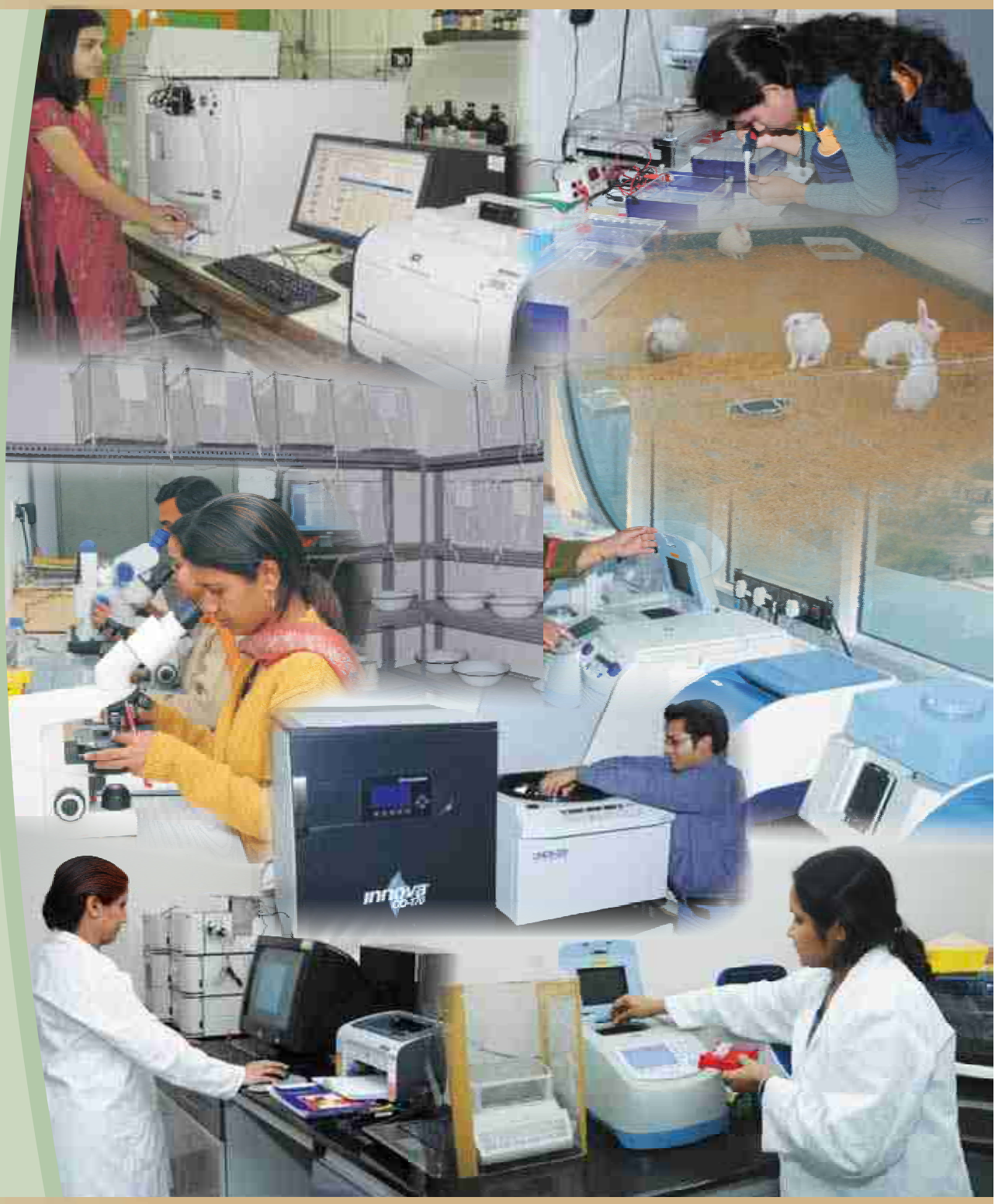
Annual Report
2008–09

NIMR



National Institute of Malaria Research
(Indian Council of Medical Research)
Sector - 8, Dwarka, New Delhi - 110 077

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Preface

It is of immense pleasure to present the Annual Report of NIMR, as the year 2009 is a memorable and most eventful year for NIMR. This year saw shifting of the Institute to our newly constructed building in Dwarka. Although it will take some time for complete establishment, NIMR will now be in a position to work as a coherent group that was not possible over the years since its inception, because of fragmented setups. Notwithstanding the pains taken by all staff of NIMR in shifting their facilities to the new building, NIMR has grown tremendously in the year of report that saw many other important achievements in terms of scientific activities of NIMR. Research support from extramural funding agencies has been increased and quality of scientific publications has also been improved remarkably.

During the year, study on bionomics of malaria vectors, evaluation of new insecticides, larvicides, insect growth regulators and long-lasting insecticidal nets were undertaken. The research work on molecular characterization of malaria parasites, chloroquine resistance monitoring, parasite immunology and biochemistry, genomics and bioinformatics of malaria vectors, parasites and humans focussed in newer areas in understanding the molecular interaction among the host, parasites and vectors. Mapping of malaria vectors using GIS & RS, validation of thematic maps, development of GIS-based malaria information management system, health impact assessment studies of development projects, taxonomic studies of different mosquito vectors, identification of epidemiological risk factors of malaria and assessment of impact of climate change on malaria were undertaken during the year in collaboration with NVBDCP and state health departments. Many plant based fractions having antimalarial and insecticidal properties were also screened.

Clinical trials on ACTs like Arterolane + piperaquine, Artesunate-Mefloquine combination therapy, pyronaridine + artesunate for the treatment of malaria were undertaken during the year. In addition, various operational research projects have also been started with World Bank assistance, and in collaboration with the National Vector Borne Disease Control Programme (NVBDCP).

In the human resource development front, more than 100 Senior Health Officers of Gujarat state, 45 District Health Officers and Entomologists of Delhi, 20 delegates from AFMC, and fellows from WHO were imparted training on malaria and other vector borne diseases for 2 to 3 weeks duration. M.Sc./B. Tech./M.Tech. students from different colleges and Universities of the country have been trained in different aspects of malaria research. Eight students were awarded Ph.D. and 38 students are currently pursuing studies leading to Ph.D. degree. NIMR is currently affiliated to five universities and an integrated M.Sc.-Ph.D. programme in Medical Entomology in collaboration with the Goa University. New linkages with other institutes for collaborative research have also been established and many training courses and workshops on various aspects of malaria have been organized. As many as 60 research papers have been published by NIMR scientists.

NIMR profile was updated during this year focusing research activities of the Institute over the last three decades. Apart from regular publication of periodicals and newsletters, during this year about six brochures were published to sensitize the scientific community by providing latest information on malaria vectors and their control. The *Journal of Vector Borne Diseases* which is being published by NIMR has been included for impact factor analysis by Thomson ISI from 2009.

All the ten field units continued to serve the national programme and catered the needs of public in providing diagnostic and treatment to the malaria and filaria patients.

I would like to place on record the contribution of Prof. AP Dash, Director NIMR, who joined WHO-SEARO in February 2009 in bringing the Institute to present level. I thank Dr VM Katoch, Secretary, Department of Health Research & Director General, ICMR for providing constant support and encouragement to this Institute.

Dr VK Dua
Director (In-Charge)

1 VECTOR BIOLOGY AND CONTROL

1.1 Studies on anopheline species complexes

1.1.1 The Culicifacies Complex

Bionomics and distribution pattern

Studies on distribution and bionomics of *Anopheles culicifacies* sibling species were undertaken in different geographical areas. In malaria endemic Dankwada district (Chhattisgarh) examination of *An. culicifacies* population revealed the prevalence of species B and C. *Anopheles culicifacies* species C, an established vector of malaria, was found to be predominant (67%) and primarily zoophagic. In Simdega and Giridih districts of Jharkhand state, *An. culicifacies* species B and C were found sympatric in the study villages with predominance of species B. A longitudinal study on the sibling species composition and their bionomics is being carried out in and around Jabalpur, Madhya Pradesh. Results obtained so far have revealed that the established vector species (C & D) together constitute ~95% of the total *An. culicifacies* population with a sporozoite rate of 0.42% indicating high malariogenic potential of the study area. Similarly, analysis of *An. culicifacies* samples from Districts Khandwa and Harda during malaria transmission season in 2008 revealed that species C and D comprised of 95% of the total *An. culicifacies* population and were found to be predominantly zoophagic.

1.1.2 Fluviatilis and Minimus Complexes (Minimus group)

Distribution and bionomics

In continuation of mapping the distribution of the members of *An. fluviatilis* complex and study of their bionomics in unexplored districts, samples examined from plain riverine areas under Upparu and Itkyal PHCs in District Mahbubnagar (Andhra Pradesh) and hilly areas of Gudalur PHC in District Nilgiri (Tamil Nadu) were found to be species T. In contrast, *An. fluviatilis* species S was predominant in villages surveyed in District Rayagada of Orissa which is highly endemic for malaria. In this district, species S was found to be highly anthropophagic as revealed by blood meal source analysis. The findings in the above mentioned area are in conformity with general distribution pattern observed for *An. fluviatilis* sibling species.

Cytogenetic characterisation of *An. minimus* populations from Districts Sonitpur, Kamrup (Assam), Jalpaiguri (West Bengal) and Sundargarh (Orissa) has led to preparation of a detailed photomap of polytene chromosomes complement of *An. minimus* species A that would serve as a standard reference. The banding pattern of the chromosomes (X, 2, 3, 4 & 5) was found homosequential to that of *An. fluviatilis* species U which suggests close phylogenetic relationship between Minimus and Fluviatilis complexes. However, *An. minimus* A and *An. fluviatilis* U have been found distinctly different in their distribution pattern, feeding preference and at molecular level.

Anopheles minimus belonging to Minimus subgroup, Myzomia series has been established as a complex of three sibling species with species A and C prevalent on the Southeast Asian mainland and species E reported from Japan. For the first time, a focus on *An. minimus* species A has been discovered in Tensa mining area of District Sundargarh of Orissa. Tensa is an iron ore mining area located at ~100 km east of Rourkela (Fig. 1). This hilly forested area is highly malarious and has streams and stream channels as major breeding

Fig. 1. Location of Tensa mining area in District Sundargarh, Orissa

sites for anophelines. In the villages surveyed, *An. minimus* was found in sympatric association with *An. fluviatilis* and *An. culicifacies*. Though *An. fluviatilis* species S was predominant in the study area, the proportion of *An. minimus* A was around 30%. The morphologically identified specimens of *An. minimus* were sequenced for D3 domain of 28S rDNA and compared with published sequence of *An. minimus* A. Alignment sequences revealed that the 335 bp fragment had 100% homology between test samples and *An. minimus* A which conclusively proved that these mosquitoes are indeed *An. minimus* species A (Fig. 2). It appears that *An. minimus* once disappeared from northern and eastern states of India during DDT-era is gradually making its appearance in these areas. A longitudinal study on the bionomics of *An. minimus* A and *An. fluviatilis* S is being carried out to ascertain the relative role of these two species in malaria transmission in the mining area. In addition, studies have been initiated to explore the possible existence of *An. minimus* C in India, particularly in the districts bordering Myanmar where this species is reported to be prevalent.

1.2 Vector-Parasite interactions

1.2.1 The immune response of *Anopheles culicifacies* against *Plasmodium vinckei petteri* infection

Immune responses of *An. culicifacies* species A was studied against rodent *P. vinckei petteri* infection. The response of immune system was studied as change in the phenol oxidase (PO) isozyme profile and/or change in the enzyme activity. Phenol oxidase is the prime enzyme of phenol oxidase cascade, a part of humoral immune system. For the purpose, haemolymph, midgut and carcass were collected from experimental group of mosquitoes fed on *Plasmodium* infected mice. Tissues were collected after 12, 18 and 24 h of feeding. To compare the effect of *Plasmodium* infection, unfed (naïve) and mosquitoes fed on healthy mice were taken correspondingly. The changes in the isozyme profile of all the three tissues in response to *P. v. petteri* infection were assessed by gradient native-polyacrylamide gel electrophoresis (Native-PAGE). The gels were photographed on 'Syngene gel documentation system' using GeneSnap™ software. Numbers of isozymes were observed in various experimental and control group. These isozymes show distinct temporal and/or physiological distribution in different stages of life span. Isozymes were designated as AcAPO 1, 2, 3, 4, 5 and 6 on the basis of their mobility. The slowest one labeled as AcAPO 1, while fastest one as AcAPO 6 in case of adult mosquitoes.

