Washington.

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Other Activities

8.1 Information, Education and Communication (IEC)

Production of video documentaries

Two video documentary films were produced. The field shooting, script writing, editing, voice-over, animation and special effects were incorporated into the films.

DDT spraying for malaria control

This documentary is related to step-by-step procedure and methodology for DDT spraying such as information to villagers, making suspension, determining discharge rate, width of swath, indoor spraying, precautions for spraymen for cleaning of equipments, etc. Duration of the film is about 15 min. This film would be useful for both supervisors and spraymen.

Chikungunya prevention and control

This documentary on Chikungunya deals with clinical symptoms of the disease, breeding sites of the vector *Aedes aegypti,* community mobilisation and involvement to control vector breeding, role of *Panchayats* for creating awareness and helping communities, mobile health facilities and the state government's efforts at all levels to confront this emergency in several states.

National Science Day celebration

The National Science Day was celebrated in Central Secondary School in Naraina, New Delhi. Health education and awareness was created through popular lectures by NIMR Scientists and Hindi Officer followed by discussion on various aspects of vector-borne diseases, their prevention and control. Other activities included live demonstrations of different stages of mosquito life-cycle and larvivorous fish, exhibitions in English and Hindi, video-film shows and demonstration of *P. falciparum, P. vivax* parasites in microscope. Pamphlets, charts and books published by NIMR were given for school's library as reference material.

Exhibition
An exhibition consisting of 10 panels (size of each panel 3 x 4 ft) depicting research activities of NIMR was conceived, designed and developed.

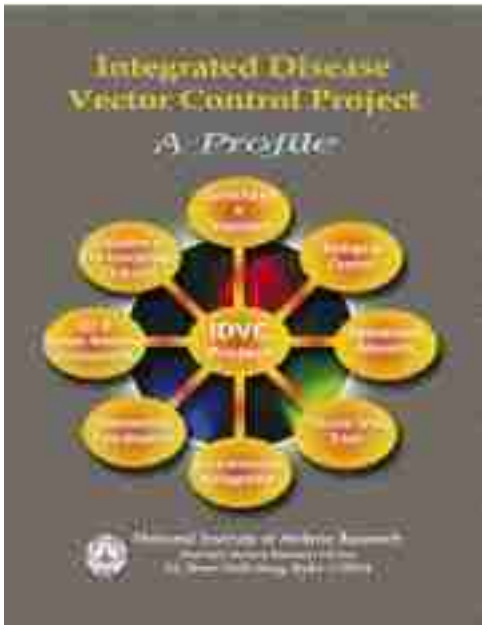
Distribution of video CDs
Video CDs of NIMR-produced films were distributed to trainees of different training programmes of NIMR, NVBDCP and NICD. All help and cooperation was extended to a team of video production highlighting the activities of NIMR in Delhi for TV channels programme "JIGYASA."

8.2 Publications

Journal of Vector Borne Diseases
The Institute has been publishing an English quarterly the *Journal of Vector Borne Diseases*. The journal publishes original research articles, research communications, review articles on various aspects of different vector borne diseases. The journal is abstracted by major abstracting agencies and is being uploaded in the web for easy access to the scientists working in the field of vector borne diseases. PDF version of all articles published in the journal can be downloaded free of cost from www.mrcindia.org/journal.

Malaria Patrika
Malaria Patrika, a quarterly periodical in Hindi language is published by the Institute to create awareness on malaria in the community. Various activities relating to the Institute, news and latest developments in the field of malaria research are being covered in this periodical apart from scientific articles. The *Patrika* is being applauded by the community.

Integrated Disease Vector Control Project: A Profile
A document on the research activities carried out under Integrated Disease Vector Control Project, highlighting the major research inputs into the programme, new technologies developed and transferred to the national programme, research support provided to the national programme since its inception in 1985. The document highlights the achievements and research activities carried out by different field units established in different eco-epidemiological settings in the country and research papers published since inception of this project.



NIMR Newsletter
NIMR has been publishing a biannual and bilingual newsletter of the institute since 2006. Activities of the institute are included in this newsletter and send to its various users— scientists, institutes and scholars working in the field in the country for reference purpose. In each issue, an article on recent malaria research by the eminent scientists is being published as guest column, malaria-related news, new research projects initiated by NIMR, publications of NIMR and other important activities are also published. It shall be renamed to "*Plasmodium*" w.e.f., July 2007.

Website www.mrcindia.org

The Institute's website contains information on research activities being carried out by NIMR, publications of the Institute, research activities of the field units of NIMR, audio-visual unit activities and profiles of scientists, etc. The documents and books published by the Institute are available in this website. A separate webpage is being maintained for Institute's english periodical, the *Journal of Vector Borne Diseases*. The website contains information on guidelines for contributors, subscribers and contact information of editorial office, etc.

