Annual Report

2009–10

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Preface

I am extremely happy in presenting the Annual Report of NIMR for the year 2009–10. The year was characterised by developments on all fronts.

In the reported year, we published more than 50 papers in reputed journals. The Institute continued to get extramural grants from various national and international agencies and also from World Bank, World Health Organization, National Vector Borne Disease Control Programme (NVBDCP), Department of Biotechnology, Narmada Valley Development Authority, etc. This is apart from various research schemes of ICMR.

The Institute is also actively engaged in translational research. This has been evidenced by various activities which have gone to the programme earlier. NIMR scientists are part of various committees taking policy decisions as regards malaria control. Based on the results of the Phase III trial conducted by NIMR, a new fixed dose combination for the treatment of falciparum malaria was registered by the Drugs Controller General of India. During this period, NIMR has been designated as the National Pharmacovigilance Centre for malaria by the CDSCO/DCGI. The NIMR has always been involved in manpower development.

The central facilities of the Institute were strengthened. NIMR has a national repository, the Malaria Parasite Bank that has more than 1000 isolates. Malaria Clinic was started in the new campus. The clinic is well attended. We also have four insectaries meant for different purposes; starting from biology of mosquito vectors to insecticide resistance studies. The central instrumentation facility is well-equipped. Our Publication Division continued the publication of the periodicals *Journal of Vector Borne Diseases*, *Plasmodium*, *Malaria Patrika* and various other publications. We also published the guidelines for diagnosis and treatment of malaria in India, which were prepared in a brainstorming meeting supported by WR India.

The Institute also continued the activity of human resource development. Many fellows are pursuing their doctoral studies at NIMR. We organised trainings for Health Officials, Vector Borne Disease Consultants, District Programme Officers, Medical Officers, Private Practitioners, Laboratory Technicians, etc.

The construction of animal house building has started in the new campus. Laboratory furniture has been installed in all the laboratories.

We organised the XI International Symposium on vector and vector borne diseases in collaboration with Goa University. This was a good opportunity for researchers to strengthen networking with those working in the field of vector borne diseases.

Dr VK Dua
Director Incharge

Vector Biology and Control

1

1.1 Studies on anopheline species complexes

1.1.1 Fluviatilis and Minimus complexes

Distribution and bionomics

In continuation of mapping the distribution of the members of *Anopheles fluviatilis/An. minimus* complexes and study of their bionomics in unexplored districts, surveys were carried out during 2009–10 in malaria endemic and tribal dominated districts, namely Gumla, Simdega, West Singhbhum (Jharkhand state); Surguja, Dantewada and Jagdalpur, Bastar (Chhattisgarh state) and Deogarh, Keonjhar and Mayurbhanj (Orissa state) (Fig. 1).

Field surveys were carried out in the above mentioned districts to select areas with prevalence of *An. fluviatilis/An. minimus* and for collection of the mosquitoes for different entomological parameters. In each selected district, Primary Health Centre (PHC) was considered as unit of study.

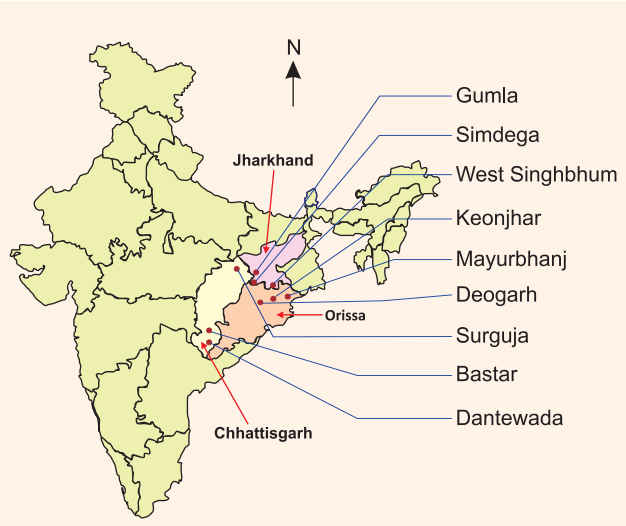

Fig. 1: Districts selected for studies on *An. fluviatilis* and *An. minimus* sibling species.

Villages in each PHC were selected keeping in view the physiogeographic features/ecosystem and epidemiological situation. In each district, surveys were carried out during transmission period after consulting local health authorities. *Anopheles fluviatilis/An. minimus* collected from different resting habitats were processed for various parameters, viz. sibling species composition, feeding preference, incrimination, insecticide susceptibility studies, etc. In study areas where *An. culicifacies* was found co-existing with *An. fluviatilis/An. minimus,* information on the above mentioned parameters was also generated with respect to *An. culicifacies* sibling species in order to establish relative role of all vector species in malaria transmission.

Vector mosquitoes collected were identified to sibling species using cytotaxonomic and molecular techniques. Feeding preference of sibling species was analyzed by counter current immunoelectrophoresis using human and bovine antisera. Incrimination of collected mosquitoes was done at sibling species level by enzyme-linked immunosorbant assay (ELISA) with *P. falciparum* and *P. vivax* 210 and 247 monoclonal antibodies. Insecticide susceptibility tests were also carried out in few districts following standard WHO procedure. Observations made so far in selected districts of three states are summarized below.

Jharkhand

Districts Gumla, Simdega and West Singhbhum were selected which are endemic for malaria with annual parasite incidence (API) of 7.03, 17.67 and 22.67 respectively in 2009. Analysis of *An. fluviatilis* populations from the study areas (having hilly terrain and dense forest) revealed prevalence of *An. fluviatilis* species T in these districts which was found polymorphic for q^1 inversion. Species T

was collected from cattlesheds/mixed dwellings and was primarily zoophagic as revealed by blood meal source analysis.

Chhattisgarh

Chhattisgarh, carved out of Madhya Pradesh has 44% of the total area under forest cover. Of the districts selected, Dantewada has the maximum area under forests (64.2%) followed by Bastar (53.7%) and Surguja (45.7%) with tribal population comprising 79, 67 and 57% respectively. The study areas were selected in hilly forested terrain of the districts. In District Surguja, *An. fluviatilis* species T was found prevalent which was primarily zoophagic and polymorphic for q^1 inversion, whereas foci of *An. fluviatilis* species S were discovered in dense forest areas of Districts Dantewada and Bastar where this species was sympatric with polymorphic species T and was found to be highly anthropophagic with human blood index (HBI) of >0.9. In District Bastar (Jagdalpur), two specimens of Fluviatilis S were also found positive for *P. falciparum* circumsporozoite antigen. In study areas of these districts, *An. culicifacies* co-existed with *An. fluviatilis* and comprised of species B and C. In District Dantewada, *An. culicifacies* outnumbered *An. fluviatilis* and was prevalent in high densities with the predominance of species C. *Anopheles culicifacies* species C was also incriminated (positive for *Pv* 210 antigen) in District Dantewada.

Orissa

Districts Deogarh, Keonjhar and Mayurbhanj selected in Orisa state are highly endemic for malaria with API ranging from 8.97 to 31.67 and *P. falciparum* cases constitute >90% of total malaria cases. These districts have 35–50% of the total area under forest cover. In these districts, the study sites were selected in hilly forest biome traversed with streams and stream channels, which support breeding of *An. fluviatilis*. Observations revealed that *An. fluviatilis* species S was predominant in study areas of these districts. Species S was found resting mainly in human dwellings and was highly anthropophagic. In District Deogarh, this species was found susceptible to DDT (4%) and malathion (5%) as revealed from susceptibility tests carried out. In District Keonjhar, *An. minimus* species A was also encountered in low numbers and was found highly anthropophagic, resting mainly in human dwellings. Incrimination studies showed sporozoite positive (*Pf*) specimens belonging to species S in Districts Deogarh and Keonjhar, which conclusively proves *Fluviatilis* S as highly efficient malaria vector. The study is in progress.

1.1.2 Ecological succession of anopheline and other mosquitoes in north-eastern states of India

A study was initiated during 2010 in 26 selected districts of Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura to carry out faunastic survey and ecological succession of *Anopheles* species and other mosquitoes with the objective of quantification of vector scenario in terms of changing biophysical, climatic and socioeconomic situation and updation of anopheline species knowledge base of north-eastern states.

First survey was carried out from 25 March to 7 May 2010 in seven districts of Assam—Sonitpur, Nagaon, Lakhimpur, Golaghat, Dibrugarh, Goalpara and Kamrup and two districts of Meghalaya—East Garo Hills and East Khasi Hills. Both adult and immature mosquitoes were collected from different habitats by using standard WHO techniques. Analysis of collected mosquitoes is in progress.

1.2 Vector-Parasite interactions

1.2.1 Transcriptional abundance and expression analysis of NOS by real time PCR in refractory *Anopheles culicifacies* species B

The mosquito immune system appears to play a fundamental role in the upregulation of nitric oxide gene known to contribute to parasite killing in malaria vector *An. culicifacies* and renders this mosquito as refractory. Our data suggest that mosquito cellular immune system may affect refractoriness via AcNOS pathway which may be an additional effector gene in addition to prophenoloxidase pathways to block the development of the malaria parasite in *An. culicifacies* mosquitoes and thus may elucidate a novel putative mechanism of refractoriness.

Since it is not possible to correlate *An. culicifacies* species A and *An. culicifacies* species B using PCR as both the species were found to be identical in terms of sequence homology of PCR amplicons, the relative expression level of AcNOS transcript in both *P. vivax* infected *An. culicifacies*

species A and *An. culicifacies* species B was analyzed by real time PCR. Real time RT-PCR was performed using SYBR Green RT-PCR kit (Roche Diagnostics, USA) and Light Cycler 480 system (Roche Diagnostics, USA) to measure the relative transcript levels of AcNOS in *An. culicifacies* species A and *An. culicifacies* species B mosquitoes. cDNA of both the species was reverse-transcribed from 500 ng total RNA using oligo (dT) primer and transcriptor reverse transcriptase (Roche), following the manufacturer's instructions. The NOS primer forward sequence of exon 1 region (5′ ATGAGGACCAACTATCGGG3′) and reverse sequence (5′ GCCTTGGTGACAATGCTC 3′) and Normalizer gene *S7 RNA* polymerase having forward primer sequence 5′ GGTGTTCGGTTCCAAGGTGA 3′ and reverse primer sequence 5′ GGTGGTCTGCTGGTTCTTATCC 3′.

The fluorescence acquisition temperature was 72°C for all genes. Amplification specificity was further validated by melting curve analysis, generated at the end of each PCR reaction (Figs. 2a & b). The threshold cycles (Ct) were recorded for AcNOS and S-7 amplicons during each experiment.

Data analysis of real time PCR was carried out by ΔΔCt method, which relies on comparing differences in Ct, values. The Ct, in which there is first detectable increase in fluorescence, serves as a tool for calculation of the starting template amount in each sample. Ct values were determined for the internal control (i.e. *S7 RNA* polymerase gene) and target genes (*NOS*) of the samples. Each target gene of a sample was then normalized in turn of its *S7 RNA* polymerase gene by computing a ΔCt value ($\Delta Ct_{gene} = Ct_{NOS\ gene} - Ct_{S7}$). The minimum ΔCt value (ΔCt value min) among the samples (both species A and species B) was used to calculate ΔΔCt values for each sample, which represents the difference between ΔCt value of a sample and minimum ΔCt value (ΔΔCt value = ΔCt $value_{sample}$ – ΔΔCt $value_{min}$). Negative of ΔΔCt value for each sample was taken and $2^{-\Delta\Delta Ct\ value}$ was plotted against each treatment; the plot represents the relative abundance of the gene with respect to each sample with lowest ΔCt value.

The Ct values show that NOS transcript in midgut tissue was 26.34 for *An. culicifacies* species A and 21.51 for *An. culicifacies* species B. The lower Ct value of NOS for species B indicates that this gene reaches the detection threshold with less

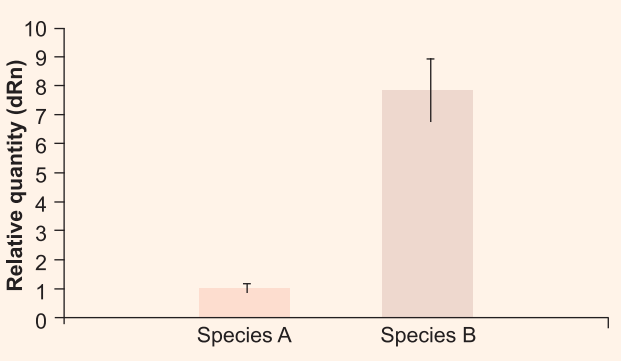

Fig. 3: Real time PCR assays: Expression pattern of NOS in midgut of *An. culicifacies* sp A and sp B (n = 25–30). Relative transcript abundance of AcNOS in *P. vivax* blood infected susceptible (species A) and refractory strain (species B). Gene transcript quantity was measured by relative RT-PCR using the internal standard *S7 RNA* polymerase gene. Error bars represent standard deviation from three independent experiments shown.

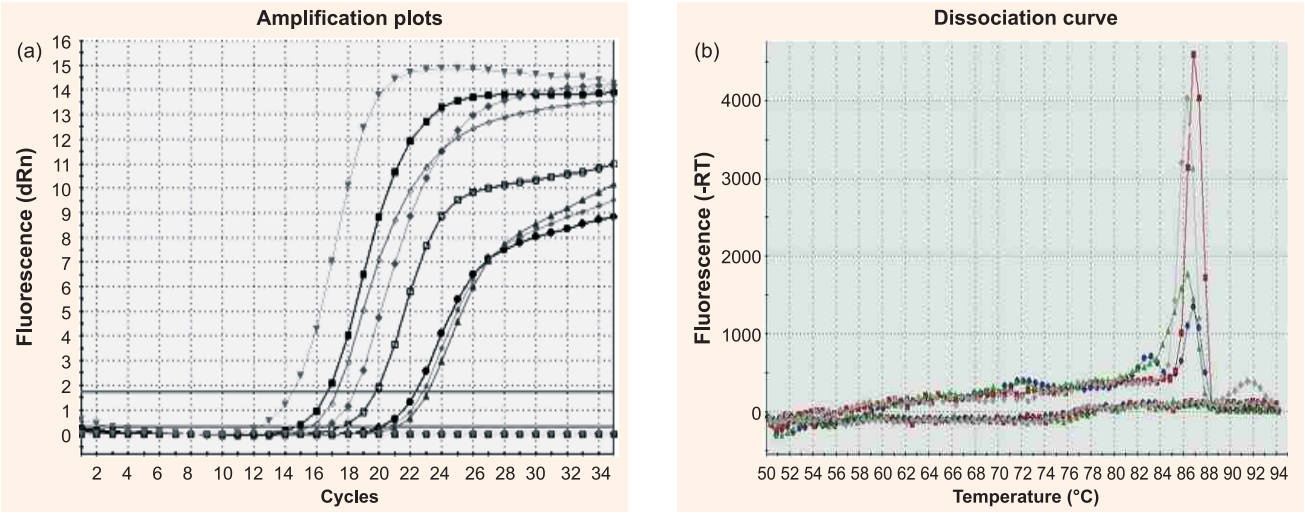

Fig. 2: Real time PCR: (a) Dissociation curve—Amplification plot for *NOS* gene of both species A and B and *S7 RNA* polymerase internal control gene; (b) Melting curve analysis, generated at the end of each PCR reaction. All genes (nitric oxide synthase, *S7 RNA* polymerase) presented single peak in the curve, which indicates absence of primer dimer formation during the reaction.

amplification cycles than species A indicating that this NOS is more abundant in the midgut of species B (Fig. 3). Ct values for reference gene *S7 RNA* polymerase was 17.47 and 17.26 for species A and species B respectively. The 24 hour post-feeding upregulation of AcNOS expression coincided with microscopic observation of encapsulated *Plasmodium* ookinetes in the midgut of the refractory strain.

1.2.2 Proteomic approach to the identification of salivary gland proteins from *Anopheles stephensi*

Mosquito salivary glands are specialized in the production of a mixture of molecules that facilitate blood feeding by the lubrication of mouth-parts and the inhibition of hemostasis. The salivary gland proteins are relevant for malaria research since the *Plasmodium* sporozoites invade the salivary glands and are injected with the saliva into vertebrate hosts during blood feeding. Main objective of the study was to identify and characterize the salivary gland proteomes from *An. stephensi* and functional annotation of salivary gland proteomes through a detailed bioinformatics analysis and data analysis by MS.

Mosquito salivary glands were isolated from mosquitoes and the proteins separated by 1D-PAGE after cell lysis. The gels were silver stained; bands were cut from the gel and subsequently digested using trypsin. The bands were analyzed by nanoLC-ESI-QTOF-MS/MS on a Bruker microTOF-Q II system. Raw data were converted into MGF format subsequently. All data were transferred into the ProteinScape database system for further data analyses. Database searches were performed on a MASCOT server using var. *propionamide* modification on *Cys* for the gel band derived samples and var. *Met* oxidation for all samples. All searches were performed as decoy searches; a minimum score of 30 for at least one peptide was required for proteins to be reported (Fig. 4).

In this experiment at least 11 mosquito proteins specific for salivary glands could be identified (Table 1). Surprisingly, not only mosquito-originating proteins were identified but also proteins originating from the nematode parasite *Brugia malayi*, which were not expected in the sample. This parasite causes the disease Elephantiasis and the combination of 1D-PAGE and nLC-MS/MS analysis of the salivary glands allowed the identification of the parasite infection in the mosquitoes. Since the accidental identification of several different proteins from a known nematode parasite can be regarded as quite unlikely, the identification of the *B. malayi* infection of the mosquitoes can be regarded as a reliable.

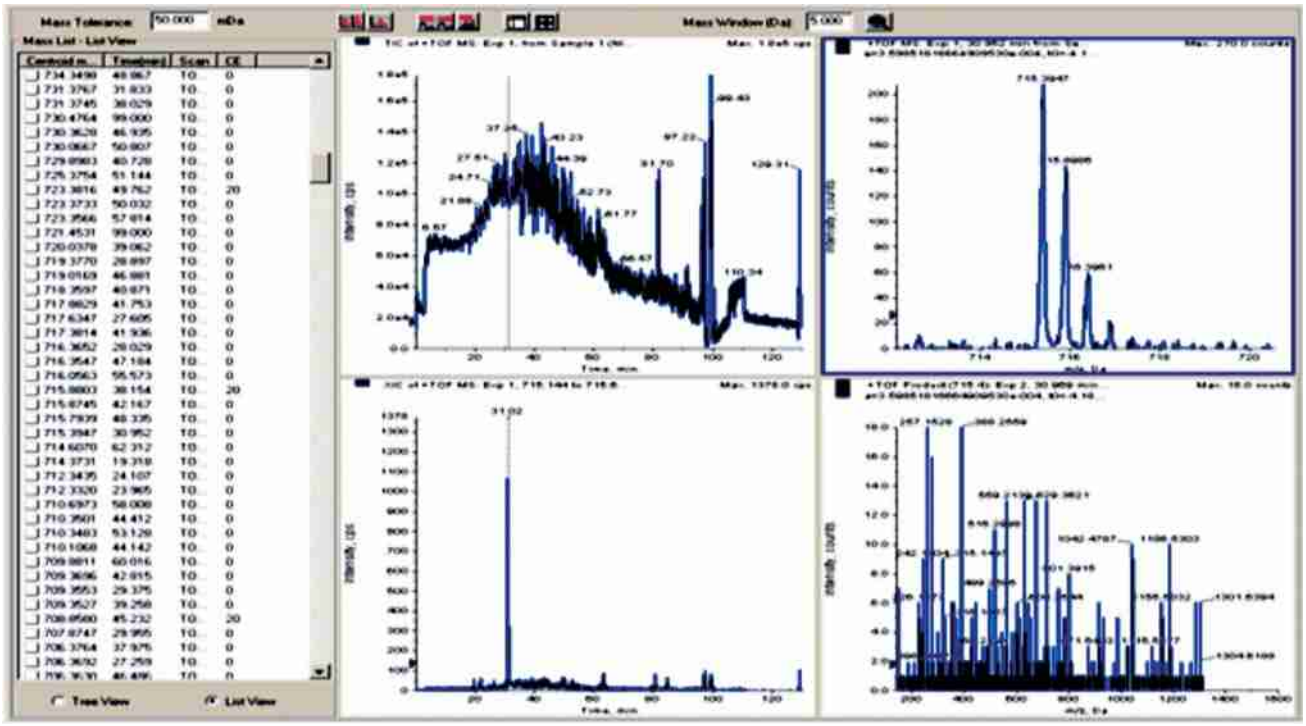

Fig. 4: Example of datasets from different origin shows insoluble digest analyzed using ABI QStar Elite.

Table 1. Proteins identified from the combined datasets. Proteins labeled in green can be assigned to mosquito salivary glands. The proteins marked in yellow originate from *B. malayi*

No.	Accession	Protein	MW (kDa)	Scores	Peptides	Seq. cov. (%)
1	gi|158299190	AGAP010147-PA [*Anopheles gambiae* str. PEST]; myosin heavy chain	224.2	1017	24	13
2	gi|37201975	GE rich salivary gland protein [*Anopheles stephensi*]	28.5	799	14	29
3	gi|15718081	D7 protein [*Anopheles stephensi*]	36.4	587	14	34
4	gi|157136033	ATP synthase beta subunit [*Aedes aegypti*]	53.9	516	10	21
5	gi|29501536	SG1D salivary protein precursor [*Anopheles stephensi*]	46.8	439	10	24
6	gi|157131823	tropomyosin invertebrate [*Aedes aegypti*]	31	396	8	26
	gi|157111829	actin [*Aedes aegypti*]	41.6	293	8	24
7	gi|27372911	salivary apyrase [*Anopheles stephensi*]	64.2	259	5	8.5
8	gi|31241663	AGAP001799-PA [*Anopheles gambiae* str. PEST]	32.5	250	5	17
9	gi|27372941	putative salivary protein SG1C [*Anopheles stephensi*]	44.3	208	5	11
10	gi|158285914	AGAP007295-PA [Anopheles gambiae str. PEST]	97.5	109	3	3
11	gi|27372939	putative salivary protein SG1A [*Anopheles stephensi*]	19.7	108	2	11
12	gi|158296846	AGAP008279-PA [*Anopheles gambiae* str. PEST]	36.8	79	2	8
13	gi|170037149	apoptosis inhibitor [*Culex quinquefasciatus*]	61.2	75	1	2
14	gi|114864734	G1 family long form salivary protein 3 [*Anopheles funestus*]	31.1	72	2	5
15	gi|27372895	salivary antigen-5 related protein [*Anopheles stephensi*]	29	63	2	8
16	gi|27372929	putative salivary protein SG1B [*Anopheles stephensi*]	48.1	60	2	4
17	gi|29501376	short D7-4 salivary protein precursor [*Anopheles stephensi*]	18.4	60	1	7
18	gi|118790507	AGAP009607-PA [*Anopheles gambiae* str. PEST]	96.7	58	2	1
19	gi|157134034	zinc finger protein [*Aedes aegypti*]	85.5	55	1	1
20	gi|158288874	AGAP000403-PA [*Anopheles gambiae* str. PEST]	19.8	53	1	6
21	gi|157126644	hypothetical protein AaeL_AAEL010587 [*Aedes aegypti*]	65.5	53	2	2
22	gi|170583521	hypothetical protein [*Brugia malayi*]	223.6	53	2	1
23	gi|157125428	hypothetical protein AaeL_AAEL001925 [*Aedes aegypti*]	51.9	48	1	2
24	gi|170038422	conserved hypothetical protein [*Culex quinquefasciatus*]	35.4	48	2	8
25	gi|158291035	AGAP002399-PA [*Anopheles gambiae* str. PEST]	70.9	45	1	1
26	gi|170591366	hypothetical protein Bm1_44895 [*Brugia malayi*]	128.8	43	1	0.6
27	gi|170589850	Abnormal embryonic partitioning of cytoplasm protein 3, isoform b [*Brugia malayi*]	119.2	42	1	1
28	gi|170055670	GM130 [*Culex quinquefasciatus*]	105.6	41	1	1
29	gi|170585830	SAP domain containing protein [*Brugia malayi*]	122.5	39	1	1
30	gi|170050108	phosphoenolpyruvate carboxykinase [*Culex quinquefasciatus*]	71.4	38	1	1
31	gi|170055800	conserved hypothetical protein [*Culex quinquefasciatus*]	47.4	38	1	2
32	gi|158287643	AGAP004077-PA [*Anopheles gambiae* str. PEST]	59.8	37	1	2
33	gi|29501528	TRIO salivary gland protein precursor [*Anopheles stephensi*]	43.7	36	1	2
34	gi|158301490	AGAP001895-PA [*Anopheles gambiae* str. PEST]	137.8	34	1	1
35	gi|58391886	AGAP009833-PA [*Anopheles gambiae* str. PEST]	30.7	34	1	3

1.3 Vector Control

1.3.1 Field evaluation of Biodart-M, an aqueous suspension of *Bacillus thuringiensis* var. *israelensis* serotype H-14 against larvae of mosquito vectors

A formulation of Biodart-M Aqueous was evaluated to determine its toxicity in laboratory bioassays against late III instar of *An. stephensi* (malaria vector), *Cx. quinquefasciatus* and *Ae. aegypti* (dengue vector) using standard protocol. LC_{50} of *An. stephensi, Cx. quinquefasciatus* and *Ae. aegypti* were 0.1002, 0.925 and 0.0381 respectively and LC_{90} were 0.4287, 0.3323 and 0.0923 respectively (Fig. 5).

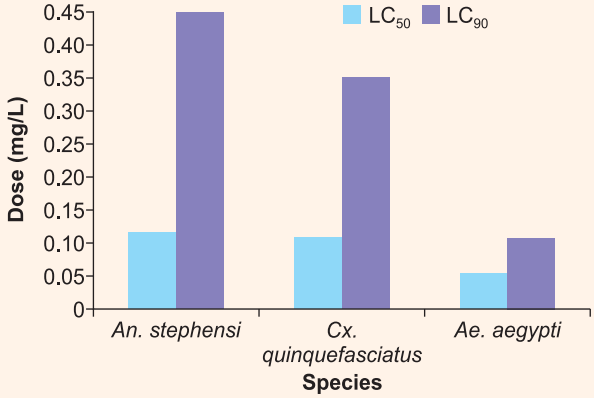

Fig. 5: Laboratory bioassay: Percent mortality of Biodart-M (Aqueous suspension) against mosquito larvae.

Small-scale field trials (Phase II) with Biodart-M aqueous was carried out in NCR, Delhi in the respective breeding habitats of *An. stephensi* and *Cx. quinquefasciatus* in clean and moderately polluted water in tanks (Fig. 6). Effective dose and frequency of application in the field was determined

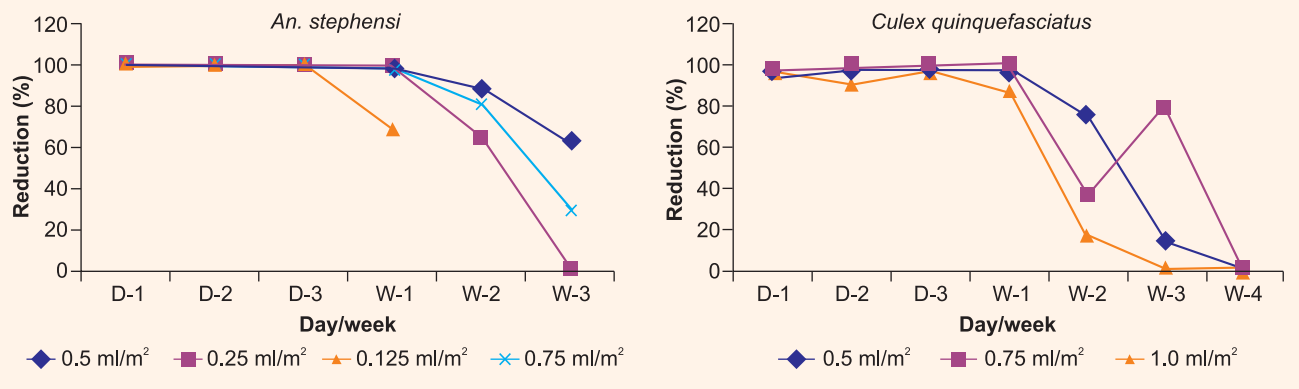

Fig. 6: Phase II—Field evaluation of Biodart-M (aqueous suspension formulation) against different mosquito species in Delhi NCR.

for Phase III trial. The percent reduction was >80% at dose of 0.5 ml/m^2 against anophelines for two week and one week against *Cx. quinquefasciatus*.

Phase III trial with Biodart-M aqueous was initiated in urban/peri-urban areas of NCR-Delhi, Raipur and Bengaluru. One square kilometer locality at each site was selected after ascertaining the breeding habitats. Dose of 0.5 ml/m^2 against anophelines in clean water and 0.75 ml/m^2 against *Cx. quinquefasciatus* in polluted water is being used for large-scale trial.

1.3.2 Phase III evaluation of Pyriproxyfen (Sumilarv 0.5G), an insect growth regulator against larvae of vector mosquitoes

Multicentric Phase III evaluation of pyriproxyfen, an IGR is being conducted at NCR Delhi, Hardwar (Uttarakhand) and Raipur (Chhattisgarh) (Figs. 7–9). Application was done at the doses of 200 and 400 mg/m^2 in clean and polluted water respectively. In addition to monitoring of immature density, the samples of pupae were collected from selected index habitats for emergence in laboratory conditions and percentage

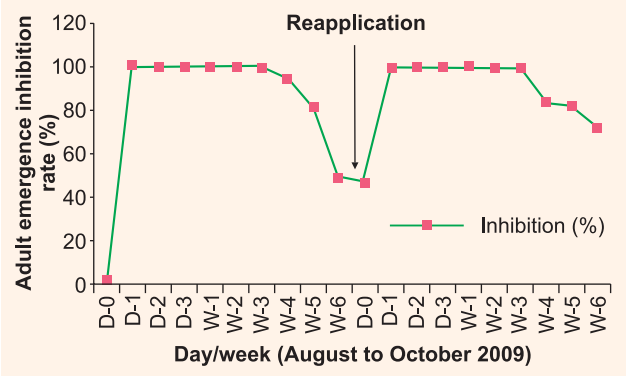

Fig. 7: Adult emergence inhibition in samples of III/IV instar larvae and pupae collected from cement tanks applied with Pyriproxyfen 0.5% Gr @ 200 mg/m^2 (*An. stephensi* and *An. subpictus*) in Delhi-NCR.

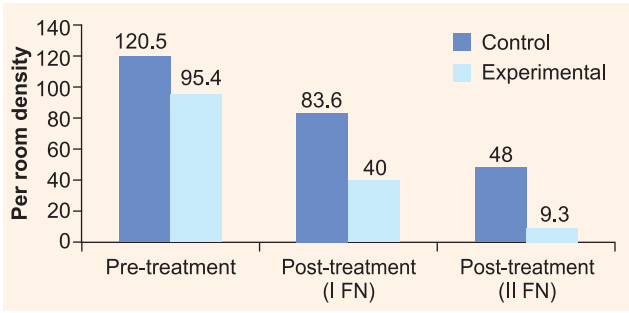

Fig. 8: Per room density of mosquitoes in control and experimental areas of Laksar PHC, Hardwar applied with pyriproxyfen.

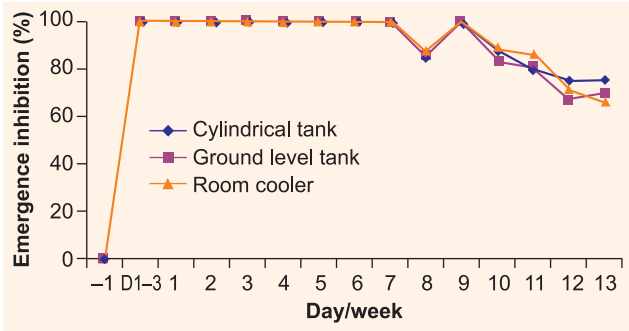

Fig. 9: Adult emergence inhibition in samples of III/IV instar larvae and pupae collected from cylindrical tanks, ground level tanks and room coolers applied with Pyriproxyfen 0.5% Gr @ 200 mg/m^2 (*An. stephensi*) in Raipur.

of adults emerged was calculated.

Results showed that pyriproxyfen was effective for minimum 5 weeks after application in clean water habitats, when applied @ 200 mg/m^2.

1.3.3 Evaluation of Icon®Life, 0.2% deltamethrin-treated LLIN against malaria vectors and disease transmission in District Gautam Budh Nagar, Uttar Pradesh

The present study on evaluation of Icon®Life 0.2% deltamethrin incorporated polyethylene nets (LLIN) against malaria vectors was undertaken in three endemic areas, District Gautam Budh Nagar (Uttar Pradesh), District Sundargarh (Orissa), and

District Karbi-Anglong (Assam), following common protocol for uniform evaluation of insecticides for use in vector control.

The study in District Gautam Budh Nagar, Uttar Pradesh was initiated in the month of May 2008 in three villages of Dadri PHC located with similar endemicity and mosquito productivity, namely Gulawati Khurd (Icon Life LLIN), Nangla Chamru (untreated net) and Nangla Nainsukh (no net). The Icon Life nets were found to be wash-resistant and bioavailability of insecticide on net fibre was effective in achieving >90% knockdown and almost 100% mortality in *An. culicifacies* even after 20 washes (Table 2). Even under field conditions the used LLIN after one year showed >90% knockdown and almost 100% mortality (Table 3). Results revealed that Icon Life nets proved to be effective personal protection tool against *An. culicifacies*. The intervention with Icon Life LLIN resulted in significant reduction in the overall entry rate of mosquitoes in houses with Icon Life nets and were found to be effective in controlling indoor resting density (Table 4) of *An. culicifacies*, the major malaria vector spp. in this area, during the post-intervention period as compared to the control (untreated net and no net) villages. The study also revealed a decline in malaria prevalence during the post-intervention period in the Icon Life village as compared to the control untreated net and without net villages (Table 5). There was significant reduction in the malaria prevalence in the study population using Icon Life in comparison to untreated net and no net study population. The community compliance and acceptance was high and no adverse health effects were reported (Table 6).

Table 3. Bioefficacy of Icon®Life long-lasting net (LLIN) against *An. culicifacies* before and after one year of use in field

Cone bioassay

Net condition	No. of mosquitoes exposed	Knockdown (1 h) (%)	Mortality after 24 h (%)
Before use (August 2008)	50	100	100
After one year of use in field (August 2009)	80	91.3	100

Ring-net bioassay

Net condition mosquitoes	No. of tested	Time for knockdown of mosquito (min)		
		1st	6th	11th
Before use (August 2008)	11 × 6	3.5	5.3	6.3
After one year of use in field (August 2009)	11 × 20	5.0	7.5	10.2

Table 2. Wash-resistance and bioefficacy of Icon® Life LLIN against *An. culicifacies* in cone bioassays after 3 min of exposure, in Dadri PHC District Gautam Budh Nagar, Western Uttar Pradesh

No. of washing	Washed IconLife net		Unwashed IconLife net		Untreated net	
	% Knockdown	% Mortality after 24 h	% Knockdown	% Mortality after 24 h	% Knockdown	% Mortality after 24 h
1	100	100	100	100	0	5.0
2	100	100	100	100	0	5.0
3	100	100	100	100	0	5.0
4	100	100	100	100	0	5.0
5	100	100	100	100	0	5.0
6	100	100	100	100	0	7.5
7	100	100	100	100	0	5.0
8	95	100	100	100	0	2.5
9	100	100	100	100	0	10.0
10	100	100	97.5	100	0	5.0
11	97.5	100	100	100	0	2.5
12	100	100	100	100	0	5.0
13	95	100	95	100	0	5.0
14	97.5	100	100	100	0	5.0
15	95.0	100	100	100	0	5.0
16	97.5	100	95	100	0	0
17	97.5	100	100	100	0	2.5
18	100	100	100	100	0	5.0
19	95	100	100	100	0	5.0
20	95	100	100	100	0	0

10 mosquitoes were exposed in each of four replicates. Wild caught blood-fed mosquitoes were used in cone bioassays.

Table 4. Efficacy of Icon®Life LLIN on indoor resting density of malaria vector *An. culicifacies* and other mosquitoes in the study villages in Dadri PHC, Distt. Gautam Budh Nagar, Western Uttar Pradesh

Period	Mosquito species	Average per man hour density		
		Gulawati-Khurd (Icon Life-LLIN)	Nangla Chamru (untreated net)	Nangla Nainsukh (no net)
Pre-intervention May–July 2008	*An. culicifacies*	20.83	26.0	28.5
	Total anophelines	138.70	203.0	110.0
	All mosquitoes	269.30	327.0	422.0
Post-intervention August 2008–July 2009	*An. culicifacies*	3.6 (–82.7)	10.2 (–60.7)	20.2 (–29.6)
	Total anophelines	81.7 (–41.0)	95.3 (–53.5)	128.4 (16.72)
	All mosquitoes	134.4 (–50.1)	194.0 (–40.6)	325.0 (–22.9)

Figures in parentheses indicate percentages.

Table 5. Efficacy of Icon®life LLIN on malaria prevalence in the study villages in Dadri PHC, Distt. Gautam Budh Nagar, Western Uttar Pradesh

Village (type of intervention)	Pop.	Study period	BSE	Total malaria positive cases	SPR	SFR	PI (% reduction/increase)
Gulawati-Khurd (Icon Life- LLIN)	1,381	Pre-intervention May–July2008	44	11	25.0	0	7.96
		Post-intervention August 2008–July 2009	133	6	4.5	0	4.34 (–45.4)
Nangla Chamru (untreated net)	1,840	Pre-intervention May–July 2008	34	7	20.6	0	3.80
		Post-intervention August 2008–July 2009	128	9	7.0	0	4.89 (28.68)
Nangla NainSukh (no net)	1,337	Pre-intervention May–July 2008	42	7	16.6	0	5.23
		Post-intervention August 2008–July 2009	100	18	18.0	1	13.46 (157)

Figures in parentheses indicate percentages.

Table 6. Cross-sectional surveys among Icon®Life net users in experimental village in District Gautam Budh Nagar Uttar Pradesh for assessment of community perceptions on adverse effects and collateral benefits of long-lasting insecticide net

S. No.	Questions	Users (%) Total N = 100
1.	Do you know why mosquito nets are used?	100
2.	Do you or your family members use mosquito nets for personal protection of your family members?	15
3.	Do you use any indigenous method for mosquito control?	40
4.	Do you sleep inside the insecticide-treated Icon®Life for personal protection or your family members?	98
5.	*Perceived side effects*	
	Do you suffered any of the following:	
	–Skin irritation	2
	–Nausea	0
	–Vomiting	0
	–Itching	2
	–Headache	0
	–Drowsiness	0
	–Eye irritation	0.25
	–Difficulty in breathing	0
	–Any other	0
6.	Do you feel suffocation while sleeping inside net?	0
7.	Do you fear of poisoning for using Icon®Life net?	0
8.	*Perception about collateral benefits*	
	Reduction in mosquito bites	92
	Reduction in nuisance of housefly, cockroaches, etc.	80
9.	Do you recommend the use of Icon®Life net in future?	100

1.3.4 Extended Phase III evaluation of PermaNet® 2.0 against malaria vectors and disease transmission in District Gautam Budh Nagar, Uttar Pradesh

The study on Phase III evaluation of PermaNet® 2.0 against malaria vectors and disease prevalence in the endemic areas of District Gautam Budh Nagar, in western Uttar Pradesh and in tribal areas of Sundargarh district, Orissa was extended for two years after the initial trial period of one year.

In Gautam Budh Nagar, the trial was initiated in April/May 2007 in three villages with population of 1187, 1165 and 1337 which were randomly selected for the distribution of PermaNet 2.0 and controls with untreated and no net. The efficacy of PermaNet 2.0 as determined by cone bioassays which revealed >80% knockdown and >90% mortality after two years of field use, but the ring-net bioassays showed an increase in the median knockdown time (Table 7). The study revealed that even after two years of use there was a reduction in the man hour density (MHD) and parity rate of *An. culicifacies* in the PermaNet 2.0 village as compared to untreated net and no net villages (Fig. 10). Month-wise incidence of malaria in the experimental villages of PermaNet 2.0 are shown in Fig. 11. The study revealed a reduction in the prevalence of malaria in the PermaNet village during the post-intervention period in 2008 and 2009 (Fig. 11). The community acceptance was as

Table 7. Efficacy of PermaNet® 2.0 against *An. culicifacies* after different intervals of use in field

Cone bioassay

Period	Exposure time (min)	Knockdown (%) after 1 h	Motality (%) after 24 h
May 2007	3	100.0	100
August 2007	3	100.0	100
November 2007	3	100.0	100
August 2008	3	95.0	100
November 2008	3	95.0	95
April 2009	3	94.8	100

Ring-net bioassay

Period	Used/ unused net	Time for knockdown of mosquito (min)		
		1st	6th	11th
Aug–October 2007	Unused net	2.37	5.00	6.5
	Used net	3.30	5.40	7.2
Sept–October 2008	Unused net	3.00	5.00	7.0
	Used net	6.00	10.25	15.5
May 2009	Unused net	3.00	5.50	8.0
	Used net	5.50	8.50	10.0

PermaNet® 2.0 distributed to the villagers in May 2007 were used for these bioassays.

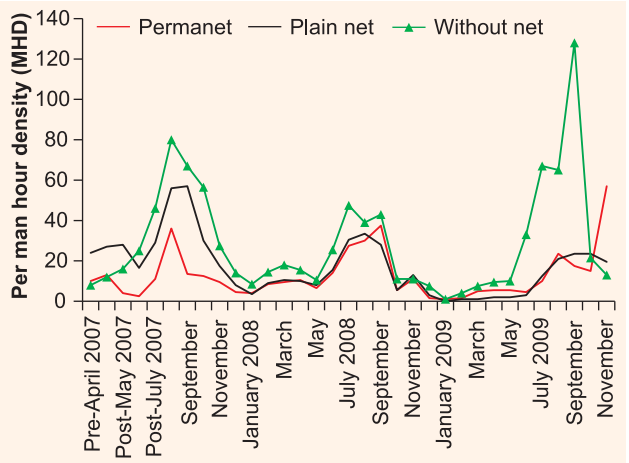

Fig. 10: Indoor resting density of *An. culicifacies* in the study villages with PermaNet® 2.0 untreated net and no net in Dadri PHC, District Gautam Budh Nagar

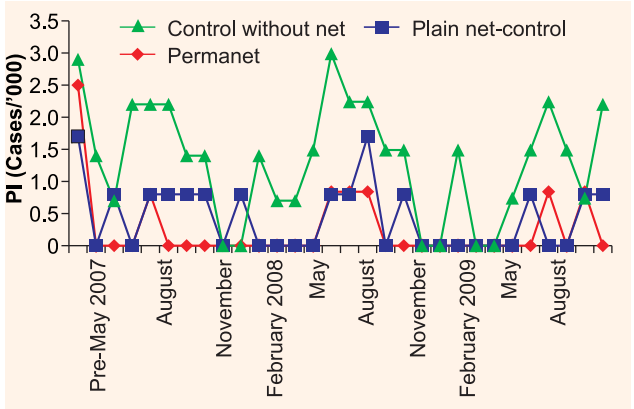

Fig. 11: Month-wise data on malaria PI (cases/'000) in the study villages provided with PermaNet® 2.0 untreated net and no net in Dadri PHC, District Gautam Budh Nagar

Table 8. Durability of PermaNet® 2.0 in the field

Month	No. of nets distributed	No. of nets damaged/ tornout	No. of nets found intact or partially damaged	In use (%)
May 2007	1,084	0	1,084	100
April 2008	1,084	14	1,070	97.4
April 2009	1,084	221	863	80.6

good as >80% nets were in use even after two years of distribution of PermaNet® 2.0 (Table 8).