Carcass of naïve mosquitoes showed two isozymes: AcAPO 1 and 4 (Fig. 3). Blood feeding on healthy mice induced a new isozyme AcAPO 5 in the carcass sample at 12, 18 as well as 24 h time points (Fig. 4). AcAPO 5 showed highest activity at 18 h than other time points studied. Further, disappearance of AcAPO 5 at 24 h and after infected blood meal, may be extrapolated for its role somewhere else than in the immune system (Fig. 5). AcAPO 1 disappeared after uninfected blood meal and again reappeared after infected blood meal. Reappearance of AcAPO 1 after infected blood meal may be because of its specific anti-plasmodium role in mosquito's immune mechanism. Whole body extract of mosquitoes also showed two isozymes; AcAPO 1 and 4 with high

```
Subject     CCAAGAAGTC TATCTTGCGC GCAAGCCAAT GGGTAAATGG TGCGGTACGC      [50]
Min_A       CCAAGAAGTC TATCTTGCGC GCAAGCCAAT GGGTAAATGG TGCGGTACGC      [50]

Subject     CGCCCATGAC TGGAAACCCA CAGGCGAAGA CAAATCGAGT GGTGCGGGAT      [100]
Min_A       CGCCCATGAC TGGAAACCCA CAGGCGAAGA CAAATCGAGT GGTGCGGGAT      [100]

Subject     TACGGGTACG GCCGATGGCG CAAGCCTTCG TCGGACCCCT CCATCCCAGG      [150]
Min_A       TACGGGTACG GCCGATGGCG CAAGCCTTCG TCGGACCCCT CCATCCCAGG      [150]

Subject     GTGTCCCGTC CGGGTGCTTG CACCCAGTGG ACATCCCCGG AGTGCGTAGG      [200]
Min_A       GTGTCCCGTC CGGGTGCTTG CACCCAGTGG ACATCCCCGG AGTGCGTAGG      [200]

Subject     ATGTGACCCG AAAGATGGTG AACTATGCCT GATCAGGTTG AAGTCAGGGG      [250]
Min_A       ATGTGACCCG AAAGATGGTG AACTATGCCT GATCAGGTTG AAGTCAGGGG      [250]

Subject     AAACCCTGAT GGAGGACCGA AGCAATTCTG ACGTGCAAAT CGATTGTCAG      [300]
Min_A       AAACCCTGAT GGAGGACCGA AGCAATTCTG ACGTGCAAAT CGATTGTCAG      [300]

Subject     AGTTGGGCAT AGGGGCGAAA GACCAATCGA ACCAT [335]
Min_A       AGTTGGGCAT AGGGGCGAAA GACCAATCGA ACCAT [335]
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Fig. 2. Sequence alignment of the D3 domain of 28S ribosomal DNA of test samples with *An. minimus* species A

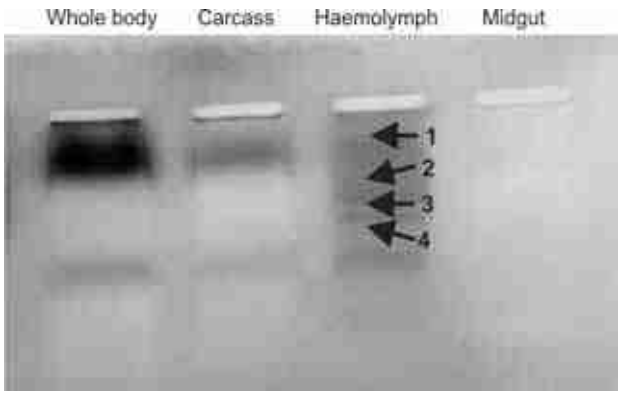

Fig. 3. Isozyme profile of naïve *An. culicifacies* A (Dehra)

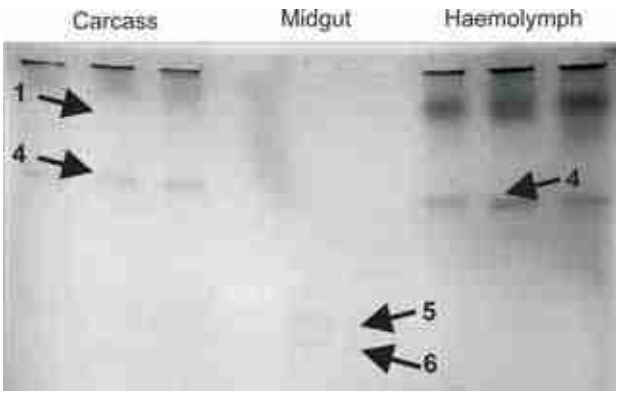

Fig. 5. Isozyme profile of *An. culicifacies* A (Dehra) fed on *P. vinckei* infected mice

PO activity than carcass. The loss of fat bodies and other body parts during dissection may be the reason for low PO activity in the carcass than the whole body sample.

Midgut of naïve mosquitoes did not show any detectable PO activity (Fig. 3), although uninfected blood feeding induced two isozymes AcAPO 5 and 6 (Fig. 4). Decrease in the activity of both the isozymes, AcAPO 5 and 6 was noticed with the time. Further, *Plasmodium* challenge also induced both the isozymes, but, without any detectable difference in the activity at any point of time (Fig. 5). Upon uninfected blood feeding, induction of isozyme activity may be because of alertness of the immune system to combat with probable pathogen, if any, coming with blood meal. Further, absence of pathogen or avirulent pathogen may calm down the immune system by switching off the expression of AcAPO 5 and 6. This may be evidenced by the unaltered activity of both the isozymes in midgut samples of infected blood-fed mosquitoes up to 24 h.

The haemolymph samples of naïve mosquito showed four isozymes: AcAPO 1, 2, 3 and 4 (Fig. 3). The disappearance of AcAPO 2 and 3, after uninfected as well as infected blood meal indicates their role in some physiological phenomena other than immunity (Figs. 4 and 5). The activity of AcAPO 4 remained unchanged in hemolymph samples of naïve as well as uninfected blood-fed mosquitoes. While, feeding on *Plasmodium* infected mice caused increase in the isozyme activity at 12, 18 as well as 24 h time points. This increase in activity may be inferred as its role in immunity. The disappearance of AcAPO 1 after uninfected blood meal and reappearance after infected blood meal, demonstrates its potent and specific role against *Plasmodium* infection. After *Plasmodium* challenge, highest PO activity was noticed at 24 h time point which may be correlated with high ookinete density towards haemolymph side of midgut.

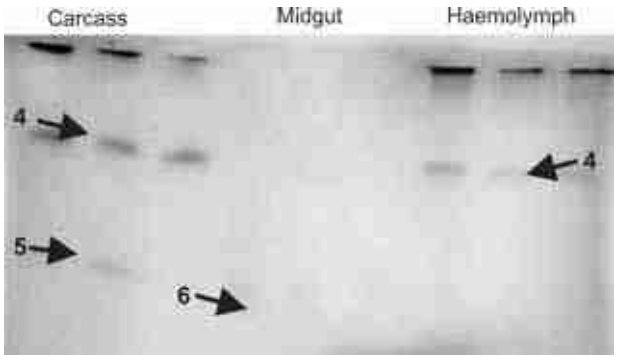

Fig. 4. Isozyme profile of *An. culicifacies* A (Dehra) fed on control mice

1.2.2 Allelic variation of phenol oxidase in isofemale cultures of *Anopheles culicifacies* Complex

In the process of re-estalishment of cyclic colony of *An. culicifacies* species B, *An. culicifacies* sensu lato were collected from Shantipuri, District Udham Singh Nagar (Uttarakhand) in November 2008. Out of 185 isofemale lines only 25 cultures could survive up to F1 generation and they were screened for phenol oxidase (PO) isozyme and sibling species composition. Female IV instar larva from each culture was used to check the allelic variation of PO in the wild population of *An. culicifacies*.

The allelic variation of PO was assessed by using 5% SDS-PAGE. Extract of single IV instar female larva (5 µl) loaded per lane irrespective of protein concentration and health of the larva.

Three different allelic forms or isozymes of PO were found (Fig. 6). These isozymes labeled as (1) Slow – towards the cathode terminal, (2) Fast – towards the anode terminal, and (3) Medium– between the slow and fast isozymes. Slow isozyme was present in all the 25 samples tested. Out of 25, 32% samples showed only slow isozyme; 32% samples showed slow and fast isozymes together; while rest (36%) showed slow and medium isozymes

(Fig. 6 a, b and c). After emergence, adult mosquitoes were checked for their sibling species status. All the isofemale cultures were identified as *An. culicifacies* species E.

Three isofemale culture samples collected from Dehra, District Gautam Budh Nagar, Uttar Pradesh were also assessed for allelic variation. All the three samples showed only one isozyme, i.e. slow. Further, on emergence adult mosquitoes were identified as *An. culicifacies* species A.

On the basis of above finding one may conclude that PO exists in multi-allelic form in *An. culicifacies*. Although the isozymes are distributed throughout the life span and under specific circumstances, as evidenced by polymorphism and feeding experiments with and without malaria parasite respectively. AcAPO 4 and 6 may have a role in defence mechanism, if any, but not specific against malaria parasite, While, the AcAPO 1 is acting specifically against *Plasmodium* invasion in mosquitoes through blood meal. The identified isozymes are candidates for future studies for characterization of their role, if any, in mosquito-parasite interaction.

1.2.3 *Anopheles culicifacies* nitric oxide synthase gene: expression profile of immune-responsive AcNOS gene

Innate immune-related antiparasite defences mounted by *Anopheles* may suppress the growth of *Plasmodium* in mosquitoes. Identification and characterized expression of *An. culicifacies* nitric oxide synthase gene (AcNOS) and their response to *P. vivax* in mosquito vectors act as an additional effector gene to block the development of the malaria parasite in *An. culicifacies* mosquitoes.

Genomic DNA was prepared from the midguts of both *An. culicifacies* species A and B. PCR assay was carried out to differentiate the sequence variations between susceptible and refractory sibling species of *An. culicifacies*. The primers were manually designed complementary to the *An. stephensi* Exon region 1 encoding for the co-factors heme. Amplification of 200 base pairs against Exon 1 was observed by using primer sequences 5' ATGAGGACCAACTATCGGG 3' and 5' GCCTTGGTGACAATGCTC 3'. The purified PCR products were cloned in pDrive cloning vector (Qiagen) and positive clones were selected by

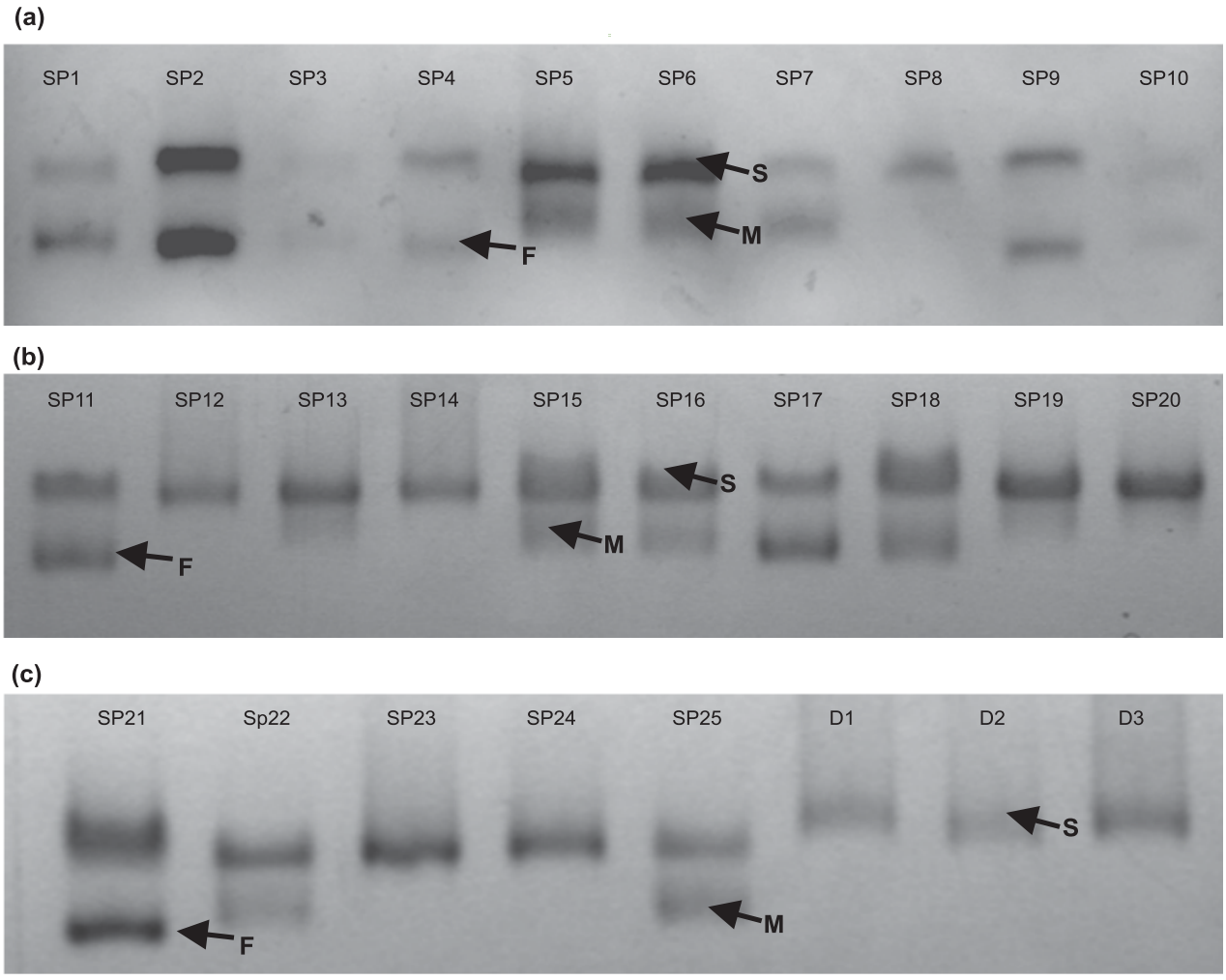

Fig. 6. *An. culicifacies* species E isofemale culture: (a) SP1–10; (b) SP11–20; and (C) SP21–25, D1–3

blue-white screening. Positive clones were sequenced in a 96 well automated DNA sequencer (Applied Biosystems). The sequence homology to other reported NOS was confirmed by BLAST homology analysis using DNA STAR software (LASERGENE). A 200 bp fragment of the *An. culicifacies NOS* gene (AcNOS) was amplified (Fig. 7). Cent percent homologous sequence of *NOS* in both *An. culicifacies* species A and species B had been obtained and the sequence was submitted to Gen Bank (FJ172998 and FJ172999 respectively). The amplified sequence encodes a 200 residue region which revealed 37–96% identical at the amino acid level to the corresponding region of these known NOS sequences, as well as 96% identical to the recently isolated *An. stephensi* NOS sequence (Fig. 8).

A phylogenetic tree (Fig. 9) was constructed on the basis of alignment of the partial AcNOS amino acid sequence and the corresponding homologous regions of several invertebrate and vertebrate NOS. Hence, in the resulting dendrogram, obtained by the Neighbour-joining method *An. culicifacies* species A and species B were in same clade and vertebrate NOS were in the different clade. The deduced amino acid sequences of *Monodelphis domestic* NOS, *Rattus norvegicus* NOS, *Homo sapiens* NOS, *An. stephensi* NOS and *An. culicifacies* species A and species B NOS showed the highest level of homology to vertebrate neuronal NOS, followed by decreasing homologies to vertebrate endothelial and inducible *NOS* genes. The homology of these distinct insect genes to a neuronal isoform should be regarded more as a reflection of gene ancestry than functional activity. The pair-wise comparison provided evidence for the evolutionary relationships between these members. Our analysis strongly suggests that AcNOS and the human NOS genes share a common ancestor.

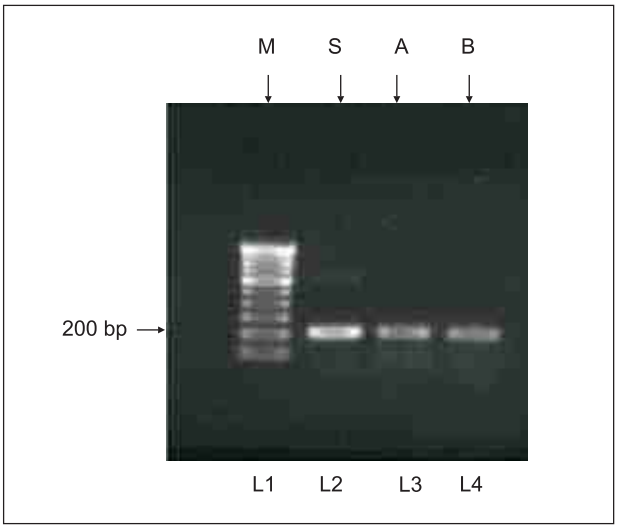

Fig. 7. PCR amplification of Exon-1 region (200 bp) of mosquito nitric oxide synthase gene (NOS). L1: 100 bp marker (M), L2: *An. stephensi* (S), L3: *An. culicifacies* sp A, L4: *An. culicifacies* sp B

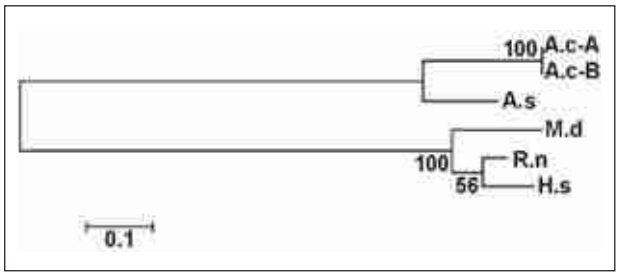

Fig. 9. Phylogenetic bootstrap consensus tree based on amino acid sequence alignment using neighbour- joining method (A.c-A–*Anopheles culicifacies* Species A, A.c-B–*Anopheles culicifacies* Species B, A.s–*Anopheles stephensi*, M.d– *Monodelphis domestic*, R.n–*Rattus norvegicus*, H.s–*Homo sapiens*)

1.2.4 MS-based proteomic approach for the identification of salivary gland proteins from the malaria vector *Anopheles stephensi*

In the female mosquito, the salivary glands play an important role in the maturation of the infective form of the malaria parasite and in malaria transmission. The salivary glands of female *Anopheles* mosquitoes secrete antihaemostatic, anti-

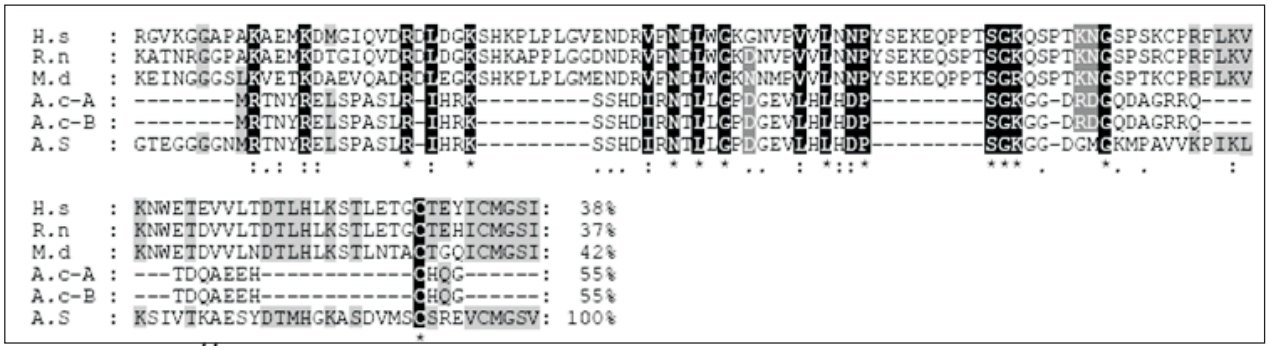

Fig. 8. Clustal alignment of AcNOS with known homologous *NOS* sequences of other insect and vertebrates. Highly conserved and similar residues have been shown in dark black and grey respectively. Relative sequence identity has also been shown

inflammatory and immune-modulator molecules. Salivary gland proteins offer attractive targets to understand dynamicity of salivary glands, feeding behaviour and strategies for control of malaria. Such novel proteins will shed more light on the biology of malaria transmission and perhaps suggest novel targets for control of malaria transmission. The complete genomic sequence of the major African malaria vector, *An. gambiae*, allows discovery of genomic/proteomic based high-throughput annotations of different proteins through experimental as well as bioinformatics' methods.

Main objective 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. Identification of several proteins and proteomes at molecular levels may provide novel targets for interrupting parasitic life cycle.

Initial study was on gel free proteomic approach using LC-MS/MS to characterize the proteome of the salivary gland extracts (SGEs) of *An. stephensi*. Salivary gland extracts were digested with trypsin using the conventional complementary in-solution approach and analyzed by LC-MS/MS This led to identification of 187 peptides and 45 total protein matches (45 unique proteins) and nine proteins were novel proteins of unknown functions (Table 1).

This is the first report describing functional annotation of salivary gland proteomes of *An. stephensi,* identified and characterized by LC-MS/MS through a detailed bioinformatics analysis (Figs. 10 and 11).

This study may provide valuable baseline information for characterizing proteomes of other mosquito vectors to provide strategies for control of disease transmission. Identification of several proteins and proteomes at molecular levels may provide novel targets for interrupting parasitic life cycle. MS provides data that can be used to validate genome annotation and to discover novel protein targets. This may serve as a basis for future strategies concerning the possible role of these novel proteins in the interaction between the human host and *Plasmodium* through Anopheline vectors for control of malaria transmission.

1.3 Vector control

1.3.1 Field evaluation of Biodart-M, a formulation of *Bacillus thuringiensis* var. *israelensis* (5% WP) against larvae of mosquito vectors

In the past two decades, number of formulations consisting of *Bacillus thuringiensis* and *B. sphaericus* have been evaluated in the laboratory and field. Among the alternative methods to insecticide based vector control, different formulations of *B. thuringiensis israelensis* (*Bti*) spores (serotype H-14) have been found effective against larvae of many mosquito species. Formulations are constantly being developed to

Table 1. A list of novel proteins identified by MS/MS using the gel-free approach

Ac	% Cov	Score	Domain/ Motif	Description	Functional prediction
gil24584051	9	11.3	No conserved domain	CG16813CG16813-PA (*Drosophila melanogaster*)	Unknown
gil157110468	3	8.4	No conserved domain	Conserved hypothetical protein	Unknown
gil125982465	6	7.7	No conserved domain	GA17685-PA (*Drosophila pseudoobscura*)	Unknown
gil158301917	1	6.6	No conserved domain	AGAP001533-PA (*Anopheles gambiae*)	Unknown
gil110758031	1	6.5	No conserved domain	Predicetd: similar to huntingtin (*Apis millifera*)	Unknown
gil9108899	1	6.4	No conserved domain	Predicted: similar to CG7044-PA (*Tribolium castaneum*)	Unknown
gil110767485	1	6.2	No conserved domain	Predicted: similar to zinc finger protein 748 isoform 1 (*Apis millifera*)	Unknown
gil110750123	4	6.1	No conserved domain	Predicted: hypothetical protein (*Apis millifera*)	Unknown
gil157117847	1	6.0	No conserved domain	Conserved hypothetical protein (*Aedes aegypti*)	Unknown

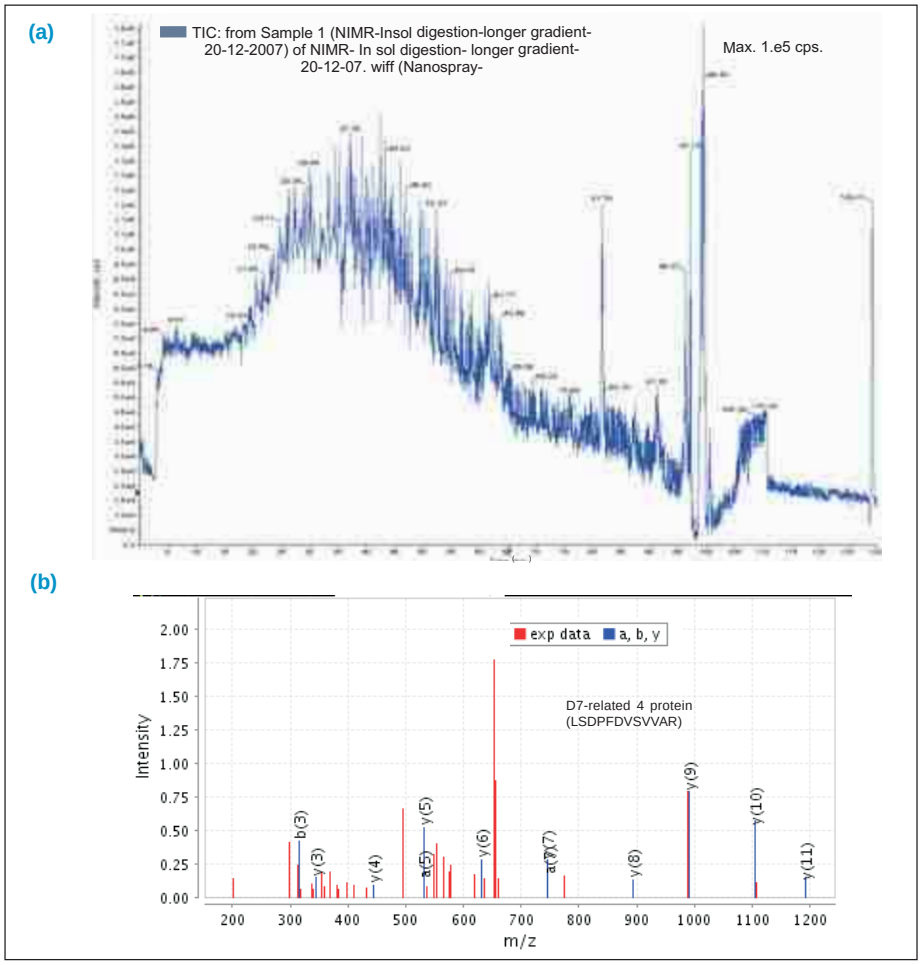

Fig. 10. A gel-free approach for characterization of salivary gland proteome A: (a) Total ion chromatogram of the salivary gland homogenate using trypsin digestion strategy in solution obtained from an LGMS/MS run; (b) Product ion MS/MS spectrum of doubly charged ion peak at m/z 654.8 corresponds to the peptide sequence LSDPFDVSVVAR, which matched a known protein designated as putative D7 related four protein precursor

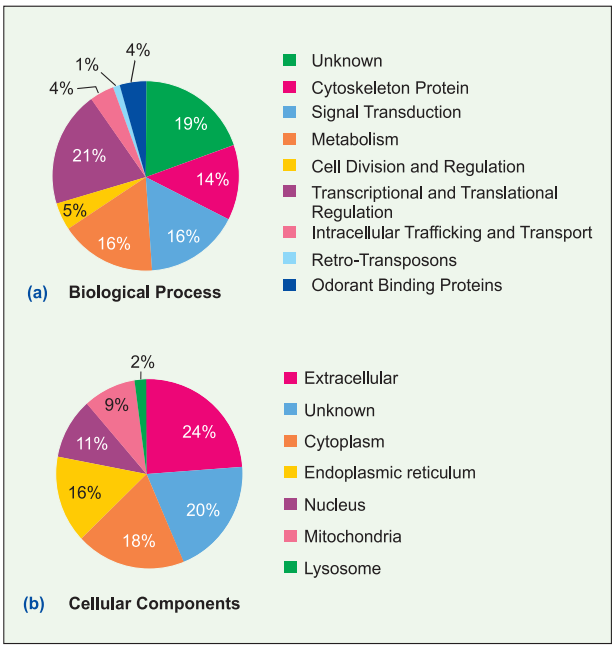

Fig. 11. Functional annotation of the salivary gland proteome of *An. stephensi* using insolution approach: (a) distribution of the MS identified proteins (%) from the salivary gland extract (SGE) grouped according to biological process; and (b) cellular component. The number of identified proteins is in percentage of total proteins

overcome the short persistence of *Bti* product. Therefore, a multicentric trial for evaluation of Biodart-M, a formulation of *B. thuringiensis* var. *israelensis* (5% WP) was carried out in three locations, viz. Raipur, Hardwar/Mathura and Sonepat. A WP formulation of Biodart-M (5%) was evaluated in laboratory at NIMR, Delhi against larvae of mosquito vectors to determine its toxicity (Fig. 12). All bioassays were carried out against late III instar larvae of *An. stephensi* (malaria vector), *Cx. quinquefasciatus* (filaria vector) and *Ae. aegypti* (dengue vector) at 26±2 °C using standard protocol for uniform evaluation of biolarvicide for use in vector control. To determine the most effective dose for the Phase III trial, Biodart-M 5% was applied at three dosages, viz. 0.5, 0.75 and 1.0 g/m^2 in different cement tanks positive for *Anopheles*, *Aedes* and *Culex* immatures in different localities of Raipur and with same doses in cement tanks in Sonepat district against *Cx. quinquefasciatus*. The study was carried out in urban/peri-urban areas of Districts Sonepat (Haryana), Raipur (Chhattisgarh), Hardwar (Uttarakhand) and Mathura (Uttar Pradesh). The habitats selected for the trial and the target

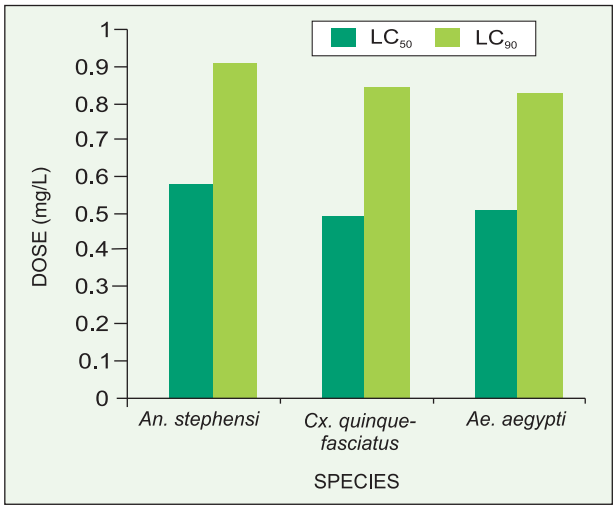

Fig. 12. Percent mortality of mosquitoes exposed to different concentrations of Biodart–M *Bti* formulation

mosquito species are shown in Table 2 and Fig. 13. Biodart-M (5%) @ 0.75 g/m^2 in phase III trial at Raipur, Sonepat and Hardwar/Mathura was effective in reduction of larval density (III+IV) of anophelines (*An. stephensi, An. culicifacies* and *An. subpictus*) (Fig. 14); *Aedes* (*Ae. aegypti* and *Ae. albopictus*) (Fig. 15); and *Cx. quinquefasciatus* (Fig. 16) in different breeding habitats. Weekly application of Biodart-M (5%) WP @ 0.75 g/m^2 against *Anopheles* and *Culex* is effective for one week but in certain highly polluted drains the dose of 1.0 g/m^2 is required weekly for effective control. Against *Aedes* in containers and tyres one time application was effective up to eight weeks period.

Table 2. Habitats and target species selected for the trial at various sites

Site	Trial habitats	Target species
Raipur	Cement tanks	*An. stephensi, An. subpictus* and *Cx. quinquefasciatus*
	Coolers	*Ae. aegypti*, Anophelines
	Drains	*Cx. quinquefasciatus*, Anophelines
Sonepat	Cement tanks	*An. stephensi*
	Pools	*An. subpictus*
	Tanks and drains	*Cx. quinquefasciatus*
Mathura	Pits, pools	*An. culicifacies*
	Drains	*Cx. quinquefasciatus*, Anophelines
Hardwar	Containers, drums and tyres	*Ae. aegypti* and *Ae. albopictus*

Fig. 13. Mosquito breeding habitats selected for Biodart–M trial

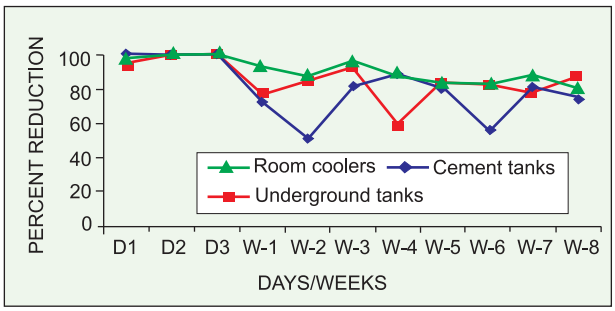

Fig. 14. Effect of Biodart–M @ 0.75 g/m² on Anopheles larvae

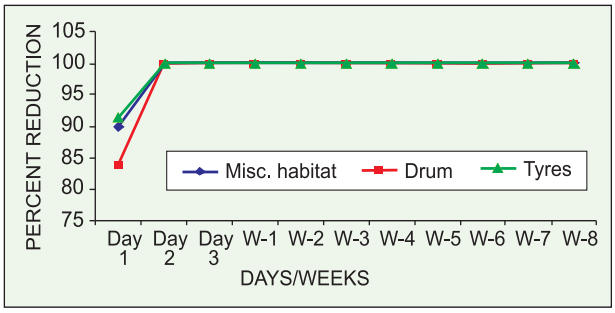

Fig. 15. Effect of Biodart–M @ 0.75 g/m² on Ae. aegypti larvae

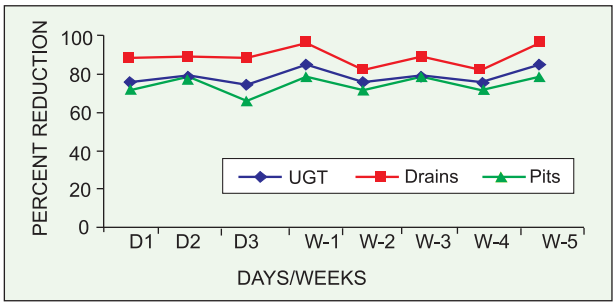

Fig. 16. Effect of Biodart–M @ 0.75 g/m² on Cx. *quinquefasciatus* larvae

1.3.2 Effectiveness of Pirimiphos methyl (Actellic 50 EC) against the mosquito vectors in clean and polluted water

Pirimiphos methyl (Actellic 50%) is an organophosphorus insecticide having contact and fumigant actions. A multicentric phase III evaluation was carried out for vector control in urban areas against *An. stephensi*, *An. culicifacies* and *Ae. aegypti* vectors in clean water habitats (Fig. 17) @100 g a.i./ha at three different sites, viz. Bengaluru, Chennai and Ahmedabad and the dose of 150 g a.i./ha was evaluated in polluted water habitats against *Anopheles, Culex* and *Aedes* immatures. Knapsack and hand compressor sprayers were used for application. After application, larval density was monitored at 24, 48, and 72 h intervals and thereafter at weekly intervals in the same habitats. The application of Pirimiphos methyl in interdomestic breeding habitats @ 100 g a.i./ha indicated >80% reduction of *An. stephensi* up to a period of seven days. The effect of Pirimiphos

Fig. 17. Breeding habitats selected for the application of Pirimiphos methyl

methyl 50% EC @ 150 g a.i./ha weekly in different peridomestic habitats with usual organic contents showed that each application produced >80% control of late instars and pupae up to one week, except in highly polluted and pipe leakage water pools, cesspits with breeding of *An. subpictus* and *Cx. quinquefasciatus,* where the percent reduction was ≥80% till Day 3 after that it declined to <80% (Fig. 18). Weekly application of Pirimiphos methyl was found effective in reducing adult density of total mosquitoes by 41% and vector mosquitoes by 49 to 100% after II fortnight.

Weekly application of Pirimiphos methyl 50% EC @ 100 g a.i./ha in clean water habitats against *Anopheles, Culex* and *Aedes* is required and in

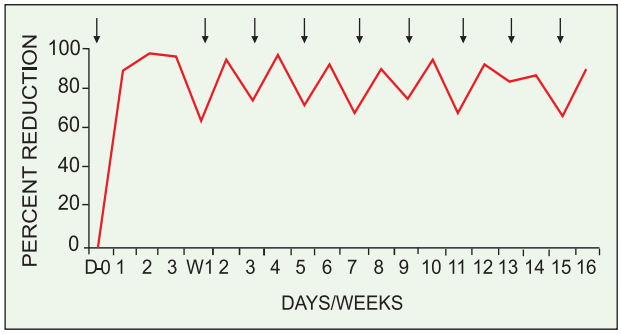

Fig. 18. Percent reduction in III/IV instar larval densities in all larval habitats applied with Pirimiphos methyl. Arrows indicate treatment with Pirimiphos methyl

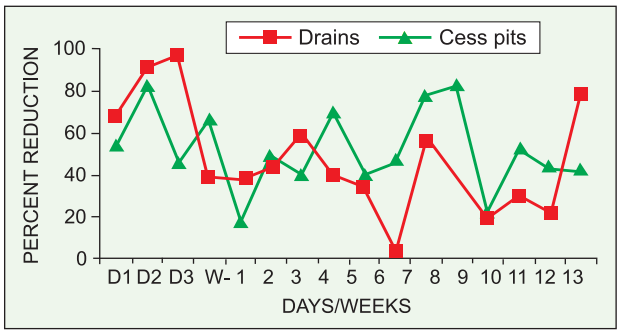

Fig. 19. Impact of Pirimiphos methyl (25% WP) on immature density of Culex mosquitoes

polluted habitats the dose of 150 g a.i./ha was effective only for three days (Fig. 19).

1.3.3 Multicentric phase III evaluation of effectiveness of diflubenzuron 25% WP (Bi-Larv), an insect growth regulator for mosquito vector control in urban setting

Among various methods of vector control, insect growth regulating (IGR) compounds are emerging as safer alternative chemical insecticides. Diflubenzuron is a potent broad-spectrum insect growth regulator that interferes with chitin synthesis at the time of moulting and has been found to be effective in arresting development of immature stage of insects. A multicentre phase III evaluation of diflubenzuron 25% WP (Bi-Larv) was conducted in urban settings in Ahmedabad, Chennai and Goa for control of vectors of urban malaria, filariasis, dengue and chikungunya. A common protocol was followed for the evaluation of diflubenzuron in three study sites. After a preparatory phase, the field trial was conducted for a period of six months in Goa, i.e. from March to August 2008 including approximate two months of preparatory phase, February to July 2008 at Nadiad and May to October 2008 at Chennai. Mosquito immatures were sampled from the treated and control habitats before the application of diflubenzuron to measure pre-treatment larval/pupal densities. Thereafter, diflubenzuron was applied at 25 g a.i./ha area in clean water and 50 g a.i./ha area in organic/polluted waters. During the baseline period, III/IV instar larvae and pupae were collected both from intradomestic water storage containers and peridomestic larval habitats. Around 10 species of mosquitoes including disease vectors were found breeding in these habitats. These included *An. stephensi*, *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus*. The other mosquito species were *An. subpictus*, *An. jamesi*, *Ae. vittatus*, *Cx. bitaeniorhynchus*, *Cx. tritaeniorhynchus* and *Ar. subalbatus*. It was interesting to note that *An. stephensi* was breeding in both peridomestic habitats and construction sites. In intradomestic habitats, *Ae. albopictus* was the dominant species, while *Cx. quinquefasciatus* was the predominant species in the peridomestic sites. Overall the diflubenzuron 25% WP formulation at dose of 25 g a.i./ha (100 g/ha) and 50 g a.i./ha (200 g/ha) showed weekly effect on immatures in pits, pools, tanks, wells, containers and barrels (Fig. 20).

1.3.4 Evaluation of safety of cyphenothrin 5% EC (Gokilaht-S 5 EC) space spray against mosquitoes to residents and spraymen

Recently, a new type II synthetic pyrethroid, Cyphenothrin 5% EC (IUPAC) name: d, d-trans-cyphenothrin with trade name, Gokilaht®-S 5% EC has been developed by M/s. Sumitomo Chemicals India Pvt. Ltd. This chemical has been classified as moderately toxic by International Programme on Chemical Safety (IPCS). Recently, entomological evaluation of Cyphenothrin 5% EC as space spray in India conducted by NIMR has shown different degrees of efficacy against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* in both indoor and outdoor conditions. In the Indian trial, two doses of Cyphenothrin 5% EC @ 0.5 mg a.i./m^2 and 1.0 mg/m^2 were used for fogging in indoors and 1.0 mg a.i./m^2 was found most effective. Similarly, out of two doses of Cyphenothrin 5% EC @ 1.0 g a.i./ha and 3.5 g a.i./ha were used for fogging outdoors and dose of 3.5 g a.i./ha was found to be more effective in producing >90% mortality in *Anopheles*. Although this insecticide was found to be very effective in killing *Anopheles* and *Culex* mosquitoes but no human safety study has been conducted so far in India. Therefore, a study was conducted on human safety of Cyphenothrin 5% EC in one of the malaria endemic area of Lakshar PHC, District Hardwar, Uttarakhand.

The study was conducted in two localities one as control and the other as experimental area in the periurban area of Lakshar PHC of District Hardwar (Uttarakhand) selected for the field study. Selection of the study site was done on the basis of

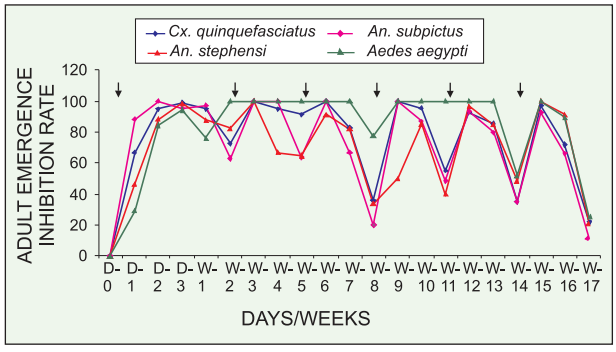

Fig. 20. Inhibition of emergence of adults of various mosquito species in habitats treated with diflubenzuron

mosquitogenic conditions of the area. In the experimental area, space spray was carried out with two dosages of cyphenothrin 5% EC, i.e. 1.0 mg a.i./m^2 in indoors and 3.5 g a.i./ha in outdoors. Fogging operation was carried out in the evening during dusk conditions with the help of Swingfog SN50 machine (Fig. 21).

Fig. 21. Cyphenothrin fogging using swingfog machine

In all 20 inhabitants (volunteers) from the experimental area where the test insecticide was sprayed, five inhabitants (volunteers) from the unsprayed area and five spraymen those who sprayed the test insecticide in the experimental area were included as study subjects for detail laboratory and clinical investigations as per the protocol.

All the study subjects were clinically examined as per the prescribed proforma on Day 0, before the spraying operation. Medical history sheets were completed as per the protocol and all the entries were made in the prescribed data sheet. All the study subjects were re-examined after 1 and 72 h of post-exposure of the insecticide fogging operation. Various parameters, viz. vital sign (pulse rate, respiratory rate, depth of respiration, temperature, chest tightness), general (weakness, fatigue, sleep, urination, sweating, nausea, vomiting, appetite, taste, abdomen pain, diarrhoea, sialorrhoea), neuro-muscular (headache, dizziness, irritability, pain, twitchings, tremors, convulsions, parasthesia, hallucinations, unconsciousness), cardio-respiratory (nasal discharge, wheeze, cough, expectoration, chest tightness, dyspnea, palpitation, heart conserveness, cyanosis, tachycardia), eye (miosis, lacrimation, double vision, blurred vision), and psychological (temperament, judgment, nervousness, insomnia) were investigated for each of the study subjects.

For liver and kidney function tests 2 ml of intravenous blood samples were collected on Day 0 before the fogging operation of test insecticide and again on Day 5 after the fogging operation from each of the study subjects.

In addition, nerve conduction studies (NCS) were carried out on Day 0 (pre-exposure), on Day 5 and Day 60 (post-exposure) for all the five spraymen, those who carried out the fogging operation in the experimental area (Fig. 22).

Detailed clinical and laboratory investigations (liver and kidney functions) and nerve conduction studies did not reveal any detectable changes in vital signs, general health status, gastrointestinal, neuro-muscular, cardio-respiratory, eye and psychological clinical symptoms after the exposure of Cyphenothrin 5% EC. Study subjects did not reveal any abnormality which could be attributed to the toxicity of the test insecticide. None of the inhabitants or spraymen involved in the experimental study had any complaint or had any discomfort or uneasiness during the study period.

Thus, all the participants in the study were well-tolerated to the exposure of Cyphenothrin 5% EC under the experimental conditions.

Fig. 22. Nerve conduction studies in study subjects

Table 3. Median knock down time (minutes) of *An. culicifacies* mosquitoes exposed to unwashed and washed Olyset nets during August 2004 to 2008

No. of washes	No. of mosquitoes tested	Median knock down time
Unwashed	220	6.8
1	44	12.5
2	44	12.5
3	44	12.7
4	44	12.0
5	44	13.5
6	44	12.5
7	44	14.0
8	44	14.7
9	44	15.5
10	44	16.0

1.3.5 Long-lasting efficacy of OlysetNet against malaria vectors and incidence of malaria in a village of District Gautam Budh Nagar, U.P.

Follow-up study on the long-lasting efficacy of Olyset nets was continued in three villages, viz. Khandera (Olyset-net village), Beel (untreated net village) and Anandpur (without net village) in Distt. Gautam Budh Nagar, U.P., beyond the initial trial period of one year. The efficacy of Olyset nets as determined by cone bioassays with 3 min exposure on nets revealed that >80% nets showed >95% knock down in *An. culicifacies* within one hour even in the 5th year of usage of Olyset nets in the field. Ring-net bioassays with *An. culicifacies* on Olyset net collected randomly after different periods of use from the field revealed no significant difference in the percent mortality in cone bioassays but median knock down time on fresh nets was 6.8 min as compared to 12–13.5 min on nets collected after three years of use and 15–17 min on nets collected after four years of use in the field irrespective of number of washes (Table 3). This indicates that washing of net did not reduce the efficacy of Olyset net to a large extent, but the overall knock down time was increased after prolonged use in the field.

The durability of Olyset nets was good, as >80% of the original nets were found intact during survey in September–October 2008. The community acceptance of Olyset nets was very high as there was still >80% usage of Olyset nets in the study population during the transmission period in the months of September–October 2008.

Fortnightly monitoring of MHD of mosquitoes and surveillance of malaria incidence was carried out. Data of MHD and malaria incidence of each fortnight in three villages were pooled and recorded month-wise. Pooled month-wise entomological data showed a reduction in the indoor resting MHD and the parity rates of the major malaria vector *An. culicifacies* in the Olyset net village, when compared with untreated net and without net villages during the post-intervention years in 2004–05 and 2005–06, but this reduction in the following years was not significant. Average MHD of this species in human dwellings with Olyset net village was 32.3 as compared to 37.8 and 33.7 in untreated net and without net villages during 2003–04 in the pre-intervention year. The average MHD of *An. culicifacies* in the Olyset net village declined to 10.3, 8.3 during 2004–05 and 2005–06 but the average MHD increased to 20 and 32.5 per man hour during 2006-07 and 2007–08. In the untreated net and without net villages, the average MHD during 2004–05, 2005–06, 2006–07 and 2007–08 was 22.6, 21.1, 26.5 and 48.3; and 39.6, 24.6, 27.7 and 64.1, respectively. The data clearly indicate the impact of Olyset nets on the reduction of resting density of *An. culicifacies* during the first two years in post-intervention period, while the difference in the MHD during the third and fourth year was much less (Fig. 23).

