

Annual Report

2013–14



National Institute of Malaria Research (Indian Council of Medical Research)

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Preface

It gives me an immense pleasure in presenting the Annual Report of the National Institute of Malaria Research for the year 2013-14. During the reporting period, research on various aspects of malaria and other vector borne diseases was carried out which included bio-ecology of vectors, molecular and proteomic studies on vector-parasite interactions, phase-III evaluation of efficacy and community acceptance of LLINs, field testing of new vector control tools, molecular studies on population genetic diversity of malaria parasites, drug resistance and host's immune responses.

Epidemiological studies focused on malaria endemic districts, microstratification of malaria in problematic districts, generation of malaria risk maps in context of climatic change, and health impact assessment of developmental projects. In clinical studies, emphasis was laid on projects with operational and translational approach. Therapeutic efficacy studies carried out by NIMR led to change in the drug policy of the northeastern states. In addition, projects on pharmacovigilance of antimalarials, effective and safe treatment of malaria in pregnancy and quality assurance of malaria rapid diagnostic tests in India were undertaken.

The Institute received extramural grants from various national and international agencies like National Vector Borne Disease Control Programme, Department of Science and Technology, Department of Biotechnology, Council for Scientific and Industrial Research, WHO Global Malaria Programme, Global Fund for AIDS, TB and Malaria, Parasight Ltd., London School of Hygiene and Tropical Medicine, Medicines for Malaria Venture, etc.

During the year 2013, NIMR scientists published 61 research papers/chapters in reputed journals/books. NIMR scientists attended national and international conferences, workshops, delivered lectures and received awards/fellowships for their valuable contribution in areas of their expertise.

The Institute continued the activities of human resource development by imparting training to various health personnel, district programme officers, VBD consultants, laboratory technicians, guiding research scholars, M.Sc. students, etc. The Journal of Vector Borne Diseases, *Malaria Patrika* and *Plasmodium* Newsletter published by the Institute disseminated knowledge on various research aspects and recent findings about vector borne diseases.

The Field Units of NIMR located in different parts of the country continued to support the national programme in evaluation of intervention tools, insecticides, larvicides, insect growth regulators, long-lasting insecticidal nets, therapeutic efficacy of antimalarials, cross-checking of blood slides, imparting training to programme personnel, investigation of malaria epidemics, etc.

I take this opportunity to thank all the scientists and staff for their valuable support in all the activities. I sincerely acknowledge the help and guidance of the Secretary, Department of Health Research, Government of India and Director General of the Indian Council of Medical Research and hope for his continuous patronage in future. I also thank the scientists and Publication Division of NIMR for their support in bringing out this report.

Neena Valecha
Director

Executive Summary

Vector Biology & Control

- Studies on ecological succession of anophelines in northeastern states reported the prevalence of *Anopheles vagus*, *An. culicifacies* and *An. maculatus* in Sikkim state as recorded in previous year survey. The species which were recorded for the first time in Sikkim state were *An. pseudowillmorei* and *An. nigerrimus*. *Anopheles culicifacies* collected from four states, namely Assam, Meghalaya, Manipur and Sikkim were incriminated as vector using ELISA and PCR techniques.
- Follow-up studies on changing ecology of anopheline mosquitoes in Dadri CHC area in District Gautam Budh Nagar, Uttar Pradesh revealed that *An. fluviatilis* has not reappeared in this area after April 2012 till date. The appearance and disappearance of *An. fluviatilis* in this area was probably due to presence of thick vegetation in NTPC canal.
- Entomological and parasitological studies on present malaria situation in villages of District Ghaziabad, Uttar Pradesh revealed prevalence of high incidence of *P. vivax* and *P. falciparum* malaria cases in some villages. Results revealed a positive correlation between *An. culicifacies* density and slide falciparum rate in the study villages.
- For the first time, a total of 18 polymorphic microsatellite markers were successfully developed for malaria vector *An. fluviatilis* species T, which will be helpful for the study of population level genetic diversity.
- Proteomic analysis of *An. culicifacies* midgut proteins using in solution and in-gel digestion approach and LC-MS/MS spectrometry analysis using MASCOT and SEQUEST algorithm identified 47 putative functional proteins involved in cytoskeletal frame energy production, signal transduction and glycolytic process. Functional role of these proteins in parasite development will be studied and identified.
- Characterization of *An. culicifacies* salivary gland proteome using in solution approach followed by LC-MS/MS and bioinformatics analysis identified 63 proteins. Their putative functional roles like signal transduction, redox function, dehydrogenases, etc have been studied to understand vector parasite interactions.
- Dual feeding associated molecular and comparative transcriptomic analysis revealed that blood meal not only alters cellular and molecular architecture of adult female salivary glands, but also enables to manage meal-specific choices and decision.
- Metagenomic analysis provided first evidence that salivary glands harbour more diverse microbial community than gut, providing strong evidence that how mosquito evolved and adapted for sugar feeding in association of diverse salivary endosymbionts.
- Preliminary molecular analysis of mosquito innate immune system suggested that antimicrobial peptides (AMPs) are constitutively synthesized by fat body, which enables fine adjustment with gut requirement to manage local infections.
- Initial characterization of mosquito hemocyte transcriptome in *An. stephensi* indicated that hemocytes are not only metabolically active but also encode several specialized products including immunity, signal transduction, etc.
- Procedural modifications in the technique of counter current immunoelectrophoresis (CCIE) for mosquito blood meal source

identification has been done. Observation suggests that certain commonly used TBE buffers can be used in place of Barbitone buffer with comparable results.

- Studies were completed on the WHOPEs sponsored phase-III randomized trial to compare insecticidal efficacy and community acceptance of long-lasting insecticidal net DuraNet® with conventional insecticide-treated nets in a malaria hyperendemic tribal area of Sundergarh district, Odisha. The trial results showed that DuraNet LNs maintained high efficacy against malaria vector for a period of three years under field conditions.
- Studies were continued on a multicentric phase-III trial on NetProtect LN impregnated with deltamethrin in Mewat area of Haryana. Studies on bioefficacy of field distributed nets were completed and surveys were conducted to assess perceived adverse effects of the NetProtect LN and epidemiological impact of the long-lasting insecticidal net in comparison to untreated or no net.
- Studies were conducted on vector incrimination in the project area of comprehensive malaria case management programme in four districts of Odisha. Vector infectivity rate at baseline has been determined and the study is in progress.
- A study on insecticide susceptibility of *Aedes aegypti* carried out in Delhi, showed development of pyrethroid resistance and presence of a knockdown resistance (*kdr*) mutation, i.e. F1534C in the population studied. Pyrethroid resistance from Delhi and *kdr* mutation from India have been reported for the first time in the species.
- A study to assess impact of insecticide resistance in malaria vectors on the effectiveness of combination of indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) in CHC Keshkal, Kondagaon district of Chhattisgarh is in progress. Census and baseline studies were carried out. *Anopheles culicifacies*, major vector was found resistant to DDT and malathion and susceptible to determine but variable resistant to bendiocarb.
- A study was conducted to determine the insecticidal efficacy of second generation neonicotinoids in the control of mosquito

vectors. Larval and topical application bioassays were carried out to assess the larvicidal and adulticidal activity on *An. stephensi*, *Ae. aegypti* and *Culex quinquefasciatus*.

- Bottle assay for monitoring insecticide resistance in adult mosquito vector species was standardized and comparative evaluation with WHO insecticide susceptibility test was made. Feasibility for use of this method in field conditions was assessed.
- Studies carried out for the development of method(s)/strategy of pyrethroid resistance in malaria vectors in India using insecticides with novel mode of action. Analysis using prediction pathway systems for three detoxification enzymes was identified for molecular docking study. *In silico* molecular modelling and docking results showed common active binding site for pyrethroids.

Parasite Biology

- Molecular characterization of 4-diphosphocytidyl-2C-methyl-d-erythritol (IspE) kinase gene of *Plasmodium vivax* samples collected from five different regions was carried out. Sequence homology and phylogenetic analysis suggested specialized role of this gene in *Plasmodium* genome as it was found highly conserved throughout.
- The expression of the *P. vivax vir* genes and drug resistance genes, *pvcr-t-o* was compared between severe and non-severe *P. vivax* infections. The increased expression levels of the *vir* and *pvcr-t-o* genes in *P. vivax* severe cases were observed that might have a role to play in the disease pathogenesis of *P. vivax* malaria.
- Merozoite surface protein–*msp1*, *msp2* and glutamate-rich protein (*glurp*) genes of *P. falciparum* were genotyped across different populations in malaria endemic regions of India. Multiplicity of infection (MOI) was found to be higher for *msp1* as compared to *msp2* gene while *glurp* did not indicate the existing genetic pattern due to a lower frequency of diverse alleles.
- Prevalence of highly diverged chloroquine-resistant (CQR)–*pfcr-t* haplotypes in Cameroonian *P. falciparum* has been observed. With PCR and DNA sequencing

approach, it was revealed that apart from *pfcr* haplotype CVIET and several other haplotypes also segregate in Cameroonian *P. falciparum*.

- DNA sequencing of four whole mitochondrial (mt) genomes of Indian *P. falciparum* and comparative genomic analyses with worldwide isolates revealed high sequence diversity in Indian *P. falciparum*. Three single nucleotide polymorphisms (SNPs) were found that are completely unique to Indian *P. falciparum*.
- Comparative assessment of the DNA sequence polymorphisms of the *P. falciparum* multi-drug resistance gene *pfmdr1* from the field and cultured Indian isolates of India indicate higher nucleotide and haplotype diversity in the field isolates, with no role of natural selection on the evolution of the *pfmdr1* gene in Indian isolates.
- Recruitment of MSCs cells during infection and infusion of these cells into naïve mice was able to confer host resistance against malaria infection. MSCs augmented interleukin (IL)-6 productions indicating that MSCs are host-protective and enhance pro-inflammatory cytokine production.
- A maximum likelihood analysis reveals genetic differentiation in *var* gene family among parasites from different geographical areas and in different clinical outcomes.
- Analysis at genome-wide neutral microsatellite (MS) loci reveals varied level of expected heterozygosity in Indian *P. vivax* from three different transmission areas.
- Studies on the mechanism of inhibition of cysteine proteases by falstatin suggested that only the BC loop of falstatin is a target for mediating inhibition of cysteine proteases of malaria parasites.

Epidemiology

- An International and multi-institutional collaborative project (India-Denmark) was initiated to study immunological correlates of protection against malaria vaccine candidates in high and low transmission malaria endemic regions of Jharkhand and Haryana states, respectively.
- The study to control dengue and chikungunya by controlling *Aedes* breeding in pre-monsoon season was conducted in selected endemic

zone of west Delhi and overhead tanks were identified as key containers. Control operations were undertaken in selected localities in collaboration with MCD and no dengue case was recorded from surveyed localities.

- Health impact assessment of Sardar Sarovar and Narmada Basin Dams was undertaken in Rajasthan and Madhya Pradesh, respectively and water habitats supporting breeding of mosquitoes were identified and mitigating measures were suggested to health authorities.
- Microstratification of malaria is being carried out in four problematic districts of Rajasthan for developing village level control strategy using satellite data and ground survey images generated from IRS LISS IV satellite data revealed that villages having proportion of water bodies >0.1% and sandy area 50% were malarious, whereas villages having water bodies nil or <0.1% and sandy proportion >50% were non-malarious/least malarious.
- A study has been initiated to generate risk map of malaria in all the states of India taking into account climatic determinants, malaria prevalence, vector distribution and ecological conditions. Distribution of *An. culicifacies* and *An. stephensi* in India was snapped using published records which revealed knowledge gaps in fauna survey.
- A study was undertaken to explore the probable role of *An. subpictus* as vector of malaria under changing climatic conditions.
- Under world bank support, cross-section population surveys were carried out in study districts of four states with objective to know the reach of National Malaria Control Programme at the community level, use of bednets, awareness, health seeking behaviour and IRS coverage.

Clinical Research

- A long-term study on efficacy of artemisinin-based combination therapy (Artesunate plus Sulphadoxine-pyrimethamine— AS+SP) in *P. falciparum* and chloroquine (CQ) in *P. vivax* was undertaken. CQ showed 100% efficacy in *P. vivax* malaria in all the eight sentinel sites.
- A multicentre research trial to detect *in vivo* resistance of *P. falciparum* to artesunate in patients with uncomplicated malaria was

carried out in District Gomati (Tripura) and District Lunglei (Mizoram). The preliminary results showed no sign of delayed parasite clearance time (PCT).

- Studies on malaria in pregnancy (MiP) were conducted in three districts of Jharkhand state. The results of the interim analysis of data on women enrolled and delivered showed that intermittent screening and treatment (IST) is beneficial in comparison to passive case detection.
- Studies on the effect of residual antimalarials in malaria patients in high malaria endemic districts showed residual antimalaria levels, namely sulphadoxine (18.2%) and chloroquine (11.2%) were present in *P. falciparum* malaria patients. An inverse-correlation between parasite density and residual levels of sulphadoxine and chloroquine on Day 0 samples of malaria patients was observed.
- Parasight P1 device (a computer-based technology for malaria diagnosis) was evaluated and its sensitivity and specificity was compared against PCR and microscopy. Sensitivity and specificity of Parasight compared to microscopy were found to be 94.4 and 95.7%, respectively as compared to microscopy.
- The project has been initiated on evaluating the safety of antimalarial by both passive and active pharmacovigilance. The data on safety of antimalarial were collected by the Medical Officers by filling up of the adverse event reporting forms. The data generated on about 6500 patients so far suggest that the antimalarials used under NVBDCP are safe and can be continued.

□

1.1 Vector Biology

1.1.1 Ecological succession of anopheline and other mosquito species in northeastern states of India

Comprehensive studies were carried out in this project on the ecological succession in Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura to update anopheline species knowledge base in the proposed areas.

During February–March 2013, a survey was

carried out by NIMR team in Sikkim state (East, West, North and South Sikkim districts) (Fig. 1). The ecological changes occurred in Sikkim state revealed that the forest cover has reduced by 52.7%. Whereas, the other parameters such as rainfall, net sown area, population/migratory population, production of rice, construction of roads/highways, and dams have also changed. The changes in ecological parameters are responsible for appearing or disappearing of species and their succession.

In Sikkim state, previously reported species,

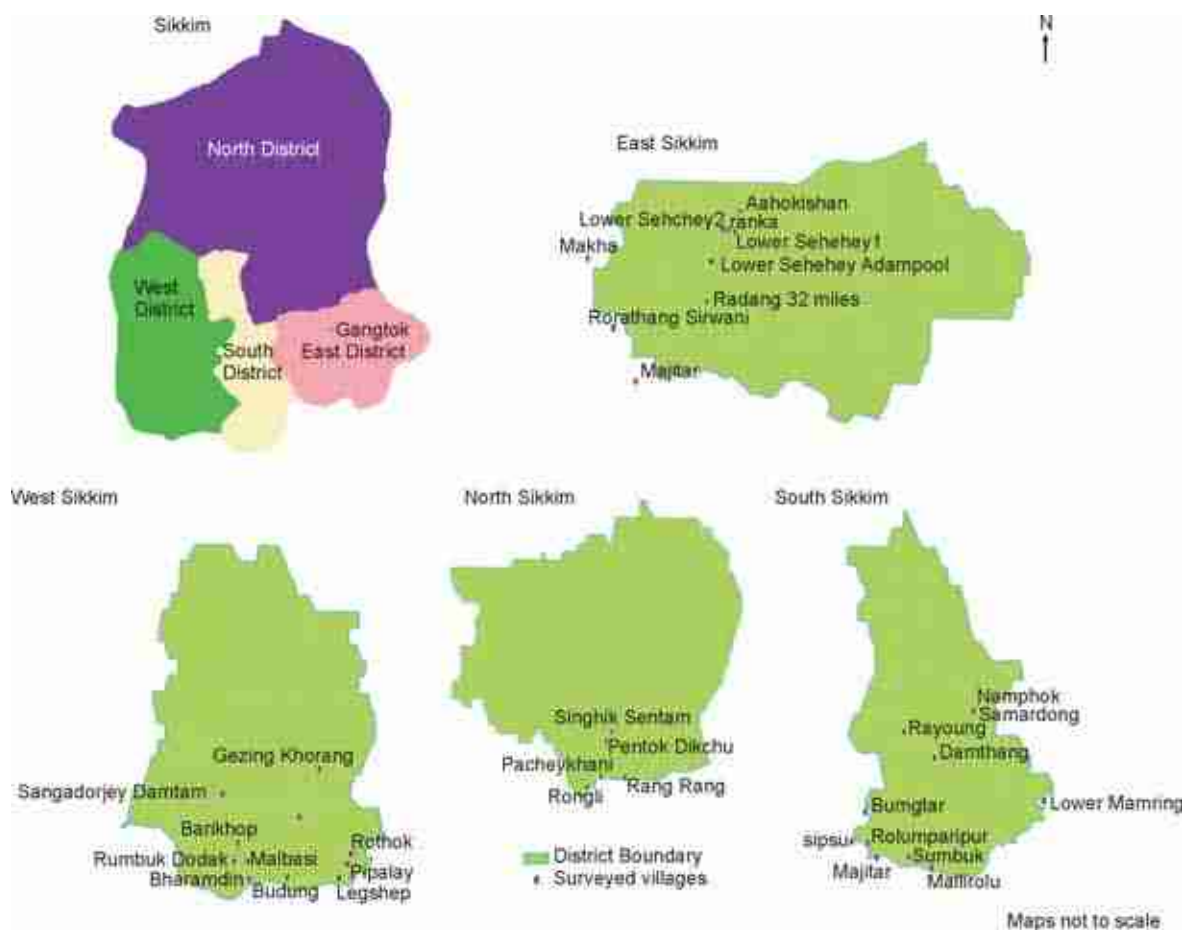


Fig. 1: Map showing districts of Sikkim state.

namely *An. vagus*, *An. culicifacies* and *An. maculatus* could be recorded in the present study. The species which were recorded for the first time in the state were *An. pseudowillmori* and *An. nigerrimus*. Different methods of mosquito collection employed are shown below.

Anopheles culicifacies collected from four states, namely Assam, Meghalaya, Manipur and Sikkim were incriminated as a vector species with the help of ELISA technique. A total of 2689 *An. culicifacies* collected from 16 districts, viz. Sonitpur, Nagaon, Golaghat, Lakhimpur, Dibrugarh, Goalpara, Kamrup, Cachar, East Khasi Hill, East Garo Hills, Chandel, East Imphal, East Sikkim, West Sikkim, North Sikkim and South Sikkim of Assam, Meghalaya, Manipur and Sikkim states of northeast India were examined, out of which 173 (6.43%) were found positive for CS antigen with the help of ELISA technique.

Remaining mosquito triturate was used for DNA extraction using the Qiagen DNA isolation kit and six specimens were subjected to PCR assay, out of which three samples showed positive band for *P. vivax*.

1.1.2 Changing ecology of anopheline mosquitoes in Dadri CHC area in District Gautam Budh Nagar, Uttar Pradesh

An. culicifacies is the primary malaria vector species in Dadri CHC area in District Gautam Budh Nagar in western Uttar Pradesh. The present study was continued to investigate the establishment of *An. fluviatilis*, a potential malaria vector, in this area. During this study regular monitoring of the indoor resting mosquito density was made by hand catch method in six indicator villages of the Dadri CHC, from where *An. fluviatilis* was also collected for the first time in November 2009.

The study revealed that *An. culicifacies* continues



Different methods of mosquito collection employed during the survey in Northeastern states.

to be the predominant malaria vector species in this area. The appearance of *An. fluviatilis* in high densities in the Dadri CHC area was observed from November–December 2009 till July 2010; and again from January–April 2012. *An. fluviatilis* has not reappeared in this area after April 2012 till date. It was also observed that the prevalence of *An. fluviatilis* in this area is not affected by seasonal changes, in contrast to other established anopheline species. An interesting observation made was the appearance of *An. fluviatilis* which was associated with the presence of thick vegetation of water hyacinth on the surface of slow moving water in the NTPC canal. It may be mentioned that NTPC canal carries water discharged from NTPC Plant after cooling of towers and ash effluents. This water is taken from irrigation channels, a tributary of Upper Ganga Canal, which receive water from the River Ganga. It may be mentioned that this river passes through the Himalayan foothills and Terai

Region, where *An. fluviatilis* is found in abundance. This has facilitated the establishment of *An. fluviatilis* in the NTPC canal. These observations indicate that the appearance and disappearance of *An. fluviatilis* in this area is probably due to the presence of thick vegetation on the surface of slow moving water in the drain. There is a declining trend in the overall density of *An. culicifacies* and other anopheline spp, except *An. subpictus*, in this area. This is probably due to land filling and increasing urbanization activities.

1.1.3 Entomological and parasitological studies on present malaria situation in certain villages of District Ghaziabad, Uttar Pradesh

Entomological and parasitological studies were carried out in some villages of District Ghaziabad (U.P.) from June 2011 to November 2013 to understand the dynamics of malaria transmission in these villages (Fig. 2). Preliminary study using

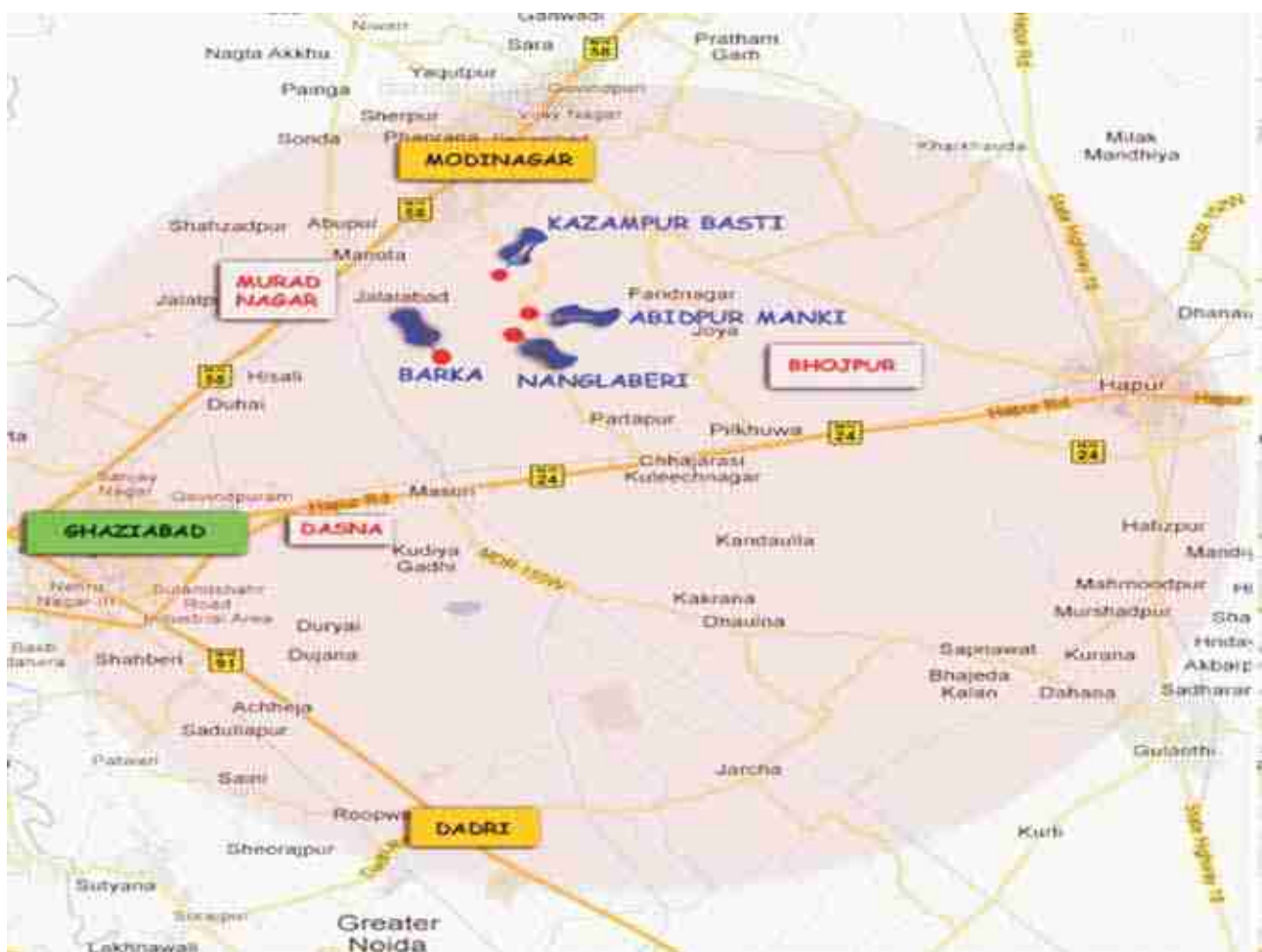


Fig. 2: Map showing the location of the study areas in District Ghaziabad, U.P.

mass blood survey revealed high incidence of *P. vivax* and *P. falciparum* malaria cases in some of these villages. Analysis of monthly entomological data collected during 2011–13 revealed prevalence of high density of *An. culicifacies*, as the only major known malaria vector species in this area (Fig. 3). Both species A and species B of *Culicifacies* Complex were found sympatric and were predominantly zoophagic in these villages. In Manki village, predominance of species A, which is the established vector of malaria was observed, while in Barka village, predominance of species B was observed (Table 1).

Monthly parasitological data from active surveillance is given in Table 2; and Fig. 4. This data revealed that Manki village in Dasna PHC had high proportion of falciparum malaria (SfR = 12.89), while Barka village in Murad Nagar PHC had lowest proportion (SfR = 3.2). Analysis of parasitological indices collected during 2012–13 revealed that Manki village in Dasna PHC had highest malaria incidence (API = 109) as well as highest proportion of *Pf* (48%), while Barka village in Murad Nagar PHC had lowest malaria incidence (API = 57.14)

Table 1. *An. culicifacies* sibling species composition in three villages of District Ghaziabad

Village	PHC	Total No. of specimen examined	Samples identified	<i>An. culicifacies</i> sibling species composition		
				A	B	C
Abidpur Manki	Dasna	56	47	15	32	0
Barka Arifpur	Murad Nagar	18	18	2	16	0
Nagla Ber	Bhojpur	19	18	7	11	0
Total		113	102	26	76	0

Table 2. Malaria transmission in certain villages in District Ghaziabad during June 2011 to May 2013

Village/PHC (Population)	Year	Total blood smears examined	<i>Pv</i>	<i>Pf</i>	Total positive	SPR	SfR	PI
Manki/Dasna (2353)	2011–12	314	53	34	87	27.7	10.8	57.2
	2012–13	570	86	80	166	29.12	14.03	109.21
	Total	884	139	114	253	28.61	12.89	166.44
Barka Arifpur/Murad Nagar (1400)	2011–12	243	64	10	74	30.4	4.1	52.8
	2012–13	256	74	6	80	31.25	2.34	57.14
	Total	499	138	16	154	30.86	3.20	110
Nagla Ber/Bhojpur (228)	2011–12	91	20	5	25	27.4	5.5	109.6
	2012–13	96	14	8	22	22.91	8.33	96.49
	Total	187	34	13	47	25.13	6.95	206.14

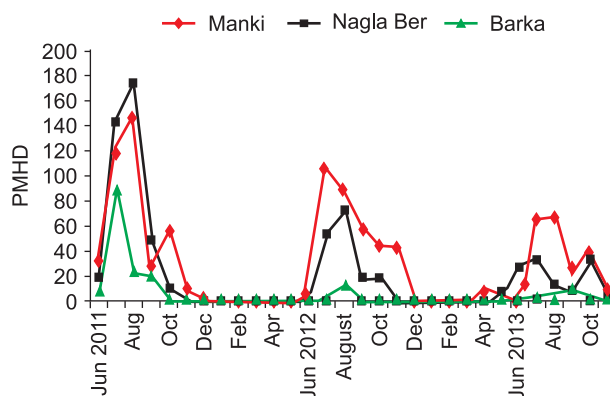


Fig. 3: Per man hour density (PMHD) of *An. culicifacies* in three different villages of District Ghaziabad during June 2011 to November 2013.

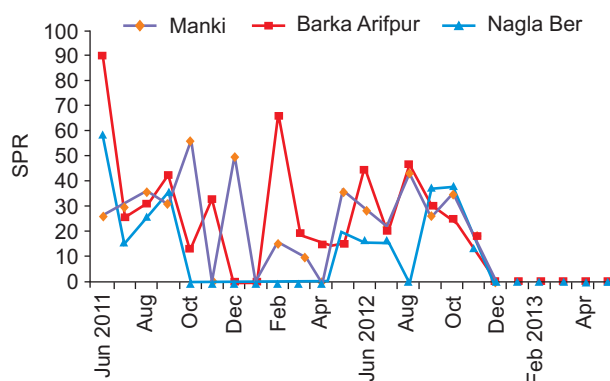


Fig. 4: Malaria transmission in three different villages of District Ghaziabad during June 2011 to May 2013.

as well as lowest proportion of *Pf* (2.6%). Similarly, Manki village had highest average MHD of *An. culicifacies* (30.1) and Barka village had lowest average MHD (2.3). Results revealed a positive correlation between *An. culicifacies* density as well as slide falciparum rates (SfR) in the three villages.

1.1.4 Isolation and characterization of polymorphic microsatellite markers from the malaria vector *Anopheles fluviatilis* species T

Anopheles fluviatilis James is an important malaria vector in India, Pakistan, Nepal and Iran. It has been now recognized as a complex of at least four sibling species S, T, U and V among which species T is most widely distributed species throughout India. The taxonomic status of these species is confusing owing to conflicting published reports. Additionally polymorphism exists in species-diagnostic chromosomal inversion of *An. fluviatilis* species T in some populations. We report the isolation and characterization of 20 microsatellite markers from microsatellite-enriched genomic DNA library of *Anopheles fluviatilis* T in order to study the population level genetic diversity. Of these primers, 18 were polymorphic and two were monomorphic. The number of alleles per locus among polymorphic markers ranged from 4 to 19, and values for observed and expected heterozygosities ranged from 0.375 to 0.857, and from 0.575 to 0.933 respectively. This study provides a promising tool for the population genetic analyses of *An. fluviatilis* T. This will help in estimating the extent of gene flow among different populations.

1.1.5 Functional annotation of midgut proteins of *Anopheles culicifacies*: A global proteomics study

An. culicifacies is an important vector of human malaria in rural India. With regards to human malaria parasites *P. falciparum* and *P. vivax*, *An. culicifacies* sibling species B tends to be refractory, implying a relatively high degree of specificity in relationship between malaria parasites and mosquito vectors. There are evidences that refractoriness of *An. culicifacies* to malaria parasite infection may be the result of multiple mechanisms operating in mosquito midgut. However, the reasons for the low levels of mature oocyst infection in *An. culicifacies* midgut remain unknown. In this post-genomic era, proteomics has emerged as a powerful tool for high-throughput analyses of proteomes. Mass spectrometry via both MALDI-TOF and LC/MS/MS are increasingly being used for biomarker discovery to compare profiles of proteins/peptides between susceptible and refractory samples. Proteins of unassigned function may also be detected and/or have functions compatible with the biogenesis and role of the midgut organelle. Understanding of these refractory

mechanisms may lead to the development of novel approaches for malaria control.

We have carried out in solution and in-gel digestion of midgut sample of *An. culicifacies* species A. These midgut digested samples were further subjected to LC/MS/MS analysis. These were further analyzed with MASCOT and SEQUEST for characterization of putative function of proteins or peptides.

Mass-spectrometry-based proteomics is now a powerful and reliable method that allows characterization of protein assemblies and when this is combined with molecular, cellular and bioinformatics techniques it provides a framework for translating complex molecules into simple molecules for in-depth analysis of expressed proteomes. The spectra obtained after LC/MS/MS were analyzed by both MASCOT (Matrix Science) and SEQUEST algorithm and matched against databases of *Anopheles*, *Aedes* and *Culex* mosquito species.

In the present study, we employed a MS-based approach to categorize different functional proteins of midgut of the rural malaria vector *An. culicifacies* species A. From in solution digestion strategy total 30 proteins were analyzed (Table 3). Among in-gel digestion strategy, 21 gel bands of midgut homogenate sample were visualized through silver staining (Fig. 5).

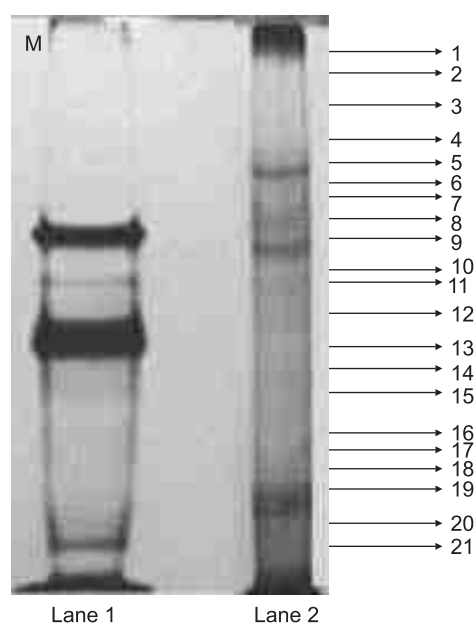


Fig. 5: Midgut protein profiling of *An. culicifacies*. Protein markers with range 14 to 100 kDa shown in Lane 1. Silver stained SDS PAGE gel of the midgut extract is shown (Lane 2).