8.3 Conferences/Workshops/Symposia/ Important meetings attended/ Lectures delivered

Prof. A.P. Dash

1. Investigators meeting of the "Multi-country clinical trial of a new synthetic antimalarial drug RBx11160" organised jointly by Swiss Tropical Institute and Medicines for Malaria Venture (MMV) at Basel, Switzerland from 26–28 April 2006.
2. "Shin Poong Pharm – MMV investigator meeting – Phase III trials of pyronaridine artesunate in Asia" at Ho Chi Minh City, Vietnam from 1–3 September 2006 .
3. Visited CDC Atlanta for discussions on collaborative activities from 18–23 September 2006.
4. National symposium on "Tribal health" at Jabalpur and chaired the session on "Malaria" from 19–20 October 2006.
5. "Insecticide resistance workshop" at Durban from 22–24 November 2006.
6. "AOAC International task force on pesticide detection in soft drinks (PSD)" at Delhi on 29 November 2006.
7. "CDC, USA Round table discussions on polio and health policy" at Delhi from 19–20 December 2006.
8. "Director's meeting on disease vectors" at Jodhpur from 20–24 December 2006.
9. "Malaria and filaria: major problem in Madhya Pradesh" at Khajuraho from 26–27 December 2006.
10. "National workshop on integrated mosquito management and future directives" 7–8 January 2007 at Hyderabad.
11. "INSERM meeting" at ICMR HQS, New Delhi on 9 January 2007.
12. "ESRI/CGIS workshop" at Delhi from 18–19 January 2007.
13. Joint monitoring mission on malaria at Bhubaneswar from 3–5 February 2007.
14. Meeting on NE Task force at ICMR (HQs), New Delhi on 17 February 2007.
15. WHO Inter country meeting at Chiangmai, Thailand on 12 March 2007.
16. Workshop on AES/JE organised by NVBDCP at India Habitat Centre, New Delhi on 20 March 2007.
17. National dissemination workshop at Delhi on 23 March 2007.

Dr. P.R. Bhattacharya

1. "XVII National conference of parasitology" at Kolkata from 22–24 November 2006 and presented a paper on "Sequence variation and immunogenicity of T-helper cell epitopes (Th-2R and Th-3R) of circumsporozoite protein of *Plasmodium falciparum* isolates from India".

Dr. Sukla Biswas

1. XLIV annual meeting of the Infectious Diseases Society of America (IDSA), at Toronto, Ontario from 12–15 October 2006 and delivered an oral presentation in the session of 'Immunology of infectious diseases' on "Immune responses to defined *Plasmodium falciparum* antigens and disease susceptibility among inhabitants from seasonal malarious areas of northern India".
2. "Finalization of standard operation procedure"

and on "Modality of quality assurance (QA) scheme for laboratory diagnosis in malaria" at NVBDCP, Delhi on 11 September 2006 and on 17 January 2007.

3. Two meetings on "Characterization of the *P. falciparum* strains prevalent in northeastern states" at Sonapur, Assam from 3–5 May 2006 and 23–25 February 2007.

Dr. Aparup Das

1. "National conference on biodiversity conservation for a sustainable society" at Nayagarh Autonomous College, Nayagarh, Orissa from 26–27 August 2006.
2. Workshop on "Modelling infectious diseases" held at the Institute of Mathematical Sciences, Chennai from 4–5 September 2006.
3. "II medical development congress on genomics and proteomics in health and disease" held at ICMR, New Delhi from 8–9 September 2006.
4. "Eastern Regional workshop on Bioethics" held at ICMR-NIH-RG Kar Medical College, Kolkata from 11–13 October 2006.
5. "Functional genomics and evolutionary biology" workshop held at the Institute of Life Sciences, Bhubaneswar from 2–25 November 2006.
6. "National session on general issues on R&D in pharmaceutical industry" at the National meeting on research and development in pharmaceutical industry: development of new drugs for emerging chronic diseases, Department of Sciences and Technology (Govt. of India)–ISHA, Bangalore from 27–29 December 2006.

Dr. R.C. Dhiman

1. International workshop on "Monsoon climate variability and change and their impacts on water, food and health in western India" held at Nirma University, Ahmedabad from 6–7 February 2006 and delivered a talk on "Impact of climate variability on vector borne diseases with emphasis on malaria in India".
2. "Workshop on women and health care: initiatives in vector control through community mobilization" on "Application of satellite remote sensing in planning malaria control", held at IIT, Delhi from 10–11 April 2006.
3. Brain storming workshop on "Aerosols and its impact on climate with reference to Indo-Gangetic plains" at Indian Institute of Technology, Kanpur from 10–11 November 2006 and delivered a talk on "Climate change and risk of malaria".
4. "Asia pacific remote sensing symposium" at Goa from 13–17 November 2006 and presented a paper on 'Potential for early warning of malaria in India using NOAA-AVHRR-based vegetation health indices'.
5. Workshop on "Balancing energy, development and climate priorities in India" held at India Habitat Centre, New Delhi from 30 November–1 December 2006.
6. "International conference on adaptation to climate variability and change: towards a strategic approach", organised jointly by the European Commission and Ministry of Environment and Forests at Delhi from 7–8 December 2006 .
7. "National review and coordination meeting on malaria control activities for malaria endemic states" at Ranchi from 24–25 January 2007.

Dr. Hema Joshi

1. Workshop on "Drug resistant malaria" at Genome Centre, Cambridge, UK from 18–22 October 2006.

Dr. Neelima Mishra

1. "Quality assurance of RDT and malaria microscopy for laboratory technicians" organised by NIMR in collaboration with NVBDCP at Delhi from 2 January to 15 February 2007.

Dr. P.K. Mittal

1. "International forum for sustainable management of disease vectors" held at, Beijing, P.R. China from 21–23 April 2006 and presented a paper entitled "Insecticide resistance in malaria vectors in India and strategies for management".