Epidemiological data of three study villages revealed almost complete interruption in the malaria transmission in the experimental village.

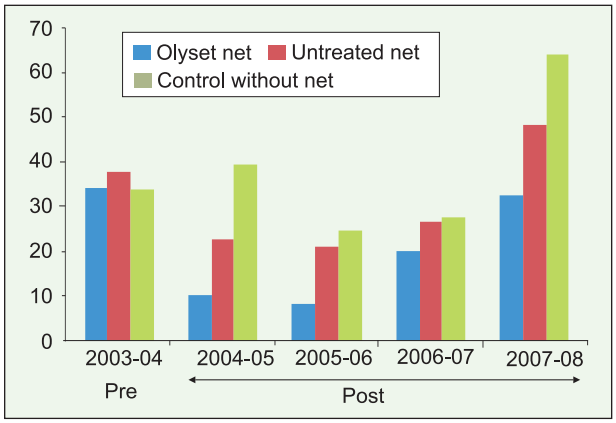

Fig. 23. Long-lasting efficacy of OlysetNet on indoor resting density of An. culicifacies in the study villages in Dadri PHC, Distt. Gautam Budh Nagar

Parasite incidence PI (cases per 1000 population) in village with Olyset net during 2003–04 (pre-intervention year) was 39.5, which declined to 1.5, 0, 2.5 and 0 during 2004–05, 2005–06, 2006–07 and 2007–08 in the post-intervention years. In the villages provided with untreated net and without net, the PI during 2003–04 during the pre-intervention year was 44 and 19 as compared to 6.1, 3.8, 8.8 and 3.8 during 2004–05, 2005–06, 2006–07 and 2007–08 in the plain net village and 19.5, 11.5, 10 and 8.5 in without net village (Fig. 24). Results indicate long-lasting impact of Olyset net in interrupting the malaria transmission.

1.3.6 Extended phase III evaluation of PermaNet® 2.0 against malaria vectors and disease transmission

Permanet® 2.0 deltamethrin-treated LLIN mosquito nets have already undergone Phase II entomological evaluations to demonstrate bio-efficacy and wash resistance in the field against malaria vectors in three different areas in India. The present study on field evaluation of Permanet® 2.0 against malaria vectors and disease prevalence was initiated in the endemic areas of District Gautam Budh Nagar, in western U.P. in April 2007 and in tribal areas of Orissa in August 2007, following uniform protocol of NIMR(ICMR). Phase III evaluation of Permanet® 2.0 against malaria vectors and disease prevalence in the endemic areas of District Gautam Budh Nagar, in western U.P. and in tribal areas of Sundargarh district, Orissa was extended for two years beyond initial trial period of one year. The trial in U.P. was initiated in April/May 2007. Three villages with population of 1187, 1165 and 1337 with similar malaria endemicity, topography and mosquito prevalence in District Gautam Budh Nagar, U.P., where malaria is transmitted mainly by *An. culicifacies* and *An. stephensi* were randomly selected for the phase III evaluation of Permanet® 2.0. Entomological and epidemiological parameters as per uniform protocol were monitored following the standard procedures.

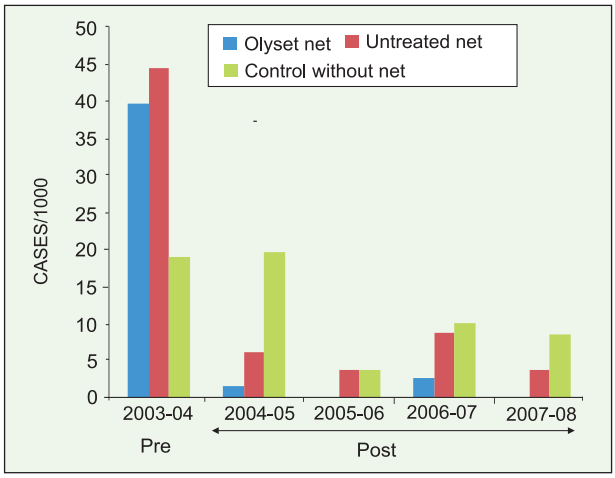

Fig. 24. Long-lasting impact of Olyset net on malaria parasite incidence (cases/000) in study villages

The efficacy of PermaNet® 2.0 as determined by cone bioassays after one and half year of field use revealed >95% knock down and 90–100% mortality in 24 hours (Table 4), but the ring net bioassays showed an increase in the median knock down time from 5 min on unwashed nets to 14 min after one and half year of use in field.

Results revealed that the per man hour density of *An. culicifacies* during pre-intervention period in Permanet, untreated net and without net villages ranged from 10–13, 24–27 and 8–12 respectively. With the commencement of intervention, there was a sharp decline in the density of *An. culicifacies* in June 2007, whereas the density in the untreated and without net control villages did not decline in June 2007. However, there was an increase in the resting density of *An. culicifacies* in all the villages during the monsoon and post-monsoon period of August to November 2007, but the build up of *An. culicifacies* density was much higher in the control

Table 4. Efficacy of PermaNet® 2.0 against *An. culicifacies* (Cone bioassay) after different intervals of use in field

Period	No. of washes	Exposure time (min)	%Knock down 1 h	%Mortaliy 24 h
Aug–Oct	0	3	100	100
2007	1	3	100	100
	2	3	100	100
	3	3	100	100
	4	3	100	100
Sep–Oct	3	3	100	100
2008	4	3	95	90
	5	3	95	95

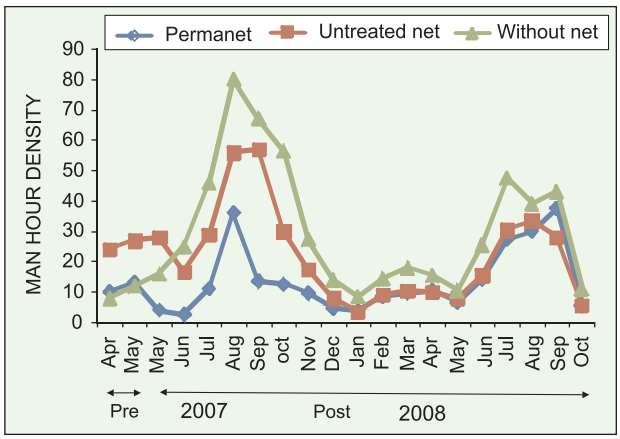

Fig. 25. Efficacy of PermaNet® 2.0 on indoor resting density of *An. culicifacies* in the study villages of Dadri PHC, Distt. Gautam Budh Nagar, U.P.

villages as compared to Permanet village (Fig. 25). The parity rate of *An. culicifacies* was low in Permanet village as compared to untreated net and without net villages. The parity rate of *An. culicifacies* in June 2007 in the first month during post-intervention period in Permanet, untreated net and without net villages was 20, 66.6 and 60% respectively.

Comparison of malaria incidence data showed that during pre-intervention period of April–May 2007, the parasite index (PI) or number of cases per 1000 population in the treated net villages was 2.5 and in the control villages with untreated nets and without nets was 1.7 and 2.9 respectively. Fig. 26 shows month-wise incidence of malaria parasite (PI) in villages with Permanet 2.0, untreated net and without net in Dadri PHC in Distt. Gautam Budh Nagar (U.P.). There was no significant difference in malaria endemicity in the trial and control villages. During intervention phase, the malaria incidence in the treated net villages was much less in the untreated net and without net population.

The compliance rate of the net usage in the

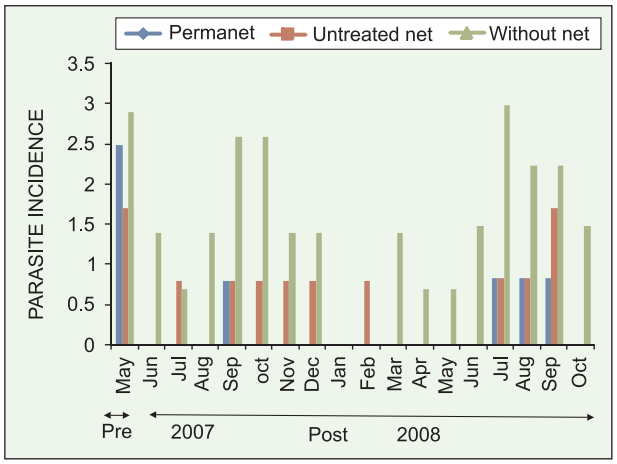

Fig. 26. Monthwise incidence of malaria parasite (PI) in villages with PermaNet 2.0, untreated net and without net in Dadri PHC in Distt. Gautam Budh Nagar (U.P.)

trial and control villages was ascertained through random checking of houses and recording of people sleeping under mosquito nets. There was 85–99% compliance rate of net usage in the study population during different months. The community perceptions on adverse effects and collateral benefits of long-lasting insecticide net Permanet 2.0 were assessed by conducting cross-sectional survey among the users (N=394, M-200, F-194) of Permanet 2.0. Almost every respondent asserted that they are sleeping under the treated nets. There were minimal complains of skin irritation (0.5%) and eye irritation (0.25%). However, these effects were only temporary lasting for few hours of the first usage. Majority of the respondents enthusiastically reported that LLIN Permanet 2.0 provided them relief not only from mosquitoes but also from other household pests such as head lice, bedbugs, cockroaches, ants and houseflies. The compliance (usage) rate was declined in the second year but still >80% usage of LLIN in the study population during the transmission period in the months of August–October 2008 was observed. The study is still in progress.

1.3.7 Evaluation of Icon-Life® 0.2% deltamethrin-treated LLIN against malaria vectors and disease transmission in District Gautam Budh Nagar, U.P.

Icon-Life is a LLIN incorporated with 0.2% deltamethrin @ 65 mg a.i./m^2 (136 mesh) and 75 mg a.i./m^2 (200 mesh) made of polyethylene with 100 denier yarn size.

The study on evaluation of Icon-Life®, against malaria vectors was undertaken in three endemic areas, viz. District Gautam Budh Nagar, U.P., District Sundargarh, Orissa, and District Karbi-Anglong, Assam, following common protocol for uniform evaluation of insecticides for use in vector control.

The present study in District Gautam Budh Nagar, U.P. was initiated in May 2008 in three villages, viz. Gulawati Khurd (Icon-Life LLIN), Nangla Chamru (untreated bed net) and Nangla Nainsukh (without bed net) of Dadri PHC, with similar endemicity and mosquito prevalence and located at least 5 km apart from each other. Cone bioassay tests carried out on unwashed and washed nets revealed 100% mortality against *An. culicifacies*, the major malaria vector species in this area, even after 20 washings (Table 5). However, ring-net bioassays showed increase in knock down time on washed nets as compared to unwashed net. The study revealed reduction in entry (Table 6) and resting density and parity rates of *An. culicifacies*, the major malaria vector species in this area, during

Table 5. Effect of washing on the efficacy of Icon-Life® LLIN against *An. culicifacies* in cone bioassays

No. of washes	Exposure time (min)	% Knock down (1 h)	% Mortality 24 h
0	3	100	100
5	3	100	100
10	3	95	100
15	3	97.5	100
20	3	95	100

Four replicates were used with 10 mosquitoes in each replicate

Table 6. Total entry and excito-repellency of *An. culicifacies* in houses with Icon-Life® Net, untreated net and without net (during Aug–Oct 2008) intervention period in study villages in District Gautam Budh Nagar, U.P.

Intervention	Total entry	Excito-repellency (%)
Icon-Life® LLIN	25	8 (32)
Untreated net	80	1 (1.25)
Without net	118	0

the post-intervention period in the Icon-Life LLIN village as compared to the control (untreated net and without 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 7). The study is in progress.

1.3.8 Evaluation of *Cyperus rotundus* Linn (Cyperaceae) hexane extract of tuber of root for repellency against mosquito vectors

Hexane extract of tuber of plant *Cyperus rotundus* (Cyperaceae) was screened under laboratory conditions for repellent activity against mosquito vector *An. culicifacies* Giles species A (Diptera: Culicidae), *An. stephensi* Liston (Diptera: Culicidae), and *Cx. quinquefasciatus* Say (Diptera: Culicidae). Mosquito cage studies with tuber extract were used to determine the repellency effect on mosquitoes, and compared with DEET (NN Diethyl 1-3 methyl Benzamide, formerly known as diethyl 1-m-toluamide). Result showed that the tuber extract was effective in repelling of mosquitoes. Clear dose response relationships were established with the highest dose of 10% tuber extract evoking 100% repellency. The data of the repellency observed in different species is given in Table 8 and it is evident that overall repellency rates varied between 80–100% for different repellents concentrations (2.5, 5, and 10%). The extract was found potent to repel mosquitoes.

1.4 Vector surveillance

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

Dengue and chikungunya are upcoming major public health problems in India and control of the breeding of their 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.

The study was initiated in three dengue and chikungunya affected states of the country, viz. Delhi, Karnataka, and Kerala. 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.

Delhi

In Delhi, a total of 3100 houses–650 in Mayur Kunj, 570 in Valmiki Colony, 625 in Netaji Nagar, 625 in R.K. Puram and 630 in Railway Colony,

IEC activities of attracticide trial in Delhi

Table 7. Efficacy of Icon-life® LLIN on malaria prevalence in the study villages in Dadri PHC, Distt. Gautam Budh Nagar, U.P.

Period	Icon-Life®			Untreated net			Without net		
	BSE	SPR	SFR	BSE	SPR	SFR	BSE	SPR	SFR
Pre-intervention (May–July 2008)	44	25	0	34	20.58	0	42	16.6	0
Post-intervention (August–October 2008)	56	8.9	0	31	16.1	0	35	22.8	0

Table 8. Percent repellency of three mosquito vectors against hexane extract of tuber of root of *Cyperus rotundus* Linn

Species	Doses %	Repellency in hours				
		0 h	1 h	2 h	4 h	6 h
An. culicifacies	Exract 2.5	80	75	88	90	95
	Extract 5	88	90	90	96	99.2
	Extract 10	97	96	97	100	100
	DEET 2.5	98	100	100	99	100
An. stephensi	Extract 2.5	84	80	85.3	90	96
	Extract 5	89	88.7	92.0	95	99
	Extract 10	99.6	93.0	93.0	99	100
	DEET 2.5	96.7	100	90	99	100
Cx. quinquefasciatus	Extract 2.5	83.5	75	82.3	91.7	95.8
	Extract 5	89.2	89.7	93	95	99
	Extract 10	89.6	96	95.5	99	100
	DEET 2.5	96.6	100	100	96.7	100

Tughlakabad were selected for ovitrap experiment setting. The ovitraps were maintained by surveillance workers and data on the positivity of ovitraps were collected from each mentioned colony. The weekly results of positivity of the ovitraps were recorded. The results revealed that the positivity of experimental and control ovitraps vary from locality to locality and the breeding has increased from the month of June 2008 in both the ovitraps.

Bengaluru

In Bengaluru City, three locations–Ashok Nagar, Kanteerava Nagar, and Narayanpura & Sanjay Gandhi Nagar were selected for the study. A total of 3043 houses, i.e. 1026 in Ashok Nagar, 1014 in Kanteerava Nagar and 1003 in Narayanpura plus Sanjay Gandhi Nagar was selected for ovitrap setting. In each house two treated and one untreated ovitraps were kept. These ovitraps were monitored for mosquito breeding and maintained on weekly basis by the surveillance workers posted in each area. The results revealed that the positivity of ovitraps in experimental and control areas vary from locality to locality. In Narayanpura, percent positivity in experimental area was found more in comparison to control. In other areas, the trends of the positivity of ovitraps in both experimental and control areas were found similar to Delhi.

Kerala

In Alapuzha district of Kerala, Ward Nos. 6, 7 and 8 of CHC Muhamma, 4, 11 and 12 of PHC Kadakkarapally and 8, 9 and 12 of PHC Vettackal area of Sherthallai taluka have been selected. House-to-house visits were made in these areas to select appropriate houses to educate householders the objective of the study and to solicit their cooperation. A total of 1421 houses were selected. Ovitraps (control and experimental) earmarked for the purpose were 2082, 1908 and 1996 for Vettackal, Kadakarapally and Muhamma respectively. In total, 5986 ovitraps were placed in houses for control and experimental areas. Bedrooms, living rooms, and bathrooms are the locations included for keeping ovitraps within houses.

Monitoring and retreatment were carried out on weekly basis to assess the usefulness of C-21 ovitraps for surveillance and control of dengue and chikungunya mosquitoes. Percent breeding in experimental and control ovitraps was recorded. Besides, entomological indices were also recorded on monthly basis by undertaking random immature sampling in Muhamma, Kadakkarapally and Vettackal to find out the seasonal breeding potential of *Aedes* mosquitoes. In Alapuzha, the positivity varied from locality to locality and the percentage positivity in control ovitraps was less as compared to experimental ones.

The field experiments were also cross-checked by the team of DRDE, Gwalior along with NIMR

Interviewing the households on attracticide

officials on regular basis and a mid-term review was also undertaken in June 2008.

Besides field trials, IEC material was also published by NIMR for community awareness. The analysis of the results of all the three study areas (Delhi, Bengaluru and Alapuzha) is in progress as the project has been extended by the Ministry up to March 2009.

1.4.2 *Aedes* breeding survey in Delhi

On the request of Municipal Corporation of Delhi (MCD) and New Delhi Municipal Corporation (NDMC), *Aedes* breeding survey was carried out during February–November 2008 in different localities, viz. R.K. Puram, Tughlakabad, Uttam Nagar, Janak Puri, Nazafgarh, PNT Colony, Govt. Offices, Schools, Nurseries, Parks, Picnic Spots, Police Stations, Bus Depots, Dispensaries, Hospitals, etc. From the survey, it was found that breeding was more in the month of July (C.I. 42.27) as compared to August (C.I. 32.40), September (C.I. 3.86) and November 2008. The survey also revealed that breeding was more in peridomestic containers (solid waste) as compared to domestic containers (coolers and OHTs).

The results of positivity of different breeding sites in different localities of Delhi were analysed.

Potential breeding habitats of Ae. *aegypti* mosquitoes in Delhi

The results of *Aedes* breeding were compared with dengue cases and the information was provided to the NVBDCP/MCD/NDMC/Delhi Administration for necessary action.

1.5 Insecticide resistance

1.5.1 Molecular basis of knock down resistance in *Anopheles culicifacies*

Anopheles culicifacies s.l., a major malaria vector in India, has developed widespread resistance to DDT and is becoming resistant to pyrethroids–the only insecticide class recommended for the impregnation of bed nets. Knock down resistance due to a point mutation in the voltage gated sodium channel at L1014 residue (*kdr*) is a common mechanism of resistance to DDT and pyrethroids. The selection of this resistance may pose a serious threat to the success of the pyrethroid impregnated bed net programme. We, therefore, molecularly characterized the voltage gated sodium channel of *An. culicifacies* spanning IIS4-IIS5 linker to IIS6 segments to find out the mutation, if any, responsible for *kdr* resistance.

1.5.2 Molecular characterization of Voltage Gated Sodium Channel

Last year we sequenced genomic DNA corresponding to IIS4-IIS5 linker to IIS6 segments of the para type voltage gated sodium channel and demonstrated L1014F mutation. This year we determined the cDNA sequences of species A, B and C of the Culicifacies Complex. We found two introns, the size of intron I in species A was 1001 bp and of B and C were 1011 bp, whereas intron II in all the three species was 62 bp only.

1.5.3 The *kdr* gene frequency in *Anopheles culicifacies* populations

Last year we demonstrated L101F mutation in *An. culicifacies* at *kdr* locus and reported development of three high throughput PCR-based methods for the identification of *kdr* mutation (L1014F), viz. Allele Specific PCR (ASPCR), Amplification Refractory Mutation System (ARMS) and Primer Introduced Restriction Analysis PCR (PIRA-PCR) assays. We screened following populations using above mentioned methods.

Surat population

An. culicifacies in District Surat is resistant to all commonly used insecticides including pyrethroids. A total of 186 samples were genotyped using ASPCR of which 167 were homozygous susceptible (SS), one was homozygous resistant

(RR) and 18 were heterozygotes. All of the RS and RR and 20 SS samples were subjected to the ARMS and PIRA-PCR assays, and results similar to the ASPCR were observed. Thus, all the three assays are specific and can be used for genotyping. The observed and expected heterozygote frequencies were 0.09677 and 0.10202 respectively, which did not differ significantly (p=0.41220) from Hardy-Weinberg equilibrium.

Bilaspur population

We screened another population of *An. culicifacies* from Bilaspur for the presence of *kdr* mutation which is susceptible to pyrethroids but resistant to DDT. Out of total 104 samples tested, we found only seven individuals (6.7%) having RS genotype, rest were SS and no homozygous RR was found. The genotypes were in Hardy-Weinberg equilibrium (p=0.93). This result suggests presence of *kdr* mutation in the population resistant to DDT only.

1.6 Genomics and phylogenetics

1.6.1 Population genomics of Indian *Anopheles minimus* species A

The study focuses on inferring population genetic structure using genetic diversity data on the single nucleotide polymorphisms (SNPs) in the most efficient malaria vector of the north-eastern states of the country, *An. minimus*. About 10 different population samples across the north-eastern India have been proposed to be used in this study and so far five population samples have been collected (Fig. 27). Since, sequence information is unknown in this species, whole genome sequence information on the most closely related species of African malaria vector, *An. gambiae* has been utilized to design DNA markers in non-coding nuclear genetic regions of *An. minimus*. For this, we scanned the whole X-chromosome of *An. gambiae* for homologous genes and designed exon priming intron crossing (EPIC) primers (Fig. 28). As many as 30 such primer pairs were designed based on *An. gambiae* sequence and tested for amplification in *An. minimus* genomic DNA, and so far we are able to successfully amplify and sequence only five fragments (Fig. 29). For the rest 25 fragments we could not get any or proper amplification for sequencing. The obtained sequences were edited and aligned to identify SNPs

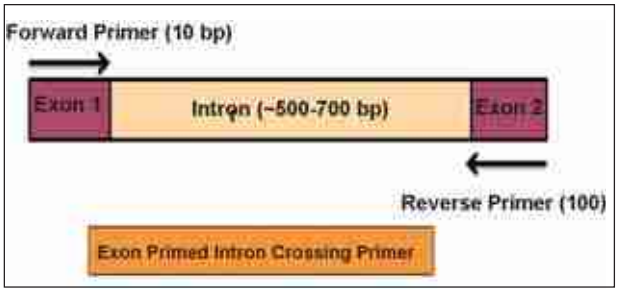

Fig. 28. Figure depicting the EPIC primer designing strategy

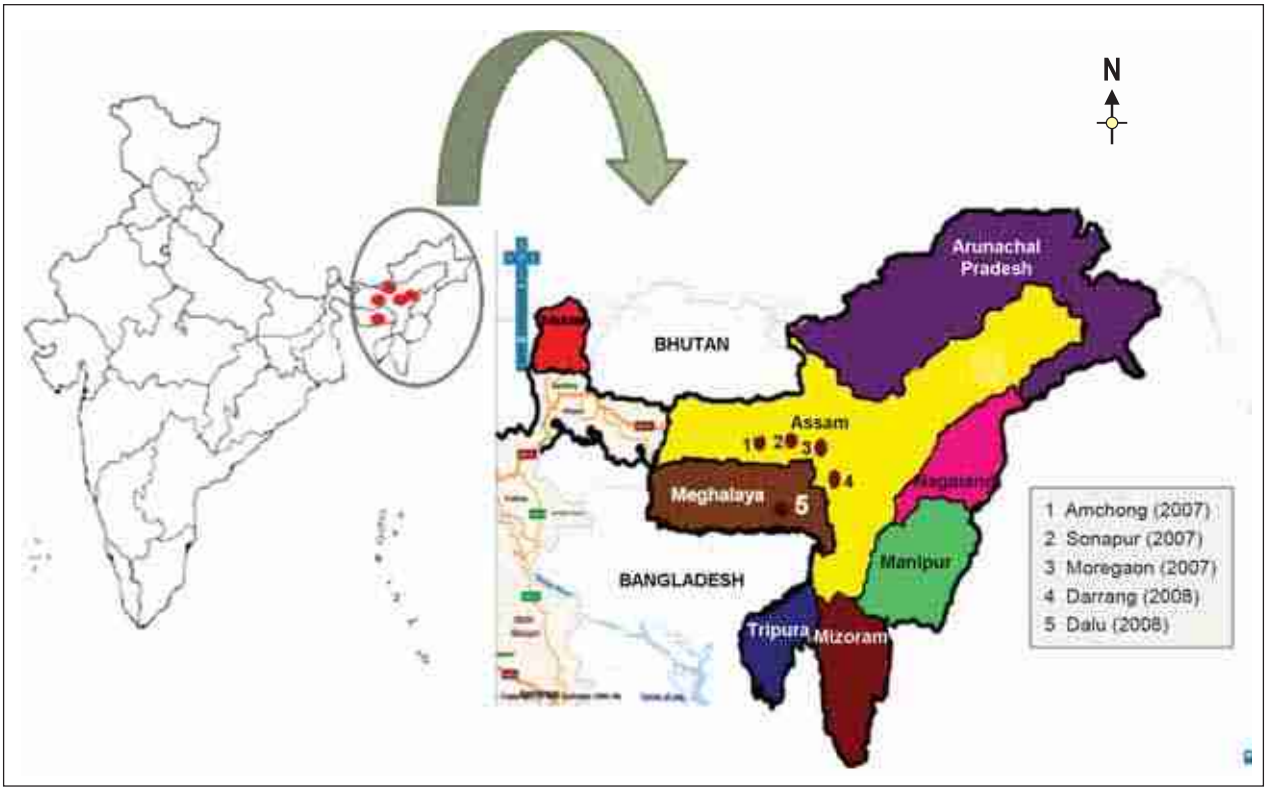

Fig. 27. Locations of An. minimus sample collection sites in the north-eastern regions of India

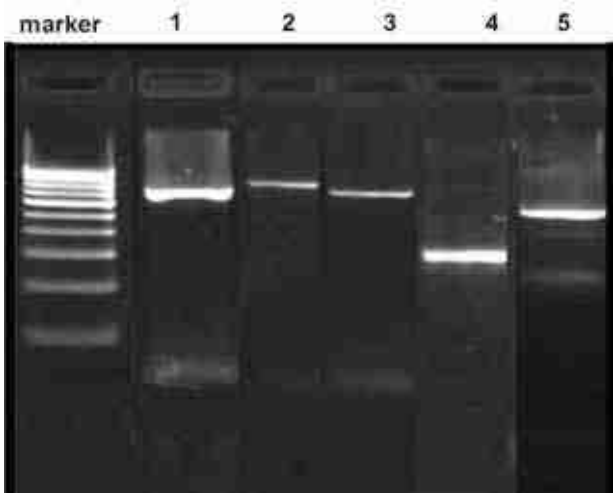

Fig. 29. PCR amplified DNA fragments in nuclear genome of An. minimus

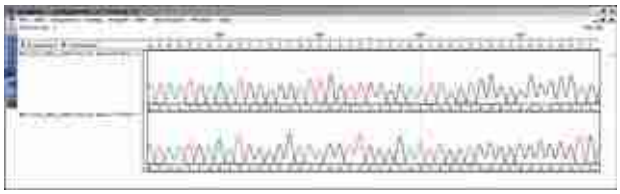

Fig. 30. Sequence chromatogram of a DNA fragment in Indian An. minimus

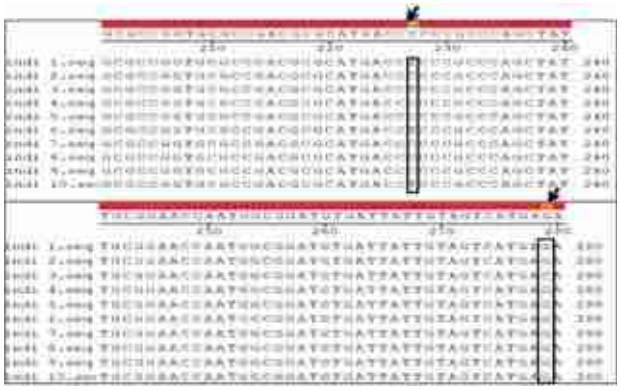

Fig. 31. DNA sequence alignment and SNP detection in Indian An. minimus

(Figs. 30 and 31). Four out of five DNA markers were found to be polymorphic (bearing SNPs) and one was completely monomorphic. Thus, we have utilized the SNP information in these four DNA markers for estimating population genetic parameters in *An. minimus* in all the five populations following different statistical methods and molecular evolutionary model testing.

1.6.2 Multilocus DNA sequences reveal phylogenetic status of Indian malaria vectors

Inference on the taxonomic positions and phylogenetic relationships among closely related species of health importance is essential to devise disease control measures. To this respect, malaria is one of the important mosquito borne diseases of tropical and sub-tropical parts of the globe. India contains one of the richest resources of mosquito species diversity, however, little molecular taxonomic information is available on Indian malaria vectors. The present study focuses on inferring the phylogenetic interrelationships among six Indian malaria vector species utilizing multilocus approach. Since, whole genome sequence information of only *An. gambiae* is available so far, we used sequence information of *An. gambiae* to amplify and sequence six orthologous nuclear genetic regions, i.e. (1) Carboxyl esterase (COE), (2) Cytochrome P450 (CYP450), (3) Nitric oxide synthase (NOS), (4) Glutathione S-transferase (GST), (5) Nicotinamide adenine dinucleotide phosphate (NADPH), and (6) Glass bottom boat (Gbb 60 A). The earlier reported sequence information of the two loci (COII and ITS2) was also utilized (making the total number of loci to eight). The sequence information of all these eight loci were used to infer the phylogenetic status of six Indian malaria vector species *An. culicifacies, An. minimus, An. sundaicus, An. fluviatilis, An. annularis* and *An. stephensi* (Table 6). Although tree topologies with COII, ITS2 and COE genes were similar, for no other five genetic regions, similar tree topologies were observed (Figs. 32 and 33). In general, the reconstructed phylogenetic status of Indian malaria vectors followed the pattern based on morphological and cytological classifications that was reconfirmed with COII, ITS2 and COE genetic regions. Further, divergence times

Table 6. Details of *Anopheles* species and sample collection sites

Anopheles species studied	Taxonomic series	Location of samples	Population coordinates
An. minimus	Myzomyia	Moregaon, Assam	26º5´ N 92º´E
An. culicifacies	Myzomyia	Hardwar, Uttarakhand	29º96´ N 78º16´E
An. fluviatilis	Myzomyia	Bilaspur, Chhattisgarh	22°02´ N 82°15´E
An. sundaicus	Paramyzomyia	Car Nicobar, A& N Islands	9°10´ N 92°452´E
An. stephensi	Neocellia	Hardwar, Uttarakhand	29º96´ N 78º16´E
An. annularis	Neocellia	Hardwar, Uttarakhand	29º96´ N 78º16´E

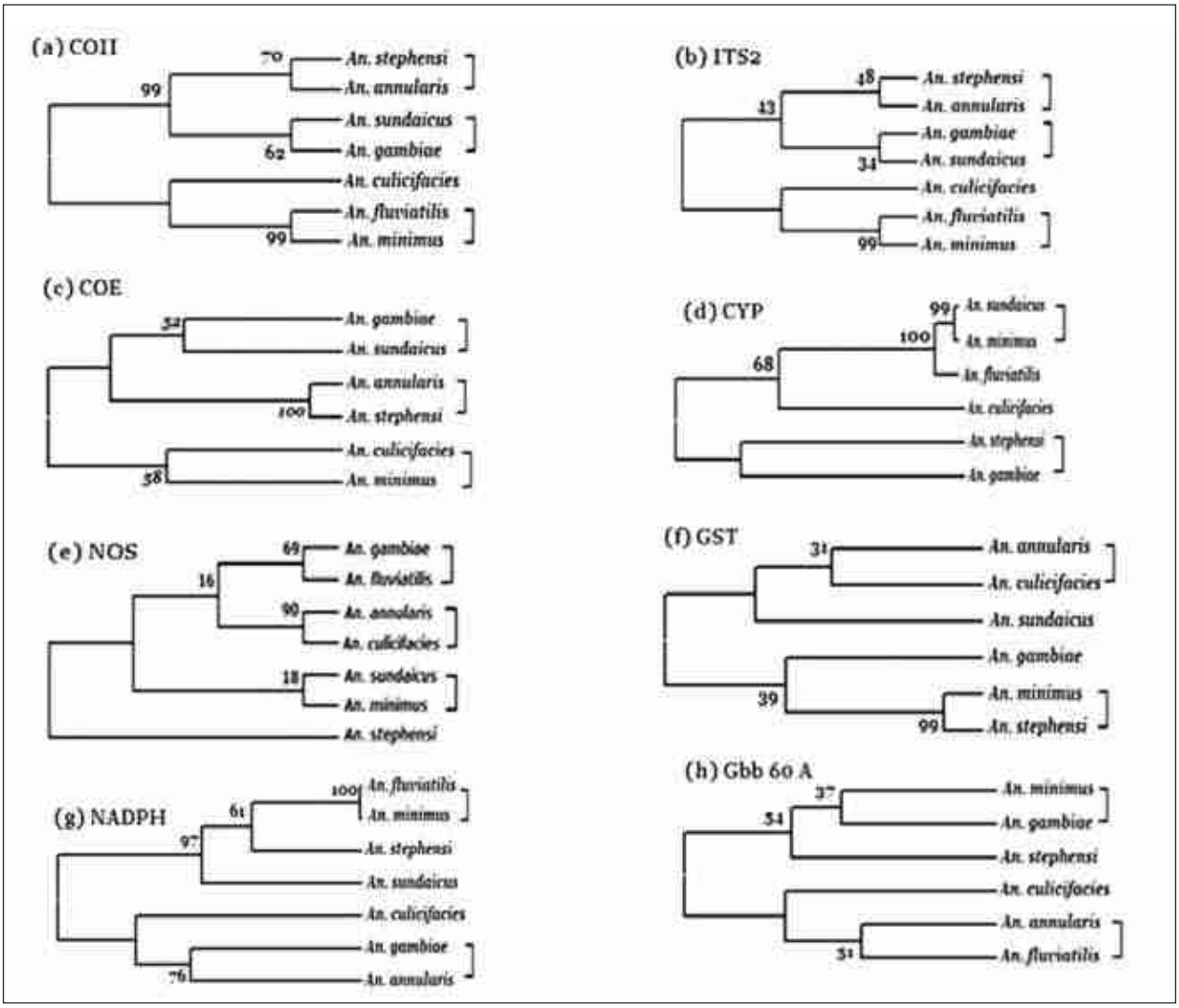

Fig. 32. Consensus trees resulting from maximum parsimony analysis based on: (a) mitochondrial COII (Cytochrome Oxidase II) gene sequences from seven species, (b) nuclear non-functional ITS2 (Internal Transcribed Spacer 2) sequences data from seven species, (c) COE (Carboxyl Esterase) gene sequences from six species, (d) CYP 450 (Cytochrome P450) gene seque nce data from six species, (e) NOS (Nitric Oxide Synthase) gene sequences from seven species, (f) GST (Glutathione S Transferase) gene sequence data from six species, (g) NADPH (Nicotinamide Adenine Dinucleotide Phosphate) gene sequence data from seven species, (h) Gbb 60 A (Glass Bottom Boat) gene sequences from six species. Tree was obtained by Max-mini branch and bound search option of MEGA 4. Bootstrap values from 1000 replications are indicated above each internode

based on COII gene sequences were estimated between each pair of Indian species which corroborate the earlier hypothesis on the radiation of *Anopheles* species during the cretaceous period.

1.6.3 Evolutionary genetics of insecticide resistance gene families in *Anopheles gambiae*

Insecticide resistance mechanism developed by vector species is one of the major obstacles in vector and disease control strategies and is known to be genetically controlled. Three major gene families (CYP, GST and COE) have been identified which encode various proteins to metabolize endogenous as well as exogenous compounds in insects and are responsible for the insecticide resistance mechanisms (Fig. 34). Since,

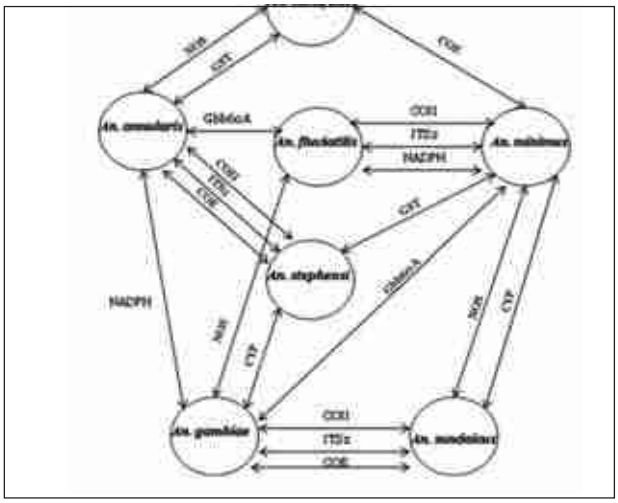

Fig. 33. Phylogenetic relationships among seven vector species (indicated as arrows). Each arrow indicates single monophyletic relationship between the two species inferred through a gene tree (named besides the arrows)

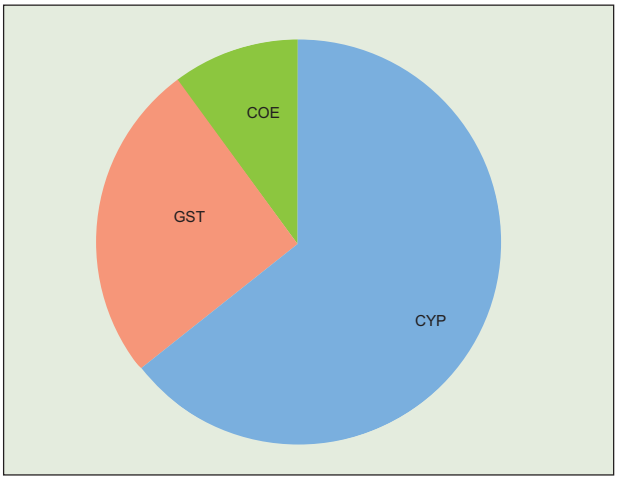

Fig. 34. Contribution of each insecticide resistance gene family in genome of *An. gambiae*

insecticides are in excessive use (and misuse) in the field putting enormous pressure for the evolution of more suitable and efficient insecticide resistance mechanisms in insects, it is important to have fair knowledge on how insecticide resistance genes have been evolving? This is of prime importance to malaria research, as vector control and thus to control malaria has been grossly hampered by emergence and evolution of insecticide resistance in malaria vectors. We herewith report the evolutionary pattern of all three insecticide resistance gene families utilizing whole genome sequence information of the African malaria vector, *An. gambiae*. The pattern of conservation of insecticide resistant genes across various taxa has been determined to infer the present evolutionary status of the three gene families (Fig. 35). Each gene of three insecticide resistance gene families was mapped at the chromosome to study the distribution in the genome of *An. gambiae* (Fig. 36). Intron organization was also studied to understand that how the non-codeing regions play a part in the evolution of these multigene families (Figs. 37 and 38). Further, phylogenetic relationship among genes of each gene family has been inferred. This predicted genetic closeness among genes, along with the identified location of genes on chromosomes has provided evidences for mode of expansion of gene families in genome (Figs. 39–41). The events of gene conversion were also detected in the insecticide resistant gene families. Further, genetic architecture of genes from all three gene families were compared to draw the differential evolution of insecticide resistance gene families.

1.6.4 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

A multiplex PCR assay was developed using the sequences of the D2 region of 28S ribosomal DNA (rDNA) to discriminate the five members of the *Anopheles culicifacies* complex provisionally designated as species A, B, C, D and E. Two minus strand primers derived from sequence differences in the D2 variable region and a universal plus strand primer derived from the conserved 28S (rDNA) has delimited five members into species A

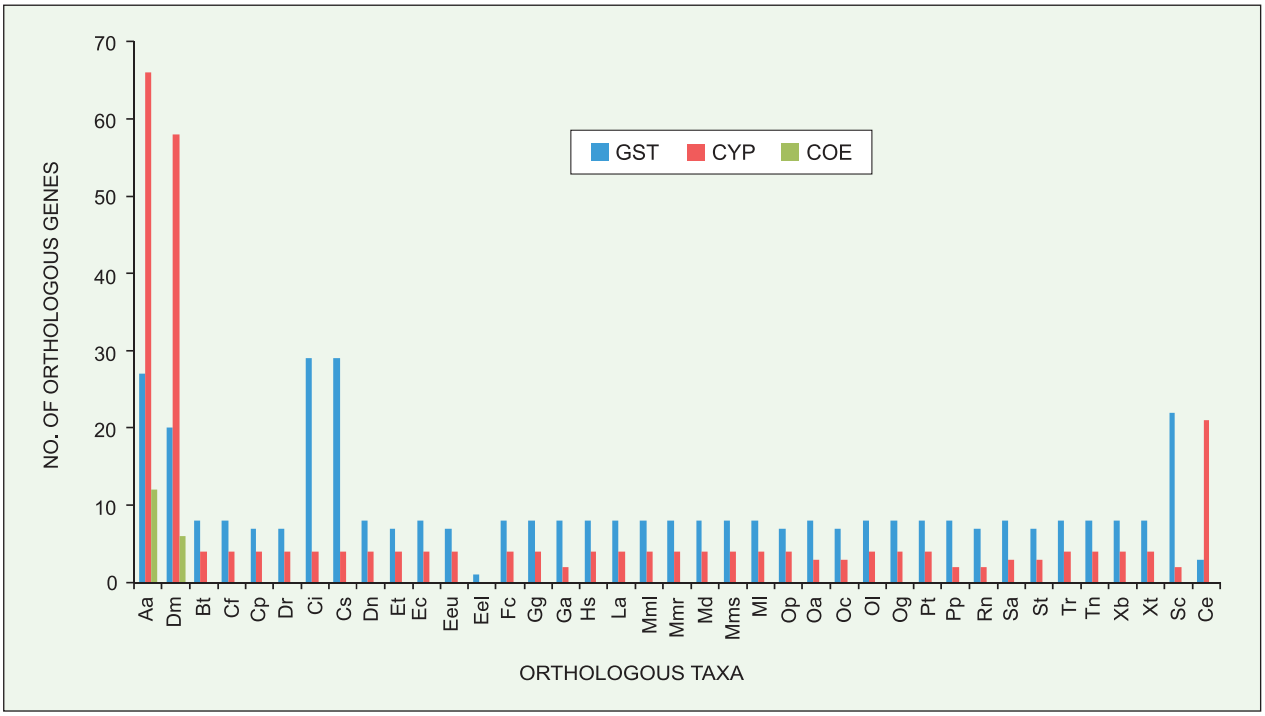

Fig. 35. Orthology of *An. gambiae* insecticide resistance genes in 39 different taxa in each gene family

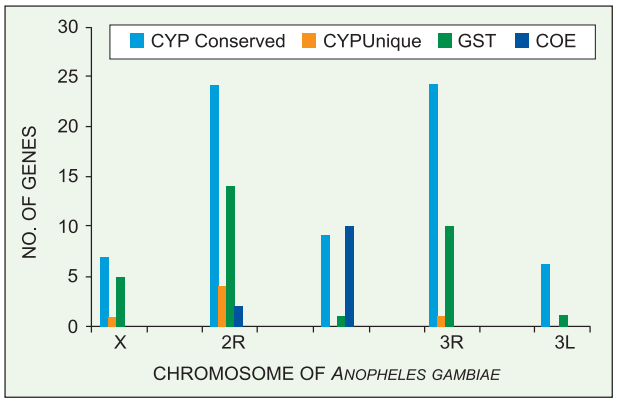

Fig. 36. Distribution of insecticide resistant genes of CYP, GST and COE gene families on different chromosome of *An. gambiae*

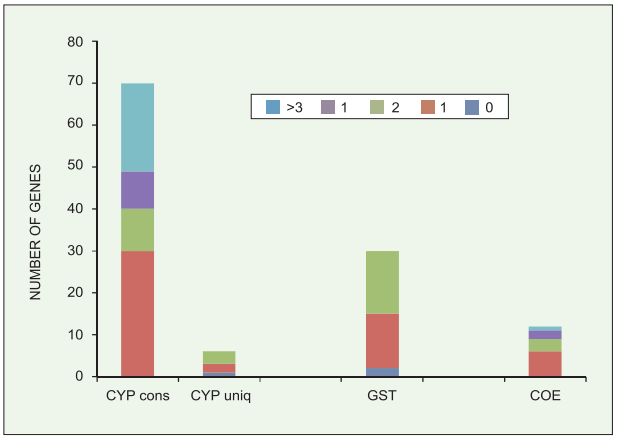

Fig. 37. Classification of insecticide resistance gene families in *An. gambiae* on the basis of intron number

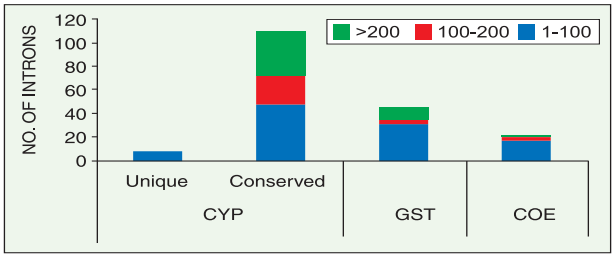

Fig. 38. Classification of introns of each insecticide resistance gene family in *An. gambiae* on the basis of intron length

and D (Group 1) and species B, C and E (Group 2) in a PCR diagnostic assay (Fig. 42). The complete 28S rDNA-D2 region sequence of *An. culicifacies* sibling species is reported for the first time. Inter-specific sequence divergence was greater than the intra-specific divergence. The phylogenetic relationships inferred from maximum likelihood, maximum parsimony and the neighbour joining analysis confirmed the presence of two unambiguous monophyly clades, one consisting of species A and D and the other of species B, C and E and that the *An. culicifacies* sibling species diverged relatively recently in evolutionary terms despite their considerable differences in bionomics.

1.6.5 Reanalysis of rDNA-ITS2 region sequences of *Anopheles cf. culicifacies* 'Bluchistan' revealed conspecificity to *Anopheles dthali*

Anopheles cf. culicifacies 'Bluchistan' was reported as a new variant of *An. culicifacies* species complex based on internal transcribed spacer 2 (ITS2) region by Djadid and Saifi in

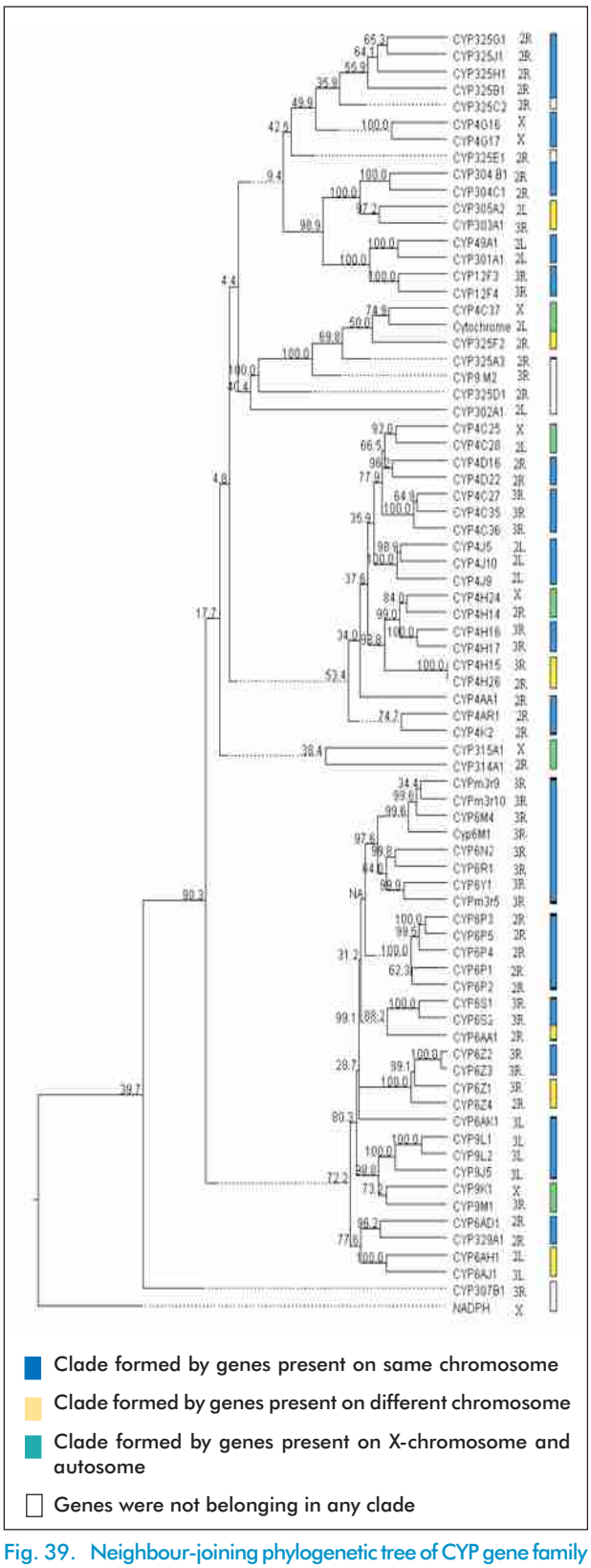

Fig. 39. Neighbour-joining phylogenetic tree of CYP gene family

Fig. 40. Phylogenetic relationship based on neighbour- joining method between genes of GST gene family

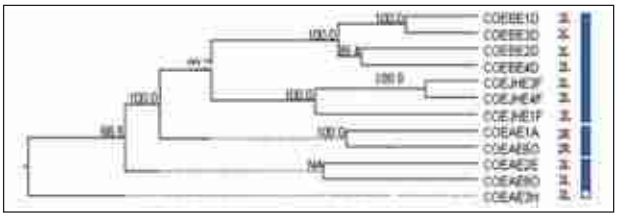

Fig. 41. Neighbour-joining phylogenetic tree showing phylogenetic relationship among all genes of COE gene family

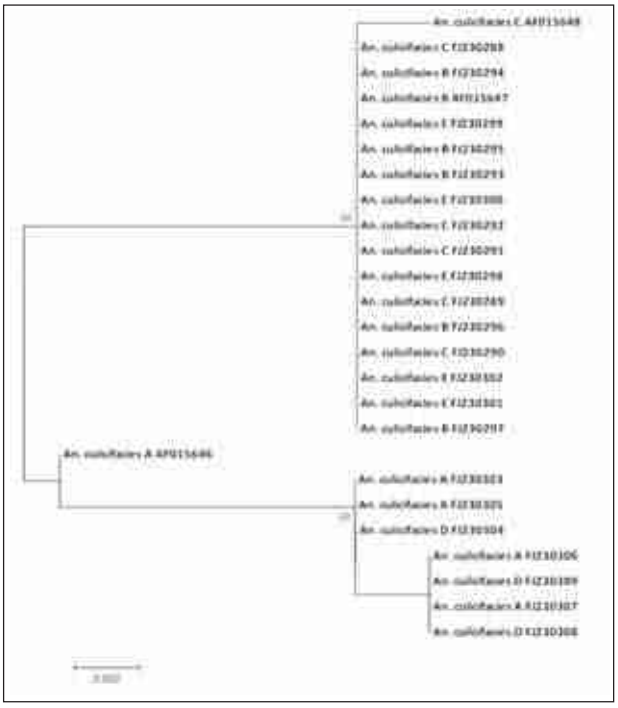

Fig. 42. Phylogenetic analysis of 28 S D2 region distinguishing five members of *An. culicifacies* complex into two groups– Group 1 (A and D), and Group 2 (B, C and E)

2001 (GenBank accession No. AF 402296) and was later stated to be phylogenetically close to *An. culicifacies* species A. Though comparison of ITS2 sequences of the members of *An. culicifacies* species complex with *An. cf. culicifacies* 'Bluchistan' revealed appreciable polymorphism to state the existence of new species, local alignment search and phylogenetic analysis results showed the conspecificity to *An. dthali*. In summary, the present study gives a case report of misidentification of a species that highlighted the importance of initial morphotaxonomical identification before conducting the computational molecular phylogenetic studies. Such misidentification may also sometimes lead to the suggestion of wrong vector control strategies for disease management.