Table 3. A catalogue of midgut proteins of *An. culicifacies* species A identified by in-solution digestion strategy and LC/MS/MS

S.No	Accession No.	Protein	Score	Peptides	Molecular weight (kDa)	Calculated pI	Function
1.	Q7PJV2 (24)	Myosin protein (Similar to <i>An. gambiae</i>)	2730	48	224.2	5.76	ATP binding
2.	E3XEC7 (16)	ATP synthase beta subunit (Similar to <i>An. darlingi</i>)	613	21	53.7	5.12	ATP binding, Ion transport
3.	T1EAN0 (27.75)	Putative Ca ²⁺ -transporting ATPase (Similar to <i>An. aquasalis</i>)	584	18	100	5.54	Cation transport, ATP binding
4.	Q7Q0D8 (49.77)	AGAP012401-PA (Similar to <i>An. gambiae</i>)	301	8	57.2	5.74	Catalytic activity
5.	Q1HRN5 (42)	Actin (Similar to <i>Ae. aegypti</i>)	250	9	41.8	5.48	ATP binding
6.	Q7PKD7 (49.77)	AGAP012081-PA (Similar to <i>An. gambiae</i>)	193.81	7	22.7	5.25	Ion transport
7.	Q8T4K0 (29.08)	AGAP009833-PA (Similar to <i>An. gambiae</i>)	181.61	6	30.7	8.56	Voltage-gated anion channel activity, Ion transport
8.	Q17PH7 (7)	AAEL000308-PA (Similar to <i>Ae. aegypti</i>)	171.09	1	18.2	9.54	Cell adhesion, Tissue regeneration
9.	T1DPL9 (29.97)	Putative cytochrome c1 (Similar to <i>An. aquasalis</i>)	139.59	5	33.3	8.54	Electron transport
10.	B0X0G3 (9)	General odorant-binding protein (Similar to <i>Cx. quinquefasciatus</i>)	132.38	1	14	8.43	Unknown
11.	Q7QFL4 (4)	AGA P000550-PA (Similar to <i>An. gambiae</i>)	130.86	6	164	5.52	Cell matrix adhesion
12.	Q7Q328 (10)	AGAP011453-PA (Similar to <i>An. gambiae</i>)	128.65	5	85.8	5.14	Iron ion transport
13.	Q27238 (13)	ADP and ATP carrier protein (Similar to <i>An. gambiae</i>)	113	4	32.8	9.67	Transport activity
14.	Q0IGB5 (2)	AAEL003046-PA (Similar to <i>Ae. aegypti</i>)	112.89	1	114.2	5.05	Sphingolipid metabolic process (lysozyme)
15.	Q7Q343 (8)	AGAP011476 (Similar to <i>An. gambiae</i>)	100.94	6	101	5.68	Peptidoglycan catabolic process
16.	Q7PUV3 (20)	AGAP001622-PA (Similar to <i>An. gambiae</i>)	100.51	3	22.9	4.78	Calcium ion binding
17.	Q5TUD8 (3)	AGAP010435-PA (Similar to <i>An. gambiae</i>)	97.57	5	513	5.55	Microtubule motor activity
18.	B0WDY8 (13)	Putative uncharacterized protein (Similar to <i>Cx. quinquefasciatus</i>)	95.4	2	32.3	5.59	GTPase activator activity, Signal transduction
19.	Q868R5 (8)	Gag-like protein (Similar to <i>An. gambiae</i>)	95.09	2	59.5	9.88	Nucleic acid binding, Zinc binding
20.	F5HJB1 (32)	Annexin (Similar to <i>An. gambiae</i>)	92.64	7	35.9	4.78	Calcium-dependent phospholipid binding
21.	B0WQN1 (7)	Ro ribonucleoprotein autoantigen (Similar to <i>Cx. quinquefasciatus</i>)	80.89	2	68.4	9.31	RNA binding
22.	E3WRC2 (8)	Uncharacterized protein (Similar to <i>An. gambiae</i>)	80.19	1	19.4	8.21	Protein kinase activity
23.	B0WEA0 (3)	cGMP-dependent protein kinase (Similar to <i>Cx.</i> <i>quinquefasciatus</i>)	79.99	1	35	6.4	Kinase activity
24.	B0XGQ7 (25)	NADH dehydrogenase iron-sulphur protein 8 (Similar to <i>Culex quinquefasciatus</i>)	79.85	4	24.3	5.67	Oxidoreductase activity
25.	E3XCW8 (3)	Uncharacterized protein (Similar to <i>An. darlingi</i>)	79.48	4	189.2	6.58	G-protein coupled receptor activity, Signal transduction
26.	H2EQR8 (23)	Calreticulin (Similar to <i>An. stephensi</i>)	75.69	6	46.2	4.54	Protein folding, Calcium ion binding, Endoplasmic reticulum
27.	E3XA88 (3)	Uncharacterized protein (Similar to <i>An. darlingi</i>)	75.22	1	57.9	6.64	DNA-directed RNA polymerase activity (nucleus)
28.	Q0PHPO (31)	Beta-1 tubulin (Similar to <i>Ae. aegypti</i>)	74.87	9	48.1	4.78	GTPase activity cytoskeleton
29.	T1E870 (22)	Putative PDSW (Similar to <i>An. aquasalis</i>)	71.75	3	19.6	6.79	Unknown
30.	Q7PSR9 (21)	AGAP011159-PA (Similar to <i>An. gambiae</i>)	70.24	3	17.3	5.41	Cytochrome C oxidase activity

Numbers in parentheses denote sequence coverage.

These gel bands were cut into 21 slices separately and subjected to digestion with trypsin and then analyzed by LC/MS/MS. A total of 17 proteins were identified by Mascot algorithm. These proteins and their characteristics like molecular weight, peptides number, calculated pI, sequence coverage and domain information are depicted in Table 4. Among the identified proteins by LC/MS/MS, further conserved domain were searched on NCBI domain programmes (<http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>), Interproscan analysis and also predicted by SMART programme (<http://smart.embl-heidelberg.de>) (Table 4).

The baseline data collection of midgut proteins of species B is in process. The data prepared from this study will be highly relevant and will serve as the basis for understanding the mechanisms of refractoriness, identification of altered protein with effect of sugar or blood feeding, etc. The hypothesis

is that the proteomic analysis leads to identification of known proteins as well as hundreds of novel proteins. Novel proteins identified from such approaches can subsequently be tested in molecular and biochemical strategies designed to control malaria transmission.

1.1.6 Proteomic analysis of salivary gland of *Anopheles culicifacies* using high resolution mass spectrometry (LC-MS/MS)

Anopheles culicifacies, a major vector of malaria parasites in India is a complex of five isomorphic sibling species, and species A is susceptible and some strains of species B are completely refractory to *P. vivax* and partially refractory to *P. falciparum*. Before taking a blood meal, mosquito females inject several salivary substances into the host skin to counteract the haemostatic reaction induced by the bite. Salivary proteins are directly involved in

Table 4. A catalogue of midgut proteins of *An. culicifacies* species A identified by in-gel digestion strategy and LC-MS/MS

S.No.	Accession No.	Protein	Molecular weight (kDa)	Peptides	Calculated pI	Sequence coverage (%)	Function
1.	G8GIB8	ATP synthase beta subunit (Similar to <i>Ae. albopictus</i>)	17.3	2	4.93	14	ATP binding
2.	Q7PJV2	AGAP010147-PA (Similar to <i>An. gambiae</i>)	224.2	3	5.76	2	ATP binding, Motor activity
3.	B0W3E5	Condensin complex subunit (Similar to <i>Cx. quinquefasciatus</i>)	158	1	6	2	Mitotic chromosome condensation
4.	E3X4B5	Uncharacterized protein (Similar to <i>An. darlingi</i>)	24.4	1	9.23	11	Unknown
5.	B0X0G3	General odorant-binding protein (Similar to <i>Cx. quinquefasciatus</i>)	14	1	8.43	10	Odorant binding
6.	E3WSY9	Ubiquitin carboxyl-terminal hydrolase (Similar to <i>An. darlingi</i>)	335.3	1	5.94	1	Ubiquitin thiolesterase activity, Peptidase activity
7.	Q1HRD0	AAEL008128-PA (Similar to <i>Ae. aegypti</i>)	10.2	1	7.74	22	Protein transport
8.	Q16LA4	AAEL012710-PB (Similar to <i>Ae. aegypti</i>)	16.6	1	8.43	5.4	Response to metal ion
9.	Q16QD5	AAEL011312-PA (Similar to <i>Ae. aegypti</i>)	172.7	1	7.21	2	Signal transduction
10.	Q7Q0D8	AGAP012401 (Similar to <i>An. gambiae</i>)	57.2	1	5.74	5	Catalytic activity
11.	Q7PJP4	AGAP003995 (Similar to <i>An. gambiae</i>)	73	3	5.31	4	Carbohydrate metabolic process
12.	Q17PH7	AAEL000308 (Similar to <i>Ae. aegypti</i>)	18.2	1	9.54	7	Cell adhesion, Tissue regeneration
13.	G9BQS9	Superoxide dismutase [Cu-Zn] (Similar to <i>An. aquasalis</i>)	17.4	1	6.13	7	Superoxide dismutase activity
14.	Q16LA4	AAEL012710-PB (Similar to <i>Ae. aegypti</i>)	16.6	1	8.43	5.41	Response to metal ion
15.	Q16N96	AAEL012054-PA (Similar to <i>Ae. aegypti</i>)	74	1	6.83	2.5	Thioloxidase activity
16.	T1DNJ3	Uncharacterized protein (Similar to <i>An. aquasalis</i>)	18.4	1	5.9	10	Uncharacterized protein
17.	Q1HRD0	AAEL008128-PA (Similar to <i>Ae. aegypti</i>)	10.2	1	7.74	21	Protein transport

human-vector contact during biting and therefore, play a key role in pathogen transmission. Hence, the proteomic study of salivary gland proteins is the essential first step towards understanding the refractory mechanisms and host-parasite relationships. In this post-genomic era, mass spectroscopy has emerged as a powerful tool for high-throughput analyses of proteomes. In case of proteomic analysis of anopheline mosquitoes, the study is mainly restricted to *An. gambiae*. Such proteomic studies have not been carried out on *An. culicifacies*. The baseline data of salivary gland proteins generated from this study will be highly relevant and serve as the basis of future research work to understand the mechanisms of refractoriness to infection.

The proteins from salivary glands of *An. culicifacies* species A were extracted and estimated. Followed by reduction and alkylation, the protein

extract was subjected to in-solution digestion with trypsin. Also, the salivary gland proteins were loaded on to SDS-PAGE gel. Each band observed on silver stained gel was cut, destained and in-gel trypsinization was carried out. Both in solution and in-gel digests were subjected to LC-MS/MS analysis. Raw data files were searched on SEQUEST and MASCOT algorithm for proteomic profiling and assignment of putative functions to the identified proteins.

A list of 63 proteins of *An. culicifacies* species A identified by SEQUEST algorithm using gel-free approach has been prepared. Few are mentioned in Table 5. Domain analysis was done for all the identified proteins by using SMART analysis software. Other details like molecular weight, calculated pI, sequence coverage and cellular component have also been depicted.

Further, 32 bands were observed on silver

Table 5. A catalogue of salivary proteins of *An. culicifacies* species A identified by in-solution digestion strategy and LC-MS/MS

S.No.	Accession No.	Description	Score	Coverage	Molecular weight (kDa)	Calculated pI	Domain/Function
1.	Q7PJV2	AGAP010147-PA (Similar to <i>An. gambiae</i>)	3966.73	33.08	224.2	5.76	ATP binding
2.	Q7PHI8	ATP synthase alpha subunit (Similar to <i>An. gambiae</i>)	789.48	27.22	59.4	9.10	ATP binding
3.	B0XB41	Actin 1 (Similar to <i>Cx. quinquefasciatus</i>)	780.55	43.09	41.7	5.39	ATP binding
4.	E3XEC7	ATP synthase beta subunit (Similar to <i>An. darlingi</i>)	717.22	57.65	53.7	5.12	ATP binding
5.	Q17H80	AAEL002759-PA Tropomyosin (Similar to <i>Ae. aegypti</i>)	304.91	27.82	32.4	4.89	Unknown
6.	Q7PKD7	AGAP012081-PA (Fragment) (Similar to <i>An. gambiae</i>)	296.34	40.09	22.7	5.25	ATP binding
7.	Q7PPA5-2	Isoform A of Calcium-transporting ATPase sarcoplasmic/endoplasmic reticulum type (Similar to <i>An. gambiae</i>)	269.28	14.73	109.5	5.45	Calcium binding
8.	Q7PUV3	AGAP001622-PA (Similar to <i>An. gambiae</i>)	252.38	27.83	22.9	4.78	Calcium ion binding
9.	Q17H74	AAEL002761-PH (Similar to <i>Ae. aegypti</i>)	204.55	25.59	29.5	4.74	Unknown
10.	F5HKJ2	AGAP002858-PB (Similar to <i>An. gambiae</i>)	201.58	16	110.8	5.45	Cation transporting ATPase activity
11.	Q7Q3F6	AGAP007852-PA (Fragment) (Similar to <i>An. gambiae</i>)	178.15	20.99	85.2	8.53	Aconitate hydratase activity
12.	E3WVT8	Uncharacterized protein (Similar to <i>An. darlingi</i>)	176.26	7.18	141.7	8.92	Nucleic acid binding
13.	T1E9F7	Putative ADP/ATP translocase 3 (Fragment) (Similar to <i>An. aquasalis</i>)	174.50	23.15	33.9	9.72	Transporter activity
14.	T1E7U1	Putative troponin T skeletal muscle (Similar to <i>An. aquasalis</i>)	168.85	14.17	43.3	4.87	Unknown
15.	Q7PSI4	AGAP010929-PA (Similar to <i>An. gambiae</i>)	164.78	28.64	50.1	4.86	GTP binding
16.	Q7PKD7	AGAP012081-PA (Fragment) (Similar to <i>An. gambiae</i>)	296.34	40.09	22.7	5.25	ATP binding
17.	Q7PYE7	AGAP001903-PA (Similar to <i>An. gambiae</i>)	135.60	30.56	35.4	9.06	Oxidoreductase
18.	B0X0G3	General odorant-binding protein 56d (Similar to <i>Cx. quinquefasciatus</i>)	127.17	9.16	14	8.43	Odorant binding

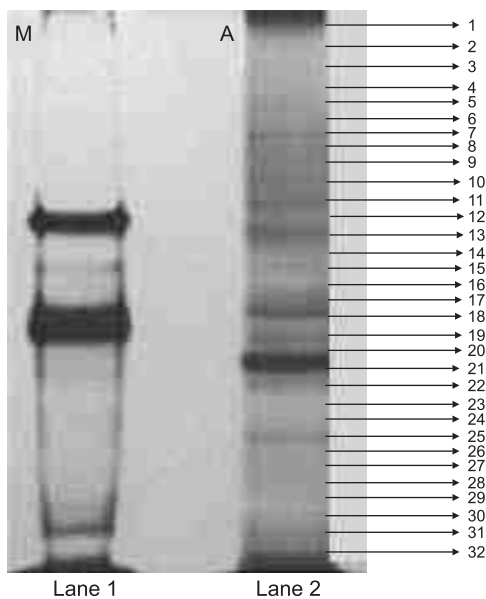


Fig. 6: Salivary gland protein profiling of *An. culicifacies*. Silver stained SDS PAGE gel of the midgut extract is shown in Lane 2 (A); and Protein markers with range 14 to 100 kDa shown in Lane 1(M).

stained gel (Fig. 6). LC-MS/MS for each band have been carried out and further investigation is in progress.

1.1.7 Microbial community structure in salivary glands and gut of mosquito *Anopheles culicifacies*

In recent years, it has been well documented that gut flora not only influence mosquito physiology,

but also significantly alter vector competency. Although, salivary glands and gut constitute key partners of the digestive system, it is still believed that salivary glands may harbor fewer flora than gut. Using a metagenomic approach, we have identified for the first time the diverse microbial community associated with these two physiologically different tissues of the digestive system in *An. culicifacies*. To estimate and compare the diversity of bacterial community, we generated a total of 1,23,325 and 96,711 sequence raw reads through pyrosequencing of 16S rDNA library for the salivary gland and the gut, respectively. Following quality filtration, total unique tags 2,18,425 that differed by not more than 3% were clustered into 6674 master OTUs dataset and compared to determine the frequency of tag distribution for inter-tissue bacterial diversity structure visualization and analysis. Normalized tag distribution frequency (0–100%) analysis revealed unique (54% SG and 35% MG) as well as overlapped (11%) microbial community between the two tissues (Fig.7a). Interestingly, the tag distribution frequency of the overlapped microbial community was dominated (Fig. 7b) by salivary gland (70%) over the gut (50%).

In addition to one Archea (0.4%) associated with salivary gland, a total of 17 different phyla of the bacterial community could be assigned to the

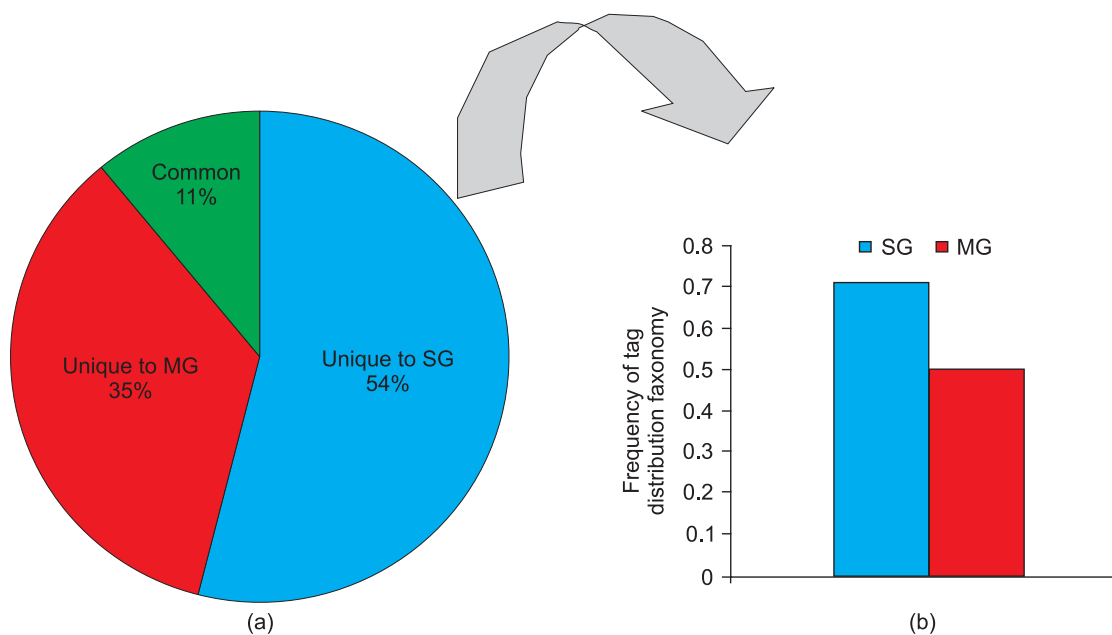


Fig. 7 (a & b): Taxonomy independent microbial community structure visualization: Tag distribution frequency (0–100%) analysis was performed to estimate the tissue associated microbial community complexity (a); and predominated by the salivary associate microbial community of the overlapped (11%) tags (b) as compared to the gut of the mosquito.

whole dataset (Fig. 8), and more than 76% of the bacteria belonged to the phylum Proteobacteria, Firmicutes; Bacteroidetes, Tenericutes and Actinomycetes (Fig. 8), while 21.4% (SG) and 19.3% (MG) clusters remain unassigned in both the tissues respectively. However, relative abundance ($>0.2\%$) analysis of the major classes revealed that salivary gland is not only dominated with γ -proteobacteria ($p < 0.01$) over other common $\alpha/\beta/\delta$ -proteobacteria, but also exclusively harbor ϵ -proteobacteria (0.3%) (Fig. 8a). Another phylum Acidobacteria (Class-Gp1/1.2%; Gp2/0.3%; Gp3/0.4% and Gp4/0.1), also significantly ($p < 0.01$) dominated in the salivary gland (2.3%) over unclassified Acidobacteria (0.3%) in the gut (Fig. 8b). We also identified other phyla ($\geq 0.2\%$), uniquely associated either with salivary gland which include Armatimonadetes (0.6%); Cyanobacteria/Chloropyta (0.2%); Chlorobi (0.2%); Chloroflexi (0.3%); Planctomycetes (0.2%); Nitrospira (0.2%); Fibrobacter (0.2%) or with gut including Fusobacteria (1.3%); Deinococcus (0.3%) and Spirochaetes (0.6%) (Fig. 8b). These findings clearly demonstrated that salivary gland is not only enriched with 'core microbiota' but also uniquely associated with more diverse bacterial taxa than gut.

In summary, we found that the salivary gland microbial community structure is more diverse than

that of the gut mosquito, probably due to differential feeding associated engagements such as food acquisition, ingestion and digestion processes, a knowledge which may guide our future investigation to better understand the feeding associated molecular relationships and design vector management strategies.

1.1.8 Unraveling molecular strategies of salivary gland for dual feeding in the mosquito *Anopheles culicifacies*

Despite the fact that mosquitoes spend longer time over sugar meal for regular energy source, the fundamental question 'how adult female salivary gland' manages molecular and functional relationship during sugar vs blood meal uptake remains unanswered. Through comprehensive molecular and functional genomics approach, we are currently engaged to unravel the molecular complexity of the salivary glands under dual feeding conditions. In recent years, next-generation sequencing has not only opened the door for functional genomics analysis, but is also emerging as an important tool to understand the evolutionary relationship of the molecular codes identified from non-model organisms.

Accordingly, we adopted Illumina based deep sequencing approach as a proof of concept for gene discovery tool and sequenced two cDNA libraries

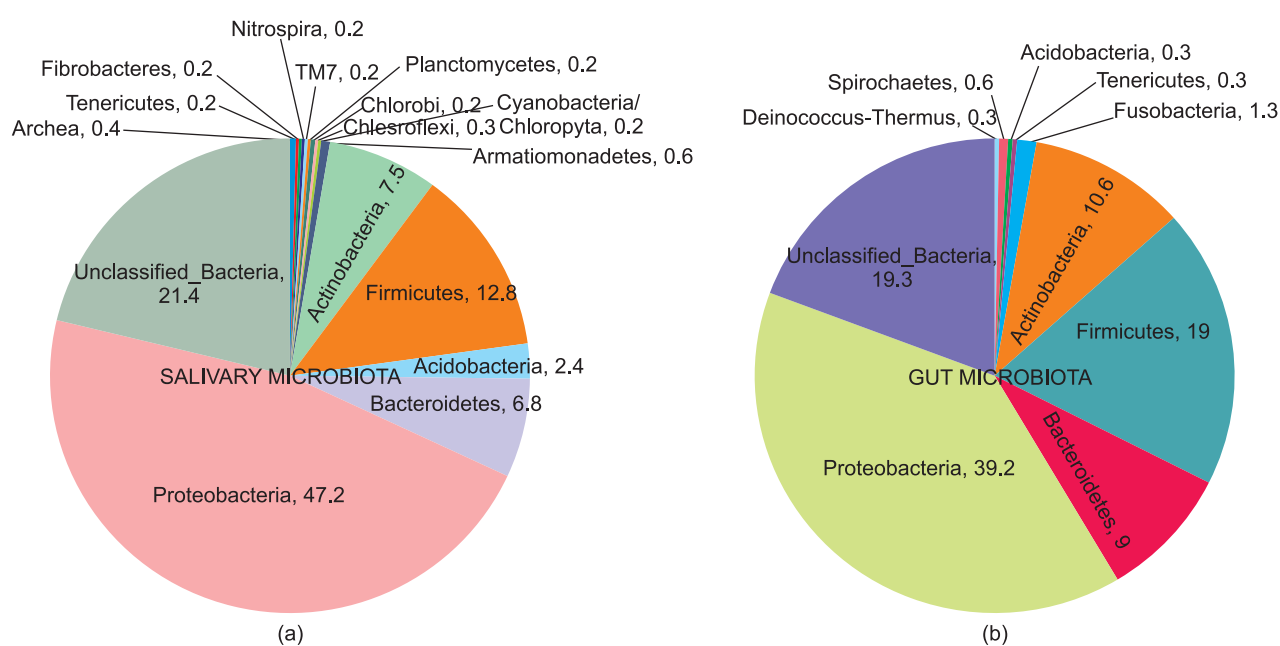


Fig. 8 (a & b): Microbial flora diversity of two physiologically different tissues of the mosquito digestive tract: Taxonomic assignment and relative percentage of the microbial community associated with the salivary glands (a); and gut (b) in *An. culicifacies*. The final high quality clustered sequences were independently analyzed through RDP classifier at the phylum level.

Table 6. Comparative annotation kinetics of mosquito salivary transcriptomes

Feature	SF	BF
Number of raw reads output	30480275	27990866
GC content (Raw reads)	33%	50%
SNPs/Indels	5546	2502
Final contigs	5808	5690
GC content (Final contigs)	39%	47%
Average size (bp)	~ 346	~ 493
Smallest contig length (bp)	195	155
Longest contig length (bp)	2223	5183

prepared independently from the salivary glands collected from 3-4 day old either sugar or immediate blood fed (within one hour) adult female mosquitoes. This protocol in fact generated a total of ~58.5 million raw reads, which were quality filtered and *denovo* assembled, yielding a set of 11,498 (5808 for sugar fed (SF) and 5690 for blood fed (BF) library contigs. Initially, the quality of the assembly was carefully examined by multiple homology search analysis of the whole transcriptome dataset against draft genome/transcript databases for *An. culicifacies* (available from: <http://www.vectorbase.org>). As expected 92% transcripts yielded significant match (10^{-5} e-value) to the draft genome of *An. culicifacies*, at nucleotide level.

To better understand the molecular complexity associated with dual feeding (sugar and blood), we annotated and compared both salivary transcriptomes. Table 6 and Fig. 9 (a & b) represent the sequencing and annotation kinetics for both the transcriptomes. Additionally, we also attempted to understand the blood meal impact on the mosquito salivary architecture.

Blood meal alters morphological, cellular and molecular architecture of mosquito salivary glands

Blood feeding is thought to be evolved independently several times among different insects, imposing direct recruitment of the salivary products for a faster way to disrupt multiple homeostasis and inflammatory responses of the vertebrate hosts. Although, molecular complexity associated with the dual feeding (sugar vs blood) behaviour evolution in mosquitoes is not well known, however, we investigated that how blood meal uptake process influence the salivary responses in *An. culicifacies*. A comparison of Nile blue stained sugar fed and blood fed salivary gland showed that first blood meal causes irreversible alteration in the morphological architecture, it primarily includes the extension, widening and swelling of the proximal lateral and median lobes. The TUNNEL assay data demonstrated that blood meal ingestion causes extensive apoptosis in the mosquito salivary glands (Fig. 10 a & b). The blood meal induced down regulation of inhibitor of apoptosis (*AcIAP*; $p \leq 0.005$) and up regulation of *AcCaspase* ($p \leq 0.005$) as well as *AcDrpr* ($p \leq 0.005$) (an important phagocytic engulfment receptor homolog of *Drosophila Draper*) indicates that blood meal induced apoptotic events are tightly controlled for the successful removal of dying cells undergoing apoptosis in the salivary glands (Fig. 10 c).

Mosquito food choice decision (sugar vs blood) is a random and complex neuro-cum-physiological and behavioural process of conflicting demand. Therefore, to better understand the role of salivary glands in this behaviour, we compared both the transcriptomes and performed digital gene

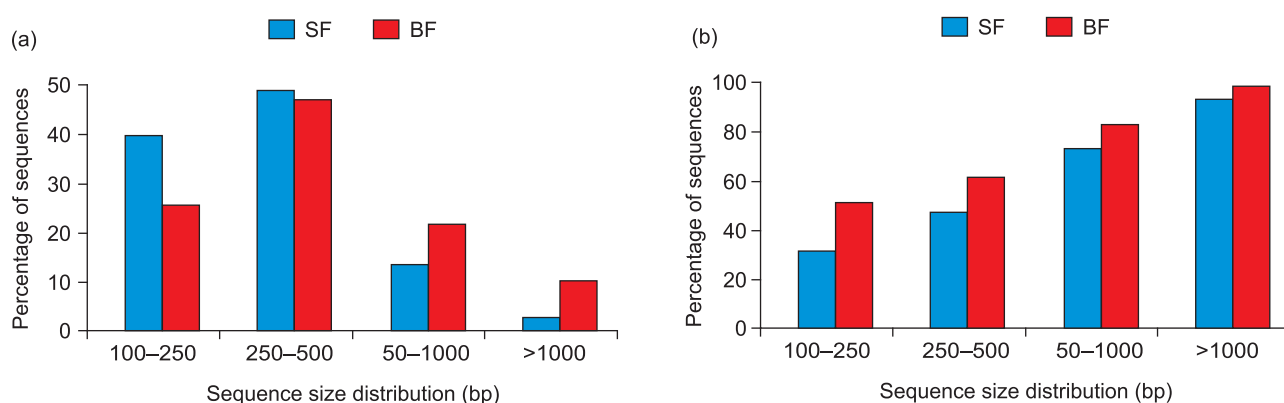


Fig. 9 (a & b): Comparative annotation kinetics of mosquito salivary transcriptomes: (a) Sequence size distribution stat; and (b) Database similarity (NR) # e-value cut-off 10^{-3} ; SF=Sugar fed; BF=Blood fed.

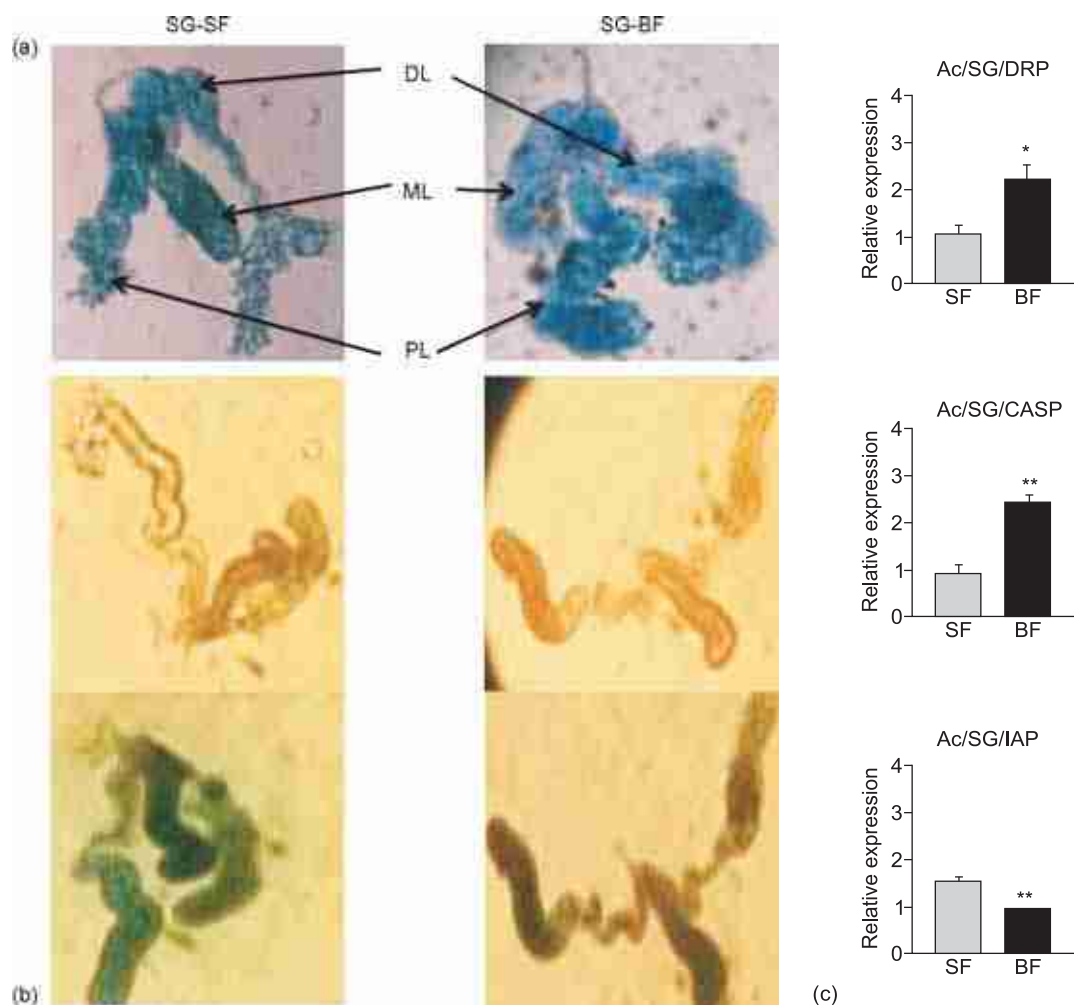


Fig.10 (a–c): Impact of blood meal on adult female mosquito salivary glands: (a) Blood meal alters the morphological architecture, resulting in the swelling and extension of the salivary lobes (Nile blue) stained glands observed under compound microscope; (b) TUNNEL assay demonstrating apoptotic response of the salivary glands following blood meal: Upper panel compares dark brown staining of the lobes in blood fed, while lower panel shows loss of green colour after staining with methylene blue, due to defragmentation of nuclear DNA; and (c) Apoptosis is a controlled cellular event resulting in the up/down regulation of positive and negative marker genes.

expression analysis (DGE). The read density plot and heat map analysis of DGE data showed meal specific restricted expression of unique tags to sugar (12%) or blood feeding (10%) as well as significant alteration of 17% tags ($p \leq 0.005$) in response to differential feeding (Fig. 11a). Together these data suggest that mosquito salivary glands may carry unique ability of “Food Choice Decision”, enabling successful uptake of desired meal through food specific switching on/off salivary gene expression. Preliminary analysis by real-time PCR expression for selected genes showed that blood meal significantly altered the gene expression through gene switching, especially anopheline and apyrase (Fig. 11b).