1.3.5 Follow-up study on the long-lasting efficacy of Olyset net against malaria vectors and incidence of malaria in a village of District Gautam Budh Nagar, Uttar Pradesh

The study was continued in three villages—Khandera (Olyset net village), Beel Akbarpur (plain net village) and Anandpur (no net village) in District Gautam Budh Nagar, Uttar Pradesh, beyond the initial trial period of one year. The efficacy of Olyset nets as determined by cone bioassays with *An. culicifacies* on Olyset net collected randomly after five years of use from field revealed reduction in the knock down time and percent mortality, in used Olyset nets irrespective of number of washes as compared to fresh nets (Table 9).

Pooled month-wise entomological data showed a reduction in the indoor resting man hour density (MHD) and the parity rates of the major malaria vector *An. culicifacies* in the Olyset net village, when compared with plain net and no net villages during the post-intervention years in 2004–05 and 2005–06, but this trend of reduction in the following years was not significant. Epidemiological data of three study villages revealed almost complete interruption in the malaria transmission in the experimental village in the post-intervention

Table 9. Bioefficacy of Olyset nets against *An. culicifacies* after five years of use in the field (Cone bioassays)

Condition of net	No. of mosquitoes tested	Knockdown (%) in 1 h after exposure for 3 min	Mortality (%) after 24 h recovery period
Unused Olyset net	110	100	100
5-year-old used Olyset net	200	82.5	88

Table 10. Impact of Olyset net on prevalence of malaria in Khandera village in District Gautam Budh Nagar, Uttar Pradesh—malaria prevalence (active surveillance) in the study villages before and after 5 years of intervention with Olyset nets

Village	Net type	TBS	SPR	SFR	PI (Cases/'000)	Pf/'000
Pre-intervention 2003–04						
Khandera	Olyset net	238	33.1	18	39.5	21.5
Beel Akbarpur	Untreated net	241	33.5	26.9	44.5	36.1
Anandpur	No net	210	18.1	9.0	19	9.5
Post-intervention 2008–09						
Khandera	Olyset net	58	3.4	0.0	1.0 (–97.4%)	0.0
Beel Akbarpur	Untreated net	69	15.9	2.8	6.1 (–86.2%)	1.1
Anandpur	No net	105	21.9	3.8	11.5 (–39.4%)	2.0

Figures in parentheses indicate percent change over pre-intervention.

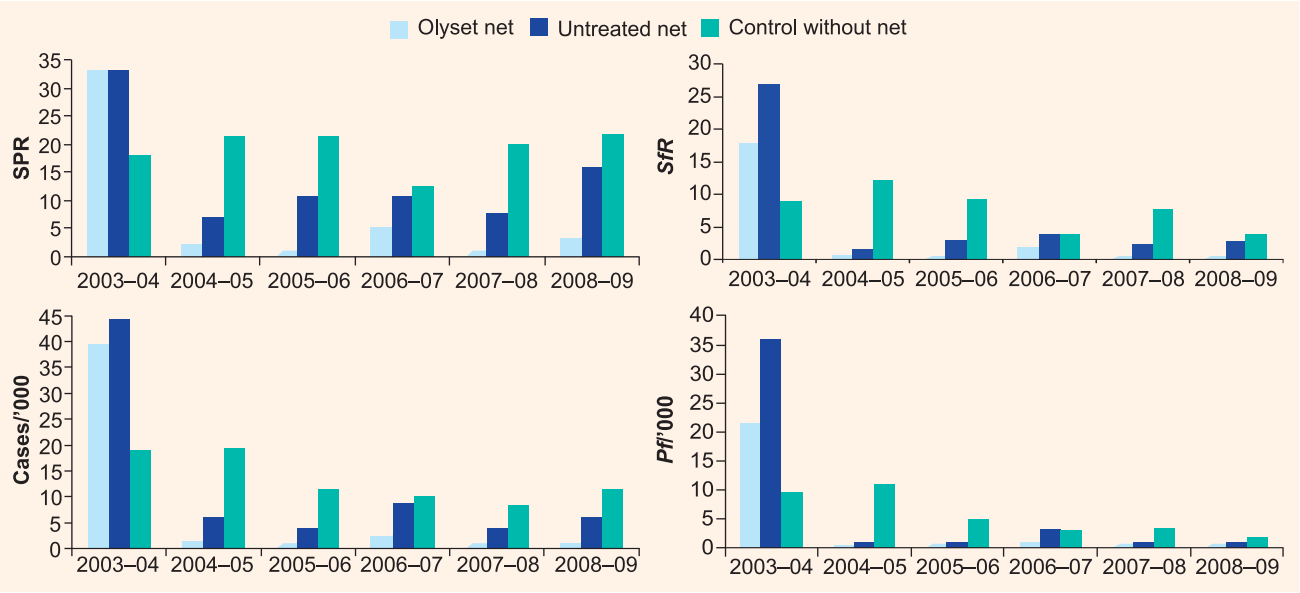

Fig. 12: Long-lasting impact of Olyset net on transmission of malaria in study villages in District Gautam Budh Nagar, Uttar Pradesh

years, as compared to the plain net village and no net village (Table 10 and Fig. 12).

The durability of Olyset nets was as good as >80% of the original nets were recovered even after five years during survey in 2009 (Table 11). A cross-sectional community-based survey undertaken in April–May 2009 covering 102 respondents in Khandera village revealed that Olyset LLIN are widely acceptable in the user community and demand for Olyset net was clearly evident as >80% respondents showed readiness to purchase the Olyset net at subsidized rates. Only 2% respondents complained about skin irritation and itching.

Table 11. Durability of Olyset Net in field after 5 years of use—Random survey for the durability of OlysetNet after five years of use

No. of bed nets distributed in August 2004	1,203
No. of houses surveyed randomly in August 2009	126
No. of nets distributed in 2004 to surveyed houses	679
No. of nets in use (recovered) after 5 years	621 (94.4)
No. of nets completely damaged	58 (8.5)
No. of nets partially damaged	350
No. of nets completely intact	283
No. of nets in actual use	621

Figures in parentheses indicate percentages.

1.3.6 Field evaluation of DuraNet LLIN incorporated with alphacypermethrin, against malaria vectors and its impact on malaria incidence at three locations in India

DuraNet incorporated with alphacypermethrin into a polyethylene netting is manufactured by M/s. Clarke Mosquito Control and approved by WHOPES. The net fibre consists of 145 denier, monofilament incorporated with technical alpha-cypermethrin complying with WHO specification @ 0.55%w/w with a target dose of 5.8 g/kg a.i. corresponding to 261 mg of alphacypermethrin per monofilament polyethylene fabric of one square metre.

The present study on evaluation of DuraNet was undertaken in three malaria endemic areas—Ranchi (Jharkhand), Raipur (Chhattisgarh) and Bijapur

(Karnataka) in India in three groups of villages provided with DuraNet, plain net and no net in each area/location. In addition, the study was also undertaken in Uttar Pradesh to determine wash-resistance and bioefficacy of DuraNet against *An. culicifacies*, the major malaria vector in this region. The bioavailability of insecticide on net fibre was effective in achieving >90% mortality in *An. culicifacies* even after 20 washes.

The study was, however, terminated halfway at the request of the sponsoring agency.

1.3.7 Capacity strengthening for laboratory testing and evaluation of public health pesticides

The second assessment of the laboratory facility for the Phase-I testing of insecticides and interventions was done in the new campus from 23 to 27 November 2009 by the World Health Organization Pesticide Evaluation Scheme (WHOPES). The assessment was made on the progress in implementation of the recommendation of first capacity assessment carried out in September 2008 on various aspects related to infrastructure facilities and through direct observation, and critical review of the organization and management of the laboratory studies. Different technologies, namely impregnation of insecticide papers, adult susceptibility tests, larval susceptibility tests, topical assay, wash resistance, cone bioassay were demonstrated. Further work on the establishment of the facilities as per the suggestions made by WHOPES for the final decision on the establishment of the laboratory.

1.4 Vector surveillance

1.4.1 Application of attracticide (oviposition pheromone in combination with insect growth regulator—IGR) for surveillance and control of dengue and chikungunya mosquitoes

Dengue and chikungunya are upcoming major public health problems in India and control of breeding of the vector *Ae. aegypti* is very difficult because of its breeding behaviour. DRDE, Gwalior developed C-21 attractant and IGR compound for surveillance and long-term control of dengue and chikungunya vector *Ae. aegypti*. Therefore, this study was undertaken to check the efficacy of these compounds in ovitraps by NIMR in collaboration with DRDE.

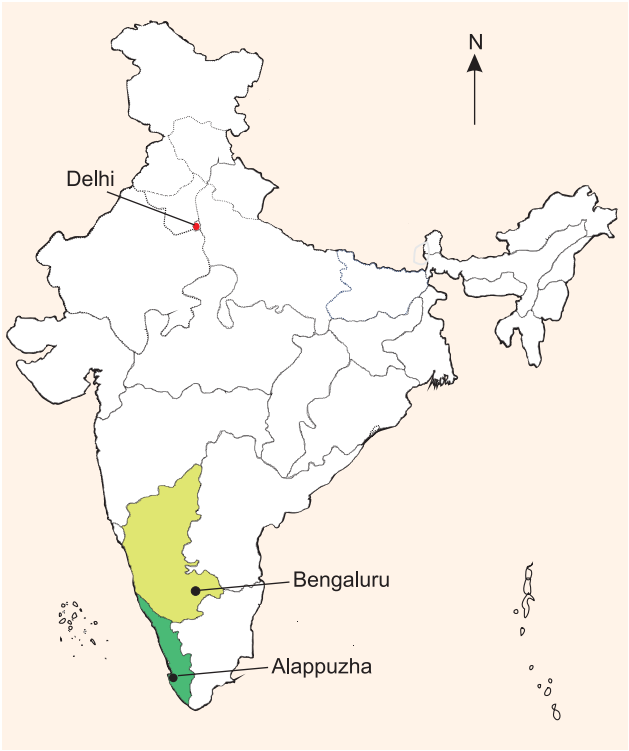

Fig. 13: Study sites of C-21 attracticide trials.

The study was initiated in three dengue and chikungunya affected states of the country—Delhi, Karnataka and Kerala (Fig. 13). The experimental ovitraps contained 395 ml water treated with 5 mg C-21, IGR and solvent. Untreated ovitraps contained 400 ml water with solvent only. Preparation and placement of ovitraps are shown in Fig. 14.

Fig. 14: Preparation and placement of ovitraps.

Delhi

In Delhi, a total of 3,100 houses—650 in Trilokpuri, 570 in Balmiki Colony, 625 in Netaji Nagar, 625 in R.K. Puram and 630 in Railway Colony, Tughlakabad were selected for ovitrap experiment setting (Fig. 15). Overall positivity in experimental and control ovitraps revealed that a total of 2,948 were found positive in experimental and control ovitraps, out of which 1,203 (41%) were experimental and 1,746 (59%) were control ovitraps. The data revealed that out of 50,513 eggs collected from 2,483 ovitraps, 20,418 (40%) eggs were collected from experimental ovitraps and 30,095 (60%) eggs were collected from control ovitraps. Locality-wise results are as follows:

- Maximum number of bowls was found positive in Tughlakabad (656) followed by Netaji Colony (600) and R.K. Puram (585), whereas percentage positivity was highest in Tughlakabad (1.81%) followed by Netaji Colony (1.34%) and Panchkuyian Road (1.26%) [Fig. 16].
- Percentage positivity of control ovitraps was higher in all the localities except Trilokpuri, where percentage positivity of experimental and control ovitraps was almost equal.
- Maximum number of eggs found in Tughlakabad was (15,528) followed by R.K. Puram (12,521) and Panchkuyian Road (10,740), whereas maximum number of eggs were collected in Tughlakabad (15,528) followed by R.K. Puram (12,521) and Panchkuyian Road (10,740) [Fig. 17].
- Number of eggs in control ovitraps was higher than experimental ovitraps in all the localities except in Trilokpuri where number of eggs in experimental ovitraps was higher than control ovitraps.
- Number of larvae in control ovitraps was higher than experimental ovitraps in all the localities.

Fig. 15: Delhi map showing localities selected for ovitraps experimental settings.

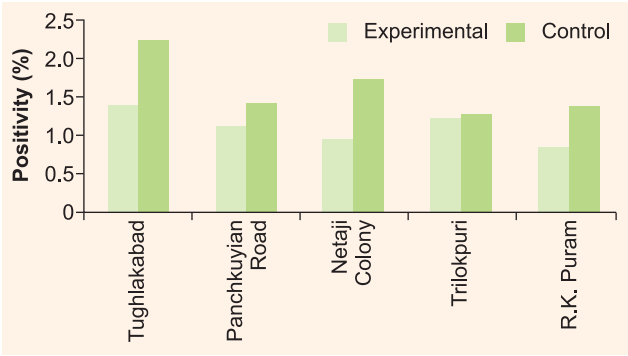

Fig. 16: Locality-wise percentage positivity in Delhi from October 2007 to March 2009.

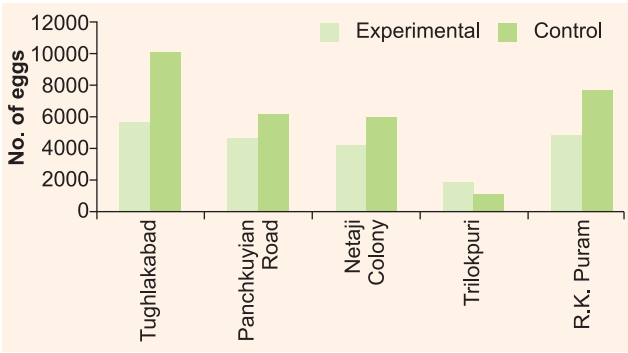

Fig. 17: Locality-wise number of eggs collected in Delhi from June 2008 to March 2009.

Bengaluru

In Bengaluru City, three locations Ashok Nagar, Kanteerava Nagar and Narayanpura & Sanjay Gandhi Nagar were selected for the study. A total number of 3,043 houses—1,026 in Ashok Nagar, 1,014 in Kanteerava Nagar and 1,003 in Narayanpura plus Sanjay Gandhi Nagar was selected for ovitrap setting. Month-wise positivity in experimental and control ovitraps revealed that a total of 5,906 ovitraps, both experimental and control were found positive, out of which 4,144 (70%) were experimental and 1,762 (30%) were control ovitraps. Eggs and larvae collection data revealed that out of 75,866 eggs collected from 4,080 ovitraps, 58,617 (77%) eggs were collected from experimental ovitraps and 17,249 (23%) eggs were collected from control ovitraps. Locality-wise results were as follows:

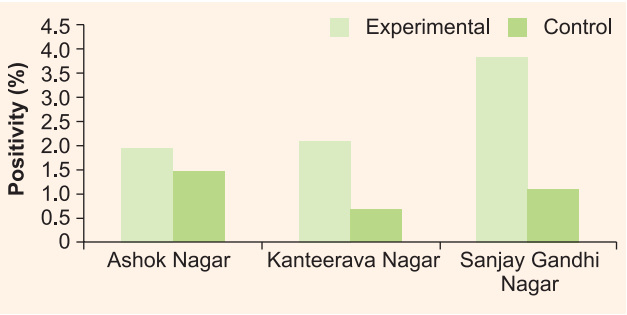

Fig. 18: Locality-wise percentage positivity in Bengaluru from January 2008 to March 2009.

- Maximum number of bowls was found positive in Sanjay Gandhi Nagar (2,707) followed by Ashok Nagar (1,975) and Kanteerava Nagar (1,224) also percentage positivity was highest in Sanjay Gandhi Nagar (2.47%) followed by Ashok Nagar (1.73%) and Kanteerava Nagar (1.40%) [Fig. 18].
- Percentage positivity of experimental ovitraps was higher than control ovitraps in all the localities of Bengaluru.
- Maximum number of eggs was collected from Sanjay Gandhi Nagar (40,503) followed by Ashok Nagar (20,331) and Kanteerava Nagar (13703) [Fig. 19].
- Number of eggs in experimental ovitraps was higher than control ovitraps in all the localities.

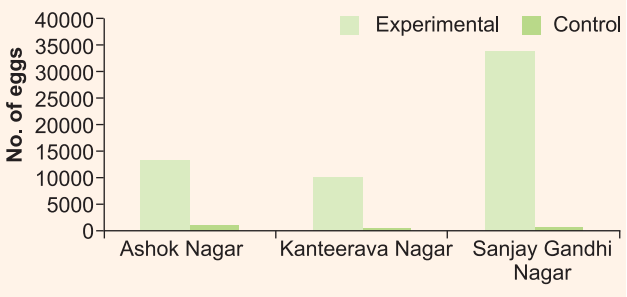

Fig. 19: Locality-wise eggs collected in Bengaluru from January 2008 to March 2009.

Kerala

In Alappuzha district of Kerala, Ward Nos. 6, 7 and 8 of CHC Muhamma, 4, 11 and 12 of PHC Kadarapally and 8, 9 and 12 of PHC Vettakal area of Sherthallai Taluka were selected. Month-wise positivity in experimental and control ovitraps revealed that a total of 21,863 ovitraps, both experimental and control were found positive, out of which 10,630 (49%) were experimental and 11,233 (51%) were control ovitraps. Eggs and larvae collection data revealed that out of 180,243 eggs collected from 15,693 ovitraps, 118,210 (66%)

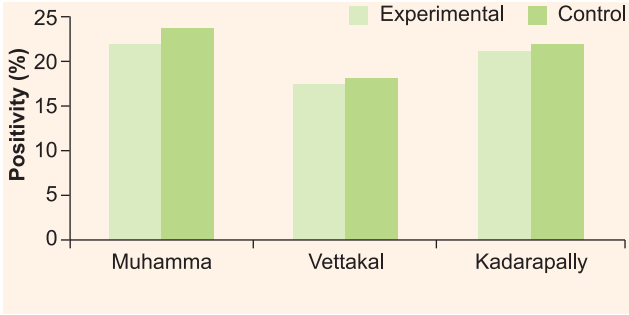

Fig. 20: Locality-wise percentage positivity in study sites of Kerala from April to December 2008.

eggs were collected from experimental ovitraps and 62,033 (34%) eggs were collected from control ovitraps. Locality-wise results are as follows:

- Maximum number of ovitrap bowls was found positive in Muhamma (8285) followed by Kadarapally (7,438) and Vettakal (34,741) also percentage positivity was highest in Muhamma (22.72%) followed by Kadarapally (21.41%) and Vettakal (17.67%) [Fig. 20].
- Percentage positivity of control and experimental ovitraps was almost equal in all the three localities.
- Maximum number of eggs found in Muhamma (70,157) followed by Vettakal (55,329) and Kadarapally (54,757), whereas maximum number of larvae found in Muhamma (9122) followed by Kadarapally (6744) and Vettakal (6557) [Fig. 21].
- Number of eggs in experimental ovitraps was higher than control ovitraps in all the localities.
- Number of larvae in control ovitraps was higher than experimental ovitraps in all the localities.

The field experiments were also cross-checked by the team of DRDE, Gwalior along with NIMR officials on regular basis and a mid-term review was also undertaken in the month of June 2008. Besides field trials, IEC material was also published

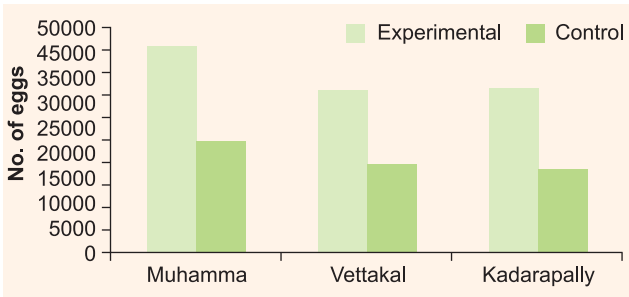

Fig. 21: Locality-wise number of eggs and larvae in C-21 ovitraps in study sites of Kerala from June to December 2008.

and distributed by the NIMR for community awareness.

Conclusion: Attracticide ovitraps can play an important role in surveillance of *Ae. aegypti*, vector of chikungunya and dengue, as the positivity of ovitraps was found almost equal but the number of eggs was more in experimental ovitraps as compared to control (statistically significant). In essence, these studies indicate that this attracticide formulation with lure and kill characteristics (C21 in combination with IGR) has potential for its use for surveillance and management of dengue and chikungunya mosquitoes.

Since some variations in efficacy of attracticide were recorded in different localities of the study areas, therefore, DRDE, Gwalior funded two more projects to find out the parameters responsible for breeding behaviour using the product C-21 attractant in ovitrap for *Ae. aegypti* in Gwalior and in combination with IGR compound in Delhi, Bengaluru and Alappuzha district of Kerala.

1.4.2 *Aedes* breeding survey in Delhi

On the request of Municipal Corporation of Delhi (MCD), New Delhi Municipal Committee (NDMC) and Delhi Administration *Aedes* breeding survey was carried out in Delhi during January–December 2009 in 106 localities (Fig. 22)—Timarpur, Sant Nagar, Roop Nagar, Gandhi Nagar, Connaught Place, Mahavir Enclave, Palam Enclave, Manglapuri, Vasant Kunj, Najafgarh, R.K. Puram, Subhash Nagar, Janakpuri, Khayala, Vikaspuri, Peeragarhi, Mangolpuri, Punjabi Bagh, Nangloi, Harsh Vihar, Vishnu Garden, Sultanpuri, Shalimar

Fig. 22: Localities in Delhi surveyed during 2009 for dengue breeding.

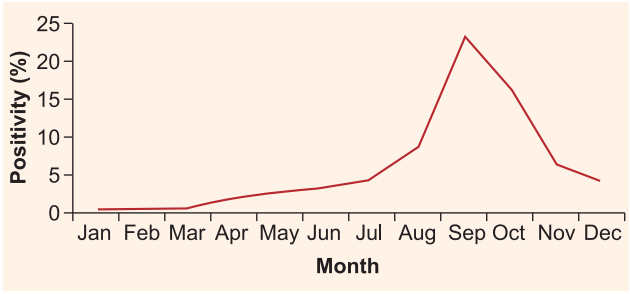

Fig. 23: Monthwise container index in Delhi (2009).

Bagh, Ashok Vihar, Uttam Nagar, Badli, Delhi Cantt., Gole Market, Panchkuyian Road, Chanakyapuri, Moti Bagh, Model Town, Azadpur, Nauroji Nagar, Keshav Puram, Tughlakabad, Lodhi Colony, Netaji Nagar, Kidwai Nagar, Nasirpur, Jhilmil, Dwarka, Sewa Nagar, Sarai Kale Khan, Kalkaji, Karol Bagh, Mehrauli, Laxmi Nagar, Mayur Vihar, Kalyanpuri, Ambedkar Nagar, Shakarpur, Lajpat Nagar, Govt. Offices, Schools, Nurseries, Parks, Picnic Spots, Police Stations, Bus Depots, Dispensaries, Hospitals, etc.

From the survey, it was found that the breeding was more in the month of September (C.I.—23.20) followed by October (C.I.—16.71) and August (C.I.—8.94) [Fig. 23]. The survey also revealed that the breeding was more in peri-domestic containers (solid waste) from the month of May 2009 as compared to domestic containers (coolers and OHTs) [Table 12]. The results of positivity of different breeding sites in different localities of Delhi were analysed and monthly locality-wise GIS maps were prepared. The results and maps of *Aedes* breeding were compared with dengue cases and the information was provided to the NVBDCP/MCD/NDMC/Delhi Administration during the fortnight meeting chaired by the Hon'ble Health Minister and Secretary Health and bimonthly meeting chaired by the Commissioner MCD for necessary action.

Table 12. Breeding sites of *Ae. aegypti* in different seasons

Month	C.I.			
	Domestic containers—overhead tanks, ground cement tanks, etc.		Peri-domestic containers—solid waste (dump tyre, gamalas, mud pots, pits, etc.	
	2008	2009	2008	2009
January	1.08	2.80	0	0
February	1.00	6.06	0	0
March	3.01	3.98	0	0

1.5 Insecticide resistance

1.5.1 Monitoring of insecticide resistance of malaria vectors in India

A project was sanctioned to assess the susceptibility status in the EMCP and GFATM implementation project areas of the NVBDCP. This area is comprised of 13 states including 7 north-east states with 156 districts. In the first phase of year-1, studies were carried out in the states of Madhya Pradesh, Chhattisgarh, Andhra Pradesh, Orissa, Jharkhand, West Bengal, Assam, Mizoram and Meghalaya. The studies were carried in the selected individual districts (where feasible) or in units comprising of a group of districts with homogeneity to ecotype, vector prevalence and other factors. Results have indicated that the vector species are mostly resistant to DDT and malathion, while in the districts of Chhattisgarh, Andhra Pradesh and Madhya Pradesh the vectors were reported resistant to pyrethroids.

In Orissa state, adult susceptibility tests were carried out in 10 districts of the total of 29 districts. It was found that *An. culicifacies* was tolerant to pyrethroid (deltamethrin) in all the districts except in District Gajapati. In Andhra Pradesh state, susceptibility tests were carried out in five districts—Srikakulam, Visakhapatnam, Vizianagaram, East Godavari and Khammam where *An. culicifacies* was resistant to pyrethroid except in District Vizianagaram. In Madhya Pradesh state, studies were carried out in districts of Shahdol, Siddhi, Mandla and Dindori. *Anopheles culicifacies* has shown resistance to malathion in all the districts surveyed except in Mandla and Dindori districts. In Chhattisgarh state, studies were carried out in Dantewada, Bastar, Kanker, Dhamtari, Raipur, Bilaspur, Korba, Korea, Jashpur Nagar and Raigarh districts (10 of the 16 districts). *Anopheles culicifacies* showed resistance to pyrethroid (deltamethrin) in all the districts surveyed except in District Dantewada. In Assam state, *An. minimus* was still susceptible to DDT, malathion and deltamethrin in Lakhimpur and Dhemafi districts. In West Bengal state, *An. culicifacies* was resistant to DDT and tolerant to malathion and susceptible to pyrethroid in Purulia and Bankura districts. In Jharkhand state, *An. culicifacies* was still susceptible to deltamethrin in Ranchi and Gumla districts but resistant to DDT and tolerant to malathion. Work for the visit II of year-1 and II visit in year-2 is in progress.

1.5.2 Analysis of exon-intron organization in P450 supergene family of *Anopheles gambiae*

Cytochrome P450 superfamily is a large, diverse in nature divided into various families and sub-families. Understanding of the functional role and evolution of *P450* genes is indispensible to develop evolution proof insecticides in future. Thus, here we made an endeavor to study the exon-intron organization CYP450 superfamily to understand the evolution of supergene family through neofunctionalization and deciphering functional role of members of gene family through conserved exon-intron organization. Cytochrome P450 superfamily is reported to be involved mainly in the developmental process and xenobiotic metabolism in insects. Analysis of *An. gambiae* genome has shown 105 putatively active *P450* genes distributed in four major clades, namely mitochondrial, CYP2, CYP3 and CYP4 clans. Analysis of exon-intron organization of P450 superfamily revealed greater diversity between the members of gene families. The conservation of exon-intron organizations of the genes has correlation with the phylogenetic relationship among them. The CYP3 clan member gene structures are highly conserved, especially the CYP6 family has highly positional conserved single, phase 1 intron. However, the functional importance of the conserved intron is to be studied in detail. The possible correlation between the total gene length and total intron length, gene clusters, orientation and gene organization, and role of intron loss and gain in the evolution of P450 superfamily are studied.

1.5.3 Molecular basis of insecticide resistance in *Anopheles culicifacies:* presence of two alternative kdr-like mutations L1014F and L1014S, and a novel mutation V1010L in the voltage gated Na^+ channel of *Anopheles culicifacies sensu lato* population from Malkangiri, Orissa

Knockdown resistance in insects resulting from mutation(s) in the voltage gated Na^+ channel (VGSC) is one of the mechanisms of resistance against DDT and pyrethroids. Earlier we reported presence of a point mutation substitution in the VGSC at residue 1014 leading to Leu-to-Phe substitution—a most common *kdr* mutation reported in insects, in an *An. culicifacies* population from Surat district of Gujarat. Further screening of

a population from Malkangiri (Orissa) revealed a total of four point mutations in VGSC leading to three amino acid substitutions including L1014SI.

Anopheles culicifacies s.l. samples, collected from Malkangiri district of Orissa (India), were sequenced for part of the second transmembrane segment of VGSC and analyzed for the presence of non-synonymous mutations which revealed presence of three amino acid substitutions in the IIS6 transmembrane segments of VGSC, resulting from a total of four point mutations. Two alternative point mutations, 3042A>T transversion or 3041T>C transition, were found at residue L1014 leading to Leu (TTA)-to-Phe (TTT) or -Ser (TCA) changes, respectively. A third and novel substitution, Val (GTG)-to-Leu (TTG or CTG) was identified at residue V1010 resulting from either of the two transversions—3028 G>T or 3028G>C. The L1014S substitution always co-existed with V1010L in the samples analyzed irrespective of the type of point mutation associated with latter. This is the first report of the presence of L1014S (homologous to the *w-kdr* in *An. gambiae*) and a novel mutation V1010L (resulting from G-to-T or -C transversions) in the VGSC of *An. culicifacies* in addition to the previously described mutation L1014F.

Allelic association of 1010L and 1014S

The IIS6 transmembrane segment of VGSC from samples heterozygote for L1014S were cloned in pGemT vector and sequenced. Sequencing of cloned product revealed that L1014S and V1010L substitutions were always found on the same haplotype whereas L1014S or L1014-wild type was always found with V1010-wild type.

Linkage disequilibrium analysis using phased data of 79 individuals revealed that the point mutation 3028G>T (V1010S) and 3041T>C (L1014S) are tightly linked (D′ = 1.000, chi-square = 158.0; $p < 0.001$). One sample with 3028G>C mutation was not tested for LD, however, in this sample 3028G>C (V1010S) and 3041T>C were found on the same haplotype.

Development of a new PIRA-PCR assay for L1014S detection

A new Primer Introduced Restriction Analysis-PCR (PIRA-PCR) was developed for the detection of new mutation L1014S (Fig. 24). The results were validated through DNA sequencing of corresponding samples which revealed that the assay is highly specific.

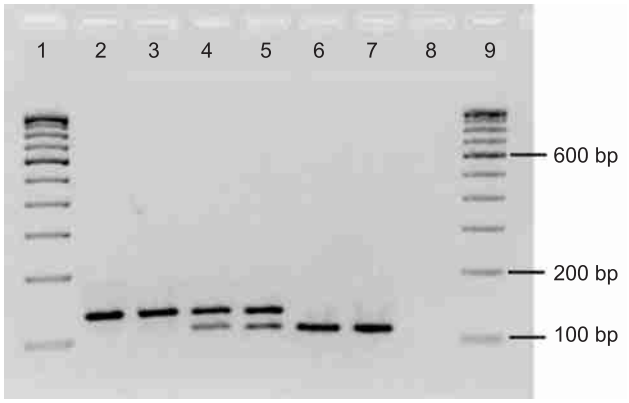

Fig. 24: Gel photograph showing results of PIRA-PCR for L1014S detection. Lanes 1&9: 100 bp DNA ladder, Lanes 2&3: homozygous wild type; Lanes 4&5: heterozygotes; Lanes 6&7: cloned products with 1014S alleles isolated from heterozygotes (1014 L/S) samples; Lane 8: negative control without DNA.

1.6 Vector Evolutionary Genetics

1.6.1 Development of multilocus nuclear DNA markers and genetic parameters in an Indian population of *Anopheles minimus*

Estimation of population genetic parameters to infer genetic structure of species populations is highly dependent on the choice of genetic markers. Furthermore, such inferences based on single gene blur the information and thus usage of multiple loci is suggested. Considering malaria as a highly fatal vector-borne infectious disease, inference of population genetic structure could be of help in future research on malaria vector control and management. Using comparative genomic approach, we first searched for most orthologous genes in *An. gambiae* and designed primers in the exons of these genes to amplify the introns in *An. minimus* nuclear genome. Eight such DNA fragments could be successfully amplified (Fig. 25) and sequenced and homology to corresponding genes of *An. gambiae* was ascertained. In order to ascertain if these markers could be used as population genetic markers, we have conducted several tests. All the eight DNA fragments were found to be polymorphic in a population sample of *An. minimus* from India (Fig. 26). Several tests of neutrality confirmed the fact that all the fragments were evolving under a standard neutral model of molecular evolution. Furthermore, all the eight fragments were found to be independently evolving

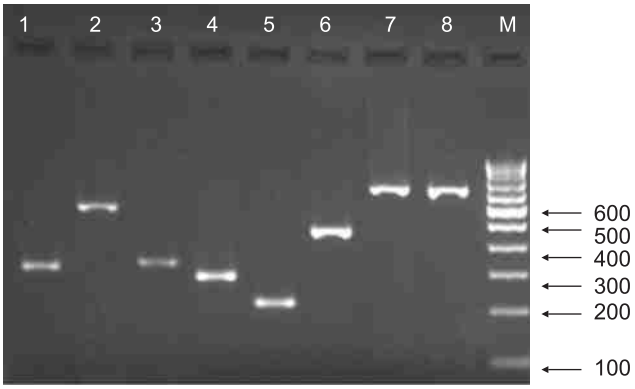

Fig. 25: Ethidium bromide stained 2% agarose gel, showing DNA fragments amplified by PCR. Lane 1–8 = M1–M8 DNA fragments; Lane M = DNA Ladder (100 base pairs).

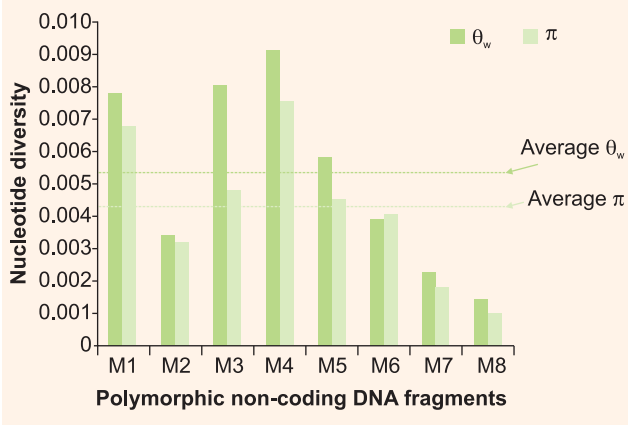

Fig. 26: Patterns of variation in nucleotide diversity across all the eight polymorphic non-coding DNA fragments in genomic DNA of Indian *An. minimus*.

in the Indian population sample. Tests of past population demographic events utilizing all the eight *loci* clearly revealed that the population follows a demographic equilibrium model, without any significant population bottleneck or expansion. The eight multilocus nuclear DNA fragments thus could be considered as 'putatively neutral' which could be used to infer population structure and demographic history of *An. minimus*, a major malaria vector in the south-east Asia and India. ❑

2

2.1.1 Wide variation in microsatellite of *pfcrt* genes in chloroquine (CQ) resistant *Plasmodium falciparum* in India

In earlier worldwide studies, microsatellites flanking the upstream and downstream of the *pfcrt* gene have been found to be fixed among the CQ resistant *P. falciparum* parasite. However, recent studies in Indian isolates, observed high diversity in microsatellites positioned in two of the introns which are next to the polymorphic exons of *pfcrt* gene. This contradicts the global studies reporting high reduction in genetic diversity surrounding *pfcrt* gene of resistant parasite and furthermore, infer an undergoing evolution in this part of genome in Indian resistant parasite. Here, we investigated the variation at microsatellite markers in introns (7 introns) of the *pfcrt* gene in a set of 86 single clone *P. falciparum* isolates comprising both CQ sensitive and resistant isolates from different malaria endemic regions of India. We measured the level of intragenic variation in terms of expected heterozygosity (H_e) between the resistant and wild type isolates and observed a low allele heterozygosity (0–0.55) at each locus of resistant *pfcrt* haplotype in comparison to the wild haplotype (0–0.94) (CVMNK$^{wild\ type}$ > CVIET$^{resistant\ type}$ > SVMNT$^{resistant\ type}$) [Fig. 1]. Intron 5 was monomorphic in all isolates. High intragenic polymorphism was observed in resistant parasites from high endemic areas in comparison to the low endemic areas indicating high selection pressure in low endemic region. The levels of variation measured show mutations in these intragenic microsatellites are still evolving in India.

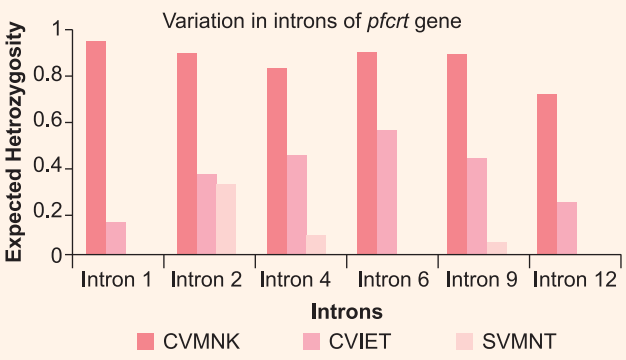

Fig. 1: Heterozygosity (H_e) of microsatellites in introns of *pfcrt* gene. H_e was compared between resistant and wild type parasite isolates.

2.1.2 Distribution and genetic relatedness of two sub-populations (subtypes) in Indian *Plasmodium vivax*

Plasmodium vivax has been categorized into two distinct lineages, Old World (Asia pacific) and New World (South and Central American isolates). These two proposed lineages were distinguishable by gene conversion in the *S-type 18S small sub unit ribosomal protein (18S SSU rRNA)*, mutations in an open reading frame (*orf470*) in the apicoplast genome, and mosquito transmission potential. New World sub-type was designated as a sub-species or separate species of Old World *P. vivax*.

We have estimated good prevalence of both Old World and New World' sub-types of *P. vivax* in Indian sub-continent, which suggests no geographical sub-division between the two proposed lineages. To understand genetic structure and relatedness of two sub-types (Old and New World), multilocus genotyping was initiated and for this we have selected 50 Old World isolates and 50 New World isolates. For the molecular characterization of two sub types of *P. vivax*, we used four different categories of genetic markers such as (i) drug resistance, (ii) antigen genes, (iii) housekeeping genes, and (iv) mini and microsatellite markers.

Putative housekeeping genes are identified from

recently sequenced *P. vivax* genome using parasite genome database PlasmoDB (*http://www.plasmodb.org/plasmo/*). We have identified 10 housekeeping genes and 17 tandem repeats (minisatellites: 10, microsatellites: 7) markers to understand the genetic relatedness between Old World and New World isolates of *P. vivax*.

Genotypic variations: Among the 100 *P. vivax* field isolates analyzed, we observed substantial amount of genotypic variations at antigen determinants (*gam-1, msp-1, msp-3α, dbp-III* and *Pv28s*), and putative housekeeping genes (*l35e, l34a, DNA gyrase,* and *acp*). To ascertain biased distribution and sub type specific genotypes in multilocus system genotyping, we compared the genotypes between S type-1 and S type-2 isolates. Analysis showed that both sub types of *P. vivax* carry identical genotypes at all the loci studied. Interestingly, various genotypes observed at drug resistant, antigen, and putative housekeeping genes were present in similar frequencies in both sub types (Fig. 2). Additionally, extensive RFLP variants observed at *Pvmsp-3α* showed similar level of genetic diversity between S type-1 and S type-2. Comparison of observed genotypes in the present study suggesting lack of genetic distance (based on genotypes and their frequencies) between Old World and New World.

Phylogenetic relatedness: Un-rooted Neighbour-Joining phylogenetic trees constructed using individual antigen and putative housekeeping genes and multilocus genetic distance data of mini and microsatellites showed consistent feature in term of phylogenetic relation between two sub types of *P. vivax,* and their genetic structure. Phylogenetic trees each derived from each set of genetic markers are given in Fig. 3. The consensus features derived from phylogenetic trees reflects that in each phylogenetic tree, there are 2–5 major clusters, and each cluster further splits into many sub clusters. Phylogenetic trees did not show a separate clade or cluster of S type-1 or S type-2 isolates. Phylogenetic tree construction showed that the two sub types of *P. vivax* randomly scattered in all phylogenetic trees, and clustering of S type-1 isolates and S type-2 isolates was not observed. Phylogenetic analysis with wide range of genetic markers (antigen and putative housekeeping genes, and tandem repeats) revealed a high degree of genetic identity between S type-1 and S type-2 isolates establishing them as single species.

Divergence analysis: Ten putative housekeeping genes selected in this study are orthologous genes that are present in human, primates, and rodent *Plasmodium*. Since, *P. vivax* is a close relative of

Table 1. Nucleotide substitution rate in *P. vivax*, and divergence rate between *P. vivax* and *P. knowlesi* for orthologous housekeeping genes

HKG	Nucleotide substitution rates in *P. vivax*		Divergence rates between *P. vivax* and *P. knowlesi*	
	dS	dN	Ks	Ka
ed	0	0.002±0.002	0.4334	0.1181
l35e	0	0.001±0.001	0.3399	0.0410
l34a	0	0	0.2907	0.0257
dg	0	0.001±0.001	0.4202	0.1423
enolase	0.004±0.003	0.000	0.3798	0.0134
RNA pol	0	0.0025±0.002	0.2332	0.0068
acp	0.002±0.002	0	0.2575	0.0320
stpk	0	0	0.6643	0.5624
ac	0.007±0.005	0	0.5496	0.0867
cdpk	–	–	0.6966	0.7130
Mean	0.004±0.001	0.0016±0.0003	0.3965	0.1142

dS: synonymous substitution rate; dN: non-synonymous substitution rate; Ks: synonymous divergence rate; and Ka: non-synonymous divergence rate.