1.6.6 *In silico* analysis of cytochrome P450 super gene family from *An. gambiae*

A total of 104 putatively active cytochrome P450 (CYP450) sequences were retrieved from *An. gambiae* genome based on conserved heme-binding sequence at C-terminal end. Of which 82% of genes belong to the CYP3 and CYP4 clans. The phylogenetic analysis grouped CYP450 sequences into the four unambiguously distinguishable clades, namely mitochondrial, CYP2, CYP3 and CYP4. The principal coordinate analysis has shown that mitochondrial and CYP2 clans share near sequence homology. The gene organization studies have shown that the mitochondrial, CYP2, CYP3 and CYP4 clans are having 6, 6, 3, and 2 families respectively. It was evident from gene cluster analysis that the CYP12, CYP4, CYP6 and CYP9 gene families have shown species-specific gene expansion. Except 20 out of 104 genes, all are localized in clusters. Two large gene clusters belong to the CYP325 and CYP6 gene family are localized on chromosome 2R (13 genes) and chromosome 3R (14 genes) respectively. The *An. gambiae* CYP450 sequences share less homology to the other related taxa except for mitochondrial and some members of CYP2 clans. While the function of CYP450 genes from *An. gambiae* is largely unknown, this super gene family was studied extensively for xenobiotic metabolism and juvenile hormone biosynthesis.

2 PARASITE BIOLOGY

2.1 Human malaria parasites: molecular characterization

2.1.1 *Plasmodium vivax* antigen genes diversity

Local antigenic variations are very crucial in the understanding of total antigenic repertoires in a country like India and in turn, planning effective vaccine-based control measures. Antigenic repertoires of *P. vivax* vaccine candidates were investigated in five widely separated geographical regions (Delhi, Panna, Kheda, Chennai and Kamrup) of India to understand the local antigenic repertoires.

2.1.1a PvMSP-1

PvMSP-1 block-5: Merozoite surface protein-1 (MSP-1) is an immunodominant antigen expressed on the surface of merozoites. MSP-1 is a potential asexual-stage vaccine candidate and induces protective immune responses in animal model. DNA sequence analysis of *MSP-1* genes derived from different *Plasmodia* species has identified conserved and semi-conserved blocks interspersed with polymorphic regions (Fig. 1). MSP-1 block-5 is characterized by three major alleles, namely Belem type, Sal-1 type and recombinant type based on amino acid sequence characteristics in the variable block-5.

Analysis of variable block-5 of *PvMSP-1* in 100 *P. vivax* isolates from five geographical regions

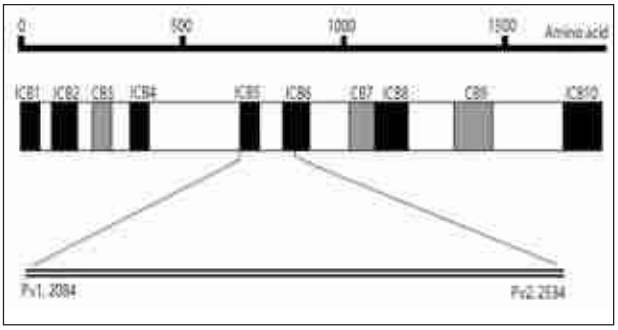

Fig. 1. Schematic representation of the *P. vivax MSP-1*. Boxes representing interspecies conserved blocks (ICBs), conserved blocks (CBs) and variable blocks are filled, hatched and opened, respectively. Sequence and location of the primers used for PCR amplification are indicated at the bottom

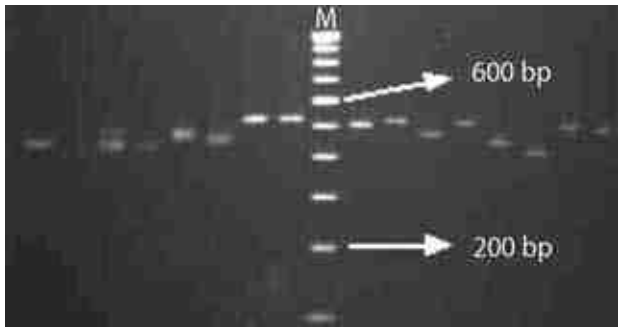

Fig. 2. Gel image of *MSP-1* block-5 in field isolates of Indian *P. vivax*. M: 100 bp DNA ladder

revealed highly polymorphic nature of *P. vivax* in the Indian populations. A total of 23 MSP-1 alleles (~400–600 bp) were found at PCR level (Fig. 2).

DNA sequence analysis of MSP-1 revealed presence of Sal-1, Belem and recombinant type alleles in the Indian population with high proportion (57.5%) of Sal-1 type, whereas, Belem (19.5%) and recombinant types (23%) were nearly equal and a novel recombinant type allele was also observed. Phylogenetic analysis revealed three significant clusters of Sal-1, Belem and recombinant type separately regardless of their origin in different populations (Fig. 3).

High sequence divergence was found in Belem and recombinant alleles of MSP-1 types than Sal-1 allele. Extensive sequence heterogeneity (Pi = 0.0975) was found at *MSP-1* block-5 revealed

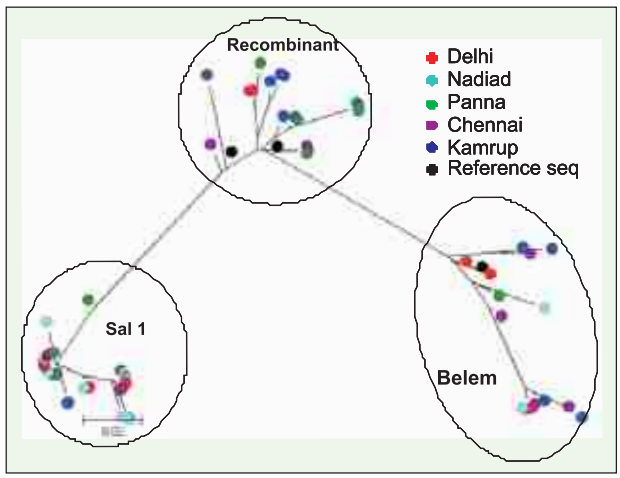

Fig. 3. Neighbour-joining phylogenetic tree derived from MSP-1 DNA sequences

substantial genetic variation in the Indian field isolates of *P. vivax*. The high rate of non-synonymous substitutions over synonymous substitutions (dN>dS) suggest that the *MSP-1* block-5 is under diversifying selection. High degree of genetic polymorphism observed in Indian *P. vivax* isolates at *MSP-1* block-5 locus indicates its potential as a molecular marker for relapse and therapeutic efficacy studies.

AMA-1 is a potential asexual stage vaccine candidate and has been well characterized from several species such as *P. falciparum, P. vivax*, and species of non-human primates and rodents. PvAMA-1 is an 82 kDa integral membrane protein with ecodomain organized in three domains stabilized by eight disulfide bonds (Fig. 4). AMA-1 is synthesized by merozoites and located in the neck of the rhoptry (electron dense organelle) and form apical complex at the time of invasion. In different malaria parasite species, nucleotide sequences of *AMA-1* are reported to be conserved between 54 and 86%.

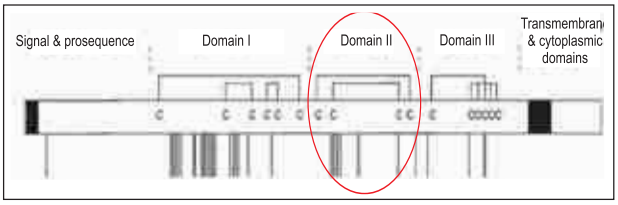

Fig. 4. Schematic representation of AMA-1 structure (domains). Analyzed domain II is encircled (red circle)

DNA sequence analysis of *PvAMA-1* from five geographical populations (100 isolates) indicated highly polymorphic nature of *AMA-1* with the signature of positive selection based on the ratio of non-synonymous and synonymous mutations in the study fragment. Data revealed high degree of haplotype diversity as well as sequence diversity. Phylogenetic analysis revealed two major clusters and several sub-clusters and each cluster was filled by isolates from each population suggesting no geographical clustering of *P. vivax* isolates (Fig. 5). We observed highest sequence divergence in Kamrup and lowest in Panna *P. vivax* 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, the New and Old world, distinguishable by gene conversion in the SSU rRNA S-type and mutations in an open reading frame (ORF 470) in the apicoplast genome. The

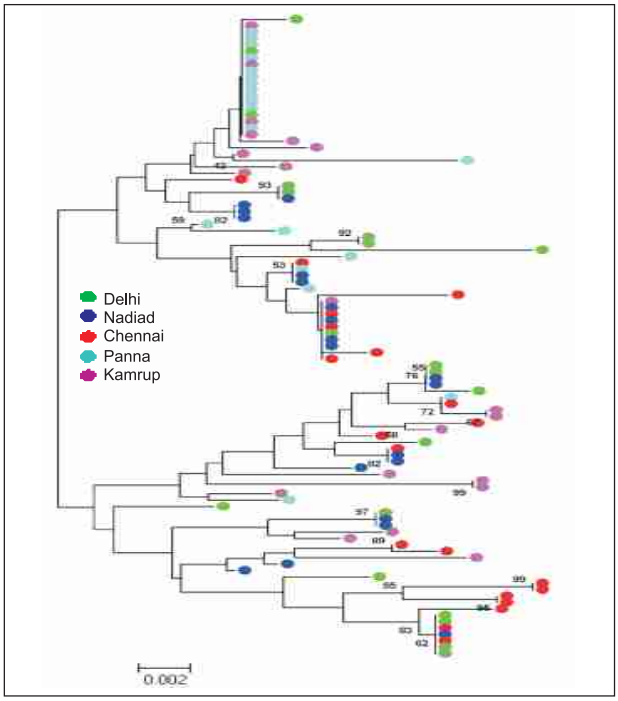

Fig. 5. Un-rooted phylogenetic tree derived from AMA-1 constructed using N-J method. Five hundred bootstrap replication simulations were set to generate consensus tree

distribution of two sub-types of *P. vivax* (Old world and New world) based on S-type 18S SSU rRNA was studied in field isolates from different locations in India. Distribution of both types of S-type 18S SSU rRNA was nearly equal among the study isolates; however, their proportions varied among isolates of different regions.

To understand the genetic structure and relatedness of two sub-types (Old world S type-1 and New world S type-2), multilocus genotyping was initiated. 'Old world' (50 isolates) and 'New world' (50 isolates) sub-types of *P. vivax* were characterized by using highly polymorphic antigen gene, *PvMSP-3α*. and Three PCR variants (Type A–1.9 kb; Type B–1.5 kb; Type C–1.1 kb) observed for *PvMSP-3α* were equally distributed in both sub-types of *P. vivax* isolates ($\chi^2 = 1.27$, df = 2, $p = 0.529$) and Type A variant was the most prevalent (Fig. 6).

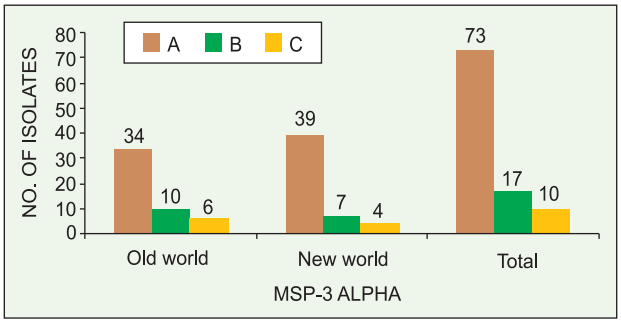

Fig. 6. Frequency of P. vivax MSP-3α PCR variants (A, B and C) among 'Old world ' and 'New world' sub-types in the Indian subcontinent

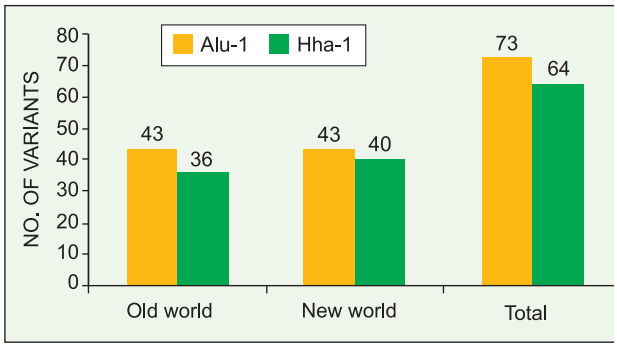

Fig. 7. Number of PvMSP-3α RFLP variants for Alu-1 and Hha-1 among 'Old world' and 'New world' sub-types in the Indian subcontinent

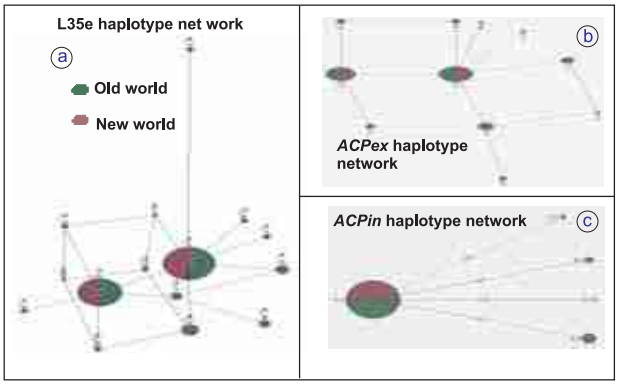

Fig. 9. Network diagram represents mutational relationship of *P. vivax* housekeeping genes haplotypes. Network diagram shows circles connected by single or more line, length of the connecting line is proportional to the number of mutational events, and size of the circle is proportional to the frequency of haplotype. Red and green colour in the circle represents proportions of 'New world' and 'Old world' isolates respectively. Figs. a, b, and c represent haplotype networks of *L35e, ACPex and ACP in,* respectively

Further, RFLP analyses of *PvMSP-3* revealed a high degree of genetic polymorphism and a total of 75 and 63 RFLP variants for Hha-1 and Alu-1, respectively, were observed (Fig. 7). Level of polymorphism observed in RFLP analysis was similar in both 'Old world' as well as 'New world' sub types of *P. vivax* (χ^2 = 1.12, df = 2, p = 0.738).

Analysis of *PvMSP-3α* suggested that both sub-types of *P. vivax* isolates are equally diverse in the population. These isolates were further characterized for single nucleotide polymorphisms (SNPs) in coding as well as non-coding regions of two housekeeping genes, *ribosomal protein L35e* and *Acyl carrier protein* (ACP) (Fig. 8).

A total of 22 SNPs were observed, of them 14 were neutral (5 synonymous and 9 non-coding) and eight were non-synonymous. Neutrality test, Tajima D and Fu & Li D revealed that both genes are neutrally evolving. SNP analysis of the isolates of the two sub-types of *P. vivax* revealed similar level of SNPs, nucleotide diversity (Old world = 0.00177, New world = 0.00201), and haplotype diversity in both sub-types.

Median-joining haplotype network of two putative housekeeping genes showed simple (single favoured haplotype and mutational path) to complex level (multiple favoured haplotypes and mutational path) of haplotype networks (Fig 9). However, mutational path (single or multiple) and favoured haplotype (single or multiple) are shared between both sub types of *P. vivax*. Specific mutational path and favoured haplotypes either for old world S type-1 or new world S type-2 was not observed in any of the haplotype network.

2.1.3 Origin and spread of CQ resistant *Plasmodium falciparum*

Our previous study on the analysis of different CQR-associated alleles (pfcrt haplotype) confirmed the spread of mutant parasite throughout the country and revealed prevalence of SVMNT pfcrt haplotype, i.e. South American type along with other haplotypes raising question on independent origin or import of prevalent South American pfcrt haplotype. To understand the origin and spread of the CQ resistant alleles in different malaria endemic areas of the country, we executed analysis of seven microsatellite loci around *pfcrt* gene (up to 24 kb upstream and +106 kb downstream) in 177 single clone *P. falciparum* isolates (Fig. 10). Microsatellite loci: –24 kb(B5M97), –20 kb(B5M77), –5 kb(2E10), +1 kb

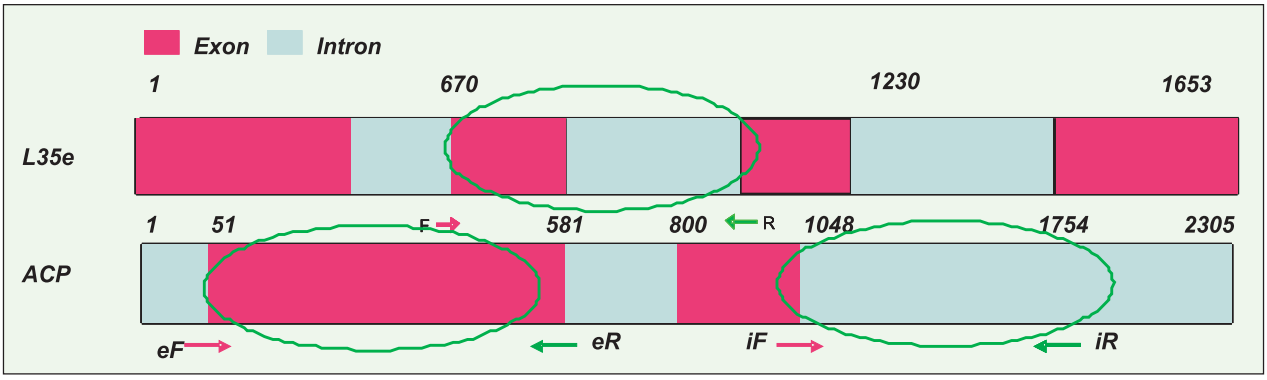

Fig. 8. Diagrammatic representation of housekeeping genes coding for *ribosomal protein L35e* and *Acyl Carrier Protein (ACP)* showing intron and exon. PCR amplified regions used for analysis are encircled (green circle) and arrows represent primer positions

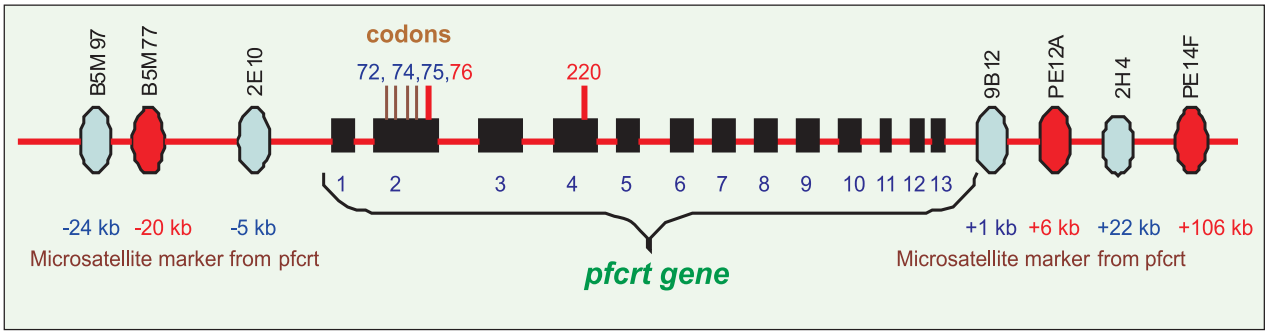

Fig. 10. Schematic representation of analyzed microsatellite loci located upstream and downstream of *pfcrt* genes

(9B12), +6 kb (PE12A), +22 kb (2H4), and +106 kb (PE14F), revealed a low allele heterozygosity at each locus of South American SVMNT pfcrt haplotype in comparasion to Southeast Asian CVIET pfcrt haplotype. We observed high allele heterozygosity in wild type CVMNK than the mutant pfcrt haplotypes. High microsatellite polymorphism was observed in parasites from high endemic areas in comparison to low endemic areas indicating high selection pressure in low endemic region. A clonal expansion of mutant SVMNT type is concluded in different low endemic regions.

2.1.4 Promotion of *Plasmodium* research and training in India

The project is primarily aiming at capacity building in malaria research at NIMR. The core aim of this proposal is the education and instruction of trainees at NIMR by a group of U.S. and Indian mentors and faculty having expertise in various areas of *Plasmodium* biology. This year we have organized the following three workshops which were attended by all grant trainees and other research students.

(1) A five-day workshop on 'Basic malaria parasitology and entomology' was conducted. The course curriculum included lectures and demonstrations on various aspects of malaria parasitology, entomology and epidemiology, including national drug policy and an introduction to the mandate and research activities of the NIMR. In addition to NIMR faculty, experts were invited from other national/ international organizations for the workshop.

(2) A workshop was held for developing 'scientific skills' among young researchers. It was conducted by Drs Steven Sullivan and Jane Carlton. The course curriculum included experimental design and troubleshooting, use of the NIH/NCBI Entrez literature database on the World Wide Web, efficient note-taking from scientific papers, preparation of figures and first drafts of scientific papers, and oral presentations.

(3) A workshop on '*P. vivax ex-vivo* maturation' was conducted by Dr Bruce Russell of the Singapore Immunology Network and A*STAR, Singapore. It aimed at practical demonstration of *ex-vivo* maturation of *P. vivax* stages from ring to trophozoite to schizont (Fig. 11). The course curriculum also included basic concepts of antimalarial drug resistance and the *ex-vivo* maturation of *P. vivax* with special reference to drug sensitivity testing.

Fig. 11. Demonstration of Leukocytes removal from blood during '*P. vivax ex-vivo* maturation ' workshop

2.1.5 Genetically diverse and allelic dominance of VK210 parasites are pivotal in paired primary and relapse infection in Indian isolates of *Plasmodium vivax*

Plasmodium vivax, although causing a less serious disease than *P. falciparum*, is most geographically widespread and the second most prevalent malaria parasite in the world. It is infecting 75 million people annually, predominantly in Asia and in Central and South America, the Middle East, and Africa, signifying that *P. vivax* malaria represents a significant public health burden in many parts of the world including India, accounting nearly 65% of total malaria annually. In contrast to *P. falciparum*, the management and control of *P. vivax* is perplexed and complicated by its ability to cause relapse infections, a phenomenon that has intrigued parasitologists for more than a century but the reason and mechanisms of which

remain enigmatic. Relapse is the result of the activation of quiescent liver-stage developmental forms, known as "hypnozoites" that remain dormant within hepatocytes for varying intervals before spontaneously dividing and developing into schizonts and subsequently releasing invasive merozoites into the bloodstream to infect red blood cell. Relapse is an important aspect of *P. vivax* life-cycle bearing upon chemotherapy and its assessment. To date, the underlying mechanism of influencing relapses and the patterns of relapses in *P. vivax* infection are remain unclear. However, relapse biology remains poorly understood at the molecular level with contrast findings on genetic relatedness among paired primary infection and relapse isolates. Earlier molecular studies indicate that parasites associated with primary infection and relapses are not genetically different whereas recent investigations have demonstrated that parasite isolates associated with relapse of infection rarely have the same genotype as the parasites that caused the primary infection.

The objective of the present study was to investigate the genetic relationship between paired primary and clinically identified relapse or recurrent isolates of *P. vivax* in an effort to provide insight into the molecular mechanisms of relapse. Our analysis is based on the extensive genetic diversity displayed by the circumsporozoite protein gene, stage-specific immunodominant surface antigen expressed by all malaria parasites examined so far during the pre-hepatic sporozoites stage, making them potentially useful markers of genotype *P. vivax* isolates.

To test the above hypothesis, we employed nested PCR strategy to amplify the regulatory domains of CSP repeat and post-repeat variable region followed by RFLP and sequencing to analyze the genetic diversity in 30 (n=64) paired and 9 (n=9) unpaired clinical isolates of *P. vivax*.

Table 1. Number and percentage of *P. vivax* paired samples with *Pvcsp* genotypes

Genotypes	Number of isolates	(%)
VK210	54	84.37
VK247	2	3.12
VK210/VK247	8	12.5

We detected VK210 parasite in 84.37% of the samples, VK247 parasite in 3.12% and mixed type infections in 12.5% of the isolates (Table 1). Analysis of CSP sequences of the VK210 type showed the variation in the alteration of alanine residue (A) or aspartic acid residue (B) in the repeat motif GDRA (A/D) GQPA along the sequence (Fig. 12). It is very interesting to observe that primary paired and clinically identified relapse or recurrent *P. vivax* population in India are genetically diverse, showing dominance of VK210 genotype infections with multiplicity of infection averaging only 0.59 found in the analyzed isolates (Fig. 13). We observed considerable degree of genetic diversity and complexity within the isolates and allelic polymorphism in paired clinical isolates collected from an area with high prevalence of vivax infection and perennial malaria transmission in northern India (Delhi, India). This also suggest that isolates' allelic polymorphism (Fig. 14) not only directly depends on parasite population, super infection and multiple different genetic parasite but could also be due to infection by single mix of genetically diverse parasites. Together with these observations and interpretations these isolates are nevertheless genetically related.

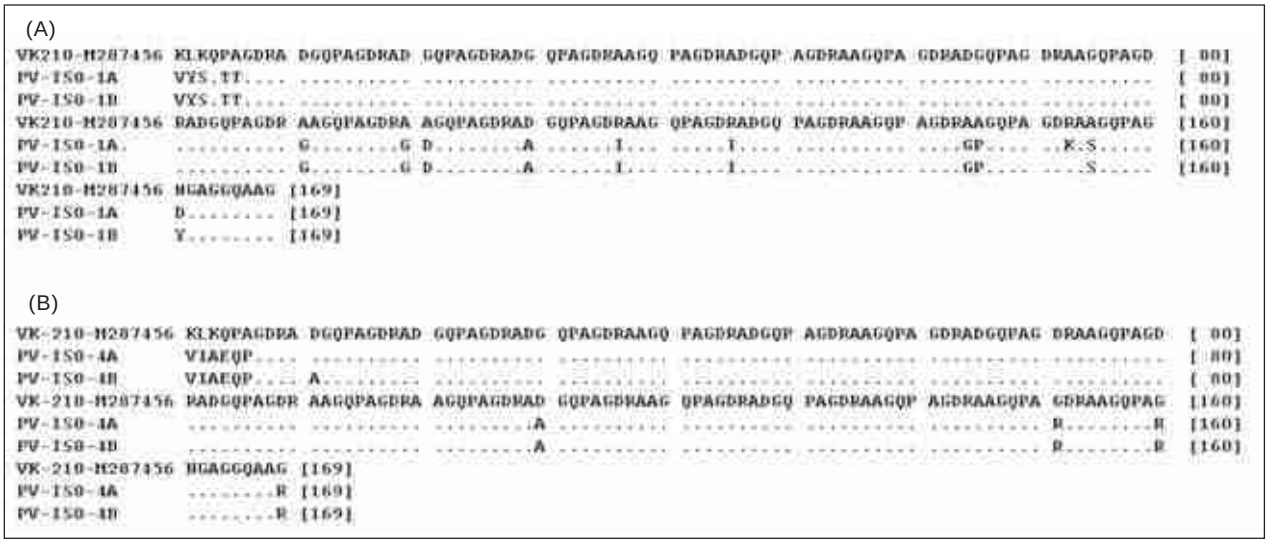

Fig. 12. Alignment of the amino acid sequences of *Pvcsp* from the paired primary and clinically identified relapse or recurrent isolates of *P. vivax* variant bearing the VK210 repeat, observed in representative Indian isolates (A and B) from which the amplified fragment was sequenced. The alignment is based on the VK210 (accession number M287456). Dots represent identical residues

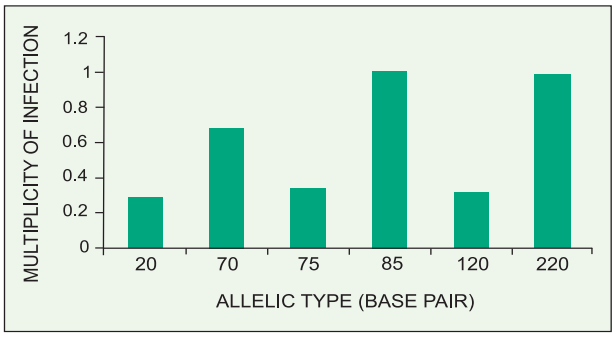

Fig. 13. Multiplicity of infection of PvCSP alleles in primary paired and clinically identified relapse isolates of vivax population

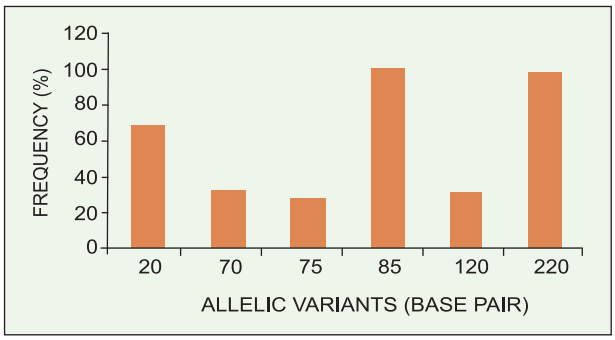

Fig. 14. Frequency distribution of the PvCSP allelic variants in primary paired and clinically identified relapse isolates of vivax population

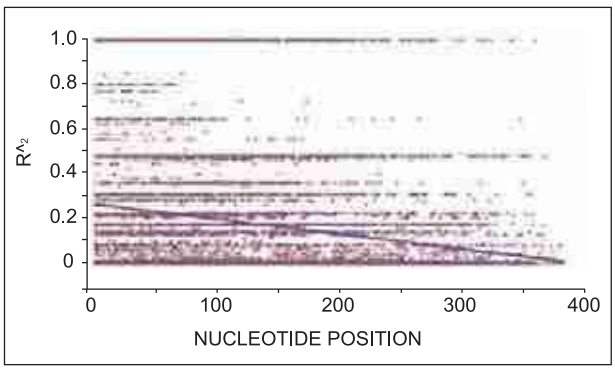

Fig.15. Linkage disequilibrium (LD) plot showing non-random association between nucleotide variants at different polymorphic sites. The R ^ 2 values plotted against nucleotide distances with two-tailed Fisher's exact test of significance. The value of LD index declines with increasing nucleotide distance, indicating that the recombination events are taking place

Further, we observed equal haplotype diversity with slightly more nucleotide diversity (1.0 and 0.67 respectively) in paired as compared to unpaired clinical isolates (1.0 and 0.52, respectively) (Fig. 15). Comparison of clinically identified relapse and unpaired Indian isolates revealed moderate level of genetic differentiation (0.6) and limited gene flow (fixation index ranging from 0.008 to 0.023) among the studied population. The difference between the rates of synonymous and non-synonymous nucleotides, Tajima's D and McDonald-Kreitman test suggest that the genetic diversity and allelic dominance in paired isolates can be attributed to the positive natural selection and some degree of regional biasness in addition to minimum recombination events, observed to be high (32) indicating the possible role of genetic recombination in generating diversity in paired isolates as compared to unpaired isolates (25).

Findings suggest that isolate's allelic dominance and genetic diversity not only directly depend on parasite population, super infection and multiple genetically different parasites but also on infection by single mix of genetically diverse parasites. Furthermore, the study provided information about how alleles are generated and maintained in the population. Specifically, it addresses the relevance of factors like intragenic recombination and natural selection. It can also assess geographic differentiation to explain the spatial distribution of alleles at a given loci. This approach answers fundamental questions related with the association of specific alleles with drug resistance and how the genetic variation may affect vaccine development and deployment. These data may also imply that a single mosquito inoculum contains a large number of genetically different parasites, most likely produced after genetic recombination within the mosquito. Findings also indicate that a single rather than multiple infections is sufficient to generate isolate complexity, one of the biggest challenges faced by malaria control and particularly by vaccine development.

2.2 Parasite Immunology and Biochemistry

2.2.1 Naturally acquired immune responses to defined *Plasmodium vivax* antigens in individuals residing in northern India

Analysis of the host-immune response to defined stage-specific peptides is useful for identification of antigens, which are most frequently recognized by infected patients. Also in clinically immune individuals, antibodies raised against specific antigens may reflect their protective properties. Identification of antigens that help in maintenance of immune response in *P. vivax* transmission season may be helpful for identifying potential candidates for vaccine design. A number of *P. vivax* antigens are under consideration in the development of vaccines. Basic studies using defined *P. vivax* antigens are limited. In this study, we used four stage-specific peptides having both B- and T-epitopes for determining immune responses. They are: circumsporozoite protein (CSP), merozoite surface protein-1 (MSP-1), apical membrane antigen-1 (AMA-1), and transmission blocking antigen (GAM-1/Pvs-24).

In the present investigation, we evaluated the prevalence of naturally acquired immune response to *P. vivax* stage-specific antigens in a group of individuals of different age groups from northern part of India (Ghaziabad, Uttar Pradesh). We measured immunoglobulin G levels to four different epitopes by using serum samples collected by a cross-sectional survey. Cellular immune response was also measured in a batch of individuals by *in vitro* lymphocyte proliferation assay to these four epitopes. Immunity in terms of antibody response and T-cell proliferation against these stage-specific peptides has been observed in the study subjects. The results demonstrated age-dependent antibody responses in this population. Forty-two patients were diagnosed with *P. vivax*. Antibody responses were higher in infected patients than in uninfected cases. In this population, 66% (201/304) cases showed seropositivity to all peptides and 13% (41/304) showed negative response. Peripheral blood mononuclear cells of more than 40% of individuals proliferated in response to stimulation by all four epitopes. We observed that these epitopes are immunogenic in a large proportion of individuals naturally exposed to *P. vivax* malaria.

2.2.2 Immunocapture based diagnostic assay for the detection of *Plasmodium falciparum* HRP-2 and LDH antigen

Antigen tests are promising tools for the diagnosis of malaria. Two such antigens are *P. falciparum* histidine rich protein (PfHRP-2) and lactate dehydrogenase (pLDH). PfHRP-2 is water soluble protein released from parasitized erythrocytes into *in vitro* culture supernatants; pLDH, a glycolytic pathway enzyme of the malarial parasite is produced by sexual and asexual stages and can be detected in culture supernatant and plasma of infected patients.

The present study is aimed to develop indigenous, rapid and sensitive immunodiagnostic method based on detection of PfHRP-2 and pLDH antigen in the blood. Unique peptide sequences of PfHRP-2 (two regions) and pLDH (three regions) antigens were synthesized by solid phase technique and purified to homogeneity. The antibodies against these sequences were raised in mice as well as rabbit using microspheres (PLGA) to generate high titer and affinity antibodies. For developing assay, peptide-specific antibodies were biotinylated, and then separated from unreacted or hydrolyzed reagent by gel filtration chromatography on Sephadex G-25 column. The extent of biotinylation and ratio of biotin to antibody was determined by the Avidin-HABA assay. The antibodies generated using microspheres were able to detect the PfHRP-2 and pLDH antigen in the culture supernatant and parasitized RBC lysate of *P. falciparum* isolates collected from malaria clinics and nearby villages of Delhi-Ghaziabad border.

2.2.3 Characterization of the *Plasmodium falciparum* strains in north-eastern states

The therapeutic efficacies of commonly used antimalarials were ascertained for the treatment of uncomplicated *P. falciparum* malaria patients who were enrolled for the follow-up of *in vivo* antimalarial response according to WHO protocol with regular clinical and parasitological assessment. The objectives of the present study were to generate data on parasite diversity using drug resistant markers for chloroquine (CQ) and sulphadoxine-pyrimethamine (SP), microsatellite markers and anchored primer amplification of DNA (APAD) for identifying and update study areas to monitor the efficacy of antimalarial drugs in northeastern states of India; to correlate clinical findings with parasite genotyping based on *msp1, msp2, glurp*, *pfcrt*, *pfmdr-1*, *dhfr,* and *dhps* genes using PCR-based assay. Study was conducted in two border areas, (i) Indo-Bhutan border area: District Nalbari (PHC Kumarikata), Assam; and (ii) Indo-Bangladesh border area: District Tura (CHC Dalu), Meghalaya. The study was carried out on uncomplicated falciparum malaria patients. All reported fever cases were clinically examined. Peripheral smears, both thick and thin were checked by microscopy. Patients diagnosed with *P. falciparum* and met the inclusion criteria were enrolled for follow-up of *in vivo* antimalarial response according to WHO protocol with regular clinical and parasitological assessment. This is in continuation of earlier work related to characterization of clinical isolates obtained from study subjects. Finger-prick blood of each patient before treatment was spotted on sterile filter paper (Whatman No. 3) strips for molecular studies. Post-treatment samples were also taken as and when the patients reported with parasitaemia during the 28-day follow-up period. Genomic DNA from blood spots pre- and post-treatment was extracted with Qiagen DNA mini kit according to manufacturer's protocol and was analyzed by PCR assay for variants in the target genes for *pfcrt*, *pfmdr-1*, *dhfr* and *dhps*. The nested mutation specific PCR methods were used to determine the prevalence of *pfcrt* K76T; *pfmdr-1*N86Y; *dhfr* A16V, N51I, C59R, S108N/T and I164L; *dhps* S436A/F, A437G, K540E, A581G and A613S/T alleles, respectively. The unequal distribution of genotypes was observed in two areas. We found some of the isolates had mixed genotypes of both wild and mutant. Majority of the isolates

had Thr_{76} mutation in *pfcrt* and wild type Asn_{86} in *pfmdr-1*. The mutant genotypes $Arg_{59,}$ Asn_{108} and Leu_{164} of *dhfr* and Gly_{437} and Glu_{540} of *dhps* found to be associated with the recrudescent parasites. Analysis of MSP-1 and MSP-2 revealed high genetic diversity in isolates of both the study areas. High degree of multiclonal infections was available in both populations. Microsatellite analysis revealed high heterozygosity in Nalbari isolates than those of Tura.

2.2.4 Production and immunological characterization of AMA-1 domain I+II from Indian *Plasmodium falciparum* alleles

The antigen/vaccine construct encompassing domains 1 and 2 accounts for generating most of the inhibitory antibodies and domains 1 and 2 are important targets of polyclonal inhibitory antibodies. Though the overall structure of AMA-1 appears to be conserved as compared to other surface proteins, numerous amino acid substitutions have been identified among different *P. falciparum* isolates. There are more than 60 residue positions having sequence polymorphism in the ectodomain, inspite of having a conserved tertiary structure. Among *P. falciparum* strains, almost 10% of amino acids in a total 622 are shown to be polymorphic present in all the three domains of the ectodomain. There is enough experimental evidence supporting the population genetic studies to show that the sequence polymorphisms in AMA-1 are to allow parasites to overcome the inhibitory activity of anti-AMA-1 antibodies.

In the present study, we have attempted the production of two allelic variant forms of domain I+II of AMA-1 ectodomain from Indian *P. falciparum* isolates and analysed their immunogenicity both in mice and rabbits. The two allelic variants differ in 18 amino acid (aa) positions among a total of ~360 aa. Among these, 12 aa difference occurs in domain I and the rest in domain II. Using simple two-step chromatography, histag affinity followed by a cation exchange chromatography; nearly 80–85% pure proteins could be generated. Under non-reducing conditions, the proteins moved as tight bands suggesting them to be monomeric single population.

This study was aimed at analyzing the immunological activity of domain I+II allelic variants and examined the strain specificity of antibodies elicited to AMA-1 from Indian *P. falciparum* isolates. For this, we have cloned, expressed and purified two allelic variants of domain I+II of AMA-1 ectodomain. The two allelic variants differ in 18 aa positions in total. The purified proteins were able to generate high titer polyclonal antibodies in mice and rabbits with *P. falciparum* growth inhibitory activity *in vitro* and these antibodies showed growth inhibition at very low concentrations.