Our current investigation suggests that mosquito

salivary glands are evolved enough to manage meal specific choices. Future studies may unravel salivary mediated nature and regulation of the dual feeding behaviour, which would be important to design molecular strategies for feeding interference.

1.1.9 Exploring fat body mediated innate immune responses in *Anopheles stephensi*

Although, fat body has been shown to be primary organ for the synthesis of the AMPs, but how it manages/coordinates with hemocytes and midgut, during any microbial pathogens exposure is largely unknown. Therefore, first to know the basal level of AMPs production by fat body, we compared it with midgut dissected from the 3–4 day old sugar-fed mosquitoes. Fat body showed a higher level

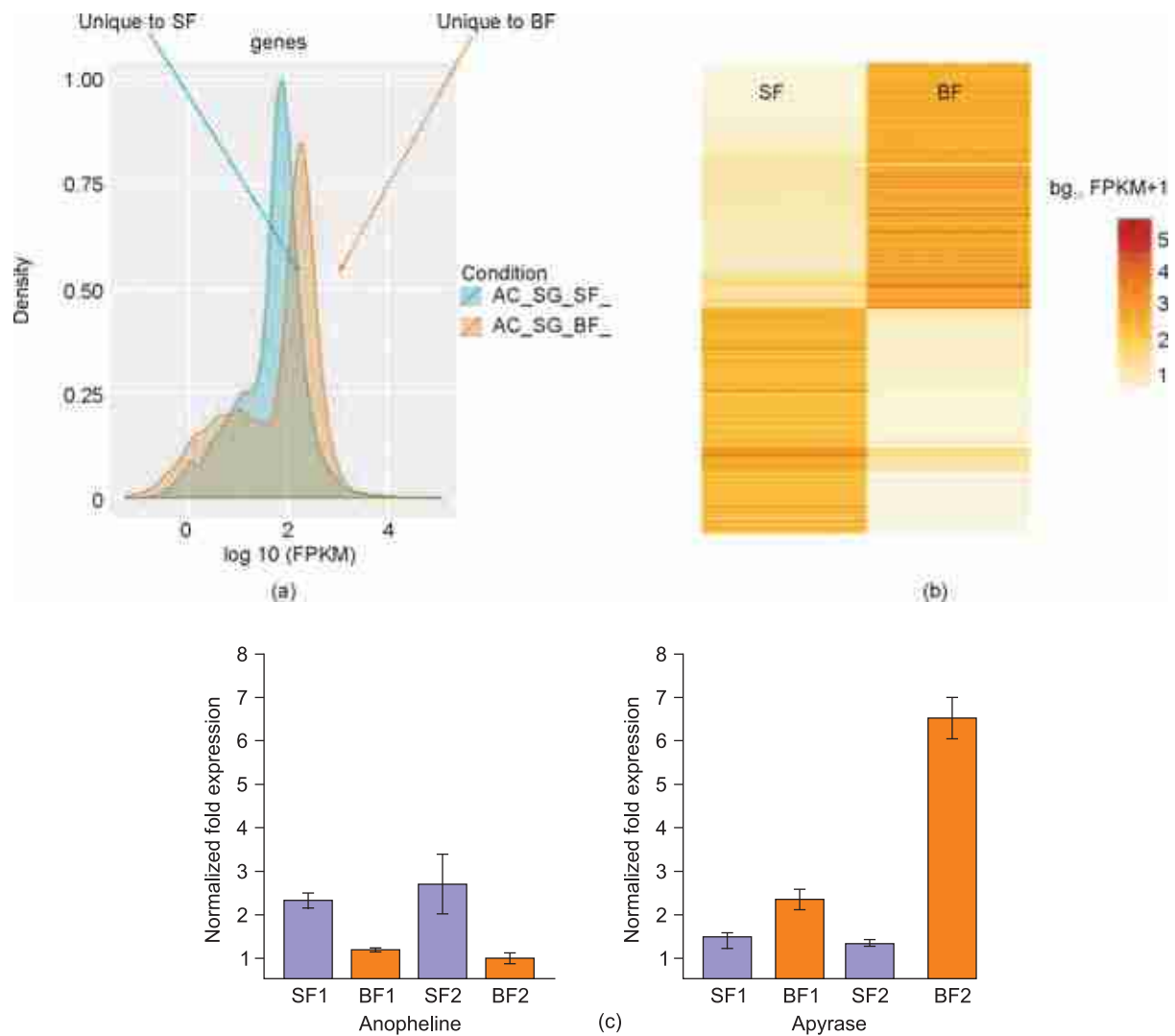


Fig. 11 (a–c): Salivary gene expression switching in response to dual feeding : Annotation of the salivary dataset showed significant differences in response to differential feeding. To further characterize their differential expression (DGE) pattern, we used merged contigs assembly to compare each transcriptome according to the number of mapped reads (FPKM). To quantify these differences, we normalized the expression level of SF and BF before calculating the read ratio of SF to BF/fold change of relative expression; (a) Read density plot of the transcriptome comparison showing unique contigs abundance restricted to feeding specific conditions (blue and orange). This analysis showed restricted expression of 1195 contigs to sugar feeding (12%) and 1021 contigs (10%) to blood feeding. While overlap region demonstrates commonly expressed genes; (b) Heat-map showing the global profiling comparison and district pattern of common salivary gene showing significant ($p \leq 0.05$) differential regulation. Relative gene abundance is defined by \log_{10} of the normalized read number followed by Z-score transformation to visualize the expression level. Yellow indicates lower expression and red indicates higher expression. At least 17% of transcriptome shows significant differential regulation of gene expression ($p \leq 0.05$), resulting in the expression alteration of 1767 contigs (847 BF and 920 SF); and (c) Real-time PCR based verification of salivary gene switching in response to dual feeding in mosquito *An. culicifacies*: To test the gene switching we screened six sets of salivary genes, which showed significant alteration in response to dual feeding. Salivary anopheline and apyrase have been shown in the figure; SF = Sugar fed; BF = Blood fed.

than midgut (Fig. 12.), pointing that FB may be a regular source of AMPs production, probably to distribute and maintain the basal level of the AMPs throughout the epithelial tissues. To test whether depletion of AMPs alters the expression in the fat body, we examined relative expression of cecropin family of AMPs after four days post dsRNA injection in the midgut and fat body tissues of the sugar fed

mosquitoes. We observed an effective depletion of all three tested cecropin1 (C1), cecropin2 (C2) and cecropin3 (C3) in the midgut, but unexpectedly fat body showed up-regulation of C1 and C3 in contrast to the down regulation of C2 as midgut. Current knowledge on cross talk/interorgan communication is too limited, however, these data provide initial evidence that fat body and midgut

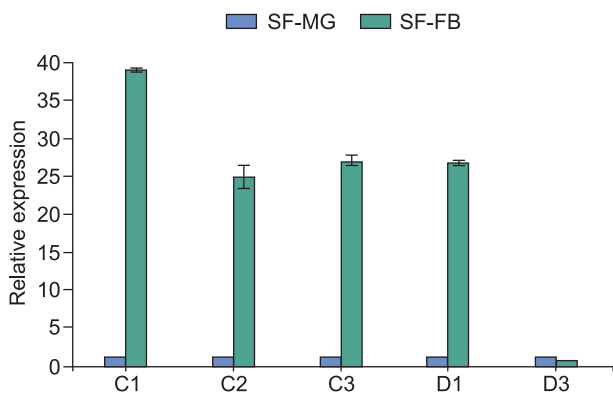


Fig. 12: Real-time PCR based relative expression analysis of AMPs in the fat body (FB) and midgut (MG) collected from 3-4 day old sugar-fed (SF) adult female mosquitoes. Total RNA was isolated using standard Trizol method and reverse transcribed using Oligo (dT) primers to prepare first strand cDNA. The expression was normalized using Actin gene as reference control in all the experiments.

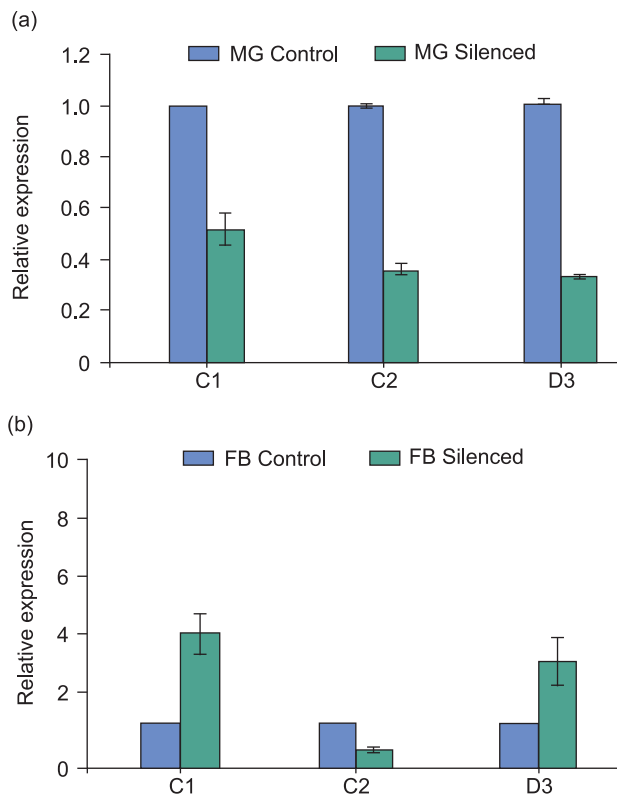


Fig. 13 (a & b): Functional relationship of fat body (FB) and midgut (MG) mediated local response: (a) dsRNA mediated gene silencing significantly ($p < 0.005$) alters the relative expression in midgut; and (b) fat body of Cecropin family proteins in the mosquito. For this test 3-4 day old sugar-fed (SF) adult female mosquitoes were injected dsRNA (3 $\mu\text{g}/\mu\text{l}$ /-69 nl/-69 nl mosquito) of respective AMPs in the thorax using nano-injector, while control mosquitoes were injected dsRNA LacZ, under similar isogenic condition 4-days post-injection; the midgut and fat body were dissected and the relative expression was monitored using real-time PCR assay.

possess an ability to manage the fine adjustment of the AMPs requirement during any alteration in the local immune response (Fig.13 a & b).

To further understand this correlation, we are examining the AMPs mediated immune regulation of the fat body and midgut during endogenous as well as exogenous exposure of gram-negative and gram-positive bacteria.

The above preliminary findings suggest that fat body manages local responses through fine adjustment of AMPs synthesis on demand basis, however, these finding also reinforces to clarify that how fat body coordinate to midgut, as both tissues are expected to work independently and does not come into direct contact at any stage of the infection. Hemocytes may be key player to communicate/coordinate for efficient immune network management and we believe our future investigation may provide better clarification of this network.

1.1.10 Initial characterization of mosquito hemocyte transcriptome in *Anopheles stephensi*

Hemocytes are metabolically active cells of the insect blood, i.e. hemolymph, which not only supply the nutrients to the body tissues but also believed to mediate multiple cellular immune responses, e.g. coagulation, phagocytosis, melanization, lysis, etc. against microbial pathogens. Although our basic knowledge on the insect hemocytes biology has largely been gained from *Drosophila* models system, we have still very limited knowledge about the molecular and cellular nature of mosquito hemocytes, which control physiological and immunological responses. Regarding post genome sequences of mosquitoes, a series of recent studies suggest that hemocytes are expected to affect multiple aspects of the mosquito biology, but how they manage the molecular and functional relationships during complex changes are yet to be understood. The key limiting factors include complex nature of synthesis and distribution, poor recovery of hemocytes and lack of hemocyte specific cellular markers that have greatly hampered our basic understanding of the molecular and cellular nature at basal level.

Therefore, to build up an initial molecular map of the 3-4 day old adult female mosquito hemocyte, we generated 13,105,858 sequencing raw reads (36 bp) using Illumina based sequencing approach. Following quality filtration, the

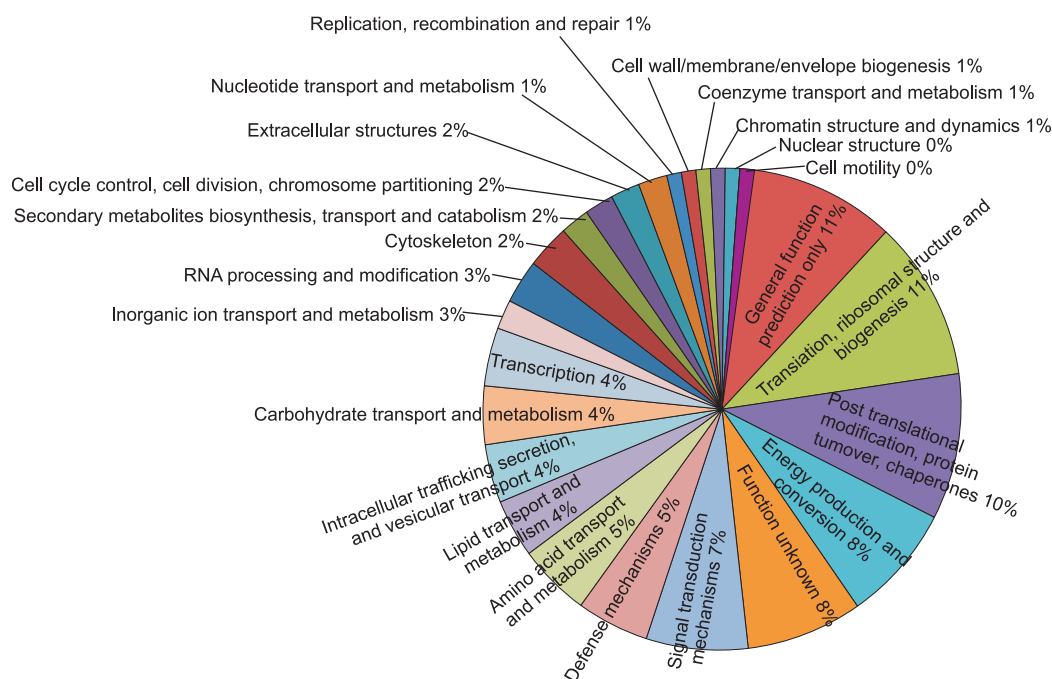


Fig. 14: Molecular map of mosquito hemocytes: To identify and predict the molecular nature of hemocytes, we compared and catalogued the whole dataset of the transcriptome to the multiple protein databases, viz. NR, GO, KOG, SMART, PFAM etc. Figures represent the predicted functional categories of the transcripts, which could be assigned as per their best homology and identity match to the known PDB database.

sequences were processed through comprehensive assembly pipeline yielding 3025 contigs in total for further analysis. Currently, an extensive molecular and functional annotation is in process to generate and characterize the molecular map of the mosquito hemocyte. The primary analysis indicate that hemocytes are not only metabolically active but also encode several specialized products including immunity, signal transduction, etc (Fig. 14).

Our ongoing functional annotation kinetics of the hemocyte database provide basis to identify and characterize the hemocyte specific molecular targets, especially signaling and immune molecules, may likely participate in the anti-plasmodial immune responses.

1.1.11 Procedural modifications in the technique of counter current immunoelectrophoresis (CCIE) for mosquito blood meal source identification

Blood meal source identification in vectors is indispensable to justify the host of the vector species which reflects their capability to transmit human or animal diseases. The vector abundances and human blood index (HBI) or anthropophagic index reflect the proxy measures of human diseases and a very useful predictive tool in study of disease transmission dynamics.

Blood meal source identification is practiced by means of different methods of choice over the years. The gold standard robust method used for blood meal source identification is based on counter-current immunoelectrophoresis using Barbitone buffer. The barbital buffer is certainly having widest alkaline pH range (6.8–9.2), less temperature sensitive and first choice for optimizing the CCIE assay at higher alkaline range. The key component of this buffer is Barbitone (diethyl malonyl urea or diethyl barbituric acid). Barbiturates are potentially habit-forming and black marketed among narcotic addicts. By habitual use it accumulates toxicity and leads to mental disorders, furthermore, it became an aspect of felony, suicide and accidents, consequently, the manufacture, distribution and sale are restricted by authoritarian laws in most of the countries. So an alternative buffer is the immediate solution rather than abandoning this robust time saving, easy and inexpensive technique. Alternative methods like ELISA and PCR are time consuming critical processes demanding expensive instruments and consumables, contain risk of contamination, false positivity, and low sensitivity when blood meal is more than half digested.

Devising a simple and reasonably competent technique for mosquito blood meal source identification by CCIE using ordinarily available

TBE buffers countering against Barbitol buffer is under process.

Six different protein buffers namely Tris-glycine buffer, Tris-tricine buffer, TBE buffer, TAE buffer, TE buffer and Tris-buffer have been tried as substitute to barbitone buffer at varying pH, voltage and time run. The promising buffers that can substitute barbitone buffer in CCIE have been tested with known human and bovine blood samples. The modified assay will be applied to detect blood meal source of field collected *An. culicifacies* and *An. fluviatilis*, the two major malaria vectors in India varying in their host feeding pattern.

1.2 Vector Control

1.2.1 Phase-III evaluation to compare insecticidal efficacy and community acceptance of long-lasting insecticidal net DuraNet[®] with conventional insecticide-treated nets in India

This study was a randomized phase III evaluation of DuraNet[®] LN in comparison with polyester nets treated with alphacypermethrin at 40 mg/m² a.i in a malaria hyperendemic tribal area of Sundargarh District, Odisha, India. Before net distribution, houses were randomized through computer generated numbers and 140 randomized houses were earmarked for distribution of ITN and rest of 300 houses for LNs. On an average two LN/ITNs were given in each household to ensure full community coverage. Over a period of three years, bioassay mortality after 24 h ranged between 85 and 96%; and 91% of DuraNet LNs showed >80% mortality in the cone bioassays with *An. culicifacies*. The remaining 9% of the failed nets have shown >90% blood feeding inhibition in tunnel test, thereby implying that 100% DuraNet LNs passed the WHO criteria of bioefficacy.

The net usage rate also depended on mosquito nuisance during certain months but every night use of net remained >53% (range 53.3–95.4%). Majority of the households (60%) washed their nets at six monthly intervals and a small proportion (6.7%) washed their nets at about monthly intervals. The proportion of washed LNs ranged from 83 to 95% after 18 to 36 months of net distribution. All the households used locally available detergent powder and cold water was used for washing the nets. The proportion of clean nets varied from 6.7 to 26.7%, whereas that of very dirty nets was 3.3% to 26% during surveys undertaken after every six

months up to three years of household use of nets. The proportion of nets with holes after one year was 26.7% which gradually increased to 43.3% and 74% after two and three years of net distribution respectively. The mean hole index increased from 2.4 after six months to 92.9 after three years. Only few nets were found with repairs in the form of stitches (0.07–0.38 per net) and knots (0.03 – 0.34), open seams (0.02/net) and none with patches. Holes due to fire ranged between 0.07 and 0.4/net during intervals. The trial results showed that DuraNet LNs maintained high efficacy against malaria vector for a period of three years under field conditions. The study has been concluded.

1.2.2 Evaluation of NetProtect LN (impregnated with deltamethrin) against malaria vectors in the states of Haryana, Uttar Pradesh and Jharkhand

This is a multicentric phase III trial. Studies at Haryana site were initiated during September 2012 because of the closure of trial at Bengaluru due to technical reasons. During last one year, studies on wash resistance showed that 100% mortality was observed in *An. stephansi* in cone bioassays on Netprotect LN up to 14 serial washings but in subsequent washings, there was a gradual decline in bioefficacy to 85% after 20 washes of Netprotect whereas 100% mortality was recorded on unwashed Netprotect nets and mortality ranged between 2.5 and 5% on serially washed untreated nets. Studies on bioefficacy of field distributed nets were completed and surveys have been conducted to assess perceived adverse effects of the Netprotect LN and epidemiological impact of the long-lasting insecticidal net in comparison to untreated or no net. The study is in progress.

1.2.3 Vector infectivity rate in project area of comprehensive malaria case management programme in Odisha

The goal of the vector survey was to provide annual information on sporozoite rates. At baseline, about 1% of the total villages in a block within strata of three ecotypes of hilly forested, foothill and plain areas were randomly selected for mosquito collections during high transmission season. Head and thorax parts of individual specimen of different vector species were processed for detection of sporozoites in the salivary glands. Homogenate of individual mosquito in grinding

buffer were tested for the presence of circumsporozoite proteins using *P. vivax* and *P. falciparum* specific monoclonal antibodies. A total of 346 specimens of *An. culicifacies* were assayed for sporozoites and eight were found positive (*Pf*-6, *Pv*-2) with an infectivity rate of 2.3% in Bolangir district. The study is in progress.

1.2.4 Insecticidal efficacy of second generation neonicotinoids, namely thiacloprid, acetamiprid, nitenpyrram, dinotefuran, sulfaxaflor and thiamethoxam in the control of mosquito vectors

This study was undertaken as an intramural activity. Larval and topical application bioassays were carried out to assess the larvicidal and adulticidal activity on *An. stephensi*, *Ae. aegypti*

and *Cx. quinquefasciatus* with differential level of susceptibility to different insecticides and larvicides. Only two insecticides, namely thiacloprid and thiamethoxam could be procured during the period. The results of bioassays revealed that thiamethoxam showed higher larvicidal activity (LC_{90}) than thiacloprid. However, the larvicidal activity of both the compounds was not promising when compared to the existing larvicides in use.

Adulticidal activity in terms of LD_{99} was more in thiacloprid against *An. stephensi* and *Cx. quinquefasciatus* than thiamethoxam. However, the LD_{99} recorded against *An. stephensi* and *Cx. quinquefasciatus* was not promising when compared to the insecticides in use in vector control programmes (Tables 7–10).

Table 7. Results of larval bioassays against Thiomethaxam

Species (n)/Susceptibility status	LC_{50} mg/l	LC_{90} mg/l	Chi-square Pearson's goodness of fit & p-value
<i>An. stephensi</i> SS strain (Nadiad) (n=752)/Susceptible to temephos	0.052	0.155	$\chi^2 = 1.787$ $p = 0.182$
<i>An. stephensi</i> (Goa)/Resistant to temephos	0.064	0.104	$\chi^2 = 9.486$ $p = 0$
<i>Cx. quinquefasciatus</i> Permethrin resistant/(n=638)/Resistant to malathion	0.343	8.53	$\chi^2 = 0186.5$ $p = 0$
<i>Ae. aegypti</i> (n=548)/Tolerant to temephos	0.298	2.278	$\chi^2 = 180.83$ $p = 0$

Table 8. Results of larval bioassays against Thiacloprid

Species (n)/Susceptibility status	LC_{50} mg/l	LC_{90} mg/l	Chi-square Pearson's goodness of fit & p-value
<i>An. stephensi</i> SS strain (Nadiad) (n=806)/Susceptible to temephos	24.232	97.5	$\chi^2 = 114.8$ $p = 0$
<i>An. stephensi</i> (Goa) (n=891)/Resistant to temephos	22.511	111.824	$\chi^2 = 62.54$ $p = 0$
<i>Culex quinquefasciatus</i> Permethrin resistant (n=800)/Resistant to malathion	6.139	16.88	$\chi^2 = 2.54$ $p = 0.77$

Table 9. Results of topical application of Thiamethoxam against different mosquito species strains

Species (n)	Resistant status (Adults)	LD_{50} (CI) ng	LD_{99} (CI) ng	Chi-square Pearson's goodness of fit	p-value
<i>An. stephensi</i> B/B	Susceptible to OC, OP and PY	0.946	241.3	0.941	0.816
<i>Cx. quinquefasciatus</i>	Selected for malthion, resistant to OC and PY	0.745	45.37	2.169	0.705
<i>An. stephensi</i> RR	Resistant to OP, OC, PY	0.302	99.75	26.19	0

Table 10. Results of topical application of Thiacloprid against different mosquito strains

Species (n)	Resistant status (Adults)	LD_{50} (CI) ng	LD_{99} (CI) ng	Chi-square Pearson's goodness of fit	p-value
<i>An. stephensi</i> B/B	Susceptible to OC, OP and PY	0.295	20.636	24.58	0
<i>Cx. quinquefasciatus</i>	Selected for malathion Resistant to OC and PY	0.491	3.743	120.3	0

1.3 Insecticide Resistance

1.3.1 Insecticide resistance in *Aedes aegypti* in Delhi

Adult bioassay for susceptibility

Two to four-day old sugar-fed adult *Ae. aegypti* female mosquitoes of F_0 (adults emerged from field collected larvae) populations were subjected to insecticide susceptibility test using WHO's standard insecticide susceptibility kit. A total of 20–25 mosquitoes in each replicate were exposed to 4% DDT, 0.05% deltamethrin and 0.75% permethrin impregnated paper alongside appropriate controls for one hour. Subsequently, the mosquitoes were kept for recovery for 24 h and were provided with 10% glucose soaked on cotton during recovery. All the bioassays were carried out at $27 \pm 1^\circ\text{C}$ and $70 \pm 10\%$ relative humidity. Mortality was recorded after 24 h of exposure and corrected percentage mortality was calculated by applying Abbott's formula. Dead and alive mosquitoes after recovery were kept in individual microfuge tubes and stored at -20°C for molecular studies. The results of insecticide susceptibility test carried out on *Ae. aegypti* in three localities of Delhi against DDT 4%, deltamethrin 0.05% and permethrin 0.75% are shown in Table 11. The results showed high percent resistance against DDT (69.83–51.85) and moderate level of resistance to pyrethroids (deltamethrin: 25.68–35.86%; and permethrin: 17.69–33.21%) in all the sites.

Molecular basis of insecticide resistance

One of the mechanisms of resistance in insects against DDT and pyrethroids is knockdown resistance (*kdr*) conferred by mutation(s) in the target

site of action, i.e. voltage gated sodium channel. Several *kdr* mutations have been reported in insects of agricultural and medical importance including *Ae. aegypti* in various parts of the world. A total of 11 non-synonymous mutations at nine different loci have been identified in *Ae. aegypti*. Among these the mutations at three loci Iso1011 (1→M/V), Val1016 (V→G/I) in domain II of segment 6 and F1534 (F→C) in domain III-S6 are most commonly reported and their role in pyrethroid resistance has been established. In *Ae. aegypti*, however, classical *kdr* mutations, i.e. L1014F/S were never found possibly due to codon constraint where two independent changes in the same codon would be necessary. However, till date there is no report of any *kdr* mutation in Indian *Ae. aegypti* population.

kdr genotyping

For genotyping of various *kdr* mutations previously reported allele-specific PCR assays were used for I1011M, I1011V, V1016G, V1016I and F1534C; and for genotyping of D1794Y, PCR-RFLP was carried out. In the absence of established PCR-based assays for mutation S989P, direct sequencing was carried out.

Results of ASPCR genotyping for F1534C mutation have been shown in Table 11. The allelic frequency of 1534C mutant is high in all the three sites ranging from 41–69%. Out of 1180 samples genotyped, a total of 54 samples representing FF (n = 15), FC (n = 20) and CC (n = 19) were sequenced to validate ASPCR results. A total of two samples showed discrepancy where homozygous CC were turned out to be FC after sequencing. A total of 166 samples were genotyped for I1011M/V and V1016G/I. Though some samples were observed

Table 11. Results of insecticide susceptibility test against DDT, deltamethrin (DEL) and permethrin (PER), genotyping result of F1534 alleles as determined by ASPCR

Locality	Percent corrected mortality			F1534 genotypes				Allelic frequencies		p-value* (HWE)
	DDT 4%	DEL 0.05%	PER 0.75%	FF	FC	CC	Total	F1534	1534C	
South Delhi I	30.17 (n = 348)	71.86 (n = 231)	82.31 (n = 373)	118	195	214	527	0.409	0.591	0
South Delhi II	37.58 (n = 165)	74.32 (n = 148)	66.79 (n = 265)	35	128	158	321	0.308	0.692	0.504
West Delhi	48.15 (n = 108)	64.41 (n = 59)	74.74 (n = 95)	139	112	81	332	0.587	0.413	0
Pooled data	35.27 (n = 621)	71.69 (n = 438)	75.72 (n = 733)	292	435	453	1180	0.432	0.568	0

HWE = Hardy Weinberg equilibrium; *Chi-square test.

to be positive for mutations (IM=7, MM=1, IV=2 for L1011 locus and VI=6 for V1016 locus) by ASPCR for both the loci but sequencing of 29 samples representing all mutant genotypes (all mutant and 13 wild genotypes) didn't confirm presence of any mutation. Sequence analysis didn't show S989P *kdr* mutation in any sample sequenced for partial domain II. The genotyping for L1011 and V1016 was therefore discontinued assuming that ASPCR is not specific and absence of L1011M/V or V1016G/I mutation in the study population. For genotyping of D1794Y, a total of 66 pyrethroid resistant mosquitoes were genotyped using PCR-RFLP but all turned out to be wild which was also confirmed by DNA sequencing performed on five samples.

1.3.2 Impact of insecticide resistance in malaria vectors on the effectiveness of combination of indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) in India: A multidisciplinary approach

The field site at Kondagaon became completely functional by June 2013 with trained staff and needed infrastructure. Census was carried in 82 of 105 villages in CHC Keshkal comprising of about 0.82 lakh population and each village was given a single digit unique identification number as V1,

V2,V82 (Fig. 15). During the census, information on different socioeconomic aspects such as education status, income, occupation, demography, etc was also collected and each household was given a display card for registering major activities during the visit. The number of sleeping units in these villages were enumerated to determine the requirement of LLINs and insecticide for the interventions. Efforts are being made to map the households by GPS for virtual information. Different entomological indices relevant to the transmission of malaria were determined. The major vector species *An. culicifacies* was found mostly resistant to DDT and malathion and but susceptible to deltamethrin (pyrethroid) and variable resistance to bendiocarb (carbamate). The species was found predominately zoophagic (0.91–0.95) with low sporozite rate of about 0.012%. Cross-sectional survey was conducted in 47 villages with around 400 slides per village to determine the prevalence of infection rate. A total of 12,000 slides were collected and the infection rate (about 0.02%) was very low. The techniques were standardized for mosquitoes blood meal identification using ELISA method and PCR method for identification of parasites. *Plasmodium malarie* infection and also mixed infection with *P. falciparum* was found. In the

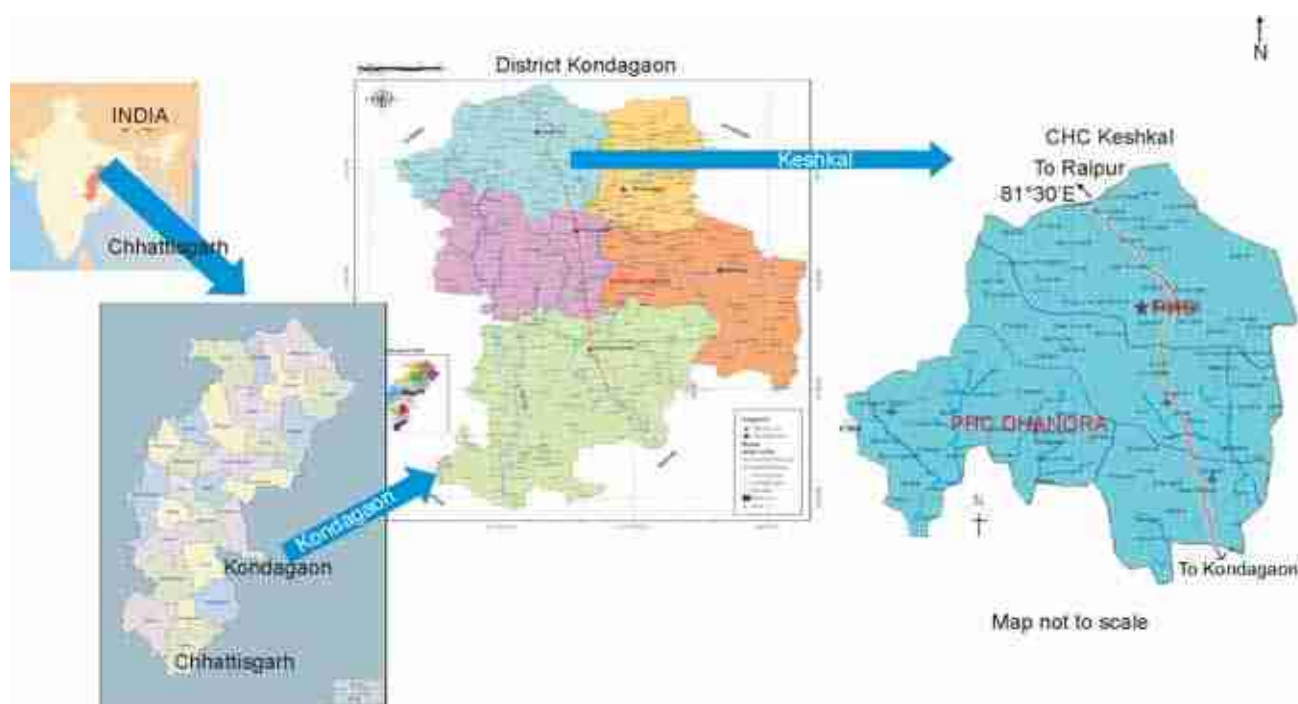


Fig. 15: Map of Keshkal block showing location of PHCs.

month of January, 2014 WHO technical consultants of the project visited the field site at Kondagaon and reviewed the progress. Later, the report of WHO consultants was submitted to WHO. The results were presented by us in National Coordinators' meeting of five participating countries and report was accepted. Study design was suggested by randomized cohort studies in 82 villages. The process of randomization is initiated. The database management is put in place and will be linked to the households.