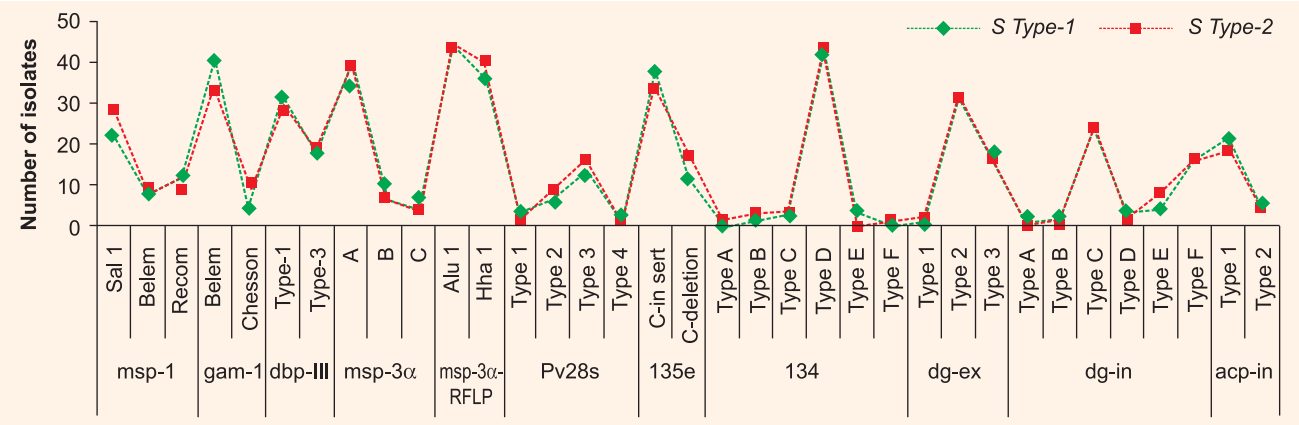

Fig. 2: Distribution of various genotypes of drug resistant, antigen, and housekeeping genes from S type-1 and S type-2 isolates of *P. vivax*.

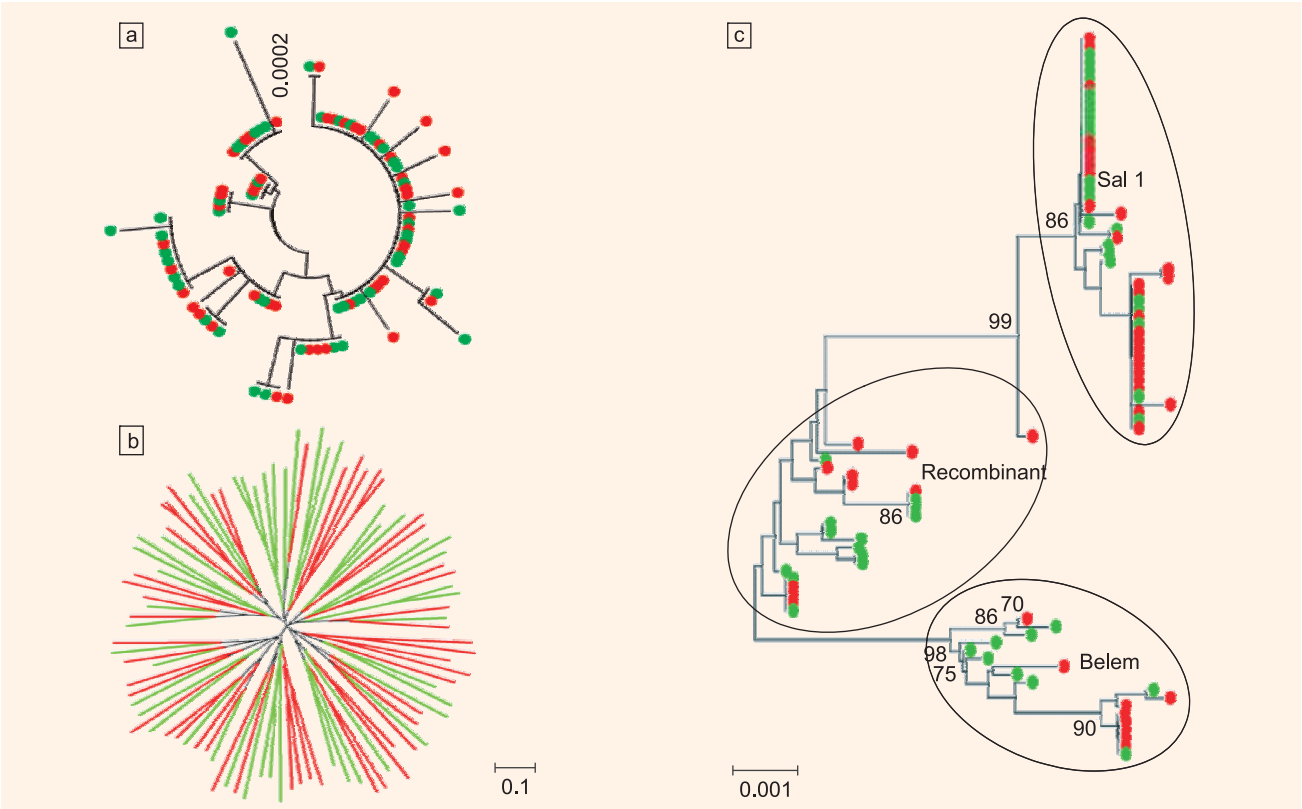

Fig. 3: N-J phylogenetic trees derived from housekeeping genes (*enolase:* a), multilocus minisatellite markers (b), and antigen gene (*msp-1*: c), and showing the phylogenetic relationship between S type-1 (green) and S type-2 (red) isolates of *P. vivax*.

Old World monkey parasites such as *P. cynomolgi* and *P. knowlesi*, the orthologous genes of *P. knowlesi* were analyzed to understand the genetic distance (divergence) with *P. vivax* and pattern of evolutionary signature (positive or negative selection) over the selected putative housekeeping genes. Approximately, 10-fold higher synonymous divergence rate was observed between *P. vivax* and *P. knowlesi* than to non-synonymous divergence rate (Table 1), thus, suggesting selected putative housekeeping genes are evolving strictly under purifying selection.

Mini and microsatellites variability and pattern of heterozygosity: Allele frequency per locus varied considerably but allele frequencies are evenly distributed between two sub types *of P. vivax*. Total allelic diversity at mini and microsatellites was high and identical in two sub types of *P. vivax*. A total of 66% (96/146) and 55% (38/69) alleles at mini- and micro-satellites, are shared between two sub-types of *P. vivax* isolates (Fig. 4). Majority of the polymorphic alleles are shared between S type-1 and S type-2 isolates with similar allele frequency. Heterozygosity analysis between two sub types of *P. vivax* revealed a similar genetic diversity pattern at both mini and microsatellites (Fig. 5). Among 18

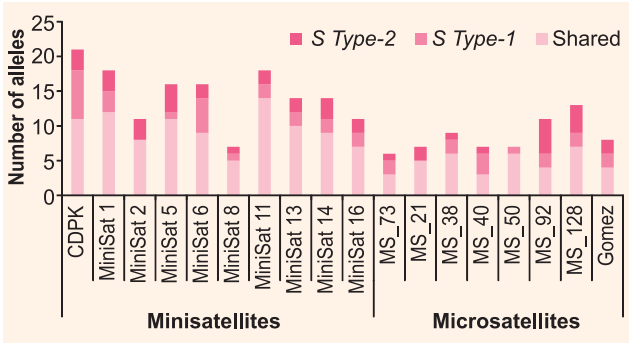

Fig. 4: Allele sharing at mini and microsatellites from two subtypes of *P. vivax*.

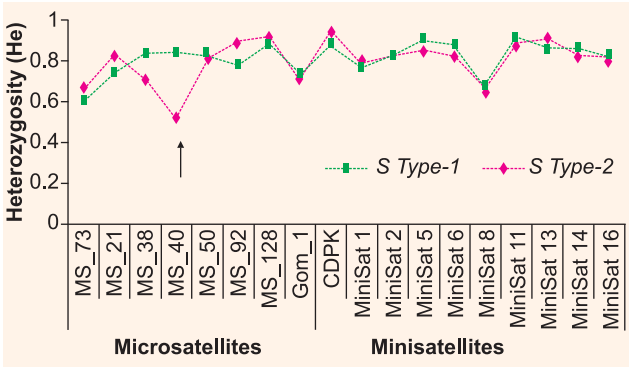

Fig. 5: Pattern of heterozygosity at microsatellite and minisatellite markers from S type-1 and S type-2 isolates of *P. vivax*. Arrow indicates loci showing heterozygosity reduction in S type-2 isolates.

tandem repeat loci, a single microsatellite (MS_40) showed differential genetic diversity pattern, i.e. high heterozygosity in S type-1 isolates (H_e = 0.847) and low in S type-2 isolates (H_e = 0.520). These observations suggest that the two proposed lineages of *P. vivax* are belonging to a single genetic pool.

2.1.3 Characterization of symptomatic and asymptomatic *Plasmodium falciparum* infection in India

Infection with *P. falciparum*, the most virulent among human malarial parasites, can cause a wide spectrum of illness ranging from symptomless infection through uncomplicated disease to life threatening conditions such as cerebral malaria and severe anemia with the pathogenesis of such infections relating to various host and parasitic factors. Therefore, a study has been taken up to understand the genotype—phenotype association of asymptomatic and symptomatic in *P. falciparum* isolates of Sundargarh district, Orissa. Asymptomatic malaria was found in 28% of the malaria positive cases and 72% had symptomatic malaria. Isolates were characterized for multiplicity of infection (MOI) using surface protein markers, namely msp-1 and msp-2. In all, 55 field isolates were involved in the study. The *P. falciparum* isolates from the study site were found to be highly diverse in respect of *msp-1* (block 2) and *msp-2* (central repeat region, block 3). Among multiclone isolates, all possible combinations of *msp-1* families were observed except K1 + RO33 combination. Isolates having MAD20 family (41.8%) was found in high proportion and K1 family (12.7%) was found to be low compared to previous study which reported the proportion of MAD20 and K1 to be 32.5 and 42.5% respectively in the same district. Both the allelic families described for *msp-2* block 3 (3D7 = 47.2%, FC27 = 16.3%) were detected in the isolates with a high proportion of 3D7. Multiple alleles of both 3D7 and FC 27 allelic variants (29%) were also detected. A high multiplicity of infection (MOI) for both *msp-1* (1.45) and *msp-2* (1.37) were observed. Analysis revealed highly polymorphic nature of the isolates on the basis of length in both the manifestations. The distribution of *msp-1* (K1, MAD20 and RO33) and *msp-2* (FC27 and 3D7) families in both the groups are shown in Fig. 6. Analysis of allele frequency distribution of *msp-1* and *msp-2* families shows a bias distribution of certain alleles in the two groups (Fig. 7). The genetic studies of parasite samples from symptomatic and asymptomatic cases will help to identify the genotypes associated with symptomatic and asymptomatic malaria.

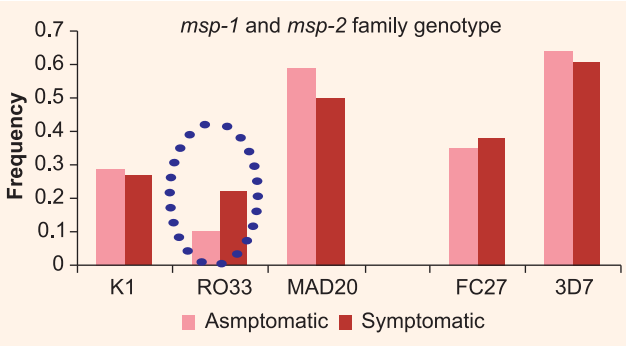

Fig. 6: Distribution of *msp-1* (K1, RO33, MAD20) and *msp-2* (FC27, 3D7) families in symptomatic and asymptomatic *P. falciparum* infection.

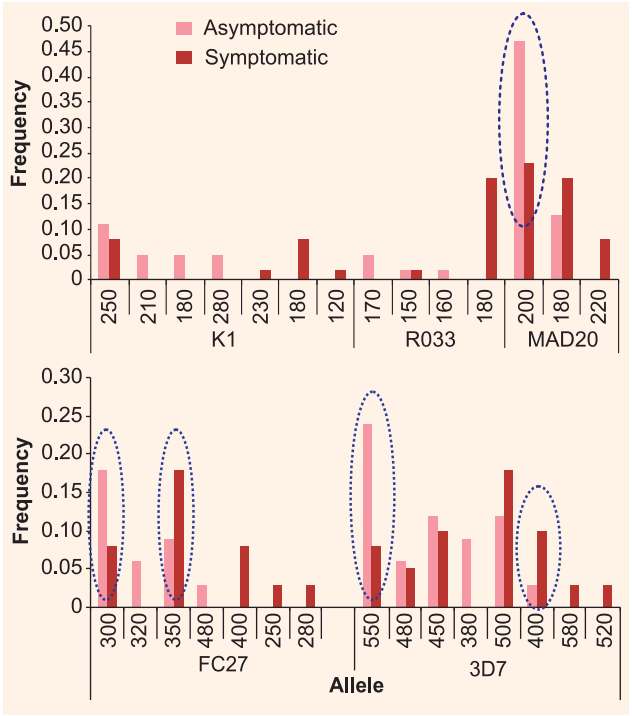

Fig. 7: Allele frequency distribution of *msp-1* and *msp-2* families in asymptomatic and symptomatic samples.

2.1.4 Characterization of the *P. falciparum* strains prevalent in north-eastern states

The present study revealed that among artemisinin-based combination therapies (ACTs) as per WHO recommendations, this AS + SP combination was safe and effective in achieving rapid parasite clearance well within 48 h of treatment initiation with cumulative cure rate of 95.3%. Owing to rapid parasite clearance, there is less possibility of development of gametocytemia, thus, large-scale deployment of ACTs could be an

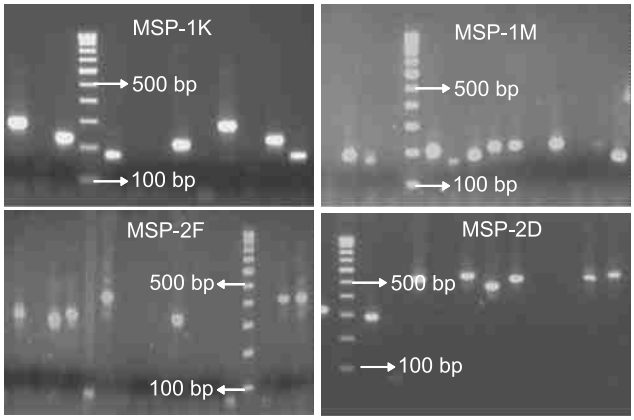

Fig. 8: Gel image showing variations in the *msp-1* and *msp-2* gene allelic families in the *P. falciparum* field isolates.

evidence-based intervention in reducing disease transmission. As this study was undertaken during peak transmission season, the possibility of reinfection was not excluded. In the present study, paired blood samples were analyzed based on PCR genotyping of alleles of *msp-1*, *msp-2* and glutamine-rich protein (*glurp*) for molecular confirmation and these were found to be reinfections (Fig. 8). Hence, the true therapeutic efficacy of this combination is validated to be >95%, thus holds good potential for treatment of drug-resistant malaria in given areas with variable transmission intensity. There being documented resistance to sulfadoxine-pyrimethamine in north-eastern states of India, its combination with artesunate should be subject to periodic monitoring for its sustained curative efficacy. It is strongly believed that drug-resistant strains of *P. falciparum* were carried into India via north-east corridor from neighbouring Myanmar where these are widely prevalent. The parasite strains of the north-east are genetically diverse and rich in point mutations that are known to confer drug-resistance. The bordering population groups that bear the brunt of disease burden serve as foci for multiplication and spread of resistant parasite strains propagated by efficient mosquito vector species of *An. minimus* and *An. baimaii* that are widely abundant. There are confirmed reports of proliferation of multi-drug resistant strains along international borders with Indo-Myanmar and Indo-Nepal. Thus, it is highly demanding to have cross-border collaborative efforts to formulate appropriate drug policy to contain the spread of drug-resistant malaria.

Nevertheless, it is the opportune time to roll out artemisinin-based combination therapy for every single case of *P. falciparum* to avert impending disease outbreaks, and saving lives. In conjunction with effective chemotherapy, it is just as important to strengthen healthcare services where there is need to provide on-the-spot diagnosis that is affordable. In rolling back malaria initiative, we strongly advocate sustained political commitment for increased allocation of resources ensuring intensive disease surveillance and case management by monitoring therapeutic efficacy and upgrading drug policy in force to thwart the development and spread of drug-resistant malaria.

We may conclude from this study that for treatment of *P. falciparum* malaria, this particular drug combination (artesunate + sulfadoxine-pyrimethamine) resulted in rapid parasite clearance well within Day 2 with cumulative cure rate of 95.3%. This drug regimen was well-tolerated, and concluded to be safe and effective. It is strongly advocated to rule out artemisinin-based combination therapy for treatment of every single case of *P. falciparum* to contain the spread of drug-resistant malaria, and saving lives. The study at this direction would be helpful in characterizing *P. falciparum* isolates by determining the changing prevalence of specific mutations over time, which may be of practical value predicting emerging patterns of drug efficacy. The prevalence of quintuple mutation (DHFR triple mutation and DHPS double mutation) can be used as a tool to screen clinical isolates by PCR-based assay for monitoring SP resistance (Figs. 9 & 10). The information generated in this study may be of direct relevance in malaria treatment and control policy. Using PCR-based assay, a large number of samples can be screened for monitoring mutant haplotypes associated with SP resistance. Interpretation of molecular data from laboratory to clinician would be helpful in the treatment of patients diagnosed with drug resistant alleles. Data generated would

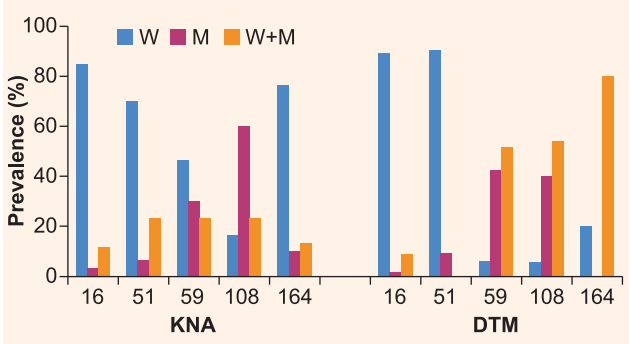

Fig. 9: Prevalence of DHFR—Wild (W), Mutant (M) and Mixed Genotypes (W+M).

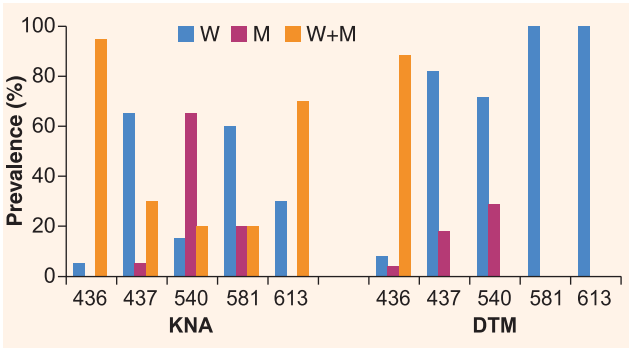

Fig. 10: Prevalence of DHPS—Wild (W), Mutant (M) and Mixed Genotypes (W+M).

be of great value as it reflect on the efficacy of antimalarials being used in the area and would be helpful in planning the appropriate drug policy for the area thus directly useful to programme strategies. In addition, clinical efficacy of drugs could be validated by molecular markers as well as prevalence of drug resistance related markers among the field isolates. Continuous surveillance to analyze the mutations of target molecules may offer a useful tool in epidemiological surveys for drug resistant malaria.

2.1.5 *Plasmodium falciparum* circumsporozoite protein variations in Indian isolates

The circumsporozoite (CS) protein is the predominant protein found on the surface of sporozoite, it has approximately 420 amino acids with a molecular weight of 58 kDa. Sequence analysis of *P. falciparum* CS gene showed two non-repetitive regions at 5′ and 3′ ends and a variable central region containing immunodominant B-cell epitopes with a multiple repeats of four amino acids motifs. The C-terminal part of the sequence contains two polymorphic T-cell epitopic regions, Th2R and Th3R flanking the highly conserved RII region and spanning amino acid residues from 326 to 343 (Th2R) and 361 to 380 (Th3R), respectively (Fig. 11). Extensive polymorphism found in T-helper cell epitope raised doubt about the feasibility of a T-helper cell epitope-based vaccine. Earlier study showed that the variants could be categorized into groups, which renewed hope about the possibility of a T-cell epitope-based subunit polyvalent vaccine. However, if the variations are restricted and are regionally unbiased, then the prototype variants could be included into a subunit polyvalent vaccine against *P. falciparum*. A recent study on vaccine formulation RTS,S based on a section of the CSP including the T-cell epitope has claimed protection against the sporozoite challenge. Though the data of variations in T-helper cell epitopic regions are available from other geographic regions of the world, the data from India, where malaria situations are very critical, are scanty. India is geographically diversified in terms of topography, climate, vectors availability and malaria endemicity. Thus, we aimed to assess the extent of variations in CSP-Th2R and Th3R of *P. falciparum* isolates prevalent at different geographic locations and at different transmission periods.

The CS protein is a potential immunogen and a suitable candidate for its inclusion in a subunit malaria vaccine. However, polymorphism in CS protein, particularly in T-helper cell epitopic regions (Th2R and Th3R) causes an impediment in designing a universally effective CS protein-based vaccine against *P. falciparum*. Global parasite populations demonstrated genetic diversity in CS gene of *P. falciparum* and genotyping studies conducted in India also showed similar findings. We investigated the extent of genetic variations in T-helper cell epitopic regions of CS protein in a large number of *P. falciparum* isolates collected from different parts of the country at different phases of malaria transmission.

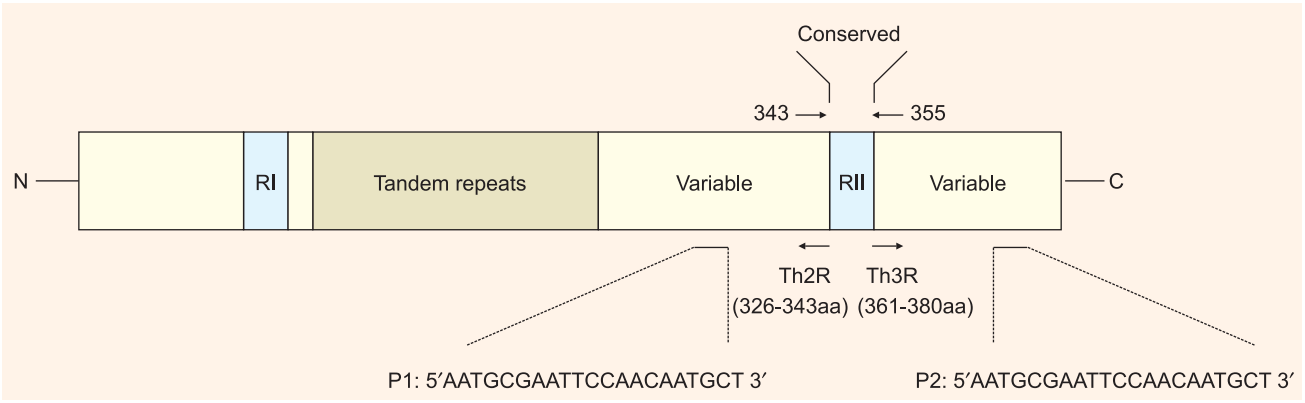

Fig. 11: Schematic diagram of the *P. falciparum* CS protein

The genomic DNA was isolated from 507 *P. falciparum* wild parasite isolates obtained from 6 geographical locations of India at 3-time points coincided with transmissions. The T-helper cell epitopic regions were PCR amplified and the products were purified and then sequenced.

The variations were found to be regionally unbiased and could be categorized into nine groups (I–IX), where group I and II were observed in all the three periods. The variants I-IV in time point (TP-1); I, II, V-VIII in TP-2, and I, II, V and IX in TP-3 were present and they showed restricted heterogeneity. During peak transmission (TP-2), parasite populations found to be more diverse and heterogeneous. Overall patterns and distribution of variants showed significance among isolates of different regions (Fig. 12). Based on sequences, nine variants were found among isolates and those were categorized into 9 groups (V-1 to V-9), where V-1 and V-2 were observed in all 3-time points. The variants V-1 to V-4 in TP-1; V-1, V-2, V-5 to V-8 in TP-2; and V-1, V-2, V-5 and V-9 in TP-3 were present and showed restricted heterogeneity. During peak transmission (TP-2), parasite populations found to be more diverse and heterogeneous and the variants were found to be regionally unbiased and restricted. However, the alleles of V-6 and V-9 in both Th2R and Th3R showed identical sequence variation with those observed in other geographical regions of the world. The remaining seven groups did not show such similarity.

The variants were found to be regionally unbiased, restricted and could be categorized into groups. However, the alleles in two groups (VI and IX) in both Th2R and Th3R showed identical sequence variation with those observed in other geographical regions of the world; rest seven groups did not show such similarity. The Th2R and Th3R epitopes are implicated in host immune response to *P. falciparum*. The polymorphism in these epitopic regions indicates antigenic diversity, which may cause adverse outcome of a subunit vaccine including CS prototype variant. Therefore, the formulation of a vaccine considering the restricted local repertoire parasite populations may be helpful.

2.1.6 Expression, purification and characterization of allelic variants of MSP-1_{42} from Indian *Plasmodium falciparum* isolates

Merozoite surface protein (MSP-1) of *P. falciparum* is the most extensively studied erythrocyte vaccine candidate antigen under consideration. MSP-1 is a 195 kDa antigen and undergoes proteolytic cleavage into smaller fragments. The C-terminal fragment MSP-1_{42} undergoes further cleavage producing 33 and 19 kDa fragments. During invasion, the MSP-1_{19} is carried on to the newly invaded erythrocyte. The C-terminal 19 kDa and 42 kDa fragments of *P. falciparum* MSP-1 have shown to be protective in animals against lethal parasite challenge. The MSP-1_{19} being highly conserved may lack sufficient number of T-cell epitopes in order to elicit a broader response in genetically diverse populations. The inclusion of additional epitopes from the N-terminal MSP-1_{42} has shown to enhance the protective efficacy of MSP-1_{19} vaccine. In an attempt to examine the strain-specific immunogenicity to MSP-1, we have cloned and expressed three diverse allelic variants of MSP-1_{42} from Indian *P. falciparum* isolates in bacteria. Among three alleles, one was extremely rare and not found earlier. These purified and refolded recombinant products were recognized by conformation specific monoclonal

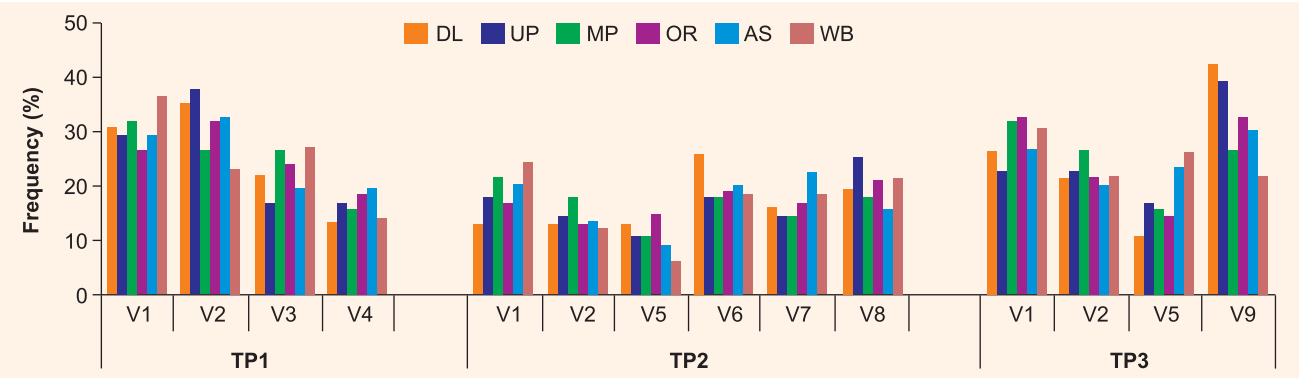

Fig. 12: Frequency distribution of different T-helper cell epitope haplotypes (V-1 to V-9) in six regions of India. V: variant; TP: time point; DL: Delhi; UP: Uttar Pradesh; MP: Madhya Pradesh; OR: Orissa; AS: Assam; WB: West Bengal.

antibodies and hyperimmune sera. Immunization of mice and rabbits with the purified proteins generated high titer biologically active polyclonal antibodies supporting further development of this vaccine candidate.

In the present study, three allelic variant forms of MSP-1_{42} were cloned and expressed from Indian isolates of *P. falciparum,* of which two were analyzed for their immunogenicity in mice and rabbits. The sequence of one of the alleles PfS76 was exactly same as that of 3D7 (E-TSR) and the other PfRK9 is E-KNG type with six amino acids different from 3D7 sequence at positions 1391 (I/F), 1582 (T/P), 1583 (N/Y), 1691 (K/T), 1700 (N/S), and 1701 (G/R) and with a deletion of K at position 1496. The third one PfS2 was completely new allele with an arginine in place of a cysteine at position 1679 and with amino acid variations at positions 1387 (G/E) and 1683 (D/N). All of the nucleotide changes seen in these isolates were non-synonymous. Among these, three variations are in the MSP-1_{33} kDa region and three in the EGF-2 domain of MSP-$1_{19,}$ the EGF-1 being completely conserved. These three allelic variants of MSP-1_{42} were cloned and expressed in *E. coli* expression vector PQE- 30 Xa.

We were interested to see any difference which may affect the growth of the two 3D7 type parasite lines with difference in chloroquine sensitivity in the presence of immune IgG. Antisera from all the rabbits immunized showed substantial inhibitory activity in both the strains at 100 µg/ml of IgG concentration. With a combination of both the proteins at 50 µg each per dose was sufficient to produce inhibitory antibodies higher than single preparation at 100 µg per dose. The combination has the effect of enhancing the response to either of the alleles, showing increased inhibitory activity. The antibodies elicited by immunization were biologically active against *P. falciparum* parasites as seen from the growth inhibition assay. In order to make sure that the inhibitory activity was specific to the antibodies alone, serum purified IgG were used in this study. These results suggest that both the allelic proteins have retained T-helper and inhibitory epitopes necessary for eliciting protective immunity against malaria and that the protective epitopes in both proteins are conserved. A majority of immunodominant T and B epitopes are localized in the conserved or dimorphic regions that are non-polymorphic in the 42-kDa protein of MSP-1.

The field parasite isolates are generally heterogeneous and contain many allelic variant forms. A combination of several variant antigens may have a greater impact as a vaccine as compared to a single one. The results suggest that both the MSP-1 allelic variants either in combination or alone by itself could induce significant immune responses in mice and rabbits with Freund's adjuvant. Furthermore, these antibodies were able to inhibit parasite growth considerably (*in vitro*) suggesting that they are functionally active. Further studies with large-scale preparations and use of human compatible adjuvant will prove the vaccine potential of these preparations for human use.

2.1.7 Polymorphism in *P. falciparum* genes encoding *Pfmsp-1* and *Pfmsp-2* in a population of parasites from eastern and north-eastern India

The study subjects included the inhabitants of eastern (Orissa) and north-eastern (Assam) India. They reported in local malaria clinics with symptoms of malaria. Finger-prick blood samples were collected by cross-sectional surveys. Thick and thin blood smears were checked after staining with JSB stain. Four to 5 drops of finger prick blood were collected from each patient. Blood drops were spotted onto sterile filter paper (Whatman No. 3) strips in triplicate for molecular studies. After drying, blood spotted filter paper strips were stored at 4°C (refrigerator) till use.

Finger-prick blood samples of a subset of 42 and 23 *P. falciparum* patients from eastern and north-eastern areas respectively were taken for molecular analysis of the parasites. Genetic diversity of *P. falciparum* field isolates from eastern and north-eastern areas were assessed using highly polymorphic surface antigen genes, namely *msp-1* and *msp-2*. In these parasite samples, band sizes for *msp-1* varied from 400–650 bp. In case of *msp-2*, band sizes varied from 400 to 950 bp and 450 to 1100 bp in *P. falciparum* isolates of eastern and north-eastern areas respectively. On comparison between the two markers, it was observed that *msp-1* showed single band pattern in 88 and 91.3% parasites respectively, whereas double and multiple bands were found in *msp-2*. The overall level of genetic diversity in polymorphic markers, *msp-1* and *msp-2* was high but almost similar in the two studied populations of *P. falciparum* indicating mostly the polyclonal infection. However, slight differences were observed in both the markers. Our

observations on these two candidate gene markers indicate that *P. falciparum* isolates from north-eastern area were comparatively more diverse than those of eastern area; however, *P. falciparum* was highly polymorphic in both the regions.

2.1.8 Prevalence of ABO blood group and G-6-PD deficiency in malaria endemic Sundargarh district, Orissa

The ABO is arguably the best known with various literatures in support of the hypothesis that *P. falciparum* malaria has shaped the distribution of ABO blood groups in humans. It has been suggested that the origin, distribution and relative proportions of ABO blood groups in humans may have been directly influenced by selective genetic pressure from *P. falciparum* infection. The protective effect of blood group O against severe *P. falciparum* malaria operates through the mechanism of reduced rosetting and that malaria is likely to be a significant factor influencing ABO blood group frequencies in tropical and subtropical regions of the world. Blood samples were analyzed for ABO polymorphisms. Allele frequencies observed for p, q and r in the population were 20.88, 25.67 and 53.66% respectively. We do not find association of blood groups with malaria endemicity. Of the hereditary disorders of blood, G-6-PD enzyme deficiency is one of the most important factors ought to have been associated with a selective survival advantage against falciparum infection. A few reports on the distribution of the genetic markers and its relation to malaria in Indian populations are available. Therefore, a study has been initiated in Sundargarh district of Orissa with the objective to correlate the frequencies of these genetic markers to malaria incidence. Blood samples from males were screened for G-6-PD deficiency by fluorescence spot test. Approximately, 10% of the study population was found to be G-6-PD deficient. Some G-6-PD deficient subjects were having asymptomatic malaria; however, none of the symptomatic subjects had G-6-PD deficiency. G-6-PD deficiency may, therefore, be providing some sort of protection against malaria and may be having some association with clinical outcome. All the G-6-PD deficient samples were subjected to molecular characterization. G-6-PD Orissa was found at a very high rate 87.09% and G-6-PD deficient samples were found to be having this mutation. Of the total G-6-PD deficient samples, 83.87% showed the 1311T/C mutation. Normal non-deficient samples also have 1311T/C mutation. G-6-PD mediterranean mutation was found in 3.22% of the study population.

2.1.9 Molecular characterization of *Plasmepsin IV*, *Falcilysin* and *Heme detoxification protein* (*HDP*) gene in *Plasmodium vivax* and their comparative analysis with primate's malaria parasites

Evolution and spread of drug resistant malaria parasites throughout the globe is the major hurdle to combating this disease. To overcome the drug resistance problem, identification of new molecules as drug targets, is of prime importance in malaria research. The first priority should be to choose a potential drug target gene which is metabolically important for the parasite's survival. *Plasmepsin IV* (*PMIV*), *falcilysin* and *heme detoxification protien* (*HDP*) all three are localised in food vacuole of the parasites playing important role in the hemoglobin degradation pathway. Expression of asexual stages between *P. falciparum* and *P. vivax* is different, however, both are human malaria parasites. *Plasmodium vivax* is very close to monkey malaria parasites, therefore, comparative study for the above three genes with simian malaria parasites would provide information about the homology and sequence conservation between species.

To understand the sequence conservation among different malaria species infecting human, primates and rodent, we have retrieved gene sequences from the *Plasmodium* genome database (www.plasmodb.org). Protein sequence alignment and N-J phylogenetic tree analysis showed high degree of sequence conservation between different plasmodium species for the *PvPMIV* (Fig. 13), *Falcilysin* (Fig. 14), and *HDP* (Fig. 15). This suggests that these food vacuole gene products could be a possible drug targets. Primers for the *PvPMIV* genes have been optimized for the amplification from field isolates (Fig. 16) to assess the sequence variations among the field isolates.

2.1.10 Chloroquine susceptibility of *P. vivax* field isolates in Chennai

Chloroquine-resistance in *P. vivax* was first reported in Papua New Guinea (1989), then in the south-western Pacific (1989) and Indonesia (1991). Since then sporadic cases of resistance have been reported in Bombay (Maharasthra, 1995), Mathura

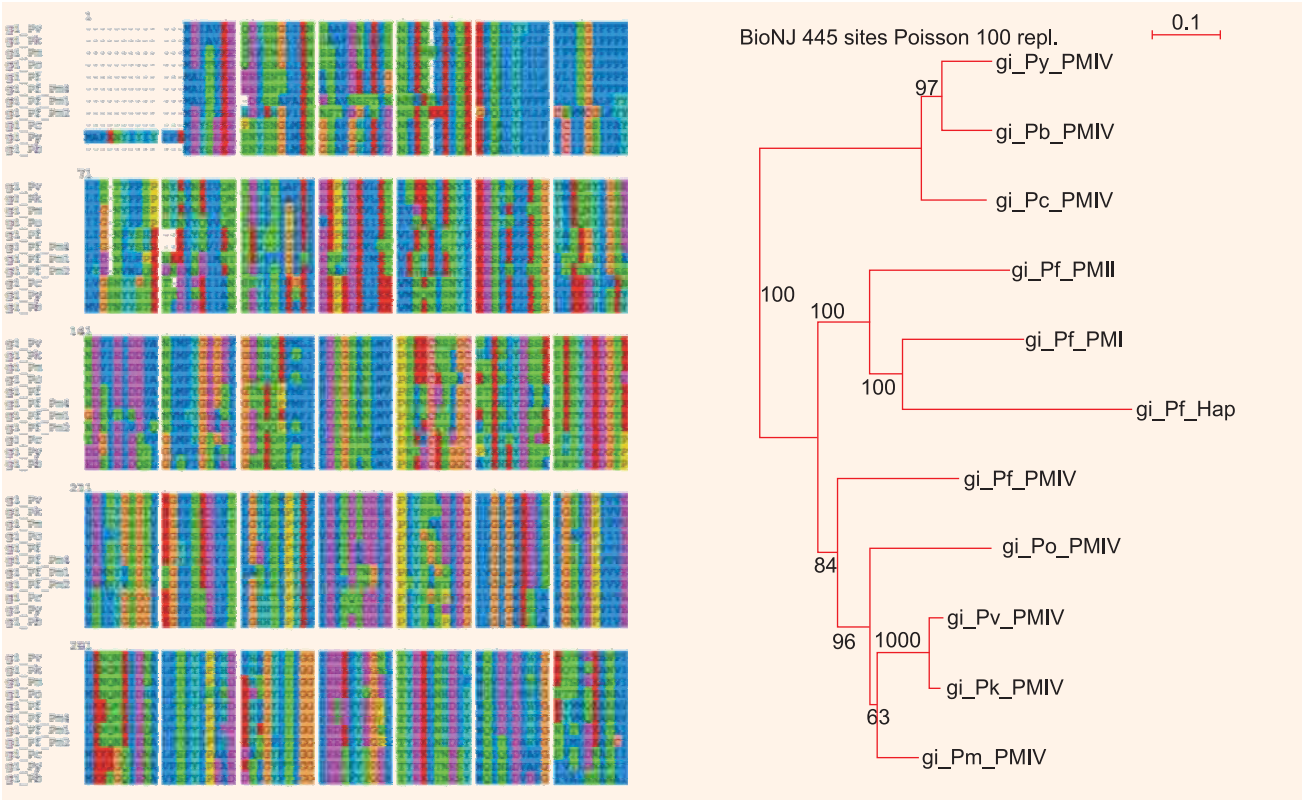

Fig. 13: Alignment and BioNJ of *PMIV* gene from different *Plasmodium* species.

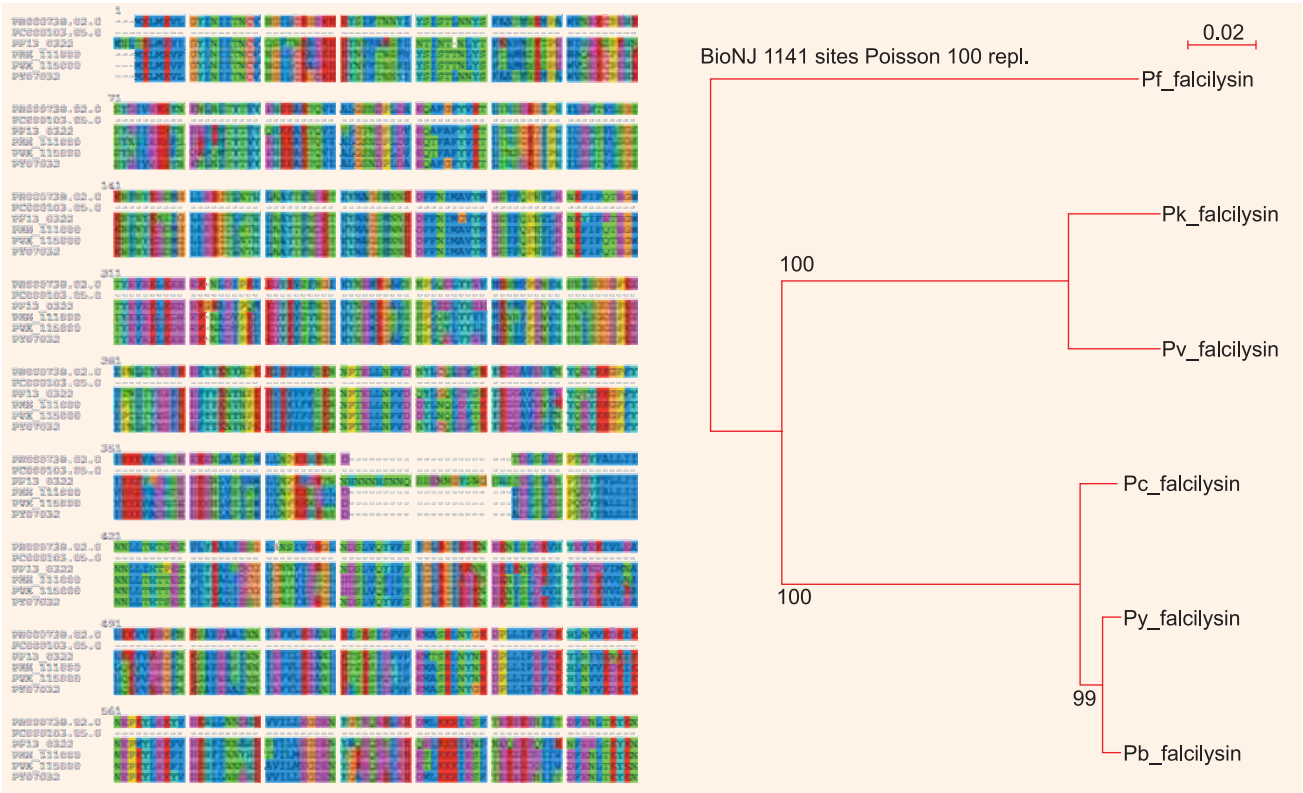

Fig. 14: Alignment and BioNJ of *Falcilysin* gene from different *Plasmodium* species.

