Annual Report
2012–13

NIMR

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

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(Indian Council of Medical Research)
Sector 8, Dwarka, New Delhi-110 077
Tel: 91-11-25307103, 25307104; Fax: 91-11-25307111
E-mail: director@mrcindia.org; Website: www.mrcindia.org

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Preface

I am delighted in presenting the Annual Report of the National Institute of Malaria Research for the year 2012–13. During the reported year, the work on Quality Assurance of Malaria Rapid Diagnostic Tests in India in collaboration with National Vector Borne Disease Control Programme and to study the Safety and Efficacy of Artesunate + Mefloquine and Artesunate + Sulphadoxine-Pyrimethamine for the treatment of falciparum malaria in pregnancy was carried out. Studies on parasite biology on aspartic protease are inhibited by NOS donors and activators. This activity may provide a new approach for the mechanism of killing malaria parasites. The Centre for the Study of Complex Malaria was established at NIMR in collaboration with New York University and Pennsylvania State University, with the National Institute of Health, USA.

The project for the Development of plant-based immersion oil for microscopy in collaboration with Forest Research Institute, Dehradun and Development of botanical insecticide formulation of essential oils extracted from *Lantana camara* and *Valeriana jatamansii* and *Psoralea corylifolia* for the control of mosquitoes with Defence Research Laboratory, Tezpur (Asom) is undergoing.

Assessment of the effectiveness of intensive intervention measures on malaria control programme in the tribal district, Balaghat, Madhya Pradesh in collaboration with Regional Medical Research Centre for Tribals, Jabalpur is being conducted. Standardization of molecular and biochemical techniques for characterization of resistance has been initiated and the study is in progress. The strain of *Anopheles stephensi* was sequenced by using voltage-gated sodium channel. Overall emphasis is being laid on research projects with operational and translational approach.

The Institute had received extramural grants from various national and international agencies like National Vector Borne Disease Control Programme, MMV, Geneva, Michigan University (USA), Institut Pasteur, Paris and State Governments of Rajasthan and Gujarat, etc.

On the request of NDMC/MCD, Delhi, NIMR undertook Entomological surveillance for *Aedes* and the breeding habitats mapped. The work on Health Impact Assessment of Narmada Basin Dam and rehabilitation of colonies was undertaken at Jabalpur, Bhopal and Narmada Nagar, and Sardar Sarovar project areas of Narmada canal in Rajasthan. The activities were continued for providing support to the programme. The *Journal of Vector Borne Diseases,* published by the Institute got an impact factor of 1.04 for the reported year.

NIMR organised a workshop on Comprehensive Case Management in Odisha. The outcome of the project is very significant in terms of demonstrating reduction in malaria with management using usual resources with better supervision. The Institute continued the activities of human resource development by imparting trainings to various health

personnel, district programme officers, VBD consultants, laboratory technicians, guiding research scholars, and M.Sc. students etc.

I take this opportunity to thank all the scientists and staff for their valuable support in all the activities. We are also grateful to the Secretary, Department of Health Research and the Director General, Indian Council of Medical Research for his continued support and encouragement. I also thank the Annual Report Committee, and the Publication Division of NIMR for bringing out this report.

Neena Valecha
Director

Executive Summary

Vector Biology & Control

- Studies on ecological succession of anophelines in north-eastern states reported the prevalence of *Anopheles fluviatilis, An. vagus, An. subpictus, An. nigerrimus* and *An. nivipes* in Chandel district of Manipur, and *An. subpictus, An. kochi, An. jamesii, An. d'thali* and *An. nivipes* in Imphal East district of Manipur for the first time. In Sikkim (East, West, North & South Sikkim districts), the previously reported species, namely *An. vagus, An. culicifacies* and *An. maculatus* were still prevalent and could be collected in the present survey. The species which were recorded for the first time in East, West, North and South Sikkim were *An. pseudowillmori* and *An. nigerrimus*.
- Information generated on the distribution and biological attributes (in terms of resting and feeding behaviour, response to insecticides and malaria transmission potential) of the members of *Fluviatilis-Minimus* group in malaria endemic tribal districts of India including north-eastern states to delineate the areas that are primarily under the influence of *Fluviatilis* and *Minimus* complexes. Observations revealed that species S of *Fluviatilis* Complex has limited distribution confined to hilly and foothill forest areas in the states of Odisha, Chhattisgarh and Andhra Pradesh. In contrast, species T was found widely distributed. In north-eastern region, only *An. minimus sensu stricto* (formerly species A) of *Minimus* Complex was found prevalent and *An. harrisoni* (*Minimus* C) was not encountered.
- Transcriptome analysis of mosquito salivary glands of *An. culicifacies* against NR and other PDB databases, viz. GO, SMART, KEEG, and PFAM showed 44% homology with the known protein databases, while another 13% sequences showed homology to the conserved hypothetical proteins of unknown function. Remaining 43% sequences were completely unmatched and categorized as unknown sequences. Through annotation of the *An. culicifacies* salivary transcriptome data, we not only identified/characterized previously described salivary-specific transcripts, like salivary peroxidase but also identified other new putative transcripts. Additionally, through BLAST analysis against insect immunoDB, we predicted 98 transcripts encoding putative innate immune proteins; majority of them belonging to CLIP domain serine proteases, PGRP, FREPs, autophagy, AMPs members (including isoforms), etc.
- Characterization of salivary proteins in *An. stephensi* (sensitive) using 1D electrophoresis and liquid chromatography mass spectrometry (LC/MS/MS) and bioinformatics analysis, showed 37 known salivary proteins and 124 novel proteins. LC/MS/MS analysis showed that majority of salivary proteins belong to categories of signal transduction, regulation of blood coagulation cascade, various energy pathways, feeding, intracellular trafficking and transport, immune properties, etc.
- Mosquito adulticidal and larvicidal efficacies of Imidacloprid, a nicotinoid were tested against different strains of mosquitoes. The order of adulticidal efficacy of imidacloprid (LC_{50}) against three species of mosquitoes was *Cx. quinquefasciatus* < *Aedes aegypti* < *An. stephensi*. The LC_{50} recorded in case of DDT-malathion-deltamethrin resistant *An. stephensi* was ~7 fold lesser than that of the DDT-malathion-deltamethrin susceptible strains. The results also indicate that the adulticidal efficacy of imidacloprid was more

pronounced in insecticide resistant strains than the susceptible strains. The larvicidal efficacy of imidacloprid was in the order of *An. stephensi* < *Cx. quinquefasciatus* < *Ae. aegypti*. Insecticide resistant strains showed lower LC_{50} values than the susceptible strains.

- Monitoring of insecticide resistance in 13 states, including 7 NE-states comprising of about 156 districts showed widespread resistance *An. culicifacies* to DDT in all the above states. The species was found resistant to malathion and deltamethrin in Andhra Pradesh and Chhattisgarh, while in other states it was mostly tolerant. *Anopheles fluviatilis,* was found resistant to DDT in Chhattisgarh, tolerant/resistant in Jharkhand but was susceptible in Odisha. In Jharkhand, this species was mostly susceptible to malathion and deltamethrin. This study has thus brought out clearly that in the mainland states there is a trend to develop pyrethroid-resistance in *An. culicifacies* in areas where pyrethroids are being used.
- **Insecticide Testing Laboratory at NIMR has been recognised as the WHO Collaborating Centre for Phase-I testing and evaluation of public health pesticides' in December 2012 for a period of four years.** This facility is the first of its kind in India and in the WHO Southeast Asia region and second such facility globally.
- Whole voltage-gated sodium channel, the target site of action for DDT and pyrethroids, of *An. stephensi* was sequenced. The distribution of *kdr* alleles in India was mapped. It was noted that L1014F is found only in Raipur whereas L1014S was found only in northern India. No mutation was found in southern India.
- Other studies included dengue vector breeding surveys in Delhi, efficacy of C-21 attracticide for surveillance and control of dengue and chikungunya in endemic zones of Delhi state.

Parasite Biology

- Studies on the role of NO in aspartic protease activity of *Plasmodium falciparum* indicated that aspartic proteases are inhibited by NOS donors and activators.
- Extensive genetic diversity of *stevor* genes in Indian *P. falciparum* isolates was reported, however, the genetic repertoire from complicated cases was less diverse. The high degree of *stevor* diversity has important implications for designing effective anti-malaria control measures.
- Successful genotyping of 18 isolates for *Pv-mdr1* revealed the mutant $M_{958}Y_{976}L_{1076}$ as the dominant haplotype (n = 17; 94.4%) and $M_{958}Y_{976}F_{1076}$ as the minor haplotype (n = 1; 5.6%) in Chennai.
- Population genetic analysis of CQ resistance in *P. falciparum* from north-eastern states and south-west India showed a large genetic break between the chloroquine resistant parasites of north-east-East-Island group and south-west group at both genome wide neutral loci and *pfcrt*-flanking (–24 kb to +22 kb) loci, suggesting a long period of isolation or a possibility of different origin among them. A pattern of significant isolation by distance was observed in low transmission areas ($r = 0.49$, $p = 0.003$, $n = 83$, Mantel test).
- Pharmacogenomic profiling of Indians based on single nucleotide polymorphisms (SNPs) at the *N*-acetyl transferase 2 (*NAT2*) gene revealed that distribution of two different acetylation phenotypes correlated well with historical dietary pattern in India. Majority of the north and west Indians are slow acetylators, whereas the central, east, north-east and south Indians are fast acetylators. Approximately 80% of the individuals categorized as fast acetylators are non-vegetarians, whereas approximately 65% of the slow acetylators are vegetarians.
- Whole mitochondrial genome of a single *P. falciparum* field isolate collected from Bilaspur (Madhya Pradesh) was sequenced using 19 novel and PCR primers and compared with the 3D7 reference sequence and one previously reported Indian sequence. While two Indian sequences were highly divergent from each other, the presently sequenced isolate was highly similar to the reference 3D7 strain.
- Genetic variations were analysed at microsatellite loci present in-and-around the *Pfcrt* gene in Indian *P. falciparum* by employing different population genetic and evolutionary approaches to conclude that

genetic drift rather than natural selection could better explain the observed data on the diversity of microsatellite alleles present in-and-around the *Pfcrt* gene in Indian *P. falciparum*.

- Studies on immuno-modulatory role of mesenchymal stem cell (MSC) in pathogenesis of malaria infection showed that there was a dramatic accumulation of $CD4^+TCRDX5^+$ cells in infected animals, which was reduced in animals that received Sca-1^+ cells.

Epidemiology

- As part of the studies on evidence-based assessment of biophysical determinants of malaria in the north-eastern states of India under climate change scenario, entomological investigations were undertaken in Mizoram, Asom, Bhimtal and Ranchi. The analysis is in progress for the development of a mathematical model for prediction of density of vectors and malaria in Indian conditions.
- A strategic plan is being developed for malaria vulnerable villages in Barmer, Bikaner and Jaisalmer districts in Rajasthan using satellite images and breeding habitats. The study warrants digital terrain modelling of vulnerable villages within 1.5 km radius of settlements.
- Three tools for early warning of malaria have been developed using cumulative rainfall, vegetation index and sea surface temperature from tropical south Atlantic Ocean. Of various models developed for early warning of malaria, rainfall was found best even at taluka level followed by NDVI as revealed in the studies undertaken in Gujarat state.
- Health impact assessment of Sardar Sarovar Narmada Project in Rajasthan and Madhya Pradesh were undertaken and mosquitogenic conditions and water habitats supporting breeding of mosquitoes were identified. Mitigating measures were suggested to respective authorities to contain the mosquito borne diseases in the command areas and rehabilitation and resettlement of colonies.
- The project "Centre for the Study of Complex Malaria in India" (funded by NIAID and NYU, U.S.A.) to study the complexity of *P. vivax* malaria and eco-epidemiology at three sites (Nadiad, Rourkela and Chennai) is being undertaken.