Immunization results showed that the two allelic products are equally highly immunogenic in mice and rabbits with IFA/CFA adjuvants. The antibody titres were around 500,000 for both the antigens in all the animals immunized, when the immunogen was 100 µg (rabbits) and 30 µg (mice) per dose at three doses. Lymphocyte proliferation assay has shown stimulatory effects on T-cells of mice immunized with the recombinant proteins**.** Vaccination of mice with both the proteins induced IgG1, IgG2a and IgG2b antibodies and also produced cytokines IL-2, IL-4, IL-10 and IFN-γ. T -cells may confer protection against erythrocytic stages either by helping in antibody production or by the secretion of effector lymphokines, such as gamma interferon (IFN-γ). The T-helper 1 (Th1) subset secrete IL-2 and IFN-γ and promote cellular responses and are associated with the IgG2a production, while the T-helper 2 (Th2) subset produce interleukin 4 (IL-4) and IL-10, which are important in promoting humoral immunity and are associated with IgG1 production. The results showed activation of mixed Th1/Th2 immune responses after the immunization of mice with both the allelic variants. The antibodies elicited by immunization were biologically active against *P. falciparum* parasites as seen from the growth inhibition/invasion assay. The antisera raised against the two alleilic proteins seemed to be inhibitory in two *P. falciparum* parasite lines, one of which is chloroquine resistant and the other chloroquine sensitive. In order to make sure that the inhibitory activity was specific to the antibodies alone, serum purified IgG were used in these studies. The effective inhibitory concentration of purified IgG found to be at ~10 µg/ml for inhibiting merozoite invasion. This suggests that the smaller antigen even at very lower concentrations may be capable of generating inhibitory antibodies. Inhibitory activity was considerably reduced or reversed when the assay was done in the presence of 0.25 µM of the recombinant antigens. It was observed that both the allelic proteins have retained T-helper and inhibitory epitopes necessary for eliciting protective immunity against malaria.

2.2.5 Molecular characterization of Aspartic protease gene of *Plasmodium vivax*

The aspartic proteinases known as plasmepsins have been officially recognized by the World Health

Organization as suitable target for the design of novel chemotherapeutic compound for the treatment of malaria. Enzymes of this class initiate the hemoglobin breakdown pathway that provide intra-erythrocytic malaria parasite with nutritional resources and inhibition of their activity results in death of the malaria parasite. Presently, we are engaged in amplification, cloning, sequencing and expression of the Aspartic protease gene of *P. vivax*. Primers were designed against Aspartic protease gene-IV (*PvPM4-1353* bp) and Aspartic protease gene-V (*PvPM5-1611* bp) genes of parasite by using Primer3 and DNA Star software and amplification of these fragments were also successfully done (Figs. 16 and 17). Following are the two primer pairs:

PvPM4 (F)– 5'-ATGGATATAGCAGTGAAAGAACAAGACTACTCAAA-3'
PvPM4 (R)–5'-TTAATTCTTTGCGATGGCAAAACCGACACTCTC-3'
PvPM5 (F)– 5'-ATGGTCGGAGCGAGCTTGGGGCCCCCCGGT-3'
PvPM5 (R)–5'-CTACGCATCCGCGGGCGCCTTGCCCTCGGAGG-3'

Aspartic protease gene-IV (*PvPM4-1353* bp) and Aspartic protease gene-V (*PvPM5-1611* bp) have been amplified and sequenced to find out homology among samples collected from different geographical regions of India and in between *P. falciparum* and *P. vivax*.

Blast results of sequences of *PvPM4* shows 100% homology among different isolates of *P. vivax* collected from different geographical regions of India. The amplified sequence (Fig. 18) revealed 89–99% identical at the amino acid level to the corresponding known *Plasmodium* Aspartic protease-IV sequences. It shows 99% homology with *P. vivax*-sal1, 98% with *P. knowlesi*-strainH, 92% with *P. ovale*, 92% with *P. malariae* and 89% with *P. falciparum*. This result suggested strongly that PvPM4 is a common plasmepsin to all *Plasmodium* species. The sequences of the enzymes were highly conserved except a small number of amino acid substitutions did not modify key residues for the function or the structure of the enzymes. All the six isolates did not contain intron within the flanking region. The six sequences had well conserved essential residues required for active site formation of aspartic proteases and the amino acids characteristically found in aspartic proteases. The high sequence conservations between the plasmepsins from the isolates support the notion that the enzymes could be reliable targets for new antimalarial chemotherapeutics. However, it is not certain whether *PvPM4* orthologs played the same functional roles in all human infecting species. Nevertheless, it seems likely to play an important role in human infecting *Plasmodium* species due to the ubiquitous presence of this ortholog in all other species. Studies regarding the cloning and expression of this aspartic protease are in progress.

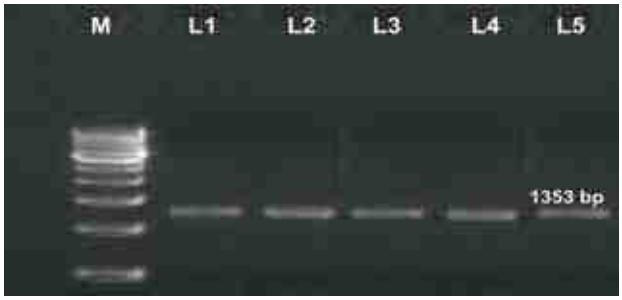

Fig.16. Amplification of the Aspartic protease gene-IV (*PvPM4*) from different geographical regions of India, viz. 500 bp Marker (M), Assam (L1), Bengaluru (L2), Chennai (L3), Delhi (L4), Goa (L5), and Nadiad (L6)

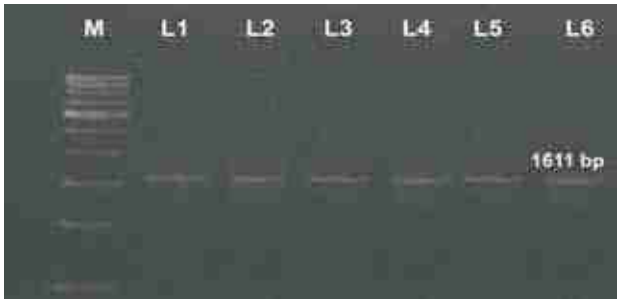

Fig. 17. The successful amplification of the Aspartic protease gene-V (*PvPM5*) from different geographical regions of India, viz. 500 bp Marker (M), Assam (L1), Bengaluru (L2), Chennai (L3), Delhi (L4), Goa (L5), and Nadiad (L6)

The pivotal role of aspartic protease in initiating hemoglobin degradation in *P. vivax* malaria parasite was demonstrated earlier by our group to develop strategies for rational drug designing for a new class of antimalarials to inhibit these critical enzymes of the parasites in order to determine if this enzyme can be used as potential drug target/ immunogen and its inhibitors as potential antimalarial drug.

2.2.6 PCR diagnostic assay revealed high mixed human malaria parasitic infections in India

Accurate diagnosis is the key to effective treatment and control of malaria. We herewith screened 180 microscopically diagnosed malaria positive cases through nested PCR assay for pure and mixed *P. vivax* and *P. falciparum* infections. The samples were collected from six different states covering almost the whole of malaria-endemic regions in India (Fig. 19). In the first step of nested PCR, a pair of primers targeting to the 18S rRNA was used. This set of primer is genus specific and amplifies 1100 bp PCR product (Fig. 20a). In the second step, two primer pairs were used; one specific to *P. vivax*, and the other specific to *P. falciparum*. *P. vivax* specific primers amplifies a

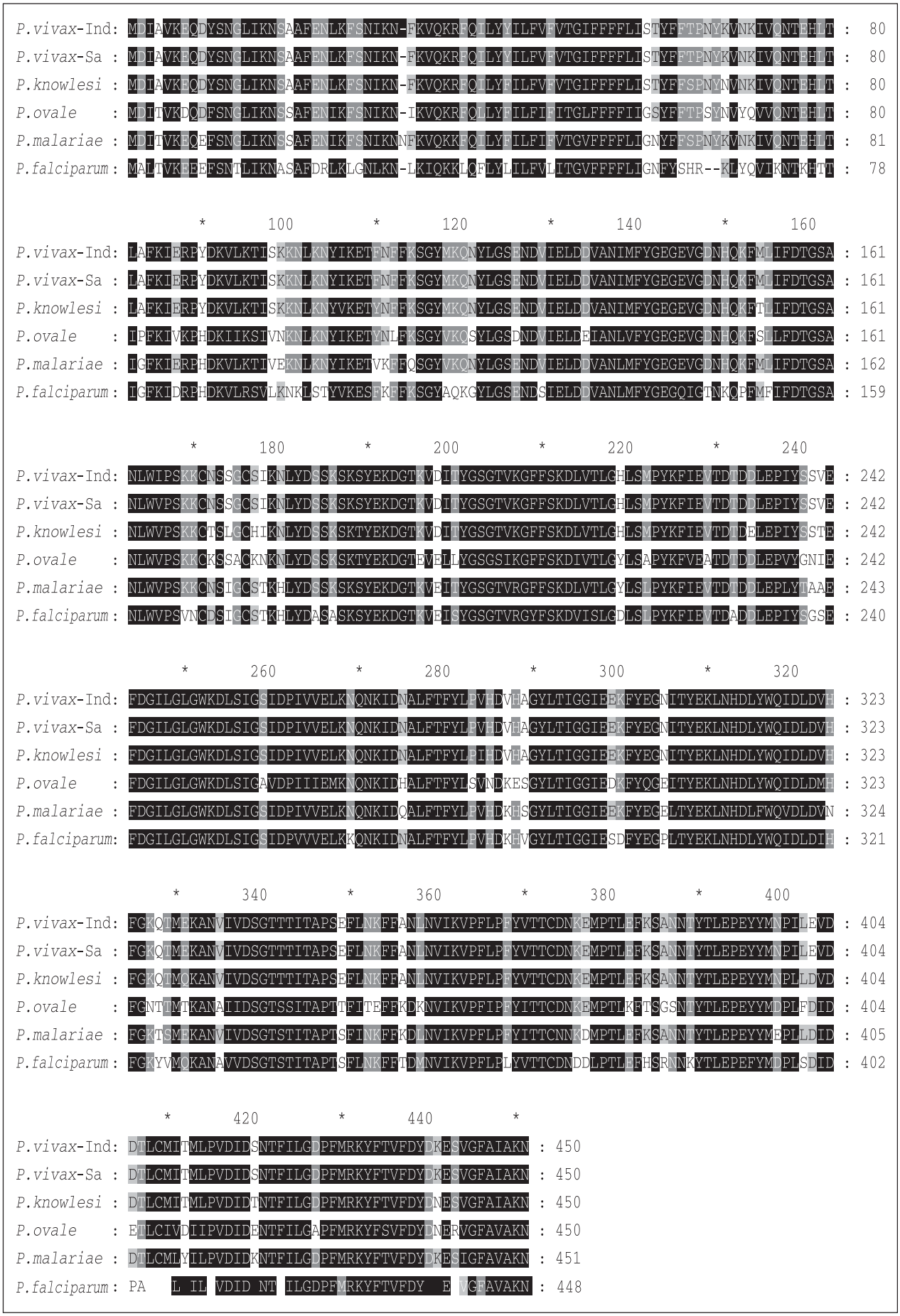

Fig. 18. Clustal alignment of Aspartic protease-IV with known homologous Aspartic protease sequences of other *Plasmodium* spp. Highly conserved and similar residues have been shown dark black and grey respectively. Relative sequence identity has also been shown

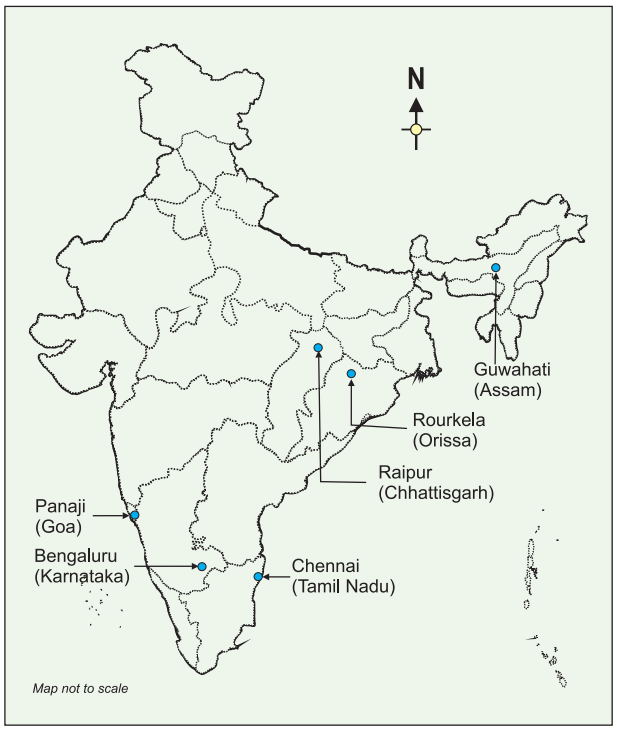

Fig. 19. India map showing sample collection sites

target sequence of 120 bp DNA whereas, *P. falciparum* specific primers amplifies 205 bp. Thus, pure *P. vivax* and *P. falciparum* samples show a clear band at 120 and 205 bp, respectively whereas mixed infected samples show bands in both the two positions (Fig. 20b). Out of 180, 143 pure *P. vivax* infections were detected with microscopy, whereas 85 with PCR assay. Similarly, 37 samples identified as pure *P. falciparum* by microscopy but with PCR assay only 13 samples were found to be pure. Interestingly, none of the 180 samples were identified as mixed infections through microscopy, but PCR assay could reveal as much as 46% of the total infections as mixed due to both *P. falciparum* and *P. vivax*. Such an alarmingly high rate of mixed malaria parasitic infection has been reported for the first time in India which should be kept in mind before applying malaria intervention in the country. For example, selection of drugs is very specific to the type of infected species, and if mixed infections are ignored, patients are only treated for one species and the other is left out.

2.2.7 Preparation of a field site for malaria vaccine trial in and around Jabalpur, Madhya Pradesh: immune responses to stage-specific *Plasmodium falciparum* and *Plasmodium vivax* antigens in a population of central India

The objective of the study was to characterize immune responses to stage-specific *P. falciparum* and *P. vivax* antigens in individuals belonging to all epidemiological age groups; to evaluate the immune mechanisms; those are involved in pathogenesis of malaria, especially anaemia, cerebral malaria and placental malaria. Study was conducted in three populations; infants, children and adults from the community; pregnant women from the community; hospitalized patients with severe malaria. Peripheral blood by repeated cross-sectional survey; placental and cord blood at delivery were taken for determining antibodies against species and stage-specific

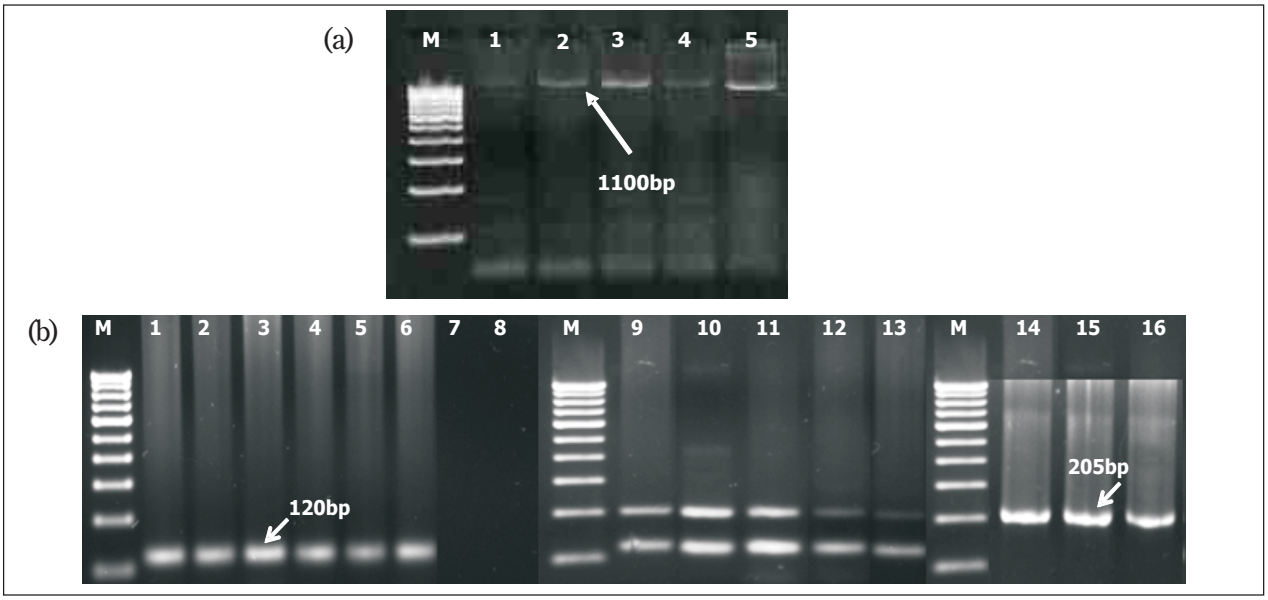

Fig. 20. Agarose gel electrophoresis of PCR products obtained after first and the second step of the nested PCR: (a) PCR products obtained after first step using *Plasmodium* genus specific set of primers— Lane M, 100 bp ladder; lanes 1–5 amplified products from parasite genomic DNA (1100 bp), and (b) PCR amplified products obtained after second step of the nested PCR using species-specific set of primers for *P. vivax* and *P. falciparum*– Lane M, 100 bp ladder; lanes 1–6, *P. vivax* single infections (120 bp product); lane 7, negative control with human DNA; lane 8, negative control without DNA; lanes 9–13 mixed infection of both *P. vivax* (120 bp) and *P. falciparum* (205 bp); and lanes 14–16 *P. falciparum* single infection (205 bp product)

antigens by enzyme immunoassay. We have measured antibody response against 13 different stage-specific peptides of *P. falciparum* (*PfCSP, PfMSP-1, PfMSP-2, PfAMA-1, PfRAP-1, PfEBA-175* and *PfG-27*) and *P. vivax* (*PvCSP, PvMSP-1, PvMSP-2, PvAMA-1, PvG-25* and *PvG-28*) in cohort samples that include pregnant women, infants, siblings, cord and maternal side placental samples. In the hospital component of the study we analyzed both *P. falciparum* and *P. vivax* cases along with healthy controls. The antibody levels were quantified using known negative and antimalarial antibody positive controls and this allowed us to estimate antibody levels in O.D. values. In a subset of adults and older children above 14 years who have past experience of malaria, cell-mediated immune response was determined in peripheral blood mononuclear cells by lymphocyte transformation test in the presence of *P. falciparum* and *P. vivax* antigens; IL-4 and IFN-γ levels were estimated in activated T-cell culture supernatant by sandwich ELISA. Peripheral blood from healthy cohort participants and *P. falciparum* malaria patients were taken for estimation of cytokines (IL-4, IL-10, IP-10, IFN-γ and TNF-α) in plasma using commercially developed two-site ELISA assay kits. Currently, we have used only peptides; now we have FALVAC-1A recombinant protein available from Bharat Biotech for *in vitro* testing for parasite growth, antibody estimation in cohort samples and CMI studies.

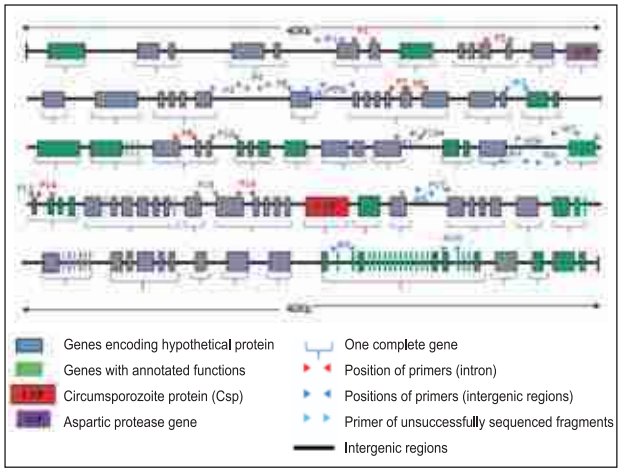

Fig. 21. Schematic overview of the ~200 kb DNA region located in chromosome 13 of *P. vivax*

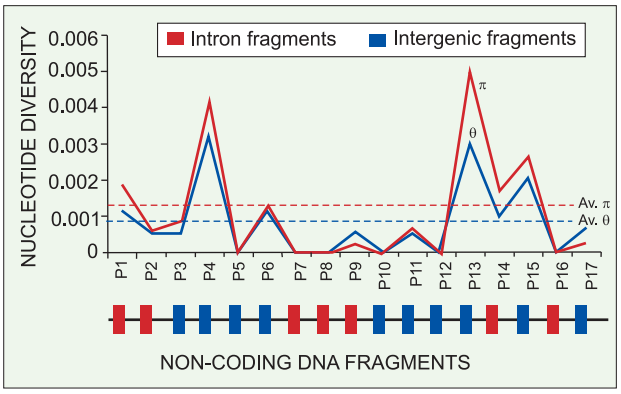

Fig. 22. Pattern of variation in nucleotide diversity across non-coding DNA fragments in the ~200 kb DNA region of Indian *P. vivax*

2.3 Bioinformatics

2.3.1 Population genetics of Indian *Plasmodium vivax* with SNP markers

Although single nucleotide polymorphisms (SNPs) are the markers of choice, the types of SNPs (coding/non-coding) play vital roles in seeking answers to specific evolutionary questions. These aspects are important in malaria, as the parasites are gradually becoming resistant to drugs and easily escaping host immunity. However, human malaria caused by *P. vivax* is less lethal in comparison to *P. falciparum*, but causes high morbidity. Comparatively less attention has been paid towards research in *P. vivax*. We have utilized a ~200 kb 13th chromosomal region of *P. vivax* (Fig. 21) and sequenced 17 non-coding DNA fragments (named as P1–P17) in a malaria endemic Indian population sample. We detected 18 SNPs (named as PVS1–PVS18) in 11 non-coding (seven intergenic and four intron) DNA fragments; the rest six were monomorphic. Data analyses under population genetic framework revealed nucleotide diversity patterns to be highly fluctuating across the whole region; in general, the intergenic regions were more variable than the introns (Fig. 22). Different tests of neutrality were performed, none of the 11 polymorphic DNA fragments were found to be deviated from neutral model. Apart from three fragments (two intergenic and one intron), Tajima's D values were positive in all other eight fragments (Fig. 23). However, non-significant linkage disequilibrium (LD) was observed between all the 17 loci but, two instances of significant LD between SNPs were observed (Fig. 24). In one case, the two SNPs involved, surround the Aspartic protease

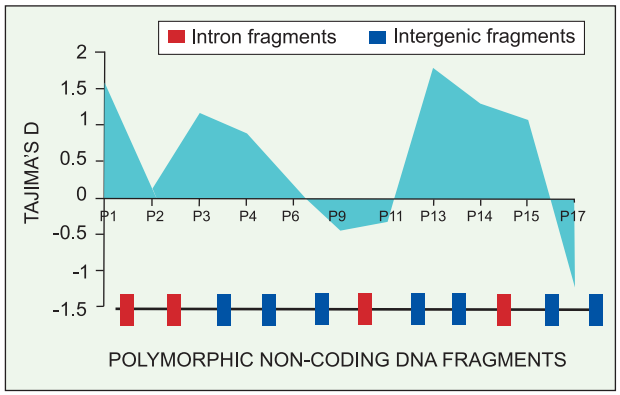

Fig. 23. Pattern of Tajima's D values across 11 polymorphic non-coding DNA fragments in Indian *P. vivax*

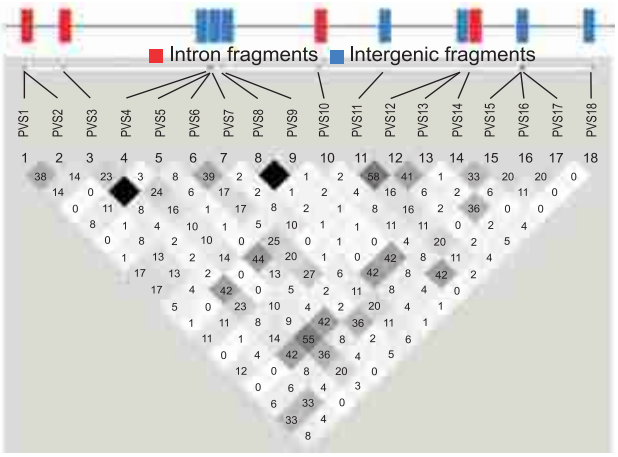

Fig. 24. LD plot (r^2) between 153 possible pairs of SNPs in Indian *P. vivax*. The strength of statistical significance of LD between a pair of SNPs is represented with the extent of darkness of the boxes. The two complete black squares thus represent statistically significant LD; one between PVS3 and PVS5 and the other between PVS8 and PVS9

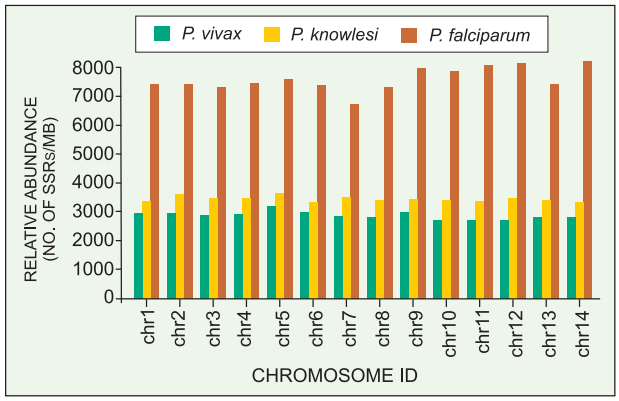

Fig. 25. Whole genome distribution of SSRs in three *Plasmodium* species

(*ASP*) gene, a potential malaria drug target. This information could be utilized for association studies between one of the SNPs and specific allele of *ASP* gene and all the 11 polymorphic fragments could be utilized as putatively neutral markers for studying the population structure and demography of *P. vivax*.

2.3.2 Comparative genomic analysis of simple sequence repeats in three *Plasmodium* species

Simple sequence repeats (SSRs) are known to be responsible for genetic complexities and play major roles in gene and genome evolution. Malaria parasites are known to have highly evolving and complex genomes with complicated and differential pathogenic behaviours. Hence, by studying whole genome comparative SSRs patterns, one can not only understand genomic complexities and differential evolutionary patterns of these species but also genome-mediated complex pathogenic characteristics. We here with utilized whole genome sequence information of three *Plasmodium* species, *P. falciparum*, *P. vivax* and *P. knowlesi* to comparatively analyze genome-wide distribution of SSRs. The results revealed that despite smallest genome size, *P. falciparum* bears the highest SSR content among the three *Plasmodium* species (Fig. 25). Further, distribution patterns of different SSRs types (e.g. mono, di, tri, tetra, penta and hexa) in term of relative abundance and relative density provide evidence for greater accumulation of di-repeats and marked decrease of mono-repeats in *P. falciparum* in comparison to other two species (Fig. 26 a–f). Overall, SSR types and distribution

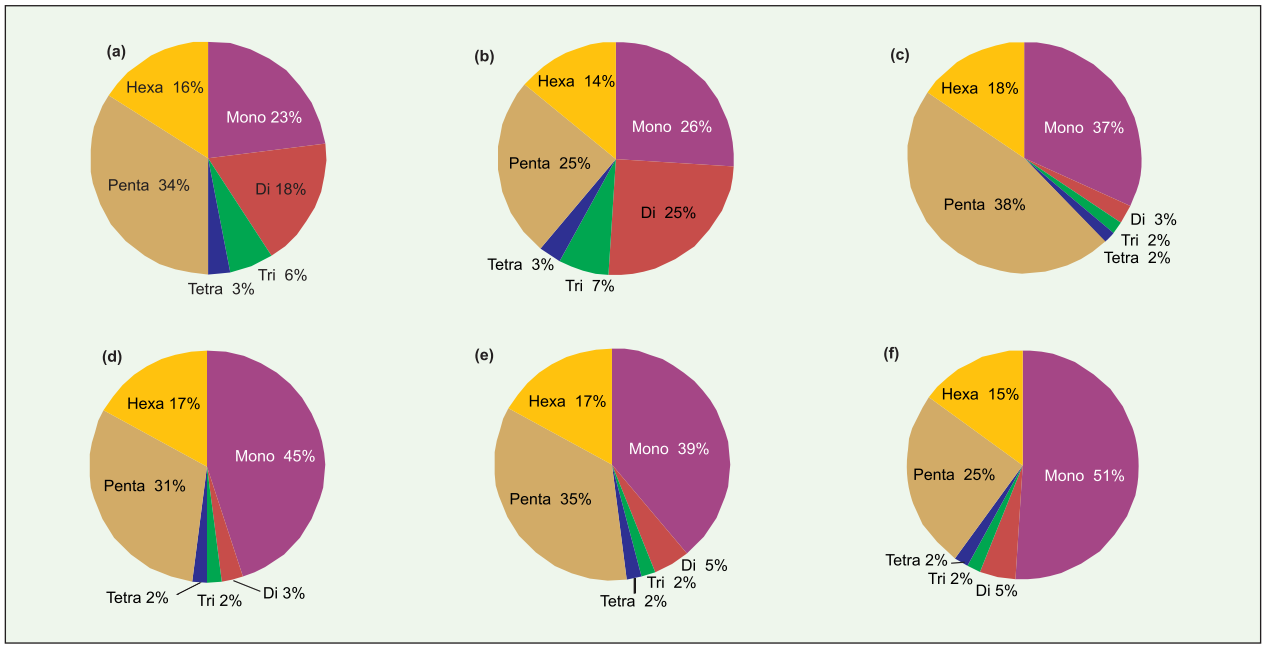

Fig. 26. (a) Relative abundance of SSRs in *P. falciparum*; (b) Relative density of SSRs in *P. falciparum*; (c) Relative abundance of SSRs in *P. vivax*; (d) Relative density of SSRs in *P. vivax*; (e) Relative abundance of SSRs in *P. knowlesi*; and (f) Relative density of SSRs in *P. knowlesi*

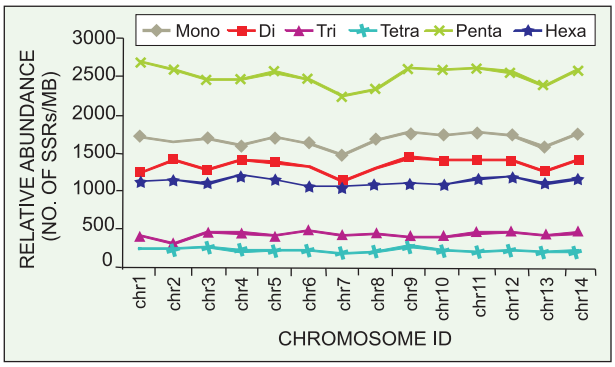

Fig. 27a. Chromosomal distribution of different SSR types in *P. falciparum*

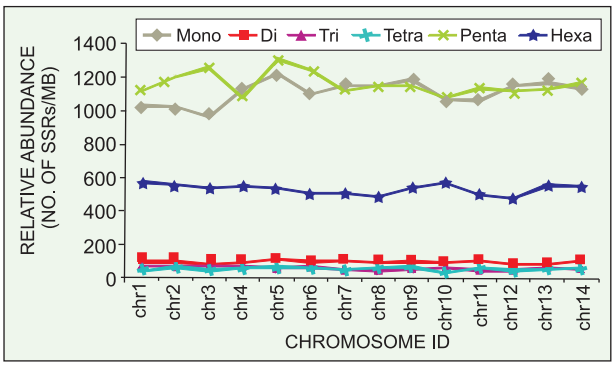

Fig. 27b. Chromosomal distribution of different SSR types in *P. vivax*

in *P. falciparum* genome was found to be different than that of *P. vivax* and *P. knowlesi* which were found to have almost similar SSR organizations. Further, chromosomal distribution of different SSR types was also studied in each of the three *Plasmodium* species. The results reflected that penta repeats were highly abundant in all chromosomes of *P. falciparum* and *P. vivax* whereas mono repeats

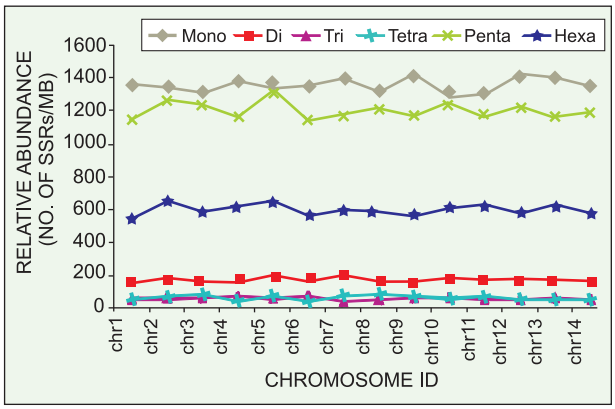

Fig. 27c. Chromosomal distribution of different SSR types in *P. knowlesi*

were highest in all chromosomes of *P. knowlesi* (Fig. 27 a–c). The study investigated the similarities and differences of SSR distribution among three *Plasmodium* species and also revealed the uniqueness of *P. falciparum* in SSR organization and evolution.

2.4 Screening of plant extracts for antimalarial activity

The study entitled, "*In vivo* study on some plant extracts for their animalarial activity in rodent malaria" model was carried out during the period. Coded plant extracts NIMR HAR VC-2, NIMR HAR HA-2, NIMR HAR LC-2 and NIMR HAR CS-1 were received and screened. The extracts NIMR HAR VC-2 and NIMR HAR HA-2 showed significant schizontosidal effect on *P. berghei* in Swiss Albino/Balb/C mice. Further work is in progress.

3 EPIDEMIOLOGY

3.1 Regional level mapping of malaria vectors using RS & GIS in north-eastern states in India to develop strategic plan for malaria control

The study was carried out in Sonitpur and Nagaon districts of Assam. IRS-1D LISS III images of 2002 and 2006 were classified to derive landuse/ land cover maps of two districts. Field surveys using GPS were carried out during October 2008 for validation of classified images and collection of entomological and epidemiological data. Survey of India topo-sheets in the scale of 1:50,000 were used to prepare thematic maps of soil, altitude and forest cover. Thematic maps of rainfall and temperature were based on standard periodical averages. Using these maps, favourable areas for malaria vector, *An. minimus* were mapped (Fig. 1).

Validation surveys were carried out in nine villages in each of Sonitpur and Nagaon districts. In Sonitpur district, out of nine villages surveyed, four were in GIS predicted favourable areas and five in non-favourable areas. The collection of species completely reconciled with the GIS

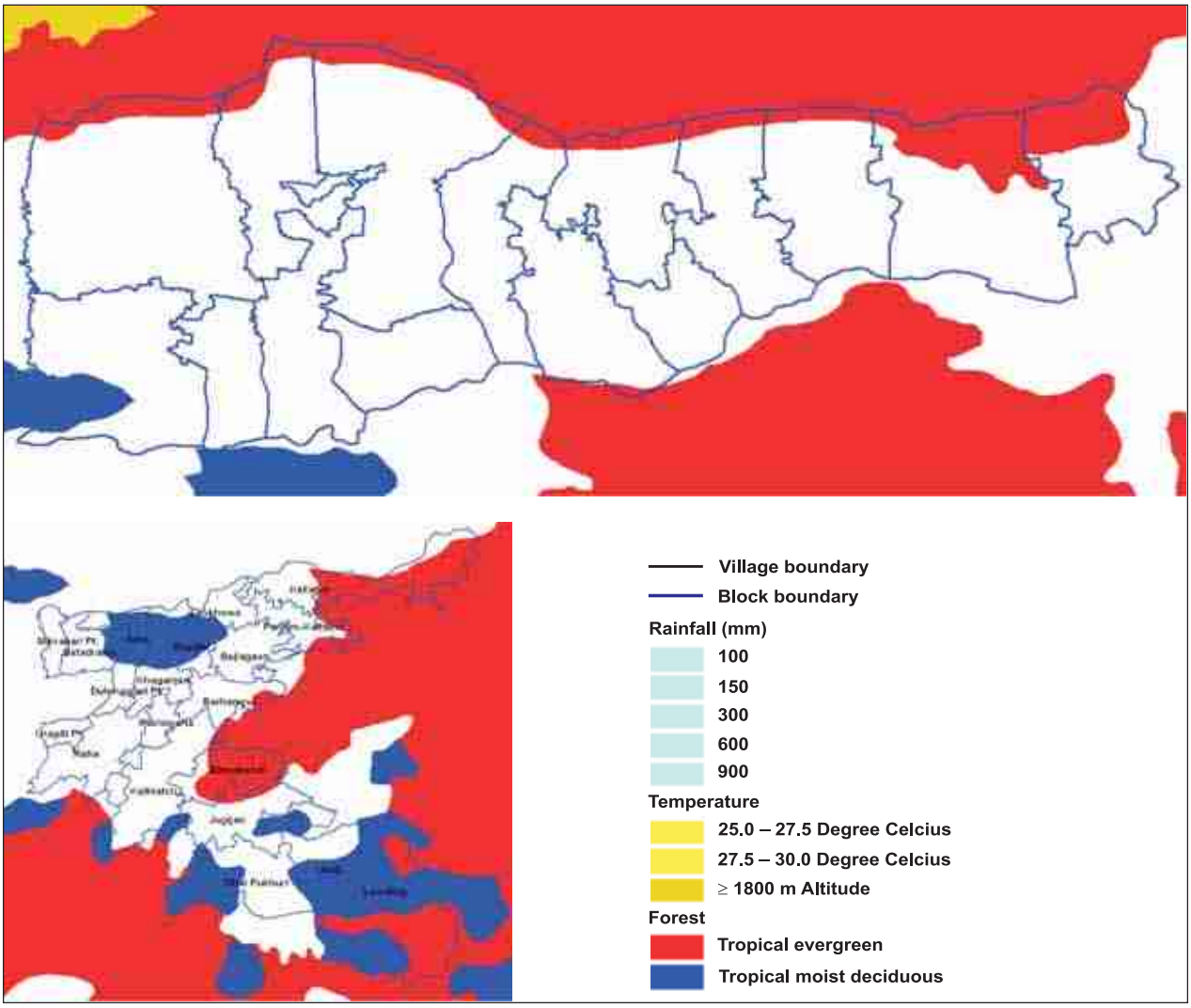

Fig. 1. GIS predicted distribution of *An. minimus* in Sonitpur and Nagaon districts of Assam. Favourable areas are depicted in red and blue colour. 'Red' are most favourable areas for *An. minimus*

prediction. In Nagaon, five villages in GIS predicted favourable area were surveyed, out of which in four villages adult/larvae of *An. minimus* could be collected. In four non-favourable villages no *An. minimus* was found.

Fever survey was carried out in villages of these two districts, out of 43 blood samples collected, two *P. falciparum* cases were found in PHC Balipara, Sonitpur where *An. minimus* was recorded in larval survey. It is worthy to report that this is the GIS predicted favourable area for *An. minimus*, i.e. an area prone to malaria. A good relation between GIS predicted vector distribution map and API was seen in District Nagaon, Assam (Fig. 2).

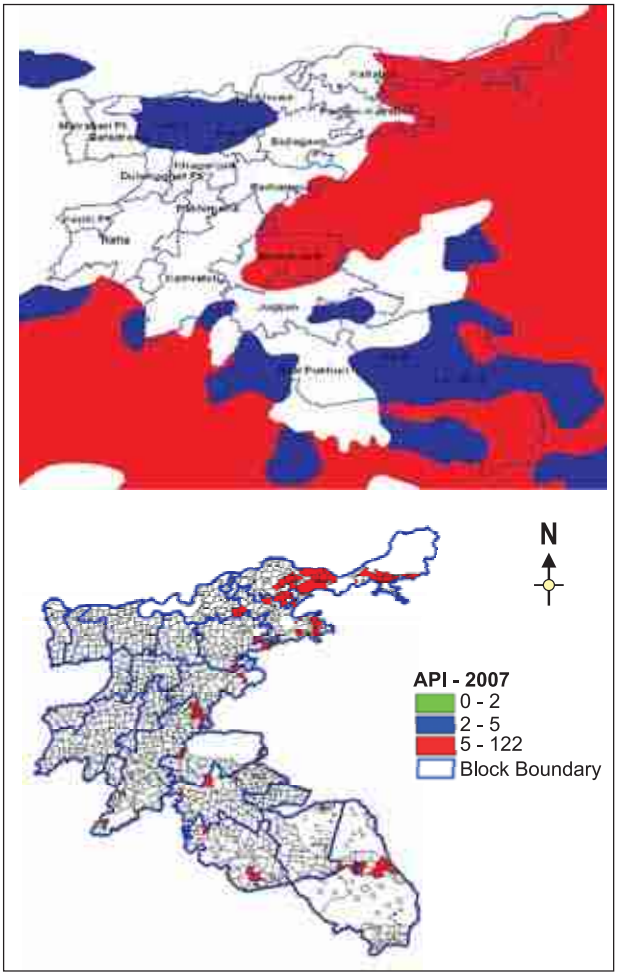

Fig. 2. GIS predicted distribution of *An. minimus* and API of District Nagaon, Assam

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

Sonitpur and Kamrup districts were selected by Defence Research Laboratory, Tezpur as study sites. Landuse/land cover classification map of Kamrup district was prepared using IRS-1D satellite image of the year 2002 and Sonitpur district using IRS-1D satellite images of 2002 and 2006. Field surveys using GPS were carried out during October and June 2008 for validation of classified images and collection of entomological (hand catch) and parasitological (active blood smear collection) data. Survey of India topo-sheets in the scale of 1:50,000 were used to prepare thematic maps of soil, altitude and forest cover. Thematic maps of rainfall and temperature were based on standard periodical averages. Using these maps, favourable areas for malaria vector, *An. minimus* were mapped. Distribution maps reconcile well with the classified imagery depicting forested areas as seen in Figs. 3 and 4.

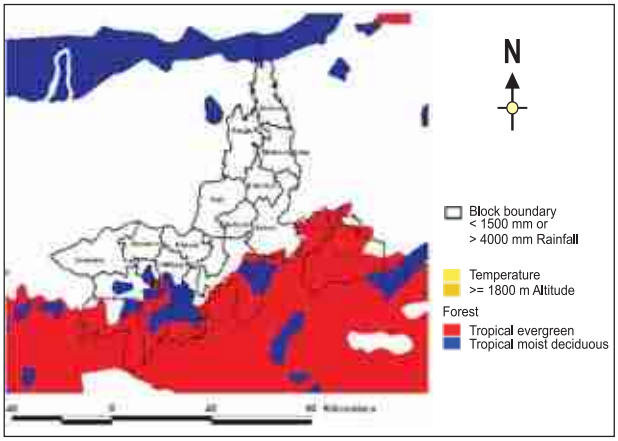

Fig. 3. GIS predicted distribution of *An. minimus* in Kamrup district of Assam. Favourable areas are depicted in red and blue colour. 'Red' are most favourable.

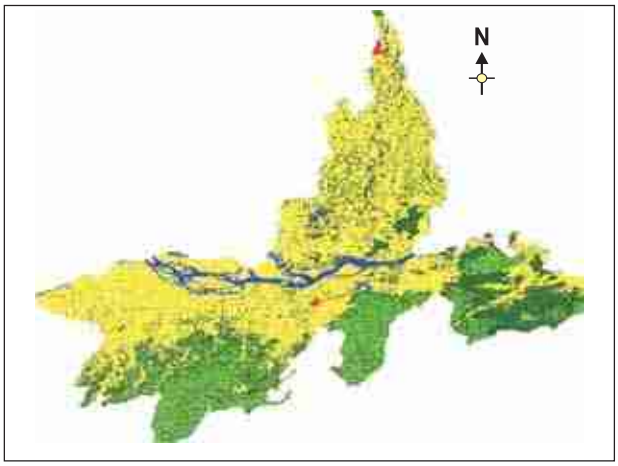

Fig. 4. GIS predicted distribution of *An. minimus* and forested area in District Kamrup, Assam

To validate GIS predictions, a total of 13 villages falling in favourable and two in non-favourable areas covering seven PHCs, viz. Sonapur, Rampur, Kamalpur, Hajo, Goreswar, Boko and Azara of District Kamrup were surveyed. In all the favourable villages, *An. minimus* adult/larvae could be collected. Most of the

specimen were collected during night. Villages in PHCs Kamalpur and Hajo were predicted to be non-favourable for *An. minimus* and the species was not recorded from these villages during this survey.

3.3 Micro level mapping of high risk districts in India for decision support of malaria control

Out of 68 NVBDCP identified high risk districts, prioritization of villages was carried out in 26 districts of India for decision support of malaria control. The districts were categorized into four strata, namely API >5 and Pf % >50; API >5 and Pf % >30 & ≤50; API >3 & ≤ 5 and Pf % >50; API >3 & ≤5 and Pf% >30 & ≤50. The names of these prioritized villages were provided to NVBDCP for designing appropriate control strategy. Also road network map consisting of highways, metalled and unmetalled roads was worked out to approach priority villages and to schedule the control operations in these areas. Mapping of high risk areas in District Chhindwara (M.P.) for focussed malaria control is shown in Fig. 5.

3.4 GIS-based dengue information system for Delhi

A GIS-based dengue surveillance system was developed for monitoring and control of dengue in Delhi. Digital map up to the level of street was used to create the GIS database. Ward-wise number of households, population, literacy rate, scheduled caste population, etc. as per 2001 census were attached. Streetwise reported dengue cases were mapped to identify locality-wise clusters requiring intense attention for disease control (Fig. 6). A routine sample survey for breeding sites supporting breeding of dengue vector was carried out by the NIMR using GPS. The data were overlaid to identify breeding sources contributing more for proliferation of dengue vectors to undertake situation-specific control measures.

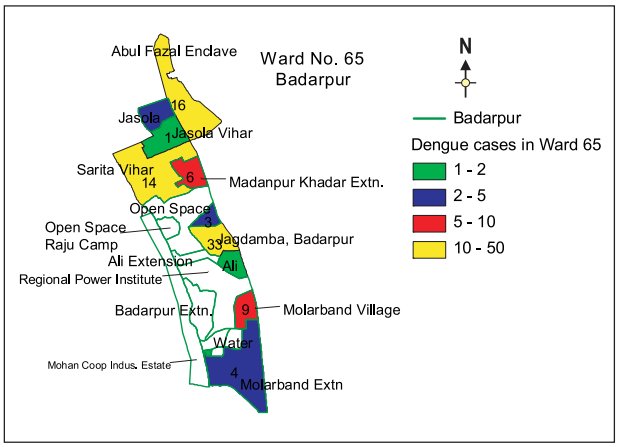

Fig. 6. Locality-wise clusters to identify the dengue problem pockets in Delhi

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

In the year 2008, three surveys for the health impact assessment of Indira Sagar Dam and RR colonies in SSP Reservoir impoundment areas in

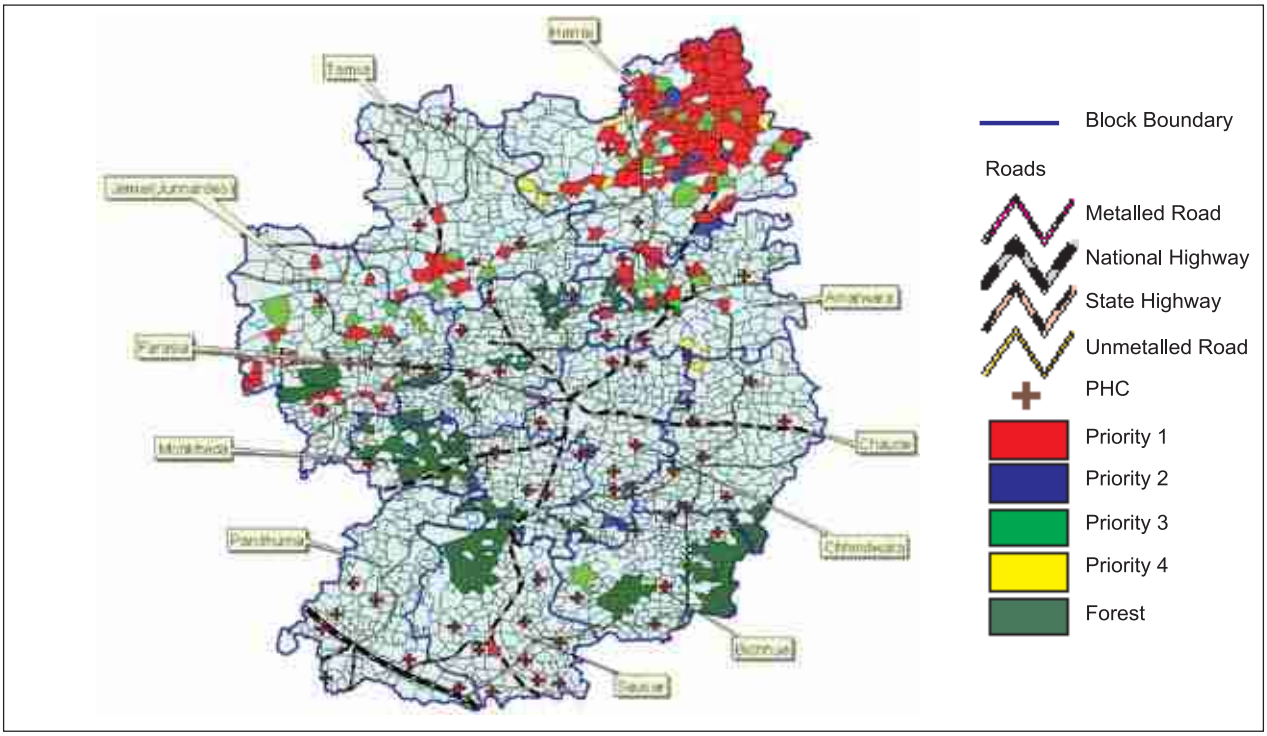

Fig. 5. Mapping of high risk areas for focused malaria control in District Chhindwara, Madhya Pradesh

Narmada Valley, M.P. were conducted. The study was undertaken in seven districts, namely Jhabua, Dhar, Barwani, Khargone, Harda, Dewas and Khandwa. Villages surveyed were in reservoir area (Dam site), rehabilitation and resettlement (RR), and canal sites.

After each survey various mitigating measures were suggested to the State Health Authorities and NVDA to reduce the disease burden in these areas. Out of 23 mitigation measures suggested, 13 have been implemented by NVDA and the State Health Authorities. With this proactive approach of all the stake holders the disease burden has been reduced to a remarkable extent. Fig. 7 shows the impact of interventions taken by the Authorities. Along with this study a survey for Schistosomiasis was also conducted and all the snail vectors found were cattle vectors. A different test for the suitability of drinking water in the affected areas is also being conducted simultaneously and the reports are being sent to the authorities immediately for necessary action.

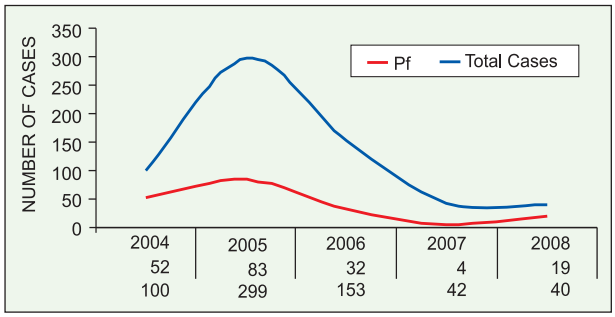

Fig. 7. Malaria incidence in intervention villages of NVDA, M.P. after implementation of mitigating measures by the Health Authorities

3.6 World bank baseline household survey for malaria control

Baseline household surveys were carried out in four states during late 2008. The states were Chhattisgarh, Orissa, Jharkhand and Madhya Pradesh. Randomly eight blocks from each state and further 10 villages from each block were selected. House listing and mapping of the villages were undertaken by one team who sketched a rough map of the village showing major landmarks and roads, and numbered each house. They also filled up the basic information of the household, names of head of the house and presence of fever (fever on the day of survey) in the appropriate questionnaire. The fever cases were directed to the place in the village where blood test was done using RDT and slides. The following next team carried out the actual household surveys. The survey was based on various questionnaires enquiring listing, fever prevalence, fever in last fortnight, health seeking behaviour, deaths, bed nets, IRS, socioeconomic aspects, role of ASHA, etc. Each questionnaire was examined by supervisors in the evening. After about a month's survey the filled-up questionnaires were collected, data were entered and analysed.

This data would serve as the baseline data for the subsequent interventions by the World Bank assisted control programme.

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

A study was undertaken in problematic districts of northern and southern Karnataka to identify epidemiological risk factors with the help of remote sensing. Based on village-wise data of past three years, villages from highest and lowest malaria endemic districts, i.e. Gulberga, Bijapur, Raichur and Bagalkot were selected for detailed survey. Field visits were undertaken in highest peak malaria month, i.e. October/November and in lowest peak, i.e. March/April from 2006 to 2008. Field surveys were undertaken in 21 selected villages 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 area was having rivers, irrigation channels, drains and borrow pits as breeding habitats. Satellite images of IRS P6 LISS IV MX were also procured for Upper Krishna Project (UKP) area. Ecoepidemiolgical risk factors were found as: introduction of irrigation channels in hitherto water scarce area, vicinity of human settlements near channels/seepage drains, local migration and settlement of rehabilitated colonies.

3.7.1 Validation of landscape features related to malaria endemicity in Tumkur and Chitradurga districts of Karnataka

To validate the relationship between Remote Sensing derived landscape features and malaria endemicity in Tumkur and Chitradurga districts, Rangnathpura and Yelldakere PHCs under Hiriyur taluka were selected from Chitradurga district while Kallembela, Tarur and Tavarakere PHCs under Sira taluka were selected from Tumkur district. Ground truth of ecological features identified from FCC was undertaken in selected

Satellite image of Upper Krishna Project area showing network of irrigation channels

villages of Ranganathpura and Kallembela PHCs. All the villages under Kallembela and Tavarakere PHCs were listed. FCCs based on merged satellite data of LISS III and PAN sensors were generated and statistics of landscape features were also generated.

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. The ground truth data in this unknown area confirmed the earlier findings.

3.8 Developing a framework for predicting malaria outbreaks in rural and urban Gujarat, India

It is a collaborative project with Michigan University. Initial analyses were focused on three districts of Gujarat, namely Kutchch, Banaskantha, and Kheda-Anand, and the time series of monthly rainfall and positive *P. falciparum* from 1986 to 2002 (or 2006). One district of Rajasthan, Barmer, has also been included for comparison purposes. Initial correlative analyses revealed significant associations between rainfall during the monsoon season and malaria during the epidemic season that follows, particularly in the more arid districts. In Kutchch 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, malaria data exhibit variability at a period of approximately two and four 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.

The 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.

In conclusion, this part of the work emphasizes the association with rainfall persists for the residuals of the model.

3.9 Assessment of the impact of climate change on malaria and dengue at national scale and adaptation strategies for short, medium to long-term scales

This is a project under NATCOM II sponsored by the Ministry of Environment and Forests for determination of transmission windows (TWs) 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, relative humidity (RH) and rainfall (January 1961 to December 1990) extracted from PRECIS (Providing Regional Climate for Impact Studies) were used as baseline. Projected scenario (A2 scenario) for 2071, 2081, 2091 and 2100 of PRECIS were used. TWs 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 as well as 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 confirmed with further analysis by incorporating landuse features and different combinations of temperature, RH and rainfall.

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

Socioeconomic status in vulnerable areas of five states selected for detailed analysis of socioeconomic conditions to arrive at possible

Workshop on Impact of climate change on malaria held at Delhi on 1 July 2008

adaptation measures, field visits were undertaken in Jodhpur (Rajasthan) and Sambalpur (Orissa) for eliciting information on socioeconomic status, knowledge and practices of the communities about malaria and existing health facilities/system. The study is in progress.

3.10 Operational research on surveillance and intervention strategies for malaria control in Gadchiroli district of Maharashtra

Malaria has been persistent in Gadchiroli district of Maharashtra and most of the area is considered as high risk area. The project was undertaken in order to evaluate the independent impact of IRS and LLIN in the area, strengthened EDPT and to find the suitable criteria for labeling the high risk malarious area. Under Dhanaura block, Muramgaon PHC being the highest malaria reporting PHCs was selected for the study. Three sub-centres, namely Pannemara, Kulbhati and Muruamgaon were categorized into (i) strengthened EDPT and IRS, (ii) strengthened EDPT and LLIN, and (iii) control. For strengthened EDPT and supervision of IRS, supervisors were appointed for experimental sub-centres. Before launching the project, meetings with DMO, Medical Officer, health workers, Anganwadi workers and village people were conducted. 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. In order to monitor malaria parasite prevalence and vector density, supervisors are visiting the selected villages daily and anopheline density is being monitored in alternate months. The study is in progress.