Dr. B.N. Nagpal

1. "Vector control linkages with voluntary agencies" presented in a workshop on "Women & health care: initiatives in vector control through community mobilization" organised by Indian Institute of Technology at New Delhi on 20 April 2006.
2. "Entomological aspects of malaria and dengue fever in reference to breeding sites especially construction sites" presented at "Workshop on sensitisation of contractors of MCD on prevention of control of dengue fever" organised by Municipal Corporation of Delhi, at Town Hall, Delhi on 22 June 2006.
3. "Life cycle of malaria parasite" presented at "Workshop on rapid assessment of malaria in pregnancy in Madhya Pradesh under USAID" organised by NIMR, Jabalpur at Katni, Madhya Pradesh on 25 July 2006.
4. "Life cycle of malaria parasite" presented at "Workshop on malaria in pregnancy" organised by NIMR, Jabalpur at Satna, Madhya Pradesh on 5 September 2006.
5. "National symposium on tribal health" organised by RMRCT, Jabalpur on 19–20 October 2006.
6. II ESRI Asia Pacific User Conference at New Delhi from 18–19 January 2007.
7. In a expert group meeting delivered a lecture on "Vector control in prevention and control of dengue fever in Delhi" organised by Municipal Corporation of Delhi at Delhi Secretariat on 29 March 2007.

Dr. K. Raghavendra

1. Meeting to review document on "Quality assurance of insecticides" at NVBDCP, Delhi on 2 May 2006.
2. Meeting of review committee on "Global funds to fight AIDS, tuberculosis and malaria (GFATM) to review for preparing projects for funding sixth round funding of WB", at Nirman Bhawan, New Delhi on 9 May 2006.
3. Technical committee meeting on "Specifications of LLINs-bednets", at DGHS, Ministry of Health and Family Welfare, Govt. of India, New Delhi on 26 May 2006.
4. "Meeting of the Society for *in vitro* biology" at Minneapolis, USA from 3–7 June 2006 and a poster on "Micropropagation of *Spilanthes acmella* L and evaluation of its larvicidal activity against vectors *Culex quinquefasciatus* and *Anopheles stephensi* Liston" was presented.
5. Expert committee meeting to deliberate on "Adulticides and larvicides and rotation" at NVBDCP, Delhi on 8 June 2006.
6. Meeting on "In-depth review committee of NVBDCP/World Bank designed team", at NVBDCP, Delhi on 12 June 2006.
7. Expert group meeting for the workshop on "Quality control and quality assurance" at NVBDCP, Delhi on 18 July 2006.
8. Review meeting on "Implementation of NVBDCP in the state of Rajasthan", and identified priorities conducted by Joint Secretary (Govt. of India), Ministry of Health and Family Welfare, NVBDCP at Jaipur on 12 September 2006.
9. "International conference on Toxicology, toxicogenomics and occupational health (ICTTOH); and XXVI annual meeting of Society of Toxicology (STOX)" at Gwalior from 9–11 October 2006. Abstract on "Malaria vector control in India by chemical pesticides".
10. "Brain storming session on vector borne diseases in India" at WHO-SEARO, Delhi, on 9 November 2006.
11. "Global Bio Pharma conference – 2006 theme:

synergies in the global biotechnology industry" at Hyderabad from 10–12 November 2006.

12. National workshop on "Integrated mosquito management and future directives" at Osmania University, Hyderabad from 8–9 January 2007 and delivered an invited lecture on "Studies on the reversion of insecticide resistance in *Anopheles culicifacies* in District Surat, Gujarat" and papers on "Genetically modified mosquitoes for vector control – a perspective" and "Isolation of four *Bacillus* strains from soil and their efficacy against *Anopheles culicifacies*"

13. Expert committee meeting on "Adulticide and larvicide in vector control–bacterial pesticide, IGRs, space sprays" at NVBDCP, Delhi on 30 January 2007.

Dr. A.M. Reetha

1. "Indo-US workshop on clinical trials and clinical research" at ITC Grand Central Sheraton and Towers, Mumbai, India from 4–6 April 2006.
2. "Ethical review for protection of human participants involved in research" at ICMR, New Delhi on 29 April 2006.
3. "Indo-US workshop on bioethics in clinical research" at New Delhi from 20–22 June 2006.
4. "Assessment of the state implementation capacities for Global fund supported IMCP" at NVBDCP, Delhi on 17 April 2006.
5. Meeting to review the current data on therapeutic efficacy of antimalaria drug and provide recommendations on malaria treatment policy state by state at NVBDCP, Delhi on 5 July 2006.
6. "Assessment of malaria treatment practices in public and private health sectors" at NICD, Delhi on 18 January 2007.

Mrs. Rekha Saxena

1. "National symposium on tribal health" organised by RMRCT, Jabalpur from 19–20 October 2006.
2. II ESRI Asia Pacific User conference organised by ESRI India at New Delhi on 18–19 January 2007.

Dr. Aruna Srivastava

1. "National symposium on tribal health" organised by RMRCT, Jabalpur on 19–20 October 2006.
2. Senior Executive seminar organised by ESRI India, New Delhi on 17 January 2007.
3. "GIS for cost-effective control of malaria" presented at II ESRI Asia Pacific User conference organised by ESRI India at New Delhi from 18–19 January 2007.