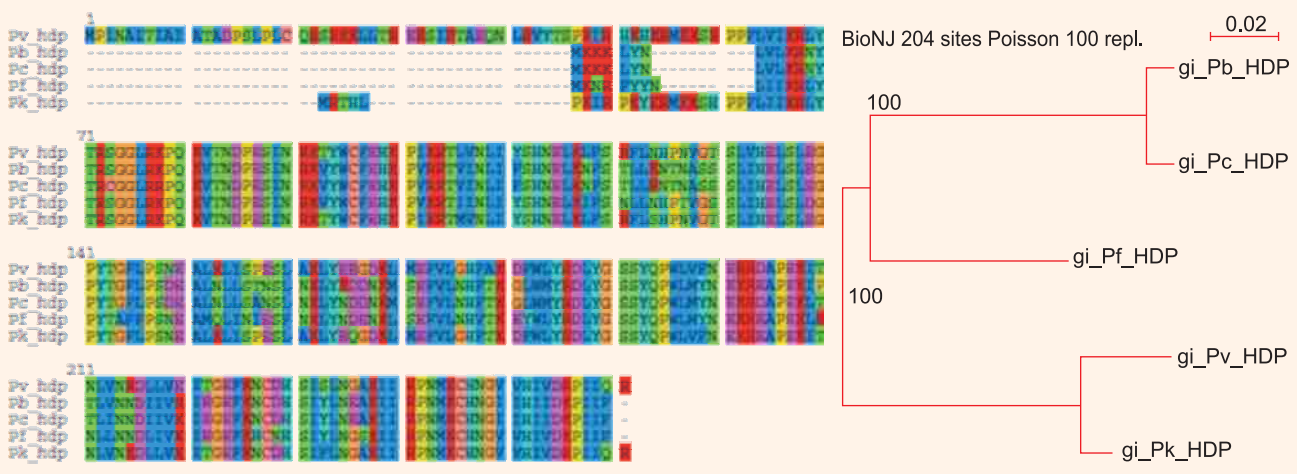

Fig. 15: Alignment and BioNJ tree of *HDP* from different *Plasmodium* species.

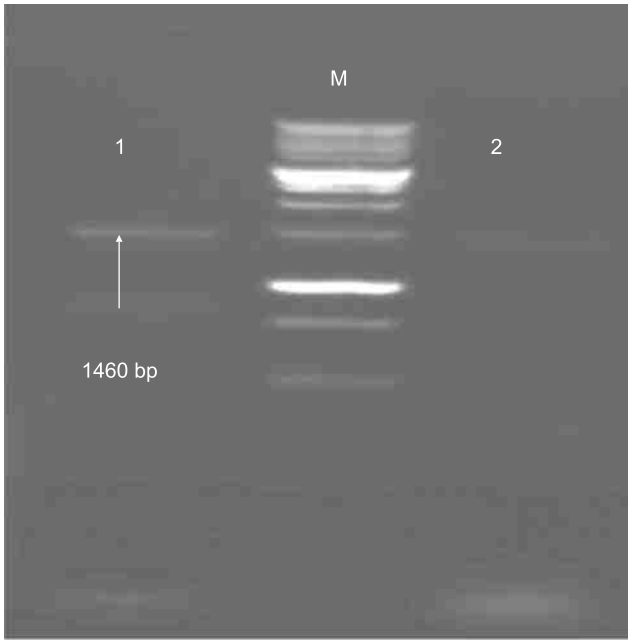

Fig. 16: Amplification of *PMIV* gene in the *Plasmodium vivax* field isolates.

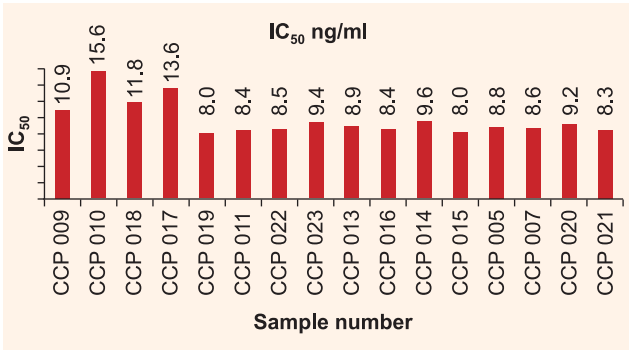

Fig 17: IC_{50} in ng/ml of the analysed 16 samples.

(Uttar Pradesh, 1996), in Daltonganj (Bihar, 2000) and recently in Nadiad (Gujarat, 2008). As chloroquine (CQ) is the first line drug against *P. vivax,* is important to examine regions for development of resistance against it. A study was designed with an aim to assess the chloroquine sensitivity of *P. vivax* isolates from Chennai (Tamil Nadu), which is an endemic region for *P. vivax* malaria. A total of 16 blood samples were collected from *P. vivax* positive patients. *In vitro* drug sensitivity test was performed by *ex vivo* maturation in the chloroquine coated 96 well culture plates. The average IC_{50} and MIC of the samples came out to be 9.7 and 219.18 ng/ml respectively. Though both the IC_{50} and MIC were found to be asserting no significant decrease in chloroquine sensitivity, it was noticed that few samples had higher tolerance for chloroquine than the rest (Fig.17).

2.2.1 Comparative profile of immune response to *P. vivax* antigens in a population of Delhi, northern India

The study subjects included the inhabitants of Delhi and adjoining areas. They reported in local malaria clinics and hospitals of Delhi city with symptoms of malaria. Finger-prick blood samples were collected by cross-sectional surveys from a group of individuals; those were reported with malarial symptom, especially fever. Some patients had earlier history of malaria but found malaria parasite negative at the time of blood collection. Both *P. vivax* and *P. falciparum* cases were recorded from the study areas. Thick and thin smears were checked after staining with JSB stain.

For this study, equal numbers (n = 47) from each

group of study subjects including children, both young and old (1 to <15 years), adults and *P. vivax* patients were taken. Serum was separated from finger prick blood samples obtained from each person. They were tested for antimalarial antibody level against eight antigens specific for *P. vivax*. High levels of antibodies were detected in almost all the sera. Substantial amount of antibodies against CSP, MSP-1, AMA1, GAM1, ESP1 and ESP2 were detected in sera of adults and *P. vivax* patients. Antibodies against LDH1 and LDH2 were detectable in almost all the sera of *P. vivax* patients (Figs. 18–20). Seroreactivity of the study subjects were compared among different groups with known malaria negative healthy individuals. A range of low, moderate and high immune responders was observed in these groups. From this study, it is observed that ESP1 and ESP2 are not good markers for differentiating the disease status of an individual. The antibodies against these molecules were detected in 98% of individuals at various intensities.

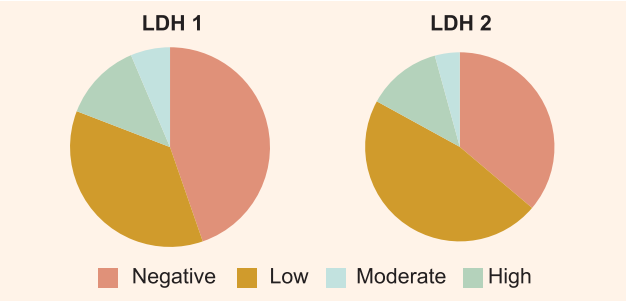

Fig. 18: Frequency of antibody responders in children.

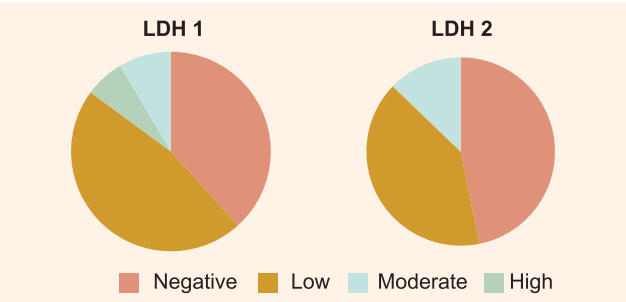

Fig. 19: Frequency of antibody responders in adults.

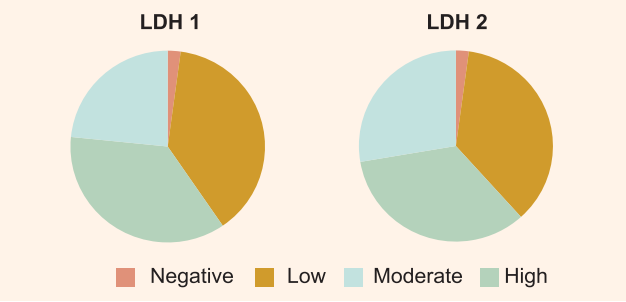

Fig. 20: Frequency of antibody responders in patients.

However, LDH1 and LDH2 were able to differentiate between symptomatic and non-symptomatic subjects. Of the 47 patients with *P. vivax* infection, 46 were detected positive for antibody. Therefore, LDH1 and LDH2 could be taken as marker antigens in serodiagnosis.

2.2.2 Naturally occurring antibody response to *P. falciparum* merozoite surface protein-1 in two sub-populations of Orissa

The study was undertaken with the following objectives: (i) to assess the antibody response of individuals in the two subpopulations of Orissa by determining serum IgG reactivity by ELISA against a defined asexual erythrocytic stage antigen of *P. falciparum*, merozoite surface protein 1 (*msp-1*); and (ii) to measure the age-wise frequency of antibody responders in individuals of two study cohorts.

Finger-prick blood samples were collected by cross-sectional surveys. Some patients had earlier history of malaria but found malaria parasite negative at the time of blood collection. Both *P. vivax* and *P. falciparum* cases were recorded from the study areas. Of the 2000 individuals, 501 were recruited for immunologic study. These 501 subjects include 250 from subpopulation-1 (SP-1) and 251 from subpopulation-2 (SP-2) areas, respectively. Thick and thin blood smears were checked after staining with JSB stain. The sera were stored at –20°C until use.

The differences in parasite positivity of SP-1 vs SP-2 were 6.8 vs 1.3%, respectively. The mean age of the four different age groups in SP-1 and SP-2 remained almost similar. All 250 and 251 sera obtained from SP-1 and SP-2 areas were tested for their reactivity to *P. falciparum* antigen, MSP-1. Overall, higher antibody responses were observed in study population of SP-1 compared to SP-2. Seroreactivity of the study subjects was compared among different age groups with known malaria negative healthy individuals. A range of low, moderate and high immune responders was observed in these groups (Figs. 21 & 22). In overall SP-1 subjects, negative, low, moderate and high antibody responders varied from 0 to 2, 0 to 4, 0 to 14 and 80 to 100%, respectively. However, >50% (52–57) subjects did not have antibody response to MSP-1 in SP-2 population. Overall, low, moderate and high responders were found in 2–6, 8–10 and 32–33% subjects of SP-2 area. Our

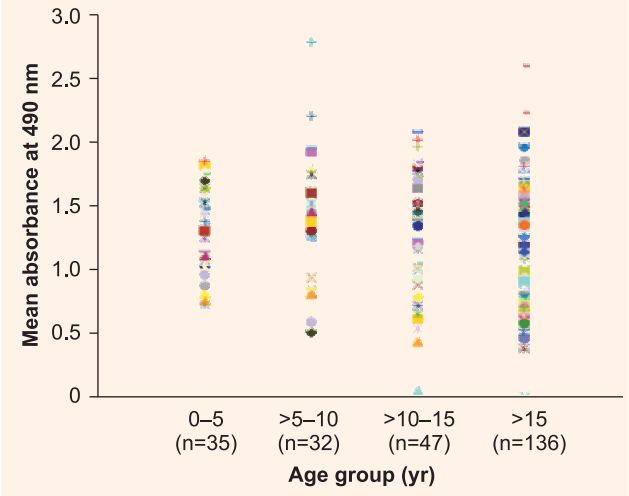

Fig. 21: Scatter diagram of antibody response in sub-population-1.

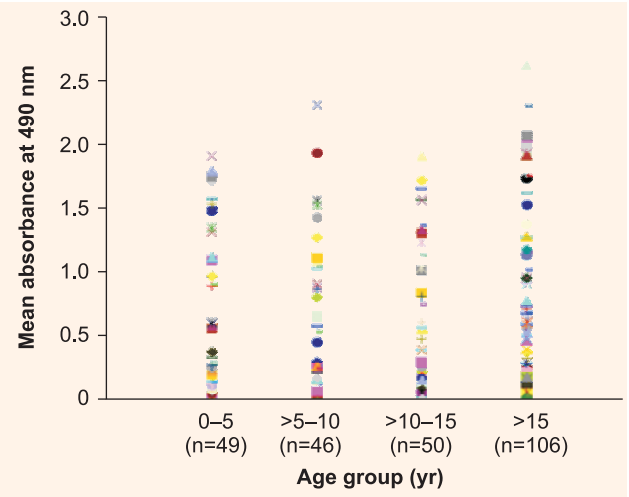

Fig. 22: Scatter diagram of antibody response in subpopulation-2.

findings revealed an overall higher ELISA IgG reactivity for samples from SP-1 compared to SP-2 against MSP-1 antigen. The MSP-1 used in antibody detection by indirect ELISA was a synthetic peptide representing major B-cell epitope of this antigen. This epitope was recognized by the sera samples showing variable degree of antigen-antibody reactions. In the present study, MSP-1 appears to be immunogenic in both SP-1 and SP-2 and it showed variable response in two subpopulations.

2.2.3 Serum cytokine profiles in *P. vivax* malaria patients

Cytokines may be the key determinants of clinical expression, severity and outcome of malaria infections. Specific cytokines reported to be of importance in human malaria include tumour necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL-4, IL-6, IL-10), etc. Balance between pro- and anti-inflammatory cytokines determines the degree of parasitaemia, level of anaemia, clinical severity and outcome of infection. Studies have shown the association between various signs and symptoms in patients suffering from *P. vivax* and their serum cytokine levels. Present study was conducted in a group of *P. vivax* patients and healthy subjects from northern India. Study patients reported with high fever, high parasite count, anaemia and liver dysfunction were compared with *P. vivax* patients without these symptoms. Peripheral blood from *P. vivax* malaria patients and healthy cohort participants were taken for estimation of cytokines in serum using commercially developed two-site ELISA assay kits.

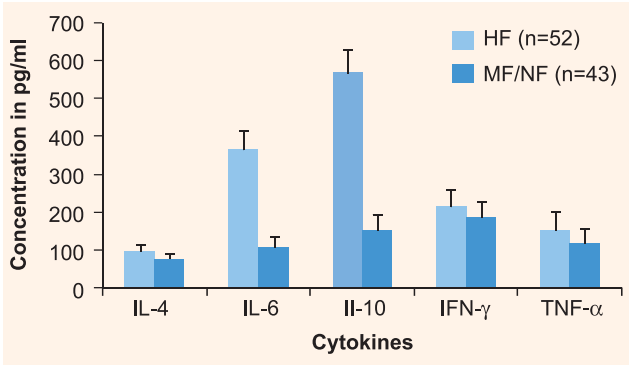

Fig. 23: Cytokine profiles in patients reported with fever.

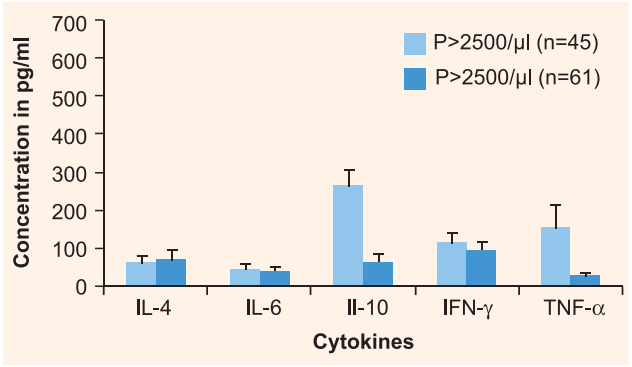

Fig. 24: Cytokine profiles in patients with high or low parasitaemia.

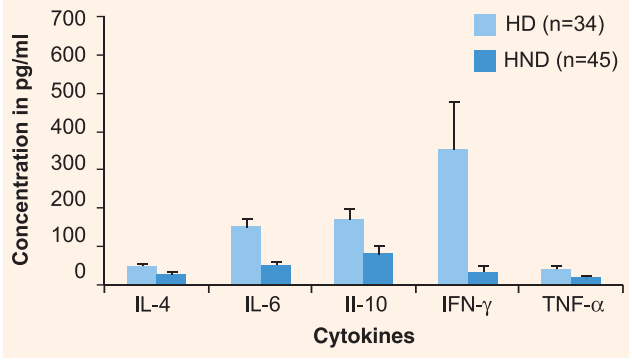

Fig. 25: Cytokine profiles in patients reported with hepatic dysfunction.

As shown in Figs. 23–25, patients reported with high fever had higher serum concentration of IL-6 and IL-10 compared with those of without fever and healthy normals. Patients with parasite count above 2500/µl had higher IL-10 and TNF-α level compared to healthy control. No association between serum cytokine concentrations and anaemia has been observed in the study cases. Patients with *P. vivax* infection, who presented with hepatic dysfunction showed higher serum concentration of IL-4, IL-6, IL-10, IFN-γ and TNF-α than those without hepatic dysfunction. The findings of this study suggest that the production of pro- and anti-inflammatory cytokines, cytokine profile and their interaction may be significant in the immune response to *P. vivax* malaria.

2.2.4 Purification and characterization of monoclonal antibodies against erythrocytic stages of *P. vivax*

The stable, well-grown, *P. vivax* antibody positive hybridoma lines were taken for further characterization. The culture supernatant of individual hybridoma was assayed for quantification of mouse-specific IgG by Pierce easy titre Mouse IgG assay kit. The cell culture fluid contained varying concentration of IgG. All 6 Immunoglobulin-G fractionated by ammonium sulphate precipitation and purification was done on Sephadex G-50 column. *P. vivax* proteins from crude parasitized erythrocyte lysate were purified by Affinity chromatography. Commercially available cyanogen bromide activated Sepharose-4B was coupled with immunoglobulin fractions of individual monoclonal antibody. Crude *P. vivax* erythrocyte lysate was passed through each preparation of gel and allowed to react for 1 hour at room temperature. Unbound proteins were eluted as fractions with 0.1 M Glycine-HCl, pH 2.8. After neutralizing pH, fractions were concentrated by Amicon ultra centrifugal filtration (Millipore). The concentrated protein fractions were stored at –20°C for further purification. The protein eluates after concentration were purified further by HPLC on a Bio-Gel column. Individual protein fractions were loaded on C18 column. The fractions were eluted with mobile phase channel A (10 mM phosphate buffered saline, pH 7.2) and channel B (Acetonitrile). The flow rate was set at 1 ml per min. The absorbance of each eluted fractions was read at 280 nm and chromatograms were analyzed. *Plasmodium vivax* proteins from crude parasitized erythrocyte lysate purified by Affinity chromatography and then by HPLC were separated on a 10% non-denaturing SDS-PAGE. Discrete bands were excised from Coomassie blue-stained gel. The *in-gel* tryptic digestion of the spots was carried out using high quality mass spectroscopy grade trypsin reagent. The digested mixture of proteins was subjected to MALDI-TOF analysis for peptide mass finger-printing. Probable peptides obtained after Peptide mass finger printing (tryptic digestion of the PvAg against six MAbs) followed by MASCOT search for MS data. The analyzed proteins were checked for homology based on the sequences available on database.

2.2.5 Toll like receptor (TLR) polymorphism in the Indian population in relation to malaria

The immune system is pronged into two major branches, the Innate immunity and the Adaptive immunity. The innate immune system recognizes the broad structural motifs conserved within the microbial species through the receptors of the innate immune system broadly called as the pattern recognition receptors (PRRs). Many different pattern recognition receptors have been identified and Toll-like receptors (TLRs) is one of them. The involvement of the Toll receptors in innate immunity was first described in drosophila. A total of 10 TLRs have been identified in humans. In recent years, role of TLRs in various diseases like tuberculosis, sepsis has been elucidated.

The role of TLRs in malaria has been recently elucidated. In malaria, TLRs 2, 4, and 9 have shown to be involved. Thus, study of polymorphism in these three TLRs in connection with malaria of the exposed population, can be of grave importance regarding the development of new treatment strategies. The polymorphisms at position 753 and 677 in TLR 2, mutations at positions 299 and 399 in TLR 4 and –1486 and –1237 in TLR 9 are associated in various diseases which prompted us to study their role in malaria.

Polymorphic studies at these positions in TLRs 2, 4 and 9 have been carried out in patients from different regions of India by employing PCR-sequencing methods.

Investigation carried out in 22 samples from Car Nicobar revealed that in TLR 2 at positions 677 (G →A) and 753 (G→A) wild type genotypes were observed. However, at position 707 (T→C) a synonymous mutation was observed only in one sample (Fig. 26).

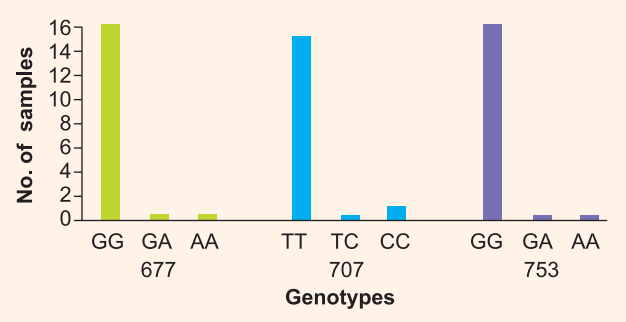

Fig. 26: Genotypes in TLR 2 position.

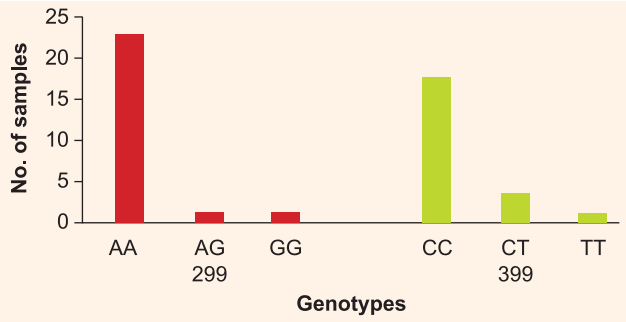

Fig. 27: Genotypes at TLR 4 position.

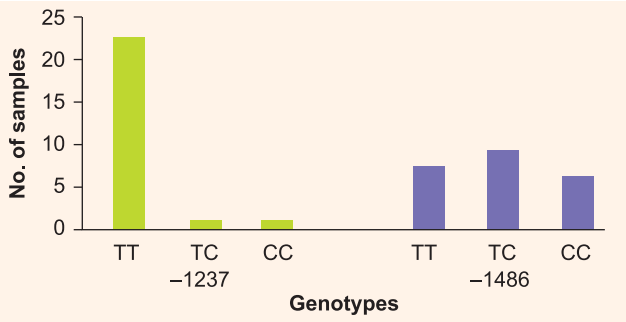

Fig. 28: Genotypes at TLR 9 position.

In TLR 4, at positions 299 (A→G) wild type genotypes were observed. However, at position 399 (C→T) 17 samples with wild type genotype (CC), 3 with heterozygote genotype (CT) were observed, following the Hardy-Weinberg law (Fig. 27).

In TLR 9, at position –1237 (T→C) wild type genotype was observed. However, at position -1486 (T→C) 7 samples with wild type genotype (TT), 6 with mutant genotype (CC) and 9 with heterozygote genotype (TC) were observed, following the Hardy-Weinberg law (Fig. 28).

This being a preliminary investigation, a complete picture of the genetic make-up of the Indian population can only be reached after the samples collected from different malaria endemic regions are analyzed.

2.2.6 Molecular evaluation of human leukocyte antigen in malaria endemic population and its association with malarial host immunity

Major histocompatibility complex (MHC) molecules play an important role in the induction of immune responses; the evolution of MHC polymorphism is often explained in terms of increased protection of hosts against pathogens. MHC is divided into highly polymorphic Class I (HLA-A, -B, -C) and Class II (HLA-DP, -DQ, and -DR) loci, which in turn are subdivided into distinct regions. Genes encoding the HLA proteins are among the most diverse in the human genome and evidences suggest that HLA molecules are considered to play a crucial role in the defense of the host against malaria infection. Different HLA alleles have been reported to be associated with reduced susceptibility to malaria or severity of malaria in different populations. The aim of the work is to study the degree of polymorphism in Indian isolates infected either with *P. falciparum* or with *P. vivax* and association of specific alleles with disease severity or resistance.

A total of 40 positive samples from Ranchi (n = 21) and Delhi (n = 19) infected with *P. falciparum* (*Pf*) and 10 control samples have been collected and processed for DNA extraction. HLA typing is done using commercially available PCR-SSP kits which include 48 alleles for HLA-B locus, 24 alleles for HLA DRB locus and 8 alleles for HLA DQB1 locus.

Isolates infected with *P. falciparum* showed higher frequency of HLA *B61* and HLA *B58* alleles 26.19 and 14.28% ($p < 0.0006$, $p < 0.03$) from Ranchi and 21.05 and 10.25% from Delhi ($p < 0.001$, $p < 0.2$) respectively compared to controls which showed only 5.55% whereas HLA *B51* allele showed lower percentage frequency for both Ranchi 4.76% ($p < 0.008$) and Delhi 7.89% ($p < 0.07$) compared to controls, i.e.16.66% and HLA *B35* also showed lower allele frequency, i.e. 14.28% ($p < 0.14$) for Ranchi and 15.78% ($p < 0.2$) for Delhi samples when compared to 22.22% in controls (Fig. 29). DRBI*1501 allele frequency increased significantly by 30.95% ($p < 0.0005$) in Ranchi and 13.15% ($p < 0.7$) in Delhi isolates in comparison to 11.11% in controls whereas DRB1*0301 showed significant decreased frequency by 4.76% ($p < 0.008$) in Ranchi and 10.52% ($p < 0.3$) in Delhi isolates in comparison to 16.16% higher frequency in control (Fig. 30).

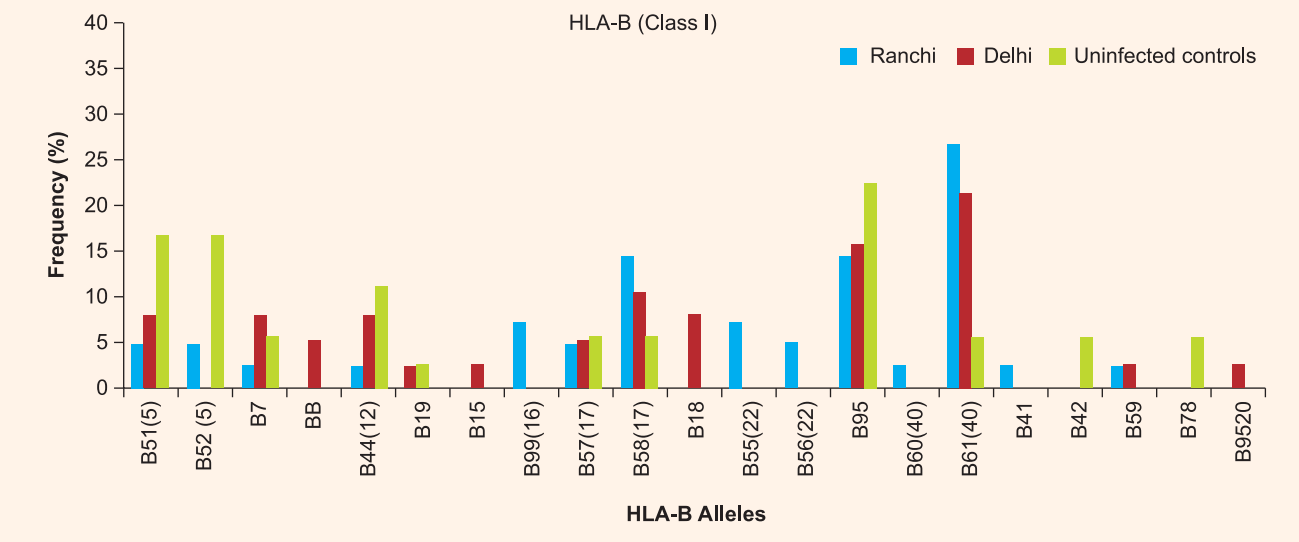

Fig. 29: Bar diagram showing distribution of variants of HLA B (Class I), alleles *loci* in malaria patients as well as in control subjects. A comparison of percentage frequency of alleles at three *loci* among infected patients from Ranchi, Delhi and uninfected controls.

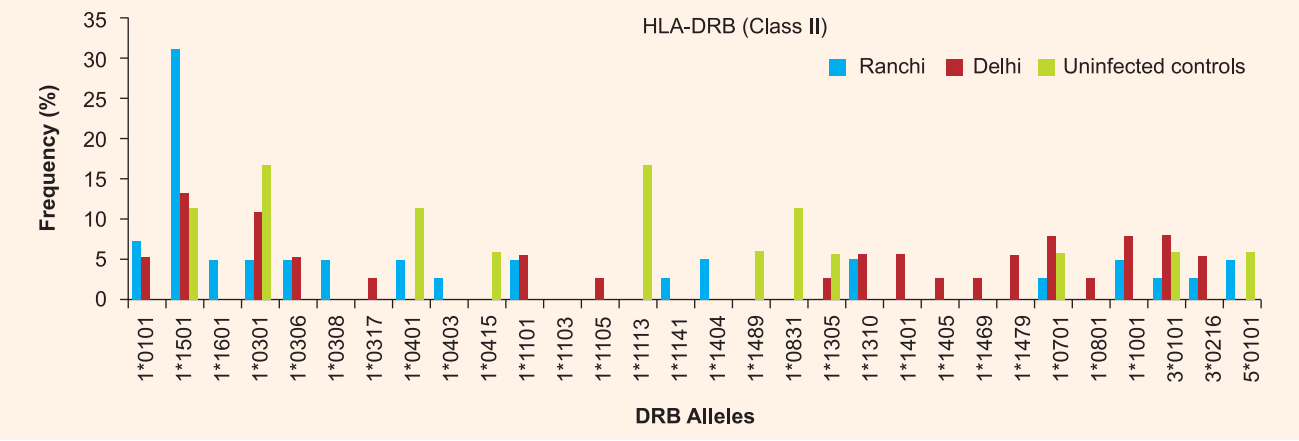

Fig. 30: Bar diagram showing distribution of variants of HLA DRB (Class II) alleles *loci* in malaria patients as well as in control subjects. A comparison of percentage frequency of alleles at three *loci* among infected patients from Ranchi, Delhi and uninfected controls.

DQB1*0501 showed significant increase of 42.85% ($p < 0.0004$) in Ranchi and 36.84% ($p < 0.0002$) in Delhi when compared to 11.11% in controls and DQB1*0301 showed a decrease in allele frequency in infected samples 16.66% ($p < 0.05$) in Ranchi and 10.52% ($p < 0.002$) in Delhi when compared to 27.77% in controls (Fig. 31).

Although from the above results, HLA*B61*, *B58*, DRB1*1501, DQB1*0501 alleles seem to be susceptible, whereas *B51*, *B35*, DRB1*0301 and DQB1*0301 seem to be resistant. HLA diversity and association studies can be utilized in elucidating the immune mechanisms involved in protective immunity, which is not fully investigated in Indian population. HLA diversity in malaria pathogenesis and protection will provide comprehensive and base line data about the genetic

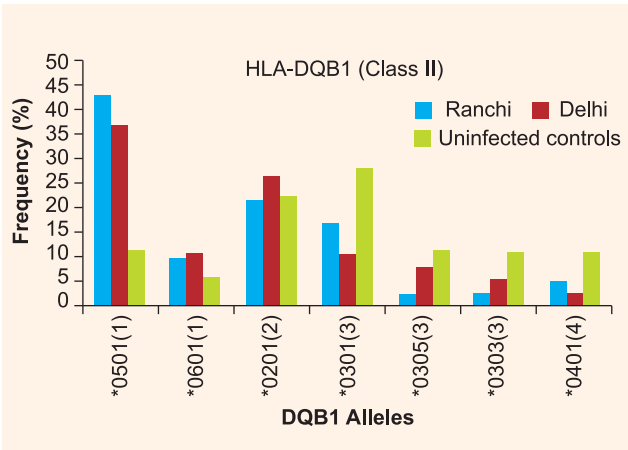

Fig. 31: Bar diagram showing distribution of variants of HLA-DQB1 (Class II) *loci* in malaria patients as well as in control subjects. A comparison of percentage frequency of alleles at three *loci* among infected patients from Ranchi, Delhi and uninfected controls.

and immunological status of the population studied from epidemic regions, which subsequently help in new vaccine designing and vaccine trial site development.

2.2.7 Molecular characterization of aspartic protease gene of *P. vivax*

Plasmepsins are aspartic proteases found in all *Plasmodium* species examined to date. Plasmepsin 4 (PM4) and Plasmepsin 5 (PM5) are essential in parasite functions and viability such as hemoglobin digestion, processing and export of proteins. In order to validate appropriate use of plasmepsins *PvPM-IV* and *PvPM-V* in systematic investigations into inhibitor drug design and development studies on genetic variations among *P. vivax* plasmepsins *PvPM-IV* and *PvPM-V* gene from different geographical areas is of utmost importance. We have already done amplification, cloning, and sequencing of the Aspartic protease-IV and Aspartic protease-V gene of *P. vivax*. The full length of the sequenced *PvPM-IV* and *PvPM-V* was deposited in the GenBank database under Accession Nos. GU569924 to GU569935 respectively.

DNA sequence analysis showed that *PvPM-IV* and *PvPM-V* represent an orthologue of PMs from other *Plasmodium* species. These plasmepsins show an open reading frame of 1353 bp and 1635 bp, which is predicted to encode proteins of 450 and 544 amino acid residues respectively. PMs from *P. berghei*, *P. ovale*, *P. vivax* and *P. malariae* show 450/451 amino acid residues while PfPMs I and II are 455 and 453 residues in length, respectively. Multiple alignment of *P. vivax PvPM-IV* and *PvPM-V* from different geographical regions of India showed 100% nucleotide sequence identity among themselves.

Phylogenetic analysis shows that each *PvPM-IV* and *PvPM-V* is clustered in a group and this is independent of host infection specificity, and in the same group, the PMs from other *Plasmodium* spp. (*P. knowlesi*, *P. malariae*, *P. ovale*) are more phylogenetically related between them compared to the human *Plasmodium* spp *P. falciparum*. Phylogenetically our sequence is highly homologous to *P. vivax,* less homologous to *P. knowlesi* and highly diverse to *P. falciparum, P. yoelli*, and *P. berghei*.

Finally for the structural analysis, 3-D structure of plasmepsins were drawn by molecular modelling PM4 and PM5 sequences modelled for 3-dimensional structure at I-TASSER server (http://zhang.bioinformatics.ku.edu/I-TASSER/) which is based on multiple-threading alignments by LOMETS and iterative TASSER simulations. Structures were further analyzed using VMD software (University of Illinois) and were selected on the basis of RMSD values and $<1\%$ deviation from Ramachandran Plot. The theoretical 3-D structure model of PM4 showing the highly conserved active site pocket from 154 to 165 amino acid residues and from 345 to 336 amino acid residues (Fig. 32). The C-score for the model is -1.98, the Exp. TM-Score is 0.48 ± -0.15 and Exp. RMSD is 11.8 ± 4.5. Three highly conserved pepsin-like domain from amino acids 145 to 165, 289 to 302 and amino acids 422 to 432 are also present. These pepsin domains are signature features for protease family.

Similarly, structural model of PM5 also shows highly conserved active site pocket from 77 to 88 amino acid residues and from 318 to 329 amino acid residues (Fig. 33). The C-score for the model is -2.59, the Exp. TM-Score is 0.41 ± 0.14 and Exp. RMSD is 13.9 ± 3.9. Three highly conserved pepsin-like domains from amino acids 68 to 88, 226 to 239 and 446 to 461 are also present.

Together with the biochemical studies our homology data on PvPMs in Indian isolates point toward a close degree of functional and structural conservation that could be exploited by our future work on plasmepsin inhibitors effective against multiple digestive vacuole plasmepsins. Therefore, blood stage attenuation of these enzymes may help identify new approaches to malaria pathologies as they are potentially promising targets for antimalarial drug development strategies.

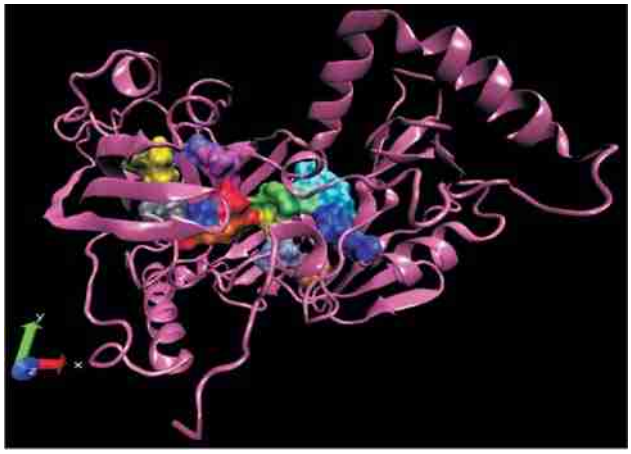

Fig. 32: Structural model of Aspartic protease-IV showing the highly conserved complete active site pocket and pepsin-like conserved domains.

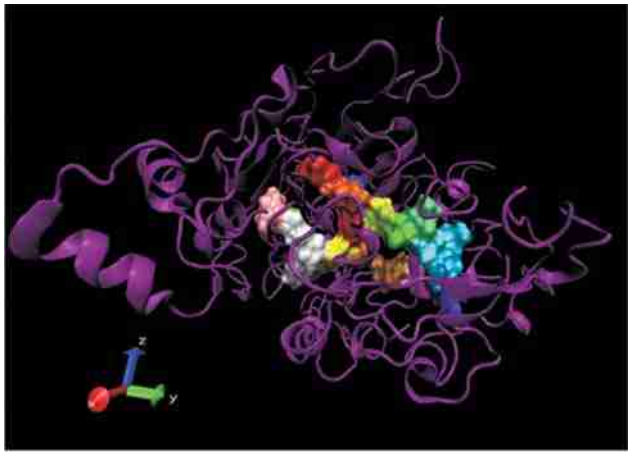

Fig. 33: Structural model of Aspartic protease-V showing the highly conserved complete active site pocket and pepsin-like conserved domains.

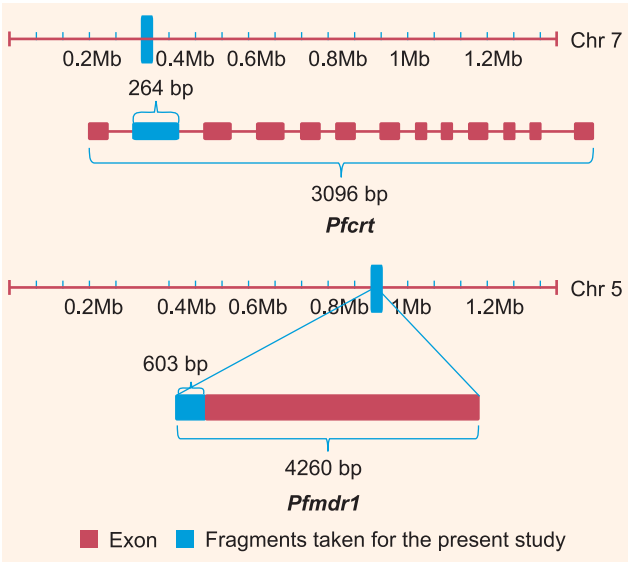

Fig. 34: Locations of *Pfcrt* and *Pfmdr1* genes on their respective chromosomes and of the genetic regions taken for sequencing.

2.3.1 Sequence analysis of coding DNA fragments of *pfcrt* and *pfmdr-1* genes in *P. falciparum* isolates from Orissa, India

Emergence and spread of the antimalarial-resistant malaria parasites across the globe is the major problem in malaria control. Genetic basis of the parasite's resistance to chloroquine (CQ) is well-documented, creating possibilities to analyze field isolates of malaria parasites for seeking evolutionary questions on the origin and spread of CQ resistance. We herewith present data on DNA sequence analyses of the second exon and the 5′ end portion of the *pfcrt* and *pfmdr1*genes (Fig. 34), respectively in 40 *P. falciparum* field isolates collected from eight different localities of Orissa, India (Fig. 35). We have first genotyped the samples for the *Pfcrt*-K76T and *Pfmdr1*-N86Y mutations in these two genes, majorly implicated for chloroquine resistance. Further, we documented the *pfcrt* haplotypes based on the amino acid changes in the 72–76th positions of the amino acid sequence (Table 2). Interestingly, both the K76T and N86Y mutations were found to co-exist in 32 out of the

Fig. 35: Map of India indicating Orissa and location of the eight places from where samples of *P. falciparum* isolates have been collected. The sample size from each location are—Sundargarh (7), Mayurbhanj (6), Keonjhar (6), Angul (5), Kandhamal (5), Rayagada (5), Kalahandi (2) and Cuttack (4).

Table 2. Nucleotide sequences and amino acid residues of codons 72–76 of the *Pfcrt* and 86 and 184 codons of *Pfmdr1* genes in *P. falciparum*

Position of codon	*Pfcrt* gene fragment					*Pfmdr1* gene fragment	
	72	74	75	76	97	86	184
Wild type	TGTCys	ATGMet	AATAsn	AAALys	CACHis	AATAsn	TATTyr
No. of isolates with wild mutations	35	13	13	8	30	8	38
Mutant type	AGTSer	ATTIle	GAAGlu	ACAThr	CTCLeu	TATTyr	TTTPhe
No. of isolates with mutant mutations	5	27	27	32	10	32	2

Table 3. Details of *Pfcrt* and *Pfmdr1* gene fragments and population genetic parameters in *P. falciparum* isolates of Orissa, India

Genes	*Pfcrt* (308526...311620)	*Pfmdr1* (957885...962144)
	3096 bp	4260 bp
No. of isolates	40	40
Size of the fragment (bp)	264	603
Nucleotide position	308619...308883	957885...958488
No. of SNPs	6	2
No. of haplotypes	4	3
Haplotype diversity	0.719	0.344
Nucleotide diversity θ	0.00541	0.00078
π	0.00866	0.00071
Tests of neutrality		
Tajima's *D*	1.6583	-0.1789
Fu & Li's *D**	1.1919	0.77124
Fu & Li's *F**	1.5621	0.57422

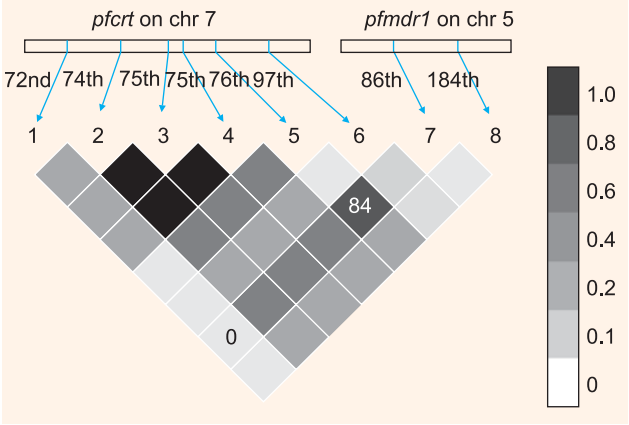

Fig. 36: LD plot (r^2) between 28 possible pairs of SNPs in *Pfcrt* and *Pfmdr1* genes in *P. falciparum*. The strength of statistical significance of LD between a pair of SNPs is represented with the extent of darkness of the boxes.

total 40 isolates, which were of either CVIET or SVMNT haplotype, while rest of the eight isolates were of CVMNK haplotype. In total, eight non-synonymous single nucleotide polymorphisms (SNPs) were observed, six in the *Pfcrt* and two were in *Pfmdr1* genes. One not-so-far well-studied SNP in the *Pfcrt* gene (A97T) was found in an appreciable frequency in many *P. falciparum* samples. Population genetic analyses of the two gene fragments revealed comparatively higher nucleotide diversity in the *Pfcrt* than the *Pfmdr1* genes (Table 3). Furthermore, linkage disequilibrium (LD) was found to be tight between closely spaced SNPs of the *Pfcrt* gene (Fig. 36). In general, both the genes were found to evolve under standard neutral model of molecular evolution.