Clinical Research

- In the pharmacovigilance of antimalarials project, about 4600 filled in AER forms have been analysed and the antimalarials used in India are found to be safe as per the inputs provided by the medical practitioners.
- NIMR and NVBDCP have developed Quality Assurance programme for malaria RDTs supplied in the national programme.
- A multi-centric randomised open-labelled clinical trial of AS + SP and AS + MQ on 210 pregnant woman revealed that the combination is safe for use in second and third trimesters of pregnancy.
- Monitoring of therapeutic efficacy of antimalarials prescribed in India revealed that the efficacy of ACT (AS + SP) for *P. falciparum* ranged between 76.3 and 100% at 12 study sites, and the efficacy of chloroquine in *P. vivax* malaria was found to be 100%.
- Presence of *Pfhrp2*/*Pfhrp3* deletions has been detected in Indian *P. falciparum* isolates collected from six different malaria endemic locations in the country, which may be the possible factor behind misdiagnosis/false negative malaria rapid diagnostic tests.

Other activities

- NIMR organized
  - (i) A workshop on "Comprehensive Case Management Pilot in Odisha" in May 2012.
  - (ii) World Bank project training on Malaria Microscopy and Molecular Studies in October 2012.
- The *Journal of Vector Borne Diseases* published by the Institute got an impact factor of 1.04 for the year 2012.

❑

Vector Biology and Control

1.1 Vector Biology

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

India represents four ecological zones, ranging from cold desert of Kashmir, arid/semi-arid hot desert of Rajasthan, deciduous dry and wet regions of Peninsula, to tropical monsoon regions of northeast and western ghats. Climatic diversity of fauna and flora is unmatched by any other geographical area. Indian region is beset with high disease burden of mosquito borne diseases like malaria, filariasis, dengue haemorrhagic fever, and Japanese encephalitis.

Countrywide insecticide spray under National Vector Borne Disease Control Programme (NVBDCP) and other development activities brought in radical changes in ecosystems, which created not only additional breeding places for anopheline mosquitoes but also resulted in ecological succession of vectors.

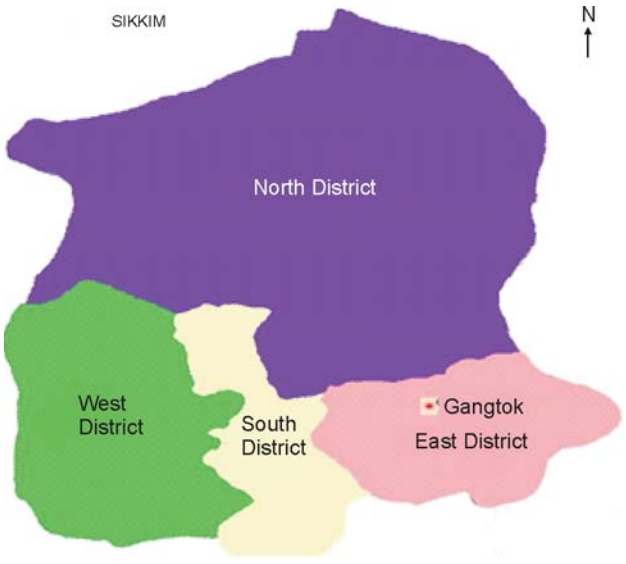


Fig. 1: Map showing districts of Sikkim state Map not to scale

Comprehensive studies were carried out in this project on the ecological succession of anophelines in Asom, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura to update knowledge on anopheline species in the proposed areas. This study was undertaken in 26 selected districts of Asom, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland and Sikkim in collaboration with the Field Unit of National Institute of Malaria Research, Guwahati, Regional Medical Research Centre, Dibrugarh and State Health Authority of Asom.

During this year, one survey was carried out by NIMR team, during March–April 2012, in two states, namely Manipur and Sikkim. East Sikkim, West Sikkim, North Sikkim and South Sikkim districts in Sikkim state (Fig. 1) and Chandel and Imphal East districts in Manipur state (Figs. 2 a & b) were surveyed and the ecological changes occurred in Manipur revealed that in Manipur forest cover was reduced by 1.91%. Besides the forest cover the other parameters such as, rainfall, net sown area, net irrigated area, population, production of rice, migratory population, construction of roads/ highways, dam construction, settlement of mining/ industry, and fish farm which affect the mosquito breeding. Survival of species and transmission of disease directly and indirectly have been increased tremendously, while in Sikkim, forest cover was reduced by 13.7%. These ecological parameters are also responsible for appearing and disappearing of species and their succession.

In Chandel district of Manipur, the following species, namely *Anopheles dirus*, *An. varuna*, *An. philippinensis* and *An. balabacensis* could not be found in the present survey. The species which were recorded from this area for the first time were, namely *An. fluviatilis*, *An. vagus*, *An. subpictus*, *An. nigerrimus* and *An. nivipes*. While in

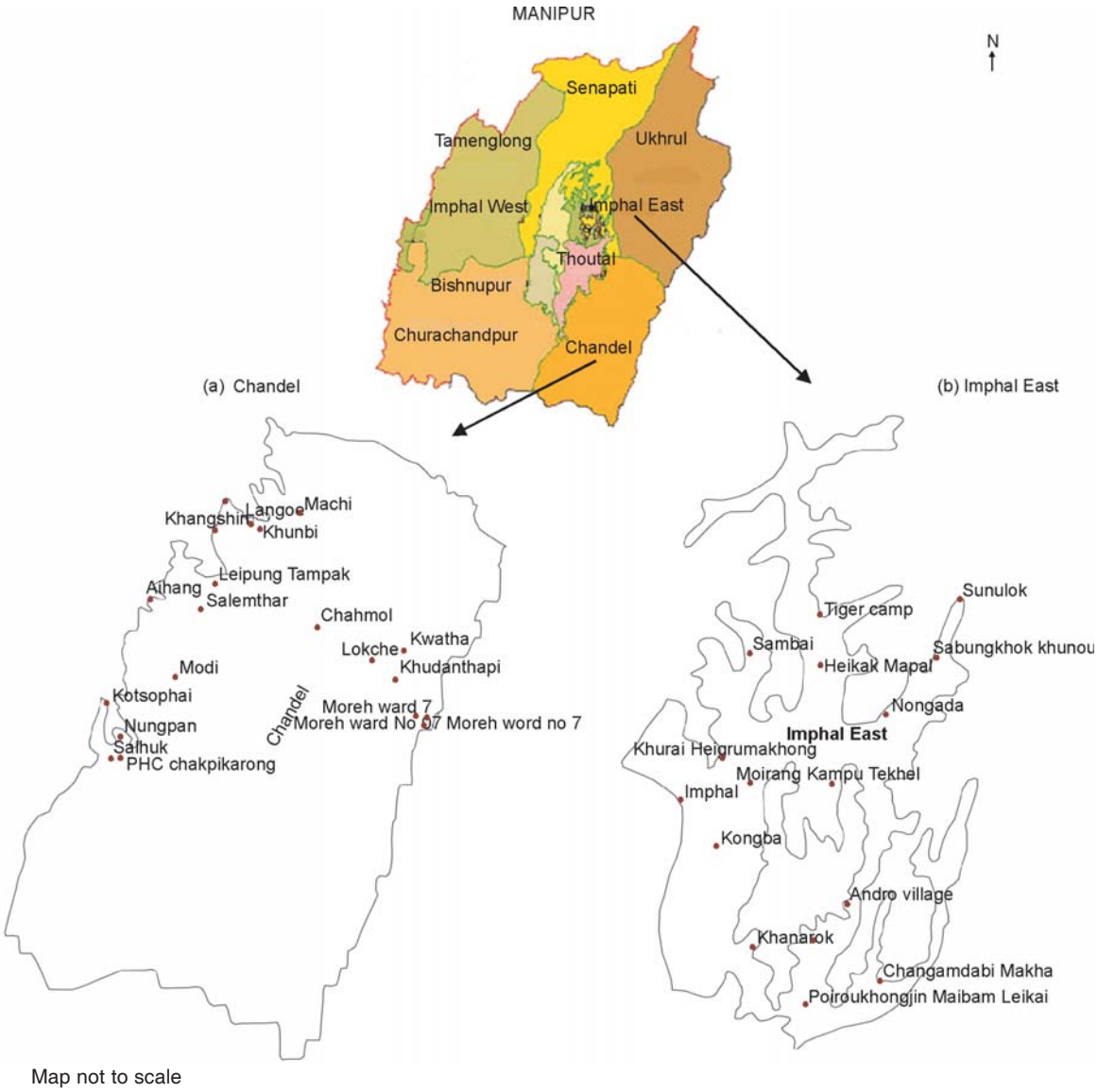


Fig. 2 (a&b): Map of Manipur state showing study sites: (a) Chandel, and (b) Imphal East districts (● denote surveyed villages).

Imphal East district of Manipur the following species, *An. gigas*, *An. maculatus* and *An. jeyporiensis* were absent while *An. subpictus*, *An. kochi*, *An. jamesii*, *An. dthali* and *An. nivipes* were recorded for the first time.

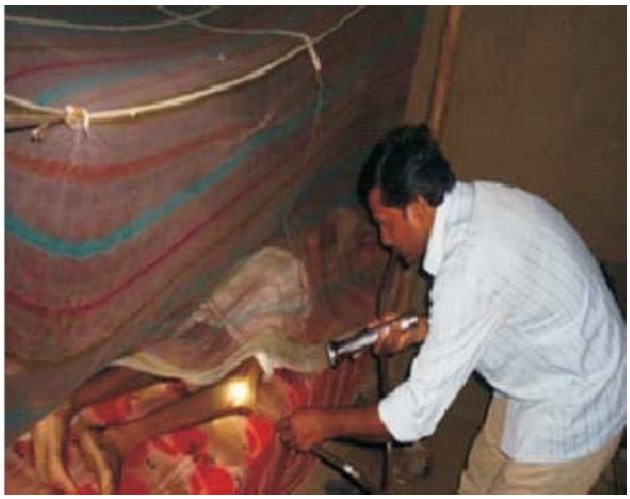
In Sikkim (East, West, North and South Sikkim districts), the previously reported species, namely *An. vagus*, *An. culicifacies* and *An. maculatus* were still prevalent and could be collected in the present survey. The species which were recorded for the first time in East, West, North and South Sikkim districts were *An. pseudowillmori* and *An. nigerrimus*.

Anopheles culicifacies collected from four states, namely Asom, Meghalaya, Manipur and Sikkim were incriminated as vector species with the help of ELISA technique. A total of 689 *An. culicifacies* dissected from 16 districts, out of which 173 were found positive with the help of ELISA.

1.1.2 Ecological succession of anopheline and other mosquitoes in northern Terai area of India

During this year, one survey was carried out by NIMR team, during October–November 2012, in two districts of Uttarakhand state, namely Udham Singh Nagar and Dehradun.

In District Udham Singh Nagar, 17 villages having different ecology were selected. The total population of the district is more than 1.6 million. The district is divided into 9 units and comprises of 327 villages and 8 towns. The peculiar geo-ecological condition in Udham Singh Nagar district consists of diverse topographic features, climate condition and other favourable factors such as rapid



Collection of mosquitoes from study sites.

industrialization and rice-fields. Dams and canals have facilitated the formation of different malaria paradigms.

In Dehradun district, 13 villages were selected from 7 CHCs having different ecology. The district is having a population of more than 1.7 million, out of which urban population is 56%. Rapid urbanization in the foothill terai region has led to decrease in agriculture land and forest cover. The new roads and building construction activities have led to changes in mosquito composition and malaria paradigms.

During the survey, more than 4000 mosquitoes using different larval and adult collection methods were collected as per protocol. The identification of species and processing of field material is under progress. During the survey, information about ecological changes were also collected from each district.

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

Malaria is one of the major public health problems in rural plain areas of India, where *An. culicifacies* Giles is the primary malaria vector species. Various studies carried out in the past in Dadri CHC area of District G.B. Nagar in western U.P. have reported *An. culicifacies s.l.* as the only established vector of malaria in this area. This study was continued to investigate the appearance and disappearance of *An. fluviatilis* in Dadri CHC area, where this species was not observed during the past three decades in various other studies undertaken in this area.