1.3.3 Adaptation of the bottle assay for monitoring insecticide resistance in adults of mosquito vector species and comparative evaluation with WHO insecticide susceptibility test

The diagnostic dose for deltamethrin bottle assay was standardized and the shelf life was determined in the laboratory for use in the field. Discriminating dose for deltamethrin and cyfluthrin was 10 µg against *An. stephensi* and 2 µg against *Ae. aegypti*. Insecticide coated bottles stored at 25 to 35°C can be used for three exposures within seven days of coating. Studies were carried out to validate the results and to assess the feasibility for use of this method in field conditions. Comparative evaluation was done with the standard WHO susceptibility tests using insecticide impregnated papers.

Presently, the kit is available from the only source in Malaysia and sometimes imports are cost prohibitive. Standardization of bottle assay was done in laboratory by using locally sourced material. The study carried out in laboratory was validated on wild caught *An. culicifacies* in the states of Odisha

(susceptible strain) and Chhattisgarh (resistant strain) against deltamethrin coated bottles and compared with WHO adult susceptibility test. The bottle assay was found practical for use in the field. And, can be used as alternative to WHO adult susceptibility test both in laboratory and field for monitoring insecticide resistance in mosquito vectors with minimal infrastructure and easily available locally sourced material.

1.3.4 Studies for development of method(s)/strategy for the management of pyrethroid resistance in malaria vectors in India using insecticides with novel mode of action

Analysis using prediction pathway system for three detoxification enzymes: malathion carboxylesterases (EC 3.1.1.1), pyrethroid hydrolase (EC 3.1.1.88), and unspecific monooxygenase (EC 1.14.14.1) were identified for molecular docking study and their interaction with the toxin pairs from eight insecticides, i.e. deltamethrin, permethrin, etofenprox (pyrethroids); and alanycarb, benfuracarb, chlorfenapyr, indoxacarb and malathion (proinsecticides) were selected for the study. *In silico* molecular modelling and docking results showed common active binding site for pyrethroids and proinsecticides. It was observed that deltamethrin shared the common binding site with chlorfenapyr, indoxacarb, benfuracarb, alanycarb, while permethrin shared with malathion, indoxacarb, benfuracarb, alanycarb while, etofenprox with chlorfenapyr, alanycarb and benfuracarb.

□

2.1 Molecular characterization of 4-diphosphocytidyl-2C-methyl-d-erythritol (IspE) kinase gene from *Plasmodium vivax*—ligand recognition in a template for antimalarial drug discovery

Erythritol kinase is essential for the parasite's survival and is the only kinase in Methyl Erythritol Phosphate (MEP) pathway. As this enzyme is absent from mammals it is therefore, considered as a potential target for antimalarial drugs. In this study, we plan to amplify, clone, sequence, express and purify recombinant *P. vivax* IspE (PvIspE). The genetic diversity of the IspE across seven different geographical regions of India will be characterized by biochemical properties and inhibition kinetic data, and structure determination of enzyme in complex with 4-diphosphocytidyl (CDP), 4-diphosphocytidyl-2C-methyl-d-erythritol (CDPME) and ADP. Novel compounds will be designed to mimic a fragment of the substrate and the complex structure will be determined with catalytic centre in culture *in vitro* and *in vivo* assays, by structural 3D modelling and docking studies *in silico*. In preliminary studies, we have isolated parasite DNA from filter paper blood spot from Bengaluru region of India. IspE gene has been amplified and amplified PCR product is shown in Fig. 1 (EK-1 and EK-2). Phylogenetic analysis and optimization of enzyme activity assay is in process.

The biochemical and molecular characterization of IspE and the models resulting from our analyses may provide compounds and templates to support the structure-based design of novel broad-spectrum antimalarial agents which may thus represent promising antimalarial drugs. Moreover, absence of erythritol kinase in human host provides the opportunity to use it as a diagnostic tool after its biochemical and molecular characterization.

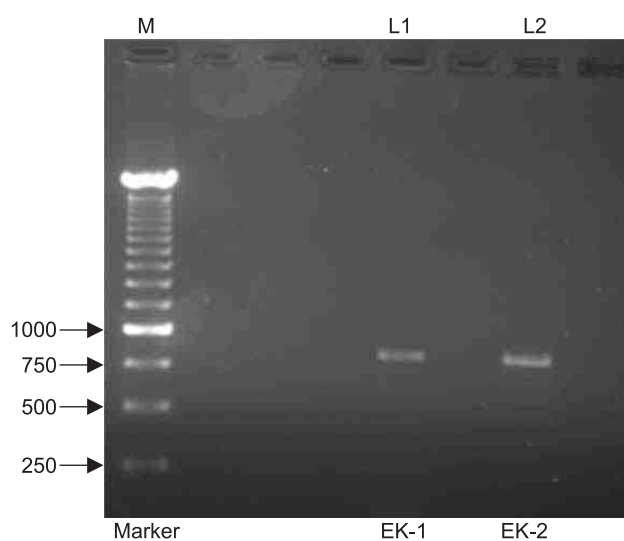


Fig. 1: PCR amplification of EK-1 gene (838 bp, L1) and EK-2 gene (829 bp, L2).

2.2 Expression of *vir* genes and drug resistance genes in severe *Plasmodium vivax*

Antigenic genes are present in malaria parasites, which encode the variant surface antigens (VSAs) providing protective immunity to the parasite against host/vector. Emergence of severe pathology like renal failure, jaundice, acute respiratory distress syndrome, cerebral malaria, seizures, anaemia, hyperparasitaemia, thrombocytopenia, pulmonary edema, splenic rupture and death, have been reported in exclusive association with *P. vivax* severity. Severe *P. vivax* and its pathogenesis has been linked to the *vir* genes and chloroquine resistance (CQR). The main transporter that has been studied with regard to CQR in *P. vivax* is the *P. vivax* chloroquine resistance transporter (*Pvcrt-o*) which has been identified as possible genetic marker of CQR. Increasing *P. vivax* severity has been known to be associated to increased

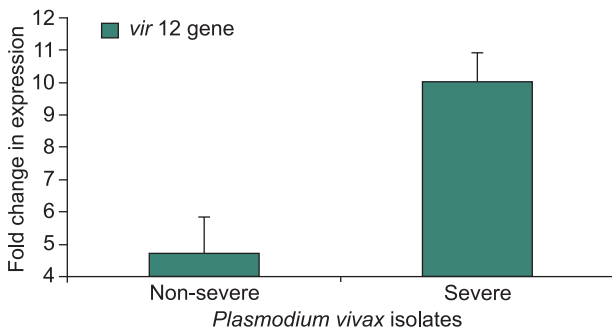


Fig. 2: Relative quantification of *vir 12* gene between severe and non-severe *P. vivax* isolates normalized with endogenous gene (*β-tubulin*); $p < 0.05$.

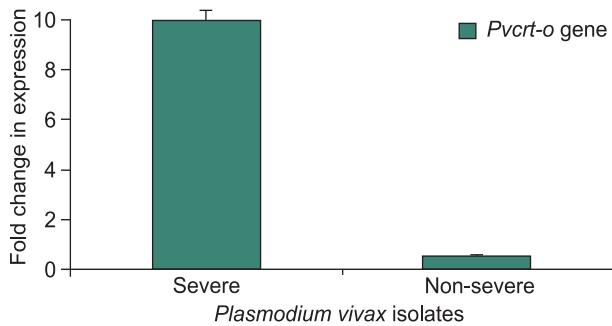


Fig. 3: Expression of *pvcr-t-o* gene in severe and non-severe *P. vivax* isolates normalized with endogenous gene (*β-tubulin*).

expression levels of *pvcr-t-o* CQR gene. In this study, the expression of five *vir* genes was compared in severe *P. vivax* infections and also the expression of *vir 12* gene along with *pvcr-t-o* gene was compared between severe and non-severe *P. vivax* cases. The severe *P. vivax* cases showed higher expression levels of *vir 12* gene and *pvcr-t-o* gene as compared to the non-severe *P. vivax* infections (Figs. 2 and 3). Studying the virulence *vir* genes and transporter of CQR, i.e. *pvcr-t-o* allows us to deduce that the increased expression levels of these genes in severe infections might be responsible for the changing trends of complicated *P. vivax*.

2.3 Multiplicity of *Plasmodium falciparum* infection in field isolates

Plasmodium falciparum attributes to about 50% of malaria infections in India but relatively little is known about the genetic structure of the parasite populations. This study reveals the genetic profile of the parasite population by molecular genotyping

with merozoite surface protein— *msp1*, *msp2* and glutamate-rich protein (*glurp*) genes in selected regions across India with varying degree of endemicity among them. Genotyping the *P. falciparum* isolates using *msp1*, *msp2* and *glurp* gene loci has been known to distinguish treatment failures of *P. falciparum* infections and *msp1* and *msp2* have been used to assess the multiplicity of infection (MOI) for detecting the number of clones per isolate. Fifty-eight single *P. falciparum* infections after the species-specific PCR were amplified for three allelic families of *msp1* gene (K1, MAD20 and RO33), two allelic families of *msp2* gene (FC27 and IC/3D7) and *glurp* gene. The study has demonstrated that more polymorphism was found in *msp2* gene than *msp1* and *glurp* genes. It was seen that 39.6% of the isolates studied were multiclonal in nature with two or more alleles present in *msp1*, *msp2* and *glurp* genes. Eleven multiple alleles were seen in *msp1* (18.9%), 16 were found in *msp2* (27.5%) and four multiple alleles were seen in *glurp* (6.8%) genes showing more genotypic variation in *msp2* than *msp1* and *glurp*. The *msp1* showed eight, *msp2* seven and *glurp* markers showed five distinct alleles present in the studied parasite population. These findings indicate that for detection of MOI, *msp1* served as a better marker as MOI with *msp1* was higher as compared to *msp2* (Fig. 4). The recent data of *msp1*, *msp2* and *glurp* markers for drug efficacy studies is highly important in malaria endemic areas for understanding the treatment criteria, etc. These genes should be monitored regularly to know the present genetic structure of the parasite population.

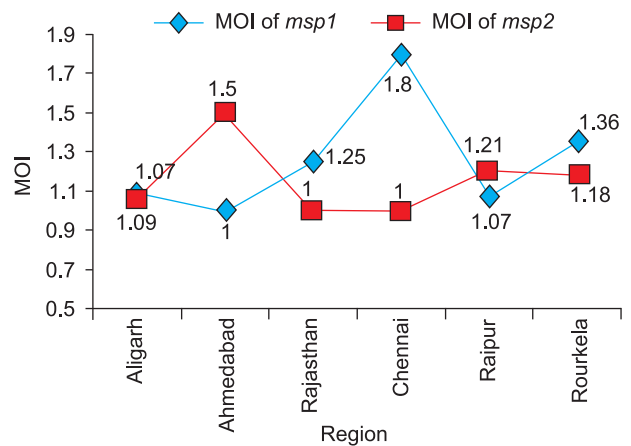


Fig. 4: Graph showing comparison of MOI for *msp1* and *msp2* genes in different regions of India.

2.4 Occurrence of multiple chloroquine-resistant *pfcr* haplotypes and emergence of the S(agt)VMNT type in Cameroonian *Plasmodium falciparum*

Cameroon is an African country located in the west-central region of the African continent, where malaria due to *P. falciparum* infections is very high. Due to massive use of chloroquine in the past and amodiaquine at present to treat *P. falciparum* malaria in Cameroon, chloroquine resistant malaria parasites are of wide prevalence. Genetic basis of chloroquine resistance is known and the mutations in the *pfcr* gene have been widely attributed to the occurrence of chloroquine-resistant *P. falciparum*. Differential distribution patterns of mutations in the *pfcr* gene result in different haplotypes. So far only one particular haplotype, CVIET has been reported from Cameroon. The main objective of this study was to unravel if other haplotypes are distributed in the central, littoral, eastern and southern regions of Cameroon and also in locations bordering Gabon and Equatorial Guinea. For this, we followed molecular approaches with DNA sequencing of the second exon of the *pfcr* gene to identify single nucleotide polymorphisms (SNPs) in 180 *P. falciparum* field isolates sampled in five different locations in

Cameroon (Fig. 5). The chloroquine-resistant *pfcr* CVIET haplotype was most abundant, followed by the wild-type CVMNK haplotype. Five hitherto unreported chloroquine-resistant *pfcr* haplotypes were detected for the first time in Cameroonian *P. falciparum*, including the surprise appearance of the S(agt)VMNT haplotype (Fig. 5). The high observed haplotype diversity of the chloroquine-resistant *pfcr* gene and the appearance of the S(agt)VMNT haplotype are daunting and can be attributed to drug pressure and/or the misuse of chloroquine and/or amodiaquine in Cameroon.

2.5 Mitochondrial genome sequence diversity of Indian *Plasmodium falciparum* Isolates

Mitochondrial genomes of the malaria parasites serve as informative genetic markers for estimating the net genetic diversity in populations of a particular species and establish evolutionary patterns both within and among different species. Several studies in the past have used this approach; however, in Indian species of malaria parasites, no information on the mitochondrial genome sequence diversity has not yet been established. We had very recently designed novel primers to sequence the ~6 kb of the whole mitochondrial genome of the Indian *P. falciparum*. Here, we have

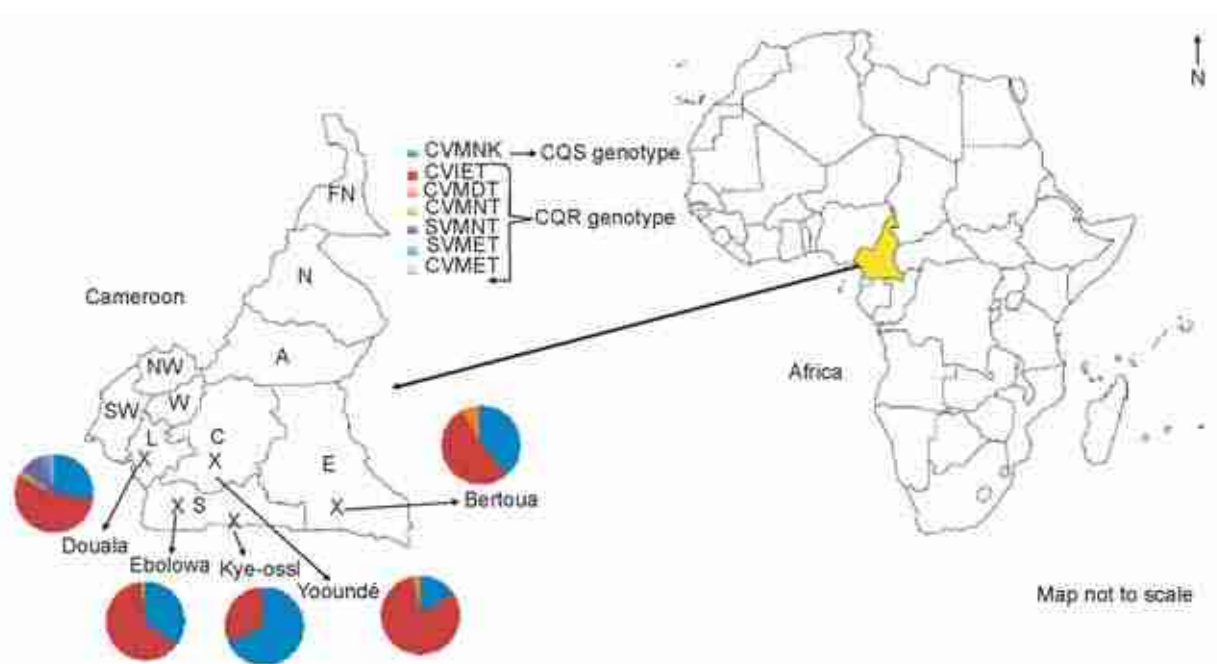


Fig. 5: A map of Africa showing the location of Cameroon, and a map of Cameroon showing the location of the different sampling sites. The pie chart against each sampling location indicates the frequencies of each of the seven *pfcr* haplotypes that were found in the respective locations. FN: far north; N: north; A: Adamaoua; C: centre; NW: north-west; W: west; SW: south-west; L: littoral; S: south; E: east; CQS, chloroquine-susceptible; and CQR, chloroquine-resistant.



Fig. 6: Map of India showing the location of collection of samples of *P. falciparum* isolates.

used these primers to sequence the ~6 kilo nucleotide base pair whole mitochondrial (*mt*) genome sequences of four field isolates of the malaria parasite *P. falciparum* collected from different locations in India (Fig. 6). Comparative genomic analyses of *mt* genome sequences revealed three novel India-specific single nucleotide polymorphisms (SNPs) (Table 1). In general, high *mt* genome diversity was found in Indian *P. falciparum*, which is comparable to African isolates. Population phylogenetic tree placed the presently sequenced Indian *P. falciparum* with the global isolates, while a previously-sequenced Indian isolate was an outlier

Table 1. Nucleotide sequence alignment of the whole mitochondrial genome sequence of the four Indian *P. falciparum* isolates (excluding the previously reported PfPH10 isolate) with the reference sequence of the 3D7 isolate

Isolates	Nucleotide positions				
	276	725	2175	2763	4952
3D7	G	C	T	C	T
Blsp1	-	-	-	-	C
Bet12*	A	T	-	T	C
Goa2*	-	-	C	-	C
Mang2*	-	-	C	-	C

*Present study; Only sites showing nucleotide variations in the alignment were shown. To be noted that due to occurrence of four SNPs in four Indian isolates, three haplotypes are formed: GCTC (Blsp1), ATTT (Bet12) and ACCC (Goa2 and Mang2).

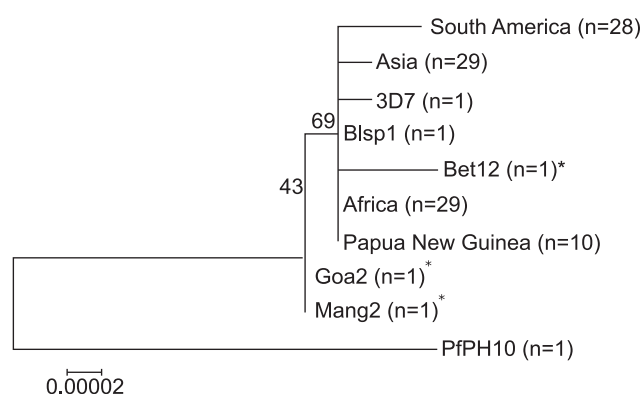


Fig. 7: Neighbour-Joining phylogenetic tree based on whole *mt* genome sequence alignment showing genetic interrelationships among *P. falciparum* isolates from different endemic localities of the globe. *Present study.

(Fig. 7). Although, this preliminary study is limited to a few numbers of isolates, the data have provided fundamental evidences on the *mt* genome diversity and evolutionary relationships of Indian *P. falciparum* with that to global isolates.

2.6 DNA sequence polymorphisms of the *pfmdr1* gene and association of mutations with the *pfcr1* gene in Indian *Plasmodium falciparum* isolates

Mutations in the *P. falciparum* multidrug resistance (*pfmdr1*) gene are known to provide compensatory fitness benefit to the chloroquine (CQ)-resistant malaria parasite and are often associated with specific mutations in the *P. falciparum* CQ resistant transporter (*pfcr1*) gene. Prevalence of the specific mutations in these two genes across different malaria endemic regions was mostly studied. However, reports on mutations in the *pfmdr1* gene and their genetic associations with mutations in the *pfcr1* gene in field parasite isolates are scarce. We have sequenced the 560 nucleotide base pairs of the coding region of the *pfmdr1* gene in 64 *P. falciparum* isolates collected from different malaria endemic populations in India (Fig. 8). Twenty out of these 64 isolates were laboratory cultured with known *in vitro* CQ sensitiveness (10 sensitive and 10 resistant). Three low frequency mutations (two non-synonymous and one synonymous) in the *pfmdr1* gene were segregating in Indian isolates in addition to the predominant Y86 and Y184 ones (Fig. 9), with high haplotype and nucleotide diversity in the field isolates in comparison to the cultured ones. No statistically

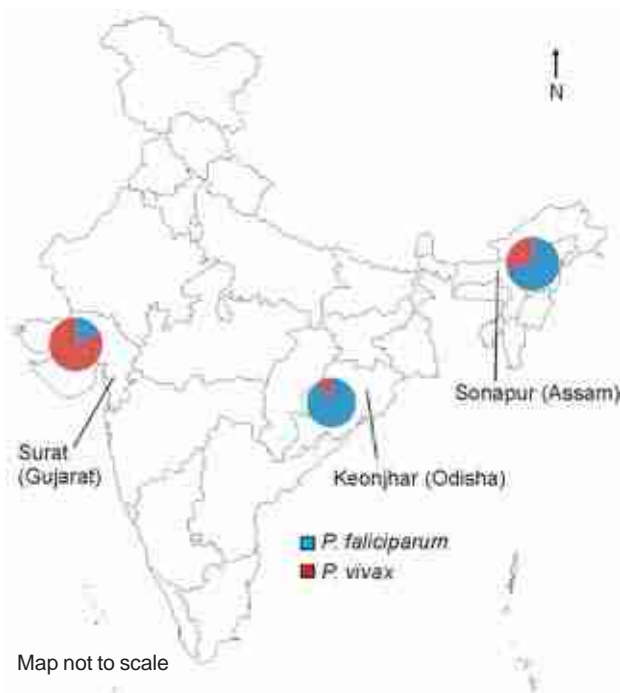


Fig. 8: Map of India showing sampling locations of the *P. falciparum* field isolates. The pie charts depict the average number of *P. falciparum* and *P. vivax* cases reported from respective states over a period of five years (2009–2013; Courtesy: NVBDCP; www.nvbdc.gov.in).

significant genetic association between the mutations in the *pfmdr1* and *pfcr1* gene could be detected (Fig. 10); almost all observed associations were intragenic in nature. Further, there was no sign on the role of natural selection in the evolution of the *pfmdr1* gene in Indian *P. falciparum*. The results on the genetic diversity of the *pfmdr1* gene were discussed in term of evolutionary perspectives in Indian *P. falciparum*, with possible future potential of gaining further insights on this gene in view of evolving malaria parasites resistant to artemisinin derivatives.

2.7 Immuno-modulatory role of mesenchymal stem cells (MSCs) in pathogenesis of malaria infection

Malaria caused by *Plasmodium* spp causes 300 million clinical episodes with approximately one million deaths annually worldwide (WHO). Malaria parasite escapes the immune response before coming out to the blood stage of infection. It is well documented that acquired immunity mounted by host limits the clinical impact of

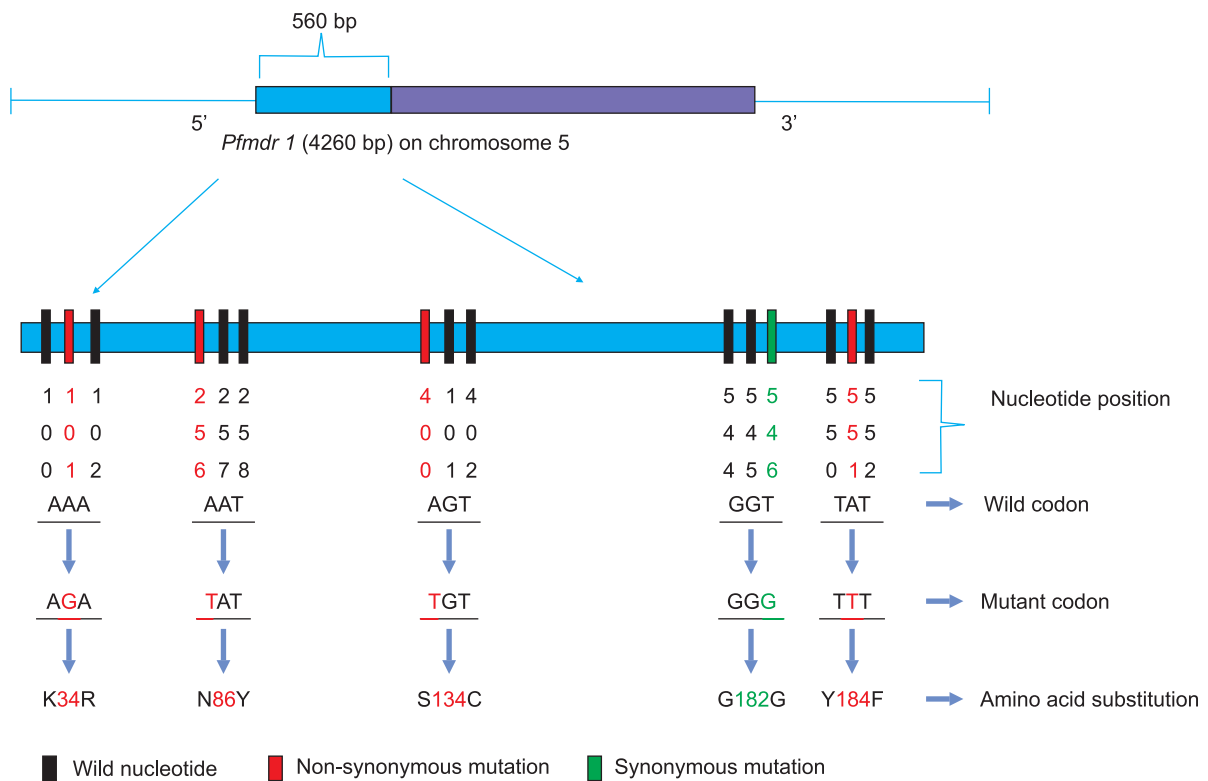


Fig. 9: Schematic representation of the location of the *pfmdr1* gene in the 5th chromosome of *P. falciparum* indicating the coding region sequenced (large light blue horizontal bar), the location of mutations, the non-synonymous mutations with the amino acid position (red) and the synonymous mutation with the amino acid positions (green). Black vertical bars: No nucleotide mutations; Red vertical bars: Non-synonymous mutations; Green vertical bar: Synonymous mutation.

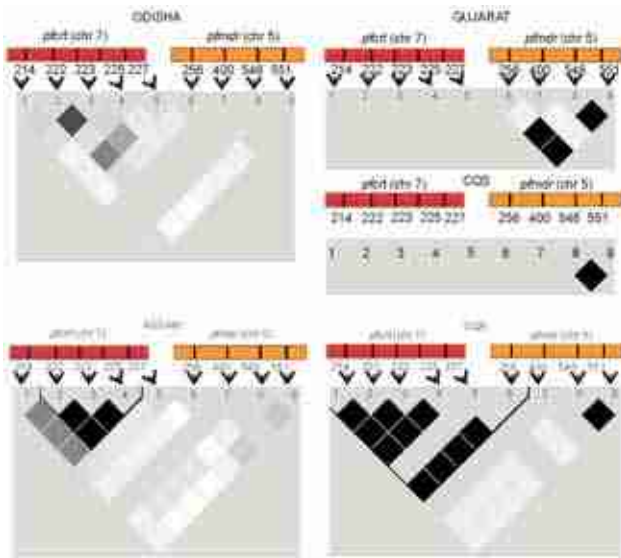


Fig. 10: The extent of linkage disequilibrium (LD) between pairs of mutations in the *pfmdr1* and the *pfcr1* gene in five population samples of Indian *P. falciparum*. The strength of LD as determined by statistical significance of the association as calculated by the r^2 values as depicted by the darkness of the boxes.

infection and provides protection against malaria. Our previous reports suggest that recruitment of MSCs cells during infection and infusion of these cells into naïve mice was able to confer host resistance against malaria infection. MSCs augmented interleukin (IL)-6 productions whereas suppressed IL-10 production in recipient animals indicating that MSCs are host-protective, enhance pro-inflammatory cytokine production, and simultaneously inhibit anti-inflammatory cytokine production, hemozoin production, and Treg-cell accumulation in the spleen. In this context, we found that MSCs that accumulate in the spleen in response to malaria infection produce inflammatory cytokines such as IL-6 and MIP-1 α as it is well known that IL-6 inhibits Treg-cell functions and differentiation; these findings provide a possible explanation for the reduced levels of Treg-cells in the spleen of infected animals. While adoptive transfer of whole splenocytes was able to give 50% protection and to extend the survival (Fig. 11 a–d).

2.8 Microsatellite markers for analysis of population genetic diversity in Indian *Plasmodium vivax*

The genetic characteristics of the malaria parasite population majorly influence the malaria transmission in endemic areas. Genome-wide

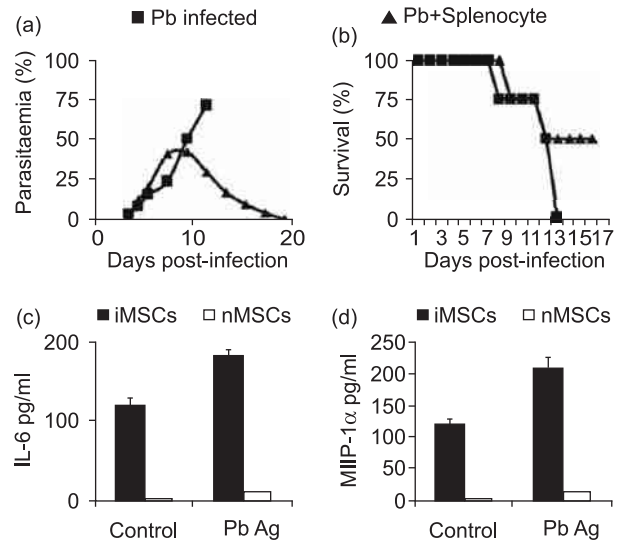


Fig. 11 (a–d): Immune response in *Plasmodium berghei* infected mice. Balb/c mice were infected with 5×10^5 *P. berghei* (Pb) parasitized erythrocytes via intraperitoneal injection and isolated whole splenocytes (5×10^6 /mouse) from Pb infected syngeneic mice were injected into Pb infected mice intravenously. Balb/c mice infected with Pb parasites were divided into two groups. One group served as the Pb infected control group (square bar) whereas the second group of mice received whole splenocytes (Triangular bar). Blood collected from tail was used to prepare smears to determine the parasitic load. Survival of mice that received whole splenocytes vs control cells (a & b). Data shown here are mean \pm SD of 12 mice from three independent experiments. The level of the pro-inflammatory cytokines IL-6 and MIP-1 α in the culture supernatant of MSCs from infected mice and normal MSCs from uninfected mice, stimulated with Pb antigen (50 ng/ml) (c & d). Values are shown as mean \pm SD of triplicate samples from one experiment representative of three experiments performed.

neutral microsatellite (MS) markers were effectively used for assessing such genetic characteristics; the multiplicity of infection (MOI) of malaria parasite infections, genetic diversity, population structure, evolutionary history and to study temporal dynamics of malaria transmission. Microsatellite is typically selected on the basis of being highly polymorphic. However, it is known that the polymorphic nature of each microsatellite can vary as much as two orders of magnitude, which radically represents how diversity changes in the genome from one marker to the next and emphasize on its careful use. We optimized protocols for genotyping highly polymorphic microsatellites sampled from across the genomes of *P. vivax* that have been extensively used in research laboratories worldwide. The study was

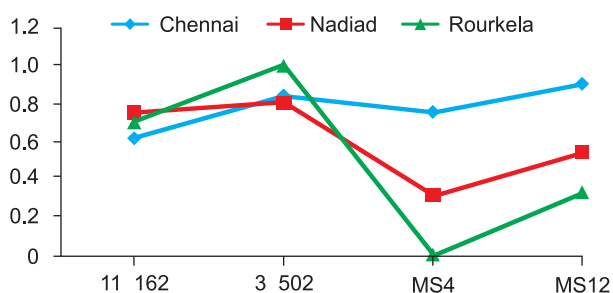


Fig. 12: Number of alleles per microsatellite locus at different study sites.