2.3.2 Characterization, comparative genomic and evolutionary inferences of a human drug metabolizing (*NAT2*) gene

The present day genetic architecture of a species bears much significance to its closely related species. Recent availability of whole genome sequence data for several closely-related species, it is now possible to infer, detect genetic similarities and differences in specific lineages and infer the role of evolutionary forces in bringing such similarities/differences. In this respect, the NAT2 that is primarily responsible for drug metabolisms in human is conserved across a few taxa and thus, comparative genomic studies could be useful for better pharmacogenetic realization. DNA sequences of human *NAT2* gene was retrieved from NCBI website and characterized (Fig. 37). Comparative and evolutionary analyses were performed with sequences from four mammalian taxa and one avian taxon with different statistical algorithms. The observed genetic architecture of *NAT2* gene was different across the six taxa. Comparisons on several genetic aspects (Table 4), viz. total gene length, intron length, length of exon containing open reading frame (ORF) (Fig. 38), number of exon and intron, schematic representation of coding region and untranslated region (UTR) (Fig. 39), non-synomous vs. synonymous substitutions (Table 5), amino acid

Table 4. Details of genetic characterization of the *NAT2* gene in six different taxa

Species	Chromosomal location	Accession No. (Gen bank)	Gene		Gene length (in bp)	Total no. of Exon	Exon length (in bp)		Coding region (in bp)	Intron length (in bp)
			Start	End			Exon 1	Exon 2		
Human (*Homo sapiens*)	8	NC_000008	18248755	18258723	9969	2	101	1417	873	8652
Chimpanzee (*Pan troglodytes*)	8	NC_006475	14595015	14609011	13997	2	101	1418	873	12478
Rhesus monkey (*Macaca mulatta*)	8	NC_007865	18284492	18294740	10249	2	101	1417	873	8731
Mouse (*Mus musculus*)	8	NC_000074	70018847	70026469	7623	2	126	1337	873	6160
Rat (*Rattus norvegicus*)	16	NC_005115	23846963	23845894	1070	1	–	1070	873	–
Chicken (*Gallus gallus*)	11	NC_006098	17492141	17493947	1807	1	–	1807	873	–

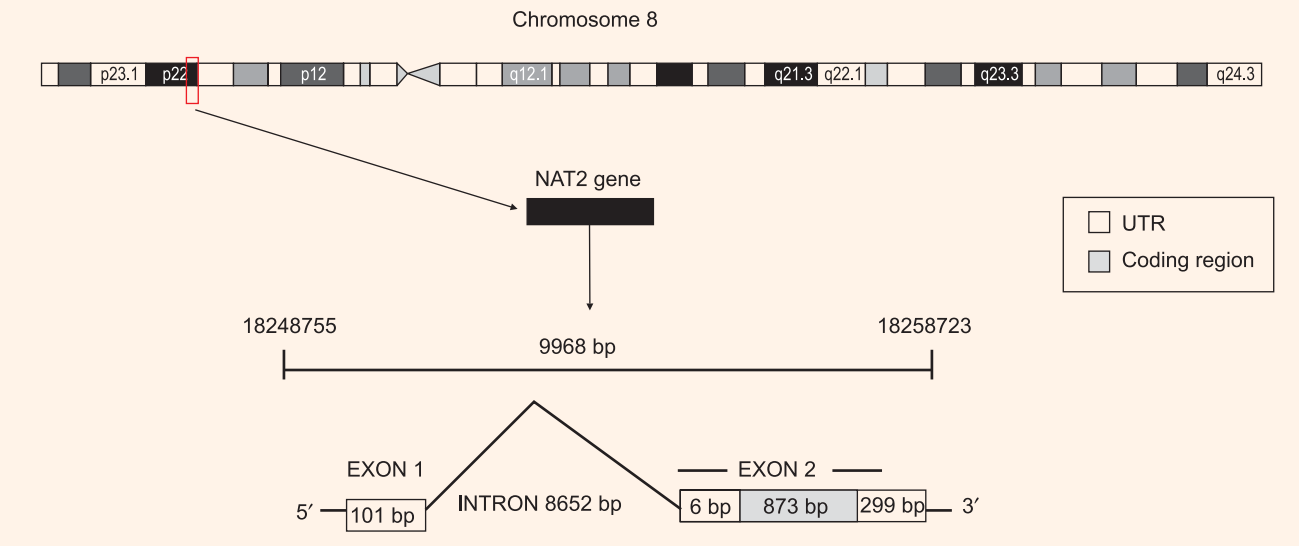

Fig. 37: Schematic representation on the positional and compositional details of *NAT2* gene.

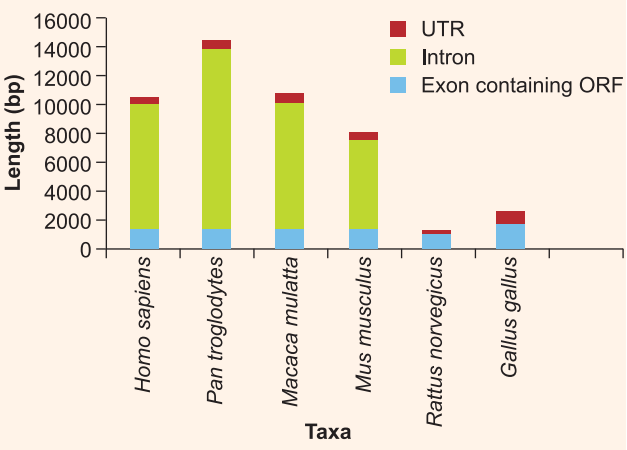

Fig. 38: Comparison on the total gene length, intron and exon containing open reading frame (ORF) of the *NAT2* gene in different taxa.

Table 5. Rate of non-synonymous (Ka) vs synonymous (Ks) mutation rate in six different taxa

Species 1	Species 2	Ks	Ka	Ka/Ks
Hs	Pt	0.0150	0.0135	0.9
Hs	Mm	0.0835	0.0493	0.590
Hs	Ms	0.6894	0.1334	0.1935
Hs	Rn	0.6443	0.1410	0.2188
Hs	Gg	n.a	0.3939	n.a
Pt	Mm	0.0858	0.0454	0.5291
Pt	Ms	0.6798	0.1288	0.1894
Pt	Rn	0.6238	0.1365	0.2188
Pt	Gg	3.0549	0.3947	0.1292
Mm	Ms	0.6730	0.1264	0.1878
Mm	Rn	0.6289	0.1376	0.2187
Mm	Gg	n.a.	0.3883	n.a
Ms	Rn	0.2391	0.0288	0.1204
Ms	Gg	3.5716	0.3701	0.1036
Rn	Gg	n.a.	0.3717	n.a.

Hs-*Homo sapiens*; Pt-*Pan troglodytes*; Mm-*Macaca mulatta*; Ms-*Mus musculus*; Rn-*Rattus norvegicus*; Gg-*Gallus gallus*

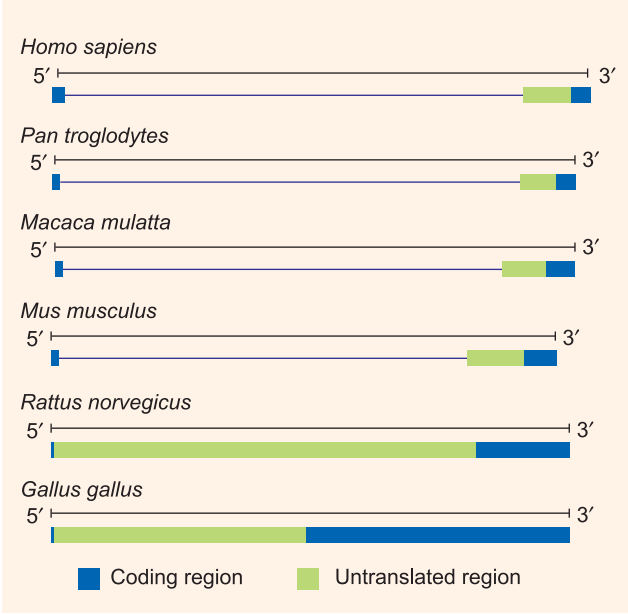

Fig. 39: Schematic representation of *NAT2* gene among five mammalian and one avian taxa (Coding region Untranslated region).

divergence in ORF of primate lineage and rodent lineage (Table 6), distribution of CpG islands (Fig. 40), microsatellites (Fig. 41) and phylogenetic inferences (Fig. 42) based on *NAT2* gene across six taxa revealed interesting patterns. The detail architecture of *NAT2* gene and its evolutionary history in different other mammalian taxa provide crucial evidence in support of the fact that this gene might have been evolving at a similar rate to other human immune system genes. Future population-based study in this gene would unravel different other aspects in relation to drug metabolization in humans.

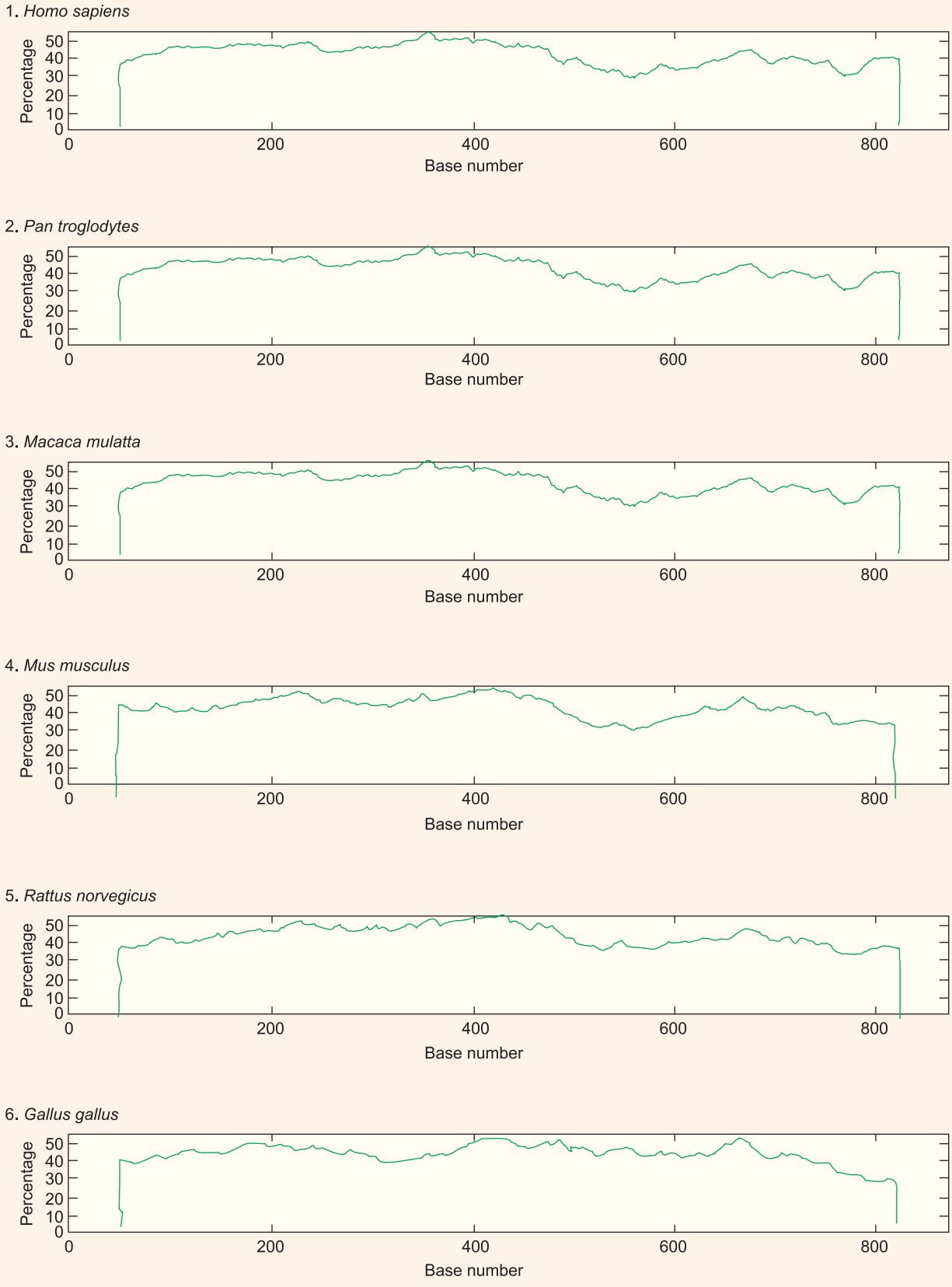

Fig. 40: CpG plots in coding region of *NAT2* gene across different taxa. The peaks above 50 are considered to be the regions containing CpG islands.

Table 6. Amino acid alignment of NAT2 protein in primate (*Homo sapiens*, *Pan troglodytes* and *Macaca mulatta*) and rodent (*Mus musculus* and *Rattus norvegicus*) lineages

Taxa	Amino acid positions (Primate lineage)							
	093	097	098	106	115	121	125	146
Homo sapiens	F	P	V	V	D	V	S	V
Pan troglodytes	F	P	A	V	E	V	S	V
Macaca mulatta	V	A	A	I	D	A	F	M

	Amino acid positions (Primate lineage)							
	148	151	155	156	171	173	179	184
Homo sapiens	C	C	E	R	T	K	H	K
Pan troglodytes	S	R	E	R	P	K	H	K
Macaca mulatta	S	R	Q	K	P	T	D	T

	Amino acid positions (Primate lineage)							
	185	189	191	198	199	216	218	222
Homo sapiens	K	I	L	T	I	S	I	F
Pan troglodytes	K	I	F	T	V	S	I	L
Macaca mulatta	T	V	S	K	I	A	T	F

	Amino acid positions (Primate lineage)							
	232	238	243	253	259	278	285	288
Homo sapiens	Y	I	K	V	T	N	D	L
Pan troglodytes	Y	I	K	V	T	K	D	L
Macaca mulatta	H	T	I	I	I	K	N	F

	Amino acid positions (Rodents lineage)						
	023	051	054	059	082	086	114
Homo sapiens	T	G	A	I	I	T	I
Mus musculus	T	S	T	I	L	T	I
Rattus norvegicus	E	N	V	V	M	A	L

	Amino acid positions (Rodents lineage)				
	149	176	205	228	250
Homo sapiens	I	L	M	P	T
Mus musculus	I	I	M	P	V
Rattus norvegicus	V	V	I	L	I

A-alanine; C-cysteine; D-aspartic acid; E-glutamic acid; F-phenylalanine; H-histidine; I-isoleucine; K-lysine; L-leucine; M-methionine; N-asparagine; P-proline; Q-glutamine; R-arginine; S-serine; T-threonine; V-valine; Y-tyrosine. The red coloured amino acids show mutational forms and the blue coloured amino acids marks the unique situation of different amino acids.

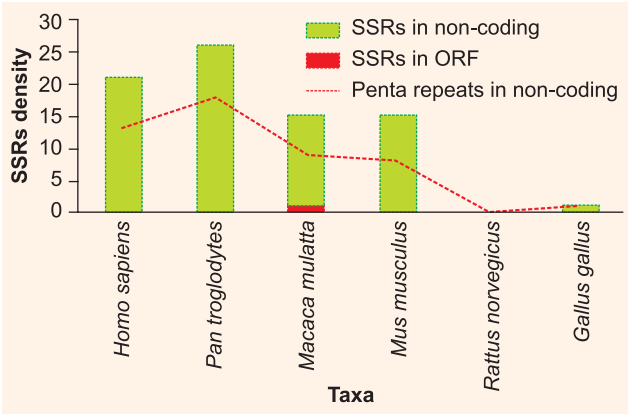

Fig. 41: Comparison of SSRs density in NAT2 coding and non-coding region across six

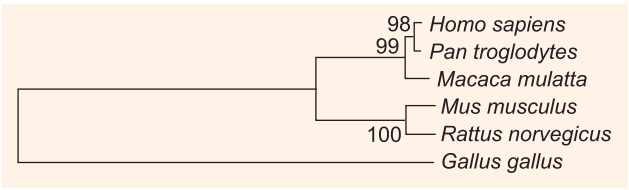

Fig. 42: Neighbour-joining phylogenetic tree in *NAT2* gene of six taxa. Figures at each internal node indicate bootstrap values obtained with 1000 simulation.

2.3.3 Evolutionary history of chloroquine-resistant malaria in India as revealed by population genetic approach

The origin and spread of chloroquine resistant (CQR) *P. falciparum* is one of the major obstacles in malaria chemotherapy and understanding on the origin and spread of CQR. *Plasmodium falciparum* is of great importance not only to unravel basic malaria epidemiological information but also in formulating apposite chemotherapeutical measures for malaria treatment. CQ resistance has a genetic basis; particularly an amino acid mutation (K→T) at the 76th position of the *Pfcrt* (*P. falciparum* chloroquine resistance transporter), otherwise known as K76T, has been directly implicated in CQ resistance. Three other mutations placed adjacent to and upstream of the 76th position (at 72nd, 74th and 75th positions), the monomorphic 73rd and the mutated 76th position form different *Pfcrt* haplotypes. Accordingly, two major CQR-*Pfcrt* haplotypes (CVIET and SVMNT) are frequently found to be distributed in *P. falciparum* endemic zones and are thus termed as CQR mother haplotypes. Since data analyses under population genetic framework provide valuable informations on evolutionary history of genes and species population, including origin and historical

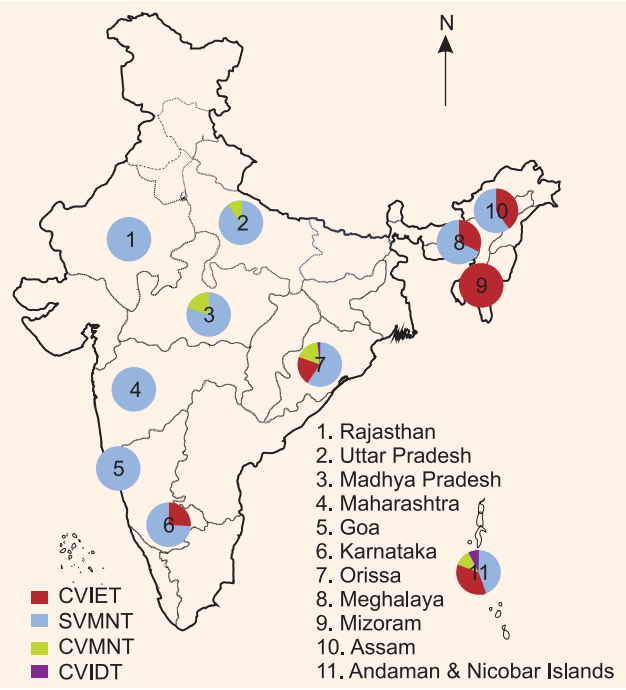

Fig. 43: Schematic representation and geographical distribution of four main haplotypes (CVIET, SVMNT, CVMNT and CVIDT) present in 11 states/union territories of India.

migration, analyzing the CQR-*Pfcrt* haplotype frequency data could provide valuable information on the migratory history of CQR *P. falciparum* and furthermore, if the population data comes from a malaria-endemic country with variable epidemiology and transmission patterns, such inferences become more practical.

Subjecting Indian CQR *Pfcrt* data from 11 population samples to rigorous statistical analyses (Awasthi *et al* 2010), the results on the distributional pattern of the two mother haplotypes of CQR in India were discussed in two important evolutionary aspects. Firstly, a clear haplotype structure was evident in CQR *Pfcrt*. Since the SVMNT haplotype is believed to confer strong CQR to *P. falciparum* fixation of this haplotype in many Indian populations is highly alarming (Fig. 43). Secondly, strong longitudinal cline provided evidence of influence of natural selection in favour of both the haplotypes in their respective zones, coupled with local adaptation. Whether or not the observed clines are responsive to usage of CQ in India

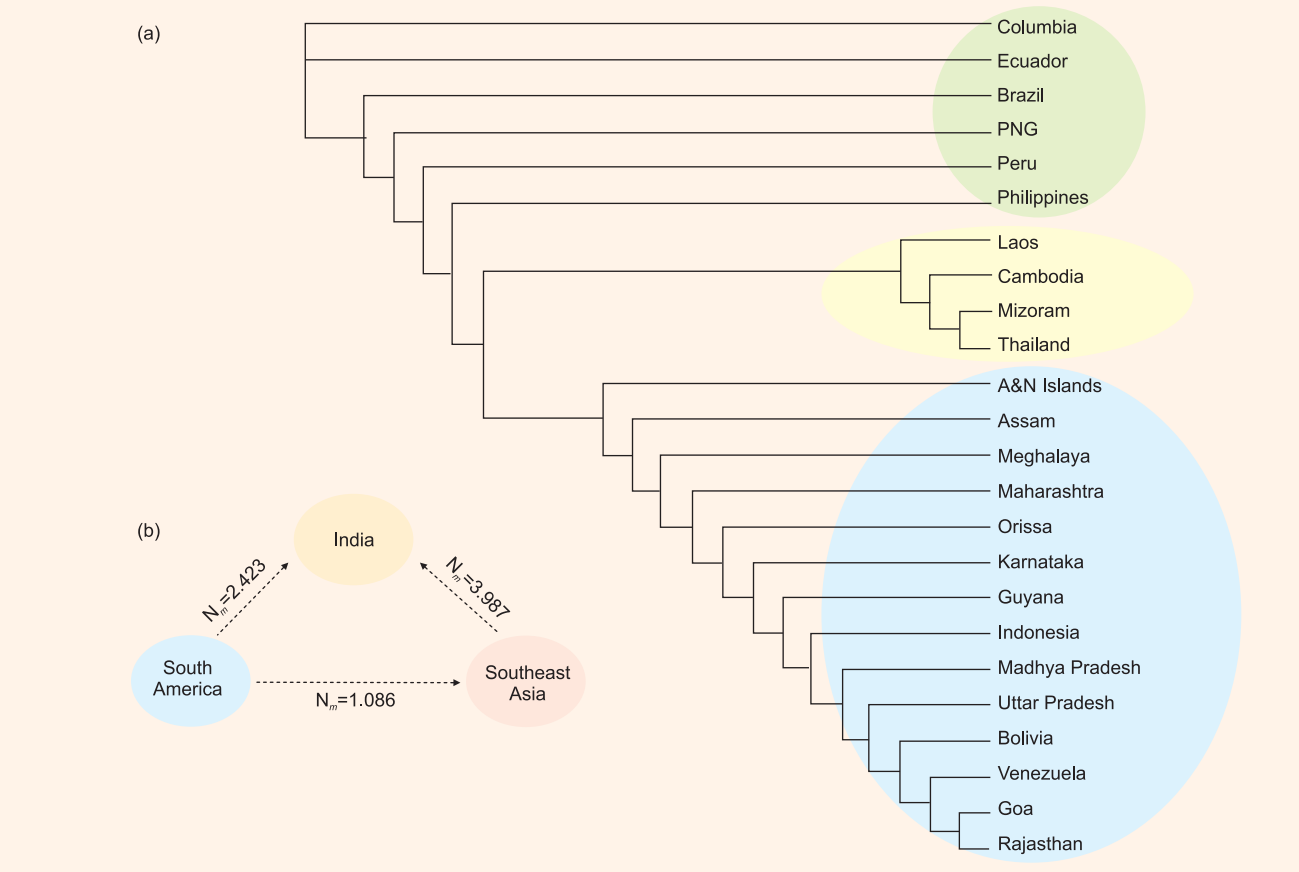

Fig. 44: (a) Neighbour joining phylogenetic tree comprising of 24 populations (11 Indian, 7 south-east Asian and six south American) and establishing phylogenetic relationships among each other. Three distinct clades have been produced separating the populations on the basis of three haplotypes, namely, CVIET (yellow), SVMNT (blue) and CVMNT (green); (b) Schematic representation depicting number of effective migrants exchanged between all three populations. As observed, the maximum number of migrants are exchanged between India and Southeast Asia and the lowest between south-east Asia and south America.

remains to be seen. To reconfirm the above contention, additional published information on the frequency and distribution of the *Pfcrt* gene haplotypes from south-east Asia and south America was merged with the Indian haplotype data and several population genetic analyses were performed and population inter-relationships from phylogenetic tree were inferred (Fig. 44 a & b). Taken together with the south-east Asian and south American data, it seemed that Indian *P. falciparum* was more genetically closer to south America than the south-east Asia at the CQR-*Pfcrt* level. However, more numbers of migration events were detected between south-east Asia and India, than between south America and India. India seemed to have received CQR *P. falciparum* from both South America and south-east Asia from two directions with two possible routes—(i) over the sea route from SEA (one of the four foci of CQR) via A&N Islands; and (ii) through Myanmar via north-eastern states. Based on phylogenetic and number of effective migrants, it could be ascertained that the CVIET haplotype came to India through Myanmar from south-east Asia directly (Model 1) and the SVMNT haplotype from SA (one of the four foci of CQR) to India (A& N Islands → Orissa) via the SEA (Philippines → Papua New Guinea (PNG) Islands → Indonesia) (Model 2). From the routes it is apparent that India might have received comparatively more migrants of SVMNT bearing *P. falciparum* than CVIET. However, it is probable that natural selection mediated local adaptation could have amplified the frequency of SVMNT haplotypes in India, which could only be revealed by thorough population genetic analyses coupled with epidemiological information.

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The study has been carried out in Sonitpur and Nagaon districts of Assam. IRS-1D/LISS III sensor imageries of January 1999 and IRS-P6/LISS III imageries of November 2008 of the mentioned districts was procured from NRSA, Hyderabad. The imageries were geo-referenced and classified using classification techniques. Landuse/land cover (LULC) was developed and a comparison of LULC classes was carried out to identify areas where major deforestation occurred in both the districts. In Sonitpur district, Biswanath Chariali and Behali were identified as deforested and forested PHCs. In Nagaon district, Lanka and Jakhlabandha PHCs were selected as deforested and forested PHCs (Fig. 1). IRS-P6/LISS IV imageries were procured for deforested Biswanath Chariali and Lanka PHCs to mark the development activities after deforestation. A field validation trip was undertaken during August–September 2009. LULC information was recorded and the validation/corrections of the classes was done for the classified satellite images.

Five villages in each PHC (total 20) were surveyed for entomological and epidemiological data collection. Daily survey track route and location of the villages surveyed were marked using Garmin GPS. Socioeconomic data were collected from all the study villages on pre-designed proforma and analysis was carried out. In Sonitpur and Nagaon districts, the purpose of deforestation was agriculture and habitation. In Sonitpur, settlement in deforested areas was low, population shift from nearby villages was observed, while in Nagaon the high settlement of migratory muslim population was observed. In deforested areas of Sonitpur, mainly paddy cultivation was observed while in Nagaon, paddy, ginger, turmeric and sugarcane cultivation was seen. In deforested areas of Sonitpur, there were *kuchcha* houses, while in Nagaon, both *kuchcha* and *pucca* houses were found. In deforested areas of Sonitpur, *kuchcha* roads were found, while in Nagaon, both *kuchcha* and *pucca* roads were seen.

In Sonitpur, during indoor resting mosquito collection, *An. philippinensis* was recorded from the deforested B. Chariali PHC while in Nagaon, *An. minimus*, *An. philippinensis* and *An. nivipes* were recorded from deforested Lanka PHC. From forested Behali PHC of Sonitpur, *An. culicifacies, An. fluviatilis* and *An. philippinensis* were recorded. From forested Jakhlabandha PHC of Nagaon, *An. nivipes* was mainly recorded. *An. vagus* was recorded as the most dominant species from the deforested and forested villages of both the districts due to rainy season during indoor resting mosquito collection (Fig. 2).

In Sonitpur, the anopheline density was little higher in forested villages (MHD 14.1) in comparison to the deforested villages (MHD 10.5) while in Nagaon, the density was observed higher in deforested villages (MHD 26.3) as compared to that of forested villages (MHD 8.5).

Rice-fields emerged as the prominent breeding sites in the deforested areas of both the districts and also the prominent larval breeding sites in forested areas of Sonitpur while in Nagaon, streams emerged as the prominent breeding sites. Larvae of *An. minimus* were recorded from the forested areas of Nagaon. Among the emerged species from the collected larvae, *An. vagus* was found to be the most dominating species collected from deforested and forested areas of both the districts. Breeding sites of the study areas are given in Fig. 3.

From deforested areas of Sonitpur, *An. philippinensis* was recorded during whole night

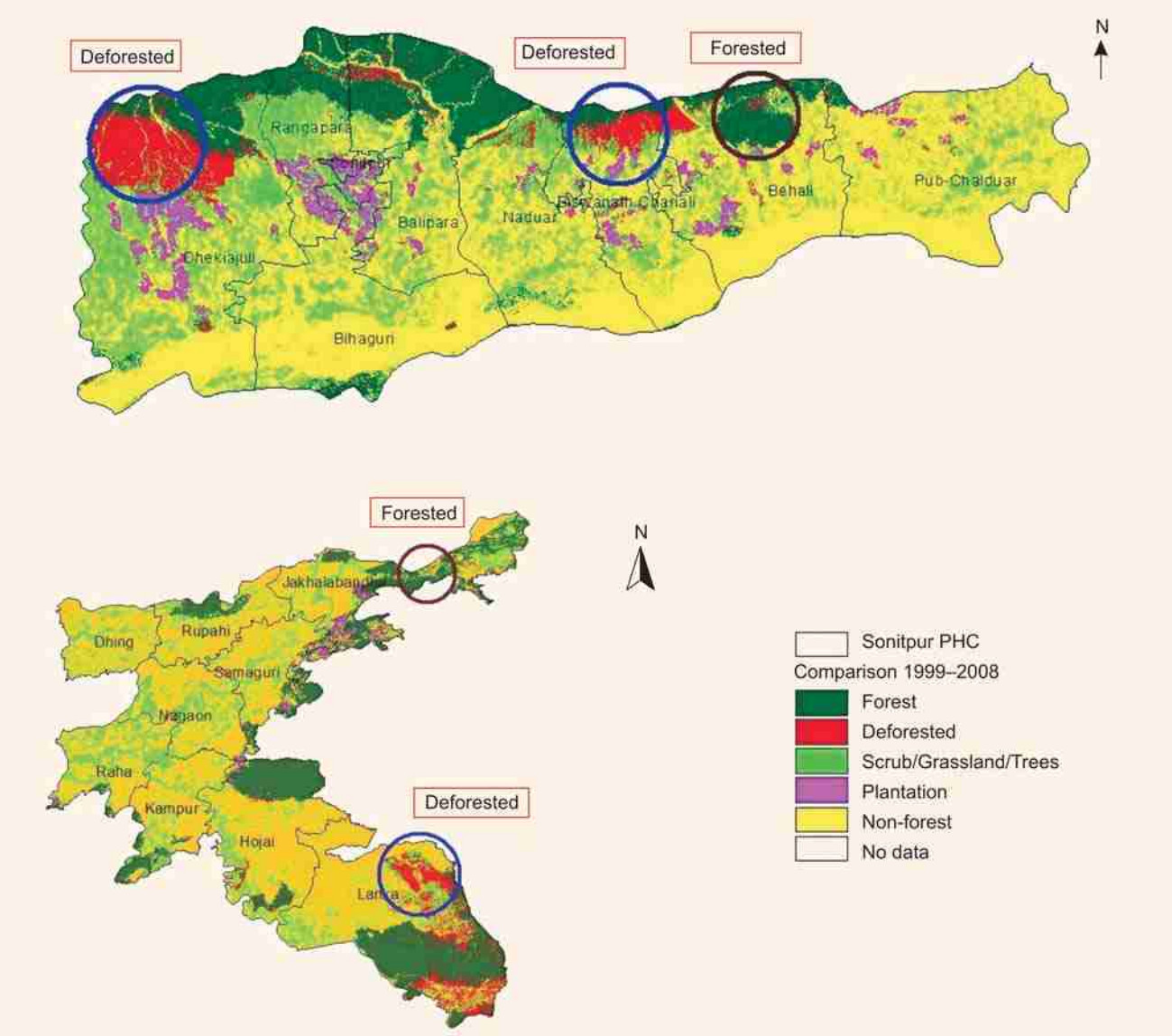

Fig. 1: Deforested and forested PHCs of Sonitpur and Nagaon districts after comparing satellite imageries of 1998 and 2008.

Fig. 2: Indoor resting mosquito collection in deforested and forested areas of Sonitpur and Nagaon districts.

Fig. 3: Breeding sites in deforested and forested areas of Sonitpur and Nagaon districts.

mosquito landing collection. In Nagaon, *An. philippinensis*/*An. nivipes* were recorded from deforested areas during these collections. *An. vagus*, the most dominating species from deforested areas of Sonitpur and Nagaon was also collected during whole night mosquito landing collection in large numbers during indoor resting mosquito collection (Fig. 4). From the forested areas of Sonitpur, *An. philippinensis* and *An. nivipes* were recorded during whole night mosquito landing collection while in Nagaon, the main vectors *An. minimus, An. dirus, An. fluviatilis* and *An. philippinensis* were recorded. *An. dirus* was recorded in highest numbers. Larvae of *An. minimus* were collected during larval survey in forested areas.

During active fever survey in these areas, no slides were found positive for malaria in the deforested and forested areas of Sonitpur and Nagaon. However, in Nagaon distrct, five *Pf* cases were recorded by *Anganwadi* workers in deforested PHC Lanka during the months of June and July 2009.

The study is in progress. In future, more extensive surveys will be undertaken to compare the species in study villages.

Fig. 4: Whole night mosquito landing collection on human and cattle baits in deforested and forested areas of Sonitpur and Nagaon.

GIS thematic layers, namely forest cover, soil type, agriculture practices, altitude, drainage network, mining areas, builtup areas, water bodies, geomorphology, PHC and village administrative boundaries related to Ranchi district, Jharkhand were prepared on 1 : 50,000 scale. Remote sensing imageries of IRS-P6/LISS III satellite, published data, topo-sheets and GPS were used to prepare these thematic layers. In preparation of soil map, reports of the National Bureau of Soil Survey were consulted. PHC-wise SC/ST and literate/illiterate population data were collected and organized in GIS format. Also malaria epidemiological data (API, *Pf*%) from 2004 to 2008 (5 years) were processed in GIS format and thematic layers were generated.

A two-day orientation workshop from 15 to 16 December 2009 on 'GIS for management and control of vector borne diseases' was conducted at Ranchi. This was the first time any GIS workshop was conducted for officials of the State Health Department of Ranchi district (Fig. 5). The study is in progress.

Sonitpur and Nagaon districts of Assam located in north-eastern part of India, have been taken for the study. Earlier based on digital datasets of IRS-1D & P6-LISS-III satellite images for the year 1999 and 2008, it was found that Sonitpur is maximum occupied by moist deciduous forest followed by tea gardens and shrubs and grasslands (Fig. 6).

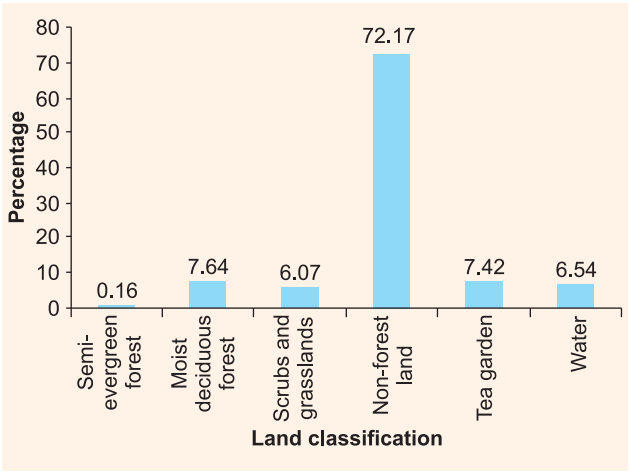

Fig. 6: Land classification in percentage of District Sonitpur, Assam.

Fig. 5: GIS workshop for state health officials at Ranchi, Jharkhand.

In Nagaon, deforestation was observed and there was an increase in the area under tea garden and settlement. Surveys were conducted for classified satellite image for validation of land use/land cover classes and the errors were rectified accordingly. PHC-wise parasitological data till 2008 and entomological parameters were also collected during the surveys. A significant correlation was found between tea gardens and API (Table 1) and it was also revealed that due to deforestation *An. dirus* was replaced by *An. minimus* and *An. fluviatilis* species.

Survey of India Topo sheets in the scale of 1:50,000 were used to digitized maps of temperature, altitude, rainfall and forest cover. Maps of rainfall and temperature are based on standard periodical averages. Using these maps favourable area for malaria vectors, namely *An. minimus, An. fluviatilis* and *An. dirus* has been mapped. To carry out validation of GIS predicted favourable area in Nagaon and Sonitpur districts, Assam entomological and parasitological surveys were carried out in May, August and November 2009.

Table 1. Correlation between Tea Garden and positive cases, *Pf* cases, SPR and API in Nagaon during 2002 and 2006

	Correlation values for 2002	Correlation values for 2006
TG / Pos. cases	0.70	0.83
TG / *Pf* cases	0.84	0.79
TG / SPR	0.74	0.80
TG / API	0.86	0.82

In Soniptur, 22 villages were surveyed, out of which eight were in the forest area. GPS recorded location of villages surveyed are shown in Fig. 7. Villages where all the three vector species have been reported are shown in Fig. 8, i.e. villages with highest potential for malaria.

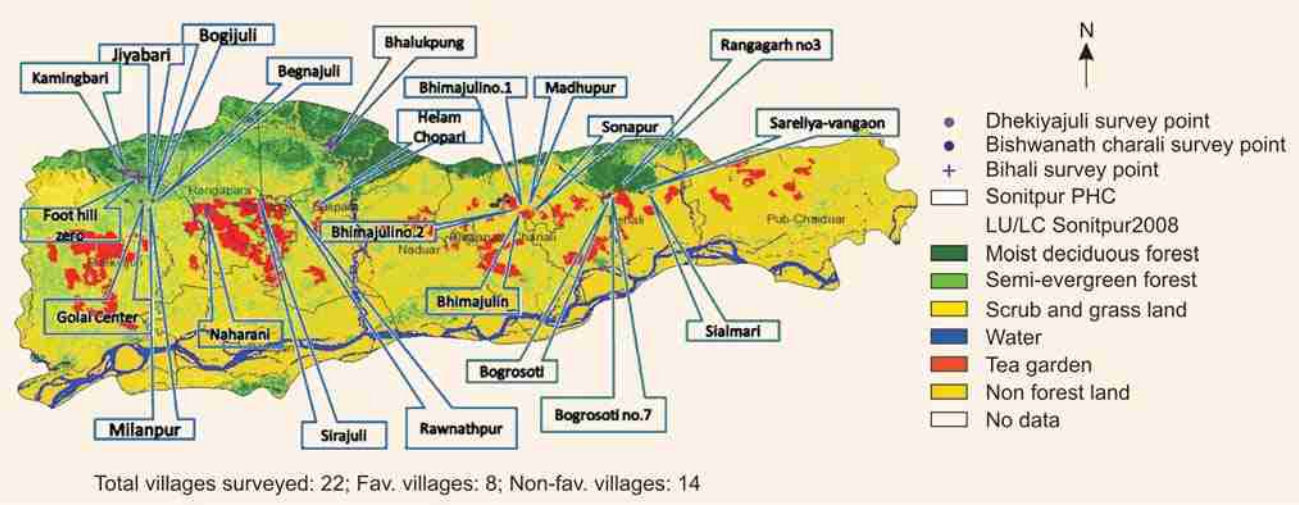

Fig. 7: Villages surveyed in Sonitpur, Assam (2009).

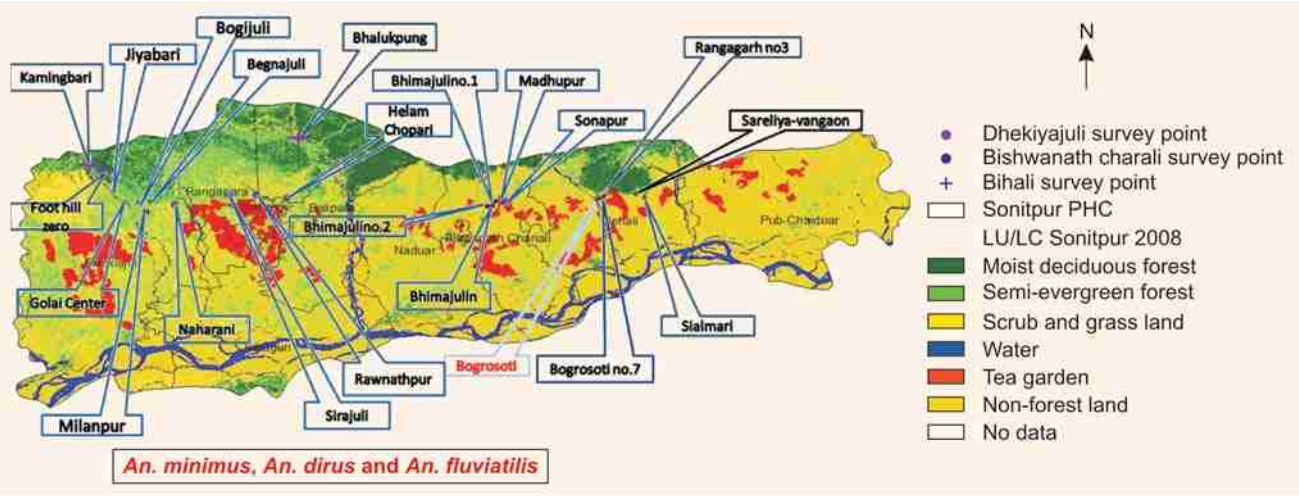

Fig. 8: Location of villages where all the three vector species—*An. minimus*, *An. dirus* and *An. fluviatilis* were collected in Sonitpur, Assam (2009).