During this study regular (fortnightly) monitoring of the indoor resting mosquito density was made by hand catch method in six villages of Dadri CHC. The study revealed the appearance of *An. fluviatilis* in high densities in Dadri CHC area during November–December 2009. The species was found to be *An. fluviatilis* species T by cytotaxonomic and molecular diagnostic techniques. The breeding as well as adult density of *An. fluviatilis* was recorded only from the villages located adjacent to the drain which carry water discharged from NTPC after cooling of towers and ash effluents. This water is taken from irrigation canal. This species was found to be totally zoophagic as revealed by blood meal source analysis. *Anopheles fluviatilis* continued to persist in these villages till July 2010. After July 2010 this species disappeared again and no specimen could be collected till December 2011 (Figs. 3 a–d). *Anopheles fluviatilis* reappeared in January 2012 till April 2012, but after this no specimen of *An. fluviatilis* was collected till March 2013. These observations indicated that appearance and disappearance of *An. fluviatilis* in this area is not a regular cyclic phenomenon. The prevalence of *An. fluviatilis* was not affected by seasonal changes. On the other hand its appearance and disappearance was found to be probably associated with the presence of thick vegetation on the surface of slow moving water in the NTPC drain. This species disappeared after removal of the vegetation cover on the surface of drain manually, and reappeared again with the appearance of this vegetation in the drain.

These results indicate the establishment of a focus of *An. fluviatilis* in Dadri CHC area, where so far this species was not present. The prevalence

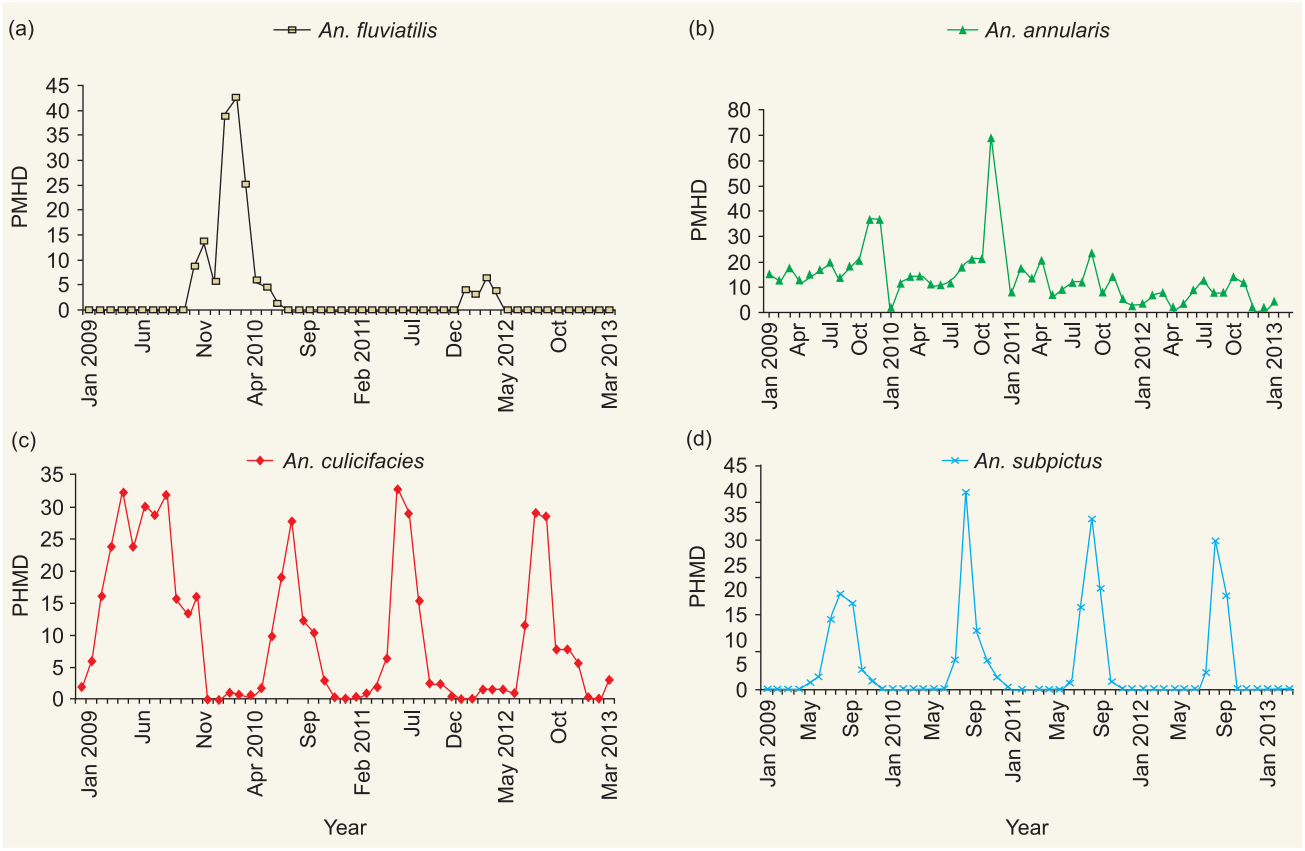


Fig. 3 (a–d): Monthly data on indoor resting density of *Anopheles* mosquito species in Dadri CHC area during 2009–12.

of this species in Dadri CHC is due to the ecological changes caused by the setting up of NTPC plant in this area without assessing its health impact. Water for cooling of chimney towers is taken from a tributary of the upper Ganga canal and this water is discharged from NTPC plant after cooling of towers and ash effluents into NTPC drain, which also carry the surplus water from the tributary of the upper Ganga canal which may also carry the larvae of *An. fluviatilis*. These larvae later got established under the vegetation cover on the surface of slow moving water in the NTPC drain.

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

Malaria is endemic in District Ghaziabad (U.P.), but the API of the district as per records of NVBDCP is <2. In contrast to this, certain villages of District Ghaziabad are highly endemic for malaria. Entomological and parasitological studies

NTPC canal showing vegetation cover.

NTPC canal after removal of vegetation cover.

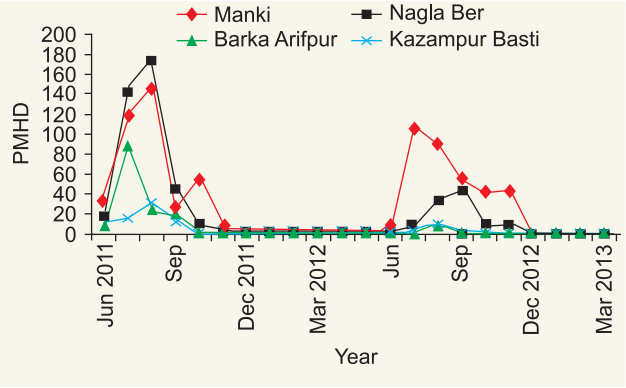


Fig. 4: Per man hour density of *An. culicifacies* in four different villages of District Ghaziabad during June 2011 to March 2013.

were continued in four villages, located in three CHCs of District Ghaziabad to understand the transmission dynamics of malaria, where malaria situation is much higher than the other villages in this area. Monthly monitoring of entomological and parasitological indices of malaria was undertaken.

The study revealed the prevalence of high density of *An. culicifacies* (178), the major known malaria vector species, during the monsoon period with a peak in the month of August, in all these villages. However, in village Manki an additional peak in October during the post-monsoon period was also observed (Fig. 4). Preliminary results revealed sympatricity of *An. culicifacies* sibling species A and species B with the predominance of species B in all these villages. Though, *An. culicifacies* is predominantly zoophagic, the socio-behavioural practice of co-existence of both human and animal population mainly poultry and relatively lesser number of cattle in these villages, make the human population more vulnerable to mosquito bites and malaria transmission. The results of fortnightly active surveillance revealed active transmission of malaria in all the villages during monsoon months from July to September, while in village Manki and Barka Arifpur active transmission of malaria continued during post-monsoon months as well (Table 1).

Prevalence of large number of *Pf* malaria cases and high density of *An. culicifacies* during monsoon and post-monsoon months in these villages indicates active transmission. A focus of *An. fluviatilis* in Dadri CHC area, where so far this species was not prevalent has been established due to the ecological changes caused by the setting up of NTPC plant in this area without assessing its health impact.

1.1.5 Studies on the distribution and biological characteristics of the members of *Fluviatilis-Minimus* group for effective vector control strategies in tribal areas of India

Information generated on the distribution and biological attributes (in terms of resting and feeding behaviour, response to insecticides and malaria transmission potential) of the members of *Fluviatilis-Minimus* group in 22 districts of 10 states of eastern region of India including northeastern states were analysed to delineate the areas that are primarily under the influence of *Fluviatilis* and *Minimus* complexes. Based on the results, effective vector control measures were suggested to scale-down malaria transmission in the study areas.

Observations revealed that species S of *Fluviatilis* complex has limited distribution confined to hilly and foothill forest areas in the states of Odisha, Chhattisgarh and Andhra Pradesh. *Fluviatilis* S was found to be highly anthropophagic with preference to rest in human dwellings. It was found to be primarily associated with the malaria transmission in the areas of its prevalence. These findings are supported by the fact that high sporozoite rate was observed in *Fluviatilis* S in all the districts where it was found prevalent (Fig. 5). Active malaria transmission was observed up to February–March in areas where species S distribution was reported.

In contrast, species T of the *Fluviatilis* complex was found widely distributed. This species was found to be primarily zoophagic with preference to rest in cattleshed-mixed dwellings. Studies revealed that resistance-tolerance to DDT has precipitated in *An.*

Table 1. Malaria transmission in certain villages in District Ghaziabad during June 2011 to March 2013

Village/PHC (Population)	Year (April–March)	T.B.S.	*Pv*	*Pf*	Total positive	SPR	SFR	PI
Manki/Dasna (2353)	2011–12	314	53	34	87	27.7	10.8	57.2
	2012–13	567	77	71	145	25.57	12.52	61.62
Barka Arifpur/Muradnagar (1400)	2011–12	243	64	10	74	26.3	4.1	52.8
	2012–13	225	69	5	71	31.55	2.22	50.7
Nagla Ber/Bhojpur (228)	2011–12	91	20	5	25	27.4	5.5	109.6
	2012–13	88	12	8	20	22.72	9.9	87.72

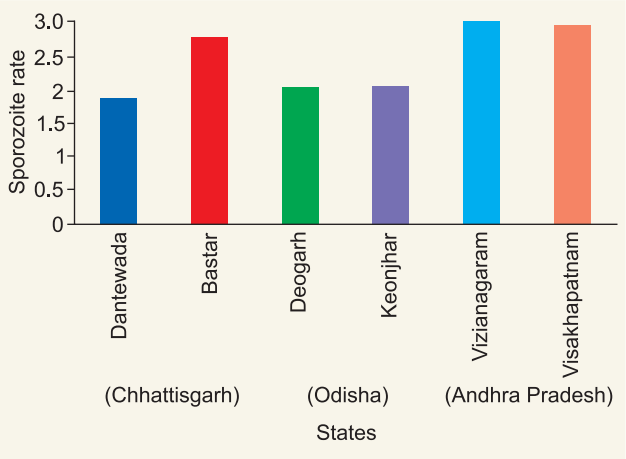


Fig. 5: Sporozoite rates in *An. fluviatilis* species S in study districts.

fluviatilis in areas where species T is predominant. Though, *Fluviatilis* T is comparatively less efficient than species S, it enhances malaria transmission during its prevalence period.

In most of the study districts on mainland *An. fluviatilis* has been found co-existing with *An. culicifacies* though their peak prevalence period varies. In these areas timely two rounds of IRS with appropriate insecticide can curb building up of vector populations and reduction in vector densities can be achieved for longer duration provided the quality of spray is up to the mark and coverage is very good. But in certain areas with prevalence of highly efficient vector (Fluviatilis S) where extended malaria transmission of high intensity is observed, an additional/special round of IRS can be carried out as short-term intervention measure to handle the epidemiological situation.