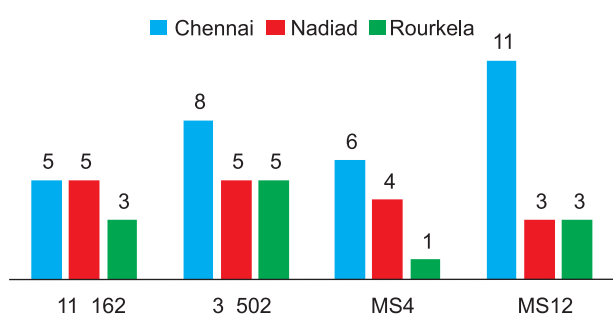


Fig. 13: Expected heterozygosity at microsatellite locus at different study sites.

performed in three different malaria transmission area of India. Till date four MS markers (11_162, 3_502, MS4 and MS12) were optimized for genetic analysis at all three study sites (Chennai; $n=38$, Nadiad; $n=18$, and Rourkela; $n=6$). The level of genetic diversity of the *P. vivax* population in Indian isolates was assessed by number of allele per locus (A) and expected heterozygosity (H_e). The H_e values for each locus were calculated using $H_e = [n/(n-1)] [1-\sum p_i^2]$, where, n corresponds to the number of isolates examined and p_i is the frequency of the i th allele. The allelic data of the four microsatellite loci were obtained from 62 isolates used in the study. In the four loci, the number of alleles (A) for each locus was 1 to 11 (Fig. 12) and the expected heterozygosity (H_e) for each of these loci was 0.0 to 0.90 (Fig. 13). As sample size (n) may affect levels of genetic diversity of living organisms, analysis in more isolates is continued to prepare concrete conclusion.

2.9 The repertoire diversity study of *var* gene family in complicated and uncomplicated *falciparum* malaria

Variable gene family (*var*) encoded protein called as *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1) is highly diverse, multi-copy genes and

have about 60 copies per haploid genome and situated on sub telomeric region of different chromosomes. Disease severity is strongly linked with the expression of *PfEMP-1* gene and different domains of this gene have very specific interaction with the endothelial cell receptors. Degenerate PCR primers have been reported to amplify *dbl- α* domain of *var* gene family and we have used this reported PCR primers, however, the PCR amplification protocol was not found suitable and hence, we re-optimized amplification condition in the laboratory. We have tested better amplification using DNA obtained from *P. falciparum* clinical and field isolate. We have successfully optimized PCR amplification condition for *dbl- α* domain of *var* gene family, which is given in Fig. 14. The samples used for *stevor* study were also used for *var* gene family repertoire diversity study. All 21 samples were successfully amplified for the *dbl- α* domain of *var* gene family. Amplified PCR products were cloned in TA cloning vector and 96 positive clones were sequenced per cloning experiment (Fig. 14 b).

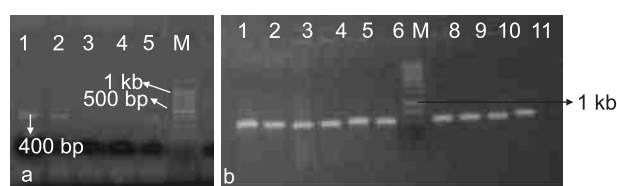


Fig. 14 (a & b): PCR amplification of *dbl- α* domain of *PfEMP-1* gene (*var*) from *P. falciparum* isolates; (a) 1–5: Severe malaria samples, M: 100 bp DNA ladder; and (b) 1–11; Colony PCR, M: 1kb DNA ladder.

A maximum likelihood phylogeny was constructed in order to understand the genetic repertoire of members of the *var* multigene family from complicated and uncomplicated malaria cases. A range of 40–60 unique sequences was obtained from 96 clones sequenced for each isolates. ML phylogenetic tree clearly indicates for distinction between geographical isolates as well as complicated samples (Fig. 15). DNA sequencing of *var* (*dbl- α* domain) gene from six complicated isolates are under analysis. Completion of sequencing of complicated samples work would lead to comprehensive analysis of complicated vs uncomplicated isolates to extend the distinction between geographical isolates to clinical isolates responsible for complicated and uncomplicated malaria phenotypes.

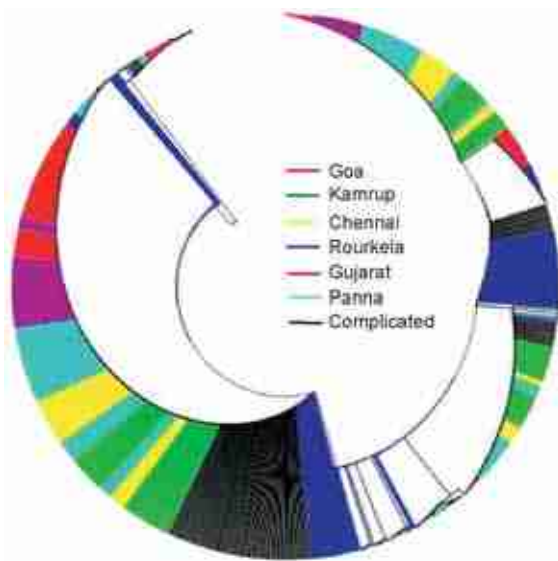


Fig. 15: Maximum likelihood phylogenetic tree showing genetic repertoire of *P. falciparum* var gene family.

2.10 Cysteine proteases as potential drug targets: A mechanism based approach for antimalarial chemotherapy

Cysteine proteases (falcipain-3, falcipain-2 and vivapain-2) play a crucial role in the development of the human malaria parasites, *P. falciparum* and *P. vivax*. Our earlier studies demonstrated that these enzymes are equipped with specific domains for defined functions. Our recent study also suggested the mechanism of activation of cysteine proteases. The sequence of events participated by these proteases are tightly regulated by a new class of endogenous cysteine protease inhibitors known as inhibitors of cysteine protease (ICP). Structural studies of cysteine protease inhibitors, chagasin and

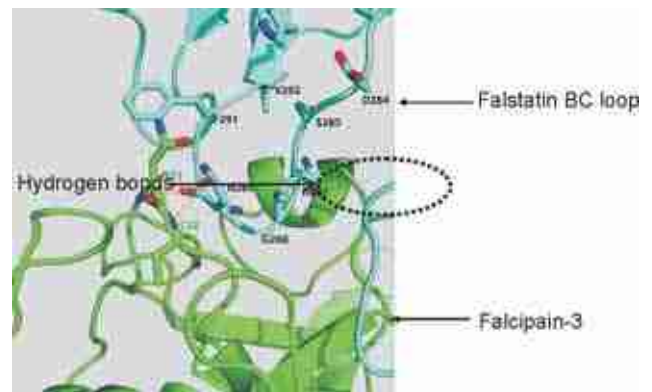


Fig. 17: Models depicting the interactions of BC loop of falstain and falcipain-3 (FP3) via hydrogen bonds [3D model depict interactions of the BC loop peptide of falstain (turquoise) with the active site (green) regions of FP3 via hydrogen bonds (yellow dashes)].

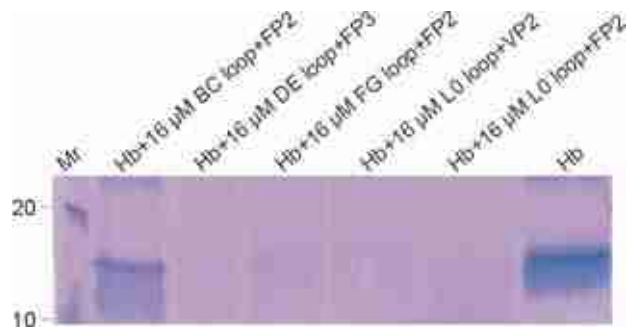


Fig. 18: Only the BC loop appears to play a central role in protease inhibition [Peptides based on the BC, DE, FG and L0 loops of falstain incubated with falcipain-2 (FP2), falcipain-3 (FP3), Vivapain-2 (VP2), and hemoglobin hydrolysis patterns were observed in 15% SDS-PAGE)].

PbICP (cysteine protease inhibitor in *P. berghei*), clearly indicated that three loops (BC, DE, FG) are crucial for binding to target proteases (Fig.16).

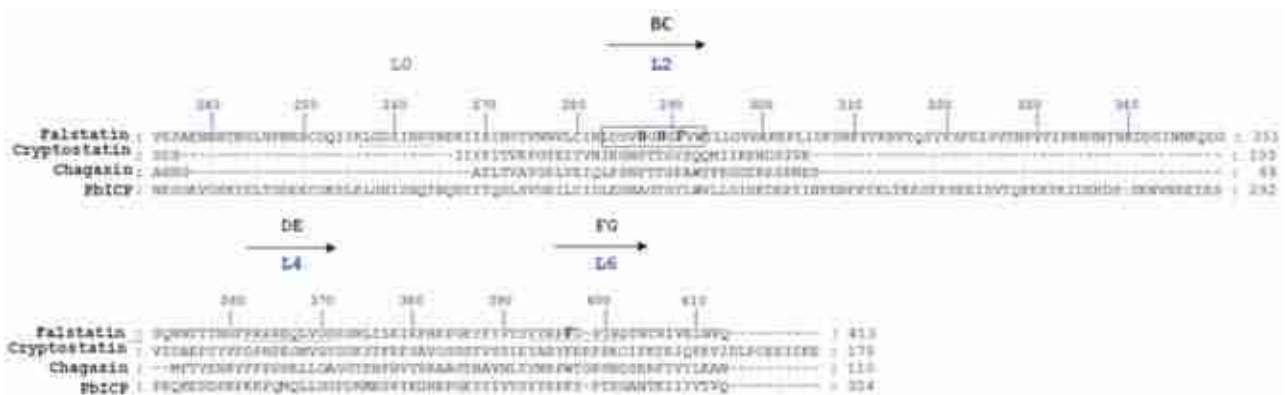


Fig. 16: Alignment of falstain with other ICPs. A multiple sequence alignment was performed with falstain and ICPs from *Cryptosporidium parvum* (cryptostatin), *Trypanosoma cruzi* (chagasin), and *Plasmodium berghei* (PbICP) (This alignment predicted three major loop regions; BC (L2), DE (L4) and FG (L6) in falstain (labelled arrows). A peptide corresponding to the BC loop is indicated, and residues mutated in the described studies are in bold type. There are other loops present in the sequence alignment named L0, L3, and L5).

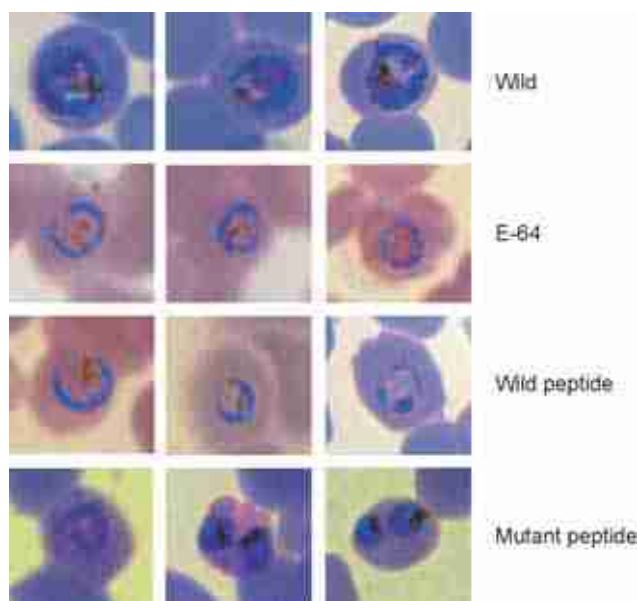


Fig. 19: Morphology of *P. falciparum* treated with wild type and mutant BC peptides [Synchronized ring-stage parasites were incubated with the wild type (LDSVNGNGFVW) and the mutated peptide (LDSVNGAGFVW) (25 μ M) or E-64 (10 μ M), and after incubation parasites were stained with Giemsa. Morphology of parasites was analyzed with two independent cultures of *P. falciparum*].

Falstatin, an endogenous inhibitor of cysteine proteases of *P. falciparum* was previously reported to play a crucial role in erythrocyte and sporozoite invasion. However, the mechanism by which this macromolecular inhibitor inhibits and regulates cysteine proteases is unknown. Our study aimed to answer this question and we have identified a crucial loop as a hot-spot (BC loop or L2 loop), which takes a center stage in the inhibitory function of falstatin (Figs. 17–19). It is noteworthy to mention that falstatin is the first known endogenous inhibitor function as multimeric form. Using site-directed mutagenesis, hemoglobin hydrolysis assay and peptide mediated inhibition studies indicated that only BC loop inhibit cysteine proteases of *P. falciparum* and *P. vivax* via hydrogen bonds. This information is useful in exploring the mechanism of falstatin inhibition, and may be exploited to design small inhibitors based on protein-protein interactions.

□

3.1 Epidemiological studies for establishing immunological correlates of protection against malaria vaccine candidates in high and low transmission malaria endemic regions in India

The malaria vaccine development is in the top priority to successfully control malaria. Currently, a series of projects are under intensive investigation for testing the immunological co-relations and efficacy of the available target vaccine candidates in the laboratory as well as under field conditions. The epidemiological studies for developing correlation with protection for these malaria vaccine candidates have been made mostly in African settings. For developing a globally successful malaria vaccine, it is essential to characterize the immune responses against it in diverse epidemiological settings. India offers diverse malaria epidemiological settings in which immunological responses to these malaria vaccine candidates are poorly understood. Both age and exposure dependent profiling of IgG responses against different malaria vaccine candidates GMZ2 (asexual blood stages), VAR2CSA (pregnancy associated malaria), Pfs48/45 and Pvs48/45 (transmission blocking malaria vaccine candidates from *P. falciparum* and *P. vivax* respectively) would be conducted in cohorts selected in high and low transmission areas in India. First investigators meeting both from India and Denmark was held in Bengaluru and a detailed work plan was made for each project partner. Field visits were made in high (Jharkhand) and low (Haryana) malaria endemic areas and preliminary information was gathered from NVBDCP to short-list PHCs. These PHCs were visited to identify suitable villages based on malaria endemicity and accessibility. Accordingly, some villages under Angada PHC of Ranchi district,

Jharkhand have been tentatively selected from high transmission area, whereas few villages of Ujhina PHC of Mewat district, Haryana representing low endemic area have been selected for immunological studies.

3.2 Control of dengue and chikungunya by controlling *Aedes* breeding in key containers in pre-monsoon season in one of the endemic zone of Delhi

A study was initiated with support from ICMR to map key containers of *Aedes aegypti*, vector of dengue and chikungunya in selected endemic zone of West Delhi and to evaluate the impact of controlling vector population in key containers on amplification of breeding, dengue and chikungunya cases during post-monsoon season.

Based on data from 2007–2013, it was observed that the average container index (CI) of Delhi is about 30%, whereas CI of West Zone of Delhi is 28% [No significant difference between CI of Delhi and CI of West Zone ($p > 0.05$)]. Four teams comprising of two NIMR and 2–3 MCD field workers, carried out surveys in 20 selected localities of West Zone in Delhi to identify *Aedes* breeding and for source reduction respectively. During non-transmission season (December–May) and during transmission season (June–November) fortnightly surveys were carried out for identification, detection and reduction of breeding in key and secondary containers. Cross-checking was done by NVBDCP officials.

Meetings and demonstrations were organized with Councillors, Resident Welfare Associations, Schools, Trade Unions, Local Personnel, etc. under *Jan Jagriti Abhiyan* and various messages were delivered to community. Source reduction was carried out and temephos was used as per WHO



Meeting and demonstration under *Jan Jagriti Abhiyan*.

Source reduction at study sites.

recommendation, i.e. 1 mg/litre and about 70 tanks with broken lids were covered.

During pre-monsoon season maximum CI was observed in tanks (6.08%) followed by coolers (5.09%) and solid waste (3.65%). Whereas, during post-monsoon season maximum CI was found in solid waste (36.92%) followed by tanks (11.84%) and mud pots (1.47%). It was also observed that breeding of vector spread from one habitat, i.e. overhead tanks in January (non-transmission season) to nine habitats, i.e. containers, overhead tanks, solid waste, curing tanks, bird pots, flower pots, cement pits, coolers, and mud pots, whereas in West Zone vector breeding spread from one habitat, i.e. overhead tanks to six habitats, i.e. overhead tanks, coolers, mud pots, containers, curing tanks, and solid waste in September (transmission season) only. Based on these observations, over head tanks were identified as key containers.

As compared to 30% CI of Delhi, 28% CI was observed in West Zone and only 2% in selected localities. Statistically, there is highly significant difference between CI of Delhi and selected localities ($p < 0.05$); and there is highly significant difference

between CI of West Zone and selected localities ($p < 0.05$). Percentage of dengue cases in West Zone also reduced from 13% (2011) to 5% (September 2013). It is noteworthy to mention that no dengue case has been recorded from surveyed localities.

3.3 Health impact assessment of Narmada Basin dams and rehabilitation and resettlement (RR) colonies in M.P.

Health Impact Assessment was initially started in 2004 in three major dam areas in Madhya Pradesh, which was extended further for five years in 2010 to cover entire Narmada Basin. Three study centres were established at Jabalpur, Bhopal and Narmada Nagar for routine survey and laboratory work in the affected areas of Narmada Basin. Pre-monsoon, monsoon and post-monsoon surveys for entomological, parasitological and microbiological parameters were carried out as per WHO guidelines.

Bhopal Unit surveyed 227 villages of five districts under 11 dam projects in Narmada Basin. In cross-sectional survey, 1991 blood slides were examined



Application of temephos in study areas.

from these villages. Total 13 *P. falciparum* and three *P. vivax* cases were detected. Per man hour density of malaria vectors, *An. culicifacies* and *An. fluviatilis* was 0–61 and 0–8 respectively, highest density was recorded at Morand, Dudhi and Shakkar projects area where rocky river pools/pits were supporting breeding of *An. culicifacies*. Of the 44 drinking water samples tested, only eight water samples were tested to be safe for drinking purpose.

Narmada Nagar Unit surveyed 208 villages of six districts under 10 dam projects in Narmada Basin area. In cross-sectional survey, 1717 blood slides were examined from the villages. Total 46 *P. falciparum* and 57 *P. vivax* cases were found. Per man hour density of malaria vectors, *An. culicifacies* and *An. fluviatilis* was 0–53 and 0–13 respectively; and *An. stephensi* was found in the range of 0–4 and *Ae. aegypti* was found in the range of 0–10. Of the 21 drinking water samples tested, none was found suitable for drinking.

Jabalpur Unit surveyed 153 villages of six districts under eight dam projects in Narmada Basin area. In cross-sectional survey, 375 blood slides were examined from the villages (up to September 2012). Total eight *P. falciparum* and nine *P. vivax* cases were found. Per man hour density of malaria vectors, *An. culicifacies* and *An. fluviatilis* ranged from 1–182 and 1–26 respectively, the highest density was recorded at Bargi and Upper Bhudhner project area where rocky river pools/pits were supporting breeding of *An. culicifacies*. Control measures were suggested to NVDA and State Health Department, i.e. dewatering, introduction of larvivorous fishes, channelization of pools in to

main river and larvicide spray to control the breeding. Of the 14 drinking water sample tested, none was found safe for drinking.

Engineering problems found were: dam seepage, damaged canals and blockage with vegetation and stones in the command areas of all the projects. Other domestic problems were mosquito breeding in stagnant pools, cemented tanks, and absence of drainage system, swamps and water logging near hand pumps, in gutter surrounding the houses etc. These result in high breeding potential of vectors and vector borne diseases. Health camps were organized involving Health Department in Narmada Basin area for the awareness on vector borne diseases and their possible control.

Recommendations were submitted to NVDA and State Health Department for necessary action for control of vector borne diseases.

3.4 Studies on health impact assessment of Sardar Sarovar project in command areas of Rajasthan

Narmada Canal Project through which Rajasthan receives water from Sardar Sarovar Dam via the name of Narmada Main Canal was inaugurated in 2008 in Districts Jalore and Barmer. This initiated in 2010 a collaborative project between NIMR and Narmada Canal Project Authority to carry out surveys and suggest control strategies regarding vector borne diseases and water borne diseases (WBDs) in the study areas.

Surveys were carried out in the study villages during pre-monsoon, monsoon and post-monsoon. The breeding of rural vector (*An. culicifacies*), urban vector (*An. stephensi*) and dengue vector (*Aedes aegypti*) was found in these mentioned sites/items. The water was also tested via HiWater™ Test Kit to ensure water free from bacterial contamination, viz. *Salmonella typhimurium*, *Salmonella enteritidis* and *Citrobacter freundii* for its potability in 20 villages.

As part of the mitigating measures, IEC activities were carried out. The release of larvivorous fishes (*Gambusia*) was recommended in pre-monsoon season in 2012 with particular emphasis on diggies, sumpwells, and outlets as the mitigation measures to check the breeding of mosquito larvae and to avoid epidemic of malaria/chikungunya/dengue. After the release of larvivorous fishes in diggies,



Release of larvivorous fishes in a diggie (a); sumpwell (b); discussion and demonstrations to villagers about VBDs (c); and discussion with NCP officials regarding implementation of suitable control strategies (d).

sump wells and outlets, many astonishing results were obtained. Then recommendation of releasing of these fishes was done in excess escape water channels too. This mitigating measure was cost-effective and eco-friendly too. The plastic sheets have also been recommended in the canal margins seepage area to stop seepage. Regular meetings were conducted with NCP officials to discuss about the newer strategies to enforce the best one for the betterment of the ongoing project.

3.5 Evidence-based assessment of biophysical determinants of malaria in the north-eastern states of India and development of framework for adaptation measures for malaria control under climate change scenario

This study is being undertaken in selected districts of Uttarakhand, Assam and Mizoram states

to generate data on biophysical, climatic and socio-economical determinants of malaria to understand the current transmission windows and ecological risk factors of malaria for development of transfer functions and simulation models; to evaluate and strengthen current adaptation measures for control of malaria; to develop projections of potential impact of climate change on seasonal transmission of malaria; and to develop a framework for adaptation measures.

Data on climatic, entomological and parasitological aspects were generated in all the study sites. Over a span of four years, changes in temperature in some months were noticeable in study areas of Almora district up to the tune of 2.69°C. The indoor temperature was more than outdoor and water bodies providing conducive micro niche for mosquitoes. Occurrence of *An. culicifacies* in the study area of Assam up to 11 man hour density (MHD) in the month of April was significant. Sporozoite positivity for *Pv* and *Pf* was found even in the month of January from Upper

Khakhara (Ramgarh) and Kannauj (Ranchi) villages of Jharkhand, at around 17°C mean temperature. Prediction of density of *An. culicifacies* using genetic programming technique was made and validated from observed density. An increase in open transmission months for malaria was projected for the years 2030 and 2070 on the basis of projected climatic conditions of PRECIS model. Indoor temperature of houses made of different material revealed that the temperature of thatched and brick made houses with tiled roof was more during winter months and less in extreme summer than the houses made of cement and mud. Study is near completion except ecological change detection and dissemination of results of study to stakeholders for capacity building and adaptation measures.

3.6 Micro stratification of malaria in problematic districts of Rajasthan for development of strategic action plan for control

The study has been undertaken with the aim of identification of epidemiological, ecological and sociological risk factors of malaria in problematic areas of Jaisalmer, Barmer, Bikaner and Jodhpur districts of Rajasthan so as to develop village level control strategy using satellite data and ground surveys. Work was continued in four sub-centres in Barmer, Bikaner and Jaisalmer districts. Entomological survey of breeding habitats, adult mosquito collection; and fever survey were undertaken twice and spot mapping of study villages was done.

Ecological maps generated through satellite images revealed that village settlements were poorly detected in LISS III resolution (23.8 m). It was found that in low malarious villages, proportion of sandy and stony land use was more than high in malarious area. On the other hand, presence of pond and fallow land was found prevalent in high malarious villages. Village level images generated from IRS LISS IV satellite data for studied villages revealed that villages having proportion of water bodies >0.1% and sandy area <50% were malarious while the villages having water body nil or <0.1% and sandy proportion >50% were non-malarious/least malarious. The accuracy in categorization was 94%. Bore wells and over ground tanks (TANKA) of different

shapes could be detected with Cartosat-1 images. Keeping in view the lack of actual malaria burden and socioeconomic attributes of communities, action plan for remedial measures has been devised.

3.7 Mapping of malaria risk in the context of climate change in India

The study aims at generating risk map of malaria in all states of India from the viewpoint of climatic determinants, malaria prevalence, anopheline vectors distribution and ecological conditions. Mapping of *P. vivax* and *P. falciparum* malaria at district level was done taking average of three years of data. Distribution of *An. culicifacies* and *An. stephensi*, the major rural and urban malaria vectors in India from published records were mapped.

The distribution map of *P. falciparum* demonstrated links with prevalence of moderate temperature (not experiencing winter).

- Distribution of malaria vector highlights knowledge gaps in fauna survey.
- The annual parasite incidence (API) map of India provides an insight for areas suitable for malaria elimination and outbreak prone areas for planning appropriate interventions.

Further work is going on to generate climatic and ecological risk map from the malaria endemicity point of view for India.

3.8 *Anopheles subpictus* as a vector of malaria in India?: Revisiting through climatic and laboratory evidence

This study was carried out to determine the role of *An. subpictus* as a vector of malaria under changing climatic conditions in India. Distribution maps of temperature and relative humidity in India during 1990 were prepared using PRECIS data of A1B scenario. Distribution map of *An. subpictus* mosquito was also prepared based on the available published records. The areas favouring distribution of *An. subpictus* were delineated and it was found that this mosquito species is widely distributed in most parts of India. Another map showing the reported vectorial role of *An. subpictus* in India was also prepared and after correlating it with the distribution maps of temperature and relative humidity. It was noted that malaria infectivity due to this mosquito was found mostly in hot and humid

areas having mean annual temperature $> 25^{\circ}\text{C}$ and mean annual relative humidity $> 60\%$. Last five year's meteorological data (monthly mean maximum temperature, monthly mean minimum temperature, and monthly mean relative humidity) from 2007–2011 for coastal districts of West Bengal and Tamil Nadu were procured from Indian Meteorological Department. Malaria epidemiological data were also collected for 2007–2011 from the corresponding districts. Both of these respective data were analyzed to correlate malaria cases with the climatic conditions. The data revealed that in South 24 Parganas (West Bengal), malaria cases increased with onset of monsoon season. During this period temperature was $28\text{--}30^{\circ}\text{C}$ and relative humidity was $> 80\%$. In Ramanathapuram district (Tamil Nadu), malaria cases were recorded throughout the year but malaria cases were higher when temperature was between 29 and 31°C and relative humidity was $> 70\%$. One field visit was also undertaken to collect *An. subpictus* mosquitoes in South 24 Parganas and Ramanathapuram districts during malaria transmission period. The collected mosquitoes were tested for the positivity of *Plasmodium* species using ELISA technique, but none of the mosquitoes was found positive. Efforts were also made to carry out study on the impact of temperature and relative humidity on sporogonic period of *Plasmodium* sp in *An. subpictus* mosquitoes but the mosquitoes could not be colonized.

Further, experimental research at different temperatures and RH is required to determine the sporogonic development of malaria parasite. Study is completed.

3.9 Baseline household survey in four malaria endemic states under World Bank support

During the period under report, the cross-sectional population survey was carried out with objectives to know the reach of National Malaria Control Programme at the community level, use of bednets, awareness, health seeking behaviour, IRS coverage, etc.

The illustrated guidelines for interviewers and supervisors were prepared and distributed. All questionnaires were translated into local languages of states and got printed. The actual field work of household survey was initiated in the states w.e.f. 1 August 2013. A liaison was developed with state



Photographs showing interaction with the households.

health authorities for seeking their assistance and cooperation for conducting this survey in all four states.

Field workers, i.e. interviewers, mappers and supervisors were selected and trained at district level to undertake surveys and fill up different types of questionnaires. Workers were deputed to various PHCs to carry out household survey in 80 randomly selected villages from each state. The work in selected villages of four states was coordinated and field cross-checked and supervision was carried out to ensure quality. Several field visits were undertaken for conducting household surveys.

The filled up performae got checked, packed and sent to NIMR, New Delhi for data cleaning, data entry and analysis.

3.9.1 Endline Household survey in five malaria endemic states, viz. Odisha, Madhya Pradesh, Chhattisgarh, Andhra Pradesh and Jharkhand

To undertake endline household population survey in five states, with the similar objectives as



Photographs of training for the trainers.

that of baseline survey, training of trainers was organized at NIMR, Dwarka from 15–17 July 2013. The participants from Odisha, Andhra Pradesh, Madhya Pradesh, Chhattisgarh, Jharkhand and Delhi participated in the training. NIMR was

also involved as the faculty along with NIMS and World Bank consultants. Field exercise in a local village was organized on the last day of training for practice.



4.1 Monitoring the therapeutic efficacy of antimalarial medicines in India

Therapeutic efficacy studies of artemisinin-based combination therapy (ACT; AS+SP) in *P. falciparum* and chloroquine (CQ) in *P. vivax* are being conducted at 15 sites in the country (13 for *P. falciparum* and 2 for *P. vivax*) in collaboration with NVBDCP and state health authorities using standard protocols of WHO.

The studies were carried out in different malaria endemic regions. These studies (2012–13) have shown the efficacy of chloroquine for *P. vivax* as 100% at two sites. The PCR corrected cure rate of ACT (AS+SP) for *P. falciparum* ranged from 74.1–100% at 12 sites.

A random 20% samples were analysed for molecular markers of partner drug-resistance. Majority of samples (53.7%) showed *dhfr* double mutation followed by single (20.1%), triple (15.4%) and quadruple mutation (0.7%). Also, majority of the samples showed *dhps* wild pattern (40.5%) followed by triple (22.9%), double (20.9%), single (9.2%) and quadruple (6.5%) mutation. The K76T mutation in chloroquine transporter *pfcr*t was observed in majority of samples (68.4%) followed by wild (12.3%) and mixed type response (9%). The samples from therapeutic efficacy study sites from Odisha and Arunachal Pradesh were further planned to be analyzed for studying artemisinin resistant trait called 'delayed PCT' (parasite clearance time) over a period of three days. This study is designed to monitor parasite clearance time (PCT) for studying artemisinin resistance by two different techniques namely, the 'gold standard-microscopy' and 'a more sensitive technique-real time PCR'. On Day 3 positivity being an important determinant to define artemisinin resistance was determined using both the techniques.

These results were shared with ICMR and NVBDCP which led to change of drug policy for malaria in north-eastern (NE) region by NVBDCP. The current ACT (artesunate+sulfadoxine-pyrimethamine) has been replaced with co-formulated tablet of artemether (20 mg)–lumefantrine (120 mg) [ACT-AL] for the treatment of uncomplicated *P. falciparum* malaria in NE region w.e.f. 30th April 2013. Although, combination therapy is fully effective in other regions of the country, regular monitoring and vigilance is required to detect any development of resistance as per WHO guidelines.

4.2 Effective and safe interventions for prevention of malaria in pregnancy in India: An assessment of burden of malaria in pregnancy, implementability of a screening strategy and barriers to scaling up interventions

The current national policy for the control of malaria in pregnancy (MiP) in India is passive case detection (PCD), i.e. testing for malaria if women report symptoms or signs of malaria. Since April 2012, the London School of Hygiene and Tropical Medicine in collaboration with the National Institute for Malaria Research, is conducting studies of MiP in three districts of Jharkhand state using an operational research approach to assess the effects of intermittent screening and treatment (IST) on the burden of MiP, when delivered during routine antenatal care (ANC).

The biomedical research component consists of a cluster randomized controlled trial with two arms. Women in the PCD arm are tested for malaria only if they complain of signs or symptoms of malaria. Women in the IST arm are screened for malaria

using a rapid diagnostic test (RDT) at each routine ANC visit irrespective of illness history. If positive, they are treated with artesunate+ sulphadoxine-pyremethamine. At delivery, peripheral blood smears and placental biopsy are collected to determine the effect of IST.