Dr. Neena Valecha

1. "Malaria burden in India" meeting at NASC, New Delhi from 29–30 March 2006.
2. "Assessment of the state implementation capacities for Global fund supported IMCP" at NVBDCP, Delhi on 4 and 17 April 2006.
3. "Interactive artesunate monotherapy" meeting at WHO-SEARO, New Delhi on 5 April 2006.
4. "Malaria in pregnancy in India: research coordination meeting" at NASC, New Delhi from 10–11 April 2006.
5. "Women and health care: initiatives in vector control through community mobilization" at IIT, New Delhi from 20 – 21 April 2006.
6. "Characterization of the *P. falciparum* strains prevalent in northeastern state, Assam" meeting at Guwahati, Assam from 3–5 May 2006.
7. "A Phase III, randomized, non-inferiority trial, to assess the efficacy and safety of Dihydroartemisinin + Piperaquine (DHA+PPQ, Artekin) in comparison with Artesunate + Mefloquine (AS+MQ) in patients affected by acute, uncomplicated *Plasmodium falciparum* malaria at Kasturba Medical Hospital, Mangalore on 8 May 2006.

8. "Assessment of the state implementation capacities for Global fund supported IMCP" at NIMR, Delhi on 23 May 2006.

9. "Evidence-base for antimalarial treatment policy in India" at US Embassy on 24 May 2006.

10. Workshop on "Drug efficacy project and disease burden project" at Simdega, Jharkhand on 7 July 2006.

11. "Task force on vaccines and diagnostics" meeting at Department of Biotechnology, New Delhi on 18 July 2006.

12. "Review meeting for therapeutic efficacy studies in the states" organised by National Vector Borne Disease Control Programme at Simdega, Ranchi (Jharkhand) on 7 and from 28–29 July 2006 .

13. "Shin Poong Pharm – MMV investigator meeting" on "Phase III trials of pyronaridine-artesunate in Asia" at Ho Chi Minh City, Vietnam from 1–3 September 2006 .

14. "National drug policy, treatment of uncomplicated cases and management of severe malaria" at NVBDCP, Delhi on 9 October 2006.

15. "Expert group meeting on drug policy" at NVBDCP, Delhi on 11 October 2006.

16. "Multicentre open-label randomised clinical trial of efficacy and tolerability of the fixed-dose artesunate/amodiaquine (AS/AQ) combination therapy versus amodiaquine (AQ) monotherapy for treatment of uncomplicated falciparum malaria in India" at Ranchi from 12–13 October 2006.

17. "Institutional assessment at NVBDCP" meeting at Nirman Bhavan, New Delhi on 19 October 2006.

18. "Consultative workshop on integrated training programme" at NVBDCP, Delhi from 24–25 November 2006.

19. "Technical Advisory Committee on malaria" at Nirman Bhawan, New Delhi on 4 December 2006.

20. "The global malaria programme (GMP)" technical and research advisory committee (TRAC) meeting at Cairo, Egypt as an expert member from 10–12 December 2006.

21. "World Bank Pre-Appraisal Mission for the proposed India-Vector Borne Diseases Control Programme" at NVBDCP, Delhi from 18 –19 December 2006.

22. "Infectious diseases" meeting with R&D delegation from Ontario, Canada meeting at New Delhi on 16 January 2007.

23. "Quality assurance of malaria diagnostics" at NVBDCP, Delhi on 17 January 2007.

24. "Mini symposium on malaria research in Orissa" at Bhubaneswar on 15 February 2007.

25. "AS/AQ launch and FACT implementation expert group" meeting at La Maison internationale de la Cite Universitaire, Paris, France from 1–2 March 2007.

8.4 Training Courses Organised

1. "Malaria in pregnancy in India: research co-ordination meeting" organised by NIMR at Pusa, New Delhi from10–11 April 2006.

2. Two training courses for laboratory technicians of MCD organised by NIMR in collaboration with NVBDCP and MCD at NIMR, Delhi from 8–12 May 2006 and 15–19 May 2006.

3. Five trainings on quality assurance of RDT and malaria microscopy for Laboratory Technicians organised by NIMR in collaboration with NVBDCP from 2–6 January 2007, 15–19 January 2007, 22–27 January 2007, 5–10 February 2007 and 12–16 February 2007.

4. Training course for entomologists/biologists jointly organised by NIMR and NVBDCP at NIMR, Delhi from 19 February–16 March 2007.

8.5 Workshop Organised

Workshop on "Development of Scientific Communication Skills"

The process of expressions of ones thoughts to others in a simple but effective way is an art known as the art of communication. The ability to communicate is a vital ladder to career development. The art of communication plays a pivotal role in the field of science also. In scientific communications both verbal and written communication skills are of great importance. Considering this aspect, a workshop was organised by NIMR on the topic "Development of Scientific Communication Skills" during 15–16 February. The resource person for the workshop was Mr. Yateendra Joshi. Thirty scientists from NIMR attended the workshop.

The main aim of this workshop was to refresh the scientists on the effective communication skills, and help them to develop and implement the same in their day-to-day activities. Both the days of the workshop was divided into three sessions. On the first day Mr. Joshi mainly proceeded with *"Introduction to measurably effective communication"*. The effective communication skill mainly constituted of the handling of numbers, handling pictures, and the handling of verbal presentation. The basics to be considered while communicating depends on the type of the audience, the purpose of presentation and whether the communication is verbal or by slides, pictures or by any other means. The written skill includes writing papers, and articles and their submission to research journals. The main area of concern while writing a manuscript is handling of references, Mr. Joshi has explained in details on it.