In study districts of northeastern region, only *An. minimus sensu stricto* (formerly species A) of *Minimus* complex was found prevalent and *An. harrisoni* (*Minimus* C) was not encountered. *Anopheles minimus s.s.* was found to have strong preference to feed on humans incriminated as malaria vector and the observations strongly indicated that a proportion of its population is exophilic. Therefore, in areas where *An. minimus s.s.* is predominant, use of insecticide-treated nets (ITNs)/ long-lasting insecticidal nets (LLINs) at community level would be very effective intervention tool. This can be achieved by large-scale distribution of LLINs by government agencies and encouraging community to buy nets at subsidised cost (social marketing). Since, *An. minimus s.s.* is highly anthropophagic, personal protection measures (protective clothings, use of repellents, coils and vaporizers) would supplement the major intervention tool in decreasing the man-mosquito contact.

1.1.6 Exploring the role of salivary glands in blood feeding of mosquito *Anopheles culicifacies*

Blood feeding, a unique characteristic of mosquitoes, possesses several physiological and immunological challenges, which not only affects the reproductive capacity, but may also affect the dynamics of *Plasmodium* development and malaria transmission by the female mosquitoes, if an infected blood meal is ingested. Mosquito salivary glands are small, dynamic and single layered, bi-lobed epithelial tissues, which play a unique role in sugar and blood meal acquisition. During blood meal intake, female mosquitoes are '*engaged in*' seeking host preference, blood vein localization and release of salivary products preventing blood coagulation, cause vasodilatation, platelet aggregation and other many salivary specific products involved in comfort uptake of vertebrate blood.

Mosquito salivary glands encodes diverse products

To understand the molecular nature and behaviour of the salivary transcripts during blood feeding, first we sequenced and analyzed the blood fed salivary gland transcriptome of the mosquito *An. culicifacies*. Primary clustering analysis of ~27 million short reads (Illumina) database resulted in the assembly of 5690 contigs carrying average size of 493bp. BLAST analysis against insect-specific database, viz. genome, ESTs, proteome, transcripts, etc. showed a match of more than 90% sequences. Analysis against NR and other PDB databases, viz. GO, SMART, KEEG, and PFAM showed 44% homology with the known protein databases, while another 13% sequences showed homology to the conserved hypothetical proteins of unknown function. Remaining 43% sequences were completely unmatched and categorized as unknown sequences (Fig. 6).

Through ongoing annotation of the *An. culicifacies* salivary transcriptome data, we not only identified/characterized previously described salivary-specific transcripts, like salivary peroxidase (Figs 7–10; Table 2) but also identified other new putative transcripts (Fig. 11). Additionally, through BLAST analysis against insect immuno DB, we predicted 98 transcripts encoding putative innate immune proteins; majority of them belonging to

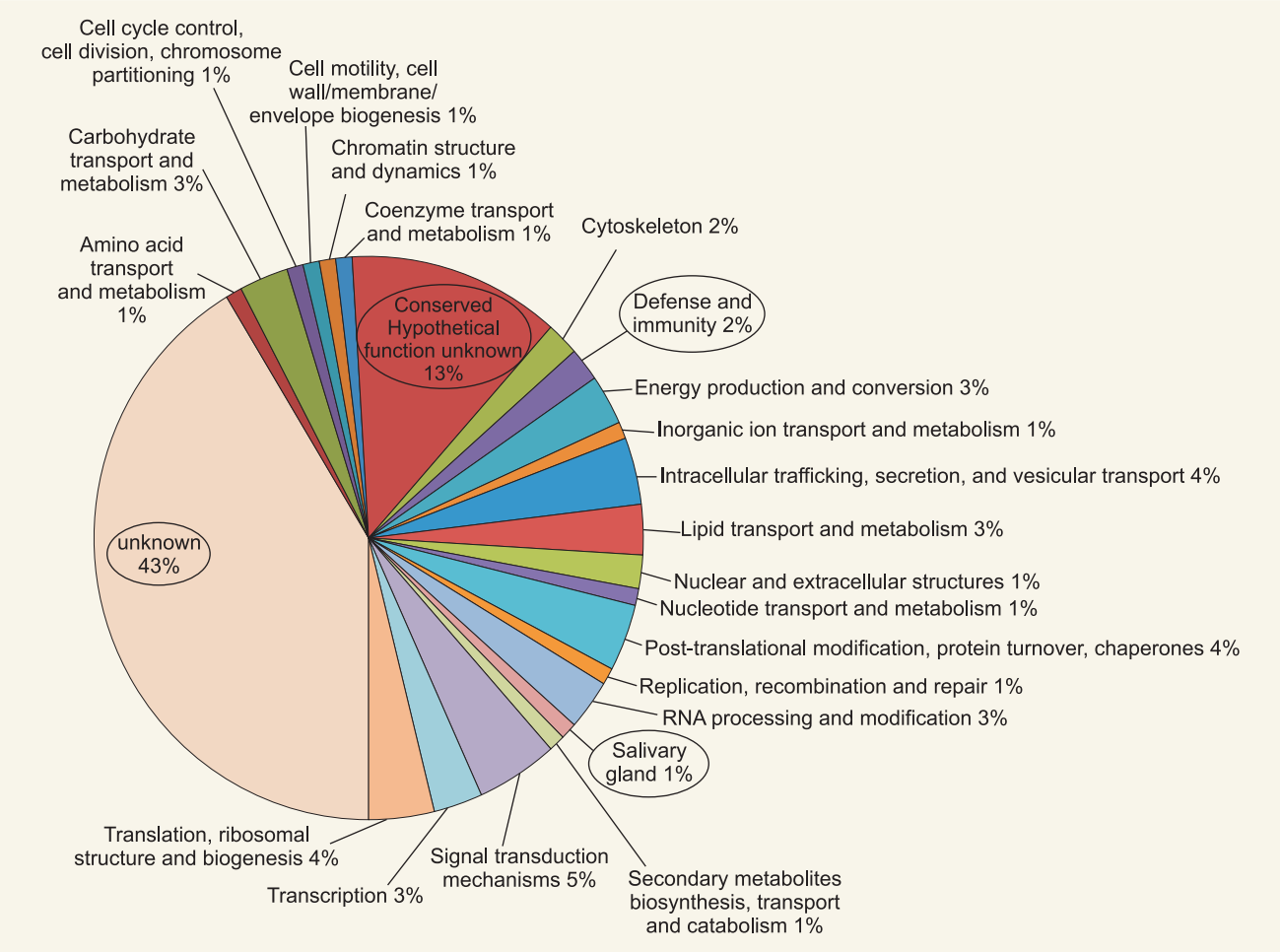


Fig. 6: Salivary glands molecular architecture: Predicted functional categories of salivary genes.

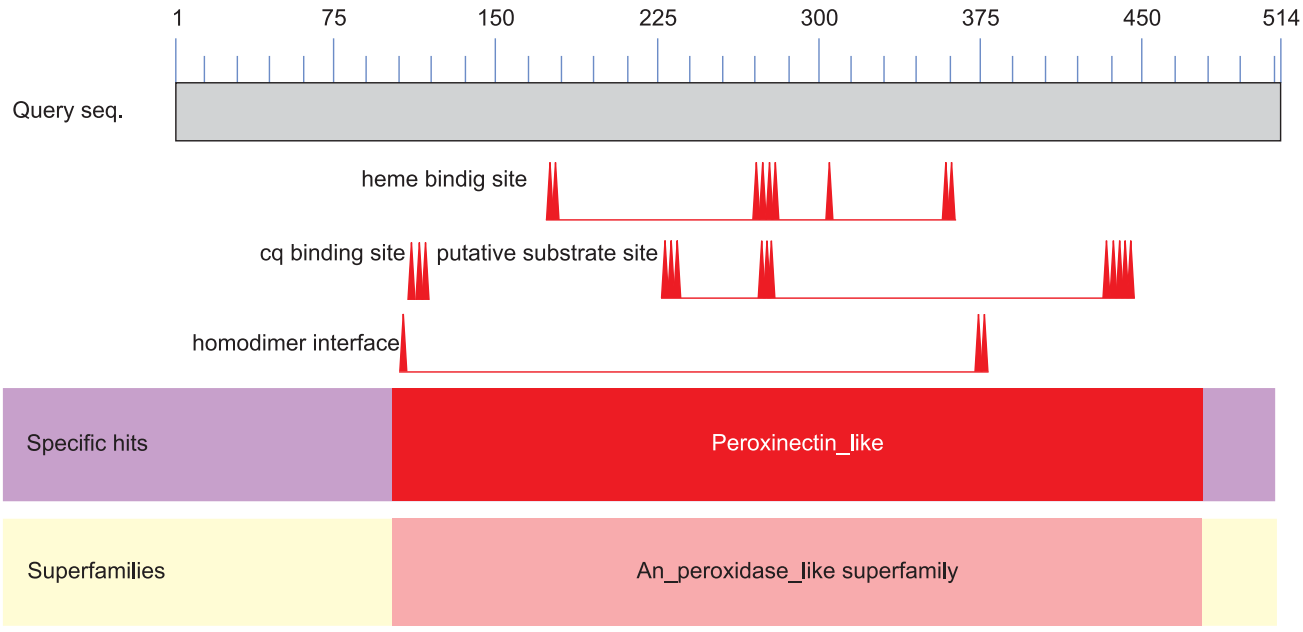


Fig. 7: Web-based domain prediction and primary identification of gene transcript encoding peroxidase like protein in mosquito *An. culicifacies.*

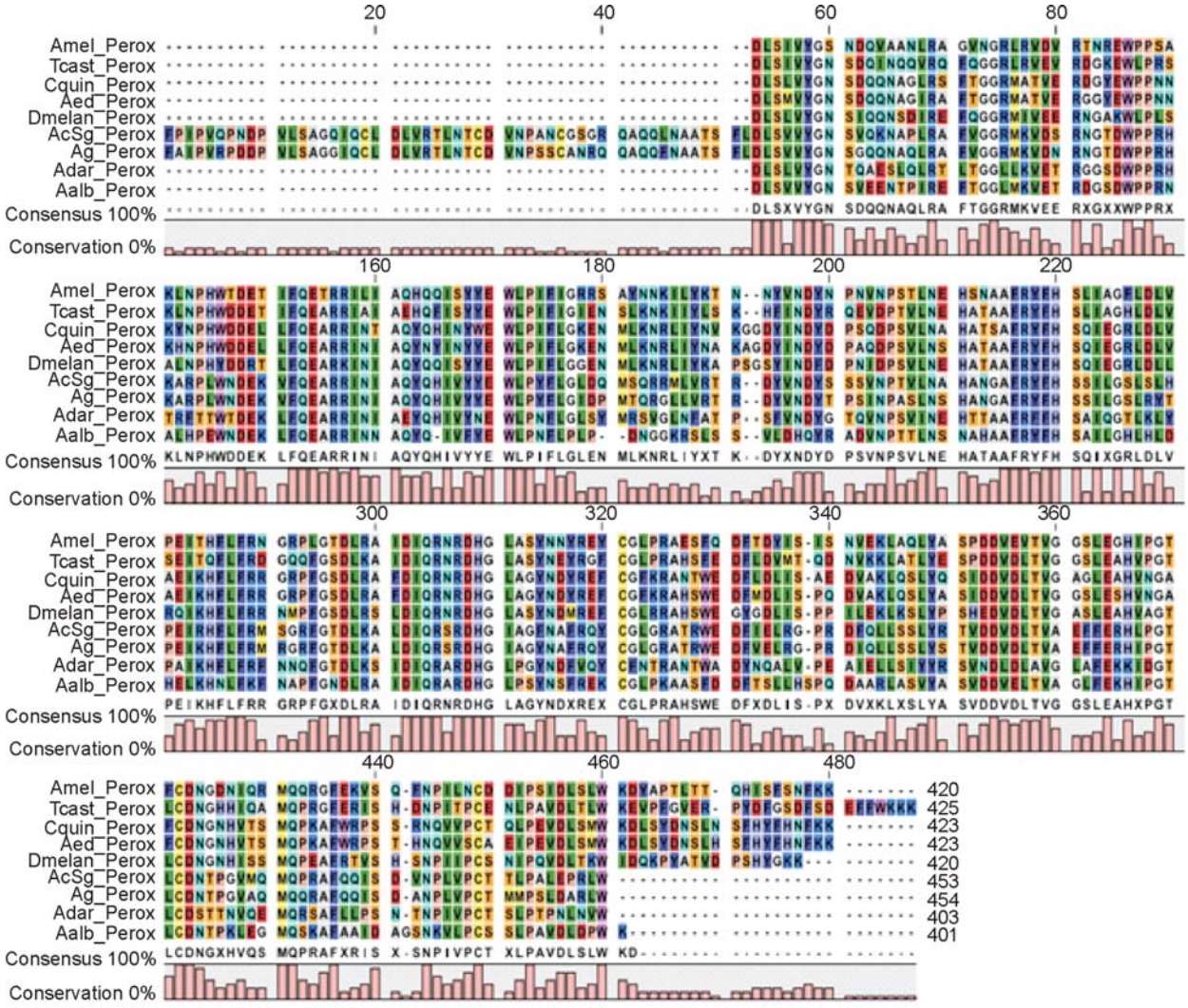


Fig. 8 : Multiple sequence alignment and molecular characterization of salivary peroxidase.