The operational research component consists of a one-year pilot implementation of IST within the routine health system in one sub district. This study involves household surveys, structured observations, indepth interviews and focus group discussions for assessing the implementability and scalability of IST within routine ANC.

Stakeholder engagement, regular feedback and updates to District, State and Central Government have been key activities within the research programme. This included an official launch of the project by national and state stakeholders in April 2012. The results of the interim analysis of data of women enrolled and delivered show that IST is beneficial in comparison to PCD.

In addition, routine and improved ANC services will increase ANC coverage and improve disease

surveillance thereby reducing the burden of malaria in pregnancy. However, logistics and training for implementing this strategy will be required before adopting in health system. This will help the national programme by better informed decision making.

4.3 A multicentric trial to detect *in vivo* resistance of *Plasmodium falciparum* to artesunate in patients with uncomplicated malaria

Antimalarial drug resistance is one of the major issues in combating malaria problem throughout the world. Presently, artemisinin based combination therapy (ACT) is recommended by WHO for the treatment of uncomplicated *P. falciparum* malaria. Artemisinin and its derivatives are the class of drugs which rapidly reduce the parasite biomass up to 10^4 fold per 48 h of life cycle. The delay in parasite clearance time (PCT) is the marker trait for artemisinin resistance which is reported to enter the world through Thai-Cambodia border. Through this study we aim to monitor *in vivo* resistance to artesunate by administration of oral artesunate for three days followed by full course treatment of recommended ACT (AL) in north-eastern sites of India.

The study was conducted in NE states of India at Silachari PHC, Gomati district, Tripura and Tlabung subdivisional hospital, Lunglei district, Mizoram. Patients reporting to local clinic were screened for *P. falciparum* infections by either microscopy or RDT or both. Those found positive were enrolled in the study and administered oral artesunate monotherapy for three days. Closely spaced blood sampling was done to monitor PCT and to define the log phase of parasite clearance curve. Blood was also withdrawn for pharmacokinetic studies and *in vitro* analyses of artemisinin susceptibility. After three days of oral artesunate monotherapy, full course of ACT (AL) for three days was administered and patients were followed up to Day 42 to monitor the clinical outcome. Enrolment of patients has been completed at the selected study sites. A total of 47 and 67 patients were enrolled at Tripura and Mizoram study sites respectively.

Northeastern states have remained the epicentre for emergence of antimalarial drug resistance in the past, latest evidence being the one which led to the change in policy in NE states from AS+SP to



Official launch of the research programme.



Pregnant women waiting at ANC clinic, Jharkhand state.

AL. Northeast states being prone to threat of developing artemisinin resistance too, as these share international borders with other countries which have already reported cases of artemisinin resistance. This study will help in monitoring to identify artemisinin resistance in selected study sites of NE states which may help in planning effective containment measures.

4.4 Effect of residual antimalarials in malaria patients enrolled for therapeutic efficacy studies and their effect on spread of drug resistant parasites in high malaria endemic districts in India

Emergence of antimalarial drug resistance is a major problem for the treatment of malaria. Antimalarial drug pressure is one of the major factors for the spread of drug resistant parasite population. The residual drug levels of previous antimalarial treatment exposing newly acquired infection to sub-therapeutic selective drug concentrations have been much debated as a possible source of spread of drug resistant parasites. Post-treatment prophylaxis of long acting antimalarial drugs, self intake of antimalarials, irrational treatment practices by the physicians, mass drug administration of antimalarials, etc. contribute to high drug pressure.

Blood samples were collected for blood smear preparation, PCR (molecular markers) and estimating residual levels of antimalarial drugs on Day 0 from *P. falciparum* infected patients enrolled under therapeutic efficacy study at Bilaspur district, Chhattisgarh, Betul district, Madhya Pradesh, Simdega district, Jharkhand and Sundergarh district, Odisha. Clinical follow-up was done as per WHO guidelines (2009).

A total of 295 samples were collected from Chhattisgarh, Madhya Pradesh, Jharkhand and Odisha. Out of 295 samples, 187 were processed for monitoring residual drug levels. Out of 187 samples, 54 (28.9%) patients had residual antimalarials on Day 0. Residual antimalarial levels namely sulphadoxine (18.2%) and chloroquine (11.2%) were present in *P. falciparum* malaria patients. An inverse-correlation between parasite density and residual levels of sulphadoxine and chloroquine on Day 0 samples of malaria patients was observed. *Pfcr* gene mutation at codon K76T was reported, which is responsible for chloroquine

resistance. Out of 295, 276 samples were analyzed for mutation in *pfcr* gene, 210 (76.1%) samples showed mutant genotype. Mutation analysis in *dhfr* gene was also done in 288 samples collected from different study sites. Results have shown that double mutation (59R+108N) was most prevalent (71.9%). For mutation analysis in *dhps* gene, 288 samples were analyzed, wild type genotype was more prevalent (51%) and triple mutant (26%), double mutant (11.8%) and single mutant (11.1%) were also observed.

Presence of residual antimalarials may be due to previous drug episodes/self intake/irrational treatment/mass drug treatment. Residual levels of antimalarial drug may increase the drug pressure in community and encourage the spread of drug resistant parasites.

4.5 Efficacy of the Parasight P1 device for malaria diagnosis

Microscopy has long been considered to be the gold standard for diagnosis of malaria despite many new tools introduced. But it has many challenges like requirement of trained microscopists and logistic issues. RDTs have revolutionized the diagnosis of malaria, polymerase chain reaction (PCR), and loop mediated isothermal amplification (LAMP) are also useful diagnostics but none is free from challenges. The Parasight P1 device aims to overcome these deficits: the computer-vision-based technology is designed for fast, accurate and cost-effective diagnosis of malaria in blood samples. Most importantly, it can also report parasitaemia levels.

This was a single centre, prospective, non-randomized, and blinded trial. This trial aimed to evaluate the efficacy of the device, as measured by the sensitivity and specificity at different levels of parasitaemia reporting for routine diagnosis for the four common species of malaria. The device was evaluated in 431 consented patients. The sensitivity and specificity of the device were compared against PCR and microscopy. Rapid diagnostic tests were used for initial patient screening. All patients were symptomatic.

Of 431 patients consented to participate, 67 samples were excluded from the study for various reasons, the study was performed with 364 samples that were evaluated by Parasight device, PCR and microscopy. The sensitivity and specificity of Parasight compared to microscopy were found to be 94.4 and 95.7%, respectively. The ability of the

Table 1. Sensitivity and specificity of P1 device vs PCR and microscopy

Comparator	Sensitivity			Specificity		
	%	n/N	95% CI	%	n/N	95% CI
Microscopy	94.4	168/178	90.3–97.1	95.7	178/186	91.7–97.8
PCR	96.5	166/172	92.6–98.3	94.8	182/192	90.6–97.1

N = Total number of patients consented; n = Study performed on the patients.

device to detect parasitaemia was within 50% in 71.3% of cases (Table 1). Moreover, the study showed that the platform also provides high sensitivity in cases of low parasitaemia. Going by the WHO standard of 95% sensitivity for malaria diagnosis, the device had a good sensitivity. Though the device showed good sensitivity and specificity against PCR and microscopy, few questions still remain to be answered. The software version used in this study is not equipped to detect mixed infections.

The Parasight device correctly identified malaria in more than 95% patients. Further, since it can estimate the parasitaemia, it can be a valuable tool in clinical practice as well as research studies.

4.6 Pharmacovigilance of antimalarials in India

The study was initiated in collaboration with AIIMS and NVBDCP. It aimed at evaluating the safety of antimalarials by both passive and active pharmacovigilance. The District Malaria Officers

Table 2. Adverse events by antimalarials administered

S.No.	Drug	No. of forms	Adverse events	No. of events
1.	Quinine	7	Gastritis Itching	4 3
2.	Chloroquine	1799	Loss of appetite Nausea Vomiting Giddiness Gastritis Itching Pain in abdomen	3 27 26 3 1 2 3
3.	Artesunate + SP, other ACT, artemisinin derivatives	4309	Headache Vomiting Jaundice Urticaria Stomatitis Gastritis Anaemia Diarrhoea Dizziness Pain in abdomen Palpitations Nausea	28 5 2 3 2 4 3 3 3 8 2 3
4.	Miscellaneous	131	–	0
Total		6246		140

from the states of Assam, Meghalaya, Arunachal Pradesh, Nagaland, Jharkhand, Odisha, Gujarat, Madhya Pradesh, Chhattisgarh and Karnataka were trained. To improve AER reporting, the component of active pharmacovigilance was also included.

The data on safety of antimalarials were collected by the Medical Officers by filling up of the adverse event reporting forms. Completed forms were sent to the NIMR/AIIMS nodal centre, which verified and validated causality analysis, analyzed data, prepared reports and passed final data to National Pharmacovigilance Cell and National Programme for necessary actions.

Till date, total 6246 filled in AER forms were evaluated. These included forms filled by the medical officers and the forms filled up during various research projects of NIMR. A total of 140 adverse events have been reported in the form of nausea, vomiting, giddiness, gastritis, etc (Table 2). The data generated in about 6500 patients so far suggest that the antimalarials used under NVBDCP are safe and can be continued.

4.7 Quality assurance of malaria rapid diagnostic tests in India

Malaria is one of the most widespread parasitic diseases all over the world. Early diagnosis, followed by prompt and effective treatment is the key to reducing malaria mortality and morbidity. During the past one decade, a number of rapid diagnostic test (RDTs) kits for malaria have been developed, evaluated and validated for improved sensitivity and specificity. They provide quick results, require less skilled persons as compared to microscopic diagnosis. In India, under the National Vector Borne Disease Control Programme (NVBDCP), PfRDTs were used for diagnosis of malaria (only for *Pf*) at peripheral level, where facility for microscopy was not available and risk of *P. falciparum* is relatively high. However, since the year 2013, bivalent RDTs which can detect both *P. vivax* and *P. falciparum* have been introduced. Like other diagnostic tests, various conditions of

Table 3. Pre-dispatch testing of RDTs

S.No.	Year	Batches tested	RDTs tested	Batches giving satisfactory results
1.	2010–11	30	1200	29
2.	2011–12	30	1140	30
3.	2013–14	37	2442	37
Total		97	4782	96

manufacture, transport, storage and the method of use may impair the accuracy of RDTs. Quality assurance (QA) and adequate monitoring of laboratory services at the peripheral level are important links. Therefore, quality assurance programme under NVBDCP is build to do monitoring of RDTs. A quality assurance programme was started by NIMR and NVBDCP jointly in August 2009. Training programmes were conducted for District Programme Officers of 12 states—Assam, Meghalaya, Manipur, Mizoram, Nagaland, Arunachal Pradesh, Odisha, Jharkhand, Chhattisgarh, Karnataka, Madhya Pradesh and Gujarat.

NIMR has been identified as the National Referral Laboratory for the quality assurance of laboratory diagnosis of malaria which includes both microscopy and rapid diagnostic tests. The National Vector Borne Disease Control Programme is the nodal agency. The regional and state referral laboratories were also identified.

The major components of the quality assurance of RDTs for malaria include preparation of quality control (QC) panels, pre-dispatch QC, post-dispatch QC, external quality assurance scheme (EQAS) and internal QC.

The details of the number of batches tested as part of pre-dispatch testing are given in Table 3. Of the 97 batches tested so far, one batch of RDTs was not upto the mark and thus was rejected.

The NIMR has been receiving RDTs from the districts. So far 4126 RDTs have been received by NIMR, the details of which are given in Table 4. Till date, 4782 RDTs were tested as part of pre-dispatch testing. As part of post-dispatch testing, 4126 RDTs were tested with a panel detection score of 95.2%. The data generated so far shows that the malaria RDTs being used in the programme are satisfactory.

4.8 Effective and safe treatment for malaria in pregnancy in India: A randomised controlled trial

Artesunate + sulphadoxine-pyrimethamine (AS + SP) is the first line of treatment for *P. falciparum* malaria in India. The combination has also been recommended for treatment of *P. falciparum* malaria in pregnancy in second and third trimesters. The study compares the safety and efficacy of artesunate + mefloquine and artesunate + sulphadoxine-pyrimethamine for the treatment of *P. falciparum* malaria in pregnancy.

This is a multicentric randomised open labelled clinical trial of AS + SP and AS + MQ. The cases of malaria in pregnancy are detected by active surveillance of a cohort of pregnant women. Cohort is visited fortnightly and screened for malaria infection by a rapid diagnostic test, if they have a history of fever within 48 h. A total of 7064 pregnant women were enrolled in this cohort and 248 pregnant women with malaria were enrolled in the

Table 4. Performance of RDTs reported in different states

State	No. of RDTs	Low positive (200/μl)			High positive (2000/μl)			Overall			Negative
		Negative	Positive	%	Negative	Positive	%	Negative	Positive	%	
Andhra Pradesh	7	0	4	100	0	2	100	0	6	100	1
Arunachal Pradesh	125	3	69	95.83	0	38	100	3	107	97.27	15
Assam	215	11	115	91.27	1	62	98.41	12	177	93.65	26
Chhattisgarh	63	0	36	100	0	18	100	0	54	100	9
Goa	7	1	3	75	0	2	100	1	5	83.33	1
Gujarat	847	11	509	97.88	0	260	100	11	769	98.59	67
Jharkhand	95	0	56	100	0	30	100	0	86	100	9
Madhya Pradesh	638	41	337	89.15	7	185	96.35	48	522	91.58	68
Maharashtra	41	0	23	100	0	13	100	0	36	100	5
Manipur	155	7	83	92.22	2	44	95.65	9	127	93.38	19
Meghalaya	163	6	86	93.48	3	45	93.75	9	131	93.57	23
Mizoram	332	20	168	89.36	3	96	96.97	23	264	91.99	45
Nagaland	358	12	193	94.15	0	103	100	12	296	96.10	50
Odisha	411	16	218	93.16	6	122	95.31	22	340	93.92	49
Total	3457	128	1900	93.69	22	1020	97.89	150	2920	95.11	387

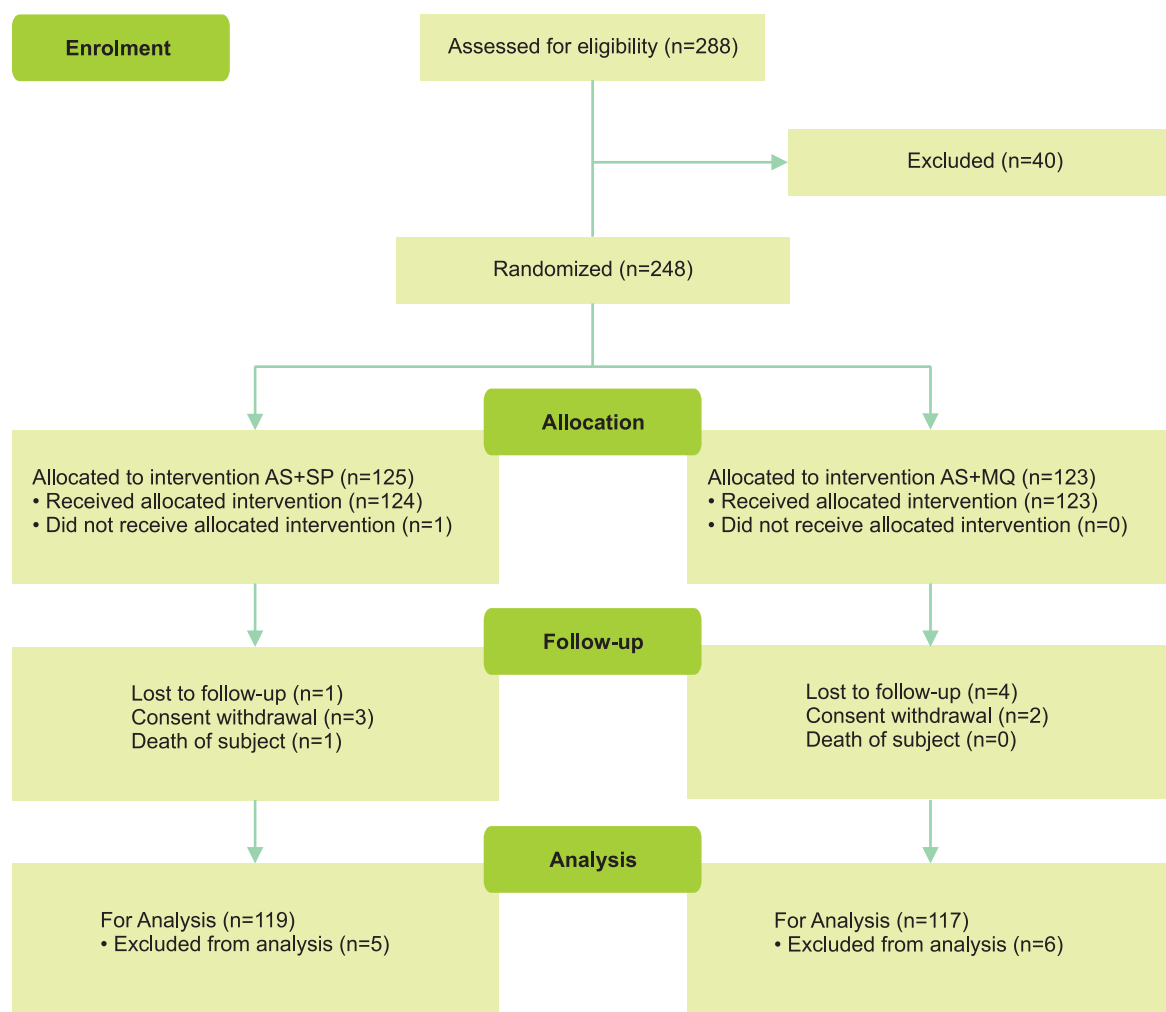


Fig. 1: Trial profile.

trial. Cure rates of both the ACTs were 100%. There were 23 severe adverse events observed during the trial (Fig. 1).

4.9 Malaria Clinic

Malaria clinic provides facility for diagnosis and treatment of malaria on OPD basis. During the year 2013, a total of 3221 fever cases were reported for diagnosis of malaria, out of which 97 were positive for *P. vivax* and six were positive for *P. falciparum*.

All malaria cases were treated as per the national treatment policy. Primaquine was given after testing the G6PD status.

NIMR has been identified as one of the sentinel surveillance sites for the diagnosis of dengue. Fever cases suspected of dengue are tested by using MAC ELISA kits supplied by NIV, Pune. During the year 2013, a total of 1085 fever cases were tested for dengue, out of which 364 cases were found positive. □

5

Highlights of Research Activities under IDVC Project

5.1 Bengaluru (Karnataka)

- Vector biology and bionomics study in the Upper Krishna project area showed that the villages situated on the banks of the river with sandy beds were having high vector abundance throughout the year as compared with non-riverine villages.
- Field trial of lambda-cyhalothrin 10CS (ICON 10CS) in Karnataka showed extended efficacy up to 12 weeks post-spray.
- The larvivorous fish, *Gambusia affinis* and *Poecilia reticulata* were found effective in containing JE vectors in Gorakhpur district (Uttar Pradesh). Here rice fields support vector breeding only during monsoon.
- Field trial of two formulations of alpha-cypermethrin against *An. stephensi* showed superior efficacy of WG formulation over WP formulation on all the surfaces tested.
- Phase-II household surveys were carried out in six districts of Karnataka for understanding the functioning of the programme implementation.
- δ -aminolevulinate synthase (ALAS) is an important enzyme for heme synthesis. This would be an important candidate for drug and vaccine candidate.
- Transcriptome analysis is being done to understand how cerebral malaria occurs in *Pf* cases.
- Two larvivorous fish, *Poecilia reticulata* and *Gambusia affinis* are being used in malaria control in Karnataka and the study has been extended to the northern districts of Karnataka.
- Transmission assessment survey was carried out in Udupi district. ICT results indicated that filariasis transmission in this district is very low and qualify for elimination.
- One dengue and one malaria outbreak

investigations were carried out. Dengue outbreak was investigated in Villages Salundi and Dhalundi, CHC Jaipura, Mysore Taluka in June 2013.

5.2 Chennai (Tamil Nadu)

- *In vivo* studies on the therapeutic efficacy of chloroquine against *P. vivax* malaria were undertaken in Rameswaram Island, Tamil Nadu.
- Studies were undertaken on the impact of new control tools and changing patterns in two PHCs and two zones in Ramanathapuram district and Corporation of Chennai, respectively.
- Assessment of malaria gametocytaemia with duration of symptoms: A potential programme monitoring tool for delay in seeking treatment was carried out among malaria patients attending the clinic.
- Field studies on transmission of *An. stephensi* in potential breeding habitats; recording of micro environmental temperature and humidity of the breeding/resting habitats besides, eco-epidemiology of malaria comprising: (a) community study to determine the incidence/prevalence rate of malaria including asymptomatic malaria; and (b) clinic study to investigate the impact of complex malaria on disease outcome in symptomatic individuals were undertaken as part of the NIH project entitled, 'Centre for the Study of Complex Malaria in India (CSCMi)'.
- Identification of the molecular marker(s) for relapse malaria in *P. vivax* to genotype for differentiation between relapse and re-infection was undertaken.
- Technical support was provided to various institutes/colleges/Govt. agencies and

collaborative research studies were also undertaken with NIMR, New Delhi.

- Malaria clinic continued to function, catering to the needs of the public by providing early diagnosis and prompt treatment.

5.3 Guwahati (Assam)

- Dengue as emerging arboviral infection in northeast India, investigations on seasonal abundance of *Aedes (Stegomyia) albopictus* and *Aedes (Stegomyia) aegypti* in Guwahati metropolis and suburban settlements revealed that both these mosquito species—the implicated vectors are widely abundant in the region and breed in a variety of habitats including discarded tyres, cement tanks, used battery boxes, etc.
- To track emergence of artemisinin resistance, study on therapeutic efficacy of artesunate + SP was undertaken along the Indo-Bangladesh border in Mizoram (Lunglei district) and Tripura (Gomti district). Based on extended follow up study, > 20% treatment failure cases to ACT (AS+SP) were recorded in both the study locations, but there was no evidence of reduced sensitivity to artemisinin, and failure was attributed to SP component. Accordingly, the drug policy has now been changed to artemether + lumefantrine combination in northeast India.
- DDT is being used ever since inception of the malaria control programme in the northeast India. A controlled study of possible adverse effects of DDT on human reproductive health was undertaken with special reference to lactation and pregnancy outcome.
- There is an increasing evidence for significant differences in concentrations of DDT residues between population groups in DDT-sprayed and unsprayed areas.
- With rapid population explosion and associated development projects in north-eastern states of India, the mosquito fauna surveys in varied ecological conditions revealed that populations of *An. minimus* are diminishing, and the niche is being occupied by *An. culicifacies*, the emerging vector resistant to most of the insecticides.
- Other activities included technical inputs to strengthen the malaria control activities

specific to northeastern region, viz. health education and capacity building measures, mass propagation and distribution of larvivorous fish (*Guppy* and *Gambusia*) in town areas, and in providing technical expertise on long-lasting insecticidal nets.

5.4 Hardwar (Uttarakhand)

- A study is being carried out for assessment of the efficiency of ASHA workers in delivery of health services. A questionnaire was prepared and survey has been initiated in Laksar PHC of District Hardwar.
- A Remote Sensing and Geographical Information System (GIS) based approach for mapping, monitoring, prediction of mosquito-genic potential and probable determinants of malaria in District Hardwar of Uttarakhand state: Using LISS IV satellite image of various landscape features and statistics of four malarious and two non-malarious areas data were generated. Spot maps of two villages have been prepared for ground truth verification. Compilation and analysis of data of malarious and non-malarious villages are in progress.
- During a focal outbreak of dengue in Hardwar during 2013, a door-to-door survey was carried out in houses and peridomestic areas in Hardwar and BHEL township to find out breeding sites of dengue vector. During 2013, a total of 1310 cases of dengue were recorded by rapid immuno-chromatographic test kit, of which 401 cases were confirmed by ELISA test by the district authorities. Four deaths due to dengue were reported.
- Field Unit is working on industrial malaria control since 1986 and successfully controlled malaria in BHEL complex. During April 2013 to March 2014, a total of 1641 blood slides were examined, out of which 38 slides were found positive for *P. vivax*, SPR being 2.3. One fish stock of *Poecilia reticulata* has been established in BHEL township.
- Evaluated NetProtect LLIN (impregnated with deltamethrin) against malaria vector in district Saharanpur of Uttar Pradesh. More than 80% mortality was recorded in mosquitoes exposed to NetProtect LLIN washed up to 20 times. Reduction in vector anopheline density was

recorded in all the villages where NetProtect nets were distributed. Community acceptance of NetProtect LLIN was high.

- Technical support to the programme was also provided. Cross-checking of blood slides received from District Hardwar for malaria parasites was performed. Entomological surveillance of dengue vector in District Hardwar was carried out.

5.5 Jabalpur (Madhya Pradesh)

- The assessment of durability of LifeNet in comparison with NetProtect and PermaNet 2.0 LNs/long-lasting insecticide-treated nets was carried out in CHC Kundam of Jabalpur, Madhya Pradesh. Baseline chemical assay for insecticidal activity carried out before distribution showed that the deltamethrin content in all the three LINs are within WHO specification tolerance limits. After distribution of all the three types of nets, the adverse events reported by the net users on week 1 were 17–47% mainly skin itching, facial burning, and eye irritation. Susceptibility tests revealed >97% mortality of *An. culicifacies* against deltamethrin 0.5%.
- The study on evaluation of the effectiveness of intensive intervention measures on malaria prevalence was carried out in two tribal districts, Dindori and Balaghat as translational research project funded by ICMR in collaboration with the Govt. of Madhya Pradesh. While in Dindori there was a sharp decline in malaria prevalence from 2011 onwards and in Balaghat the decreasing trend was observed only in 2013 in villages where Zero Vector Durable Lining (ZVDL) was installed.
- Study on bionomics of malaria vectors was initiated in the month of October 2013 in two Districts of Chhattisgarh state. Korea district showed relatively higher man hour density of *An. fluviatilis* than Bastar. *Anopheles culicifacies* in Bastar district is resistant to DDT, malathion and synthetic pyrethroid while, in Korea *An. culicifacies* is susceptible to alpha-cypermethrin.
- On the request of Govt. of Madhya Pradesh, malaria outbreak investigation was carried out in Katni and Anoopur districts of M.P.
- Two training workshops on malaria and other

vector borne diseases were organized for Medical Officers from various districts of Madhya Pradesh during the year.

5.6 Nadiad (Gujarat)

- The mosquito breeding in containers was found very high in Phase-II districts under the command area of Sardar Sarovar Dam as a result dengue and chikungunya cases have increased.
- A pilot epidemiological study indicated no significant difference between microscopy and PCR. Seasonal transmission, infections possibly are relapsing vivax in summer possibly once in past 12 months.
- Considering persistence, efficacy and impact on the elements of vectorial potential of both formulations, pirimiphos-methyl CS was found more effective than pirimiphos-methyl EC.
- In order to know the current status of intervention coverage and challenges faced by the malaria control programme at community level household surveys in four districts of Gujarat have been completed.
- Assessed disease prevalence and control activities undertaken by VBD teams in different areas. Also organized trainings of medical and nursing students and laboratory technicians.

5.7 Panaji (Goa)

- Studies were carried out on proteome of *An. stephensi* and annotation of genes responsible for these proteins with particular focus on those involved in *Plasmodium* infection. Eight putative proteins were identified from the mid gut of female *An. stephensi*, which are involved in vector-parasite interactions.
- Studies were carried out on the characterization of proteome of *Ae. aegypti*. In all, 944 and 1912 proteins were identified from the salivary gland and mid gut, respectively using in-gel digestion approach. About 628 proteins were found common in both the salivary gland and mid gut. Further, analysis is in progress.
- Under NIH sponsored MESA project, the field unit has initiated studies on seasonal vector abundance and prevalence of *Plasmodium* infection in vectors. Cyclic colony of *An.*

stephensi (type form) has been established for vector competence studies.

- Larvicidal and pupicidal activity of methanol and chloroform extracts of dried leaves of plant code named GIC was evaluated against urban malaria vector *An. stephensi* Liston, filariasis vector *Cx. quinquefasciatus* Say and dengue and chikungunya vector *Ae. aegypti* Linn. with promising results. Fractionation of active compounds is in progress.
- Bioassays with LLINs revealed that up to 21 months, there was absolute mortality in the vectors after washing the nets and after 24 months of usage and 7 washes declined to 73.5% in the laboratory-bred *An. stephensi*, 57.5 and 87.5% in the case of *Cx. quinquefasciatus* and *Ae. aegypti*, respectively. Without washing the LLINs, the percent mortalities remained at 95, 97.5 and 97.5% in *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*, respectively.
- Insecticide susceptibility studies with DDT, malathion and deltamethrin revealed that mortality in *An. stephensi* females was 53.2, 73 and 92% respectively; in *Cx. quinquefasciatus* mortality was 25, 40 and 93%, respectively; in *Ae. aegypti* it was 87.2, 83.7 and 100% respectively; whereas, in *Ae. albopictus* mortality was 15.95% against DDT and 80% against malathion.
- Vector monitoring and evaluation is being carried out in six high risk PHCs/UHCs in Goa and based on which action is being taken to control malaria transmission by the state health services.
- Field Unit undertakes monitoring of vector populations at seaport and international airport in Vasco-da-Gama, Goa as per the International Health in Ports of entry and ground crossings and in 400 m perimeter of these establishments. Reports were submitted for interventions to the Port Health Officer and state health authorities on regular basis.
- Twelve training programmes were organized for medical officers, students, laboratory and field staff.

5.8 Raipur (Chhattisgarh)

- Study on the implications of insecticide resistance on the effectiveness of combination of indoor residual spraying (IRS) and long-

lasting insecticidal nets (LLINs) is being carried out. The study is exploring appropriate strategy to manage insecticide resistance for effective malaria control.

- Monitoring of the epidemiological impact of rotation of insecticides for indoor residual spraying (IRS) in malaria endemic areas was done. The study compared the effectiveness of IRS with DDT on multi-resistance vector population and malaria incidence in comparison to IRS with synthetic pyrethroid.
- Monitoring of resistance in malaria vectors to various insecticides in 11 districts of the state was done to help the state government to plan and rationalize the insecticide spraying strategy.
- Evaluation of a new insecticide molecule chlorfenapyr with novel mode of action against multi-resistant malaria vectors was done to use it as a tool to effectively manage the problem of insecticide resistance and malaria control in tribal forested areas.
- Large-scale, community-based, household randomized trial of long-lasting nets treated with alpha-cypermethrin, deltamethrin and permethrin was carried out.
- Induction and refresher training was organized for the malaria laboratory technicians from various health facilities all over the state.

5.9 Ranchi (Jharkhand)

- Mosquito fauna survey was undertaken with particular reference to anophelines in West Singhbhum district of Jharkhand state.
- Field evaluation of NetProtect LLIN (impregnated with deltamethrin) against malaria vectors and its impact on malaria incidence was completed in Jharkhand state.
- A randomized controlled trial of artesunate + sulphadoxine-pyrimethamine (ASP) vs ASP + primaquine for decreasing malaria transmission was completed in Jharkhand state.
- Filariasis survey was carried out in the tribal population of Santhal Pargana (Dumka, Godda, Pakur, Sahibganj and Jamtara districts) of Jharkhand state.
- MDA assessment as per NVBDCP guidelines for elimination of lymphatic filariasis was conducted in Deoghar and Godda districts of the Jharkhand state.
- Monitoring of insecticide resistance in malaria

vectors was undertaken in different ecotypes of different districts of Jharkhand state and the results were provided to the SPO.

- Technical support was provided to NVBDCP in the areas of malaria microscopic surveillance and capacity building on entomological aspects.
- Diagnostic and treatment services were provided to malaria and filarial patients attending the Field Unit Clinic.

5.10 Rourkela (Odisha)

- The MMV funded Comprehensive Case Management Programme was launched in collaboration with the Government of Odisha, in four districts, namely Bolangir, Dhenkanal, Angul and Kandhamal categorized as low, moderate, high and hyper-endemic to malaria, respectively.
- Studies on eco-epidemiology and transmission of complex malaria were continued in highly endemic *P. falciparum* dominated Sundergarh district of Odisha as a part of multi-centric

study being carried out simultaneously in other centres under NIH funded CSCMi project.

- The Phase-II/III randomized clinical trial on the efficacy and safety of artesunate + sulphadoxine-pyrimethamine and artesunate + mefloquine to treat uncomplicated falciparum malaria in pregnancy was continued.
- Study on the assessment of treatment seeking behaviour, LLIN usage and IRS acceptance by the tribal communities of Odisha was continued.
- Study on the impact of new tools and changing patterns in the control of malaria in Odisha was continued.
- A study on *Aedes* mosquitoes in arboviral epidemic prone areas of Sundergarh district, Odisha was undertaken.
- A study on insecticide susceptibility status of malaria vectors in six western districts of Odisha was undertaken.