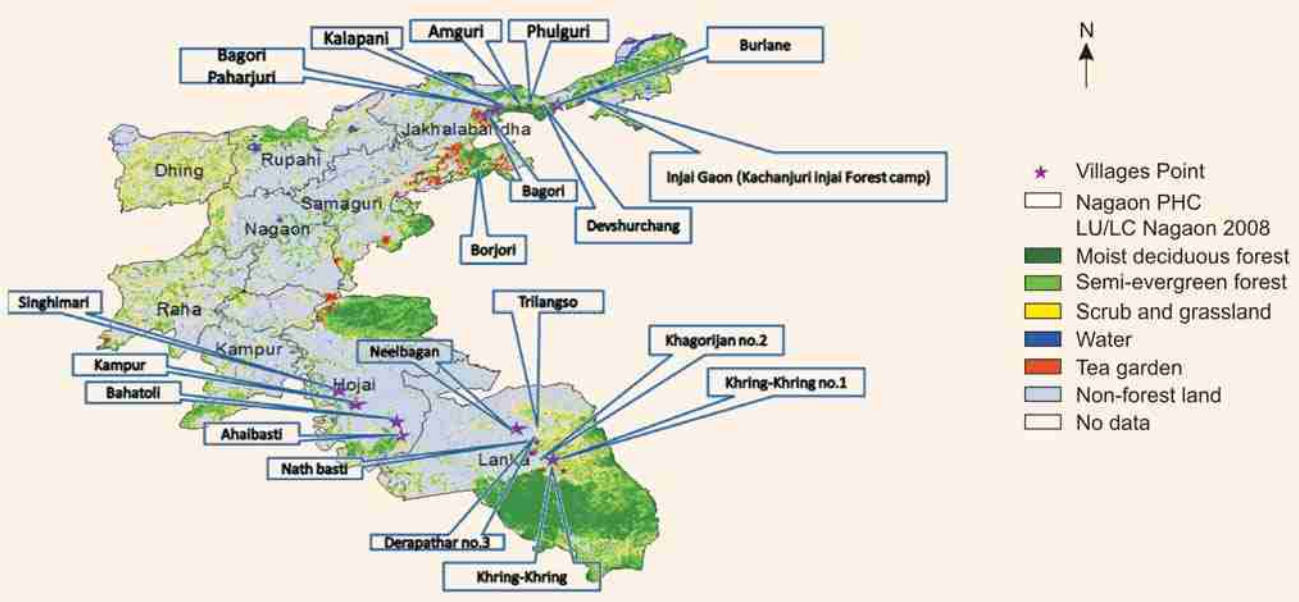

Fig. 9: Villages surveyed in Nagaon, Assam (2009).

In Nagaon, 21 villages were surveyed, out of which 10 were in the forest area. The GPS recorded location of villages surveyed are shown in Fig. 9. Villages where all the three vector species have been collected are shown in Fig. 10, i.e. villages with highest potential for malaria.

Conclusion: Distribution of malaria vectors, viz. *An. minimus, An. dirus* and *An. fluviatilis,* in villages surveyed and village-wise malaria situation was compared and it was found that malaria is high in villages falling in favourable zone. It was also found that malaria is high in areas with good network of stream and favourable forest area. The three PHCs, Balipara and Rangapara, Nadaur and Dhekiajut in Sonitpur, and Simonabasti and Jakhalabandha (Kalibor) in Naogaon were identified as hot spots. In Sonitpur, out of 1,792 villages, 972 villages and in Nagaon out of 1,391 villages, 874 villages were identified with no distribution of major vector, extracted and were designated as malaria risk free villages (Figs. 11 & 12). From the above data, it is revealed that GIS predicted the correct location of the vector species which help in identifying malarious and

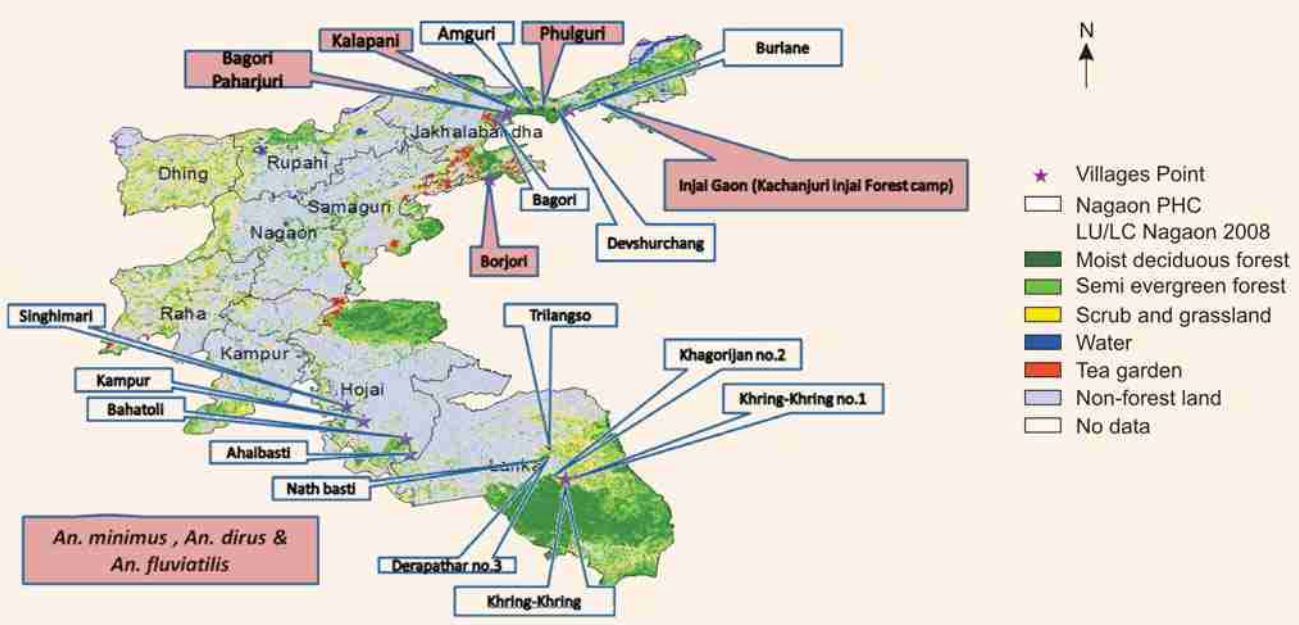

Fig. 10: Location of villages where all three vector species—*An. minimus, An. dirus* and *An. fluviatilis* were collected in Nagaon, Assam (2009).

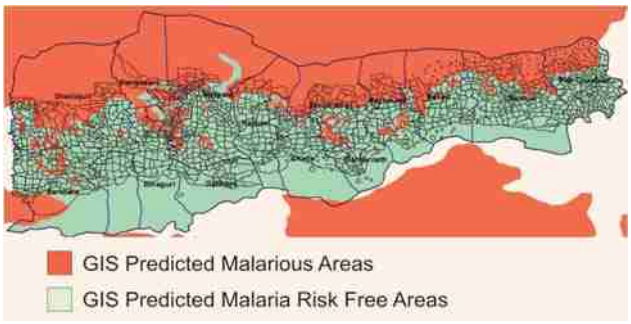

Fig. 11: Malaria risk-free areas in Sonitpur.

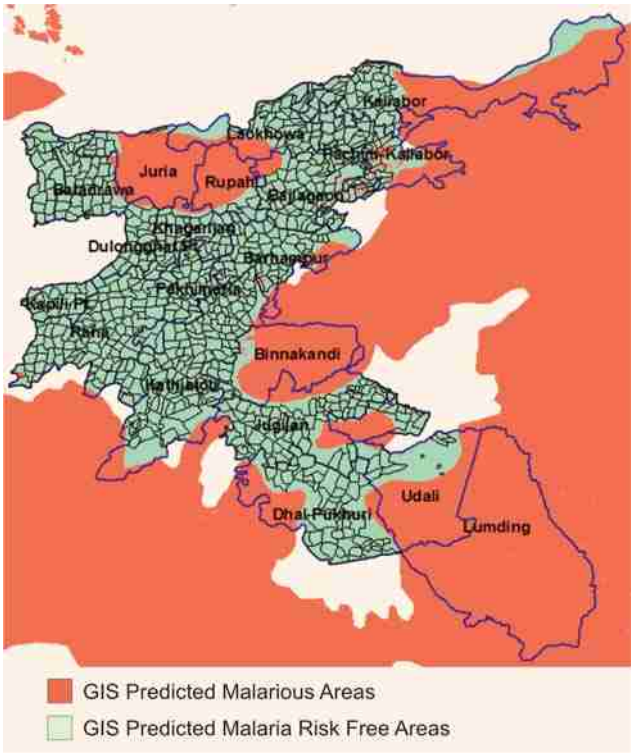

Fig. 12: Malaria risk-free areas in Nagaon.

non-malarious areas to formulate the appropriate control strategy.

The change in environment affects the population on a large-scale. Construction of big dams such as ISP and OSP has widely affected the entire ecosystem. Man's impact on Narmada Basin environment is producing critical situation to the ecosystem. Since exposure to new environment possesses new threats to the people, therefore, effective actions through preventive, curative and promotional health services are to be taken. The project on Health Impact Assessment on Indira Sagar Dam and RR Colonies in SSP Reservoir was submitted to NVDA, Bhopal in August 2003 and study was initiated in January, 2004. Till February 2010, 19 surveys in three different seasons—pre-monsoon, monsoon and post-monsoon have been carried out in seven districts– Khandwa and Dewas (ISP & OSP), Khargone and Harda (ISP), Badwani, Dhar and Jhabua (SSP). Mosquitogenic conditions created due to dam construction, viz. seepage of the reservoirs, pits and pools of down streams, new canals created, curing tanks, etc (Fig. 13) have been identified. Surrounding to these, a total of 32 villages, 18 rehabilitation and resettlement centres and 5 command area villages and 6 labour colonies under 7 districts have been surveyed for entomological and epidemiological data for all the vector-borne diseases—malaria, dengue, JE and filariasis.

Man hour density/room density of malaria vector *An. culicifacies* and *An. stephensi*, filaria vector *Culex quinquefasciatus,* JE vector *Culex vishnui* and dengue vector *Ae. aegypti* were calculated in all surveys. Impact of dam construction was observed in nine villages as the vector density was reported high till July-August 2005. To establish the transmission, other entomological parameters, viz. 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 pre- and post-monsoon and winter seasons. *Anopheles culicifacies* was found resistant to DDT and susceptible to synthetic pyrethroids. Report of the vector breeding and malaria positive cases was sent to the State Health Department and NVDA Health Authorities.

GIS mapping of all the seven districts have been completed. Digital maps of villages were prepared attached with attribute and malaria data. Trend analysis of epidemiological data from 2002—05 has been done. The data on various entomological and parasitological parameters being collected through periodic surveys are regularly put into GIS-based frame work to view the impact of the construction of dams in space and time.

Fig. 13: Potential mosquito breeding sites at ISP impoundment areas.

Water samples collected from taps, hand pumps, tube-wells in each survey were analyzed by the Public Health Engineering Department and were found safe for drinking. Recently, in April 2008, tap and hand pump water samples from 11 villages of 3 districts, viz. Jhabua, Barwani and Khargone were tested for coliform and other harmful bacteria. The reports of tested samples were sent to the concerned PHCs for further necessary action.

A special survey in October 2008 focusing on schistosomiasis was also carried out and snail species (Fig. 14) collected were sent to the Defence Research Development Organisation (DRDO), Gwalior for identification and the species found were reported to be specific vectors of cattle. No cases for dengue, JE and filariasis were found in the conducted surveys.

Fig. 14: Snail species (vector or schistosomiasis) collected at the Dam site

After completing each survey, meetings were held with the Vice-chairman, NVDA and the state Authorities and survey highlights and actions required for developing situation-specific mitigating measures, i.e. engineering, epidemiological and entomological to control the vector-borne diseases were suggested.

From October 2005, the following suggested mitigation measures were implemented in the field by State Health Department NHDC and NVDA. De-weeding of canals, release of larvivorus fishes in tanks of Narmada Nagar, RR Centre, and ponds and wells of villages, source reduction, spray of pyrethroids in Narmada Nagar and DDT in problematic villages/RR Centres, cleaning and oiling of drains in Narmada Nagar on weekly basis, fogging in power houses, construction of mosquito-proof house in Omkareshwar Dam Site, IEC activities in villages/RR Centre & Narmada Nagar (Fig. 15) and Radical treatment to all the *Pf* cases.

Blood samples were also collected for dengue, JE and filariasis but none was found positive. It is also worth mentioning that no vector species of kala-azar was recorded from these project areas.

In a cross-sectional survey carried for the JE, dengue and filaria from 2004–09, a total of 6790 blood samples on filter papers were collected and all were found negative. About 351 intravenous blood samples (I/V routes) from fever cases were collected from 2004 to 2008 and the result was found negative for all the cases. For filaria 1041 blood slides were collected during night survey in

Fig. 15: IEC activities.

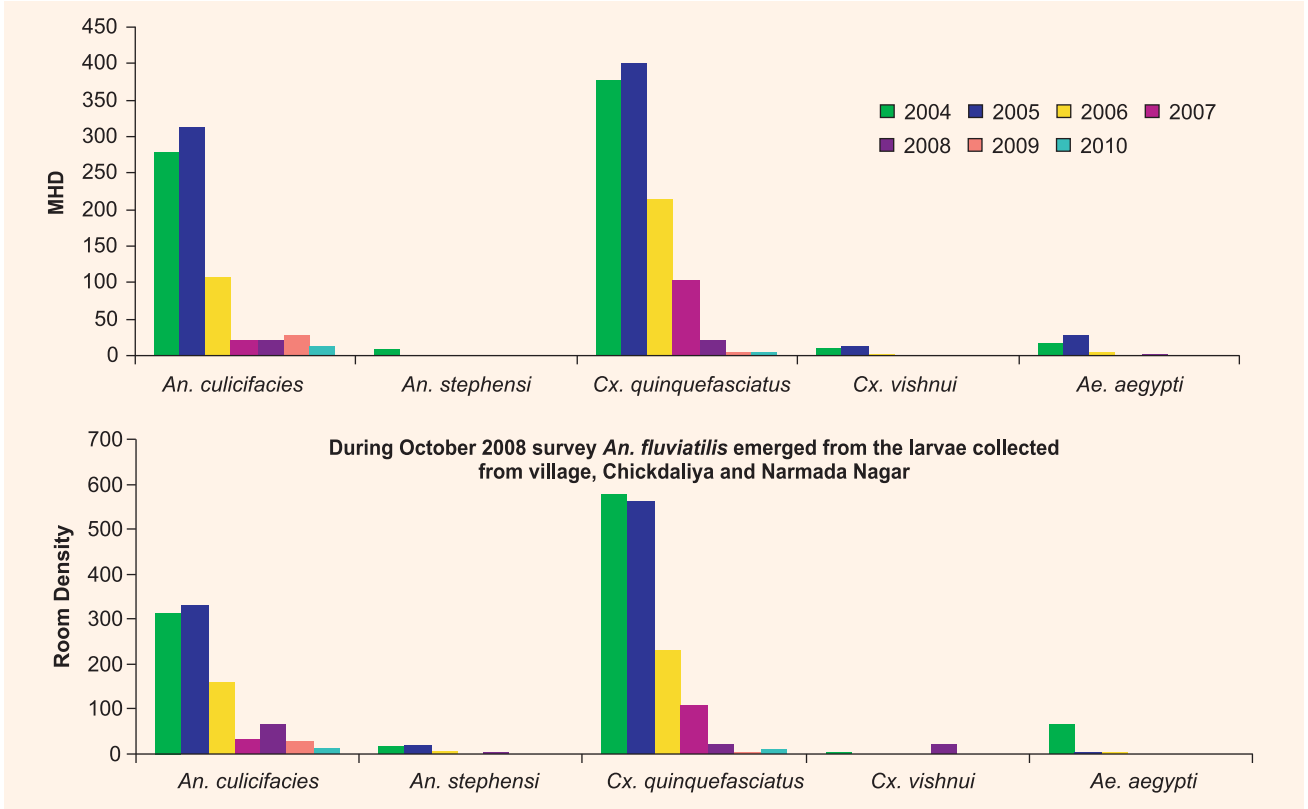

Fig. 16: Impact of interventions on vector density (MHD) 2004–10.

pre- and post-monsoon seasons (2004–08), and these all were also found to be negative.

The results of interventions taken by the NVDA are apparent from the reduction in vector density (MHD and room density). The data collected in each survey show a drastic change in the time span of five years with constant efforts of all the stakeholders (Fig. 16).

A survey for percentage positivity of the containers of *Ae. aegypti* was also carried out in each survey and the results are summarized in Fig. 17. The results suggest drastic reduction in percentage positivity of *Aedes* breeding habitats after intervention.

Due to the effective actions taken by the NVDA as per suggested mitigation measures, the vector density of malaria, dengue, chikungunya, JE and filariasis reduced drastically and the impact of this density was also observed on the number of malaria cases which has been reduced from 299 in 2005 to 1 case till February 2010. It is important to note that in Sharda Canal area within four years (1924—28) about 38,000 malaria cases and thousands of deaths were recorded whereas in this study, within three years (2004—07), about 500 malaria cases were recorded with no death.

In the initial observatory period the number of malaria cases was high but after the implementation of suggested mitigation measures there was a remarkable decrease in the disease load.

Two workshops were also organized for all the stakeholders at NVDA, Bhopal premises (Fig. 18) to acknowledge the progress of the work and to train the CMOs and DMOs of the concerned districts under study to make them aware of the vector species and other disease dynamics. The health impact study is in progress and will continue till December 2010.

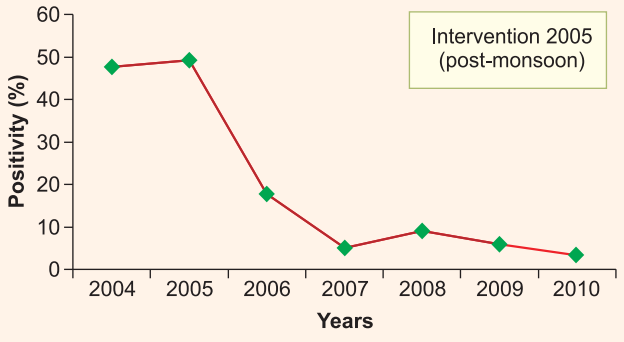

Fig. 17: Percentage positivity of *Ae. aegypti* in Narmada Nagar including all types of breeding sites of *Aedes*— OHTs, ground tanks, tyres, coolers, mud pots, drums, containers, etc. (2004 – Jan 2010).

Fig. 18: Workshop in progress at NVDA, Bhopal.

After receiving encouraging results, the NVDA has now extended the HIA survey to cover the entire Narmada basin in Madhya Pradesh which includes 30 major dams, command areas, RR centres and labour colonies till 2014.

Study was continued in problematic districts of northern and southern Karnataka to identify ecological and epidemiological risk factors of malaria with the help of remote sensing and ground surveys. Based on village-wise data of past three years, villages from highest and lowest malaria endemic districts, i.e. Gulbarga, Bijapur, Raichur and Bagalkot were selected for detailed survey. Field visits were undertaken in highest peak malaria

months— October/November and in lowest peak March/April from 2006 to 2009. Field surveys were undertaken in 21 selected villages altogether for types of breeding habitats, man hour density of adult malaria vectors for mosquitogenic potential in and around each village and fever surveys for parasite load in the community. In addition to generation of entomological, parasitological and ecological data, data on socioeconomic attributes of villages were also generated through questionnaires. The areas were having rivers, irrigation channels, drains and borrow pits as breeding habitats. Satellite images of IRS P6 LISS IV MX were procured for Upper Krishna Project (UKP) area. Ecoepidemiological risk factors were found as: introduction of irrigation canals in hitherto water scarce area, vicinity of human settlements near channels/seepage drains, local migration and settlement of rehabilitated colonies.

Validation of landscape features related with malaria endimicity in Tumkur: To validate the relationship between Remote Sensing (IRS LISS IV) derived landscape features and malaria endemicity in five PHCs of Tumkur and Chitradurga districts in 153 villages, a study was carried out. In an earlier study, the presence of water bodies, vegetation cover, low barren area and scrubs were found as significant landscape features associated with high malaria endemicity. Based on classified landscape features, 153 villages were grouped into low (52), moderate (45) and high (56) which validated the earlier findings.

In Narayanpur, location of villages close to seepage drains, river bed pools, relocation of villages, staying in field huts, migration and variable surveillance are the main risk factors. Ecological risk factors are less discernible in Almatti as compared to Narayanpur.

The findings were shared with Karnataka State Government and the results of reclassification of villages for labelling of high risk based on criteria of NVBDCP were disseminated to NVBDCP for necessary action.

This is a project under NATCOM II sponsored by the Ministry of Environment & Forests for determination of transmission windows (TW) of malaria and dengue in terms of climate and socioeconomic parameters, GIS-based outputs indicating the extent of disease spread under current and based on climate change, landuse and socioeconomic conditions and formulation of adaptation framework.

Monthly temperature (T), relative humidity (RH) and rainfall (January 1961 to December 1990) extracted from PRECIS (Providing Regional Climate for Impact Studies) were used as base-line. Projected scenario (A2 scenario) for 2071, 2081, 2091 and 2100) of PRECIS were used. Transmission Windows of malaria were determined using lower and upper thresholds of temperature and 55–90% RH. TWs were determined for dengue also. Details of projected scenario in respect of India specifically for Assam, Orissa, Rajasthan, Uttarakhand and Delhi states were generated.

In 3–9 months TW open categories, appreciable increase in months of TWs is expected leading towards stable malaria. In baseline 128 pixels show no transmission which may reduce to 90 pixels by 2091. Baseline TWs in 10-12 months (546) are likely to be reduced to 322 by the year 2091. Results are yet to be firmed up with further analysis by incorporating landuse features and different combinations of T, RH and rainfall.

Projected scenario of TWs of dengue by the year 2071, 2081, 2091 and 2100 were also determined at national as well some for specific states like Delhi, Uttarakhand, Assam, Orissa and Rajasthan.

For socioeconomic status in vulnerable areas of the five states selected for detailed analysis of socioeconomic conditions to arrive at possible adaptation measures, field visits were undertaken in Jodhpur (Rajasthan) and Sambalpur (Orissa) for eliciting information on KABP of the communities about malaria and existing health facilities/ system.

Malaria has been persistent in Gadchiroli district of Maharashtra state and most of the area is considered as high risk area. In order to evaluate the independent impact of IRS and LLIN in the area,

strengthened EDPT and to find the suitable criteria for labelling the high risk malarious area, the project was undertaken. Under Dhanora block, Murumgaon PHC (being the highest malaria reporting PHC) was selected for the study. The project was initiated in the month of August 2009. After going through the epidemiological data of past three years, three sub-centres (SC), namely Murumgaon, Pannemara and Kulbhati with the population of 2292, 2095 and 2237 respectively were selected for three categories of intervention: (i) Control; (ii) Strengthened EDPT and LLIN; and (iii) Strengthened EDPT and IRS.

Before launching the project, meetings with DMO, Medical Officer, Health Workers, *Anganwadi* workers and village people were conducted for sensitization of work.

After collecting the baseline data on number of households, demographic variables, malaria parasite prevalence and man hour density of malaria vectors, intervention was undertaken from November 2008. Indoor residual spray was supervised by the team. About 1500 long-lasting nets were distributed in the selected villages under SC Pannemara.

For strengthened EDPT and supervision of IRS, supervisors were appointed for each SC. Supervisors are visiting the selected villages for blood slide collection and coordination between community and PHCs. Anopheline density is being monitored in alternate months by the NIMR team.

For detecting sporozoites in field collected mosquitoes, the technique of detection of sporozoites by ELISA is under progress.

It is a collaborative project with Michigan University. Initial analyses were focused on three districts of Gujarat, Kutchh, Banaskantha, and Kheda-Anand, and the time series of monthly rainfall and positive *P. falciparum* from 1986 to 2002/2006. One district of Rajasthan, Barmer has also been included for comparison purpose. Initial correlative analyses revealed significant associations between rainfall during the monsoon season and malaria during the epidemic season that follows, particularly in more arid districts. In Kutchh district, rainfall and malaria cases cumulated during the respective season. Similar patterns emerge if one considers specific months and specific lags (typically of 2 months). These associations are also evident in the frequency domain, that is in the spectra of frequencies (or periodicities) present in rainfall and malaria. Dominant cycles present in the data using wavelet spectra were also determined. In Barmer (Rajasthan), malaria data exhibit variability at a period of approximately 2 and 4 years; similar dominant periods are present in the rainfall anomalies and importantly, that the timing of these cycles correspond to that of the malaria cases. This illustrates similar patterns of variability in rainfall and malaria, consistent with an important role of rainfall as a driver of epidemics.

This work so far has developed two epidemiological models of increasing complexity that incorporates vector dynamics through a simplification. This allows us to consider variations in the vector abundance, as well as the delay due to the development of the parasite in the vector and the survival of the vector.

Monthly epidemiological data in respect of 10 districts of Gujarat and 11 districts of Rajasthan and corresponding meteorological data are being collected for expanding the work. Data on retrospective irrigation practices, changes in demography, crop patterns, etc. procured from district statistical books are being considered for further analysis. Vegetation indices derived through remote sensing are also being analysed in respect of 10 selected districts of Gujarat.

This project is multi-institutional, multi-disciplinary and multi-locational. The study is planned to be undertaken in selected districts of Uttarakhand, Assam and Mizoram states to generate data on biophysical, climatic and socioeconomical determinants of malaria to understand the current TWs and ecological risk factors of malaria for development of transfer functions and simulation models; to evaluate and strengthen current adaptation measures for control of malaria; to develop projections of potential impact of climate change on seasonal transmission of malaria and

finally to develop a framework for adaptation measures. Keeping in view the additional institutional measures, technological interventions required to combat the adverse impacts of climate change and mainstream climate change adaptation concerns through capacity building of various categories of the state health personnel vis-à-vis climate change.

Three sites in Bhimtal, Kolasib and Bokajan have been identified and two districts in each state have been selected, i.e. Nainital and Almora in Uttarakhand, Kolasib and Aizwal West in Mizoram and Karbi-Anglong and Jorhat in Assam. Project field units for continuous monitoring of entomological and parasitological parameters have been set up at Bhimtal in Uttarakhand, Kolasib in Mizoram while staff has to be posted at Bokajan in Assam. Three sites in each district at varying altitude have been identified for generation of entomological and parasitological data.

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Clinical Research 4

A total of 637 patients including those referred from hospitals for blood examination and treatment of malaria attended the Malaria Clinic at Dwarka, Delhi during April 2009 to March 2010. In all, 17 patients were found positive for malaria and all were diagnosed as *P. vivax* infections.

4.2.1 A Phase III, double-blind, randomised, multicentre trial comparing the safety and efficacy of fixed dose combination tablets of arterolane maleate and piperaquine phosphate (PQP) with Coartem® (artemether-lumefantrine tablets) in patients with acute uncomplicated *Plasmodium falciparum* malaria

Arterolane (RBx 11160) maleate is a synthetic trioxolane. It is easy to synthesize, inexpensive, and orally active. Clinical studies conducted till date with arterolane suggest that the drug is well-tolerated and has rapid action. Piperaquine phosphate (PQP) has antimalarial activities against both *P. vivax* and *P. falciparum* malaria including strains of chloroquine resistant *P. falciparum*. The tolerability, efficacy and pharmacokinetic profile and low cost of PQP makes it a promising partner drug for use with short and rapidly acting antimalarial agents. In Phase II studies, combination of arterolane maleate and PQP has shown that the safety and efficacy of arterolane maleate and PQP combination is comparable to Coartem®, the current gold standard fixed dose combination for the treatment of *P. falciparum* malaria. The data showed that the initial parasite clearance has been achieved in all the patients treated with the arterolane maleate + PQP combination. There has not been any incidence of reappearance of parasites by Day 28 in the followed-up patients. Both the regimens have been well-tolerated by the patients in the trial. For further development, a Phase III, double-blind, randomised, multicentre trial comparing the safety and efficacy of fixed dose combination tablets of arterolane maleate and piperaquine phosphate (PQP) with Coartem (artemether-lumefantrine tablets) in patients with acute uncomplicated *P. falciparum* malaria has been initiated. The study is being conducted at Thailand, India and Bangladesh. In India, the study was initiated in November 2009 at four study sites and patients recruitment at different sites is as follows: Rourkela (n = 49), Jamshedpur (n = 13), Ranchi (n = 11) and Mangalore (n = 52). So far the results indicate high efficacy and safety.

4.2.2 Effective and safe treatment for malaria in pregnancy in India: a randomised controlled trial

The study on "Malaria in Pregnancy in India" is sponsored by the Malaria in Pregnancy Consortium, Liverpool School of Tropical Medicine, United Kingdom. The primary objective of this study is to assess the efficacy of artesunate-mefloquine compared to artesunate + sulphadoxine-pyrimethamine for the treatment of falciparum malaria in pregnancy in India. In addition, assessment of the safety and tolerability of AS + MQ and AS + SP and establishment of the pharmacokinetics of AS + MQ in pregnancy will also be carried out. The study will be conducted in three hospitals, namely Mahadevi Birla Hospital, Ranchi, Tata Hospital, Jamshedpur and ISPAT Hospital, Rourkela. These three hospitals have experience in conducting Phase II/III clinical trials of antimalarial drugs. Pregnant women of all parities in 2nd and 3rd trimester having *P. falciparum* parasitemia (mono infection) will be enrolled in the study. Pregnant women with haemoglobin levels <7 or gestation <12 or >36 weeks or age <18 year will be

excluded from the study. Abnormal liver or renal function, history of taking an antimalarial within the last 7 days, history of allergy to any of the study drugs, taking part in any other clinical trials of drugs or vaccines; severe malaria (based either on clinical presentation or parasitological grounds), other conditions requiring hospitalization or evidence of severe concomitant infection, known chronic disease (cardiac, haemoglobinopathy), history of convulsions during the present illness or history of psychiatric disorder or seizures and infection with vivax malaria or mixed infection patients will be excluded from the study. The primary endpoint will be adequate clinical and parasitological response corrected for new infections by PCR by Day 63 post-treatment (ACPR63). A sample size of 500 pregnant women with *P. falciparum* peripheral parasitemia (250 per treatment group) will be enrolled in the study. The staff has been recruited and investigators meeting will be held in September 2010.

4.3.1 Antimalarial drug use practice in children with emphasis on ACT drugs in selected study sites of Jharkhand state, India

Blister packs of antimalarials are used in the national programme for the treatment of adult malaria patients (aged >15 yr) to improve acceptance of anti-malarial drugs and compliance of the full course of radical treatment. However, such blister packs were not available for children in the national programme during 2007–08 and also no fixed dose combination of ACT is recommended in the programme for pediatric use. Therefore, the exact information on the malaria drug use practices in children and compliance with prescribed drug policy across the public health facilities is lacking. The drug use practice in children in public health facilities like primary health centres (PHCs) were evaluated to obtain information on the prevailing practices for antimalarial drug use. The above study was undertaken in Jharkhand state in districts with ACT as the first line of treatment for *P. falciparum* malaria. To ascertain the prescription practices, audit of OPD records at the PHC for a period of one year was also carried out in selected districts (Fig. 1).

As per the national malaria drug policy 2008, ACT for the treatment of uncomplicated *P. falciparum* malaria has been implemented in all the areas of 12 districts in the Jharkhand state. Based on this information, preliminary surveys were carried out in above districts. Antimalarial consumption of above districts was collected from the State Malaria Office, Jharkhand state. However, ACT supply was physically present in only five districts of Jharkhand state, namely Latehar, Saraikela, Simdega, Gumla and Ranchi. To evaluate the current prescription practices, exit interviews were also conducted in patients who were prescribed antimalarials at Outpatient Department in above five districts in the state.

Fig. 1: Prescription practices in children.

Exit interview data showed that majority of the patients were malaria negative (41–83%) and 21% patients were unware about the results of malaria test in Ranchi district (Fig. 2).

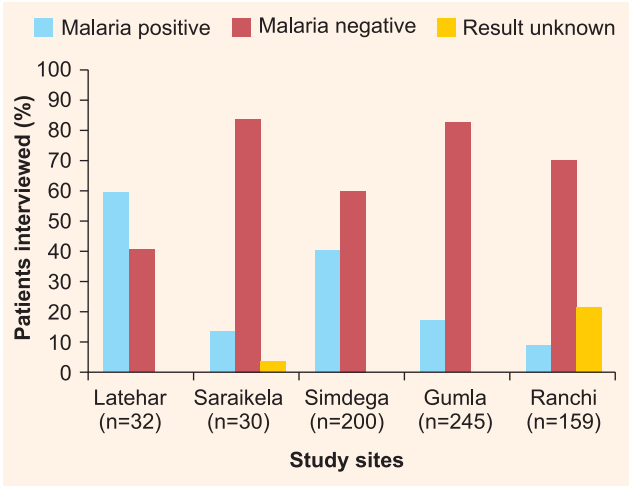


Fig. 2: Exit interview details.

Antimalarial drugs were prescribed to malaria positive cases with majority receiving antimalarial in combination with other drugs (35–100%) (Fig. 3). However, antimalarials were also prescribed to

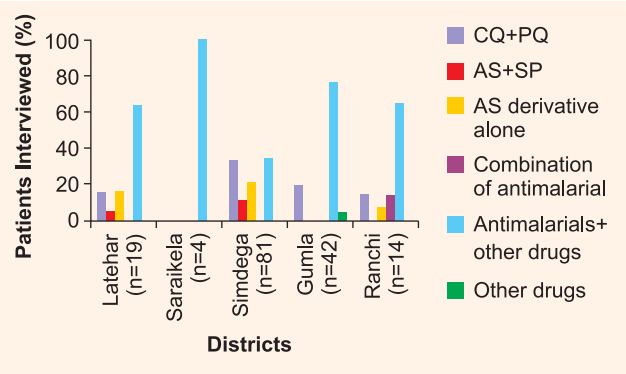


Fig. 3: Drugs prescribed to malaria positive cases.

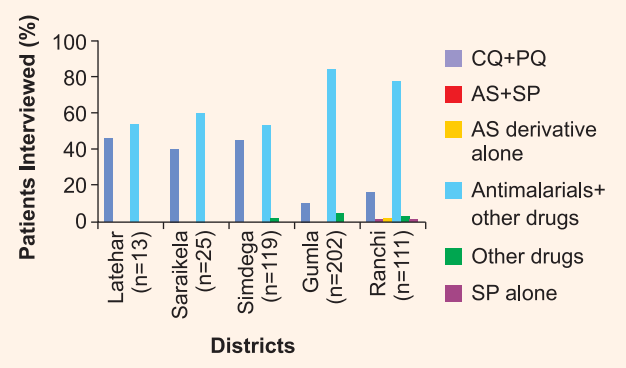


Fig. 4: Drugs prescribed to malaria negative cases.

malaria negative cases at all the study sites with majority receiving antimalarials in combination with other drugs (54–85%) (Fig. 4).

Despite changes in drug policy in the areas, implementation and prescription by the clinicians is inadequate and requires logistic support and proper training. There is an urgent need of imparting training to health personnel at all levels for the use of ACT and also timely supply and availability should be ensured for the ACT drugs (including pediatric blister packs) in the implemented areas.

4.3.2 Monitoring therapeutic efficacy of antimalarial medicines in India

This is a collaborative study between NIMR and NVBDCP funded by the World Bank for monitoring the therapeutic efficacy of antimalarials used in the national programme for *P. falciparum* and *P. vivax* malaria. ACT has been introduced by the national programme for all *Pf* cases in the country and continuous monitoring of its efficacy is required, since there is limited data available. In addition, there are several reports of drug resistance in vivax malaria. Therefore, studies are also needed to validate the observations scientifically. This will help in early detection and limit drug resistance and exploring alternatives.

During the first year, study was conducted at 13 sites. Therapeutic efficacy protocols which were recently modified by the World Health Organization were followed. The project was initiated with the investigators' meet on 28 April 2009 held at NVBDCP, Delhi.

Regional Directors, State Programme Officers from all the 15 study sites attended the meeting. This was followed by the launch of projects by Dr V.M. Katoch, Secretary, Department of Health Research, Government of India and Director General, Indian Council of Medical Research, New Delhi. Dr Katoch emphasized on the importance of close collaboration between research Institutes and national programme for the study. Orientation meeting was conducted and enrolment of patients was initiated at each site.

Orientation meetings held at Dadra & Nagar Haveli and Mangalore

All the patients reporting to local clinic with the complaint of fever were examined for prevalence of parasites in blood smear. The temperature, body weight and other demographic information were recorded. Peripheral blood smear was examined and positive cases for *P. falciparum* or *P. vivax* were enrolled. Informed consent was obtained and case

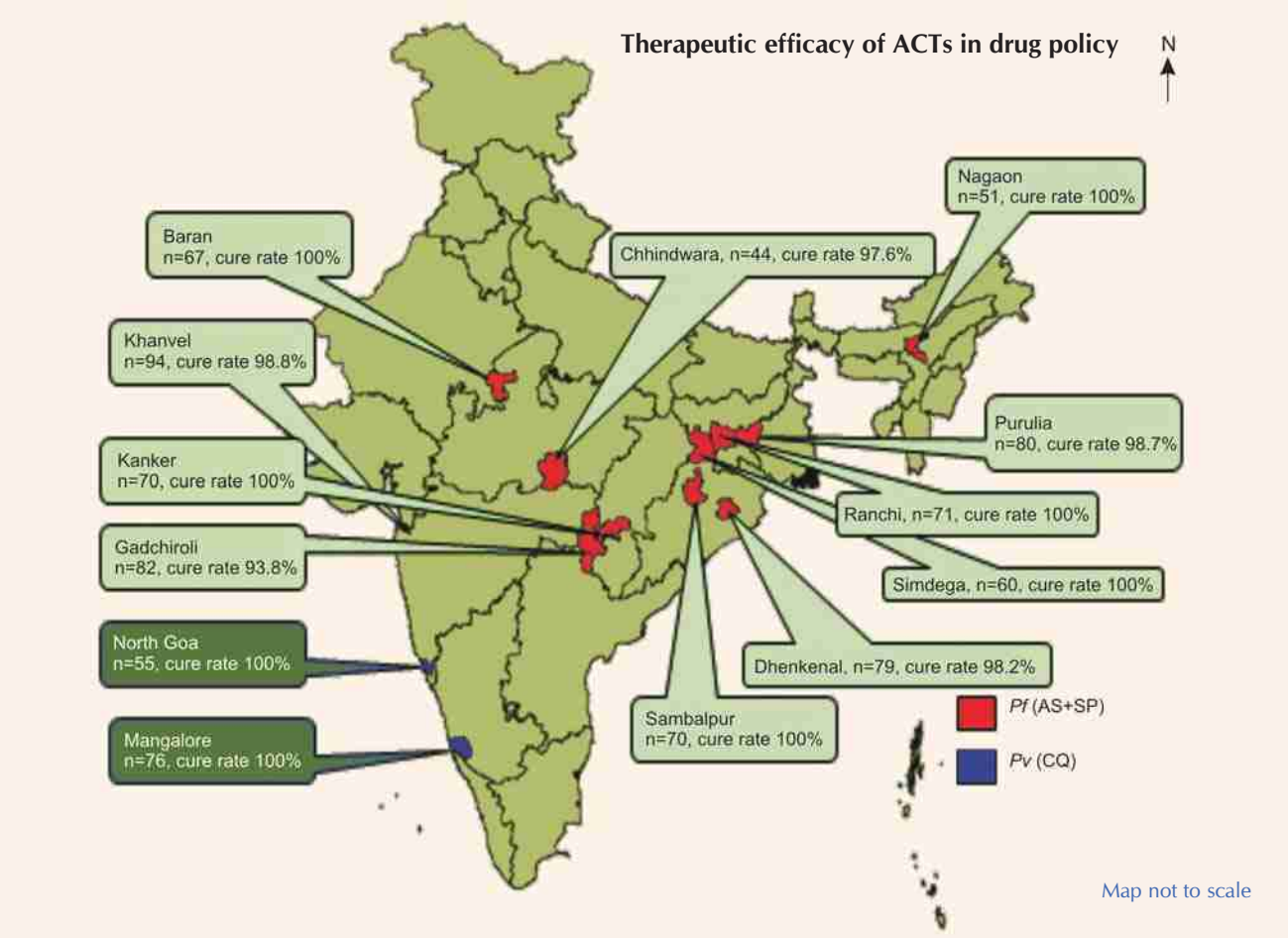


Fig. 5: Study sites of ACT (*Pf*) and CQ (*Pv*) efficacy studies 2009–10.

record form was completed for each patient. Patients aged above six months with mono-infection with *P. falciparum* and parasitemia in the range of 1,000 to 100,000/µl with auxiliary temperature ≥ 37.5ºC or history of fever during the previous 24 h and who were able to come from stipulated follow-up visits with easy access to health facility were included for the therapeutic efficacy studies for AS + SP in *P. falciparum* infections. Informed consent by patients/guardians for children was obtained. For chloroquine efficacy in *P. vivax* infection, asexual parasitemia of 250 parasites/µl of blood or more was taken as cut-off for inclusion criteria. The lower limit of parasitemia was selected to minimize error in the microscopic diagnosis. All other inclusion criteria as followed for *P. falciparum* infection remain the same. Presence of one or more of the general danger signs or any of severe malaria, mixed infection, severe malnutrition and pregnancy were excluded from the study. Presence of G-6-PD deficiency and a history of poor tolerance of the drug were ruled out before prescribing antimalarials.

The studies conducted during the year 2009–10 have shown the efficacy of chloroquine for *P. vivax* as 100% in Goa and Mangalore, while the efficacy of ACT (AS + SP) for *P. falciparum* ranged from 97.6 to 100% (PCR corrected) at 10 sites (Fig. 5). However, cure rate was 93.8% at Gadchiroli, Maharashtra (PCR corrected). Molecular genotyping (MSP2/MSP1) was done in paired samples of treatment failure cases. Out of 13 study sites, a total of 14 treatment failure cases were reported. All the cases were either late

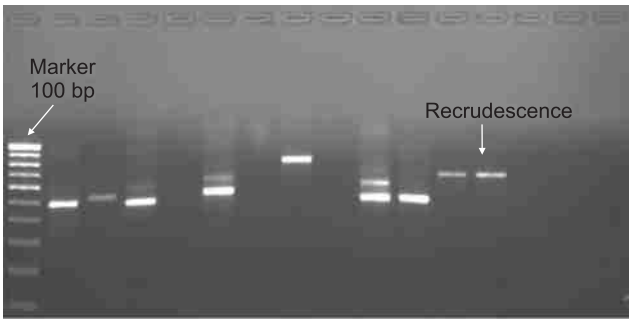


Fig. 6: MSP2('F'allele) genotyping of paired samples from Dhenkanal (Orissa) and Purulia (West Bengal) study sites.

parasitological failure or late clinical failure. Out of these cases, one was reinfection and four were with other species which were withdrawn from analysis. Nine treatment failure cases from 5 sites, namely Dhenkanal, Orissa (n = 1), Purulia, West Bengal (n = 1), Chhindwara, Madhya Pradesh (n = 1), Silvasa, Union Territory (n = 1) and Gadchiroli, Maharashtra (n = 5) showed recrudescence through MSP2 genotyping (Fig. 6).

To monitor the chloroquine resistance pattern in the samples, molecular marker (*Pfcrt*) was done for the samples obtained on Day 0. Samples were randomly selected from 10 sites, namely Dhenkenal, Orissa (n = 11), Purulia, West Bengal (n = 12), Raipur, Chhattisgarh (n = 34), Sambalpur, Orissa (n = 31), Silvasa, U.T. (n = 22), Simdega, Jharkhand (n = 14), Ranchi, Jharkhand (n = 14), Gadchiroli, Maharashtra (n = 14), Baran, Rajasthan (n = 14) and Bhopal, Madhya Pradesh (n = 14) and analyzed for *Pfcrt* mutation. Out of the total 172 samples analyzed, 127 (73.8%) samples showed K76T mutations and 6 (3.5%) showed mixed type of response, whereas 39 (22.7%) were wild (Fig. 7).