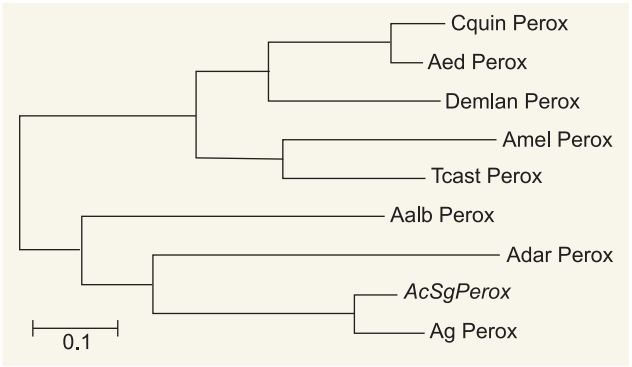


Fig. 9: Phylogenetic analysis of salivary peroxidase transcript identified from blood fed transcriptome of *An. culicifacies*.

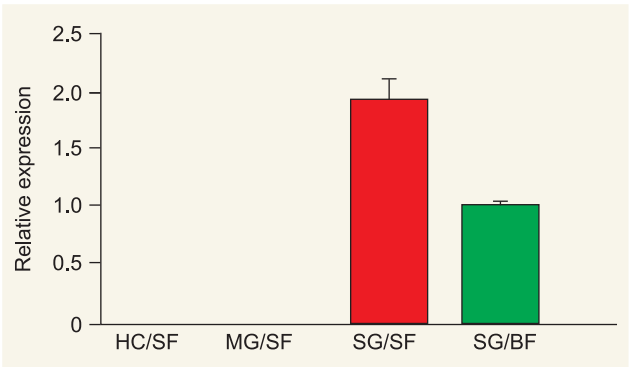


Fig. 10: Relative expression of peroxidase transcript in different tissues, viz. hemocyte (HC), midgut (MG), sugar fed (SF), blood fed (BF), and salivary glands (SG).

CLIP domain serine proteases, PGRP, FREPs, autophagy, AMPs members (including isoforms), etc. from the salivary glands transcriptome of the mosquito *An. culicifacies* (Table 3). Currently, these salivary-specific secretary molecular factors are under investigation to understand their role in mosquito bite/vertebrate exposure as well as *Plasmodium* transmission dynamics.

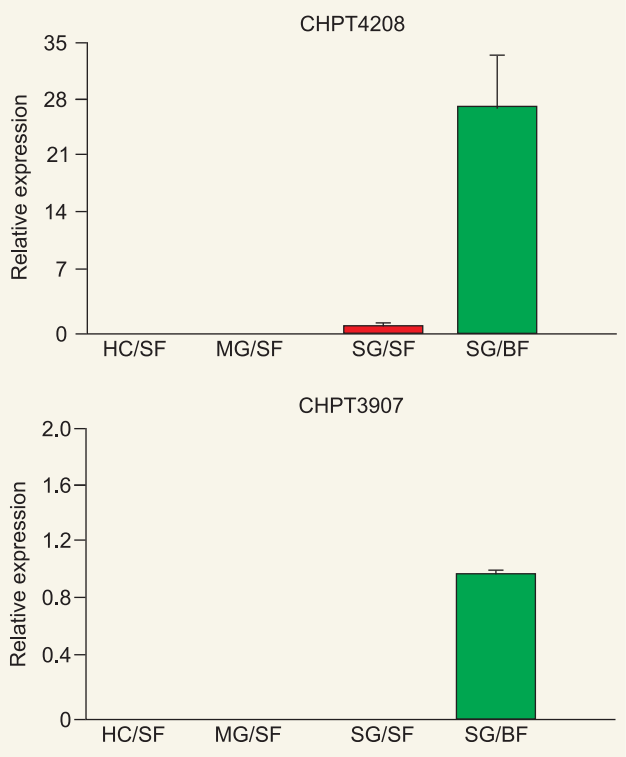


Fig. 11: Relative expression of conserved hypothetical transcripts (contig No. 4208 and 3907) in different tissues, viz. hemocyte (HC), midgut (MG), sugar fed (SF); blood fed (BF); and salivary glands (SG).

Salivary peroxidase

Mosquito salivary mediated vasodilation allows comfort uptake of blood meal from the vertebrate host. In the present genetic screen of salivary genes, we identified (1562 bp) partial cDNA, encoding (514aa) long peptide, similar to previously described salivary peroxidase from the mosquito *An. culicifacies* (*AcPerox*). Detailed molecular analysis including multiple sequence alignment/ phylogenetic analysis showed high degree of conservation with other known family of insect peroxidases. Real-time PCR analysis indicated that *AcSGPerox* expresses specifically in the salivary glands significantly undergoes down regulation in response to blood feeding, suggesting that *AcSG-Perox* could play an important role in vasodilator activity, as reported in mosquito *An. albimanus* (Figs. 7–10).

Novel salivary transcripts (Conserved hypothetical proteins—CHPT)

In the current salivary genetic screen, we identified at least 13% conserved hypothetical proteins, which have also been previously identified from other insects, but no function has been predicted

Table 2. Putative mosquito salivary proteins identified from salivary transcriptome

S.No.	Salivary gene family	Subfamily members	Total no. of transcripts identified
1.	Apyrase	Salivary apyrase (2)	2
2.	D7 related protein	D7 related 1 protein	1
		Short form D7 related protein 2 (2)	2
		Long form of D7 related protein (2)	2
3.	Galectin	Putative salivary galectin (2)	2
4.	Salivary gland protein	SG2A salivary protein	1
		Putative salivary protein SG2B	1
		gSG7 salivary protein (2)	2
		GE rich salivary gland protein precursor	1
		Putative salivary protein SG1B	1
		gSG1b salivary protein	1
		SG1-like 3 salivary protein	1
		SG1D salivary protein precursor	1
		gSG6 salivary gland protein precursor	1
		Putative salivary protein SG1A	1
		Putative 13.4 kDa salivary protein	1
		trio 2 salivary protein	1
		Putative 41.9 kDa basic salivary protein	1
		Putative 23.4 kDa salivary protein	1
5.	Secreted protein	Secreted protein	1
		hyp 37.3 putative secreted salivary gland protein	1
		Putative 56 kDa salivary secreted protein	1
6.	Phospholipase	Phospholipase D	1
7.	Salivary antigen	Salivary antigen-5 related protein AG5-1	1
		Antigen 5-related 2 salivary protein	1
8.	Peroxidase	Salivary peroxidase	1
9.	Sensory	Methyl-accepting chemotaxis sensory transducer	1
10.	Ion channel	Ion channel	1

Table 3. Summary of putative immune transcripts from salivary transcriptome

S.No.	Immune gene family	Subfamily members (No. of isoform)
1.	Antimicrobial peptides (AMPs)	Ac_DEF1
2.	Autophagy (APHACs)	Ac_APG3 Ac_APG7A Ac_APG7B (2) Ac_APG8 Ac_APG18B
3.	Inhibitors of apoptosis	Ac_IAP6 (4)
4.	Caspase activators (CASPAs)	Ac_CASPAR (3) Ac_CASPS5 (1)
5.	Catalases (CATs)	Ac_CAT (3)
6.	Clip domain serine proteases (CLIPs)	CLIP3 (1) CLIPA6 (1) CLIPA7 (6) CLIPA9 (7) CLIPA10 (1) CLIPA15 (1) CLIPB1 (1) CLIPB3 (1) CLIPB6 (1) CLIPB13 (2) CLIPB15 (1) CLIPB20 (1) CLIPC1 (1) CLIPC3 (2) CLIPC6 (1) CLIPC10 (1) CLIPD2 (2) CLIPD3 (1) CLIPD6 (4) CLIPD8 (1) CLIPE8 (1)
7.	C-type lectins	Ac_CTL6 (3) Ac_CTLGA3 (2) Ac_CTLMA2 (3) Ac_CTLSE2 (1) Ac_REL1 (3)
8.	Fibrinogen-related proteins (FREPs)	Ac_FREP34 (1) Ac_FREP40 (2) Ac_FREP63
9.	Galactoside-binding lectins (GALEs)	Ac_GALE5 (3)
10.	IMDPATH	Ac_TAK1(2)
11.	JAK-STAT pathway	Ac_HOP (2)
12.	Peptidoglycan recognition receptors (PGRPs)	Ac_PGRPLB1 Ac_PGRPLD
13.	Peroxidases (PRDXs)	Ac_GNBPB (1) Ac_GPXH1 (1) Ac_GPXH2 (1) Ac_HPX12 (1) Ac_HPX3 (1) Ac_TPX1 (1) Ac_TPX3 (1) Ac_TPX5 (2)
14.	Phenoloxidase	Ac_PPO3 (1)
15.	Scavenger receptors (SCRs)	Ac_SCRAC1 (4) Ac_SCRB6 (1) Ac_SCRBQ2 (3)
16.	Thioester containing proteins	Ac_TEP3 (1)

or reported so far. To understand their role in blood feeding, we examined expression of randomly selected CHPT proteins through real-time PCR. Unexpectedly, we observed induced expression in the blood-fed (BF) salivary glands, while these do not express in other sugar-fed (SF) mosquito tissues, viz. hemocytes/midgut (Fig. 11). Together, these data suggest that these proteins may have important role in the blood meal acquisition, demanding further functional studies.

In summary, transcriptome sequence analysis revealed that mosquito salivary glands secrete broad spectrum of products carrying diverse evolutionary adaption values for blood meal acquisition. Our ongoing functional analysis will provide more insight to understand the molecular basis of mosquito/human bite exposure and malaria transmission dynamics.

1.1.7 Transcriptional responses of antimicrobial peptides in mosquito *Anopheles stephensi*

Due to strong evolutionary adaptation to almost all ecological niches, insects are regularly exposed to diverse microbes which allows insect to mount an effective innate immune response against any infectious microbial pathogen including virus, bacteria and protozoan etc. Antimicrobial peptides constitute a first line LOCAL immune defense and also activate the cellular components of the SYSTEMIC defenses.

AMPs constitutively express during mosquito development

To characterize the molecular local and systemic responses of mosquito AMPs, first we identified, characterized and analyzed a set of four family members totaling eight transcripts belonging to Cecropins (C1, C2, C3), Defensins (D1, D3),

Table 4. Molecular details of the selected antimicrobial peptides mosquito—*An. stephensi*

S.No.	AMP family	Transcripts	Nucleotide size	Peptide size	% Identity to *An. gambiae*
1.	Cecropins	C1	469	87	95
		C2	387	76	87
		C3	617	60	98
2.	Defensins	D1	607	92	80
		D3	377	67	70
3.	Gambicin	G	466	88	90
4.	Lysozymes	L1	685	163	84
		L7	776	148	86

Gambicin (G) and Lysozymes (L1, L7) (Table 4). Initially, RT-PCR analysis revealed that all AMPs constitutively expresses throughout the development of the mosquito, suggesting that their important role for maintaining the mosquito free from any microbial infection, encountered during feeding and/or any other injuries (Fig. 12).