6

Research Support Facilities

6.1 Animal House Facility

The animal house facility at NIMR is maintained as per the CPCSEA guidelines. Majorly it maintains small laboratory animals like mice and rabbits for research activities such as screening the antimalarials, parasite maintenance, insectary maintenance, immunological studies, etc. The projects involving the animals are only undertaken after their approval by the Scientific Advisory Committee (SAC) and Institute Animal Ethics Committee (IAEC) of the Institute. The new animal house is under construction and to be completed soon. The animal facility has dedicated technical staff for its smooth functioning.

6.2. Repository of Biological Materials

6.2.1 Mosquito species

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

6.2.2 Malaria Parasite Bank (MPB)

Malaria Parasite Bank at NIMR is functioning as a National Resource with a variety of human and

non-human plasmodia (Table 2). *Plasmodium falciparum* *in vitro* cultivation, characterization of the isolates for susceptibility to different antimalarials; and cryopreservation of isolates adapted to *in vitro* culture and those non-adapted and their revival are routine activities at the Bank.

Collection of biological materials

Till now, a total of 1309 isolates collected in parasite bank, including 940 *P. falciparum*, 364 *P. vivax* and 5 *P. malariae* has been collected (Tables 3–4). Providing malaria parasites to the scientific community has been one of the major activities of the Malaria Parasite Bank.

Supply of biological materials

Till now, 1400 vials/samples of positive sera/plasma and human and non-human malaria parasites have been supplied to 84 Institutes/Universities and Research Organizations.

Resource generation

As per SAC recommendations we have already started charging for the biological materials

Table 1. Details of mosquito species being maintained in the Insectary of NIMR

Species	Strain/Origin	Year of establishment	Isolated from
<i>An. stephensi</i>	Sonepat	Since 2000	Haryana
	Nadiad	2007	Gujarat
	Panjim	2009	Goa
	Alwar	2013	Rajasthan
<i>An. culicifacies</i>	Burari	2013	Delhi
	Rameswaram	2013	Tamil Nadu
	Dehra	2013	Himachal Pradesh
	Dadri	2013	Uttar Pradesh
	Beel Akbarpur	2013	Uttar Pradesh
	Manki	2013	Uttar Pradesh
<i>Cx. quinquefasciatus</i>	Raipur	2013	Chhattisgarh
	RR Permethrin (0.05%)	1999	Mewat (Haryana)
	RR Lambda-cyhalothrin (0.05%)	1999	Mewat (Haryana)
	RR Deltamethrin (0.05%)	1999	Mewat (Haryana)
	RR Malathion (5%)	2000	Mewat (Haryana)

Table 2. Non-human malaria parasites preserved in the Malaria Parasite Bank

Parasite	Species	Susceptibility to antimalarials
Simian malaria	<i>P. cynomolgi bastianelli</i> (CDRI)	Not done
	<i>P. cynomolgi bastianelli</i> (NICD)	Not done
	<i>P. knowlesi</i> (NICD)	Not done
	<i>P. knowlesi</i> (CDRI)	Not done
	<i>P. fragile</i> (CDRI)	Not done
Avian malaria	<i>P. gallinaceum</i>	Not done
	<i>P. relictum</i>	Not done
Rodent malaria	<i>P. berghei</i> (CDRI)	CQ-Resistant
	<i>P. berghei</i>	CQ-Sensitive
	<i>P. berghei</i>	Quinine-Resistant
	<i>P. berghei</i> ANKA	Not done
	<i>P. berghei</i> (NK65) PGI Chandigarh	Not done
	<i>P. chabaudi</i> (Paris)	Not done
	<i>P. yoelii nigeriensis</i> (ICGEB)	Not done
	<i>P. yoelii nigeriensis</i> (CDRI)	Multi-resistant
	<i>P. yoelii nigeriensis</i> (London S.H.T.M.)	Not done
	<i>P. yoelii yoelii</i> (265 By) Paris	Not done

Table 3. Total parasite samples collected year-wise

Year of collection	<i>P. falciparum</i>	<i>P. vivax</i>	<i>P. malariae</i>	Total
1992–2003	601	52	5	658
2004	17	1	—	18
2005	4	6	—	10
2006	59	9	—	68
2007	27	9	—	36
2008	55	88	—	143
2009	9	16	—	25
2010	42	75	—	117
2011	75	47	—	122
2012	11	45	—	56
2013	40	16	—	56
2014	—	—	—	—
Total	940	364	5	1309

supplied from Parasite Bank and till now ₹ 4,24,500/- collected up to 2013.

Manpower development

As per part of manpower development parasite bank is actively involved in imparting training to Scientists/Research Fellows/WHO Fellows/students in *in vitro* cultivation of *P. falciparum* and drug sensitivity testing. A total of 235 students have taken training from Parasite Bank.

Table 4. Details of characterized *P. falciparum* parasites

• Adapted isolates susceptible to chloroquine	54
• Adapted isolates resistant to chloroquine	52
• NF-54, an infective gametocytes producing strain of <i>P. falciparum</i>	1
• 3D 7A : A clone of NF-54	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	110
• Isolates genotyped for virulence genes	74
• Isolates genotyped for <i>msp3α</i> genes	46
• Isolates adapted <i>in vitro</i> producing gametocytes	5
• Isolates characterized for drug resistance genes	47
• Field isolates sequenced for various genes	92

Training facilities available in Malaria Parasite Bank

- 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*.
- *In vitro* testing for sensitivity of *P. falciparum* isolates to antimalarials.
- *In vitro* screening of medicinal plant extracts for antiplasmodial properties.

Cell lines available at Malaria Parasite Bank

- Hepatoma cell line: Hep G2 A16 used in the *in vitro* cultivation of exo-erythrocytic stage of 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 and Information Centre

Library and Information Centre is a resource centre which provides an access to documentation

in the field of malaria and other vector borne diseases. It serves as a bank of information.

The Library and Information Centre of NIMR endeavours to acquire process, organize and disseminate global information to fulfil the information needs of the administrators, policy makers, scientists, research scholars, outside visitors and foreign delegates. The Library and Information Centre uses LIBSYS software package, which consists of modules on acquisition, cataloguing, circulation, serial, OPAC, membership and article indexing. The collection of Library and Information Centre is completely computerized. The Library and Information Centre of NIMR is probably one of the best in India in this field.

Library collections

Books	8500
Bound Journals	4650

Journals (P+O)	29
Newspapers	10
Magazines	19
CD/DVD	30
Reprint documents	3700
Theses	25
Reports (National and International)	115

Special collections

- Census of India Publications
- WHO Publications
- National Survey Reports on Malaria and other Vector Borne Diseases
- NIMR Publications

Library services

- Circulation of Books
- Inter Library Loan



7

Inter-Institutional Collaboration

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

1. Comprehensive case management pilot programme in Odisha in collaboration with Government of Odisha and Medicines for Malaria Venture.
2. Effective and safe interventions for prevention of malaria in pregnancy in India: An assessment of burden of malaria in pregnancy, implementability of a screening strategy and barriers to scaling up interventions in collaboration with London School of Hygiene and Tropical Medicine, London, U.K., TMH Jamshedpur, IGH Rourkela and St. Ursula Mission Hospital, Gumla, Jharkhand.
3. Effective and safe treatment for malaria in pregnancy in India: A randomized controlled trial in collaboration with London School of Hygiene and Tropical Medicine, London, U.K., TMH Jamshedpur, IGH Rourkela and Mahadevi Birla Hospital, Ranchi.
4. Quality assurance of malaria rapid diagnostic tests in India in collaboration with National Vector Borne Disease Control Programme (NVBDCP).
5. Monitoring the therapeutic efficacy of antimalarial medicines in India in collaboration with NVBDCP.
6. Pharmacovigilance of antimalarials in India in collaboration with NVBDCP and AIIMS, New Delhi.
7. Efficacy/Evaluation of the Parasight P1 device for malaria diagnosis in collaboration with Parasight, Israel and Wenlock Hospital, Mangalore.
8. Establishment of WHO recognized, laboratory for quality assurance of malaria RDTs in collaboration with Caritas India and WHO.
9. Monitoring the therapeutic efficacy of antimalarial medicines across international borders of India in collaboration with WHO and State Programme Offices of Tripura, Mizoram and Arunachal Pradesh.
10. Health impact assessment of development project: Impact of Sardar Sarovar Project on vector borne diseases in Gujarat in collaboration with Sardar Sarovar Narmada Nigam, Govt. of Gujarat.
11. A randomised village-scale evaluation to compare the efficacy of pirimiphos-methyl CS (300 g a.i./l) with pirimiphos-methyl EC (500 g a.i./l) by indoor residual spraying for malaria vector control in Gujarat state, India in collaboration with WHOPES (WHO, Geneva).
12. Development of plant-based immersion oil for microscopy in collaboration with Forest Research Institute, Dehradun.
13. Development of botanical insecticide formulation of essential oils extracted from *Lantana camara*, *Valeriana jatamansi* and *Psoralea corylifolia* for the control of mosquitoes in collaboration with Defence Research Laboratory, Tezpur, Assam.
14. Differential expression of salivary proteins between susceptible and insecticide-resistant mosquitoes of *Anopheles stephensi* in collaboration with JALMA, Agra and IMTECH, Chandigarh.
15. Dynamics of gametocytogenesis among *Plasmodium falciparum* isolates from areas of seasonal malaria transmission: Correlation with antimalarial drug resistance in collaboration with LHMC, New Delhi.
16. Deciphering the functional significance of Rab-mediated vesicular trafficking processes in malaria parasites in joint collaboration on malaria research with the Department of

- Biochemistry, Indian Institute of Science, Bengaluru.
17. Exploring the new facets of *Plasmodium* biology to identify potential drug targets in joint collaboration on malaria research with the Department of Biochemistry, Indian Institute of Science, Bengaluru.
 18. Malaria parasite biology: An avenue to discover new drug targets. Study on heme biosynthesis in malaria parasite and development of new antimalarial drug targets in joint collaboration on malaria research with the Department of Biochemistry, Indian Institute of Science, Bengaluru.
 19. Synthesis, pka determination and *in vivo* toxicity of a new promising antimalarial 6-methoxy-5,8-d-(4-amino-1)-quinoline in collaboration with Jamia Hamdard University, Delhi and NIPER, SAS Nagar, Punjab.
 20. Phase-II baseline household survey for malaria in World Bank Project districts of Maharashtra, Gujarat, West Bengal and Karnataka in collaboration with NVBDCP.
 21. Larvivorous fish in vector control in JE prone areas in Gorakhpur district, eastern U.P. in collaboration with National Institute of Virology, Pune.
 22. Spatio-epidemiological analysis of dengue (*Aedes*) in New Delhi in collaboration with Institute of Pasteur, Paris, France.
 23. Comprehensive case management malaria MMV project/ vector infectivity rate in project area of comprehensive malaria case management programme in Odisha in collaboration with Stephan Duparc, Penny Grewal Daumerie, Jaya Banerji and MMV.
 24. Operationalization of larvivorous fish for biological control of mosquito breeding in Guwahati metropolis and suburban settlements in collaboration with State Vector Borne Disease Control Programme, Assam.
 25. Assessment of mass drug administration in districts of Assam in collaboration with State Vector Borne Disease Control Programme, Assam.
 26. Mapping of malaria risk in the context of climate change in India, Mapping of malaria CCP-DST in collaboration with NVBDCP.
 27. Molecular and morphological characterization of members of Minimus group of mosquitoes in northeastern states and bordering areas MMC project in collaboration with DRL/DRDO.
 28. Establishing immunological correlates of protection against malaria vaccine candidates using functional bioassays and proteomic deciphering of host-parasite interactions in joint collaboration with IIM, Jammu; Malaria Group, ICGEB, New Delhi; Department of Biochemistry, IISc, Bengaluru; Bioklone Pvt Ltd., Kanchipuram; CBI&G Statens Serum Institute, Denmark; University of Copenhagen, Copenhagen and Expression Biotechnologies, Horshlm, Denmark.
 29. Accreditation of the laboratory for quality assurance of malaria RDTs in collaboration with NVBDCP.
 30. Generation of prototype lateral flow assay kit using antigen-specific hybridomas to develop rapid diagnostic test for clinical diagnosis of malaria in joint collaboration on malaria research with the Department of Biochemistry, Indian Institute of Science, Bengaluru, Karnataka.
 31. Monitoring the therapeutic efficacy of antimalarial medicines across International borders of India in collaboration with NVBDCP and State Health Authorities.
 32. Large-scale (Phase-III) evaluation of efficacy, fabric integrity and community acceptability of PermaNet[®] 3.0 long-lasting insecticidal nets compared with PermaNet[®] 2.0 in India in collaboration with WHOPEP, WHO, Geneva.
 33. Malaria research training in south India in collaboration with Biological Science Group, Birla Institute of Technology; Deptt. of Biochemistry, Jawaharlal Nehru Centre for Advance Scientific Research, Bengaluru; National Institute of Malaria Research, Bengaluru; LightHouse Polyclinic, Mangalore; Father Muller's Medical College, Mangalore; Wenkick District Hospital, Mangalore and Deptt. of Biochemistry and Molecular Biology, Pennsylvania, and State University College of Medicine, Hershey, U.S.A.
 34. Analysis of *in vivo* transcription of *Plasmodium falciparum* from Indian patients suffering from cerebral malaria and its comparison with that from patients infected with severe malaria (with MOD symptoms) in collaboration with JNCASR, Jakkur, Bengaluru.

35. Proteomic analysis of *Anopheles culicifacies* using high resolution mass spectrometry (LC-MS/MS) in collaboration with Imptech, Chandigarh and Central JALMA, Institute of Leprosy, Agra.
 36. Monitoring residual bio-efficacy of field distributed long-lasting insecticidal nets in malaria endemic districts of Assam in collaboration with the State Health Deptt. of Assam.
 37. Epidemiology of lymphatic filariasis in tribal areas of Jharkhand state in collaboration with State Health Department, State VBDCP, Ranchi, Jharkhand.
 38. Joint monitoring mission on vector borne diseases in collaboration with WHO/WR India.
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8

Human Resource Development

8.1 Ph.D. Programme

NIMR provides facilities for pursuing Ph.D. degrees to the students. The institute is affiliated to the Jiwaji University, Gwalior; Goa University, Goa; Kumaun University, Nainital; and M.D. University, Rohtak. More than 12 students are working for their Ph.D. degree under the supervision of NIMR scientists.

8.2 Ph.D. Awardees

1. Dr Hemlata Srivastava was awarded Ph.D. by Jiwaji University, Gwalior.
2. Dr Naazneen Khan was awarded Ph.D. by Kumaun University, Nainital.
3. Dr B.P. Niranjana Reddy was awarded Ph.D. by Jiwaji University, Gwalior.
4. Dr Sonam Vijay was awarded Ph.D. by Jiwaji University, Gwalior.
5. Dr Manmeet Rawat was awarded Ph.D. by Jiwaji University, Gwalior.

8.3 M.Sc. Projects

This year, more than 16 students of M.Sc. in Life Science/Biotechnology/Bioinformatics successfully completed their projects/dissertations under the supervision of NIMR scientists.

8.4 Training Courses/Workshops organized

NIMR has conducted regular training programmes as under:

- Organized training courses on malaria

surveillance and patient care for three batches of first year students (five in each batch) of Nursing College, Civil Hospital, Nadiad in January–February 2013. The training included active surveillance, blood smear preparation, treatment and patient care. A live demonstration on mosquito life cycle was also organized.

- Two training courses organized on Vector Borne Diseases Consultant Induction in collaboration with NVBDCP from 13 May–21 June 2013, and 29 July – 6 September 2013, respectively.
- A 3-day training for 45 students (Master of Social Works) as interviewers/supervisor and seven NVBDCP consultants was organized at Gujarat Vidhyapeeth, Ahmedabad from 10–12 September 2013 under “Phase-II baseline household surveys project” supported by NVBDCP (World Bank) to carry out household surveys in 80 villages of Gujarat.
- Two training courses were organized (for 22 and 27 participants, respectively) in collaboration with Municipal Corporation of Delhi (MCD) for Laboratory Technicians of SDMC on Skill up-gradation hands on training on Diagnosis of malaria from 20–24 January 2014, and 27–31 January 2014, respectively.
- Organized workshop on Antimalarial drug resistance monitoring in collaboration with NVBDCP from 19–23 March 2014.



9

Research Papers Published (January–December 2013)

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Chapter/Book

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10

Other Activities

10.1 Information Education and Communication

During the antimalaria month (June), prior to monsoon and transmission season, general information about vector borne diseases (VBD) especially malaria and dengue was provided to the residents of Mansarovar Park area of East Delhi. This area was selected for IEC

activities as most by the houses have over head tanks, people use coolers and keep potted plants which are the preferred breeding sites of dengue vector.

Residents were told how to prevent and control mosquito breeding inside the houses and nearby areas.



IEC activities carried out at Mansarovar Park, East Delhi.

10.1.1 Documentation Cell

In Documentation Cell, the following information was collected and compiled.

1. Up to date list of intramural and extramural projects undertaken by NIMR and their status, i.e. whether ongoing or completed, extension period (if any) granted, funding agency and budget was compiled following 33rd Scientific Advisory Committee Meeting's minutes for the year 2013–14 as well as inputs provided by individual principal investigator/co-principal investigator.
2. List of NIMR research publications published in national and international journals was updated.
3. In the service section of documentation cell following information was provided to various Divisions of NIMR, ICMR, NVBDCP/ WHO and other agencies.
 - (i) Updated list of ongoing Intramural/ Extramural projects was provided to Store Division and repository for equipment purchasing purpose.
 - (ii) List of ongoing projects for the year 2008 onwards pertaining to insecticide trials was provided to Environmental Epidemiology Division.
 - (iii) List of ongoing Intramural/Extramural projects for the year 2006 onwards running at Rourkella Field Unit of NIMR was provided to Finance Consultant, IDVC and Vector Biology and Control Division of NIMR.
 - (iv) Research publications of NIMR for the years 2008–13 were provided to Environmental Epidemiology Division and the last ten years research publications of NIMR related to vector borne diseases were also provided to Dr HG Thakur of NVBDCP/WHO, New Delhi.
 - (v) List of completed and ongoing projects was provided to Dr OP Singh, Scientist 'F', NIMR, New Delhi for preparation of Result-Framework Document (January–March) 2013–14.

10.1.2 Photography

The following photography work was carried out on various occasions/meetings/workshops/trainings/field surveys and award functions organized by NIMR/ICMR/WHO. Photography coverage of *Jan*

Jagriti Abhiyan held from 4–26, April, and 2 May 2013; WHO workshop on capacity strengthening for pesticide specifications held from 10–12 April 2013; Interim SAC meeting of NIMR; Workshop on Bioethics in reference to recent changes in the regulatory scenario in India held on 6 May 2013; Household survey training programme held during July 2013; Hindi workshop organized by NIMR, New Delhi on 6 August 2013; Induction training for District VBD consultants held during July to September 2013; Independence Day celebration & Annual Day celebration of NIMR; *Hindi Pakhwada* held during September 2013; ICMR award ceremony held at India Habitat Centre, New Delhi; Photography of Central Instrumentation Facility of NIMR for SAC meeting; RAC/SAC meetings held during December 2013; Republic Day celebration; and JMM project meetings held during February and March 2014 were also undertaken.

10.2 Publications Division

The Publication Division of the NIMR brought out Institute's scientific quarterly periodical *Journal of Vector Borne Diseases* (JVBD) during the reporting year. The JVBD is already included in Thomson ISI indexing— the abstracting agencies, which award Impact Factor to scientific journals. The articles published in JVBD are being made available full text through PubMed and DOAJ. The Division is also bringing out a popular Hindi Magazine *Malaria Patrika*, quarterly for educating the community on malaria and other vector borne diseases, and the *Plasmodium*—a biannual newsletter of the Institute. Besides above, the Division also undertook the publication of NIMR and IDVC Annual Reports 2012–13 during the reporting year.

10.3 Workshops/Seminars/Conferences/ Training Courses/Important Meetings attended

Anvikar Anup

1. Attended leadership course on Science of eradication: Malaria at Barcelona, Spain from 5–10 May 2013.
2. Attended advanced course on Managing the end of malaria held at Sitges, Spain from 11–13 May 2013.

3. Attended Malaria in pregnancy consortium annual meeting at Durban, South Africa from 3–5 October 2013.
4. Attended the Multilateral initiative on malaria (MiM) meeting at Durban, South Africa from 6–10 October 2013.
5. Attended meeting on Collaborative projects at Desert Medicine Research Centre, Jodhpur on 27 October 2013.
6. Attended 2nd meeting of the Writing Committee for the Development of the global strategic action plan on *Plasmodium vivax* control and elimination at World Health Organization, Geneva, Switzerland from 4–6 November 2013.
7. Delivered talk on Epidemiology and control of malaria at National Congress of the Indian Association of Medical Microbiologists at Hyderabad on 24 November 2013.
8. Attended Stakeholder Consultation on Enhanced harmonization of malaria rapid diagnostic tests at Antwerp, Belgium from 3–5 December 2013.
9. Delivered series of Training Course Curriculum for Laboratory Technicians at South Delhi Municipal Corporation offer from 27–31 January 2014.
10. Attended meeting to Revise guidelines for diagnosis and treatment of malaria on 12 February 2014.
11. Attended workshop of EIS officers at National Centre for Disease Control, Delhi from 26–28 February 2014.
12. Attended Joint Monitoring Mission meetings held at New Delhi from 1–10 March 2014.
13. Attended meeting to Prepare guidelines for Apex Referral laboratories and Sentinel Hospitals for dengue and chikungunya organized by the National Vector Borne Disease Control Programme at New Delhi on 4 March 2014.
14. Attended workshop on Prevention and control of vector borne disease at Municipal Corporation of Delhi, New Delhi on 15 March 2014.
15. Delivered series of lectures at workshop on Antimalarial resistance monitoring at NIMR, Delhi from 19–23 March 2014.
16. Attended Consultation on delaying artemisinin resistance at New Delhi from 24–25 March 2014.
17. Attended meeting of mentors of EIS officers at US Embassy, New Delhi on 29 March 2014.

Das Aparup

1. Participated in XII International conference on Vector and Vector Borne Diseases entitled “Vector Borne Diseases challenges in 21st century: Their global impacts and strategic management” at Udaipur, Rajasthan from 16–18 September 2013.

Das Jyoti

1. Completed the NIH-web-based training course on “Protecting Human Research Participants” at NIMR, New Delhi on 2 April 2013.
2. Participated in a training on ICH-GCH guidelines at NIMR, New Delhi on 6 May 2013.

Das Ram

1. Participated in training on ICH-GCP guidelines at National Institute of Malaria Research (ICMR), New Delhi on 6 May 2013.
2. Attended workshop on *In situ* hybridization at National JALMA Institute for Leprosy and other Mycobacterial Diseases (ICMR), Agra from 14–18 October 2013.

Dev Vas

1. Participated in a brain-storming conference on Dengue scenario in India: Disease burden, surveillance and control held at Madurai from 25–26 July 2013, and presented a paper on Dengue, an emerging arbovirus infection in Assam, northeast India.
2. Participated in a workshop on Millennium alliance conference held at Guwahati on 22 October 2013 organized by FICCI/USAID, and for DST-Lockhead Martin, innovation growth programme (by invitation) in the FICCI held at Guwahati on 26 December 2013.
3. Participated in NASI conference held at Goa from 5–7 December 2013 and presented a paper on Epidemiology of malaria transmission in Tripura, northeast India.
4. Participated in an International conference on Entomology held at Punjabi University, Patiala from 21–23 February 2014, and presented a paper on The dominant mosquito vectors of

human malaria in India and also chaired a session on Medical Entomology.

5. Participated in an International conference of tropical medicine and parasitology held at Kuala Lumpur and presented a paper on Seasonal abundance of *Aedes (Stegomyia) albopictus* and *Aedes (Stegomyia) aegypti* at Guwahati metropolis and suburban settlements, northeast India.
6. Participated in a workshop on Delaying artemisinin resistance in India held at New Delhi from 24–25 March 2014 jointly organized by NIMR and Public Health Foundation of India (PHFI).

Dhiman RC

1. Delivered an invited lecture at Training workshop on Climate change and WASH jointly organised by the National Institute of Administrative Research (NIAR) and Lal Bahadur Shastri National Academy of Administration (LBSNAA) at Mussoorie from 26–27 June 2013.
2. Participated as an invited speaker in XII International conference on vectors and vector borne diseases organised by Mohanlal Sukhadia University, Udaipur, Rajasthan from 16–18 September 2013.
3. Participated as invited speaker at the training programme organised by TERI, New Delhi for IAS Officers, and delivered lecture on Impacts and vulnerabilities of health sector in the context of climate change at Delhi on 24 September 2013.
4. Acted as a Temporary Advisor, WHO, Geneva for Planning meeting for operational research on malaria elimination from 17–18 October 2013.
5. Participated in ASEAN meeting to present proposal for ASEAN-India Flagship Programme on Science and Technology combating malaria: A public health challenge at Kuala Lumpur (Malaysia) from 9–10 November 2013.
6. Delivered an invited lecture at the International conference on entomology held at Patiala from 17–18 February 2014.
7. Reviewer of chapter on Human health: Impacts, adaptation and co-benefits (AR5, Working Group II) of Intergovernmental Panel on Climate Change (IPCC), 2014.

Dixit Rajnikant

1. Attended an International conference on Entomology at Punjabi University, Patiala from 21–23 February 2014.
2. Participated in the investigators meeting held at Indian Institute of Science, Bengaluru from 29–30 June 2013.

Eapen Alex

1. Attended and presented research/scientific paper on Advances in *Plasmodium vivax* malaria research at Barcelona, Spain from 28–29 May 2013.
2. Visited Puducherry for discussion with Dr P Jambulingam, Director and Dr Rajavel, Scientist 'D', Vector Control Research Centre (ICMR), Puducherry on the collaborative protocol on Ecology and distribution of *Aedes albopictus* with special reference to *albopictus* subgroup of the subgenus *Stegomyia* in Kerala, India on 13 June 2013.
3. Discussion with Chief Vector Control Officer, Corporation of Chennai regarding malaria cases reported in Chennai for the proposed research proposal on Dynamics of malaria transmission in endemic areas of Chennai-Thiruvottriyur, Tamil Nadu, India on 14 June 2013.
4. Visited Chennai for discussion with Dr Kannan, Additional Director (M&F) and Chief Entomologist, DPH & PM (Govt. of Tamil Nadu), Chennai regarding the proposed *in vivo* study on *P. vivax* malaria in Rameswaram Island on 17 June 2013.
5. Visited Chennai for discussion with Director of Public Health & Preventive Medicine and Chief Entomologist, DPH & PM (Govt. of Tamil Nadu), Chennai regarding the logistics and technical local support for the proposed *in vivo* study on *P. vivax* malaria in Rameswaram Island, Tamil Nadu on 26 July 2013.
6. Discussed with Senior Entomologist (ZET), DPH & PM, Vellore regarding the indigenous malaria scenario of Vellore town for proposed study of Bionomics of malaria vectors in urban areas on 1 August 2013.
7. Attended and presented research findings of the ongoing project on Centre for the Study of Complex Malaria in India at the III Annual SAG meeting of International Centres of

- Excellence in Malaria Research (ICEMR) of NIH at New Delhi, India on 14 August 2013.
8. Attended and presented the research findings of the ongoing project on Centre for the Study of Complex Malaria in India at the III Annual workshop of International Centres of Excellence in Malaria Research (ICEMR) of NIH at Guilin, China from 21–23 August 2013.
 9. Discussed with Entomologists of Zones I and V, Corporation of Chennai regarding malaria cases reported in the respective zones for the proposed research proposal on Dynamics of malaria transmission in endemic areas of Chennai-Thiruvottriyur, Tamil Nadu, India with Corporation of Chennai, Chennai on 3 September 2013.
 10. Attended and presented a research/scientific paper on Challenges for sustainable control of malaria in India in an International conference on Vector borne diseases combat and control at Chennai, India from 22–23 January 2014.
 11. Attended and presented a research/scientific paper on Epidemiology and transmission of perennial malaria in Chennai: A metropolitan city in India in Keystone Symposium on The science of malaria eradication at Merida, Yucatan, Mexico from 2–7 February 2014.
 12. Delivered a lecture to the staff and PG students of the PG and Research Department of Advanced Zoology and Biotechnology, Loyola College, Chennai on 21 February 2014.
 13. Visited Chennai for discussion with HODs, Community Medicine and Internal Medicine, Sri Ramachandra University, Chennai on the proposed project proposal on Multi-site observational study to explore the clinical spectrum, outcome and management of severe malaria in selected tertiary health facilities of India on 7 March 2014.
3. Participated in a state-level meeting with Principal Secretary, Health, Government of Karnataka on Vector borne diseases held at Vikas Sadan, Bengaluru on 9 July 2013.
 4. Attended a video conference on Vector borne diseases at Vikas Sadan, Bengaluru on 5 August 2013.
 5. Attended a workshop on Clinical proteomics at Institute of Bioinformatics, Bengaluru, India from 26–30 August 2013.
 6. Attended XII International conference on vectors and vector borne diseases at Udaipur, Rajasthan, India from 16–18 September 2013.
 7. Attended 62nd Annual meeting of the American Society of Tropical Medicine and Hygiene held at Washington DC from 13–17 November 2013.
 8. Attended a meeting with the Chief Executive Officer, Zilla Parishad, Dakshina Kannada district at Mangalore on 20 March 2014.

Kumar Ashwani

1. Attended Science of Malaria Eradication and Managing the end of malaria advanced courses in Barcelona and Sitges organized by ISGlobal of Barcelona University, Spain from 5–13 May 2013.
2. Attended the Goa State Task Force meeting of NVBDCP, Directorate Health Services, Goa held at Conference Hall, Secretariat, Porvorim on 15 May 2013 under the Chairmanship of Principal Secretary (Health).
3. Invited as Temporary Advisor to Regional Director, SEARO for Inter-country training on Ship sanitation inspection and issuance of ship sanitation certificate organized by WHO, SEARO at Kochi from 24–26 June 2013.
4. Invited as Temporary Advisor for regional meeting on International health regulations (2005): Core capacities at the point of entry, organized by WHO, SEARO at Kochi from 27–28 June 2013.
5. Attended 3rd meeting of Evidence Review Group (MPAC) in WHO HQs at Geneva Switzerland from 8–9 July 2013.
6. Invited to be a Member of Vector Control Advisory Group by the Department of Neglected Tropical Diseases, WHO, Geneva, Switzerland and attended its first meeting from 10–12 July 2013.
7. Attended a brain-storming conference on

Ghosh SK

1. Organized three workshops on Household surveys in Bengaluru, Raichur and Tumkur on 12 August 2013; from 26–29 August 2013, and from 2–3 September 2013, respectively.
2. Organized a workshop on Transmission assessment survey for ELF at Bengaluru from 30 September to 1 October 2013.

Dengue scenario in India: Disease burden, surveillance and control organized by Centre for Research in Medical Entomology (ICMR) at Madurai from 25–26 July 2013 and presented a paper entitled, “Impact of insect growth regulating (IGR) compound, Diflubenzuron 25% WP (BI-Larv) spraying on *Aedines* in Goa, India”.

8. Attended III Annual workshop of International Centres of Excellence in Malaria Research at Guilin, China from 21–23 August 2013.
9. Presented Plenary talk on IVM in malaria elimination: Implementation, evidence and impact assessment at VI Multilateral Initiatives on Malaria (MiM) conference held at Durban, South Africa from 6–10 October 2013.
10. Participated in FICCI meeting on DST-Lockheed Martin, FICCI India innovation growth programme 2014 at Hotel Cidade De Goa, Goa on 23 December 2013.
11. Presented (Invited: Plenary Session) a paper entitled “Impact of insect growth regulating (IGR) compound, Novaluron 10% EC (mosquiron), spraying in Goa, India: Phase-III evaluation of the effectiveness on mosquito vector control in urban settings” in an International Conference on Entomology organized by the Department of Zoology and Environmental Sciences, Punjabi University, Patiala, Punjab, India from 21–23 February 2014.

Mishra AK

1. Attended NIMR RAC and SAC meetings at New Delhi on 26 November 2013.

Mishra Neelima

1. Attended a workshop on Recent changes in the regulatory scenario of India, with respect to serious adverse events, compensation and registration of Ethics Committees at Convention Centre, Jamia Hamdard Deemed University, New Delhi on 10 April 2013.
2. Attended a meeting on Tribal Health Research Forum at VCRC, Puducherry on 15 April 2013.
3. Attended a workshop on Bioethics in reference to recent changes in the regulatory scenario in India at NIMR, New Delhi on 6 May 2013.
4. Attended a meeting with Senior Regional Directors of Assam, Meghalaya and Tripura

states under “A multicentric trial to detect *in vivo* resistance of *Plasmodium falciparum* to artesunate in patients with uncomplicated malaria” at Guwahati, Assam from 13–14 May 2013.