Sulphadoxine-pyrimethamine resistance markers (*dhfr* and *dhps*) were analyzed in the samples obtained on Day 0. A total of 185 samples (randomly selected from each site) have been analyzed from 10 different sites, namely Dhenkenal (Orissa), Purulia (West Bengal), Raipur (Chhattisgarh), Sambalpur (Orissa), Dadar & Nagar Haveli (Union Territory), Simdega and Ranchi (Jharkhand), Gadchiroli (Maharashtra), Baran (Rajasthan) and Bhopal (Madhya Pradesh). Out of the total 185 samples, 158 samples could be amplified by PCR. Remaining 27 samples could not be amplified. In most of the cases, *dhfr* (Codon 108: 71.89% and Codon 59: 56.75%) mutations

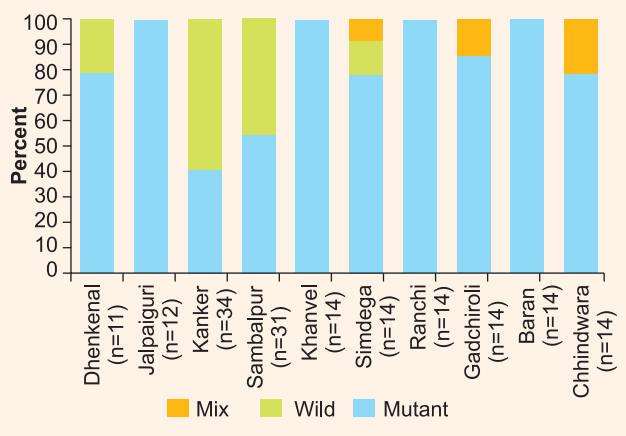


Fig. 7: Chloroquine resistance pattern (*Pfcrt*) at 10 study sites.

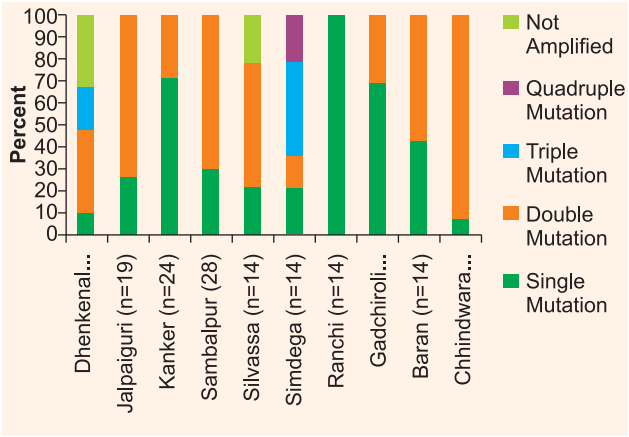


Fig. 8: Sulphadoxine-pyrimethamine resistance pattern (*dhfr*) at study sites.

were prevalent (Fig. 8). *dhfr* [Codon 51(8.64%), codon 164 (4.32%) and codon 16 (4.32%)] mutations have also been observed in some of the samples. However, in the case of *dhps* mutations, only wild type pattern was observed in most of the cases.

The results indicate that AS + SP is well-tolerated and is highly effective for *P. falciparum*. Chloroquine remains effective in vivax malaria. The molecular studies indicate presence of double mutations in *dhfr* gene in half of the samples and a high prevalence of chloroquine resistance.

4.3.3 Pharmacovigilance of antimalarial medicines in India

Pharmacovigilance for antimalarial medicines in India was jointly funded by the NVBDCP and the World Bank with the objectives "Assessment of benefit, harm, effectiveness and risk of ACTs in the treatment of malaria". The project was initiated in the month of June 2009 along with collaborating institutes—All India Institute of Medical Sciences (AIIMS) and NVBDCP. ADR form was developed in consultation with the Department of Pharmacology, AIIMS, New Delhi and the NVBDCP. Review workshop was organized on 14 September 2009 at AIIMS, New Delhi. Representatives of all significant stake holders, WHO, NIMR, NVBDCP, AIIMS, WR India and World Bank attended the meeting. The sample size of patients in this cohort study was finalized to be 10,000 and practical experiences of pilot study and inputs of all the participants were incorporated to develop Version 1.2 of ADR form.

Sensitization meeting of SPOs was held at NVBDCP on 22 December 2009 and attended by the representations of WHO, AIIMS, NVBDCP,

Investigators meeting held at AIIMS, New Delhi.


Sensitization meeting held at NVBDCP, Delhi.

NICD and 26 State Programme Officers. For the validation of ADR forms, a pilot study was undertaken at three sites, namely Raipur, Goa and Rourkela. The physicians actually used ADR format version 1.1 to collect patients' details, both initial as well as follow-up. Their feedback was proactively sought, where 21 filled up forms were received and were analyzed to detect any reporting hindrances/speed breakers.

The programme was reviewed periodically by the principal investigators and collaborating institutes. Training programmes for DMOs were conducted in Guwahati NE region, Ranchi (Jharkhand), Bengaluru (Karnataka) and Jabalpur (Madhya Pradesh).

Till date, 272 forms were received and analyzed. Mostly, forms were received from north-east region. Frequent telephone calls were made to the DMOs, PHCs, RDs and Consultants for the submission of more ADR forms.

4.3.4 Quality assurance of malaria rapid diagnostic tests

NIMR has been identified as the national referral laboratory for the quality assurance of laboratory diagnosis of malaria. The NVBDCP is the nodal agency. The regional and state referral laboratories have been identified. Major components of the quality assurance of RDTs for malaria include preparation of quality control (QC) panels, pre-dispatch QC, post-dispatch QC, external quality assurance scheme (EQAS) and internal QC.

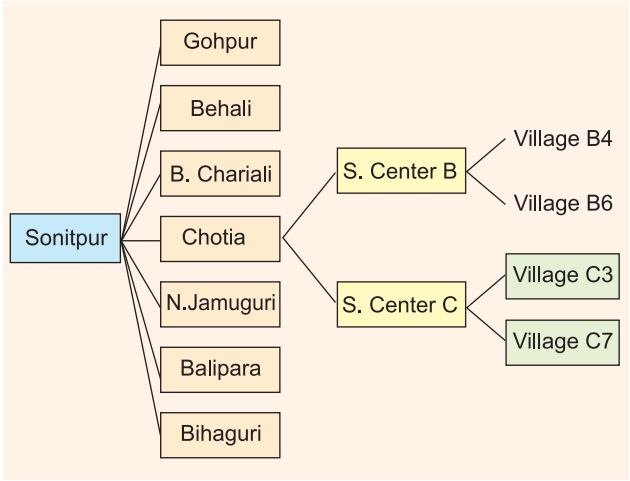
Preparation of QC test panels

NIMR staff were trained in the preparation of panels with samples at the NIMR Field Unit, Rourkela, Orissa. Patients with parasitemia of >20,000/µl, from different areas were selected as donors. Parasitized blood was diluted with 'O' group blood cells and 'AB' group fresh frozen plasma to attain parasite density of 200/µl (low positive) and 2000/µl (highly positive). Desired numbers of panels (13 samples) in appropriate quantity (200 aliquotes per panel) have been prepared, and the procedure will be repeated every quarter since the shelf-life of panels is 180 days.

Testing of RDT kits using QA panels

Pre-dispatch QC (national level): This was achieved by lot testing of the kits. From each RDT lot, 50 RDTs were drawn and tested using positive and negative controls for immediate QC. In all 29 batches have been tested till date and were found to be acceptable.

QA of the RDTs used by health workers at periphery: RDT samples are being drawn from the



Flowsheet showing the pattern of collection of RDTs from the field

RDTs being tested.

Orientation meeting held at Jabalpur.

representative Primary Health Centres/subcentres/ ASHAs and tested for their quality. The District Malaria Officers (DMOs) have been collecting RDT samples from their districts and sending the same to the referral laboratory every quarter.

Progress

A sensitization meeting of the State Programme Officers was organized on 13 May 2009. Training programmes had also been conducted for District Programme Officers of 12 states—Assam, Meghalaya, Manipur, Mizoram, Nagaland, Arunachal Pradesh, Orissa, Jharkhand, Chhattisgarh, Karnataka, Madhya Pradesh and Gujarat.

Kits procured by the NVBDCP through UNOPS India were sent to the NIMR for evaluation. Till date, 29 batches have been evaluated. The post-dispatch QA has also been initiated. The District Malaria Officers have started picking up seven RDTs at random from different levels (PHC, Subcentre, ASHAs) every quarter. Of the 199 districts, training was conducted for 179 districts which was attended by District Programme Officers of 138 districts and 52 districts have started sending RDTs to the NIMR. So far, 779 RDTs had been received by NIMR. The RDTs received from the field were tested for their quality by standard panels. Till date, RDTs from 52 districts of seven states have been received (Table 1).

Table 1. Results of RDTs tested so far

S. No.	State	No. of RDTs tested	Results (satisfactory/tested)*		
			2000p/µl	200p/µl	Negative
1.	Nagaland	217	61/62	104/124	31/31
2.	Manipur	14	4/4	6/8	2/2
3.	Mizoram	126	32/36	69/72	18/18
4.	Meghalaya	98	25/28	49/56	14/14
5.	Assam	14	3/4	6/8	2/2
6.	Madhya Pradesh	137	38/42	61/78	19/19
7.	Orissa	21	6/6	10/12	3/3

*Correct results/number tested.

Two types of RDTs have been sent to NIMR: (i) Wondfo (Manufactured by Wonfo Biotech Ltd.); and (ii) ParaHit 'F' (Manufactured by Span Diagnostics Ltd.). Of the 779 RDTs received, 641 have been tested so far. Overall sensitivity was 86.6% and specificity was 100%.

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5 Highlights of the Research Activities under IDVC Project

Bengaluru (Karnataka)

- Experimental studies showed that both the sexes of *Poecilia reticulata* (Guppy) were equally capable to consume IV instar larvae of *Anopheles* and *Culex*.
- Production of recombinant HRP2 and pLDH monoclonal and polyclonal antibodies is underway for the development of diagnostic kit for malaria.
- Clinical trial of Arterolane is underway and all the 31 *P. falciparum* patients who received the treatment responded satisfactorily up to 42-day follow-up in Mangalore City.
- Evaluation of Biodart-M, a liquid formulation of *Bti* is underway.
- Micro-PCR-based RT-PCR showed better detection level of malaria than RDT and microscopy.
- Alpha-cypermethrin-based LLIN failed to show the required level of susceptibility.
- Synthetic pyrethroids in Andhra Pradesh showed reduced susceptibility in *An. culicifacies*—a cause of concern.
- The second phase of C-21 trial, an attractant for surveillance and control of *Ae. aegypti* is underway.
- Malaria is under control in all the project areas undertaken in 1992 onwards. Efforts have been made to replicate the same in the northern districts of Karnataka.
- As a part of technical support to the NVBDCP and local health authorities, chikungunya outbreak investigations were carried out in Haveri, Gadag and Tumkur and also in a slum area in Bengaluru City.

Chennai (Tamil Nadu)

- Phase III trials on the application of Attracticide for surveillance and control of dengue and chikungunya mosquitoes (C-21 project) were undertaken in Alappuzha district, Kerala, besides evaluation of Novaluron 10% SC (Mosquiron), an insect growth regulator for mosquito vector control in urban settings.
- Monitoring of insecticide resistance in mosquitoes and drug resistance on malarial parasites was carried out.
- Studies on purification and identification of active compounds from the selected plants for vector mosquitoes, mosquitocidal activities of medicinal plant extracts, effect of Kitazin (fungicide) and Hostathion (insecticide) on the larvivorous potential of *Oryzias carnaticus*; isolation, screening and identification of microflora associated with gastrointestinal tract of larvivorous fishes were also carried out.
- Technical support was provided to various centres/institutes/government agencies and collaborative research/scientific work was also undertaken with NIMR, Delhi. Health education and training programmes to students, officials and public were also conducted. Malaria Clinic continued to function catering to the needs of the public by providing early diagnosis and prompt treatment.

Guwahati (Assam)

- The major research projects included field evaluation of alternate technologies for vector control including: (i) Follow-up field evaluation and extended follow-up investigations of long-lasting insecticidal nets (LLINs) impregnated with pyrethroids against malaria transmitting mosquitoes in Assam, and associated disease transmission; (ii) Monitoring insecticide

resistance against disease vectors in north-eastern states; (iii) Bio-monitoring of organochlorine residues in human populations and their relation with food intake; and (iv) Regional level mapping of malaria vectors using RS and GIS in north-eastern states in India to develop strategic plans for malaria control.

- A new project was initiated on "Evidence-based assessment of biophysical determinants of malaria in the north-eastern states of India and development of framework for adaptation measures for malaria control under climate change scenario".
- Other activities included technical inputs to strengthen the malaria control activities specific to the north-eastern region, i.e. health education and capacity building measures, mass propagation and distribution of larvivorous fishes (Guppy and *Gambusia*) in town areas, and building public-private partnership/intersectoral convergences for promoting community-based action to combat malaria illness. In addition, sentinel malaria sites were established in Sonapur Primary Health Centre of Kamrup district as well as in Gauhati Medical College Hospital to ascertain disease transmission trends and monitoring of drug-efficacy investigations.

Hardwar (Uttarakhand)

The following studies were undertaken during the year.

- Antimalarial properties of some plants from Garhwal region of north-west Himalaya.
- Organochlorine residues in soil, water, whole blood and major local food products from low and high malaria endemic areas of Assam.
- LC/MS/MS assay for the determination of lumefantrine and its metabolite desbutyl-lumefantrine in plasma using high performance liquid chromatography.
- Studies on the transmission dynamics of encephalitis in District Saharanpur of Uttar Pradesh: An action plan for the prevention and control.
- Epidemiological investigation of malaria in District Saharanpur, Uttar Pradesh.
- Monitoring of insecticide resistance of malaria vectors in some districts of West Bengal.
- Phase III evaluation of Pyriproxyfen (Sumilarv 0.5G) an insect growth regulator against larvae of mosquito vectors.
- Technical support to NVBDCP and Govt. of Uttarakhand was provided through activities like Epidemiological investigation of malaria in NTPC, Rihand Nagar; Monitoring of industrial malaria control at BHEL, Hardwar and IOC, Mathura and consultancy was provided to control malaria at NTPC, Rihand Nagar, District Sonbhadra, Uttar Pradesh.

Jabalpur (Madhya Pradesh)

- Under the ICMR National task force project on "Preparation of a field site for malaria vaccine trial in and around Jabalpur", 46 pregnant women, 50 siblings, 28 fathers and 55 infants were found positive for malaria during follow-up. Age dependent increase was observed in humoral response against different *Pf* and *Pv* specific antigens. Vector incrimination of malaria vectors showed eight *An. culicifacies* positive for sporozoites by ELISA technique. All *An. fluviatilis* were of sibling species T.
- Epidemic investigations were carried out in eight districts of Madhya Pradesh which were previously considered to be free of infection due to low malaria transmission. Rapid fever surveys carried out in these districts revealed very high percentage of malaria. In each district, epidemic claimed several lives.
- A study on intensive monitoring of insecticide residual spray (IRS), insecticide-treated mosquito nets (ITNs), verification of ASHA and implementation of programme strategies under NVBDCP project was carried out in three districts of Madhya Pradesh. The observations were recorded and recommendations sent to the NVBDCP.
- The vector susceptibility tests carried out in nine districts of Madhya Pradesh revealed that *An. culicifacies* mosquitoes of these districts are resistant to DDT 4% and susceptible to malathion 5% and deltamethrin 0.05%.
- On the request of Govt. of Madhya Pradesh, three training workshops on malaria and other vector-borne diseases for Medical Officers of various districts of Madhya Pradesh were conducted from November 2009 to March 2010 at NIMR Field Unit, Jabalpur.

Nadiad (Gujarat)

- Health Impact Assessment of Sardar Sarovar Project on vector-borne diseases in Gujarat was continued.
- Scaling-up of use of larvivorous fishes and capacity strengthening in Gujarat.
- Phase III evaluation to compare insecticidal efficacy and community acceptance of long-lasting insecticidal nets with conventional insecticide-treated nets and Multi-centre Phase II and III evaluations of the effectiveness of Novaluron 10% SC (Mosquiron), an insect growth regulator for mosquito vector control in urban settings were undertaken.
- A collaborative project for developing a framework for predicting malaria outbreaks in rural and urban areas of Gujarat was initiated.
- Monitored insecticide resistance of malaria vector in Mizoram state.
- Provided technical support and training to the NVBDC Programme in terms of reviewing dengue situation in Ahmedabad, Surendra-nagar and Bhawnagar districts, in liquidating the malaria foci in Gujarat, Monitoring of susceptibility of malaria vector in Gujarat, Assessment of dengue situation and control measures, Entomological investigations and review of the control measures in Vadodara Municipal Corporation, urban malaria scheme (UMS) and providing societal benefits to the communities through Malaria Clinic.

Panaji (Goa)

- Estimation of Malaria disease burden in Jharkhand, India under WHO funded project was continued.
- Studies on Burden of malaria in pregnancy: a one year longitudinal study in Ranchi district, Jharkhand, India were undertaken.
- Studies on characterization and efficacy of mosquito pathogenic bacteria from mangrove and paddy-fields in Goa, India were under-taken.
- Larvicidal activities of leaf and stem powder of *Ipomoea carnea* Jacq. against *An. stephensi* Liston, *Cx. quinquefasciatus* Say and *Ae. aegypti* Linn were determined.
- On the request of NVBDCP Goa, testing of the efficacy of ultra-low volume fogging machine for vector control was undertaken.
- Monitored therapeutic efficacy of antimalarial in the treatment of *P. vivax* malaria.
- Enrolled 59 patients of *P. vivax* in the age groups of 4–70 years (median age 25.5 yr) to test the efficacy of chloroquine as treatment of vivax malaria in Candolim PHC of Goa.
- Monitored insecticide resistance of mosquito vectors in the districts of East and West Garo Hills of Meghalaya.
- Investigations and rapid response to chikungunya outbreak in Goa Medical College Complex at Bambolim, Goa and in areas of Quepem PHC, South Goa district, Goa, were undertaken. Invetsigation of outbreak in areas of PHC-Cansarvarnem, North Goa district, Goa was also undertaken.
- Provided technical support to the local health authorities in training, and technical inputs.

Raipur (Chhattisgarh)

- Phase III evaluation was undertaken to compare insecticidal efficacy and community acceptance of long-lasting insecticidal nets (LNs) with conventional insecticide-treated nets in India.
- Evaluated the bioefficacy of field distributed alpha-cypermethrin-treated LLINs (Inter-ceptor®) against malaria vectors in Assam, Chhattisgarh and Orissa states.
- Evaluated DuraNet, a LLIN incorporated with alpha-cypermethrin, against malaria vectors and its impact on malaria incidence.
- Field evaluation of Biodart-M, aqueous suspension of *Bacillus thuringiensis* var. *israelensis* serotype H-14 against larvae of mosquito vectors was undertaken.
- Phase III evaluation of Pyriproxyfen (Sumilarv 0.5G) an insect growth regulator against larvae of mosquito vectors was undertaken.
- Monitored insecticide resistance of malaria vectors in selected areas of Chhattisgarh.
- Studies were undertaken on distribution and biological characteristics of the members of *fluviatilis-minimus* groups for effective vector control strategies in tribal areas of Chhattis-garh.
- Monitored the epidemiological impact of rotation of insecticides for indoor residual spraying in malaria endemic areas of Chhattis-garh.
- Monitored the therapeutic efficacy of anti-

malarial medicines in India.
- Provided technical support to NVBDCP in monitoring of malaria control activities, training support, examination of malaria/filaria blood slides, cross-checking of blood slides and running Malaria Clinic for societal benefits.

Ranchi (Jharkhand)

- Studied anopheline mosquito fauna, biology and bionomics of malaria vectors in Ranchi district.
- Incrimination of *An. culicifacies, An. fluviatilis* and *An. annularis* was done by detection of sporozoites in mosquitoes.
- Insecticide susceptibility of malaria vectors *An. culicifacies*, *An. fluviatilis* and *An. annularis* was undertaken in Ranchi, Gumla and West Singhbhum districts of Jharkhand.
- Field evaluation of DuraNet a long-lasting insecticidal net (LLIN) incorporated with Alpha-cypermethrin against malaria vectors and its impact on malaria incidence was undertaken.
- Investigations on malaria outbreaks in Mahuadan PHC, District Latehar and in Giridih districts were undertaken.
- Filariasis survey in Gumla, Garhwa and Ranchi districts was undertaken.
- Monitored the therapeutic efficacy of ACT (Artesunate+Pyrimethamine and Sulphadoxine) against uncomplicated *P. falciparum* malaria in tribal area of Ranchi district.
- Provided support to NVBDCP in training, evaluation of programme activities like MDA, IRS and bed nets.

Rourkela (Orissa)

- Studies on development of field site for malaria vaccine trial were continued.
- WHOPES Phase III evaluation (household randomized trial) to compare insecticidal efficacy and community acceptance of long-lasting insecticidal net (DuraNet®) with conventional insecticide-treated nets in five hamlets under Bisra PHC in Sundargarh district in India is under progress.
- Studies were completed on extended evalua-tion of the bioefficacy of field distributed alpha-cypermethrin-treated LLINs (Interceptor®) against malaria vectors in Orissa.
- Phase III studies as per NIMR common protocol were completed on Evaluation of Icon®Life — a LLIN treated with deltamethrin against malaria vectors and disease transmission.
- Extended evaluations of PermaNets and Olyset LLINs were undertaken.
- Monitoring of insecticide resistance in malaria vectors was undertaken in four districts of Orissa.
- Studies were initiated to monitor the epi-demiological impact of rotation of insecticides for indoor residual spraying in malaria endemic areas of Sundargarh district, Orissa.
- A GCP trial was initiated on Phase III, randomized, open label, multicentre study to assess the antimalarial efficacy and safety of arterolane (RBx 11160) maleate and piperaquine phosphate co-administration and Coartem® in patients with acute uncomp-licated *P. falciparum* malaria.
- Assessment of therapeutic efficacy of Arte-sunate + Sulphadoxinepyrimethamine (ACT) in uncomplicated *P. falciparum* patients in Charmal PHC of Sambalpur district, Orissa.
- Investigated the malaria outbreak in residential school children in Jharsuguda, Orissa.
- Technical support to the NVBDCP was provided in training, monitoring of pro-gramme implementation for malaria control and IRS operations and assessment of district readiness for implementation of World Bank Project etc.

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6 Research Support Facilities

6.1 Animal house facility

NIMR has an animal house facility which maintains laboratory mice and rabbits as per CPCSE guidelines. Laboratory mice are used for screening the antimalarials, host-parasite relationship, and maintenance of rodent plasmodia. There is an experienced veterinarian looking after the same. Experiments are performed with the approval of the Scientific Advisory Committee and the Animal Ethics Committee of the Institute.

6.2 Repository of biological materials

6.2.1 Mosquito species

The details of mosquitoes being maintained in the NIMR Insectary are furnished in Table 1.

Table 1. Details of mosquito species maintained at NIMR Insectary

Mosquito species	Strain/Origin	Mitotic karyotype/ Y-chromosome	Sibling species
Anopheles culicifacies	Burari	Sub-metacentric	A
An. culicifacies	Dehra	-do-	A
An. culicifacies	Rameswaram	-do-	A
An. culicifacies	Jabalpur	-do-	C
An. culicifacies	Rourkela	-do-	C
An. culicifacies	JP-2	-do-	C
An. stephensi	Haryana		
An. stephensi	Punjab		
An. stephensi	Delhi		
An. stephensi	Okhla, Delhi		
An. stephensi	Goa		
An. stephensi	Sonepat		
An. stephensi	Mewat		
An. fluviatilis	Rourkela		
Culex quinquefasciatus	BSSS (Sensitive to biocide)		
Aedes aegypti	Delhi		
Cx. quinquefasciatus	Insecticide resistant		
Mutant Lines			
An. stephensi	Black larva with white eye		
Cx. quinquefasciatus	Red eye		

6.2.2 Parasite species

Parasite Bank is supporting a large number of organizations working on various aspects of malaria. Biological materials including non-human and human plasmodia preserved/maintained in Malaria Parasite Bank were supplied to various research organisations. Till now, a total of 1004 isolates of human malaria parasites *P. falciparum, P. vivax* and *P. malariae* were collected and cryopreserved in the Malaria Parasite Bank. A total of 57 isolates including 45 *P. vivax* and 12 *P. falciparum* were collected during the year 2009–10. All these parasites are cryopreserved in liquid nitrogen. Details of human and non-human malaria parasite isolates collected are shown in Tables 2 and 3.

Details of characterized *P. falciparum* parasites are shown in Table 4. Screening of medicinal plant extracts/fractions for their antiplasmodial activity against CQ sensitive and resistant *P. falciparum* isolates is a routine activity of the Malaria Parasite Bank. Since 1993, a total of 287 *P. falciparum* samples from different regions were tested for chloroquine sensitivity and 187 (65.16%) were found to be resistant to chloroquine. Details of chloroquine resistant and susceptible *P. falciparum* isolates available at the Parasite Bank are shown in Table 5.

Cell lines available at the Malaria Parasite Bank

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

Table 2. Human malaria parasites preserved in the Parasite Bank

Parasite species	Collection sites		No. of isolates collected/years of collection			Total
	State	District	1992–2008	2009	2010	
P. falciparum	Andhra Pradesh	Visakhapatnam	12	–	–	12
	Assam	Sonapur	20	–	–	20
		Tezpur	6	–	–	6
		Nalbari	1	–	–	1
	Chhattisgarh	Jagdalpur	14	–	–	14
		Bilaspur	26	–	–	26
	Delhi		198	–	–	198
	Gujarat	Anand	4	–	–	4
		Kheda	7	–	–	7
	Goa	Panaji	18	–	–	18
	Haryana	Gurgaon	25	–	–	25
	Karnataka	Mangalore	28	1	–	29
	Madhya Pradesh	Mandla/Jabalpur	14	–	3	17
	Meghalaya	Tura	18	–	–	18
	Mizoram	Kolasib	6	–	–	6
	Orissa	Rayagada	29	–	–	29
		Sundargarh	42	–	–	42
		Rourkela	13	5	–	18
	Rajasthan	Alwar	25	–	–	25
		Bharatpur	35	–	–	35
		Jaisalmer	38	–	–	38
	Tamil Nadu	Chennai	9	–	–	9
		Ramanathapuram	20	–	–	20
	Uttar Pradesh	Baharaich	22	–	–	22
		Gautam Budh Nagar	37	–	–	37
		Ghaziabad	17	–	–	17
		Allahabad	60	–	–	60
	Uttarakhand	Haldwani	–	3	–	3
	West Bengal	Kolkata	18	–	–	18
		Midnapur	1	–	–	1
	Total		763	9	3	775
P. vivax	Assam	Sonapur	2	–	–	2
	Delhi		38	–	–	38
	Goa	Panaji	23	–	–	23
	Karnataka	Mangalore	38	14	–	52
	Madhya Pradesh	Jabalpur	3	–	7	10
	Orissa	Rourkela	12	2	–	14
		Bissam Cuttak	4	–	–	4
	Rajasthan	Jaisalmer	–	–	15	15
	Uttar Pradesh	Shankargarh	4	–	–	4
		Mirzapur	11	–	–	11
		Baharaich	2	–	–	2
		Gautam Budh Nagar	2	–	–	2
		Ghaziabad	–	–	7	7
	Tamil Nadu	Ramanathapuram	9	–	–	9
		Chennai	16	–	14	30
	West Bengal	Kolkata	1	–	–	1
	Total		165	16	43	224
P. malariae	Orissa	Sundargarh/Rayagada	4	–	–	4
	Delhi		1	–	–	1
Total isolates			933	25	46	1004

Table 3. Non-human malaria parasites preserved in the Parasite Bank

Parasite	Species	Susceptibility to antimalarials
Simian malaria	*P. cynomolgi bastianelli* (CDRI)	Not done
	P. cynomolgi bastianelli (NICD)	-do-
	P. knowlesi (NICD)	-do-
	P. knowlesi (CDRI)	-do-
	P. fragile (CDRI)	-do-
Avian malaria	*P. gallinaceum*	Not done
	P. relictus	-do-
Rodent malaria	*P. berghei* (CDRI)	CQ-resistant
	P. berghei+**	CQ-sensitive
	P. berghei	Quinine-resistant
	P. berghei ANKA	Not done
	P. berghei (NK65) PGI Chandigarh	-do-
	P. chabaudi (Paris)	-do-
	P. vinckei petteri 279 BY	-do-
	P. yoelii nigeriensis (ICGEB)	-do-
	P. yoelii nigeriensis (CDRI)	Multi-resistant
	P. yoelii nigeriensis (LSHTM)**+	Not done
	P. yoelii yoelii (265 BY) Paris**	-do-

+Infective gametocyte producing strain; *Oocyst positive in *An. stephensi*; **Oocyst and sporozoite positive in *An.stephensi*

Table 4. Details of characterized *P. falciparum* isolates available at the Parasite Bank

Adapted isolates susceptible to chloroquine	54
Adapted isolates resistant to chloroquine	52
NF-54, an infective gametocyte producing strain of *P. falciparum*	1
3D 7A : a clone of NF-54	1
A-4 : a clone with binding property to CD36	1
Dd2: a clone which can invade trypsin-treated erythrocytes	1
Field isolates which can invade trypsin-treated erythrocytes	3
Field isolates which can invade neuraminidase-treated but not trypsin-treated erythrocytes	3
Field isolates which can invade normal erythrocytes but not in neuraminidase or in trypsin-treated erythrocytes	3
Field isolates which can invade both in neuraminidase-treated and in trypsin treated-erythrocytes	5
Field isolates which can form rosettes	3
Field isolate which can bind to CSA	1
Field isolates which can bind to CD36	9
Field isolates which can bind to ICAM-1	2
Isolates with isoenzyme profile of GPI, GDH, ADA & LDH markers	22

Table 5. Chloroquine susceptible and resistant strains of *P. falciparum* at the Parasite Bank

Place of collection	No. tested	Sensitive	Resistant*
Delhi	74	17	57
Jaisalmer (Rajasthan)	22	1	21
Shankargarh (U.P.)	10	2	8
Gurgaon (Haryana)#	66	44	22
Sonapur (Assam)	18	5	13
Baharaich (U.P.)	11	6	5
Visakhapatnam (A.P.)	4	–	4
Gautam Budh Nagar (U.P.)	33	14	19
Bissam Cuttack (Orissa)	16	–	16
Rourkela (Orissa)	4	–	4
Jagdalpur (M.P.)	5	1	4
Tura (Meghalaya)	10	2	8
Mangalore (Karnataka)	1	–	1
Kheda (Gujarat)	1	–	1
Bilaspur (Chhattisgarh)	4	–	4
Kolasib (Mizoram)	6	6	–
Ramanathapuram (T.N.)	2	2	–
Total	287	100	187

*WHO methods/kits were used; #Out of 66 samples tested from Gurgaon we could preserve only 25 *P. falciparum* samples.

6.3 Library

The Institute has one of the best libraries in the country in the field of malaria having more than 7550 books, 4300 bound journals, 3700 reprints, 18 video cassettes, 27 audio cassettes, 20 micro-films, 19 theses and 106 national and international reports. About 52 journals (39 Foreign and 13 National) are being subscribed besides eight journals which are received on complimentary and exchange basis.

It provides information services to the scientists, research-scholars and outside visitors. The Library is the support centre for researchers of 10 field units of NIMR located in different parts of the country. Library provides other necessary services such as paper clipping, citation search, photocopying and reference collection. NIMR Library has been participating in resource sharing works like Union Catalogue of Biomedical Journals developed by the National Informatics Centre-ICMR and a member of Developing Library Networks (DELNET) to fulfil the users' need for information. The general house-keeping activities are automated using *Libsys* software and a dedicated server is developed with compatibility for multilingual records— English and Hindi. The documents are classified and database is updated regularly. The books are all barcoded for automation of issue/return and issue of barcoded library membership card has been done. Library web portal is developed and circulated among

scientists to maximize the use of subscribed and freely available journals and other internet-based information. Around 1000 biomedical journals are also available through consortia such as J-GATE@ERMED of National Medical Library (NML), ICMR e-journals consortia, JCCC@ICMR of ICMR.

6.4 Equipments and facilities at NIMR

The following equipments and facilities are available at NIMR Headquarters, New Delhi.

Equipments

1. Mortorized Research Fluorescence Microscope
2. Multi-Channel Liquid Handling Workstations
3. Mortorized Stereozoom Microscope
4. Lyophilyzer
5. Auto Analyzer
6. LCMS/MS
7. High speed centrifuge with 96 well plate rotor
8. Nano DROP Spectrophotometer
9. Data logger (for monitoring temperature and humidity with sensor)
10. Flow Cytometer
11. Binocular stereo microscope
12. Binocular compound microscope
13. Microscope with teaching aids
14. Microscope with LCD Monitor
15. Ice Flaking Machine
16. Control Rate Freezer
17. High Performance Liquid Chromatography
18. UV-VIS Spectrophotometer
19. Ultracentrifuges
20. DNA Sequencer
21. Gel-doc system
22. Laminar Flow
23. Chemical hood
24. Climatic Chambers
25. Ultra-deep Freezers
26. Mass Spectrophotometer
27. Real Time PCR

Facilities

1. Milli-Q Water Purification System
2. Central Auto-clave facility
3. Animal house facility
4. Insectarium
5. Cold & Hot room facilities
6. Liquid Nitrogen Plant
7. Dark Room facility
8. Dawn and Dusk control system
9. Bioinformatics
10. GIS facility
11. Audio-visual facility

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7 Inter-Institutional Collaboration

Collaborative projects were undertaken with the following ICMR/non-ICMR Institutes and Medical Colleges of the country.

1. 'Studies on the distribution of members of *Anopheles dirus* species complex in north-eastern states' in collaboration with Defence Research Laboratory (DRL), Tezpur, Assam.
2. 'Engineering Indian malaria vector *An. culicifacies* mosquito genetically using transposable element' in collaboration with M.D. University, Rohtak, Haryana.
3. 'Application of attracticide (oviposition pheromone in combination with insect growth regulator) for surveillance and control of chikungunya and dengue mosquitoes in collaboration with Defence Research and Development Establishment (DRDE), Gwalior, Madhya Pradesh, Municipal Corporation of Delhi and NVBDCP, Delhi.
4. 'Micro-level mapping of malaria vectors using GIS in bordering districts of Assam and Arunachal Pradesh to assist malaria control' in collaboration with DRL, Tezpur, Assam.
5. 'Developing epitope-based immunogen selecting different stages of *Plasmodium vivax* using in-built immunoadjuvants and delivery in microspheres' in collaboration with All India Institute of Medical Sciences (AIIMS), New Delhi.
6. 'Immunocapture-based diagnostic assay for the detection of *P. falciparum* HRP-2 and LDH anti-gen' in collaboration with AIIMS, New Delhi.
7. Complement receptor-1, TNF-α, nitric oxide and the respective gene polymorphisms in relation to the pathophysiology and susceptibility to severe malaria in collaboration with AIIMS, New Delhi, and funded by ICMR, New Delhi.
8. 'Promotion of *Plasmodium* research in India' in collaboration with New York University, New York, USA, funded by NIH Fogarty.
9. 'Identification of epidemiological risk factors of malaria for development of strategic action plan for malaria control in problematic districts in Karnataka' in collaboration with Govermment of Karnataka, Bengaluru.
10. 'HRP-2 based rapid detection on *P. falciparum* using agglutination latex-based system' in collaboration with DRDE, Gwalior.
11. 'Evaluation of therapeutic efficacy of anti-malarials' in collaboration with the NVBDCP, Delhi and funded by the World Bank.
12. 'Therapeutic efficacy of Artemether-Lumefantrine combination in Orissa' in collabora-tion with AIIMS, New Delhi and WHO.
13. 'Pharmacovigilance of antimalarials in India' in collaboration with AIIMS, New Delhi and NVBDCP, and funded by the World Bank.
14. 'Clinical trials of antimalarial agents' in collaboration with Medical Colleges, Guwahati and Goa; Wenlock Hospital, Mangalore; Tata Main Hospital, Jamshedpur; Mahadevi Birla Hospital, Ranchi; Ispat General Hospital, Rourkela; Community Welfare Hospital, Rourkela; and funded by agencies like Medicines for Malaria Venture, Geneva, Drugs for Neglected Diseases *initiative* (DND*i*), Geneva and Ranbaxy.
15. 'Primary screening of medical plants from north-eastern states of India for their antimalarial activity' in collaboration with DRL, Tezpur, Assam.
16. 'Screening of chloroquine sensitivity status of *P. falciparum* parasites from western border areas of India' in collaboration with DRDE, Gwalior, Madhya Pradesh.

17. '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.
18. '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.
19. 'Characterisation of *P. falciparum* strains prevalent in north-eastern states' in collaboration with Regional Medical Research Centre, Dibrugarh, Assam.
20. 'Screening of antimalarial activity of synthetic compounds in *P. falciparum* culture lines' in collaboration with the Department of Chemistry, University of Delhi, Delhi and Indian Institute of Chemical Technology, Hyderabad.
21. 'Development of site for malaria vaccine trial at Sundargarh district, Orissa' in collaboration with International Centre for Genetic Engineering and Biotechnology, New Delhi and the State Government of Orissa.
22. 'Preparation of a field site for malaria vaccine trial in and around Jabalpur' funded by ICMR task force and Center for Disease Control and Prevention (CDC), Atlanta, USA.
23. 'Assessing the burden of malaria in pregnancy in India (Chhattisgarh)' in collaboration with Boston University School of Public Health, funded by ICMR, New Delhi and NIH, Washington, U.S.A.
24. 'Rapid assessment of burden of malaria in pregnancy in Madhya Pradesh, India' in collaboration with CDC, Atlanta, USA, Liverpool School of Medicine, UK, and funded by USAID, New Delhi.
25. 'Assessing the burden of malaria in pregnancy in east India (Jharkhand)' in collaboration with Boston University School of Public Health, and funded by USAID, Washington, U.S.A.
26. 'Monitoring micro-action plan to control *P. falciparum*' in collaboration with NVBDCP, Delhi.
27. 'Developing a framework for predicting malaria outbreaks in rural and urban areas of Gujarat and Rajasthan in India' in collaboration with Michigan University, Princeton University, London School of Hygiene and Tropical Medicine, London, BISAG, Gandhinagar, Govt. of Rajasthan, Govt. of Gujarat, and funded by Michigan University, U.S.A.
28. 'Quality assurance of rapid diagnostic kits for malaria in India' in collaboration with NVBDCP, Delhi and funded by the World Bank.
29. 'Phase II/III clinical trials of ACT to treat uncomplicated *P. falciparum* malaria in pregnancy' in collaboration with London School of Hygiene and Tropical Medicine and funded by MiP Consortium.
30. 'Phase III clinical trial of Arterolane maleate and piperaquine phosphate' in collaboration with Ranbaxy Laboratories Limited, Gurgaon, Tata Main Hospital, Jamshedpur, Ispat General Hospital Rourkela, Wenlock Hospital Mangalore, Community Welfare Society Hospital, Rourkela.
31. 'Identification of malaria risk factors in different ecosystems of Assam, using remote sensing' in collaboration with Defence Research Laboratory, Tezpur, Assam.
32. 'Evidence-based assessment of biophysical determinants of malaria in the north-eastern states of India and development of framework for adaptation measures for malaria control under climate change scenarios in collaboration with the State Governments of Assam, Mizoram and Uttarakhand, IARI, IIT and Ministry of Environment and Forests, New Delhi and funded by ICMR, New Delhi.
33. 'Impact of climate change on dengue in Delhi and environs' in collaboration with Municipal Corporation of Delhi, and funded by the Ministry of Environment and Forests, Govt. of India, New Delhi.
34. 'Operational research on surveillance and intervention strategies in Gadchiroli district of Maharashtra' in collaboration with Govt. of Maharashtra, and funded by NVBDCP, Delhi.
35. 'Developing sensitive, inexpensive and hand-held diagnostic point of care (POC) instrumentation to detect malaria and other pathogens' in collaboration with Genomix Molecular Diagnostics (India) Pvt. Ltd, funded by DBT (SBIRI).

36. 'Proteogenomic Analysis of malaria vectors in India' in collaboration with the Institute of Bioinformatics, Bengaluru.
37. 'Antilarval activities of plant extracts' in collaboration with the Department of Zoology, Panjab University, Chandigarh, Goa College of Pharmacy, National Institute of Oceanography, Goa and Department of Botany, Parwatibai Chowgule College, Margao, Goa.
38. 'Characterization of indigenous strains of Bacilli' in collaboration with the Department of Microbiology, Goa University, Goa.
39. 'Exotic fish and the biological control of malaria in a biodiversity hotspot' in collaboration with the St. Andrew's University, UK, and funded by the Royal Society of London.
40. 'Development of HRP2 and pLDH-based diagnostic kits for the differential diagnosis of malarial parasites' in collaboration with M/s Bhat Biotech (I) Ltd. Bengaluru and funded by the Department of Biotechnology, Govt. of India.
41. 'Differential diagnosis of malaria using micro-PCR device in collaboration with Bigtec Labs., Bengaluru'.

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Human Resource Development

8

8.1 Ph.D. Programme

NIMR provides facilities for pursuing Ph.D. to the students. The Institute is affiliated to: University of Delhi, Delhi; Guru Govind Singh Indraprastha University, Delhi; Rani Durgavati University, Jabalpur; Sambalpur University, Burla; Bangalore University, Bengaluru; Jamia Millia Islamia, New Delhi; Jiwaji University, Gwalior; Goa University, Goa; and M.D. University, Rohtak. About 30 scientists of NIMR are recognised as guides by the different universities. Details of Ph.D. students working at NIMR and its Field Units are furnished in Table 1.