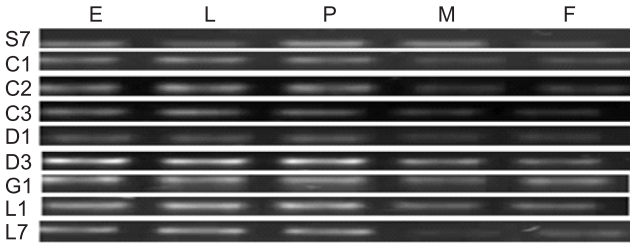


Fig. 12: RT-PCR profiling of innate immune genes during development expression of mosquito *An. stephensi*. E: Egg; L: Larva; P: Pupa; M: Male; F: Female; *S7*: Ribosomal protein *S7* gene; C1–C3: Cecropin 1, 2, 3; D1: Defensin 1; D3: Defensin 3; G: Gambicin; L1: Lysozyme 1; L7: Lysozyme 7.

AMPs locally express in epithelial tissues of digestive tract

The activation of a strong anti-*Plasmodium* response is believed to be mediated by microbial flora enrichment during blood meal digestion in the adult female mosquitoes, however, the underlying molecular mechanism involving the role of AMPs has not been investigated in detail. Therefore, to understand the role of AMPs, first we examined the relative expression of all the eight transcripts among different tissues, viz. hemocytes (HC), midgut (MG) and salivary glands (SG) collected from 3–4 days old sugar-fed adult female mosquitoes. The real-time PCR analysis revealed that all the AMPs are abundantly expressed in the salivary glands and midgut, keeping digestive tract free from any harmful microbes ingested during sugar meal uptake (Fig. 13).

Blood feeding induces local response of AMPs

Midgut is one of the major tissues, where a maximal metabolic activities, as well as interaction with microbial growth occur during first 24–30 h post-blood meal (PBM). Therefore, we monitored the impact of blood feeding on the AMPs expression in the midgut dissected 30 h post-blood meal. The real-time PCR analysis showed that mostly the AMPs expression is significantly induced during blood meal digestion (Fig. 14), however, our further ongoing studies should clarify whether this AMPs induction is due to increased microbial gut flora and/or any other physiological responses.

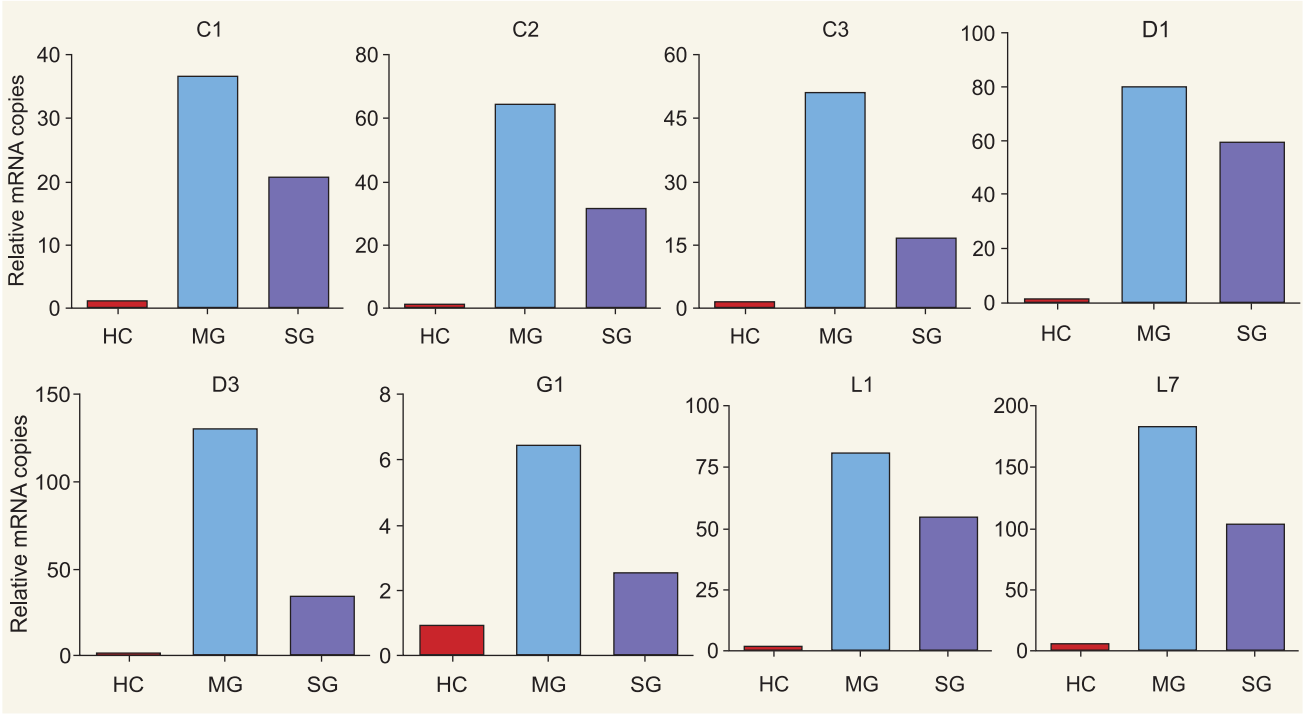


Fig. 13: Examination of relative expression of innate immune genes in hemocyte (HC), midgut (MG), and salivary gland (SG) tissues collected from mosquito *An. stephensi*.

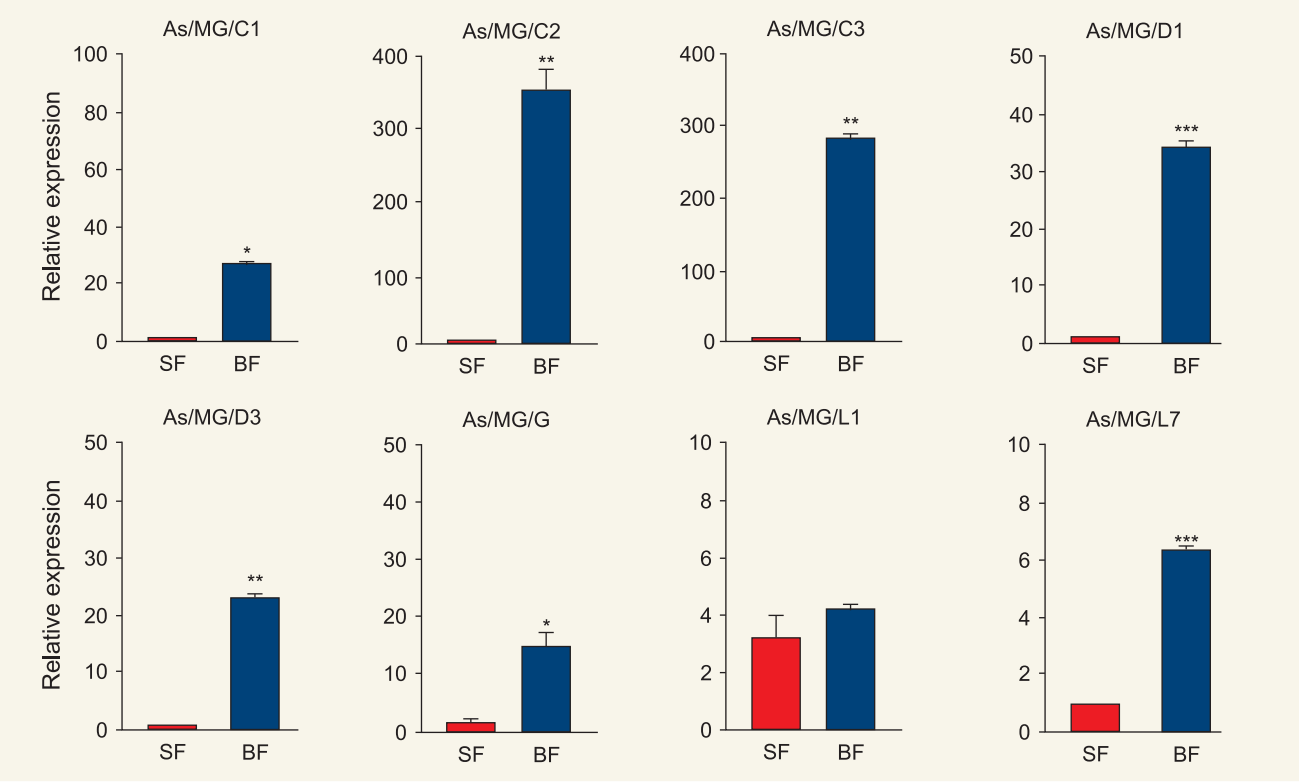


Fig. 14: Effect of blood meal on the mosquito AMPs expression in the midgut (MG) tissue collected 30 h PBMS (SF: Sugar fed; BF: Blood fed).

Exogenous microbial challenge induces systemic response of AMPs in the hemocytes

To understand 'Systemic' responses of AMPs, first we exogenously challenged the 3–4 days sugar-fed adult mosquitoes with either gram negative or gram positive bacterial paste by pin-prick method. Following immune challenge, we monitored early (30 min, 2 h); medium (12 h) and late (24 h) responses of AMPs in the hemocytes. The preliminary results revealed induced expression of AMPs post 24 h challenge, indicating hemocytes may play important role of late clearance of antigens in the hemolymph (Fig. 15).

The current data suggest that mosquito AMPs constitute an important immune arm to fight against any microbial infection. Our ongoing studies should provide new information for selecting crucial anti-parasitic AMPs, an important target for vector-based malaria control strategy design.

1.1.8 Differential expression of salivary proteins between susceptible and insecticide-resistant mosquitoes of *Anopheles stephensi*

Salivary proteins are directly involved in human-vector contact during biting and, therefore, play a key role in pathogen transmission. The salivary

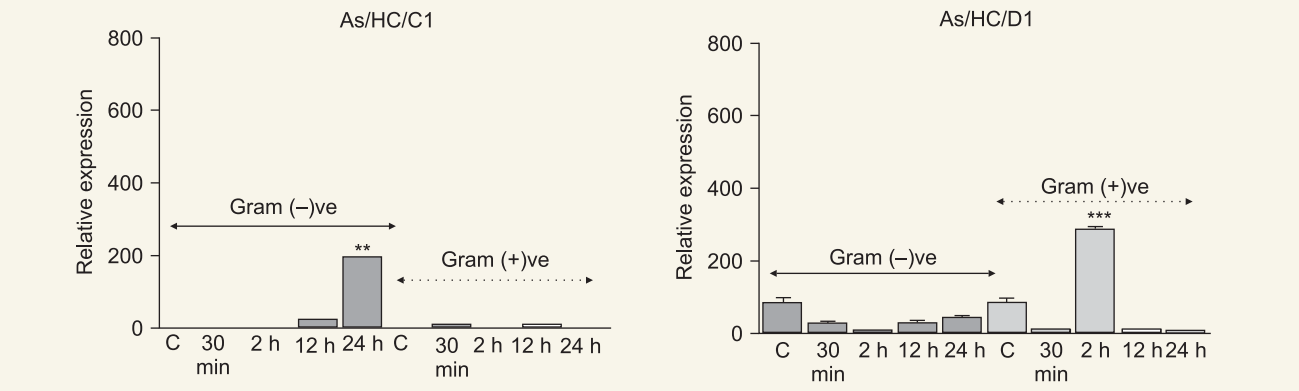


Fig. 15: Systemic response of hemocytes: Exogenous microbial challenge with Gram (–)ve and Gram (+)ve bacteria results a distinct response of AMPs in the hemocytes collected (C: Control, 30 min, 2 h, 12 h, and 24 h) post-challenge from sugar-fed mosquitoes.

proteins can also be involved in insecticide metabolism. Advancement in proteomic tools has opened up unprecedented opportunities to understand host-vector biology, refractory mechanisms and insecticide metabolism pathways. The present study will result in expanding the knowledge about the salivary gland proteins in insecticide susceptible and resistant *An. stephensi* species determined by proteomic approaches. We have carried out in gel digestion of salivary gland sample of insecticide susceptible strain of *An. stephensi* and further subjected to LC/MS/MS analysis. These were further analysed with MASCOT and OMSSA for characterizing proteins or peptides functionally. We have also analyzed protein-protein interactions with string analysis alongwith 1D, 2D electrophoresis of both insecticide resistant and susceptible samples of *An. stephensi* were also carried out. To characterize the salivary proteins in *An. stephensi* (sensitive) 1D electrophoresis and liquid chromatography mass spectrometry (LC/MS/MS) was employed. Function characterization was done using bioinformatics analysis. We identified total 36 known salivary proteins (Table 5) and 124 novel proteins. LC/MS/MS analysis showed that majority of the salivary proteins belong to the categories of signal transduction, regulation of blood coagulation cascade, various energy pathways, feeding, intracellular trafficking and transport, immune properties, etc. (Fig. 16). String network of Thioredoxin 1 and Histone H2A with other protein interactions is also shown in Fig. 17.