The second day session was carried out with the topics such as communicating through letters, e-mails and fax messages. According to Mr. Joshi the writing should be as simple as possible, using simple words and straight forward language so that even a lay audience can understand. Most important aspect of writing is handling of words; some common errors should be avoided such as, spelling mistakes, grammatical errors, punctuations, idiom usage and style. Some principles which should be well thought-out while writing for Newsletters and annual reports were also discussed.

8.6 Trainings Imparted

Dr. P.R. Bhattacharya

1. Ms. Harmeet Kaur and Staffi Satija, Allahabad Agricultural Institute, Allahabad (Uttar Pradesh) received training on "Studies on sequence variation and immunogenicity of T-helper cell epitopic regions of circumsporozoite protein of *Plasmodium falciparum* isolates from India".

2. Mr. Gaurav Arora, Department of Biotechnology, Jiwaji University, Gwalior (Madhya Pradesh) received training on "Studies on genome display and genotype of *Plasmodium falciparum* isolates from India".

3. Ms. Deepali Khandelwal, IET, Biotechnology, M.I.A., University of Rajasthan, Jaipur (Rajasthan) received training on "Studies on genotype of *Plasmodium falciparum* isolate from India by Anchored Primer Amplification of DNA (APAD)".

4. Mr. Nitesh Jadon, Department of Biotechnology, Jiwaji University, Gwalior (Madhya Pradesh) received training on "Allelic variation of T-helper cell epitopic regions of circumsporozoite protein of *Plasmodium falciparum* isolates from India".

5. Ms. Rashmi Agarwal, Department of Biotechnology, Institute of Applied Medicine and Research, C.C.S. University, Meerut (Uttar Pradesh) received training on "Studies on sequence variation and cloning and expression of T-helper cell epitopic regions of circumsporozoite protein of *Plasmodium falciparum* isolates from India".

Dr. Sukla Biswas

1. Guidance provided to Mr. Rakesh Patel, Department of Biomedical Technology, Jiwaji University, Gwalior (Madhya Pradesh) on "Development of immunochromatographic test for determining antimalarial antibody in patients' blood".
2. Guidance provided to Ms. Arti Yadav, Department of Biochemistry, Jiwaji University, Gwalior (Madhya Pradesh) on "Differential effect of hyper immune sera and immunoglobulin-G in presence of monocytes on the growth of *Plasmodium falciparum in vitro"*.
3. Guidance provided to Mrs. Nidhi Varshney, Department of Biomedical Sciences, Bundelkhand University (Uttar Pradesh) on "Source of mosquito blood meal and determination of antimalarial antibodies in injested human blood meal"
4. Guidance provided to Ruchi Jain, Department of Microbiology, Chaudhary Charan Singh University, Meerut (Uttar Pradesh) on "Effect of immune sera and immunoglobulin-G and monocytes in *Plasmodium falciparum* growth *in vitro*".
5. Guidance provided to Ms. Thangjam Premabati, Department of Biosciences, Jamia Millia Islamia, New Delhi on "Antimalarial antibody profile in a group of individuals belonging to malaria endemic areas".
6. Guidance provided to Richa Maheshwari, School of Studies in Biotechnology, Jiwaji University, Gwalior, M.P on "Antimalarial IgG responses and genotyping of *Plasmodium vivax* in a group of patients from northern India"
7. Guidance provided to Varsha Gurjar, Department of Biomedical Technology, Jiwaji University, Gwalior, M.P. on "Antimalarial IgG responses and dihydrofolate reductase gene polymorphism in a group of *Plasmodium falciparum* malaria patients"
8. Training provided to Bharat Bhusan and Ambuj Ashok, students of B.E. (Biotechnology), Institute of Engineering & Technology, Dr. B. R. Ambedkar University, Agra on Microscopic diagnosis of malaria parasites and ELISA technique for Detection of antimalarial antibodies in patients' blood.

Dr. Aparup Das

Supervised M.Sc. and B. Tech. dissertation of:

1. Ms. Abha Srivastava, Jiwaji University, Gwalior
2. Ms. Meenu Sharma, Jiwaji University, Gwalior
3. Ms. Lily Basu, College of Biotechnology, Dehradun
4. Ms. Garima Goyal, Jiwaji University, Gwalior
5. Ms. Deepsikha Lal, Amity University, Noida
6. Ms. Priti Kumari, Banasthali Vidyapeeth, Rajasthan
7. Ms. Surabhi Verma, Banasthali Vidyapeeth, Rajasthan
8. Ms. Ruchi Bajaj, Gurukul Kangri Vishwavidyalaya, Hardwar
9. Ms. Rama Rajendran, Bharathidasan University, Tiruchirappalli
10. Ms. Shashi Rajput, Boston College, Gwalior

Dr. Hema Joshi

Training imparted for project/thesis work of the following M.Sc. students:

1. Mr. Pankaj Sharma, Jiwaji University, Gwalior, (Madhya Pradesh)
2. Ms. Shabana Parveen, Jiwaji University, Gwalior (Madhya Pradesh)

3. Mr. Rajneesh Sharma, Chaudhary Charan Singh University, Meerut (Uttar Pradesh)
4. Ms. Garima Pandey, H.N.B. University, Srinagar (Uttarakhand)
5. Ms. Nidhi Ralli, CET-IILMAHL (U.P. Technical University), Noida (Uttar Pradesh)
6. Mr. Shameemul Haque, Jamia Millia Islamia, New Delhi
7. Ms. Ramie Begum, SRF, NIMR Field Unit, Sonapur (Assam)
8. Mr. Ankit Sharma, M. Tech., Amity Institute of Biotechnology, Amity University, Noida.
9. Anshu Aggarwal, B.Tech., NSIT, University of Delhi, Delhi

Dr. Nutan Nanda

1. Mr. Y.V.S. Rao, Scientist B and Mr. N.G. Das, Technical Officer from Defence Research Laboratory, Tezpur, Assam were provided training in processing of mosquito for cytotaxonomic studies, identification of polytene chromosomes and mosquito blood meal analysis in July 2006.
2. Dr. Sunita Singh, DST-Women Scientists, DRDO, Gwalior was trained in cytotaxonomic techniques for the identification of sibling species of malaria vectors in September 2006.