Table 1. Details of students who are pursuing Ph.D. at NIMR during 2009–10

Name	Title of Thesis	University
O.P. Singh	Molecular characterization of different chromosomal forms of *Anopheles fluviatilis*	Jiwaji University, Gwalior
Nandini Korgaonkar	An epidemiological study on risk factors responsible for enhanced receptivity and vulnerability to malaria in Goa (India)	Goa University, Goa
Prashant K. Mallick	Studies on drug resistance	University of Delhi, Delhi
Ajaz A. Bhat	Developing epitope based immunogen using different stages of *Plasmodium vivax* with in-built immuno-adjuvants and delivery in microspheres	AIIMS, New Delhi
Mayank Madhukar	Complement receptor 1 (CR1) and its gene polymorphisms in relation to the pathophysiology and susceptibility to severe malaria	AIIMS, New Delhi
Sanghamitra Verma	Studies on sequence variation and immunogenicity of recombinant fusion proteins of T-helper cell epitopes of circumsporozoite protein of *Plasmodium falciparum* isolates from India: relevance for vaccine development	Jiwaji University, Gwalior
Jai Prakash N. Singh	Studies on genetic polymorphism and immunogenicity of synthetic peptides of T-helper cell epitopic regions of circumsporozoite protein of *Plasmodium falciparum* isolates from India: relevance for vaccine development	Jiwaji University, Gwalior
Suresh Yadav	Study of acute and sub-acute toxicity of some plant extracts against malaria vector *Anopheles stephensi*	Dr B.R. Ambedkar University, Agra
A.K. Upadhyay	Studies on the mosquito fauna and bioecology of malaria vectors in the malaria endemic tribal area of northern Orissa	Jiwaji University, Gwalior
Gaurav Verma	Antimalarial properties of some plants from Garhwal region of north-west Himalaya	Jiwaji University, Gwalior
Prerna Sethi	Determination of some new antimalarials by using high performance liquid chromatography and their application to malaria cases	Jiwaji University, Gwalior

Table 1. *contd...*

Name	Title of Thesis	University
Mahesh B. Kaliwal	Bioecology of *Culex quinquefasciatus*, the principal vector of *Lymphatic filariasis* in Goa	Goa University, Goa
Deeparani Prabhu	Studies on mode of action and bioefficacy of fungi pathogenic to larvae of *Anopheles stephensi* (Liston), *Culex quinquefasciatus* (Say) and *Aedes aegypti* (Linnaeus)	Goa University, Goa
Ratanesh K. Seth	Isolation and characterization of monoclonal antibodies against erythrocytic stages of Indian *Plasmodium vivax* isolates	Jiwaji University, Gwalior
A.S. Pradeep	Development of more specific and sensitive Histidine rich protein 2 (HRP2) based diagnostic system for *Plasmodium falciparum* malaria	Jiwaji University, Gwalior
Gauri Awasthi	Genetic diversity of the 7th chromosomal genes in Indian *Plasmodium falciparum*	Jiwaji University, Gwalior
Jyotsana Dixit	Population genetic studies of malaria vector *Anopheles minimus* in north-eastern parts of India using bioinformatic and evolutionary approaches	Jiwaji University, Gwalior
Hemlata Srivastava	The effect of natural selection on immune response genes of *Anopheles minimus* species	Jiwaji University, Gwalior
Bhavna Gupta	Population genetic studies of Indian *Plasmodium vivax*	Jiwaji University, Gwalior
Anita C.	Population genetic and evolutionary studies of duffy gene in Indian humans	Jiwaji University, Gwalior
Sonam Vijay	Characterisation of nitric oxide synthase (NOS) in *Anopheles culicifacies* : implication for an innate immune mechanism of refractoriness	Jiwaji University, Gwalior
Manmeet Rawat	Molecular characterisation of aspartic protease gene from *Plasmodium vivax*	Jiwaji University, Gwalior
B. Prasad Rao	Biochemical and molecular characterization of insecticidal resistance in *Anopheles culicifacies*	Jiwaji University, Gwalior
Vaishali Verma	Studies on insecticide resistance and its management: biochemical and molecular approaches for characterisation	Jiwaji University, Gwalior
B.P. Niranjan Reddy	Characterisation of insecticide resistance mechanisms in Indian malaria vectors	Jiwaji University, Gwalior
Ajeet Kumar Mohanty	Midgut proteome analysis of *Anopheles stephensi* Liston a vector for human malaria in India	Goa University, Goa
Sompal Singh	Low dose radiation induced molecular changes in human blood cells	CCS University, Meerut
Bijayalaxmi Sahu	Molecular epidemiology of drug resistance in *Plasmodium falciparum* in Orissa, India	Jiwaji University, Gwalior
Hardev Parasher	Comparative study of enzyme phenoloxidase in the members of *Anopheles culicifacies* complex upon *Plasmodium* infection	MD University, Rohtak
Prerna Bali	Toll like receptor (TLR) gene polymorphism in relation to malaria in Indian isolates	University of Delhi, Delhi
Sandeep Kumar	Organochlorine residues in soil, water, whole blood and major local food products from Assam	Jiwaji University, Gwalior
Ripu Daman Sood	Comparative efficacy of different long-lasting insecticidal nets against malaria vectors, *An. stephensi* and *An. culicifacies* in India	Indira Gandhi National Open University, New Delhi
L. Dolie Devi	Molecular characterization of symptomatic and asymptomatic *P. falciparum* malaria in India	Panjab University, Chandigarh

Table 1. ***contd...***

Name	Title of Thesis	University
Sneh Shalini	Molecular characterization of Plasmepsin IV falcilysin and heme detoxification protein (HDP) gene in *Plasmodium vivax* and their comparative analysis with primate's malaria paraites	Jiwaji University, Gwalior
Devender Dhayal	Immune alteration by *Plasmodium vinckei* in *Anopheles stephensi*	MD University, Rohtak
Divya Sharma	To map the current status of chloroquine and sulphadoxine-pyrimethamine drug resistance in India with respect to *Plasmodium falciparum* and *Plasmodium vivax*	MD University, Rohtak
Manila Lather	To study the proteomics of insecticide resistant and susceptible in *Anopheles culicifacies*	MD University, Rohtak
Naazneen Khan	Single nucleotide polymorphisms (SNPs) and evolutionary history of a drug metabolizing gene (NAT2) in Indian humans	Banasthali Vidhyapeeth, Rajasthan
Kshipra Chauhan	Evolutionary history of *Pfcrt* gene in Indian *P. falciparum*	Kumaon University, Nainital
Deepa Jha	Proteome analysis during blood stage of *Plasmodium falciparum* under treatment with free and liposomal antimalarial drugs	University of Delhi, Delhi

8.2 Ph.D. Awardees

During the year the following candidates were awarded Ph.D. degrees.

1. Surendra Kumar Prajapati was awarded Ph.D. degree from Jamia Millia Islamia, New Delhi on "Molecular studies on house-keeping genes of *Plasmodium vivax*" in October 2009.
2. Swapnil Rai was awarded Ph.D. degree from Gurukul Kangri Viswa Vidhyalaya, Hardwar on "Profile of persistent organochlorine insecticide residues in sub-Himalayan region of north-west Himalaya" in 2009.
3. Praveen Kumar Bharti was awarded Ph.D. degree from Rani Durgavati University, Jabalpur on "Study of nature and extent of polymorphism in vaccine candidate antigen (MSP-1, MSP-2 and MSP-3) and drug resistance gene (*Pfcrt*) of *Plasmodium falciparum* in central India" in 2009.
4. Mohammad Sohail was awarded Ph.D. from Vinoba Bhave University, Hazaribagh on "Study on immuno-modulatory & inflammatory cytokine and cytokine gene polymorphism in malaria in Indian isolates" in 2009.

8.3 M.Sc. Projects

This year, more than 20 students of M.Sc. in Life Sciences/Biotechnology/Bioinformatics successfully completed their projects/dissertations under the supervision of NIMR scientists.

8.4 Trainings Imparted

NIMR conducts regular training programmes which are as under:

- Collection, cryopreservation, revival and transportation of malaria parasite isolates/ strains.
- *In vitro* cultivation of erythrocytic stages of *P. falciparum*.
- *In vitro* testing for sensitivity of *P. falciparum* isolates to antimalarials.
- *In vitro* screening of medicinal plants for antiplasmodial properties.
- Microscopic diagnosis of malaria parasites and cytological identification of sibling species of mosquitoes.
- Field oriented training on mosquito collection, preservation, dissection, etc.
- NIMR has conducted series of training programmes for microscopists, district malaria officers, entomologists, technicians on various aspects of malaria.

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9 Research Papers Published

(January to December 2009)

1. Alfredo M, Rovira-Vallbona E, Srivastava A, Sharma SK, Pati SS, Puyol L, Quinto L, Bassat Q, Machevo S, Mandomando I, Chauhan VS, Alonso PL, Chitnis CE. Functional and immunological characterization of a duffy binding-like alpha domain from *Plasmodium falciparum*-erythrocyte membrane protein-1 that mediates resetting. *Infect Immun* 2009; *77:* 3857–63.
2. Anand PP, Okiro EA, Gething PW, Guerra CA, Sharma SK, Snow RW, Hay SI. Defining the relationship between *Plasmodium falciparum* parasite rate and clinical disease: statistical models for disease burden estimation. *Malar J* 2009; *8:*186.
3. Anvikar AR, Rao VG, Savargaonkar DD, *et al*. Seroprevalence of sexually transmitted viruses among tribal population of central India. *Int J Infect Dis* 2009; *13*(1): 37–9.
4. Awasthi G, Dash AP, Das A. Characterization and evolutionary analysis of human CD36 gene. *Indian J Med Res* 2009; *129:* 534–41.
5. Awasthi G, Dash AP, Das A. Evolutionary insights into duffy gene in mammalian taxa with comparative genetic analysis. *J Vector Borne Dis* 2009; *46:* 230–6.
6. Bhat AA, Seth RK, Babu J, Biswas S, Rao DN. Induction of mucosal and systemic humoral immune responses in murine system by intra-nasal immunization with peptide antigens of *P. vivax* and CpG oligodeoxynucleotide (ODN) in microparticle delivery. *Int Immuno-pharmacol* 2009; *9:* 1197–208.
7. Das A, Sharma M, Gupta B, Dash AP. *Plasmodium falciparum* and *P. vivax*: so similar yet very different. *Parasitol Res* 2009; *105:* 1169–71.
8. Diamond-Smith N, Singh N, Gupta RD, Dash A, Thimasarn K, Campbell OM, Chandramohan D. Estimating the burden of malaria in pregnancy: a case study from rural Madhya Pradesh, India. *Malar J* 2009; *8*(1): 24.
9. Dua VK, Pandey AC, Raghavendra K, Gupta A, Sharma T, Dash AP. Larvicidal activity of neem oil (*Azadirachta indica*) formulation against mosquitoes. *Malar J* 2009; *8:* 214.
10. Dua VK, Verma G, Dash AP. *In vitro* anti-protozoal activity of some xanthones isolated from the roots of *Andrographis paniculata*. *Phytother Res* 2009; *23:* 126–8.
11. Gandhi M, Singh A, Dev V, Adak T, Dash AP, Joshi H. Role of CR1 Knops polymorphism in the pathophysiology of malaria: Indian scenario. *J Vector Borne Dis* 2009; *46*(4): 288–94.
12. Garg A, Dhiman RC, Bhattacharya S, Shukla RP. Development, malaria and adaptation to climate change: a case study from India. *Environ Manag Res* 2009; *43:* 779–89. DOI10.1007 s00267–008 9242-z.
13. Ghosh SK, Dash AP. Larvivorous fish against malaria vector. *Trans R Soc Trop Med Hyg* 2009; *103:* 210–1.
14. Hamer DH, Singh MP, Wylie BJ, Yeboah-Antwi K, Tuchman J, Desai M, Udhayakumar V, Gupta P, Brooks MI, Shukla MM, Awasthy K, Sabin L, MacLeod WB, Dash AP, Singh N. Burden of malaria in pregnancy in Jharkhand state, India. *Malar J* 2009; *8:* 210.
15. Jain V, McClintock S, Nagpal AC, Dash AP, Stiles JK, Udhayakumar V, Singh N, Lucchi NW. Macrophage migration inhibitory factor is associated with mortality in cerebral malaria patients in India. *BMC Res Notes* 2009; *2:* 36.
16. Kar PK, Bhatt RM, Anvikar A, Dua VK. Malaria–change in therapeutic policy. *Indian J Med Res* 2009; *129*(2): 203–4.
17. Kar PK, Das A, Nagpal BN. Raghavendra, K. Ghosh SK, Dua VK, Dash AP. Molecular characterization of chikungunya virus from

Andhra Pradesh, India: possible avian virus linkages. *Indian J Med Res* 2009; *129:* 230–2.
18. Kar PK, Dua VK, Gupta NC, Gupta A, Dash AP. *Plasmodium falciparum* gametocytaemia with chloroquine chemotherapy in relation to persistent malaria in an endemic area of India. *Indian J Med Res* 2009; *129*: 299–304.
19. Kar PK, Nagpal BN, Dua VK, Ghosh SK, Raghavendra K, Bhatt RM, Anvikar A, Das A. Molecular characterization of chikungunya virus from Andhra Pradesh, India. *Indian J Med Res* 2009; *129*(3): 335–7.
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22. Lumb V, Das MK, Singh N, Dev V, Wajihullah, Sharma YD. Characteristics of genetic hitchhiking around dihydrofolate reductase gene associated with pyrimethamine resistance in *Plasmodium falciparum* isolates from India. *Antimicrob Agents Chemother* 2009; *53*(12): 5173–80. Epub 2009 Sep 28.
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24. Raghavendra K, Cornel AJ, Reddy BPN, Collins FH, Nanda N, Chandra D, Verma V, Dash AP, Subbarao SK. Multiplex PCR assay and phylogenetic analysis of sequences derived from D2 domain of 28S rDNA distinguished members of the *Anopheles culicifacies* complex into two groups, A/D and B/C/E. *Infect Genet Evol* 2009; *9:* 271–7.
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27. Rani A, Sharma A, Rajagopal R, Adak T, Bhatnagar RK. Bacterial diversity analysis of larvae and adult midgut microflora using culture-dependent and culture-independent methods in lab-reared and field-collected *Anopheles stephensi*—an Asian malarial vector. *BMC Microbiol* 2009; *9*: 96.
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Other Activities 10

10.1 Health camp organized

A health camp was organized at the Kendriya Vidhyalaya in February 2010 for students. Demonstration of various live stages of mosquitoes like eggs, larvae, pupae and adults and displaying of charts showing life cycle of malaria parasites (*P. falciparum and P. vivax)* was carried out along with the discussion on the various aspects of vector borne diseases (VBD). General awareness regarding prevention and control of vector-borne diseases was created among students and faculty.

The causative agents of malaria, viz. *P. falciparum* and *P. vivax* were demonstrated to children using microscope. Video film on health education programme "*Agyan Mitao Mukti* Pao" was shown. The students were also involved in discussion during the question sessions.

10.2 Exhibitions & Photography

10.2.1 Exhibition at X International Symposium on Vectors and Vector Borne Diseases, Goa, 4–6 November 2009

An exhibition consisting of 14 panels depicting malaria and its control was displayed at the symposium site for two days. Books and brochures were also distributed.

10.2.2 Distribution of Video CDs

Video CDs on malaria, mosquitoes, bed nets and related subjects produced at NIMR were distributed to the participants of different training programmes organized by the NIMR, NVBDCP and NCDC. The CDs were also sent to the states and given to the interested visitors.

10.2.3 Photography

In the Photography section, following photography work was carried out on occasion of various meetings/workshops/functions and field work activities etc. held at NIMR or other places.

Launching World Bank, Project release of treatment guidelines (booklet) at India International Centre, New Delhi; Training course for Gujarat state, Senior Officer's on prevention and control of vector-borne diseases; Consultation of malaria elimination at Taj Hotel, New Delhi; Photography of NIMR Building for DDA; Induction training programme for District VBD consultants organized by the Public Health Foundation of India in collaboration with NIMR and NVBDCP; Entomology training; ICMR Awards presentation ceremony at Vigyan Bhawan, New Delhi; Hindi Week Prize distribution; Training of Armed Forces Medical College, Pune, students; Photography of NIMR Laboratories for Annual Report; World Bank meeting held at Sham Nath Marg, Delhi. Photographs were also taken at various stadium sites of Common Wealth Games at New Delhi. These images were taken to highlight the mosquito breeding sites in these areas and to suggest methods to control breeding.

10.3 Documentation work

In Documentation Cell, the following tasks were carried out during the period under report :

- Contents of *Journal of Vector Borne Diseases* (formerly *Indian Journal of Malariology*) from the year 1981–2009 were compiled.
- List of the published research papers by NIMR scientists for the year 2009 was prepared and updated.
- Reprints of published research papers by NIMR scientists for the year 2009 were collected.
- Complete list of the Projects undertaken by NIMR from the year 1977 to 2009 was prepared and updated.

- Information on aims and objectives of major research activities of NIMR was compiled.

10.4 Publications

The Publication Division of the NIMR has been bringing out its scientific quarterly journal *Journal of Vector Borne Diseases (JVBD)* regularly during this year 2009–10. The JVBD also included in Thomson ISI indexing the abstracting agencies, which award Impact factor to scientific journals. The articles published in JVBD are being made available full text through PubMed and DOAJ. The number of citations of the articles published in the journal has been increasing day-by-day. During the year 2009, all the articles published in the journal (from 2003) have been uploaded in PubMed and DOAJ for providing open access to the full text articles.

The Division is also bringing out a popular Hindi Magazine *Malaria Patrika*, a Hindi quarterly for educating the community on malaria, and the *Plasmodium*, a biannual newsletter of the Institute in both English and Hindi languages. Besides producing NIMR and IDVC Annual Reports (2008-09) in the year 2009 the Division had also initiated the process of publishing four booklets and one folder namely, Expanded polystyrene beads for mosquito control, Larviorous fish for mosquito control, Insecticide-treated nets, long-lasting nets and material for malaria control, *An. culicifacies* and *An. fluviatilis* complexes and their control, and Biolarvicides for mosquito control.

10.5 Workshops/Training courses organized

1. Organized 'X International symposium on vectors and vector borne diseases' in collaboration with Goa University, Goa held at Goa from 4–6 November 2009.
2. Conducted a series of training programmes/ workshops at NIMR, New Delhi for DMOs/ Induction VBD Consultants/Entomologists/ Laboratory Technicians from Delhi, Gujarat and other States.

10.6 Awards/honours received

1. Dr VK Dua received Godrej Sara Lee Ltd. Award for distinguished contributions in the field of research on personal protection from mosquito vectors in India at 'X International symposium on vectors and vector borne diseases' held at Goa from 4–6 November, 2009.
2. Dr RC Dhiman was Elected as co- leader of GEO Community of Practice "Health and Environment" by Global Earth Observation, Geneva.
3. Dr RC Dhiman was elected as member (Academic and research constituency) of Country Coordinating Mechanism (CCM-India) for Global Funds and participating in the meetings held at Ministry of Health & Family Welfare, Govt of India.
4. Dr Ruchi Singh was awarded with Shakuntala Amirchand Prize by ICMR for best published work in biomedical sciences.

10.7 Conferences/workshops/ meetings attended/lectures delivered/trainings imparted

Anvikar Anup

1. Participated in Consultation on Malaria Elimination from 25–27 June 2009 at New Delhi.
2. Participated in the meeting for the preparation of Guidelines for Diagnosis and Treatment of Malaria in India, NIMR, 2009.
3. Attended X International Symposium on Vectors and Vector Borne Diseases and delivered a talk on '*In vitro* sensitivity" from 4–6 November 2009 at Goa.
4. Delivered lectures on "Rapid diagnostic tests for malaria" at Training Course for MOs, ADHOs, CDHOs, and Regional Deputy Directors of Gujarat at NIMR on various occasions.

Biswas Sukla

1. Guidance provided to M.Sc. Microbiology; Dissertation submitted on "Comparative profile of immune response to *Plasmodium vivax* antigens in a population of northern India (Delhi)" by Ms. Vaibhavi Joshi to the Department of Microbiology, Barkatullah University, Bhopal, Madhya Pradesh.
2. Guidance provided to M.Sc. Biotechnology; Dissertation submitted on "Naturally occurring antibody response to *Plasmodium falciparum* merozoite surface protein-1 in two subpopulations of Orissa" by Ms. Urvashi to the School of Life Sciences, Jaipur National

University, Jaipur, Rajasthan.

3. Guidance provided to M.Sc. Biotechnology; Dissertation submitted on "Polymorphism in *Plasmodium falciparum* gene encoding *Pfmsp-1, Pfmsp-2, Pfcrt* and *Pfmdr-1* in a population of parasites from eastern and north-eastern India" by M. Ashok Kumar to the Department of Biotechnology, Bharathidasan University, Tiruchirappalli, Tamil Nadu.
4. Training provided to Ms. Aayushi Garg, student of B.Sc. Instrumentation (Hons.) III year, Shaheed Rajguru College of Applied Sciences for Women, Delhi University; during her training she has been acquainted with various instruments and their use in bio-medical research.
5. Training provided to Hemant Sahal, student of B.Tech (Biotechnology), III year, VIT University, Vellore, Tamil Nadu; during his training he has learnt microscopic diagnosis of malaria parasites, PCR assay, Gel electrophoresis and ELISA technique for detection of antimalarial antibodies in patients' blood.
6. Training provided to Ms. Akanksha Panwar, student of B.Sc (Biotechnology), III year, IET Biotechnology Institute, Alwar, Rajasthan; during her training she has learnt Microscopic diagnosis of malaria parasites, PCR assay, Gel electrophoresis and ELISA technique for detection of antimalarial antibodies in patients' blood.

Dua VK

1. Dr VK Dua, Dr NC Gupta and Miss Prerna Sethi attended a training on LC/MS organized by Waters India Ltd. at Bengaluru from 15–17 June 2009.
2. Dr VK Dua, Dr T Sharma, Ms. Prerna Sethi, Mr. Gaurav Verma, and Mr. Sandeep Kumar attended the X International Symposium on Vectors and Vector Borne Diseases held at Goa from 4–6 November 2009.
3. Dr VK Dua, Ms. Prerna Sethi, Mr Gaurav Verma and Mr Sandeep Kumar attended the IV Uttarakhand State Science Congress held at Pantnagar from 10–12 November 2009.
4. Two week training on "Molecular biology techniques" given to Mr. Mzohd Ashfaque, Ph.D. student (Microbiology), AMU Aligarh in March 2010.
5. Attended meeting with DG, ICMR for land acquisition in Medical college campus, Guwahati for NIMR Field Unit on 3 September 2009.
6. Attended review workshop on Operational research projects in malaria at AIIMS, New Delhi on 14 September 2009.
7. Attended the Fogarty annual network meeting and annual meeting of the American Society of Tropical Medicine & Hygiene at Washington DC from 16–22 November 2009.
8. Attended a meeting in ICMR HQ with DG, ICMR on creating Data Repository of data generated in ICMR on 8 December 2009.
9. Attended a meeting on SPO's in World Bank Programme at NVBDCP, Delhi on 22 December 2009.
10. Attended a meeting to review the operational research with DG, ICMR in ICMR HQ on 6 January 2010.
11. Attended Estimation of malaria burden meeting at NVBDCP, Delhi on 3 February 2010.
12. Attended SAG meeting of ICMR at ICMR HQ from 19–20 February 2010 as a member of selection committee for selection of suitable candidates for the award of PDF of ICMR at ICMR HQ on 26 March 2010.

Dhiman RC

1. Served as WHO temporary Advisor at SEARO-WHO, New Delhi from 18–21 August 2010 regarding technical discussion on protecting human health from climate change prior to 62 session of WHO Regional Committee for Southeast Asia, SEARO and contributed a working paper on "Ongoing research on climate change impacts on health in south-east Asia (Working paper No. 4).
2. Worked for SEARO-WHO under APW for Development of Generic Protocols for: (i) Prospective study on the impact of climate change on vector-borne diseases; (ii) Retrospective study on the correlation of climatic/meteorological change and vector-borne disease patterns; and (iii) Assessing the national/local preparedness to respond to the impact of climate change on health, specifically vector-borne diseases.
3. Participated as an expert for Consultation meeting of SEARO-WHO on Climate change and health for discussing generic protocols at

Kolkata from 24–26 August 2009.
4. Participated in Consultation on malaria elimination at Delhi from 25–27 June 2009.
5. Participated as invited delegate in Indo-US workshop on climate change and health held at Goa from 30 August to 2 September 2009 and contributed ongoing activities of NIMR.
6. Participated on behalf of Ministry of Health & Family Welfare, Govt. of India, in a national workshop on Climate change, organized by Ministry of Environment & Forests, at Delhi on 14 October 2009.
7. Attended X International Symposium on vectors and vector borne diseases held at Goa from 4–6 November 2009 and delivered a lecture.
8. Delivered a lecture on Climate change and vector borne diseases at the National symposium on weather, climate and sustainable development, organized by Indian Meteorology Department, New Delhi on 17 December 2009.
9. Participated in CCM (Global Fund) meetings held at New Delhi on 25 May 2009, 23–24 July 2009 and 27 January 2010.
10. Participated in a workshop conducted by CCM-India from 18–19 February 2010.
11. Participated as an expert of European Union in Global Earth Observation Satellite System workshop for discussion on Development of environmental information system for vector borne diseases at Geneva (Switzerland) from 7–9 July 2009.
12. Participated as an invited expert for DFID workshop on 'Why does the health sector need to engage in protecting human health from climate change? at Hyderabad from 17–18 September 2009.
13. Chaired a scientific session on GEOSS sponsored "Ecosystems, climate and health – policy, science, and applications" and participation in break out session on Vector borne disease—malaria at Washington (USA) from 12–13 November 2009.
14. Participated in Institutes' training programme for District VBD Consultants, MO/CDHO/ADHO and Regional Deputy Directors from Gujarat on various occasions.

Malhotra MS

Attended the Symposium on 'Vectors and vector borne diseases' held at Goa and presented a paper on Baseline household survey in Jharkhand from 4-6 November 2010.

Mittal PK

Attended the Inaugural function of the V International Conference on Biopesticides organised by the Society for Innovation and Promotion of Biopesticides and TERI at India Habitat Centre, New Delhi on 26 April 2009.

Nagpal BN

1. Attended "Anopheline biodiversity shift in current scenario". National seminar on climate change and vector borne diseases organized by the Asiatic Society, Kolkata and NAVBD on 6 February 2010.
2. Attended a workshop on Mosquitoes and transmitted diseases at Ranchi from 14–16 December 2009.
3. Attended workshop on Health impact assessments of Indira Sagar Dam at Ranchi from 14–16 December 2009.
4. Attended workshop on Bioecology of vectors of malaria, dengue and chikungunya and control, medicine update 2009 at Bareilly from 23–27 November 2009.
5. Attended X International symposium on vector borne diseases at Goa from 4–6 November 2009 and presented a paper on Health impact assessment of Indira Sagar Dam, Madhya Pradesh: a case study.
6. Attended workshop on Mosquito vectors in India at Gwalior on 21 July 2009.
7. Attended meeting on Health impact assessment of Indira Sagar Dam at Bhopal on 4 April 2009.
8. Attended a meeting on Health impact assessments of Indira Sagar Dam and RR Colonies in Narmada Valley, in Madhya Pradesh at Jabalpur on 27 February 2009.
9. Attended and presented lecture in a stake holders meeting organized by NVBDCP at Bhopal on 4 April 2009.
10. Delivered series of lectures at "Training course for Dy. Health Officers and Epidemiologists of MCD on Prevention and control of vector borne diseases" organized by NIMR and MCD from 20–25 April 2009.
11. Attended orientation meeting and launching ceremony of the World Bank funded study "Monitoring the therapeutic efficacy of antimalaria medicines in India" at India

International Centre, New Delhi on 28 April 2009.

12. Attended review meeting regarding "Induction training programme for three months for district vector borne disease consultations" at NVBDCP on 8 May 2009.
13. Attended Inter-sectoral coordination committee meeting for Prevention and control of vector borne diseases under the chairmanship of Commissioner MCD at Ambedkar Stadium, New Delhi on 6 May 2009.
14. Attended meeting for developing session plan towards Induction training programme for district vector borne disease consultation at NVBDCP on 18 May 2009.
15. Attended meeting of the committee to review action plan and activities undertaken for Prevention and control of dengue under the chairmanship of Dr Shiv Lal, Spl. DGHS (PH) organized by NVBDCP at Nirman Bhawan, New Delhi on 19 May 2009.
16. Attended meeting to review the implementation of NVBDCP (malaria, dengue and chikungunya) and water borne diseases control activities in Delhi under the chairmanship of Prof. Kiran Walia, Minister of Health, Woman & Child Development organized by the Directorate of Health Services at Delhi Secretariat on 19 May 2009.
17. Delivered series of lectures at Training courses for DMOs, Biologists, Entomologists and Sr. Officers of Gujarat on Prevention and control of vector borne diseases organized by NIMR and the Commissionerate of Health Medical Services & Medical Education, Gujarat from 1–12 June, 15–26 June and 29 June to 10 July 2009.
18. "Finding out of different breeding places in Schools/colleges" presented at Role of Principals/Nodal Officers of School in NDMC area for containment of dengue/chikungunya/malaria organized by NDMC on 17 July 2009.
19. Delivered series of lectures at Training courses for MOs, ADHOs, CDHOs, and Regional Deputy Directors of Gujarat on Prevention and control of vector borne diseases organized by NIMR and the Commissionerate of Health Medical Services & Medical Education, Gujarat from 20 to 25 July, 27 July to 1 August and 10–12 August 2009.
20. "Remarks" at Inauguration of collaborative induction training of district VBD consultants at Indian Institute of Public Health, Gurgaon on 8 September 2009.
21. Delivered series of lectures at "Induction training programme for district VBD consultants" organized by Public Health Foundation of India (PHFI), National Institute of Malaria Research (NIMR), National Center for Disease Control (NCDC) and National Vector Borne Disease Control Programme (NVBDCP) from 15–24 September and 16–23 October 2009.
22. "Bioecology of vectors of malaria, dengue and chikungunya and control" presented at Bareilley from 3–4 October 2009.
23. "Mosquitoes & transmitted diseases" presented at Orientation workshop on Geographical Information System (GIS) for management and control of vector borne diseases for Sr. Officers of State Health Department, Ranchi, Jharkhand organized by NIMR, Delhi on 12 December 2009.
24. "ERDAS- India user conference" participated at Epicentre, Gurgaon on 18 January 2010.
25. "Anopheline biodiversity shift in current scenario" presented at National seminar on climate change and vector-borne diseases organized by the Asiatic Society in collaboration with Post-graduate Department of Zoology, Asutosh College, Kolkata and National Academy of Vector Borne Diseases (NAVBD) on 6 February 2010.
26. "New Trend regarding the breeding of *Aedes* mosquito" at training of Sanitary Inspectors, Surveillance Workers and Anti Malaria Jamadars organized by Health Department, NDMC at Chanakyapuri, New Delhi on 20 February 2010.
27. Attended meeting of the Committee to review action plan and activities undertaken for prevention and control of dengue under the chairmanship of Dr Shiv Lal, Spl. DGHS (PH) organized by NVBDCP at Nirman Bhawan, New Delhi on 22 February 2010.
33. "Vector surveillance specially in reference to games village and CWG sports sites" at Workshop on vector borne diseases and water quality surveillance in respect to CWG-2010 organized by MCD at India Habitat Center, New Delhi on 20 March 2010.

Nanda Nutan

1. Delivered lecture on "Phylogenetic analysis of Indian malaria vectors based on D3 domain of 28S ribosomal DNA" in 'X International Symposium on Vectors and Vector Borne Diseases' held at Goa from 4–7 November 2009.
2. Delivered lecture on "Genetic characterization of *Plasmodium falciparum* field isolates from malaria endemic Sundargarh district of Orissa", in 'X International symposium on vectors and vector borne diseases' held at Goa from 4–6 November 2009.
3. Participated as faculty member and chief resource person in the training course for Dy. Health Officers and Epidemiologists of MCD for management of vector borne disease control programme organized by NIMR from 20-25 April 2009. Delivered lectures on life cycle and morphology of human malaria parasites.
4. As one of the experts to develop course curriculum and comprehensive training modules for district VBD consultants appointed by NVBDCP under World Bank project, attended meetings with officials/ scientists of Directorate of NVBDCP, NICD and Public Health Foundation of India (PHFI) during 2009 to develop and finalize the training module for entomological section of the training course.
5. As one of the chief course coordinators organized entomology training of District VBD consultants at NIMR from 15–25 September 2009. Delivered lectures, conducted practicals and entomological exercises pertaining to vector biology and bionomics.
6. Imparted training to Mr. Pallab Kumar Rana, Senior Research Fellow and Mr. Narayani Prasad Kar, Project Fellow on various aspects of malaria entomology and the techniques used for differentiation of the members of Fluviatilis/ Minimus complexes, to study their host feeding behaviour and vectorial potential in September-October 2009.

Saxena Rekha

1. Delivered two lectures on 'Introduction to GIS' and 'Introduction to GIS tools and GPS-based field surveys and field data incorporation into GIS system' during workshop on 'GIS for management and control of vector borne diseases' organized at Ranchi, Jharkhand from 15–16 December 2009.
2. Delivered a lecture on 'Basics of Geographical Information System' to Dy. Health Officers and Epidemiologists of MCD on prevention & control of vector borne diseases from 20–25 April 2009.
3. Delivered series of lectures on 'Basics of Geographical Information System & Global Positioning System (GPS)' to four batches of DMOs, Biologists, Entomologists and Sr. Officers of Gujarat from I–12 June, 15–26 June, 29 June–10 July 2009, and 20–25 July 2009.
4. Imparted training on 'Application of GIS and RS in management and control of VBDs' to a B.Tech. (IT) student of Lingaya Institute of Management & Technology, Faridabad from 10 June to 28 July 2009.
5. Imparted training on 'Geographical information system & its tools' to MPH scholars from NICD on 30 April 2009. Practical training on GIS software was provided on 1 May 2009. Handouts were prepared and distributed.

Singh Ruchi

1. A Ph.D. student Ms. Olasetinde Grace from Covenant University, Ota, Ogun State, Nigeria was guided to carry out project work on "*In vitro* and molecular studies on drug resistant *Plasmodium falciparum*" under TWOWS-TWAS postgraduate fellowship.

Valecha Neena

1. Lecture on "Chemotherapy of malaria with special reference to drug resistance and its management" at Training course for Dy. Health Officers of MCD from 20–25 April 2009.
2. Lecture on "Treatment of malaria" at Training course for MOs, ADHOs, CDHOs, and Regional Deputy Directors of Gujarat at NIMR from 27 July to 1 August 2009.
3. Guest lecture on "Research on drugs and vaccines by NIMR" at WHO-SEARO, New Delhi from 23–24 April 2009.
4. Lecture on "Best practices in Pharmacovigilance" at AIIMS, New Delhi from 24–25 April 2009.
5. Guest lecture on "New modalities of diagnosis

and treatment: a step forward" at Consultation on Malaria Elimination at New Delhi on 25–27 June 2009.

6. Invited Lecture on "Drug resistance in malaria in India" at Global anti resistance network meeting held at Habitat Centre on 24 August 2009.
7. Key note address on "Artemisinin-based combination therapy: Indian perspective at third Joint WHO/MMV Artemisinin conference held at Mumbai, India from 28–30 September 2009.
8. Invited lecture on "Chemotherapy of malaria" for VBD consultants at NVBDCP on 14 October 2009 .
9. Invited lecture on "Challenges in malaria diagnosis" in Expert consultation on disease control in India: Strategic pathways for the control of measles, malaria, and chronic diseases at New Delhi on 27 October 2009.
10. Invited lecture on "Malaria treatment in India: Journey from Chloroquine to Artemisinin" in X International symposium on vectors and vector borne diseases held at Goa, India on 4–6 November 2009.
11. Invited lecture on "Pharmacovigilance and public health programmes: Antimalarials" in "National Pharmacovigilance Programme (NPVP): The way ahead" at AIIMS, New Delhi from 29–30 December 2009.
12. Invited lecture on "Infectious diseases: Novel strategies for design and development of vaccines & drugs in Malaria Treatment in India: Past, present and the future" at TIFR, Mumbai from 5–8 January 2010.
13. Invited lecture on "Clinical management of severe malaria cases" in Malaria treatment: Past, present and future at NIHFW, Munirka, Delhi from 19–20 March 2010.
14. Invited lecture on "Pharmacovigilance—A primer in Pharmacovigilance of the special drug groups" at CME at Rohtak, Medical College, Haryana on 20 March 2010.

By Ph.D. students

1. Mrs L. Dolie Devi attended the X International symposium on vectors and vector borne diseases held at Goa from 4–6 November 2009 and presented a poster entitled "Genetic characterization of *Plasmodium falciparum* field isolates from malaria endemic Sundargarh district of Orissa".
2. Mrs Sneh Shalini presented a paper "Comparative evolutionary analysis of food vacuole drug candidate genes in *Plasmodium* species" in the "X International symposium of vector

A TRIBUTE TO DR HEMA JOSHI

Staff of NIMR, New Delhi expresses their deep condolence on the untimely and sad demise of Dr Hema Joshi, an eminent scientist of the Institute who passed away on 23 March 2010 in New Delhi at the age of 60 years.

Dr Joshi had obtained Ph.D. degree from the Indian Institute of Technology, New Delhi in Biochemical Genetics (1979) and joined National Institute of Malaria Research on 25 March 1982 as Assistant Research Officer and served in various capacities for about 28 years. She was Scientist 'E' when she passed away. Apart from having a bright student career, Dr Joshi had an illustrated scientific career exemplified by establishing and contributing to the field of vector bionomics, human and malaria parasite population genetics, and molecular biology. She guided several Ph.D. students. She got two international projects funded by World Health Organization (WHO, Geneva) and National Institute of Health (NIH, US). She had received several national and international awards and published more than 55 research papers in peer reviewed journals. She was also a visiting scientist at Oxford University (2003), where she worked on microsatellite markers of *Plasmodium falciparum.*

With the passing away of Dr Joshi, India has lost an eminent scientist on malaria research. Her services to the Institute shall always be remembered and may the Almighty grant peace to her departed soul.

borne disease 2009" which was held at Goa from 4–6 November 2009.

3. Mrs Sneh Shalini attended a workshop on "EuPathDB" database at University of Georgia from 7–10 June 2009.
4. Mrs Sneh Shalini attended a conference on "Vivax malaria beyond 2009" organised by University of South Florida at Panama Central America from 24–28 June 2009.
5. Mrs Sneh Shalini attended US-India—Joint training workshop on "Bioinformatics of Intracellular Pathogens" supported by Fogarty International Centre, USA from 7 –9 February 2009 in School of Life Sciences at Jawaharlal Nehru University, New Delhi.
6. Surendra K Prajapati presented a paper in the "Sequence variations in the receptor-binding domain of *Plasmodium vivax* Duffy Binding protein-II: implications for a malaria vaccine development X International symposium on vectors and vector borne diseases held at Goa, India from 4–6 November 2009.

10.8 Trainings received

1. Dr K. Raghavendra received training on Laboratory procedure for testing insecticides at World Health Organization (WHO CC), LIN, Montpellier, France from 2–13 March 2009.
2. Mrs Sneh Shalini received Four month training in the Department of Medical Parasitology Student at New York University under exchange programme of NIMR and NYU collaborative project "Promotion of *Plasmodium* research and training in India" from 17 February to 15 June 2009.

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संस्थान में राजभाषा विकास संबंधी गतिविधियाँ

11

संस्थान में राजभाषा अधिनियम के अनुपालन की दिशा में वर्ष 2009-10 में भी राजभाषा हिन्दी के प्रसार एवं विकास और सरकारी काम-काज में हिन्दी के प्रगामी प्रयोग के क्षेत्र में प्रगति के उद्देश्य से राजभाषा अधिनियम 1963 की धारा 3(3) के अंतर्गत आने वाले दस्तावेजों और संस्थान में प्रयुक्त प्रपत्रों का अनुवाद संबंधी कार्य पूर्ण किया गया। इसके साथ ही राजभाषा स्थिति की समीक्षा हेतु तिमाही बैठकें आयोजित की गईं। यहाँ यह भी उल्लेखनीय है कि विज्ञान को हिन्दी से जोड़ने की दिशा में प्रति वर्ष संस्थान द्वारा मलेरिया पत्रिका (त्रैमासिक) एवं *प्लाज़्मोडियम* नामक समाचार दर्शना (द्विवार्षिक) प्रकाशित किया जाता है। इसके साथ ही प्रति वर्ष की भाँति इस वर्ष भी संस्थान में हिंदी दिवस पूर्ण उत्साह के साथ मनाया गया। इस उपलक्ष्य में दिनांक 30 सितंबर 2009 को एक व्याख्यान एवं पुरस्कार वितरण समारोह का आयोजन किया गया। इस अवसर पर केन्द्रीय अनुवाद ब्युरो के पूर्व-निदेशक (सेवानिवृत) डॉ. विचारदास सुमन को आमंत्रित किया गया। डॉ. विचारदास ने अपने व्याख्यान में राजभाषा अधिनियम पर प्रकाश डाला। इसके साथ उन्होंने प्रशासनिक अनुवाद के संबंध में विस्तार से जानकारी देते हुए बताया कि सरकारी काम-काज में किस प्रकार प्रशासनिक शब्दों का प्रयोग किया जाना चाहिए एवं टिप्पण के प्रारूपण के कई उदाहरण देते हुए उन्होंने बताया कि शब्दों के मामूली हेर-फेर से अर्थ का अनर्थ हो सकता है।

उनके इस रूचिकर व्याख्यान के पश्चात् संस्थान में हिन्दी में अधिकाधिक कार्य करने हेतु लागू वर्ष 2008-09 की प्रोत्साहन योजना के पुरस्कारों की घोषणा डॉ. नूतन नंदा वैज्ञानिक 'ई' द्वारा की गई। संबंधित पुरस्कार मुख्य अतिथि डॉ. विचारदास सुमन एवं प्रभारी निदेशक डॉ. वीरेन्द्र कुमार दुआ के कर-कमलों द्वारा प्रदान किए गए, जिनमें प्रथम

डॉ. विचारदास व्याख्यान देते हुए

प्रोत्साहन योजना का प्रथम पुरस्कार लेते हुए श्री मोहनलाल

पुरस्कार श्री मोहनलाल, श्री मोहन सिंह बिष्ट, द्वितीय पुरस्कार श्री के.सी. सेहरा, श्री जितेन्द्र कुमार, श्री रामफूल मीणा और तृतीय पुरस्कार श्री राम देव, श्रीमती सुदर्शना छाबड़ा और श्री रघुवेन्द्र कुमार शर्मा को दिए गए।

अंतत: कार्यक्रम का विधिवत् समापन करने हेतु संस्थान के प्रशासन प्रभारी डॉ. भूपेन्द्र नाथ नागपाल ने इस कार्यक्रम के सफलतापूर्वक आयोजन करने में प्रभारी निदेशक महोदय

प्रोत्साहन योजना का प्रथम पुरस्कार लेते हुए श्री मोहन सिंह बिष्ट

धन्यवाद ज्ञापित करते हुए डॉ. नागपाल

तथा संस्थान की हिन्दी अधिकारी के नेतृत्व में काम कर रहे हिन्दी अनुभाग के योगदान की सराहना करते हुए उन्हें हार्दिक धन्यवाद ज्ञापित किया। यही नहीं उन्होंने सभी उपस्थित श्रोताओं एवं विजेताओं का भी समारोह में पधारने के लिए विशेष रूप से आभार प्रकट किया।

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Committees of the Institute

12

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Dr RC Dhiman
Dr SK Ghosh
Dr MS Malhotra
Dr Arun Sharma
Dr Neena Valecha

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Dr RM Bhatt
Dr Sukla Biswas
Dr Vas Dev
Dr Hema Joshi (Expired on 23 March 2010)
Dr Ashwani Kumar
Dr PK Mittal
Dr BN Nagpal
Dr Nutan Nanda
Dr K Raghavendra
Dr AM Reetha
Dr MC Sharma
Dr SK Sharma
Dr MM Shukla
Mr OP Singh
Dr HC Srivastava

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Dr PK Atul
Dr Aparup Das
Dr Jyoti Das (Joined on 2 March 2010)
Dr AK Mishra
Dr Neelima Mishra
Mrs Rekha Saxena

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Dr Ruchi Singh
Dr Alex Eapen (Joined on 2 April 2009)

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Dr VP Singh
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