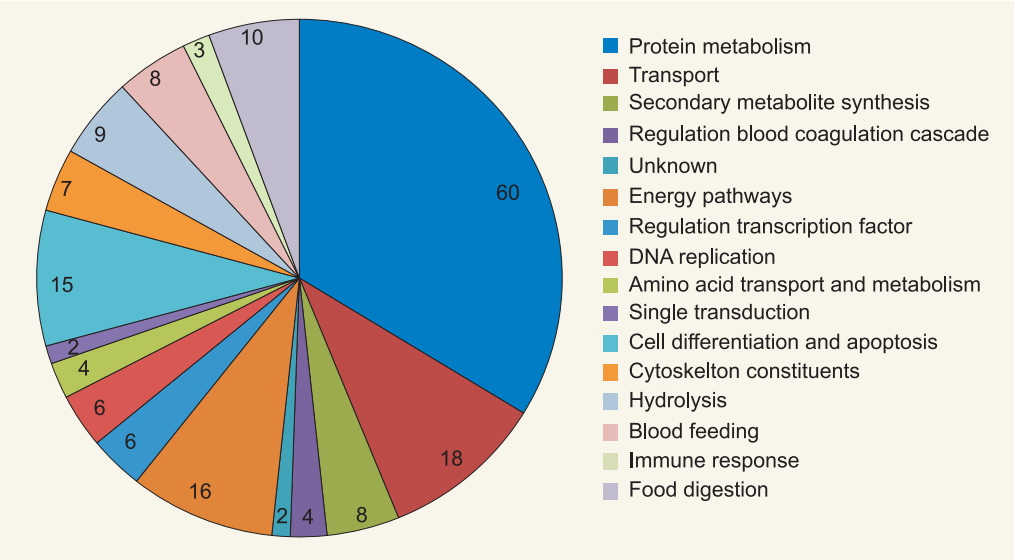
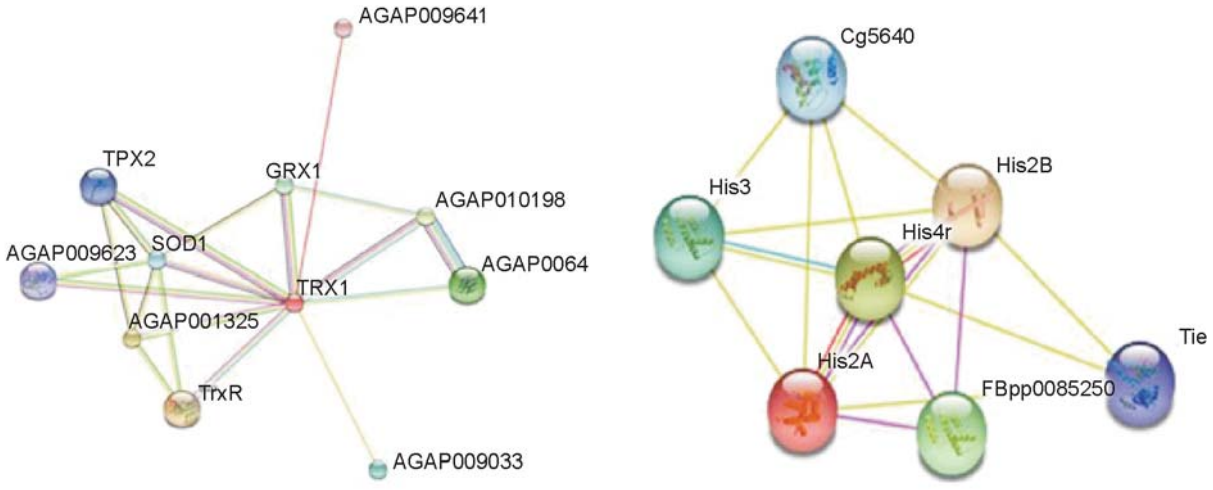


Fig. 16: Functional classification of identified known and novel protein by gel digestion strategy using gene ontology.



(a) Protein Q9NGZ1_ANOGA is shown as TRX1 in the network (Thioredoxin 1).

(b) Protein H2A_DROME is shown as His2A in the network.

Fig. 17: STRING network of protein-protein instructions identified by MASCOT and OMSSA algorithm: (a) Thioredoxin 1; and (b) Histone H2A (Different line colours represent the types of evidence for the association).

Table 5. A catalogue of known proteins identified by using in-gel digestion strategy and LC/MS/MS using MASCOT algorithm

S.No.	Accession No./ Vector base accession No.	Protein	Molecular weight	Peptides	Calculated PI	Sequence coverage %	Domain/Function
1.	GI: 37201975	GE rich salivary protein	15214	8	5.15	56	No conserved domain
2.	GI: 15718081	D7 protein	36396	14	8.79	34	Protein binding/ Glycoprotein
3.	GI: 29501536	SG1D Salivary protein precursor	46811	10	9.38	23	No conserved domain
4.	ASTM013042-PA	G1 family long form salivary protein 3	45829	9	6.85	22	No conserved domain
5.	GI: 27372941	Putative salivary protein SG1C	44292	8	6.73	16	No conserved domain
6.	ASTM005454-PA	Tropomyosin 2	30832	5	4.82	17	No conserved domain
7.	GI: 27372911	Salivary apyrase	64248	8	6.77	12	No conserved domain
8.	ASTM006960-PA	Alpha amylase	67923	2	5.27	4	Alpha amylase domain/ Catalytic activity
9.	ASTM007102-PA	Salivary peroxidase	67504	6	8.75	10	Heme binding/ Peroxidase activity
10.	ASTM006933-PA	Myosin heavy chain	135972	5	5.2	4	Motor activity
11.	GI: 27372939	Putative salivary protein SG1A	19725	2	4.94	11	Nucleotide transport and metabolism
12.	ASTM007096-PA	Sulfate transporter protein	88306	2	9.27	2	Transport
13.	ASTM014183-PA	Hypothetical protein/ ATP synthase alpha subunit	80465	2	9.81	2	ATPase enzyme
14.	GI: 27372929	Putative salivary protein SG1B	48120	2	–	4	No conserved domain
15.	GI: 29501376	Short D7-4 salivary protein precursor	18412	1	–	7	No conserved domain
16.	GI: 339649279	Glutathione-S-transferase E4	25076	1	6.53	5	No conserved domain
17.	ASTM000527-PA	Hypothetical protein	66553	1	8.09	1	Uncharacterized
18.	GI: 27372895	Salivary antigen-5 related protein	28974	2	9.05	8	CTD-interacting domain (Polypeptide binding)
19.	ASTM017293-PA	Porin (voltage dependent anion selective channel)	42907	1	9.12	1	Anion transport
20.	ASTM005798-PA	Elongation factor1 alpha 1	59939	1	8.71	1	GTP binding
21.	ASTM010693-PA	Short chain dehydrogenase	27502	2	7	8	Oxidoreductase function
22.	ASTM014983-PA	Histone H2A	13355	2	10.72	11	Nucleosome assembly/ DNA binding
23.	ASTM009772-PA	Actin	41852	2	5.36	7	Actin protein HSP70/ ATP binding
24.	GI: 29501528	TRIO salivary gland protein precursor	44013	3	7.01	3	SCP-like extracellular protein domain
25.	ASTM015472-PA	Hypothetical protein	21893	1	8.29	10	Uncharacterized
26.	ASTM005771-PA	Peptide N-glycanase	85828	2	8.77	1	Signal transduction
27.	ASTM009670-PA	Prefoldin subunit	18939	1	5.66	4	Post-translational modification, protein turnover and chaperones
28.	ASTM017244-PA	JmjC domain-containing histone demethylation protein	48571	1	9.07	2	Protein binding
29.	ASTM000815-PA	Hypothetical protein	181818	2	8.47	1	Signal transduction
30.	ASTM001058-PA	Hypothetical protein (Rhoeffector protein)	15505	1	9.16	5	Signal transduction
31.	GI: 373880220	Calreticulin	46514	2	4.37	2	Unfolded protein binding
32.	ASTM007078-PA	Axonemal dynein light chain	28779	1	5.5	2	Motility
33.	ASTM017761-PA	Type-2 keratin	56560	2	7	3	Cell division and chromosome partitioning
34.	ASTM012154-PA	Hypothetical protein	7046	1	6.9	11	Uncharacterized
35.	GI: 380865625	BZIP1 OS	41596	3	8.85	4	DNA binding
36.	GI: 66863200	Putative reverse transcriptase (Fragment)	13461	1	9.93	4	RNA-directed DNA polymerase activity

2D electrophoresis of *An. stephensi* insecticide sensitive/resistant samples analysis is in process (Fig. 18). About 168 spots were clearly identified and total 44 matches and 5 annotations were found and analysed by MALDI-ToF between resistant and susceptible strains with the help of Protein PAGE software. Spectra of 5 annotations are clearly shown in Fig. 19. We are in the process of making catalogue of proteins that are differentially expressed in insecticide resistant and sensitive proteins of *An. stephensi*.

These initial studies with LC/MS/MS comprise molecular and biologically characterized proteins from salivary glands of *An. stephensi* identified proteins that can be used to understand the concept of feeding, insecticide resistance mechanisms, immunological properties and various aspects of vector-parasite-host interactions for development of novel control strategies for improving host protection against malaria.

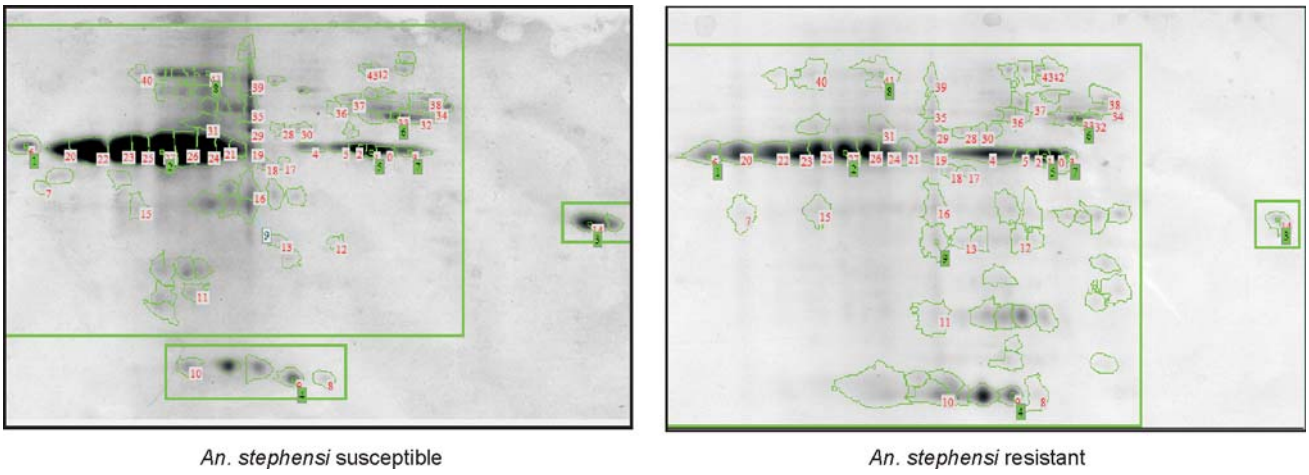


Fig. 18: 2D spots (differentially expressed) selected among *An. stephensi* (susceptible and resistant species) with the help of protein PAGE software.