4 CLINICAL RESEARCH

4.1 Malaria Clinic

A total of 835 patients attended the Malaria Clinic at 22 Sham Nath Marg, Delhi or were referred from hospitals for blood examination and treatment of malaria during April 2008 to March 2009. Out of 91 patients found positive for malaria, 20 were diagnosed as *P. vivax*, 69 as *P. falciparum* and two as mixed infections.

4.2 Clinical trials

4.2.1 A phase II randomized, open label, multicentre study to assess the antimalarial efficacy and safety of arterolane (RBx 11160) maleate and piperaquine phosphate coadministration and Coartem® in patients with acute uncomplicated *Plasmodium falciparum* malaria

Arterolane (RBx 11160) maleate is a synthetic trioxolane. It is easy to synthesize, inexpensive, *achiral* and orally active. *In vitro* and *in vivo* studies with arterolane have demonstrated its antimalarial activity in pre-clinical models and has a substantial safety margin between an effective dose for malaria and the toxic dose. Clinical studies have also shown that the drug is well-tolerated and has a rapid parasiticidal action. Current WHO guidelines recommend combination therapy for falciparum malaria. Piperaquine phosphate (PQP) has therefore been chosen as a partner drug. It has antimalarial activity against both *P. vivax* and *P. falciparum*. In phase I studies, combination of arterolane maleate and PQP has shown that the combination is safe and well-tolerated.

Work in progress at Malaria Clinic

The aim of the present study was to assess the antimalarial efficacy and safety of arterolane (RBx 11160) maleate 150 mg + PQP 750 mg administered once daily for three consecutive days versus Coartem® in patients with acute uncomplicated *P. falciparum* malaria. It was a phase II, randomized, open label, multicentre study and carried out at different sites in India and Thailand. Blood sampling was also done for pharmacokinetic analysis of arterolane (RBx 11160) and piperaquine. A total of 240 patients were recruited according to predefined inclusion criteria outlined in the protocol. Initial parasite clearance was achieved in all the patients treated with the arterolane maleate+ PQP combination. There was no reappearance of parasites by Day 28 in arterolane maleate + PQP group while there was one treatment failure in Coartem® group. Both the regimens were well-tolerated. Pharmacokinetic analysis showed that all the patients receiving tested treatment were sufficiently exposed to arterolane and piperaquine.

The safety and efficacy of arterolane maleate and PQP combination is, therefore, comparable to Coartem®, the current gold standard fixed dose combination for the treatment of *P. falciparum* malaria.

4.2.2 Assessment of efficacy, safety and population pharmacokinetics of the fixed-dose combination of Artesunate-Mefloquine in the treatment of acute uncomplicated *Plasmodium falciparum* malaria in India

The Indian national drug policy recommends chloroquine (CQ) as the drug of choice for both *P. vivax* and *P. falciparum* malaria with the Artesunate+Sulphadoxine-pyrimethamine (ACT) combination as the second line of treatment in areas showing >25% of CQ resistance. However, resistance to the recommended antimalarial drugs has become a challenge and is a public health problem. An intensive effort is required to search for a new therapy (single or in combination), which could be an alternative to the standard

treatment. One of the best known ACTs is the three-day regimen of Artesunate (AS) and Mefloquine (MQ).

The present study was undertaken in two endemic districts of Mangalore and Goa to evaluate the efficacy of Artesunate-Mefloquine (AS-MQ) combination in India. The primary objective of this clinical study was to evaluate the clinical and parasitological efficacy of AS-MQ fixed-dose combination in adult patients with uncomplicated falciparum malaria, by determining the proportion of patients achieving a negative parasitaemia without recrudescence before 63 days (cure rate).

The study was a multicentric, single-arm, open-label clinical trial. All patients recruited in the study were given full, supervised treatment with oral AS-MQ 100 + 220 mg tablets (2 tablets daily for 3 days). After treatment for three days, follow-up was done on Day 7, 14, 21, 28, 35, 42, 49, 56 and 63 for assessments. Male and female patients aged ≥18 years, having microscopically confirmed monoinfection of acute uncomlicated *P. falciparum* (asexual forms parasitaemia ≥1000/µl to ≤100,000/µl), history of fever or presence of fever (temperature ≥37.5°C) were included. Patients with signs and symptoms of severe/complicated malaria requiring parenteral treatment or having mixed *Plasmodium* infection with known history or evidence of clinically significant disorders were excluded.

A total of 77 patients with uncomlicated falciparum malaria were enrolled according to predesigned inclusion/exclusion criteria. Ten patients had recrudescence during 63 days of follow-up and eight patients were lost to follow-up. Overall, the drug was effective (cure rate >95%) and well tolerated. PCR data are being analyzed to ascertain whether these were reinfections or due to recrudescence.

4.2.3 A phase III comparative, open-label, randomised, multicentre, clinical study to assess the safety and efficacy of fixed dose formulation oral pyronaridine artesunate (180 : 60 mg tablet) versus mefloquine (250 mg tablet) plus artesunate (100 mg tablet) in children and adult patients with acute uncomplicated *Plasmodium falciparum* malaria

Artemisinin-based combination therapies (ACTs) are effective and are today considered by the World Health Organization to be the best antimalarials in terms of efficacy and a lower propensity to resistance. In order to prevent the occurrence of drug resistance to artemisinins and to address the issue of its relatively short half-life, artemisinins are recommended to be given in combination with another antimalarial agent.

This new ACT, artemisinin as artesunate is being partnered with an established antimalarial agent pyronaridine. The action of artesunate is a rapid knock down of the parasites, after which the drug is rapidly cleared as it has a short systemic half-life. Pyronaridine is also effective in the short-term but has a long blood half-life, thus, providing a sustained schizontocidal effect. The aim of the fixed dose combination of pyronaridine and artesunate in the treatment of uncomplicated acute malaria is to provide a rapid reduction in parasitaemia with a three-day regimen, thereby improving compliance and reducing the risk of recrudescence through the slower elimination of pyronaridine. Fixed combination of pyronaridine and artesunate as a once daily treatment, enables the course of treatment of artesunate to be reduced from 7 to 3 days has been shown to provide high cure rates and low recrudescence. This well-designed and controlled clinical study will be used for regulatory approval and registration purposes and will compare safety and efficacy of the fixed combination of pyronaridine artesunate with that of mefloquine plus artesunate in malaria endemic countries in Southeast Asia and India.

The primary objective of this clinical study was to compare the efficacy and safety of the fixed combination of pyronaridine artesunate with that of mefloquine plus artesunate in patients with acute, uncomplicated *P. falciparum* malaria. The study was multicentric and conducted in Thailand, Vietnam, Cambodia and India. Patients were randomly assigned in 2 : 1 ratio to pyronaridine artesunate (180 : 60 mg tablets) or mefloquine (250 mg tablet) plus artesunate (100 mg tablet). Patients were followed for safety for 42-days after the first study drug administration. The primary efficacy endpoint was at 28 days (D28).

In India, Mangalore was the study site and 59 patients were enrolled and followed-up for 28-days. Male and female children (≥20 kg body weight) and adult patients, aged between 3 and 60 years having microscopically confirmed monoinfection of *P. falciparum* (asexual forms parasitaemia ≥1000/µl to ≤100,000/µl or mixed infection), history of fever or presence of fever (temperature ≥37.5°C) were included. Patients with signs and symptoms of severe/complicated malaria requiring parenteral treatment or having mixed *Plasmodium* infection with known history or evidence of clinically significant disorders were excluded. There was no treatment failure at Indian site. Data are being analyzed from all the study sites.

4.2.4 A phase III multicentre, randomised, double-blind, double-dummy, comparative clinical study to assess the safety and efficacy of a fixed-dose formulation of oral pyronaridine artesunate (180 : 60 mg tablet) versus chloroquine (155 mg tablet) in children and adult patients with acute *Plasmodium vivax* malaria

Plasmodium vivax represents a major health problem throughout the tropics. Outside of Africa, it accounts for over 50% of malaria cases, affecting an estimated 70–80 million people per year, notably in Southeast Asia and Central and South America.

The *P. vivax* infection is rarely life-threatening, but it is responsible for an important morbidity in all age groups. Indeed, *P. vivax* forms persistent hypnozoite parasite stages in the liver that can result in multiple relapses of infection, weeks to months after the primary infection. The blood schizontocide chloroquine and the tissue schizontocide primaquine have been the mainstay of *P. vivax* treatment in most areas of the world for the past 50 years. However, susceptibility of *P. vivax* to chloroquine has significantly decreased in various countries within the past two decades. Reports of chloroquine-resistance have emerged from several geographical regions, including India. Therefore, the evaluation of alternative therapies for the treatment of the blood stage of *P. vivax* is strongly needed.

A single tablet strength containing 180 : 60 mg pyronaridine artesunate has been selected for development and licensing, to ease the administration, dosing regimen as well as the maintenance of inventory in low income countries. Taken together, this forms the rationale for developing the fixed-dose combination pyronaridine artesunate (3 : 1) 3-day regimen for the treatment of blood stage *P. vivax* malaria, in parallel to its development in *P. falciparum* malaria.

The primary objective of this clinical study was to compare the efficacy and safety of the fixed combination of pyronaridine artesunate (180 : 60 mg) with that of standard chloroquine therapy in children and adults with acute uncomplicated *P. vivax* malaria.

A phase III multicentre, randomised, double-blind, double-dummy, comparative clinical study was conducted on over 450 adult and children patients with acute *P. vivax* malaria. Patients were aged between 3 and 60 years having weight 20–90 kg with acute uncomplicated *P. vivax* mono-infection confirmed with fever and positive microscopy of *P. vivax* with parasite density ≥250/µl of blood (including at least 50% of asexual parasites) and a rapid test negative for *P. falciparum*. Primary efficacy endpoint was cure rate on Day 14. On completion of follow-up on Day 28, non-G-6-PD deficient patients were given a 14-day course of primaquine (15 mg/day). Patients who were deficient in G-6-PD were treated according to the national policy. All patients were followed-up to Day 42. Secondary efficacy endpoints included: proportion of patients with treatment failure, parasite clearance time, fever clearance time and the proportion of patients aparasitaemic on Days 7, 21 and 28. Safety was assessed through regular assessment with 12-lead ECG; clinical safety laboratory evaluations for haematology, biochemistry and urinalysis. Monitoring of all safety was ensured through a Safety Review Board and a centralised reading of ECGs conducted concurrent with the trial to ensure quality of recording and interpretation. Quality control for microscopy on the primary efficacy parameter was conducted both locally as well as centrally at an independent laboratory.

In India, Mangalore was the study site and 80 patients were enrolled and followed-up for 42 days. The combination was safe, well-tolerated and resulted in high cure rates (>95%).

4.3 Completed studies

4.3.1 Assessment of therapeutic efficacy of Artemether-Lumefantrine (AL) in uncomplicated *Plasmodium falciparum* patients in Sundargarh district, Orissa

AL is one of the ACTs recommended by WHO. The drug has been registered in India in 2006. Currently, coformulated tablets containing 20 mg of artemether and 120 mg of lumefantrine are available in India. The total recommended treatment is six dose regimen twice a day for three days. During efficacy studies in 2007 in Orissa, late treatment failure was observed in few cases. Since lumefantrine absorption is increased by coadministration with fat, low blood levels leading to treatment failure that can result from low fat intake or in malnourished patients. It was therefore proposed to correlate the therapeutic concentration with the treatment outcome. The work was initiated in September 2008 in Sundargarh district where the first line of treatment is ACT as per the revised drug policy of 2007. In all, 56 patients of uncomplicated *P. falciparum* malaria who were fulfilling the inclusion criteria were enrolled in the study. High 28-day cure rates were observed. The data are being analyzed for drug concentration.

4.3.2 *In vitro* sensitivity of Indian *Plasmodium falciparum* strains to antimalarial agents

In vitro sensitivity profiles were assessed in 218 *P. falciparum* isolates from five different sites of India, viz. Jharkhand, Chhattisgarh, Karnataka, Orissa and Goa. Parasite isolates were obtained from the patients with acute falciparum malaria having a parasitaemia ranging from 1000 to 80,000 asexual parasites per microlitre.

In vitro cultures of these isolates were set up in predosed WHO plates of chloroquine, monodesethylamodiaquine, dihydroartemisinin and mefloquine. Plates were incubated at 37°C for 24–30 h. Post-culture blood slides were examined and the counts read in the drug wells. The mean inhibitory concentrations of individual samples for each drug were determined by non-linear regression analysis.

In all, 218 samples were studied from five different sites as well as parasite bank of NIMR; the proportions of successful assays were 58.8 and 36.8% in fresh and cultured isolates respectively. The geometric mean IC_{50} were 21.6, 3.8, 2.4 and 24.7 nmol/L respectively for chloroquine, monodesethylamodiaquine, dihydroartemisinin and mefloquine respectively. The level of resistance in chloroquine was 50%, while that of in amodiaquine was 25%. One isolate showed higher IC_{50} and was resistant to dihydroartemisinin. The isolate also showed resistance to chloroquine as well as amodiaquine. No isolate showed resistance to mefloquine. A high degree (71.4%) of chloroquine resistance was seen in Orissa, followed by Jharkhand (33.3%) and other states. The study shows a high degree of resistance to chloroquine among the Indian *P. falciparum* isolates.

4.3.3 Rapid assessment of extent of unregulated use of artemisinin monotherapy in public and private sectors in India

The study has been completed in six states with funding from World Bank. Questionnaire based survey was carried out to obtain information on prescription practices of artemisinin monotherapy, sale of antimalarials with special reference to artemisinin monotherapy. The World Health Assembly has urged all WHO members states to deploy Artemisinin combination therapies (ACTs) and progressively withdraw oral artemisinin monotherapies from the market. The National Drug Policy of the country has been revised to include artemisinin-based combination therapy for the treatment of falciparum malaria in chloroquine resistant areas. However, antimalarials including artemisinin are prescribed irrationally and this practice increases the chance of development of resistance to artemisinin in malaria parasites. Artesunate monotherapy is prescribed to diagnosed cases in all the states with highest in Jharkhand (40%) followed by Gujarat (11%), Orissa (10%), Delhi (7%), Assam (3%) and Goa (0.5%). Combinations of two or more antimalarials are also prescribed to patients with highest in Goa. Antimalarials were rampantly prescribed to undiagnosed cases in all states with different frequency. Chloroquine + primaquine (CQ + PQ) is prescribed to 20% undiagnosed cases in Orissa. CQ alone is prescribed to 63% undiagnosed cases in Delhi, followed by Orissa (17.5%), Gujarat (14%) and Assam (12%). Artesunate alone is prescribed to undiagnosed cases in Jharkhand (38%), followed by Gujarat (14%) and Delhi (8%).

Prescription pattern of antimalarials by the clinicians showed that antimalarial monotherapy was prescribed by majority of the clinicians, particularly artemisinin monotherapy prescribed by large number of medical practitioners in Jharkhand, Gujarat, Assam, Orissa and Delhi states. The availability of artemisinin derivative alone, at the chemist shops is reported by 75% of the chemists. The sale of antimalarials showed that 42% chemists sell one or the other type of antimalarials without prescription and the most common amongst them was chloroquine.

This study reveals that artemisinin monotherapy is prescribed at all levels of health facilities in the studied states and is available with large number of chemists. An effort to stop the prescription and sale of artemisinin monotherapy is urgently required.

4.4 Operational studies initiated

4.4.1 Therapeutic efficacy studies in selected sentinel sites in India

Antimalarial drug resistance is a major obstacle in the fight against malaria. Establishment or strengthening of systematic surveillance system with periodic updating is essential for containment. As such 13 *Pf* monitoring teams of the National Vector Borne Disease Control Programme (NVBDCP) and also some research institutions do the monitoring of drug resistance. However, the data are still limited and need updating. In addition, a standardized system needs to be adapted so that the data generated by different agencies can be utilized to change the drug policies.

As against *P. falciparum* where regular monitoring of resistance is done by the National Anti Malaria Programme, there has not yet been any systematic and prospective study to detect

chloroquine resistance in *P. vivax* in our country. Therefore, it is important at this early stage to detect, and limit further spread of resistance in *P. vivax* to chloroquine and evaluate the efficacy of alternative drug regimen to treat resistant cases of *P. vivax*.

The studies have been planned at 15 sites with funding from World Bank. Staff recruitment, site selection, disbursement of funds has been done in collaboration with NVBDCP. A national workshop has been planned for orientation of site investigators.

4.4.2 Pharmacovigilance for antimalarial medicines in India

Once marketed a medicine leaves the secure and protected scientific environment of clinical trials and is legally set free for consumption by the general population. By this time, data for only short-term safety and efficacy on a limited number of carefully selected individuals is available. Therefore, it is essential that new evolving treatments are monitored for their effectiveness and safety, and also post release. The switch over to artemisinin combination therapies (ACTs) has necessitated pharmacovigilance system in the countries.

Sentinel sites for therapeutic efficacy studies will be utilized (20 peripheral centres, four for each of five regional units). The data will be collected by MO/LT who are posted for drug efficacy studies using standard format for recording ADR with necessary modifications. Completed forms will be forwarded to the District Centre.

Project has been approved in principle for funding by the World Bank and site selection has been done.

4.4.3 Quality assurance for laboratory diagnosis of malaria

Malaria is one of the most widespread parasitic diseases all over the world. The disease is prevalent in 102 countries and is responsible for over 100 million reported cases annually and 1–2 million deaths, especially in children. Often, diagnosis of malaria is based on clinical symptoms such as presence of chills and rigors, intermittent fever, etc. which are non-specific, leading to false diagnosis and over use of antimalarial drugs, thus increasing the potential of drug resistance, as well as the number of malaria cases. Laboratory diagnosis of malaria greatly facilitates the management of the disease by confirming the clinical diagnosis. Microscopy being the "Gold standard" for malaria diagnosis has got its limitations.

Quality assurance (QA) and adequate monitoring of laboratory services at the peripheral level are important links in the programme. Therefore, it is essential to build and incorporate a quality assurance programme under NVBDCP.

Although in India, under the NVBDCP cross-checking of examined slides is done for ensuring the quality of microscopy, there is no structurred programme for QA of malaria and RDT at present. It is important to develop, implement and establish a quality assurance programme for rapid diagnostic tests as an integrated part of malaria control under NVBDCP.

During the past decade, number of rapid diagnostic test kits (RDTs) for malaria have been developed, evaluated and validated for improved sensitivity and specificity. Like other diagnostic tests, various conditions of manufacture, transport, storage and the method of use may impair the accuracy of RDTs.

The NIMR and NVBDCP will work together for quality assurance of diagnosis of malaria. The objectives of this study are to establish quality assurance for rapid diagnostic tests for malaria in India, to assess the quality of the sample collection and processing, validity of the test methods, monitor reagents, stains, equipments, the performance of test procedures and personnel, to review the test results and to provide feedback for corrective action. The NVBDCP will act as a nodal centre and NIMR as the National Referral Laboratory, which would assess the quality of testing of the Regional Referral Laboratories and the State Referral Laboratories.

This study will involve training of the personnel for QA of both microscopy and rapid diagnostic test kits. The project will help to assure the quality of malaria diagnosis in the field, thus, help to implement the programme effectively. Project has been approved in principle for funding by the World Bank and site selection has been done.

5 HIGHLIGHTS OF THE RESEARCH ACTIVITIES UNDER IDVC PROJECT

Bengaluru (Karnataka)

- C-21 attracticide for control and surveillance of *Aedes* mosquitoes was found very effective in Bengaluru City. There was significant forced breeding in experimental bowls than the control ones ($p<0.01$).
- Production of native antibodies of HRP2 and pLDH is underway. The native HRP2 antigen is 66–68 kDa while recombinant is 37.2 kDa.
- Clinical trial of Pyramax (Pyronaridine artesunate combination) against 80 vivax and 59 falciparum cases showed highly effective results in Mangalore.
- Clinical trial of artesunate-mefloquine combination against 35 falciparum cases indicated high effective results in Mangalore.
- DDT was found totally ineffective against *An. culicifacies* in Andhra Pradesh. Predominance of species C over species B in DDT sprayed areas in Andhra Pradesh is the resultant of low level malaria transmission.
- Pirimiphos-methyl – an organophosphate compound was effective against mosquito larvae for one week.
- Larvivorous fish is very effective for malaria control. Now, this is being implemented in Karnataka through NRHM and *Panchayat Raj* Institutions.
- One chikungunya outbreak investigation was carried out in Dakshina Kannada district. Both *Aedes aegypti* and *Ae. albopictus* were found in the affected areas. Control of breeding has been implemented involving the local *Panchayats*.

Chennai (Tamil Nadu)

- Phase III trials on the application of C-21-attracticide for surveillance and control of dengue and chikungunya mosquitoes were undertaken in Alapuzha district, Kerala.
- Evaluation of Diflubenzuron 25% W.P. (Bi-Larv), an insect growth regulator and Pirimi-phos-methyl (Actellic 50 EC) against mosquito vectors in clean and polluted water was undertaken in Chennai.
- Evaluation of rapid card test for malaria (Pan+Pf) to find out the relative sensitivity and specificity of the kit was also undertaken.
- Studies pertaining to environmental, social and behavioural risk factors related to persistent malaria transmission in Chennai and screening of plant extracts for antimosquito activities have been initiated and are in progress.
- Technical support was provided to various centres/institutes/government agencies and collaborative research/scientific work was also carried out.
- Health education and training programmes on malaria were also imparted to students and people.
- Malaria clinic continued to cater the needs of the people by providing early diagnosis and prompt treatment.

Guwahati (Assam)

- Follow-up field evaluations of long-lasting insecticidal nets (LLINs) impregnated with pyrethroids against malaria transmitting mosquitoes in Assam and associated disease transmission were undertaken.
- Characterization of *Plasmodium falciparum* strains prevalent in north-eastern states was undertaken.
- Malaria transmission and vector incrimination in west Garo hills of Meghalaya was studied.
- Microfilariae (mf) and entomological surveys in Kamrup district of Assam were undertaken.
- Operationalization and impact assessment of larvivorous fish as biological intervention for control of malaria in Assam is in progress.
- Regional level mapping of malaria vectors using RS and GIS in north-eastern states in India to develop strategic plans for malaria control was undertaken.
- Biomonitoring of organochlorine residues in human populations from Assam and their

correlation with food intakes was undertaken.

- Other activities included technical inputs to strengthen the malaria control activities specific to north-eastern region, viz. health education and capacity building measures, mass propagation and distribution of larvivorous fishes (Guppy & Gambusia) in town areas, and malaria outbreak investigations in affected districts of Assam.
- Effective linkages were established with the NGOs and other major establishments for providing evidence-based interventions, and with collaborating agencies/scientists for basic research on disease epidemiology and control.

Hardwar (Uttarakhand)

In addition to ongoing activities on providing consultancy to industrial malaria control, outbreak investigations and malaria clinic, research work was focussed on:

- Antimalarial properties of some plants from Garhwal region of north-west Himalaya.
- Accumulation of organochlorine compounds in sub-Himalayan region of north India.
- Organochlorine residues in soil, water, whole blood and major local food products from low and high malaria endemic areas of Assam.
- Simultaneous determination of curcumin and piperine 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 PHC Gangoh and Nukur of District Saharanpur.
- Evaluation of human safety to residents and spraymen of cyphenothrin as space spray against mosquitoes.
- Field evaluation of Biodart-M, a formulation of *Bacillus thuringiensis* var. *israelensis* (5% WP) against larvae of mosquito vectors.

Jabalpur (Madhya Pradesh)

- Under the ICMR National Task Force project on 'Preparation of a field site for Malaria Vaccine trial in and around Jabalpur', 541 new pregnant women were included this year in the community cohort. Immunological profile of all the subjects against 13 different stage-specific peptides was drawn. The genetic poly-morphism in the vaccine candidate antigen genes (MSP1, MSP2, MSP3, TRAP, RAP1, CSP, EBA-175 & AMA1) and drug resistance genes (*pfcrt*, *pfdhfr* and *pfdhps*) were studied.
- Vector incrimination of malaria vectors showed one *Anopheles culicifacies* positive for sporozoites by ELISA technique. All *An. fluviatilis* were of sibling species T.
- Epidemic investigations were carried out in four 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.
- The study on intensive monitoring of insecticide residual spray (IRS), insecticide-treated mosquito nets (ITMN), 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 the recommendations were sent to the NVBDCP.
- 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 organized in November 2008 and January 2009 at NIMR Field Unit, Jabalpur.

Nadiad (Gujarat)

In addition to technical support to the state health programme in providing training, assessing malaria situation in Anand and Kutchch, independent assessment of ITN programme, research activities were concentrated on the following:

- Phase III evaluation to compare insecticidal efficacy and community acceptance of long-lasting insecticidal nets with conventional insecticide-treated nets in India.
- Health impact assessment of development project: Impact of Sardar Sarovar Project on vector borne diseases in Gujarat.
- Scaling-up of use of larvivorous fishes, particularly *Aphanius dispar* and capacity strengthening in Gujarat.
- Participating in a collaborative project with Michigan University (USA) on developing a framework for predicting malaria outbreaks in rural and urban Gujarat.
- Multicentre, Phase III evaluation of effectiveness of Diflubenzuron 25% WP (Bi-

Larv), an insect growth regulator for mosquito vector control in urban settings.

Panaji (Goa)

- Community randomized evaluation of the effectiveness of the insecticide-treated nets for malaria control was undertaken in construction workers in the urban slums of Goa.
- Molecular characterization and efficacy of mosquito pathogenic bacteria from mangrove and paddy-fields in Goa was done.
- Assessed the efficacy, safety and population-pharmakokinetics of the fixed-dose combination of Artesunate-Mefloquine in the treatment of acute uncomplicated *P. falciparum* malaria in India.