Dr. K. Raghavendra

1. Imparted one week training and demonstration of "Molecular method for identification of *An. dirus*" to Mr. N.G. Das, T.O. and Mr. Y.V.S. Rao, Scientists from Defence Research Laboratory (DRDO), Tezpur, Assam in July 2007.
2. Ms. Mehar Darukhshan Kalim, Deptt. of Biochemistry, Jiwaji University, Gwalior

Mr. O.P. Singh

1. Miss Garima Sharan, Department of Biochemistry, Jiwaji University, Gwalior

Dr. Vineeta Singh and Dr. C.R. Pillai

1. Imparted training to 22 trainees from various Institutes and Universities on different techniques at the Parasite Bank for one week to four months.

Dr. Aruna Srivastava, Dr. B.N. Nagpal and Mrs. Rekha Saxena

1. Training on 'Geographical Information System for mapping malaria receptivity' to Mr. Mayuri Panditrao, Mr. P. Jeevan and Mr. T. Akbar from Berkeley University, California under United Nation Industrial Development Organization (UNIDO) fellowship from 22 May to 9 June 2006.
2. Training on 'GIS based mapping of malaria receptivity, distribution of malaria vectors and dengue vector surveillance' to Mr. Sein Thaung, WHO fellow from Department of Medical Research (Lower Myanmar) from 5 to 23 March 2007.

8.7 Ph.D. Programme

The following students are working for their Ph.D. degrees under the supervision of NIMR scientists.

Name of the Supervisor	Name of the Student
Prof. A.P. Dash	Mr. S. Mishra Mr. N. Marai Ms. Priyanka Kar Mr. U. Sreehari
Dr. T. Adak	Mr. Anil Sharma Ms. A. Mehrunnisa Ms. Prerana Bali Mr. Hardev Parashar Mr. Mohammed Sohail
Dr. P.R. Bhattacharya	Mr. Jay Prakash Narayan Singh Ms. Sanghamitra Verma
Dr. V.K. Dua	Mr. N.C. Gupta Mr. A.C. Pandey Mr. Firoz Alam Mr. Swapnil Roy
Dr. R.C. Dhiman	Mrs. Sharmila Pahwa

(*contd...*)

Name of the Supervisor	Name of the Student
Dr. Ashwani Kumar	Mrs. Deeparani Prabhu Mrs. Nandini Korgaonkar Mr. M.B. Kaliwal
Dr. Arun Sharma	Mr. Suprio Ray Miss Neha Chauhan
Dr. P.K. Mittal	Mr. Suresh Yadav
Dr. Hema Joshi	Mr. S.K. Prajapati Ms. Gertrude Kiwanuka Mr. P.K. Mallick
Dr. Sarala K. Subbarao (Former Director)	Mr. O.P. Singh Mr. Dinesh Chandra
Dr. Aparup Das	Ms. Hemlata Srivastava

8.8 NIMR Building Complex at Dwarka, New Delhi

Construction of the Research Block

The construction of Research Block of NIMR started in February 2006 by M/s Rajasthan State Road Development and Construction Corporation Limited under the supervision of M/s Gherzi Eastern Ltd., the consultant for this project. The civil work for the superstructure (basement + ground, I, II & III floors) has been completed. Utility works like electrical installations, plumbing, fire fighting, HVAC etc. are progressing at a fast pace. The external development works like construction of the boundary wall, approach road to the research block, laying of sewer lines etc. are being carried out simultaneously. The process for procuring furniture and fixtures for the Research Block has been initiated. It is likely that this building would be ready to move in during the IVth quarter of 2007.

Creation of Liquid Nitrogen facility at this new campus

Construction of the room for Liquid Nitrogen Plant was initiated in August 2006 after obtaining necessary approvals from the Council. This work

was also awarded to M/s Rajasthan State Road Development and Construction Corporation Ltd. Construction of the room was completed in November 2006 and the equipment was installed and tested by M/s Stirling Cryogenics India Pvt. Ltd. in November–December 2006. The Liquid Nitrogen facility was inaugurated by Prof. N.K. Ganguly, DG, ICMR on 10 January 2007. On this occasion Prof. Ganguly addressed the staff of NIMR and congratulated the Director for his sincere efforts for creating such facility for the scientists of NIMR and other institutes.