5. Attended a meeting on Tribal Health Research Forum at DMRC, Jodhpur from 9–10 August 2013.
6. Poster presented on Effect of residual anti-malarial drugs on spread of drug resistant parasites in malaria endemic district in India in the XII International Conference on Vector and Vector Borne Diseases entitled, “Vector borne diseases challenges in 21st century: Their global impact and strategic management”, held at Udaipur, Rajasthan from 16–18 September 2013.
7. Poster presented on Qualitative analysis of antimalarials in selected sites of India in XII International Conference on Vector and Vector Borne Diseases entitled “Vector borne diseases challenges in 21st century: Their global impact and strategic management”, held at Udaipur, Rajasthan, India from 16–18 September 2013.
8. Poster presented on Monitoring the levels of sulphadoxine-pyrimethamine on Day 7, better determinant of ACT (AS+SP) efficacy in the XII International Conference on Vector and Vector Borne Diseases entitled, “Vector borne diseases challenges in 21st century: Their global impact and strategic management”, held at Udaipur, Rajasthan from 16–18 September 2013.
9. Poster presented on Studying artemisinin resistance in selected malaria endemic sites of India in the XII International Conference on Vector and Vector Borne Diseases entitled, “Vector borne diseases challenges in 21st century: Their global impact and strategic management”, held at Udaipur, Rajasthan from 16–18 September 2013.
10. Poster presented on Detection of *Plasmodium falciparum* mature gametocytes from filter paper samples in clinical trial using molecular techniques in the XII International Conference on Vector and Vector Borne Diseases entitled, “Vector borne diseases challenges in 21st century: Their global impact and strategic management”, held at Udaipur, Rajasthan from 16–8 September 2013.

11. Attended MiP Consortium Annual Meeting at Durban, South Africa from 3–5 October 2013.
12. Attended Multilateral Initiative on Malaria (MiM) meeting at Durban, South Africa from 6–11 October 2013.
13. Attended meeting for discussion on Proposed research studies including multi-site observational study to explore the clinical spectrum, outcomes and management of severe malaria in selected tertiary health facilities of Rajasthan and other part of country at Jodhpur, Rajasthan from 6–7 December 2013.

Mishra Shobhna

1. Attended training on Next generation sequencing (Ion^{PGM}) given by Life technologies at NIMR, New Delhi from 28–31 October 2013.
2. Attended training on Next generation sequencing data analysis given by Life technologies at NIMR, New Delhi on 9 December 2013.

Nagpal BN

1. Attended training of trainers on Management of DF/DHF/DSS for physicians and pediatricians of sentinel surveillance hospitals organized by SDMC Health Department on 24 May 2013.
2. Delivered lecture on Breeding of *Aedes* mosquitoes during launch of antimalaria/dengue month organized by MCD on 1 June 2013.
3. Delivered lecture on Ecology of important vectors of public health importance in India vector lifecycle, habitats, bionomics, disease transmission, vulnerability and receptivity in 5-days district level training organized by NVBDCP at Government Medical College, Amritsar on 11 June 2013.
4. Delivered lecture on Indoor residual spray and space spray-principles, methods and equipment used for long-lasting insecticidal nets (LLINs) – Principles, merits and demerits in 5-days district level training organized by NVBDCP at Government Medical College, Amritsar on 11 June 2013.
5. Delivered lecture on Principles of vector control (Integrated vector control of diseases)

in a 5-days district level training organized by NVBDCP at Government Medical College, Amritsar on 11 June 2013.

6. Delivered lecture on Entomological/vector surveillance includes insecticide susceptibility in a 5-days district level training organized by NVBDCP at Government Medical College, Amritsar on 12 June 2013.
7. Delivered lecture on Breeding sites of mosquitoes during a meeting organized by NDMC on 19 July 2013.

Nanda Nutan

1. Attended meetings of the Task Force project on Biology and bionomics of vectors, under Vector Borne Disease Science Forum of ICMR held on 20 May and 21 November 2013.
2. Participated as faculty member in an Induction training for district VBD consultants organized by National Institute of Malaria Research, New Delhi in collaboration with NVBDCP from 13 May–21 June 2013; and 29 July–6 September 2013; and delivered lectures on Conducted practicals related to malaria entomology.
3. Participated as faculty member in two training courses on Skill up-gradation/hands on training programme on Diagnosis of malaria for Laboratory Technicians of SDMC, held at NIMR, New Delhi from 20–24 January 2014; and 27–31 January 2014.
4. Participated as faculty member in the workshop on Antimalarial drug resistance monitoring for clinicians/research officers/technical officers organized by National Institute of Malaria Research in collaboration with NVBDCP from 19–23 March 2014; and delivered lecture on Life-cycle and morphology of human malaria parasites.
5. Attended and delivered invited lecture on Species complexes in malaria vectors: Its implication in malaria control in India in a National Symposium on Vector Biology and Vector Management held at Deshbandhu College, University of Delhi on 21 June 2013.

Prajapati SK

1. Attended Molecular characterization of the *Plasmodium vivax vir* multigene family in the Indian populations. Advances in *Plasmodium vivax* malaria research held at Barcelona from 28–29 May 2013.

2. Attended next generation sequencing-bioinformatics and data analysis at AU-KBC Research Centre, Anna University, Chennai from 17–21 September 2013.
3. Participated in the training — Next generation sequencing (Ion^{PGM}) given by Life Technologies at NIMR, New Delhi from 28–31 October 2013.
4. Participated in the training — Next generation sequencing data analysis given by Life Technologies at NIMR, New Delhi on 9 December 2013.

Raghavendra K

1. Nominated to represent the Institute in the Technical Advisory Committee (GoI) meeting on Vector Borne Diseases, Nirman Bhawan Govt. of India, New Delhi on 30 April 2013.
2. Nominated to represent the Institute in the Technical Advisory Committee (GoI) Sub-Committee meeting on Field trial on bio-efficacy of *Bti* 12AS conducted by NIMR at Nirman Bhawan (GoI), New Delhi on 5 March 2013.
3. Participated as Technical Advisor of 16th WHOPES Working Group Meeting on Review of testing/evaluation of pesticides at Geneva, Switzerland from 22–30 July 2013.
4. Attended “Implications of insecticide resistance (IR) National Project Coordinators meeting at Nairobi, Kenya from 27–30 January 2014.
5. Attended the following meetings also:
 - Member expert committee on Insecticides for use in vector control by NVBDCP.
 - Member Secretary for Translational Research Cell of NIMR.
 - Member of the Expert Committee on Clearance of *Bti* technology, ICMR.
 - Member Technical Committee for specifications, Directorate General of Health Services, Government of India.
 - Member of the ICMR sub-committee for revision of Common protocol for evaluating insecticides.

Ravindran K John

1. Discussed with Chief Vector Control Officer, Corporation of Chennai regarding the ongoing studies on Impact of new control tools and

changing patterns in Chennai City from 19–22 April 2013.

2. Discussed with Chief Entomologist, Directorate of Public Health and Preventive Medicine to appraise him on the ongoing studies on Impact of new control tools and changing patterns in Ramanathapuram district, Tamil Nadu on 18 July 2013.
3. Discussed with the Director of Translation, Tamil Development, Religious Endowments, Information Department, Secretariat, Chennai regarding translation of consent forms for the study entitled, “Comparative study on the susceptibility of *Anopheles stephensi* from geographically diverse ecotypes in Tamil Nadu to *Plasmodium* species” on 18 December 2013 and 20 January 2014.

Savargaonkar Deepali

1. Acted as Consultation on Delaying artemisinin resistance in India convened by the Public Health Foundation of India and National Institute of Malaria Research, New Delhi from 24–25 March 2013.
2. Training of trainers on Management of DF/DHF/DSS for physicians and paediatricians of sentinel surveillance hospitals organized by Public Health Department, South Delhi Municipal Corporation on 24 May 2013.
3. Attended Fundamentals of International Clinical Research workshop sponsored by NIAID at Bangkok, Thailand from 15–20 September 2013.
4. Attended training at NIV, Pune on Virus culture and RT-PCR for dengue from 21–25 October 2013.
5. Attended a workshop as a faculty on Antimalarial drug resistance monitoring for clinicians/research officers/technical officers organized by NIMR, New Delhi from 19–23 March 2014.

Sharma SK

1. Participated in the investigators meeting held at Indian Institute of Science, Bengaluru from 29–30 June 2013.
2. Attended the meeting on Technical specifications of insecticides at Nirman Bhawan, New Delhi on 7 January 2014. The meeting was organized by the Ministry of Health & Family Welfare and NVBDCP.

Shukla MM

1. Attended NIMR RAC and SAC meetings at Delhi on 26 November 2013.

Singh Neeru

1. Organized a Tribal Health Research Forum quarterly meeting on 15 April 2013 as Coordinator, THRF at VCRC, Puducherry.
2. Attended a brain-storming meeting under Vector Science Forum at ICMR, New Delhi on 1 May 2013.
3. Attended Malaria Group meeting at NIMR, New Delhi on 3 May 2013.
4. Attended Expert Group meeting on Insecticides at NIMR, New Delhi on 8 May 2013.
5. Attended Task Force meeting to review protocols received under Task Force on Insecticide resistance monitoring in different disease vectors at NIMR, New Delhi on 10 May 2013.
6. Attended 2nd Malaria RTAG meeting as Temporary Adviser to the Regional Director, WHO, SEARO at New Delhi from 14–18 May 2013.
7. Attended a Task Force meeting on Biology and bionomics of malaria and dengue/chikungunya vectors under Vector Science Forum at ICMR, New Delhi on 20 May 2013.
8. Attended 3rd Iron and Malaria Research Review Committee (RRC) meeting at Rockville, Maryland, University of Toronto, Canada from 13–14 June 2013.
9. Delivered a lecture and made discussion on Indo-Canada collaborative project at Rockville, Maryland from 15–18 June 2013.
10. Attended Project Review Committee meeting on Tribal Health Research (ECD) at Bengaluru from 24–25 June 2013.
11. Attended Vector Science Forum meeting at ICMR, New Delhi on 24 July 2013.
12. Attended Project Review Committee Meeting of Tribal Health (ECD) at ICMR, New Delhi on 25 July 2013.
13. Attended 4th Annual Meeting of THRF at DMRC, Jodhpur from 8–11 August 2013.
14. Attended meeting on Regulatory check for issuing commercial licence of Malaria Rapid Diagnostic Tests at Central Drugs Standard Control Organization, Food and Drugs Administrative Bhawan, New Delhi from 9–11 September 2013.
15. Attended meeting on Priority areas of research for malaria, leishmaniasis, filariasis, dengue, chikungunya and Japanese encephalitis under Vector Borne Disease Science Forum at ICMR HQs, New Delhi from 8–10 October 2013.
16. Attended meetings at Geneva, Switzerland: (i) World Health Organization (WHO) Global Technical Strategy (GTS) for Malaria Control and Elimination 2016–25; (ii) Country Typology meeting from 14–15 October 2013; (iii) Roll Back Malaria Partnership Work Plan (PWP) Dialogue meeting on 16 October 2013, and (iv) Operational Research Planning meeting from 17–18 October 2013.
17. Attended PRC meeting of Tribal Sub-plan at ICMR, New Delhi on 5 November 2013.
18. Attended meeting on Task Force on Biology and Bionomics of vectors at MoHFW, Govt. of M.P., NRHM on 21 November 2013.
19. Attended meeting on Operational research to support accelerating malaria elimination in the context of artemisinin resistance falciparum malaria at Bangkok, Thailand from 9–10 December 2013.
20. Attended meeting of Need for regulatory check for issuing commercial license to manufactures of Malaria Rapid Diagnostic Tests at ICMR, New Delhi on 18 December 2013 .
21. Attended Expert Group meeting to review proposals on Insecticide at ICMR, New Delhi on 20 December 2013.
22. Attended brain-storming meeting on Diseases in elimination phase at Tanda, H.P. from 27–28 December 2013.
23. Attended annual review meeting at ICMR HQs, New Delhi to review the Progress of the VDLs at RMRIMS, Patna; RIMS, Ranchi; KIPM, Chennai; SMS, Jaipur and RMRC, Jabalpur on 28 January 2014 and regarding establishment of new laboratory at Raipur and field unit visit at District Hospital Korea and Janakpur from 29–30 January 2014.
24. Attended a meeting on Malaria situation in Madhya Pradesh with the Principal Secretary, Ministry of Tribal Welfare, Govt. of M.P., Bhopal on 12 February 2014.
25. Attended Joint Monitoring Mission meeting at WHO, SEARO on Vector borne diseases in India from 1–10 March 2014.

Singh OP

1. Attended the conference on Molecular characterization of the *Plasmodium vivax vir* multigene family in the Indian populations: Advances in *Plasmodium vivax* malaria research held at Barcelona from 28–29 May 2013.

Singh Vineeta

1. Participated in the training on ICH-GCH guidelines at NIMR, New Delhi on 6 May 2013.

Srivastava HC

1. Attended a meeting for Inter-sectoral coordination in vector borne disease control, organized by the Commissionerate of Health at Gandhinagar on 24 April 2013.
2. Facilitated on the topic Invest in future in defeating malaria on World Malaria Day at District Malaria Office, District Panchayat, Kheda on 25 April 2013.
3. Participated as faculty in a workshop organized by the Joint Director, Office Dadra and Nagar Haveli on Prevention and control of malaria and other vector borne diseases for Supervisor and Multi Purpose Health Workers at Dadra and Nagar Haveli from 14–15 May 2013.
4. Participated in a meeting on Intersectoral collaboration for prevention and control of mosquito borne diseases at Ahmedabad Mahanagar Seva Sadan, Ahmedabad on 28 May 2013.
5. Attended Review meeting to discuss issues related with High Court PIL in 2011. The meeting was called by the Commissionerate of Health, at Gandhinagar on 28 May 2013.
6. Delivered lecture on Conquering malaria— with special reference to industrial estates/ township in short-term training programme on Updates in public health for Factory Medical Officers of Gandhinagar, Thaltej and Ahmedabad at Indian Institute of Public Health on 24 June 2013.
7. Attended pre-mission field visit meeting for World Bank Mission in connection with Malaria control project review at the Commissionerate of Health, Gandhinagar on 9 July 2013.
8. Attended 24th NVBDCP sub-committee

meeting to review the VBDC activities in various districts in Gujarat and provided input on IRS and alternative methods for vector control organized by the Additional Director (Health), Gandhinagar on 30 July 2013.

9. Attended review meeting on Vector borne diseases control in Gujarat and presented the results of assessment of mosquito control activities in Ahmedabad Municipal Corporation, at NVBDCP, Regional Office for Health & Family Welfare Ahmedabad on 12 September 2013.

Tiwari SN

1. Attended ELF training programme at Udupi district from 17–20 March 2014.

Valecha Neena

1. Attended Tribal Health Research Forum on Progress of ongoing studies in tribal areas at VCRC, Puducherry on 15 April 2013.
2. Attended Selection Committee meeting as a Member for selection of suitable candidates for award of ICMR-PDF at ICMR HQs, New Delhi on 17 April 2013.
3. Attended Household survey meeting at NVBDCP, Delhi on 22 April 2013.
4. Attended Technical Advisory Committee meeting of Vector Borne Diseases at Nirman Bhawan, New Delhi on 30 April 2013.
5. Attended Expert Group Meeting on Insecticides at NIMR, New Delhi on 8 May 2013.
6. Attended Malaria Regional Technical Advisory Group meeting at WHO, SEARO, New Delhi from 14–16 May 2013.
7. Participated in Malaria Vaccine Development Programme at Sitges, Barcelona, Spain from 26–27 May 2013.
8. Attended Advances in *Plasmodium vivax* malaria research held at Barcelona, Spain from 28–29 May 2013.
9. Attended a workshop on Assessing the *Plasmodium vivax* research agenda: Interdisciplinary workshops held at Barcelona, Spain 30 May 2013.
10. Attended writing committee meeting on Global strategic plan on *Plasmodium vivax* malaria control and elimination at Barcelona, Spain on 31 May 2013.

11. Attended meeting of NIH funded project South Asia ICEMR programme at University of Washington and TAG meeting at Path HQs at Seattle, USA from 9–17 July 2013.
12. Attended Selection Committee meeting for the post of Scientist-E at ICMR HQs, New Delhi on 30 July 2013 .
13. Attended Sub-committee meeting of Technical Advisory Committee of NVBDCP at Nirman Bhawan, New Delhi on 2 August 2013.
14. Attended MESA-ICEMR Scientific Advisory Group meeting at Panaji, Goa from 11–13 August 2013.
15. Attended Technical Advisory Committee meeting under the chairmanship of DGHS at Nirman Bhawan, New Delhi on 19 September 2013.
16. Participated as Temporary Advisor for Malaria Policy Advisory Committee (MPAC) at Geneva, Switzerland from 11–13 September 2013.
17. Participated in the XII International Conference on Vector and vector borne diseases at Mohanlal Sukhadia University, Udaipur from 16–18 September 2013.
18. Attended a meeting on Priority areas of research for malaria and dengue under Vector Science Forum at ICMR HQs, New Delhi from 8–10 October 2013.
19. Attended Expert Scientific Advisory Committee (ESAC) meeting of MMV at Amsterdam, Netherlands from 29 October–1 November 2013.
20. TEG, Technical Consultation meeting on the 3rd edition of the Guidelines for the treatment of malaria at Geneva, Switzerland from 6–8 November 2013.
21. Attended a Technical Committee meeting regarding Procurement of scientific equipments at ICMR HQs, New Delhi on 13 November 2013.
22. Attended a Technical Committee meeting on Good laboratory practices at DST, New Delhi on 28 November 2013.
23. Attended Scientific Advisory Committee (SAC) meeting of VCRC at VCRC, Puducherry from 3–4 December 2013.
24. Acted as Informal Consultation on Operational research to support accelerating malaria elimination in the context of artemisinin resistance falciparum malaria by the Southeast Asian Ministers of Education Organization (SEAMEO), Regional Tropical Medicine and Public Health Network (TROPMED) at Bangkok, Thailand from 9–10 December 2013.
25. Acted as Malaria Stakeholders consultation on Malaria control in India at Le Meridien Hotel, New Delhi on 17 December 2013.
26. Attended a meeting to discuss Regulatory issues for registration of rapid diagnostic tests at Nirman Bhawan, New Delhi on 18 December 2013.
27. Attended Technical Specification Committee meeting of Miltefosine capsule under the chairmanship of DGHS (PH), at Nirman Bhawan, New Delhi on 16 January 2014.
28. Attended a meeting of the committee on Working Group for developing national priority areas in pharmaco-epidemiology at ICMR HQs, New Delhi on 31 January 2014.
29. Attended 25th Scientific Advisory Committee meeting of CRME at CRME, Madurai from 4–5 February 2014.
30. Attended Access to quality medicines and others technologies Task Force meeting at Sydney, Australia from 11–14 March 2014.
31. Attended a workshop on Prevention and control of vector borne diseases at India Habitat Centre, New Delhi on 15 March 2014.
32. Attended Stakeholder consultation meeting— Compensation in case of inquiry/death in clinical trials at ICMR, New Delhi on 20 March 2014.
33. Attended a meeting on Working group of pharmaco-epidemiology network on creation of representative database with a view to provide a framework and road map and advice and guidance for capturing information for pharmaco-epidemiology at ICMR HQs, New Delhi on 21 March 2014.
34. Attended a meeting of three ICMR Institutes (VCRC & NIMR, RMRC) to develop a comprehensive action plan proposal of vector related to malaria transmission, identifying appropriate insecticide for supporting local government to assist malaria control programme in Odisha at RMRC, Bhubaneswar, Odisha from 28–29 March 2014.

By Ph.D. Students

Gupta Purva

1. Attended a conference on Studies on disease pathogenesis in *Plasmodium vivax* and molecular characterization of *P. vivax vir* genes in the Malaria Gordon Research Conference held at Italy from 4–9 August 2013.

Kale Sonal

1. Participated in a training — Next generation sequencing (Ion^{PGM}) given by Life technologies at NIMR, New Delhi from 28–31 October 2013.
2. Participated in a training — Next generation sequencing data analysis given by Life technologies at NIMR, New Delhi on 9 December 2013.

Mallick Prashant Kumar

1. Participated in the training — Next generation sequencing (Ion^{PGM}) given by Life technologies at NIMR, New Delhi from 28–31 October 2013.
2. Participated in the training — Next generation sequencing data analysis given by Life technologies at NIMR, New Delhi on 9 December 2013.

Shalini Sneha

1. Attended a conference on *Plasmodium vivax* chloroquine efficacy studies confirm drug susceptibility in Chennai, India for Advances in *Plasmodium vivax* malaria research held at Cosmo Caixa, Barcelona, Spain from 28–29 May 2013.
2. Attended a workshop on Assessing the *Plasmodium vivax* research agenda: Interdisciplinary workshops, held at Cosmo Caixa, Barcelona, Spain on 30 May 2013.

10.4 Awards and Prizes

Dr Alex Eapen

Awarded with 'The Global Health Travel Award' funded by the Bill and Melinda Gates Foundation



Dr Ashwani Kumar being presented with Bio-Tech International Award for Excellence for the promotion of bio-control of malaria.

to attend the Keystone Symposium on 'The Science of Malaria Eradication' at Merida, Yucatan, Mexico from 2–7 February 2014.

Dr Ashwani Kumar

Awarded Biotech International Award 2013 for excellence in promotion of bio-control of vectors in an International Conference on Vectors organized by the National Academy of Vector Borne Diseases at Udaipur, Rajasthan, India on 18 September 2013 at the hands of Dr VM Katoch, Secretary, DHR and DG, ICMR.

Dr K Raghavendra

Awarded with "Vestergaard Frandsen Award-2013" by National Academy of Vector Borne Diseases for contributions in the field of Insecticide resistance, Vector bionomics and Vector control in XII International Conference on Vector and Vector Borne Diseases, held at Mohanlal Sukhadia University, Udaipur, Rajasthan on 18 September 2013.

Dr Neena Valecha

Honored with Fellowship of the National Academy of Medical Sciences (India) in the NAMSCON held at AIIMS, Jodhpur on 26 October 2013. The research carried out by her was useful in revising the national drug policy for malaria from time-to-time.

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संस्थान में राजभाषा विकास संबंधी गतिविधियाँ

संस्थान में वर्ष 2013-14 के दौरान राजभाषा अधिनियम के अनुपालन के उद्देश्य से राजभाषा हिन्दी के प्रगामी प्रयोग को बढ़ावा देने हेतु कई कदम उठाए गए हैं। जिसके अंतर्गत तिमाही बैठकें, मलेरिया पत्रिका (हिन्दी) का प्रकाशन करने के साथ ही राजभाषा विभाग द्वारा लागू प्रोत्साहन योजनाएं कार्यान्वित की गईं जिनके अन्तर्गत निदेशक महोदया द्वारा लागू की गई अधिक शब्द सीमा की प्रोत्साहन योजना जारी रही एवं संस्थान के प्रवेश स्थल पर एक नवीन अंग्रेजी हिन्दी शब्द एवं सुविचार लिखने का नवीन प्रयास इस वर्ष भी जारी रहा जो कि राजभाषा के प्रति रूचि जागृत करने का प्रयास था।

यही नहीं, वर्ष 2013-14 के दौरान हिन्दी कार्यशालाएं भी आयोजित की गईं, जिनका संचालन हिन्दी अधिकारी डॉ. वंदना शर्मा द्वारा किया गया। जिसके अन्तर्गत दिनांक 5 अप्रैल 2013, 28 मार्च 2014 एवं हिन्दी दिवस के दौरान पूर्णकालिक कार्यशाला आयोजित की गईं। दिनांक 5 अप्रैल 2013 को आयोजित की गई हिन्दी कार्यशाला में अतिथि व्याख्याता के रूप में स्वास्थ्य एवं परिवार कल्याण मंत्रालय के उपनिदेशक श्री आर.एन. शुक्ला को आमंत्रित किया गया। संस्थान की निदेशक महोदया डॉ. नीना वलेचा द्वारा इस कार्यशाला की अध्यक्षता करने के साथ ही सभी अनुभाग अधिकारियों को राजभाषा संबंधी हिन्दी पुस्तकों का वितरण भी माननीय निदेशक महोदया द्वारा इस आशय से किया गया कि वह अपने अधीनस्थों में उन पुस्तकों को वितरित कर उन्हें राजभाषा में कार्य करने हेतु प्रेरित करें। डॉ. शुक्ला ने अपने अनुभव एवं ज्ञान से न केवल कर्मचारियों की आत्मा को झकझोरा वरन् अनेक उदाहरणों के माध्यम से राजभाषा हिन्दी में अपना सरकारी कामकाज करने हेतु प्रेरित किया।

इसके साथ ही प्रतिवर्ष की भांति इस वर्ष भी हिन्दी पखवाड़ा दिनांक 16 से 25 सितम्बर 2013 तक पूर्ण उत्साह के साथ



दिनांक 5 अप्रैल 2013 को आयोजित हिन्दी कार्यशाला



कार्यशाला में व्याख्यान देते हुए डॉ. आर.एन. शुक्ला



हिन्दी कार्यशाला में पुस्तक वितरण करती हुई निदेशक महोदया

मनाया गया, जिसमें कि जहां एक ओर प्रशासन वर्ग के अधिकारियों एवं कर्मचारियों हेतु पूर्णकालिक हिन्दी कार्यशाला का आयोजन था जिसका उद्घाटन डॉ. नीना वलेचा एवं संचालन डॉ. आर.सी. धीमान द्वारा किया गया। वहीं दूसरी महत्वपूर्ण गतिविधि पुरस्कार वितरण समारोह का आयोजन था, किन्तु इसके साथ-साथ निबन्ध प्रतियोगिता, वाद-विवाद (कर्मचारी वर्ग), वाद-विवाद (अधिकारी वर्ग) एवं टिप्पण प्रारूपण प्रतियोगिताओं का आयोजन भी किया गया।

इस पखवाड़े के दौरान उल्लेखित गतिविधियों के अलावा दिनांक 25 सितम्बर 2013 को एक और गतिविधि— पुरस्कार वितरण समारोह का आयोजन किया गया जिसका संचालन हिन्दी अधिकारी डॉ. वंदना शर्मा द्वारा किया गया। इस समारोह में डॉ. (प्रो.) आर.सी. महाजन को मुख्य अतिथि तथा डॉ. शैलेन्द्र कुमार, निदेशक (राजभाषा एवं औषध), स्वास्थ्य एवं परिवार कल्याण मंत्रालय को सम्मानित अतिथि के रूप में आमंत्रित किया गया था। इस समारोह का शुभारंभ मुख्य अतिथि, सम्मानित अतिथि और संस्थान की निदेशक महोदया को पुष्प एवं स्मृति चिन्ह भेंट कर किया गया। स्वागत समारोह के पश्चात् संस्थान के डॉ. रमेश चन्द्र धीमान द्वारा डॉ. (प्रो.) महाजन एवं डॉ. शैलेन्द्र कुमार को शॉल भेंट कर सम्मानित किया गया। इसके बाद संस्थान की निदेशक महोदया ने अपने संबोधन में कहा कि गृह मंत्रालय द्वारा प्रेरणा एवं प्रोत्साहन की नीति द्वारा भी सफलता तभी मिलेगी जब आपके भीतर हिन्दी में काम करने की प्रबल इच्छा शक्ति होगी। जिस प्रकार हम कार्यालय के अन्य नियमों का पालन करते हैं उसी प्रकार राजभाषा नियमों का पालन करना भी हमारा कर्तव्य है।

तत्पश्चात् डॉ. शैलेन्द्र कुमार (सम्मानित अतिथि) ने सभा को संबोधित करते हुए कहा कि वर्तमान समय में हिन्दी तेजी से अपना एक विशेष स्थान बनाते हुए आगे बढ़ रही है। इस संबंध में उन्होंने अपना उदाहरण प्रस्तुत करते हुए बताया कि सिविल सेवा परीक्षा में मेरा माध्यम हिन्दी ही था और मेरा चयन होना दर्शाता है कि हिन्दी के माध्यम से हम ऊंचाइयों को छू सकते हैं। उन्होंने हिन्दी भाषा के सरकारी कामकाज में बढ़ते हुए वर्चस्व स्थापित होने की बात भी कही।

पुरस्कार वितरण के पश्चात् मुख्य अतिथि प्रो. आर.सी. महाजन ने सभा को संबोधित करते हुए संस्थान में आयोजित



संबोधित करते हुए डॉ. (प्रो.) आर.सी. महाजन



संबोधित करती हुई निदेशक महोदया



संबोधित करते हुए डॉ. शैलेन्द्र कुमार

पुरस्कार वितरण समारोह में सभी अधिकारियों एवं कर्मचारियों के उत्साह को देखकर हर्ष जाहिर किया। उन्होंने राजभाषा हिन्दी के प्रयोग संबंधी सवैधानिक प्रावधानों पर प्रकाश डालते हुए सरकारी कामकाज में हिन्दी का अधिक से अधिक प्रयोग करने हेतु प्रेरित किया। अंततः कार्यक्रम का विधिवत् समापन संस्थान के डॉ. रमेश चन्द्र धीमान, वैज्ञानिक

‘एफ’ द्वारा किया गया। यहां यह भी बताना उल्लेखनीय होगा कि संस्थान ही नहीं वरन् संस्थान की क्षेत्रीय इकाइयों में भी राजभाषा कार्यान्वयन के प्रति रूचि जागृत करने के उद्देश्य से हिन्दी दिवस के उपलक्ष में विभिन्न प्रतियोगिताओं का आयोजन किया गया, जिसमें नडियाड, बंगलुरु, गुवाहाटी एवं गोवा मुख्य हैं।

इसके अतिरिक्त दिनांक 28 मार्च 2014 को भी राजभाषा के प्रयोग को बढ़ावा देने के उद्देश्य से एक हिन्दी कार्यशाला आयोजित की गई, जिसका उद्देश्य प्रशासन वर्ग में काम कर रहे अधिकारियों एवं कर्मचारियों में राजभाषा हिन्दी में कार्य करते हुए आने वाली समस्याओं एवं मुश्किलों का समाधान करना था और उन्हें राजभाषा में कार्य करने हेतु प्रेरित करना था। इस कार्यशाला का विषय ‘राजभाषा में काम करना आसान फिर मुश्किल क्यों?’ था। इस विषय पर व्याख्यान हेतु डॉ. महेश चन्द्र गुप्त, पूर्व निदेशक, रेलवे को आमंत्रित किया गया था।

कार्यशाला में सर्वप्रथम संस्थान की हिन्दी अधिकारी डॉ. वंदना शर्मा ने डॉ. महेश चन्द्र गुप्त का स्वागत करते हुए डॉ. आर.सी. धीमान, निदेशक प्रभारी से कार्यशाला के उद्घाटन का अनुरोध किया। डॉ. धीमान ने सभी का स्वागत करते हुए डॉ. महेश चन्द्र गुप्त का परिचय दिया एवं सभी को उनके विचारों, अनुभव एवं ज्ञान का लाभ उठाने हेतु कहा तथा अपने कार्य में आने वाली समस्याओं का समाधान करने की बात कहते हुए कार्यशाला हेतु शुभकामनाएं दीं और डॉ. महेश चन्द्र गुप्त को व्याख्यान हेतु आमंत्रित किया।

डॉ. गुप्त ने हिन्दी भाषा के सरलीकरण के बारे में जानकारी



दिनांक 28 मार्च 2014 को आयोजित हिन्दी कार्यशाला

देते हुए अंग्रेजी एवं हिन्दी शब्दों की तुलनात्मक व्याख्या की। इसके साथ ही उन्होंने कर्मचारियों एवं अधिकारियों को एक विषय देकर अभ्यास करवाया और उनकी जाँच की एवं उसमें सुधार करते हुए कर्मचारियों को राजभाषा में कार्य करने हेतु प्रेरित किया। डॉ. गुप्त के व्याख्यान, प्रश्नोत्तर, अभ्यास एवं अनुवाद की सभी कर्मचारियों ने भूरि-भूरि प्रशंसा की। अन्ततः डॉ. गुप्त के एक घण्टे के संक्षिप्त किन्तु विस्तृत जानकारी वाले व्याख्यान हेतु हिन्दी अधिकारी ने उन्हें धन्यवाद ज्ञापित किया। इस प्रकार यह कहने में कोई अतिशयोक्ति नहीं होगी कि वर्ष 2013-14 के दौरान संस्थान एवं क्षेत्रीय इकाइयों में राजभाषा के प्रयोग को बढ़ावा देने हेतु सृजनात्मक, रचनात्मक एवं व्यावहारिक कार्य एवं कार्यक्रमों के माध्यम से हर संभव प्रयास किया गया। संस्थान एक विज्ञानीय अनुसंधान संस्थान होने के बावजूद राजभाषा नियम, अधिनियमों का अनुपालन करते हुए राजभाषा के प्रयोग को बढ़ावा देने में प्रयासरत है और इसका साक्षात् प्रमाण राजभाषा संबंधी गतिविधियों का उल्लेखित सारांश है।

□

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