- Rapid response for preventing an impending JE outbreak in Goa by monitoring mosquito density and instituting engineering intervention measures for preventing vector breeding.
- Estimation of malaria burden in Jharkhand state was continued.
- Investigations into the *P. vivax* deaths in Goa was confirmed using clinical, parasitological, molecular and pathological evidences.
- Multicentre Phase III evaluation of effectiveness of Diflubenzuron 25% WP (Bi-Larv), an insect growth regulator for mosquito vector control in urban settings.

Raipur (Chhattisgarh)

The Field Unit carried out the following during the report period.

- Evaluation of bioefficacy of field distributed long-lasting insecticidal nets (LNs) impregnated with alpha-cypermethrin (Interceptor®) against *An. culicifacies* is in progress in villages of Amoda CHC in District Kanker and observation of the usage pattern and washing practices by the communities.
- WHOPES Phase III evaluation (household randomized trial) to compare insecticidal efficacy and community acceptance of long-lasting insecticidal nets (Interceptor) with conventional insecticide-treated nets in India is under progress in seven villages of District Kanker.
- Selection and preparation of sites for studies on efficacy of artesunate-based combination therapy (ACT) (artesunate and sulphadoxine-pyrimethamine) for the treatment of uncomplicated *P. falciparum* malaria with special reference to bioavailability of artesunate and sulphadoxine-pyrimethamine in Chhattisgarh.
- Evaluation of Biodart-M 5% WP, a formulation of *Bacillus thuringiensis* var. *israelensis* in various habitats in a peri-urban locality in Raipur City.
- Entomological surveys in various parts of the state for collection of biological material to study the distribution of sibling species of *An. culicifacies* and *An. fluviatilis*.
- Technical support to the programme by: (i) monitoring of malaria control activities in seven high *Pf* endemic districts of Chhattisgarh; (ii) entomological survey for identification of sibling species complexes of *An. culicifacies* and *An. fluviatilis* in various districts of Chhattisgarh; (iii) monitoring of insecticide resistance in malaria vectors in various parts of the state; (iv) facilitating the cross-checking of blood slides (for malaria and filaria parasites) received from various districts; (v) facilitating the quality check of blood slides to compare various rapid diagnostic kits in Dantewada district by MSF; (vi) one-day orientation training to III-year MBBS students from Govt. Medical College and Homoeopathic Medical College, Raipur; and (vii) undertaking baseline survey for malaria control activities as per the requirement of NVBDCP for World Bank aided malaria control project in 112 villages of six malaria endemic districts in the state.

Ranchi (Jharkhand)

In addition to technical support to local health department in providing training, monitoring of programme implementation and IEC activities, running malaria and filarial clinics, the following research activities were undertaken:

- Mosquito fauna and bionomics of malaria vectors.
- Incrimination of *An. annularis* as vector of malaria.
- Insecticide susceptibility of malaria vectors *An. culicifacies, An. fluviatilis* and *An. annularis* in Gumla district.
- Insecticide susceptibility status of *Ae. agypti* (Linn) in Ranchi City.
- Malaria outbreak investigations in Giridih district.
- Filariasis survey in the primitive tribe Oraon in villages Purio, Khatri Khatinga, Murto and

Khukhara of Bero PHCs of District Ranchi

- Therapeutic efficacy of chloroquine against uncomplicated falciparum malaria in Car Nicobar Island revealed early treatment failure in 23 cases (47.9%).
- Evaluation of use of DDT spray in Bihar state against kala-azar

Rourkela (Orissa)

In addition to technical support to the national programme in providing training, monitoring of malaria situation and IRS operations, research activities were focussed on the following:

- Work on development of field site for malaria vaccine trial was continued.
- Evaluation of Icon-Life–a long-lasting insecticidal net (LLIN) treated with deltamethrin against malaria vectors and disease transmission.
- Extended evaluation of PermaNet, Olyset and Interceptor LLINs was undertaken.
- A Phase II, randomized, open label, multicentre study to assess the antimalarial efficacy and safety of arterolane (RBx 11160) maleate and piperaquine phosphate coadministration and Coartem® in patients with acute uncomplicated *P. falciparum* malaria.
- Operational study for the assessment of therapeutic efficacy of artesunate + sulphapyrimethamine (ACT) in uncomplicated *P. falciparum* patients in Sundargarh district.
- Assessment of therapeutic efficacy of Coartem (Artemether + Lumefantrine) in uncompli-cated *P. falciparum* patients in Sundargarh district.
- Evaluation of rapid card test for diagnosis of malaria (Pan + *Pf*).
- Action plan for intensive malaria control programme (Zero transmission) in two pilot districts of Orissa.

6 RESEARCH SUPPORT FACILITIES

6.1 Animal house facility

NIMR has an animal 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 material

6.2.1 Mosquito species

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

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

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	Sub-metacentric	A
An. culicifacies	Rameswaram	Sub-metacentric	A
An. culicifacies	Jabalpur	Sub-metacentric	C
An. culicifacies	Rourkela	Sub-metacentric	C
An. culicifacies	JP-2	Sub-metacentric	C
An. stephensi	Haryana		
An. stephensi	Punjab		
An. stephensi	Delhi		
An. stephensi	Okhla, Delhi		
An. fluviatilis	Rourkela		T
Culex quinquefasciatus	BSSS (Sensitive to biocide)		
Aedes aegypti	Delhi		
Mutant Lines			
An. stephensi	Black larva with white eye		
Culex quinquefasciatus	Red eye		

human plasmodia preserved/maintained in Malaria Parasite Bank were supplied to various research organisations. During 2008, a total of 143 isolates were collected from different parts of the country. Out of these collected isolates, 55 were *P. falciparum* and 88 were *P. vivax* isolates. 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

Table 2. Human malaria parasites preserved in the Parasite Bank

Parasite species	Collection sites		No. of isolates collected/years of collection			Total
	State	District	1992–2006	2007	2008	
P. falciparum	Andhra Pradesh	Visakhapatnam	12	—	—	12
	Assam	Sonapur	20	—	—	27
		Tezpur	6	—		
		Nalbari	1		—	
	Chhattisgarh	Jagdalpur	14	—	—	40
		Bilaspur	26	—	—	
	Delhi		191	2	5	198
	Gujarat	Anand	4	—	—	11
		Kheda	7	—	—	
	Goa	Panaji	—	—	18	18
	Haryana	Gurgaon	25	—	—	25
	Karnataka	Mangalore	14	—	14	28
	Madhya Pradesh	Mandla/Jabalpur	14	—	—	14
	Meghalaya	Tura	18	—	—	18
	Mizoram	Kolasib	—	6	—	6
	Orissa	Rayagada	29	—	—	84
		Sundargarh	42	—	13	
	Rajasthan	Alwar	25	—	—	98
		Bharatpur	35	—	—	
		Jaisalmer	38	—	—	
	Tamil Nadu	Chennai	4	—	—	29
		Ramanathapuram	1	19	5	
	Uttar Pradesh	Baharaich	22	—	—	136
		Gautam Budh Nagar	37	—	—	
		Ghaziabad	17	—	—	
		Allahabad	60	—	—	
	West Bengal	Kolkata	18	—	—	19
		Midnapur	1	—	—	
	Total		681	27	55	763
P. vivax	Assam	Sonapur	2	—	—	2
	Delhi		20	—	18	38
	Goa	Panaji	—	—	23	23
	Karnataka	Mangalore	6	—	32	38
	Madhya Pradesh	Jabalpur	3	—	—	3
	Orissa	Rourkela	4	—	8	16
		Bissam Cuttak	4	—	—	
	Uttar Pradesh	Shankargarh	4	—	—	19
		Mirzapur	11	—	—	
		Baharaich	2	—	—	
		Gautam Budh Nagar	2	—	—	
	Tamil Nadu	Ramanathapuram	—	9	—	25
		Chennai	9	—	7	
	West Bengal	Kolkata	1	—	—	1
	Total		68	9	88	165
P. malariae	Orissa		4	—	—	4
	Delhi		1	—	—	1
	Total		5	—	143	5

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*

extracts/fractions for their anti-plasmodial activity against CQ sensitive and resistant *P. falciparum* isolates is a routine activity of the Malaria Parasite Bank. 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)

6.3 Library

The Institute has one of the best libraries in the country in the field of malaria having more than 7500 books, 4283 bound journals, 3673 reprints, 18 video cassettes, 27 audio cassettes, 20 microfilms, 19 theses and 106 national and international reports. About 52 journals (39 Foreign and 13 National) are being subscribed besides eight journals which are received on complimentary and exchange basis.

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

Adapted isolates susceptible to chloroquine	54
Adapted isolates resistant to chloroquine	52
NF-54, an infective gametocytes 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
Isolates with MSP-1, MSP-2 and GLURP markers	40

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

Place of collection	No. tested	Sensitive	Resistant*
Delhi (UT)	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.

From July, 2008 the library is functioning in the newly constructed campus at Dwarka. During the year 2008–09 around 175 books are newly added to the collection. Journals of reputed publishers such as Elsevier, Wiley, American Society of Microbiology (ASM) and Entomological Society of America (ESA) are subscribed both in print and online mode. Library is the regular subscriber of World Health Organization (WHO) publications. It provides information services to the scientists, research-scholars and outside visitors. The library is the support centre for researchers of ten 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 National Informatics Centre-ICMR and a member of Developing Library Networks (DELNET) to fulfill 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, i.e. 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 Equipment and facilities at NIMR

The following equipment and facilities are available at NIMR headquarters, New Delhi.

Equipment

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

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 antigen' 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 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 Govt. 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 antimalarials' in collaboration with the NVBDCP, and funded by the World Bank.
12. 'Therapeutic efficacy of Artemether-Lumefantrine combination in Orissa' in collaboration with AIIMS, New Delhi and WHO.
13. 'Pharmacovigilance of antimalarials in India' in collaboration with AIIMS, New Delhi and NVBDCP, and funded by 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 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 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 (BUSPH) funded by ICMR 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, 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, 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 Hygeine and Tropical Medicine, London, BISAG, Gandhinagar, Govt. of Rajasthan and Gujarat, funded by Michigan University, U.S.A.
28. 'Quality assurance of rapid diagnostic kits for malaria in India' in collaboration with NVBDCP, funded by World Bank
29. 'Phase II/III clinical trial 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.
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 State Governments of Assam, Mizoram and Uttarakhand, IARI, IIT and MoEF, funded by ICMR.
33. 'Impact of climate change on dengue in Delhi and environs' in collaboration with Municipal Corporation of Delhi, funded by Ministry of Environment and Forest, Govt. of India, New Delhi.
34. 'Operational research on surveillance and intervention strategies in Gadchiroli district of Maharashtra' in collaboration with Govt. of Maharashtra, funded by NVBDCP.
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).

8 HUMAN RESOURCE DEVELOPMENT

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 different universities. Details of Ph.D. students working at NIMR and its Field Units are furnished in Table 1.

8.2 Ph.D. Awardees

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

1. Dr Alex Eapen was awarded Ph.D. on the topic, 'Systematics and larvivorous potential of Indian fishes of the genus *Aplocheilus* McClelland (Pisces: Cyprinodontiformes) with special reference to *Aplocheilus parvus* Raj' by the University of Madras, Chennai.
2. Dr Gertrude N. Kiwanuka was awarded Ph.D. on the topic 'Characterization of *P. falciparum* parasites of normal as well as sickle-cell children among Uganda population using molecular markers' by the Mbarara University, Uganda.
3. Dr U. Sreehari was awarded Ph.D. on the topic, 'Efficacy of long-lasting insecticide treated net technology against *Anopheles culicifacies*, a principal malaria vector in India' by the Jamia Millia Islamia, New Delhi.
4. Dr Sharmila Pahwa was awarded Ph.D. on the topic, 'Environmental epidemiology of malaria in three states of India' by the CCS University, Meerut.
5. Dr Anil Sharma was awrded Ph.D. on the topic, 'Immune response of *Anopheles culicifacies* sibling species complex (Diptera: Culicidae)' by the Maharshi Dayanand University, Rohtak, Haryana.
6. Dr Mehrunissa Khan was awrded Ph.D. on the topic, 'Comparative susceptibility of *Anopheles fluviatilis* species T and U to plasmodial infection' by the Aligarh Muslim University, Aligarh.
7. Dr Mohammad Sohail was awarded Ph.D. on the topic, 'Study on immuno-modulatory and inflammatory cytokine and cytokine gene polymorphism in malaria in Indian isolates' by the Vinoba Bhave University, Hazaribagh.
8. Dr. B.N. Parkash was awarded Ph.D. on the topic, 'Traditional plant based remedy for prevention of malaria' by the Manipal University, Manipal.

8.3 M.Sc. Projects

This year, more than 30 students of M.Sc. in Life Sciences 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*.
- Short-term cultivation of *P. vivax* and other species of *Plasmodium*.
- *In vitro* cultivation of exo-erythrocytic stages of *P. vivax*.
- *In vitro* testing for sensitivity of *P. falciparum* isolates to antimalarials.
- *In vitro* and *in vivo* 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.

Table 1. Details of students who were pursuing Ph.D. at NIMR during 2008-09

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
Swapnil Roy	Accumulation of organochlorine residues in sub-Himalaya region of north India	Gurukul Kangri Viswavidhyalaya, Hardwar
Praveen Kumar Bharti	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	Rani Durgavati University, Jabalpur
Surendra K. Prajapati	Molecular studies on house keeping genes of *Plasmodium vivax*	Jamia Millia Islamia, New Delhi
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
AK Upadhyay	Studies on the mosquito fauna and bio-ecology 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
Mahesh B. Kaliwal	Bio-ecology of *Culex quinquefasciatus*, the principal vector of *Lymphatic filariasis* in Goa	Goa University, Goa
Deeparani Prabhu	Studies on mode of action and bio-efficacy 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 northeastern 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
Geeta Sharma	Target antigens of immunity to *Plasmodium vivax*: characterization in areas of north-eastern 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
L. Dolie Devi	Molecular characterization of symptomatic and asymptomatic *P. falciparum* malaria in India	Goa University, Goa
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	Goa University, Goa
Sandeep Kumar	Organochlorine residues in soil, water, whole blood and major local food products from Assam	Jiwaji University, Gwalior
Ripu Dawan Sood	Comparative efficacy of different long-lasting insectidal nets against malaria vectors, *An. stephensi* and *An. culicifacies* in India	Indira Gandhi National Open University, New Delhi

9 RESEARCH PAPERS PUBLISHED

(January to December 2008)

9.1 In Scientific Journals

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11. Dash AP, Valecha N, Anvikar AR, Kumar A. Malaria in India: challenges and opportunities. *J Biosci* 2008; *33*(4): 583–92.
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12. Dev V, Dash AP, Hojai D. Fishing out malaria in Assam, north-eastern India: hope or hype? *Trans R Soc Trop Med Hyg* 2008; *102:* 839–40.
13. Dev V, Doley GC, Dash AP. Rolling back malaria is possible. *Indian J Med Res* 2008; *128:* 82–3.
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9.2 In Books and Proceedings

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3. Singh N, Sinha S, Awadhiya SB, Singh MP, Dash AP. Malaria in pregnancy–a neglected disease in India. In: Sharma RS, Rajanna A, Rajalaxmi M, editors. *Recent advances and challenges in reproductive health research*. New Delhi: ICMR 2008; p. 241–51.

9.3 Publications in National language

1. Nagpal BN, Srivastava Aruna. Sukhagrast chetron mein *Anopheles stephensi* ghatko ki jaivparisthiki. *Malaria Patrika* 2008; *16*(1): 12–5.
2. Tyagi Padmawati. Malaria mein raktalpta ki samasya evam nivaran. *Malaria Patrika* 2008; *16* (4): 5–7.

10 OTHER ACTIVITIES

10.1 Preparation of health education materials

10.1.1 Pamphlets/Booklets

Material for following scientific pamphlets were updated in consultation with experts. Essential materials and illustrations, etc. were incorporated in the pamphlets. Pamphlets were designed and illustrations were placed at appropriate places.

- *Anopheles culicifacies* and *Anopheles fluviatilis* complexes and their control
- Biolarvicides for mosquito control
- Larvivorous fish for mosquito control
- Insecticide treated nets (ITNs/LNS) and long-lasting materials (LM) for malaria control
- Major vectors of India
- Expanded polystyrene beads to control mosquito breeding

10.1.2 Posters

Several posters depicting the research work of different laboratories and field studies were designed, printed and mounted in 3 x 4 feet size for display in exhibitions and conferences.

10.1.3 Distribution of video CDs

Video CDs/Films on malaria/mosquito related subjects produced at NIMR were distributed to participants of different training programmes organized by NIMR, NVBDCP and NICD.

10.2 Health camps organized

Health camp was organized at the Center for Rural Development and Technology, IIT, Delhi in September 2008 for students, scholars and faculty. Demonstration of different live stages of mosquitoes and displaying of charts showing life cycle of malaria parasites (*Plasmodium falciparum* and *Plasmodium vivax*) was carried out along with the discussion on the various aspects of vector borne diseases (VBD) and general awareness regarding the prevention and control of VBD to scholars and faculty. Video-films showing intervention methods of mosquito control and life-cycle of *Plasmodium* were shown. The students were also involved in question session and discussion.

10.3 Photography

In the photography section following photography work was carried out on occasion of various meetings /workshops/functions, etc. held at NIMR and other places. Photographs were taken on the occasion of workshops on 'Basic malaria parasitology and entomology', 'Impact of climate change on health', 'Artesunate monotherapy', 'Developing basic scientific skills', 'Indian Medical Association workshop', field photography under 'Evaluation of C-21 Attracticide in the control of mosquito breeding', visit of WHO scientists at NIMR, Dwarka, Health awareness camps, Hindi pakhwara celebration, Goa University's team's visit, Release of NIMR publications function; Meeting on climate change



at NIMR, RAC and SAC meetings, Annual Day function at NIMR, Dwarka, and Mosquito breeding sites at venue of Common Wealth Games, Delhi.

10.4 Documentation work

A documentation cell was established for easy access to published materials, project reports and other related information of NIMR. In this direction following tasks have been initiated:

- All the issues of *Indian Journal of Malariology/ Journal of Vector Borne Diseases* from the year 1981 to 2009 were collected.
- Contents of *Indian Journal of Malariology/ Journal of Vector Borne Diseases* from the year 1981–2009 were arranged yearwise.
- Complete list of the published research papers by NIMR scientists from the year 1980 to 2009 was prepared.
- Complete publication list of the individual NIMR Scientist was prepared.
- Reprints of published research papers by NIMR scientists from the year 1980 to 2009 were collected.
- All the publications of NIMR such as Annual Reports, Brochures/Pamphlets, Books (authored/edited by NIMR scientists), Profiles, Proceedings, *Malaria Patrika*, Newsletters, etc. were collected.
- Complete list of the Projects undertaken by NIMR scientists from the year 1977 to 2009 was prepared.

10.5 Publications

The Publication Division of NIMR has been bringing out a scientific quarterly journal, *Journal of Vector Borne Diseases (JVBD)*. During this year the issues were brought out regularly on time. The JVBD was included in Thomson ISI indexing and abstracting agencies, which award Impact factor to scientific journals. In addition we have tied up with another two firms for the promotion of the journal. The number of citations of the articles published in the journal is increasing progressively. During the year 2008, all the articles published in the journal (from 2003) were uploaded in PubMed and DOAJ for providing open access to the full text articles.

The Division is also bringing out *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, in the year 2008, the Division has published NIMR Profile, focussing the research activities of the Institute since its inception and various IEC documents, namely Exapanded polystyrene beads for mosquito control, Larvivorous fish for mosquito control, Insecticide treated nets, Long-lasting nets and materials for malaria control, *Anopheles culicifacies* and *An. fluviatilis* complexes and their control, and Biolarvicides for mosquito control.

These publications were released by Dr V.M. Katoch, Secretary, Department of Health Research, Government of India and Director General, Indian Council of Medical Research, New Delhi on 26 March 2009 at NIMR. Prof. A.P. Dash, Regional Adviser, WHO-SEARO and Former Director, NIMR and Prof. R.C. Mahajan, Chairman, SAC also attended the Publications release function.

10.6 Workshops/Training courses organised

- A five-day workshop was organised by NIMR from 28 April to 2 May 2008 on 'Basic malaria parasitology and entomology' under NYU-NIMR collaboration.
- A workshop was held for developing 'Scientific skills' among young researchers from 18 to 20 August 2008. It was facilitated by Drs Steven Sullivan and Jane Carlton, New York University School of Medicine.
- Training was organized on Treatment of malaria for resident doctors of Department of Community Medicine, B.J. Medical College, Ahmedabad in collaboration with Vector Borne Disease Control Programme in India from 23 to 29 July 2008 at the Institute.
- A workshop on '*P. vivax* ex-vivo maturation' was conducted from 25 August to 5 September 2008. Faculty members were Dr Bruce Russell of the Singapore Immunology Network and A*STAR, Singapore apart from scientists of NIMR.
- NIMR organized a symposium on malaria and dengue in collaboration with the Janakpuri Chapter of the Indian Medical Association and Municipal Corporation of Delhi, for clinicians on 27 September 2008.
- A seminar was organized on National malaria drug policy for Post Graduate students of Community Medicine Department, NHL Medical College, Ahmedabad on 17 October 2008.
- A workshop on malaria and other vector borne diseases was organized for Medical Officers of Municipal Corporation of Delhi on 3 December 2008.

10.7 Awards received

- Dr V.K. Dua was felicitated with Dr V.P. Sharma Oration Award at the symposium on 'Recent advances in vector biology and control' held at DAV College, Dehradun, Uttarakhand from 3–4 December 2008.
- Ms. Gauri Awasthi visited Ludwig Maximilian University, Munich, Germany for three months to study the selective forces operating in and around *pfcrt* in *P. falciparum*. She was awarded prestigious travel fellowships from the Journal of Cell Sciences, U.K. and Boehringer Ingelheim Funds, Germany. She was also awarded Geprufte Wissenschafliche Hilfskraft fellowship from LMU, Munich.
- Ms. Prerana Sethi and Mr. Gaurav Verma were awarded Young Scientist Awards for Oral and Poster presentations respectively at the symposium on 'Recent advances in vector biology and control' held at DAV College, Dehradun, Uttarakhand from 3–4 December 2008.
- Ms. Prerana Sethi and Ms. Kumkum Mishra were awarded Young Scientist Awards for their best posters in Chemistry and Environment Science respectively in III Uttarakhand Science Congress held at IIT, Roorkee.

10.8 Conferences/Workshops/ Meetings attended

Adak T

- Attended International conference on "Cryo-preservation practices of various biological materials used for different research purposes and the usages of liquid nitrogen as cryo-preservation material for research and industry" held at Netherlands from 18 to 24 May 2008.
- Attended the Technical meeting on the

occasion of World Health Day at National Institute of Communicable Diseases, Delhi on 7 April 2008.
- Attended the conference of CPCSEA at Hyderabad from 28 to 30 Januray 2009.

Anvikar Anup
- Attended meeting on Antimalarial treatment in India at WHO-SEARO on 14 October 2008.
- Attended the XV RBM Partnership Board Meeting at New Delhi on 10 November 2008.
- Participated in the Brainstorming meeting to form treatment guidelines for diagnosis and treatment of malaria in India at New Delhi from 17 to 19 March 2009.
- Participated and presented a paper at the International symposium on Tribal Health held at RMRCT, Jabalpur from 27 February to 1 March 2009.
- Attended a training programme on HRP II technique for *in vitro* sensitivity of antimalarials at Malaria Research initiative, Bundarban, Bangladesh from 13 to 21 July 2008.

Atul PK
- Attended CPCSEA National Conference held at Hyderabad from 30 to 31 January 2009.

Dash AP
- Attended first meeting of investigators of TDR Business Line of Innovative Vector Control International (BL 5) TDR BCV SAC meeting at Geneva from 1–4 April 2008.
- Attended a meeting on Protecting health for climate change, Indian Association of Epidemiologists at NICD, Delhi on 7 April 2008.
- Attended Basic malaria parasitology and entomology workshop at NIMR, Delhi on 28 April 2008.
- Attended Central Insecticide Board meeting on 29 April 2008.
- Attended Scientific Advisory Committee meeting of Institute of Life Sciences, Bhubaneswar on 1 May 2008.
- Attended CCM Orientation workshop at New Delhi on 6 May 2008.
- Attended Expert Committee meeting on Climate change at Vigyan Bhawan, New Delhi on 15 May 2008.
- Attended a meeting on LLINs at Nirman Bhawan, New Delhi on 6 May 2008.
- Attended CIB meeting at Faridabad on 12 June 2008.
- Attended symposium on Capacity building for malaria vaccine development at Pune on 21 June 2008.
- Attended meeting on trial of C-21 attracticide and climate change on malaria at Nirman Bhawan, New Delhi on 25 June 2008.
- Visited CDC, Atlanta in connection with collaborative studies on malaria from 8 –11 July 2008.
- Attended ICMR sponsored projects review committee meeting at New Delhi on 24 July 2008.
- Attended a meeting on Climate change on malaria at Nirman Bhawan, New Delhi on 25 July 2008.
- Delivered a lecture on Climate change and malaria on the occasion of NICD Foundation Day on 30 July 2008.
- Attended a meeting at DBT on 8 September 2008.
- Attended a meeting on Ecological succession of anopheline species in North Eastern States of India at New Delhi on 18 September 2008.
- Attended Indian Medical Association workshop on dengue and malaria at New Delhi on 27 July 2008.
- Attended Scientific Advisory Committee meeting of Centre for Research in Medical Entomology at Madurai on 6 October 2008.
- Attended meeting on Climate change on malaria at Nirman Bhawan, New Delhi on 17 October 2008.
- Attended Expert Group meeting on assessment of impact of climate change on vector borne diseases at New Delhi on 24 October 2008.
- Attended Roll Back Malaria meeting at New Delhi on 9 September 2008.
- Attended World Bank Technical Review Mission, NVBDCP Support Project meeting on 14 November 2008.
- Attended ISPOR India Chapter meeting at India Habitat Centre, New Delhi on 21 November 2008.
- Attended WHO Consultation on Development of a Global Operational Plan for Integrated Vector Management (IVM) at WHO, Geneva from 1–3 December 2008.
- Attended Technical Specification Committee meeting at Nirman Bhawan, New Delhi on 11 December 2008.
- Attended Central Insecticide Board meeting at ASRB, Krishi Anusandhan Bhawan, New Delhi on 19 December 2008.
- Attended Scientific Advisory Committee meeting of RMRCT, Jabalpur on 22 December 2008.
- Attended PRC meeting of Malaria & Filaria and Leishmaniasis and task force meeting at ICMR on 2 January 2009.

- Attended ICMR meeting on Environment and malaria at Goa on 12 January 2009.
- Attended *Plasmodium vivax* Vaccine Board meeting at London from 15–16 January 2009.
- Attended Malaria Elimination meeting at Goa on 27 January 2009.
- Attended Estimation of malaria burden meeting at NVBDCP, Delhi on 3 February 2009.

Dhiman RC

- Delivered invited lecture at International conference on Tribal Health held at Jabalpur from 27 February to 1 March 2009.
- Delivered invited lecture on 'Impact of climate change on health' at TERI conference held at TERI Gram, Gurgaon on 13 November 2008.
- Delivered invited lecture on 'Threat of global warming to vector borne diseases' in TROPACON-2 held at AIIMS, New Delhi on 30 October 2008.
- Participated in NATCOM Training workshop on climate change scenarios held at IITM, Pune from 13 –14 October 2008.
- Delivered invited lecture at National Conference on climate change organized by Lions Club, Bhubaneswar on 'Climate change: a challenge for vector borne disease' on 12 July 2008.
- Delivered invited lecture on World Health Day seminar organized by PGIMER, Chandigarh on 'Climate change and its impact on vector borne disease' on 7 April 2008.
- Was invited to participate and conduct a session on Malaria in a training programme organized by UNICEF on 'Managing public health in disasters' on 18 March 2009.

Joshi Hema

- Attended the 'Grants policy and management training' conducted by National Institute of Allergy and Infectious Diseases (NIAID), NIH, DGHS at New Delhi from 30 July–1 August 2008.

Mishra Neelima

- Attended WHO-ICMR sponsored training programme on Good clinical laboratory practices at RMRI, Patna from 27–30 May 2008.