8.9 राष्ट्रीय मलेरिया अनुसंधान संस्थान में राजभाषा विकास संबंधी गतिविधियाँ

संस्थान में राजभाषा अधिनियम के अनुपालन की दिशा में वर्ष दर वर्ष प्रगति दृष्टिगोचर हुई है। इसी क्रम में वर्ष 2006-07 में एक ओर जहाँ राजभाषा अधिनियम के अनुपालन के उद्देश्य से संस्थान में राजभाषा अधिनियम-पत्राचार, प्रशासन संबंधी कार्य, आदि में राजभाषा की स्थिति की समीक्षा हेतु तिमाही बैठकें आयोजित की गई, प्रोत्साहन योजना लागू की गई। वहीं दूसरी ओर विज्ञान दिवस मनाए जाने के साथ ही हिन्दी पखवाड़े के अवसर पर विभिन्न गतिविधियाँ आयोजित की गई जो कि वर्ष की मुख्य गतिविधियाँ रही। उल्लेखनीय है कि इस वर्ष संस्थान में हिन्दी पखवाड़ा दिनांक 14 से 29 सितम्बर 2006 तक पूरे हर्षोल्लास के साथ मनाया गया। हिन्दी पखवाड़ा के अवसर पर हिन्दी कार्यशाला, टिप्पण-प्रारूपण प्रतियोगिता, निबन्ध प्रतियोगिता, कर्मचारियों और अधिकारियों के

लिए पृथक-पृथक वाद-विवाद प्रतियोगिताओं का आयोजन किया गया। संबंधित प्रतियोगिताओं का आयोजन संस्थान की हिन्दी अधिकारी एवं राजभाषा कार्यान्वयन समिति के विभिन्न सदस्यों द्वारा किया गया।

हिन्दी पखवाड़े की प्रथम गतिविधि टिप्पण-प्रारूपण प्रतियोगिता का संचालन संस्थान के सहायक अनुसंधान अधिकारी श्री आर. एन. यादव द्वारा किया गया। पखवाड़े की दूसरी गतिविधि हिन्दी कार्यशाला का आयोजन दिनांक 18.9.2006 को पूर्वान्ह 10 बजे किया गया। यह कार्यशाला संस्थान के प्रशासन वर्ग के कर्मचारियों के लिए आयोजित की गई थी जिसमें संस्थान के निदेशक महोदय ने भी भाग लिया था। संस्थान के वरिष्ठ प्रशासनिक अधिकारी के संचालन में इस पूर्णकालिक कार्यशाला में विभिन्न विषयों पर व्याख्यान हेतु विभिन्न मंत्रालयों के उच्चाधिकारियों को आमंत्रित किया गया था। इसी क्रम में दिनांक 20.9.2006 को पखवाड़े की तीसरी गतिविधि निबन्ध प्रतियोगिता का आयोजन एवं संचालन

डॉ. चन्द्र प्रकाश बत्रा, सहायक निदेशक द्वारा किया गया। प्रतियोगिता का विषय था— "बालश्रम: एक विडम्बना एवं उपाय" अथवा "आतंकवाद: एक स्थायी हल"। दिनांक 22.9.2006 को अपराह्न 2 बजे कर्मचारी वर्ग के लिए संस्थान के सहायक निदेशक डॉ. नूतन नंदा के संचालन में आयोजित वाद-विवाद प्रतियोगिता का विषय था—"दीर्घ आयु: वरदान या अभिशाप"। इस पखवाड़े के दौरान उल्लेखित गतिविधियों के अलावा दिनांक 29 सितम्बर 2006 को डॉ. भूपेन्द्र नागपाल, उपनिदेशक ने एक और गतिविधि अर्थात् वाद-विवाद प्रतियोगिता (अधिकारी वर्ग) का आयोजन किया जिसमें संस्थान के प्रशासनिक एवं विज्ञानीय अधिकारियों ने भाग लिया। प्रतियोगिता का विषय था—"कोल्ड ड्रिंक: कितना सही कितना गतल"।

वाद-विवाद प्रतियोगिता की समाप्ति के पश्चात् अपराह्न 4 बजे से पुरूस्कार वितरण समारोह का आयोजन किया गया जिसमें मुख्य अतिथि के रूप में श्री एम.एल. गुप्ता (संयुक्त सचिव), गृह मंत्रालय को आमंत्रित किया गया था। अतिथिगणों के स्वागत समारोह के पश्चात् माननीय श्री एम.एल. गुप्ता को संस्थान के निदेशक महोदय द्वारा शॉल भेंट कर सम्मानित किया गया। तत्पश्चात् श्री एम.एल. गुप्ता ने उपस्थित वैज्ञानिकों एवं समस्त अधिकारियों व कर्मचारियों को अपना सरकारी कामकाज राजभाषा हिन्दी में करने का अनुरोध करने के साथ ही मातृभाषा एवं राजभाषा के संबंध में विस्तृत जानकारी दी। साथ ही संस्थान के निदेशक ने सभी को संबोधित करते हुए कहा कि संस्थान में आयोजित गतिविधियों, प्रतियोगिताओं आदि को आयोजित करने का मूल उद्देश्य राजभाषा हिन्दी में कार्य को बढ़ावा देना है। उन्होंने मुख्य अतिथि महोदय को संस्थान से प्रकाशित होने वाले प्रकाशनों की जानकारी प्रदान की और अंत में कहा कि यदि आप में राजभाषा में कार्य करने का उत्साह है तो सफलता है, नहीं है तो सिर्फ बहाने हैं।