- Invited lecture on Rapid assessment of unregulated use of artemisinin monotheraphy in public and private sector in India at WHO country office in a meeting on Antimalaria treatment in India at WHO Regional Office for the South East Asian Region, New Delhi on 14 October 2008.
- Invited lecture on 'Malaria case management at Point of Care' at XV RBM Partnership Board meeting on 10 November 2008.
- Invited lecture in NVBDCP and World Bank on "Rapid assessment of unregulated use of artemisinin monotheraphy in public and private sector in India" on 14 November 2008.

Mittal PK

- Attended the dissemination workshop on "Profile of health research" organized by NISTADS, Pusa, New Delhi on 30 May 2008 and Chaired the session.

Nagpal BN

- Attended meeting on the completion of project and preparation of final report of the project entitled, "Application of attracticide (Oviposition in combination with insect growth regulator) for surveillance and control of dengue and chikungunya mosquitoes" organised by NIMR at DRDO Guest House, New Delhi on 31 March 2009.
- Attended a training course on "Disease surveillance and use of new tools in planning and management of vector borne diseases" for Dy. Health Officers and Epidemiologists of MCD on Prevention and control of vector borne diseases on 24 March 2009.
- "General morphological characters of mosquitoes and difference between anophelines and culicines followed by video-film on morphology of mosquitoes" at training course for Dy. Health Officers and Epidemiologists of MCD on Prevention and control of vector borne diseases on 23 March 2009.
- "Demonstration of vectors of vector borne diseases" at training course for Dy. Health Officers and Epidemiologists of MCD on Prevention and control of vector borne diseases on 23 March 2009.
- "Bioecology of disease vectors" at training course for Dy. Health Officers and Epidemiologists of MCD on Prevention and control of vector borne diseases on 23 March 2009.
- Attended Publication Advisory Committee meeting of *Journal of Vector Borne Diseases* at NIMR, New Delhi on 14 March 2009.
- Attended and presented paper "Health impact assessment of vector borne diseases in project/ developmental area in workshop on preparation of action plan for prevention and control of vector & water borne disease during common wealth games 2010" organised by MCD, Delhi on 7 March 2009.

- Attended and presented paper entitled, "Health impact assessment of Indira Sagar Dam in M.P. in International symposium on Tribal health organized by RMRC, Jabalpur on 27 February 2009.
- Attended and received awards to "Recognize and honour the usage of geospatial technology in health sector" organised by GIS Development (P) Ltd on 12 February 2009.
- Attended meeting to "Review the water borne diseases and the vector borne disease control measures being undertaken in Delhi" under the chairmanship of Dr. Yoganand Shastri, Hon'ble Minister of Health, GNCTD at Delhi Secretariat, Delhi on 14 May 2008 and subsequently every second Tuesday of the Month from May to December 2008.
- Attended and presented presentation "*Aedes* breeding in Delhi" at MCD Commissioner Office, Delhi Gate on 17 November 2008.
- Attended meeting "Discussion on manpower and training status of VBD Consultants with partner institutions – PHFI, NICD, NIMR" organized by World Bank Technical Review Mission, NVBDCP on 15 November 2008.
- Attended meeting on "Structure-function analysis of malarial cysterine proteases– Falcipains" delivered by Dr. Kailash Chand Pandey, Deptt. of Medicine, SFGH-UCSF, San Francisco on 23 October 2008.
- Attended a meeting to review the vector borne diseases in Delhi under the chairmanship of Commissioner MCD, at Commissioner Office, Delhi Gate on 10 October 2008.
- Attended a meeting "Applications of GIS to public health" at PHD House, Delhi organized by Public Health Foundation of India on 30 September 2008.
- Attended a meeting for assessment of survey/ studies/planning and implementation of the plans on environmental safeguard measures for Sardar Sarovar Project & Indira Sagar Project at Indore organized by NVDA on 25 September 2008.
- Attended a meeting to discuss the role of Civil Engineering Department for containment of Dengue/Chikunguniya diseases in NDMC area organised by Health Department, NDMC on 8 September 2008.
- Attended meeting on "GIS based vector surveillance in collaboration with NIMR for prevention and control of vector borne diseases in Delhi" organized by MCD Anti Malaria Operations (HQ) on 21 August 2008.
- Attended meeting on the work progress of the project entitled, "Application of attracticide (Oviposition in combination with insect growth regulator) for surveillance and control of dengue and chikungunya mosquitoes" organised by NIMR at DRDO Guest House, New Delhi on 19 June 2008.
- Attended a meeting to discuss issues relating to launch of a Short-term Certificate Programme (3-months) for malaria managers by PHFI/AIIH&PH under the chairmanship of Sh. Deepak Gupta, Spl. Secretary, Ministry of H & FW on the behalf of Director at Nirman Bhawan, New Delhi.
- Attended meeting to "Review the vector borne diseases in Delhi" under the chairmanship of Commissioner, MCD at Commissioner Office, Delhi Gate on 29 April 2008.
- "Identification of malaria and dengue vectors — lecture-cum-demonstration in workshop on "Basic malaria parasitology and entomology" organized by NIMR, Delhi on 28 April 2008.
- "Morphology & taxonomy of mosquitoes" in workshop on "Basic malaria parasitology and entomology" organized by NIMR, Delhi on 28 April 2008.
- "Live demonstration of adult and immature stages of *Anopheles*, *Culex* and *Aedes*" in workshop on "Basic malaria parasitology and entomology" organized by NIMR, Delhi on 28 April 2008.
- Attended meeting to "Review the dengue action plan/contingency plan and its activities undertaken for the prevention and control of dengue fever" under the chairmanship of Addl. DG & Director, NICD at Nirman Bhawan on 15 April 2008.
- Attended workshop on "Community participation for prevention and control of dengue fever and its legal provisions" organized by MCD Health Department on 12 April 2008.
- Attended a meeting on "Geographical information system based surveillance" under the chairmanship of MHO organized by MCD on 10 April 2008.

Nanda Nutan

- Attended International symposium on tribal health entitled, "Bionomics of malaria vectors in potential malaria vaccine trial sites in central India, Jabalpur Malaria Project-II" held at RMRC, Jabalpur from 27 February – 1 March 2009.
- Attended International symposium on tribal health entitled, "Prevalence of ABO blood groups, G6PD deficiency and haemoglobin variants in malaria endemic Sundargarh

district of Orissa" held at RMRC, Jabalpur from 27 February – 1 March 2009.

Raghavendra K

- Presented an invited lecture entitled, "Malaria control: present needs for vector control" in symposium–150 Birthday celebrations of Sir Ronald Ross. Ross perspective: Global warming/ malaria alarming at Sir Ronald Ross Institute, Hyderabad.
- Presented a paper on "Insecticide resistance and malaria control" in the symposium "Essence of malaria research even after a century of its discovery", organized by Osmania University, Hyderabad on 13 May 2008.
- Attended inception meeting on World Bank Project for approval of projects on Insecticide resistance at NVBDCP, Delhi on 14 November 2008.
- Attended meeting on Insecticide resistance at WHO HQs, Geneva, Switzerland from 2–3 February 2009 .
- Presented a paper on 'Use of LLINs and IRS in vector control in India–prospects for combination' in Technical Consultant meeting for combining indoor residual spray (IRS) and long-lasting insecticidal nets (LLINs) intervention at WHO HQs, Geneva, Switzerland from 4–6 February 2009.

Saxena Rekha

- Attended symposium on "Climate change: emerging public health challenge" organized by National Institute of Communicable Diseases, Delhi on 45th Foundation Day celebration on 30 July 2008.
- Delivered a lecture on 'Narmada Valley development project and vector borne diseases (VBD)" during a scientific session organized by NIMR under the theme "Development and VBD" on 19 September 2009.

Sharma A

- Attended 20th International symposium on "Pharmaceutical and biomedical analysis' at Agra from 1–4 March 2009.
- Presented a research poster "Identification of mosquito salivary gland proteins and determination of parasite infection" (Marchus Macht, A.P. Dash, Romano Hebeler, Arun Sharma) in the Association of Bimolecular Resource Facilities (ABRF 2009) at Memphis, Tennessee.

Singh Ruchi

- Attended the 'Grants policy and management training' conducted by National Institute of Allergy and Infectious Diseases (NIAID), NIH, DGHS at New Delhi from 30 July–1 August 2008.

Valecha Neena

- Attended Board Meeting of World Wide Antimalarial Resistance Network (WWARN) meeting at Paris from 16–19 April 2008.
- DND*i*, FACT Advisory Group Meeting in Geneva, Switzerland on 22 April 2008.
- Expert Group meeting on the choice of the best drug(s) to be combined with Tafenoquine at Oxford, U.K. on 29 April 2008.
- Temporary Advisor for "Technical Expert Group Meeting to review and upgrade the WHO guidelines for the treatment of malaria" at WHO Geneva, Switzerland from 11–14 November 2008.
- Meeting of "Technical specification committee for drugs used under NVBDCP" under the chairmanship of Special DGHS (PH) at Nirman Bhawan, New Delhi on 10 July 2008.
- Expert committee meeting to review use of rapid diagnostic tests in India at Nirman Bhawan, New Delhi on 8 May 2008.
- Meeting on "ACT use in India" at Nirman Bhawan, New Delhi on 22 May 2008.
- Meeting of Climate change on malaria for Global fund at Nirman Bhawan, New Delhi on 12 February 2009.
- Meeting of "Technical specification committee" at Nirman Bhawan, New Delhi on 19 March 2009.
- Meeting for regulatory approval of new antimalarial formulations organized by DCGI at FDA Bhawan, New Delhi on 20 March 2009.
- Delivered a lecture on "Malaria chemotherapy and National Drug Policy" for Malaria Training Course at NIMR, Delhi on 2 May 2008.
- Delivered a lecture on "Clinical trials" for MPH students at NICD, Delhi on 15 May 2008.
- Invited speaker for DHA-PQP symposium, at 13th International Congress on Infectious Diseases, Kuala Lampur, Malaysia from 19–22 June 2008.
- Invited lecture on "Diagnosis treatment of malaria" for Doctors at IMA meeting, Janakpuri Branch organized by NIMR and MCD on 27 September 2008.
- Poster presentation for "An open label randomized comparison of dihydroartemisinin-piperaquine versus artesunate - mefloquine in falciparum malaria in Asia" at Jeju Island,

Korea from 29 September – 3 October 2008.

- Invited Speaker at symposium on Pyramax entitled, "Artemisinin derivatives in combination (ACT) are recommended for treatment of falciparum malaria. Dihydro-artemisinin - Piperaquine (DHA-PQP) is a fixed dose combination with excellent efficacy and simple dosing schedule. The present study compares its efficacy with Artesunate-Mefloquine (AS-MQ) in a randomised phase III trial in Asia" at ICTM, Jeju Island, Korea from 29 September–3 October 2008.
- Invited Speaker for DND*i* symposium for lecture entitled, "Fixed-dose artesunate/ amodiaquine combination: a viable option for treatment of *P. falciparum* malaria in India" at XVII International Congress for Tropical Medicine and Malaria at Jeju Island, Korea from 29 September–3 October 2008.
- Invited lecture entitled, "Efficacy of chloroquine and ACT's for treatment of malaria in India" at WHO meeting on antimalarial treatment in India from 13–14 October 2008.
- Invited lecture entitled, "Malaria diagnosis and treatment" at meeting of DHO's organized by MCD on 3 December 2008.
- Invited lecture entitled, "Dihydroartemisinin/ Piperaquine: an innovative ACT for the treatment of *P. falciparum* malaria" symposium at 57th Annual Meeting of ASTMH, New Orleans, U.S.A. from 7–11 December 2008.
- Invited lecture "Artemisinin based combination therapy for treatment of malaria in India: current strategy and new developments" in symposium at 41st Annual Conference of India in Pharmacological Society at AIIMS on 18 December 2008.
- Meeting to discuss launch of a short-term certificate programme (3-months) for Malaria Mangers by PHFI/AIIH & PH at Nirman Bhawan, New Delhi on 30 May 2008.
- Indo-US vaccine action programme workshop at Delhi on 17–18 June 2008.
- Site initiation meeting for trial of ASMQ organized by DND*i* at Mangalore from 14–15 July 2008.
- Site initiation meeting for trial of ASMQ organized by DND*i* at Goa from 17–18 July 2008.
- Meeting of the International Advisory Panel (IAP) to the Ministry of Health regarding the National Rural Health Mission (NRHM) of India on 4 August 2008.
- Meeting with State Health Authorities at Kanpur for malaria outbreak investigation from 11–12 September 2008.
- Meeting entitled, "India: catalyst in drug development for neglected diseases" organized by DND*i* at Delhi on 13 October 2008.
- Review meeting for therapeutic efficacy studies monitoring under the Chairmanship of the Director, NVBDCP at NVBDCP, Delhi from 2–3 July 2008.

10.9 Trainings imparted

Mittal PK

- Participated as faculty member in the training course for Dy. Health Officers and Epidemiologists of MCD for Management of Vector Borne Disease organized by NIMR in collaboration with MCD Health Department, Delhi and delivered lecture on 'Methods of vector control'.

Nagpal BN

- Two days training on "Introduction to GIS and hands on in GIS" to seven medical and three non-medical scholars of MPH(FE) from NICD, Delhi from 14–15 May 2008.
- Training on identification of mosquitoes and sandflies to Scientist 'B' from DRDO, Gwalior from 26 May–7 June 2008.
- Training to newly joined Research Assistant Mr. Gaurav Kumar of NIMR from 23–29 June 2008.
- Training to newly joined Research Assistants of NIMR from 7–11 July 2008.
- Training to newly joined Research Assistant Mr. Vikram Kumar of NIMR from 23–31 July 2008.
- Training on 'Use of spatial technology in malaria research and control' imparted to Dr Olajumoke Morenikeji, TWAS-DBT Post Doctoral Fellow from Nigeria from 4–8 August 2008.
- Twelve days training on "Laboratory diagnostic aspect of malaria" to two Laboratory Technicians (DMLT) from Hope Multi Services Society from 18–29 August 2008.
- Training on GIS to Scientists from NIOH from 24–28 November 2008.
- Two days of training to WHO-Fellows on GIS technology and tools being used in malaria epidemiology from 23–24 December 2008.
- WHO training in malaria entomology from 24 November 2008– 2 January 2009.

Nanda Nutan

- Imparted training to WHO Fellows Mr. B.R. Mane, Zonal Entomologist, Mr. P.J. Oza, Malaria Officer and Mr. R.R. Jha, Research

Assistant on identification of sibling species of malaria vectors and their epidemiological significance, techniques of mosquito blood meal source analysis during November 2008.

- Imparted training to Ms SB Suby, Scientist 'B', DRDE, Gwalior on cytological techniques for differentiating members of malaria vector complexes and techniques to analyse host feeding preference of field collected mosquitoes for one week in June 2008.
- Under NIH/FIC funded project "Promotion of *Plasmodium* research and training in India" guided the project fellows Mr. Narayani Prasad Kar and L. Dolie Devi to develop research proposals for their Ph.D. degree and for field work under the project.

Saxena Rekha

- Training on 'Introduction to GIS" to II semester scholars of MPH from NICD from 14–15 May 2008.
- Training to newly joined Research Assistants of NIMR from 7–11 & 23–31 July 2008.
- Training on 'Use of spatial technology in malaria research and control' imparted to Dr. Olajumoke Morenikeji, TWAS-DBT Post Doctoral Fellow from Nigeria from 4–8 August 2008.
- Training on "Understanding GIS and its tools for health related studies" to Dr. S. Raghavan and Dr. Vijay Kumar Shivgotra both Scientist 'B' from National Institute of Occupational Health, Ahmedabad from 24–28 November 2008.
- Training on 'Introduction to GIS" to I semester scholars of MPH from NICD from 1–2 December 2008.
- Two days of training to WHO-Fellows on GIS technology and tools being used in malaria epidemiology from 23–24 December 2008.

Sharma A

- Training provided to Ms Neha Chanana, the student of M.Sc. Biochemistry final year of Jamia Millia Islamia, New Delhi on "Protein identification by SDS PAGE of Mosquito *Anopheles stephensi*" from 1 July to 30 September 2008.

10.10 Trainings received

Mittal PK

- Participated in the workshop on "Basic malaria parasitology and entomology" organised by National Institute of Malaria Research, sponsored by NIH training grant and delivered a lecture on Anti-larval methods of vector control on 30 April 2008.

Raghavendra K

- Obtained two weeks training on "Laboratory procedures for testing and evaluation of public health pesticides in phase-I according to WHOPES guidelines" at World Health Organization (Collaborating CenExtract) , LIN, IRD, Montpellier, France from 2–13 March 2009.

Saxena Rekha

- Training acquired on 'Introduction to ERDAS 9.3–a remote sensing software' organized by Leica Geosystems, Delhi from 19–21 November 2008.
- Attended "Orientation in medical research methodology programme" organized by Institute of Cytology and Preventive Oncology, Noida from 3–5 December 2008.
- Training acquired on Map/Info Professional 9.5 and Vertical Mapper –the GIS softwares and Map Basic-GIS programming language organized by Lepton Software Export & Research (P) Ltd, Delhi from 17–19 March 2009.
- Attended orientation programme conducted by National Medical Library (NML) regarding ERMED *e*-resources to facilitate optimum utilization of electronic journal resources provided by the ERMED *e*-journal consortium on 17 November 2008.

10.11 Brainstorming meeting to prepare treatment guidelines for malaria in India

The National Institute of Malaria Research organised a Brainstorming Meeting to prepare treatment guidelines for malaria in India from 17–19 March 2009 in collaboration with the National Vector Borne Disease Control Programme. The meeting was sponsored by the World Health Organisation Country Office in India. Scientists and faculty from NIMR, WHO, NVBDCP, WR India, Ministry of Health and Family Welfare, Government of India and eminent physicians participated in the meeting.

10.12 Annual Day Celebration

The Institute organized its Annual Day on 26 November 2008. Dr G.C. Mishra, Director, National Centre for Cell Sciences delivered the Annual Day lecture. Dr V.M. Katoch, Secretary, Department of Health Research, Ministry of Health and Family Welfare, and Director General, Indian Council of Medical Research presided over the

Dr V.M. Katoch addressing the audience

function. Dr Katoch emphasized the need of translational research that would help to improve diagnosis and treatment of malaria in India. Prof. N.K. Ganguly, distinguished Biotechnology Fellow and Adviser, Translational Health Science & Technology Institute and Former DG, ICMR,

Dr G.C. Mishra delivering the Annual Day lecture. On the dias, Mr Sanjeev Dutta, Prof. R.C. Mahajan, Dr V.M. Katoch, Prof. N.K. Ganguly, Prof. A.P. Dash and Mr M. Rajamani.

Mr M. Rajamani, Senior Deputy Director General (Admin) and Mr Sanjeev Dutta, Financial Advisor, ICMR were the guests of honour. Prof. R.C. Mahajan, S.N. Bose INSA Research Professor and Emeritus Professor, PGIMER, Chandigarh introduced the speakers. On this occasion, employees completing 25 years of service were felicitated. Dr S. Pattanayak, Prof. M.K.K. Pillai, Dr V.P. Sharma, Dr Sarala K. Subbarao, Mr N.L. Kalra and other dignitaries were also present in the function.

10.13 Visit of WHO scientists

A delegation from World Health Organization Pesticide Evaluation Scheme from WHO, Geneva visited NIMR for assessing the infrastructure facilities for establishing a Collaborating Centre

for Testing of Public Health Pesticides at NIMR in September 2008. The delegation reviewed the infrastructure, techniques and laboratory practices and accepted the first assessment improvements. ❐

Two scientists from NIMR Joined WHO

Dr Rajpal Singh Yadav

Dr Rajpal Singh Yadav, Scientist 'F' joined as Scientist in WHO Pesticide Evaluation Scheme, World Health Organization, Geneva, Switzerland in July 2008.

Prof. Aditya Prasad Dash, Scientist 'G' and Director, joined WHO-SEARO, New Delhi, India in February 2009 as Regional Adviser-VBN.

Prof. Aditya Prasad Dash

11 संस्थान में राजभाषा विकास संबंधी गतिविधियाँ

संस्थान में राजभाषा अधिनियम 1963 की धारा 3 (3) के अनुपालन की दिशा में वर्ष 2008-09 में भी राजभाषा हिन्दी के प्रसार और विकास की गति बढ़ाने के लिए और सरकारी काम-काज में हिन्दी के प्रगामी प्रयोग के क्षेत्र में प्रगति हेतु आने वाले दस्तावेजों, अनुसंधान लेखों और संस्थान में प्रयुक्त प्रपत्रों का हिन्दी अनुवाद संबंधी कार्य पूर्ण किया गया। इसके साथ ही राजभाषा स्थिति की समीक्षा हेतु तिमाही बैठकें आयोजित की गई।

यहाँ यह भी उल्लेखनीय है कि विज्ञान और राजभाषा हिन्दी के संबंध को मजबूत बनाने की दिशा में प्रति वर्ष संस्थान द्वारा मलेरिया पत्रिका (त्रैमासिक) एवं न्यूज लैटर (द्विवार्षिक) प्रकाशित किया जाता है। वहीं दूसरी ओर विज्ञान दिवस मनाए जाने के साथ ही हिन्दी पखवाड़े के अवसर पर विभिन्न गतिविधियाँ आयोजित की गई जो कि वर्ष की मुख्य गतिविधियाँ रही। इसके साथ ही इस वर्ष संस्थान में हिन्दी पखवाड़ा पूरे हर्षोल्लास के साथ मनाया गया। हिन्दी पखवाड़ा के अवसर पर हिन्दी कार्यशाला, वैज्ञानिक संगोष्ठी, श्रुतलेख प्रतियोगिता, टिप्पण-प्रारूपण प्रतियोगिता, निबन्ध प्रतियोगिता तथा कर्मचारियों और अधिकारियों के लिए पृथक-पृथक वाद-विवाद प्रतियोगिताओं का आयोजन किया गया। संबंधित प्रतियोगिताओं का आयोजन संस्थान के निदेशक प्रो. ए.पी. दाश के निर्देशन में संस्थान की हिन्दी अधिकारी एवं राजभाषा कार्यान्वयन समिति के विभिन्न सदस्यों द्वारा किया गया।

इस पखवाड़े का आरंभ दिनांक 15 सितम्बर 2008 को हिन्दी कार्यशाला के आयोजन के साथ किया गया। यह कार्यशाला प्रशासनिक वर्ग के अधिकारियों एवं कर्मचारियों के लिए आयोजित की गई, जिसमें संस्थान के निदेशक महोदय ने भी भाग लिया। इस कार्यशाला के प्रथम व्याख्याता एवं मुख्य अतिथि के रूप में पूर्व-प्रबंधक, भारत हैवी इलैक्ट्रिकल्स लिमिटेड (भेल) के श्री गोपेश गोस्वामी को आमंत्रित किया गया था। श्री गोपेश गोस्वामी ने अपने व्याख्यान में हिन्दी के ऐतिहासिक महत्व पर प्रकाश डालते हुए इसे संपर्क भाषा के रूप में अपनाकर एवं ज्ञान-विज्ञान को हिन्दी में शामिल करके इसके प्रयोग को बढ़ावा देने हेतु प्रेरित किया।

कार्यशाला के द्वितीय चरण जिसमें श्री अशोक सचदेवा, उपनिदेशक वित्त मंत्रालय (राजभाषा) को आमंत्रित किया गया। उन्होंने 'ससदीय राजभाषा समिति की प्रश्नावली' विषय के माध्यम से उल्लेखित प्रश्नावली के विभिन्न बिन्दुओं के विषय में रोचकतापूर्ण ढंग से विस्तृत जानकारी दी। चूंकि यह कार्यशाला पूर्णकालिक थी इसलिए भोजनावकाश के बाद कार्यशाला को पुनः आरंभ किया गया, जिसमें श्री सतेन्द्र सिंह सहायक निदेशक, केन्द्रीय अनुवाद ब्यूरो को आमंत्रित किया गया था। श्री सिंह के व्याख्यान का विषय था-"सरकारी कामकाज में राजभाषा (टिप्पण-प्रारूपण)"। उन्होंने अपने व्याख्यान में अनुवाद की बारीकियों से परिचित कराते हुए बताया कि गलत अनुवाद से किस प्रकार अर्थ का अनर्थ हो सकता है। इसके साथ ही उन्होंने अपने व्याख्यान द्वारा सभी को हिन्दी में कार्य करने हेतु प्रोत्साहित किया।

हिन्दी पखवाड़े की दूसरी गतिविधि के अंतर्गत दिनांक 16 सितम्बर 2008 को टिप्पण-प्रारूपण प्रतियोगिता एवं श्रुतलेख प्रतियोगिता का आयोजन किया गया। इनका संचालन क्रमशः श्री आर.एन. यादव, सहायक अनुसंधान अधिकारी एवं डॉ. अरूण शर्मा, वैज्ञानिक 'एफ' द्वारा किया गया।

हिन्दी कार्यशाला

निबन्ध प्रतियोगिता

वैज्ञानिक संगोष्ठी

डॉ. बलवंत सिंह को सम्मानित करते हुए निदेशक महोदय

दिनांक 17 सितम्बर 2008 को पखवाड़े की तीसरी गतिविधि निबंध प्रतियोगिता का आयोजन किया गया, जिसका सफलतापूर्वक संचालन डॉ. नूतन नंदा, वैज्ञानिक 'ई' द्वारा किया गया। संस्थान में प्रतियोगिता का विषय था – ''जनजागरण में मीडिया की भूमिका'' या ''बच्चों में बढ़ती अपराधिक प्रवृति: कारण एवं निदान''। इसी क्रम में चलते हुए दिनांक 18 सितम्बर 2008 को कर्मचारी वर्ग के लिए वाद-विवाद प्रतियोगिता का आयोजन किया गया जिसका संचालन श्रीमती रेखा सक्सेना, वैज्ञानिक 'डी' द्वारा किया गया। इस प्रतियोगिता में निर्णायक के रूप में श्री दिनेश चन्द्र त्रिपाठी एवं श्री शम्भुनाथ सिंह, प्रशिक्षक, दैनिक जागरण को आमंत्रित किया गया था। प्रतियोगिता का विषय था- 'सेवा-निवृत्ति की आयु में वृद्धि सही/गलत'। इस विषय पर संस्थान के कर्मचारियों ने जोशपूर्ण ढंग से अपने-अपने विचार प्रस्तुत किए। प्रतियोगिता के अंत में श्री त्रिपाठी एवं श्री शम्भुनाथ सिंह ने परिणाम घोषित कर कर्मचारियों के उत्साह एवं विचारों की प्रशंसा की एवं उल्लेखित विषय पर विचार विमर्श किया।

दिनांक 19 सितम्बर 2008 को हिन्दी पखवाड़े की पाँचवी गतिविधि वैज्ञानिक संगोष्ठी का आयोजन किया गया, जिसका संचालन डॉ. रमेश चन्द धीमान, वैज्ञानिक 'एफ' द्वारा किया गया। संबंधित संगोष्ठी में मुख्य अतिथि के रूप में प्रो. सोमदत्त दीक्षित पूर्व निदेशक, हिन्दी निदेशालय को आमंत्रित किया गया था। संगोष्ठी का विषय था- 'विकास और रोगवाहक जन्य रोग'। सर्वप्रथम माननीय मुख्य अतिथि प्रो. सोमदत्त दीक्षित का विधिवत् स्वागत करते हुए संगोष्ठी का प्रारंभ श्रीमती रेखा सक्सेना द्वारा उक्त विषय पर स्लाइड शो से किया गया। अपने स्लाइड शो के माध्यम से श्रीमती सक्सेना ने नर्मदा घाटी विकास बोर्ड के अन्तर्गत बांध निर्माण से रोगवाहकों की संख्या में हुई वृद्धि पर संस्थान द्वारा किए गए सर्वेक्षण एवं उनके नियंत्रण हेतु उठाए गए कदमों पर विशेष जानकारी प्रदान की। इसके उपरान्त उपस्थित वैज्ञानिकों तथा प्रो. सोमदत्त दीक्षित ने विषय के विभिन्न पहलुओं पर अपने विचार प्रकट किए।

इस पखवाड़े के दौरान उल्लेखित गतिविधियों के अलावा दिनांक 23 सितम्बर 2008 को वाद-विवाद प्रतियोगिता (अधिकारी वर्ग) का आयोजन किया गया जिसमें संस्थान के प्रशासनिक एवं विज्ञानीय अधिकारियों ने भाग लिया। संबंधित प्रतियोगिता में निर्णायक एवं मुख्य अतिथि के रूप में कुँमाऊ विश्वविद्यालय एवं गढ़वाल विश्वविद्यालय के पूर्व-कुलपति डॉ. बलवंत सिंह एवं वरिष्ठ वैज्ञानिक डॉ. एस. पट्टनायक को आमंत्रित किया गया था। प्रतियोगिता का विषय था-"भारत का परमाणु करार: पक्ष/विपक्ष"। वाद-विवाद प्रतियोगिता की समाप्ति के पश्चात् पुरस्कार वितरण समारोह का आयोजन किया गया था। डॉ. बलवंत सिंह ने उपस्थित वैज्ञानिकों एवं समस्त अधिकारियों व कर्मचारियों को अपना सरकारी कामकाज हिन्दी में करने का अनुरोध करने के साथ ही मातृभाषा एवं राजभाषा के संबंध में विस्तृत जानकारी दी। तत्पश्चात् संस्थान के निदेशक ने सभी को संबोधित करते हुए कहा कि संस्थान में आयोजित की गई गतिविधियों का उद्देश्य राजभाषा के प्रयोग को बढ़ावा देते हुए हमें उसका उचित दर्जा दिलाना है एवं राजभाषा अधिनियम का अनुपालन करने की दिशा मे प्रयासरत रहना है। इसके साथ ही उन्होंने संस्थान में राजभाषा हिन्दी की उन्नति एवं प्रगति में अधिकारियों की इच्छा शक्ति एवं कर्मचारियों की लगन की महत्वपूर्ण भूमिका का उल्लेख किया। साथ ही मलेरिया संबंधी पुस्तिकाओं का विमोचन भी माननीय मुख्य अतिथि एवं निर्णायक महोदय के कर-कमलों द्वारा किया गया। तदोपरान्त पखवाड़े के दौरान आयोजित प्रतियोगिताओं के पुरस्कारों की घोषणा की गई।

सर्वप्रथम संस्थान में हिन्दी में अधिकाधिक कार्य करने हेतु लागू वर्ष 2008-09 की प्रोत्साहन योजना के पुरस्कारों की घोषणा डॉ. चन्द्र प्रकाश बत्रा, वैज्ञानिक 'ई' द्वारा की गई। संबंधित पुरस्कार मुख्य अतिथि डॉ. एस. पट्टनायक के कर-कमलों द्वारा प्रदान किए गए, जिसमें प्रथम पुरस्कार श्री मोहनलाल, श्री के.सी. सेहरा, द्वितीय पुरस्कार श्री रामदेव, श्री एस.पी. पाण्डेय, श्रीमती सुदर्शना छावडा, तृतीय पुरस्कार श्री रमेश कुमार झंडवानी, श्री जितेन्द्र कुमार को प्रदान किए गए। इसके अलावा

मलेरिया संबंधी पुस्तिकाओं का विमोचन

हिन्दी में अधिकाधिक डिक्टेशन देने वाले अधिकारी का पुरस्कार श्री जय प्रकाश वर्मा, वरिष्ठ प्रशासनिक अधिकारी को प्रदान किया गया।

इसके साथ ही श्रुतलेख प्रतियोगिता के पुरस्कारों की घोषणा डॉ. अरूण शर्मा, वैज्ञानिक 'एफ' द्वारा की गई जिसमें प्रथम पुरस्कार श्री जितेन्द्र कुमार, द्वितीय पुरस्कार श्री एस.पी. पाण्डेय, तृतीय पुरस्कार श्री दयानंद विश्वकर्मा एवं सांत्वना पुरस्कार श्री रमेश बुधोड़ी को निर्णायक महोदय डॉ. बलवंत सिंह द्वारा प्रदान किए गए। इसके साथ ही टिप्पण-प्रारूपण प्रतियोगिता के पुरस्कारों की घोषणा आर.एन. यादव, सहायक अनुसंधान अधिकारी द्वारा की गई, जिसमें प्रथम पुरस्कार श्री जी.एल. पुरी, द्वितीय पुरस्कार श्री आर.एस. भारद्वाज, तृतीय पुरस्कार श्री के.सी. सेहरा एवं सांत्वना पुरस्कार श्री सुनील गुप्ता को प्रदान किए गए। निबन्ध प्रतियोगिता के पुरस्कार की घोषणा डॉ. नूतन नंदा, वैज्ञानिक 'ई' द्वारा की गई, जिसमें प्रथम पुरस्कार श्रीमती कमला नेगी, द्वितीय पुरस्कार श्री ए.के. द्विवेदी, तृतीय पुरस्कार सुश्री स्नेह शालिनी एवं सांत्वना पुरस्कार श्री जितेन्द्र कुमार परिहार को प्रदान किए गए। वाद-विवाद प्रतियोगिता (कर्मचारी वर्ग) के पुरस्कारों की घोषणा श्री जय प्रकाश वर्मा, वरिष्ठ प्रशासनिक अधिकारी द्वारा की गई, संबंधित पुरस्कार निदेशक महोदय द्वारा क्रमशः श्रीमती कल्पना वर्मा, श्री जितेन्द्र परिहार, श्री शैलेन्द्र पाण्डेय एवं सांत्वना पुरस्कार श्री जितेन्द्र कुमार को प्रदान किए गए।

इसके साथ ही पखवाड़े के दौरान आयोतिज अंतिम प्रतियोगिता वाद-विवाद प्रतियोगिता (अधिकारी वर्ग) के पुरस्कारों की घोषणा डॉ. भूपेन्द्र नाथ नागपाल, वैज्ञानिक 'ई' द्वारा की गई, जिसमें प्रथम पुरस्कार डॉ. के. राघवेन्द्रा, द्वितीय पुरस्कार श्री जी.पी. माथुर, तृतीय पुरस्कार डॉ. आर.सी. धीमान तथा सांत्वना पुरस्कार डॉ. वी.पी. सिंह को अतिथि महोदय के कर-कमलों द्वारा वितरित किए गए।

अंततः कार्यक्रम का विधिवत् समापन करने हेतु संस्थान के वरिष्ठ प्रशासनिक अधिकारी श्री जय प्रकाश वर्मा ने पखवाड़े के दौरान आयोजित गतिविधियों का सफलतापूर्वक संचालन करने हेतु सभी संचालकों को धन्यवाद ज्ञापित करने के साथ ही समग्र कार्यक्रम के आयोजन में संस्थान के निदेशक महोदय, संस्थान की हिन्दी अधिकारी के योगदान की सराहना करते हुए उन्हें हार्दिक धन्यवाद ज्ञापित किया। यही नहीं निर्णायक-गणों का भी समारोह में पधारने के लिए विशेष रूप से आभार व्यक्त किया गया और इसके साथ ही उपस्थित प्रतियोगियों, श्रोताओं एवं विजेताओं को भी धन्यवाद दिया गया, जिनके सहयोग से इस कार्यक्रम का सफलतापूर्वक आयोजन किया जा सका।

12 COMMITTEES OF THE INSTITUTE

12.1 Scientific Advisory Committee

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12.5 Human Ethics Committee

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12.6 Animal Ethics Committee

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Prof. AP Dash (Retired on 24 February 2009)

Director In-Charge

Dr VK Dua (From 24 February 2009)

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Dr RC Dhiman
Dr SK Ghosh
Dr MS Malhotra
Dr Arun Sharma
Dr Neena Valecha
Dr RS Yadav (Retired on 2 July 2008)

Scientists 'E'

Dr CP Batra
Dr RM Bhatt
Dr Sukla Biswas
Dr Vas Dev
Dr Hema Joshi
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

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Dr PK Atul
Dr Aparup Das
Dr AK Mishra
Dr Neelima Mishra
Mrs Rekha Saxena
Dr RP Shukla (Retired on 30 June 2008)
Dr HC Srivastava

Scientists 'C'

Dr MK Das
Dr Ruchi Singh

Scientists 'B'

Mr Bhagirath Lal
Dr Vineeta Singh
Dr VP Singh (Joined on 4 August 2008)

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Dr PK Tyagi

Research Scientists

Dr SK Chand
Dr GDP Dutta
Dr Alex Eapen
Dr Ashish Gupta
Dr S Haq
Dr PK Kar
Dr AK Kulshrestha
Dr Raj Kumar
Dr K Padhan
Dr B Shahi
Dr SN Sharma
Dr SP Singh
Dr SN Tiwari

Names are listed in alphabetical order by surname; Staff position as on 31 March 2009.