मुख्य अतिथि महोदय एवं निदेशक महोदय के संबोधन के पश्चात् पूरे सप्ताह के दौरान आयोजित विभिन्न प्रतियोगिताओं के पुरूस्कारों की घोषणा की गई। इसमें सर्वप्रथम संस्थान में हिन्दी में अधिकाधिक कार्य करने हेतु लागू वर्ष 2005-06 की प्रोत्साहन योजना के पुरूस्कार निदेशक महोदय प्रो. ए.पी. दाश के कर-कमलों द्वारा श्री रामदेव, अनुभाग अधिकारी, श्री के.सी. सेहरा, श्री मोहन सिंह विष्ट, श्री राघवेन्द्र शर्मा, श्रीमती मोनिका मल्होत्रा, श्री गोरी दत्त, श्री रमेश चन्द्र बुधोड़ी और श्री मोहन लाल को प्रदान किए गए। इसके अलावा हिन्दी में अधिकाधिक डिक्टेशन देने वाले अधिकारी का पुरूस्कार श्री जय प्रकाश वर्मा, वरिष्ठ प्रशासनिक अधिकारी को प्रदान किया गया। इसके बाद निबंध प्रतियोगिता का प्रथम

पुरूस्कार श्री रमेश झंडवाणी, द्वितीय पुरूस्कार श्री यू. श्रीहरि, तृतीय पुरूस्कार श्री दानसिंह सोंटियाल और प्रोत्साहन पुरूस्कार श्री जी.एल. पुरी व श्री एम.डी. तिवारी को निर्णायक के रूप में पधारे डॉ. विचार दास जी के कर-कमलों द्वारा वितरित किए गए।

इसके साथ ही टिप्पण-प्रारूपण प्रतियोगिता के पुरूस्कारों की घोषणा निदेशक प्रो. ए.पी. दाश द्वारा की गई जिसमें प्रथम पुरूस्कार श्री के.सी. सेहरा, द्वितीय पुरूस्कार श्री जी.एल. पुरी एवं तृतीय पुरूस्कार श्रीमती वीना और प्रोत्साहन पुरूस्कार श्री सुनील कुमार गुप्ता व श्री जय कुमार वी को डॉ. आर.सी. धीमान, वरिष्ठ उपनिदेशक के कर-कमलों द्वारा प्रदान किए गए।

तत्पश्चात् दिनांक 22 सितम्बर 2006 को आयोजित वाद-विवाद प्रतियोगिता (कर्मचारी वर्ग) का प्रथम पुरूस्कार डॉ. पदमावती त्यागी, द्वितीय पुरूस्कार श्रीमती वीना, तृतीय पुरूस्कार श्री सत्यपाल सिंह और प्रोत्साहन पुरूस्कार श्री के.सी. सेहरा और पंकज सिंह को श्री जय प्रकाश वर्मा, वरिष्ठ प्रशासनिक अधिकारी द्वारा वितरित किए गए। इसके साथ ही सप्ताह के दौरान आयोजित अंतिम प्रतियोगिता अर्थात् वाद-विवाद प्रतियोगिता (अधिकारी वर्ग) का प्रथम पुरूस्कार डॉ. के. राघवेन्द्र, द्वितीय पुरूस्कार श्रीमती रेखा सक्सेना, तृतीय पुरूस्कार डॉ. आर.सी. धीमान और प्रोत्साहन पुरूस्कार श्री जय प्रकाश वर्मा और श्री प्रवीण कुमार अतुल को निदेशक महोदय के कर-कमलों द्वारा वितरित किए गए। अंतत: कार्यक्रम का विधिवत् समापन करने हेतु संस्थान के वरिष्ठ प्रशासनिक अधिकारी श्री जय प्रकाश वर्मा ने सभी संचालकों को धन्यवाद ज्ञापित किया। यहाँ यह बताना भी प्रासंगिक होगा कि संस्थान में ही नहीं अपितु निदेशक महोदय द्वारा विभिन्न क्षेत्रीय इकाइयों में हिन्दी दिवस मनाए जाने संबंधी निर्देश का पालन करते हुए जबलपुर, गोआ, बैंगलोर और सोनापुर में भी हिन्दी दिवस पर विभिन्न प्रतियोगिताएं आयोजित की गई। राष्ट्रीय मलेरिया अनुसंधान संस्थान, क्षेत्रीय इकाई जबलपुर में भी हिन्दी दिवस पूरे उत्साह के साथ मनाया गया। इस उपलक्ष्य में निबंध प्रतियोगिता एवं वाद-विवाद प्रतियोगिता का आयोजन किया गया तथा क्षेत्रीय इकाई गोवा में भी दिनांक 14 सितम्बर 2006 को हिन्दी दिवस पूरे उल्लास के साथ मनाया गया तथा निबंध एवं भाषण प्रतियोगिता का आयोजन किया गया। ठीक इसी प्रकार क्षेत्रीय इकाई सोनापुर, असम में दिनांक 6 नवम्बर 2006 को हिन्दी दिवस बड़े उत्साह के साथ मनाया गया। इस उपलक्ष्य में एक हिन्दी कार्यशाला एवं विभिन्न प्रतियोगिताओं का आयोजन किया गया जिसके अंतर्गत निबंध प्रतियोगिता और कर्मचारी एवं अधिकारी वर्ग के लिए पृथक-पृथक भाषण प्रतियोगिताओं का आयोजन किया गया। संस्थान की क्षेत्रीय इकाई, बैंगलोर में भी दिनांक 21 सितम्बर 2006 को हिन्दी दिवस के उपलक्ष्य में विभिन्न प्रतियोगिताओं का आयोजन किया गया।

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