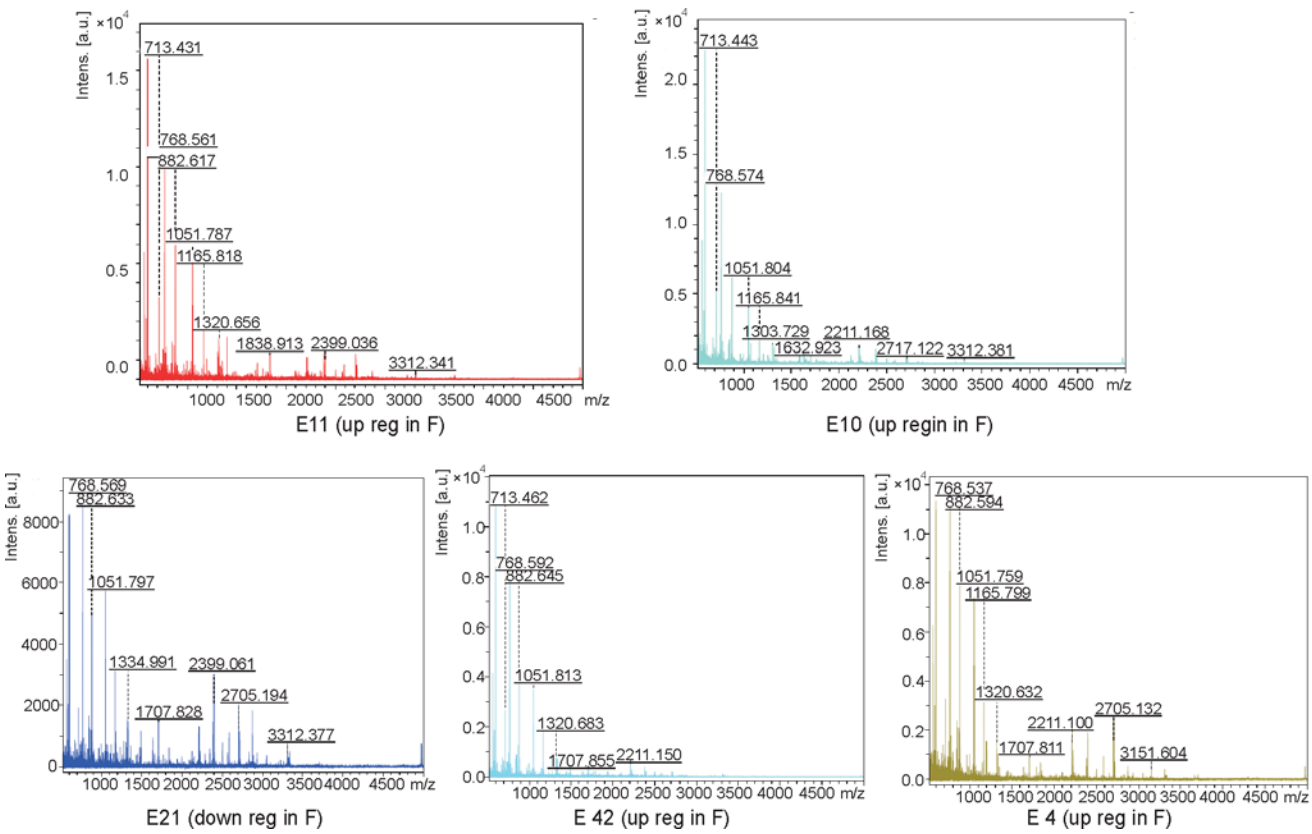


Fig. 19: Analysis of 5 annotated sequences.

1.1.9 Proteogenomic analysis of *Anopheles culicifacies* using high resolution mass spectometry (LC/MS/MS)

Proteome analysis of invasive stages in the midgut and salivary glands of *Anopheles* mosquitoes is highly specialized and may reveal by the presence of a high number of species-specific proteins. In our study, we plan to provide a first detailed baseline data for proteomic analysis of the midgut and salivary glands of the adult female *An. culicifacies* species. We speculate that mass spectrometry (MS) will provide valuable proteomic catalogue database that may be used to validate genome annotations and to discover novel functional proteins in a high-throughput manner. For preliminary studies, dissection of midgut and salivary gland tissues of *An. culicifacies* species A mosquitoes (susceptible) for proteomic studies were carried out. However, due to limited availability of species B (refractory) dissection of tissues of *An. culicifacies* species B is in process. After collection of species B sample, we will process the samples soon because we have to process both the samples simultaneously for proteomic studies.

1.2 Vector Control

1.2.1 Insecticidal efficacy of imidacloprid, a neonicotinoid in the control of mosquitoes

Imidacloprid is a systemic, chloro-nicotinyl insecticide used in agriculture for soil, seed and foliar treatments for the control of sucking insects. The chemical acts by interfering with the transmission of stimuli in the insect nervous system. According to the primary site of action (main group) imidacloprid falls in the category of nicotinic acetylcholine receptor (nAChr) agonists based on the Insecticide Resistance Action Committee—Mode of Action (MOA) Classification No. 4A (*http://www.irac-online.org*). A laboratory study was undertaken to assess the insecticidal and larvicidal activities of imidacloprid against laboratory strains of insecticide susceptible and resistant *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. Adulticidal activity was tested using topical application method and larval bioassays were conducted for determining the lethal doses following standard WHO methods.

Comparative results of topical application of different concentrations on adult mosquitoes are shown in Table 6. The results revealed that DDT-malathion-deltamethrin susceptible for *An. stephensi* strains showed LC_{50} and LC_{90} of 2.217 and 5.78 ng/mg female mosquitoes, respectively. Interestingly, the DDT-malathion-deltamethrin resistant strain of *An. stephensi* showed lower LC_{50} and LC_{90} than the susceptible strains. DDT-malathion-resistant and deltamethrin-tolerant strains of *Cx. quinquefasciatus* showed lower LC_{50} and LC_{90} values than the susceptible *An. stephensi* strains. *Aedes aegypti* showed LC_{50} and LC_{90} of 0.558 and 6.135 ng/mg of female mosquitoes, respectively. The order of efficacy of imidacloprid (LC_{50}) against the three species of mosquitoes was *Cx. quinquefasciatus* < *Ae. aegypti* < *An. stephensi*. The LC_{50} recorded in case of DDT- malathion-deltamethrin resistant *An. stephensi* was ~7 fold lesser than that of the DDT-malathion-deltamethrin susceptible strains. The results also indicate that the adulticidal efficacy of imidacloprid was more pronounced in insecticide resistant strains than the susceptible strains.

Table 6. Results of topical application of imidacloprid against different mosquito strains

Species (No.)	LC_{50} ng/mg Female mosquito	LC_{90} ng/ mg Female mosquito	Chi-square Pearson's goodness of fit	*p*-value
An. stephensi—Nadiad strain DDT-malathion-deltamethrin susceptible (469)	2.217	5.78	1.837	0.398
An. stephensi—Sonepat DDT-malathion-deltamethrin susceptible (464)	2.216	5.781	1.837	0.399
An. stephensi—Goa DDT-malathion-deltamethrin resistant (248)	0.297	1.847	6.701	0.082
Cx. quinquefasciatus DDT-malathion resistant and deltamethrin tolerant (368)	0.016	6.688	0.730	0.948
Cx. quinquefasciatus DDT-malathion resistant and deltamethrin-tolerant (238)	0.009	12.17	0.919	0.821
Ae. aegypti DDT-malathion-deltamethrin susceptible (445)	0.558	6.135	27.364	0.0002

Table 7. Results of larval susceptibility tests with imidacloprid (72 h mortalities)

Species (No.) Susceptibility status*	LC_{50} mg/l (95% CI)	LC_{90} mg/l (95% CI)	Chi-square Pearson's goodness of fit & p-value
An. stephensi SS strain (Nadiad) Fenthion-temephos-malathion susceptible (725)	0.049 (0.017–0.09)	0.086 (0.39–0.525)	$\chi^2 = 29.304$; p = 0
An. stephensi Black brown (Sonepat) Fenthion-temephos-malathion susceptible (685)	0.015 (0.005–0.028)	0.03 (0.002–0.3)	$\chi^2 = 33.893$; p = 0
An. stephensi (Goa) Fenthion-malathion susceptible and temephos resistant (632)	0.066 (0.016–0.11)	0.77 (0.048–0.5)	$\chi^2 = 7.235$; p = 0.124
Cx. quinquefasciatus Fenthion-temephos susceptible and malathion resistant (638)	0.02 (0.001–0)	0.07 (0.012–0.29)	$\chi^2 = 0.741$; p = 0.98
Cx. quinquefasciatus Fenthion-temephos susceptible and malathion resistant (480)	0.13 (0.10–0.16)	0.41 (0.035–0.56)	$\chi^2 = 0.491$; p = 0.921
Ae. aegypti Fenthion-malathion susceptible and temephos tolerant (548)	0.21 (0.01–0.214)	0.27 (0.2–0.29)	$\chi^2 = 4.634$; p = 0.327

*WHO criterion of susceptibility — Susceptible: 98–100% mortality; Verification required/tolerant: 81–97% mortality; Resistant: <80 mortality. Figures in parentheses are 95% confidence intervals.

The comparative results of larval tests of imidacloprid against III and early IV instar larvae of different strains of mosquito species are shown in Table 7. The results showed that the LC_{50} values against the larvae of *An. stephensi* found susceptible to fenthion, malathion and temephos ranged from 0.015 to 0.049 mg/l, whereas in case of *An. stephensi* which is resistant to temephos, the LC_{50} of imidacloprid was 0.066 mg/l, which is higher than that recorded for susceptible strains. The larvicidal efficacy was more against the *An. stephensi* strains (both susceptible and resistant) when compared to *Ae. aegypti* and *Cx. quinquefasciatus*. In case of two *Culex* strains, the LC_{50} values were not comparable. The larvicidal efficacy of imidacloprid is in the order of *An. stephensi* < *Cx. quinquefasciatus* < *Ae. aegypti*. Insecticide resistant strains showed lower LC_{50} values than the susceptible strains

1.2.2 Insecticidal efficacy of second generation neonicotinoids, namely thiacloprid, acetamiprid, nitenpyram, dinotefuran, sulfxaflor and thiamethoxam in the control of mosquito vectors

The nicotinoids are a new class of compounds with neurotoxic action unique from other insecticidal compounds which are generally used in the national programmes, namely organophosphates, organochlorines, carabamates and synthetic pyrethroids. The nicotinoids are segregated into nitroquanidines, nitromethylenes, chloronicotyinyles, and neonicotinoids. These are more toxic to insects than mammals due to differences in their mode of action in their binding site interactions at the corresponding nicotinic acetylcholine esterase receptors. The present study was undertaken to investigate the insecticidal efficacy of second generation neonicotinoids in the control of both insecticide susceptible and resistant strains of *An. stephensi*, *Cx. quinquefasciatus*, and *Ae. aegypti*. During the year thiamethoxam and thiacloprid could be procured and the compounds were tested for adulticidal and larvicidal efficacy. Results of larval bioassays against thiamethoxam was in the order of *An. stephensi* < *Ae. aegypti* < *Cx. quinquefasciatus*. Topical assays are in progress.

1.3 Insecticide Resistance

1.3.1 Field studies on persistence and reversal of insecticide resistance in *Anopheles culicifacies* population after sequential withdrawal of insecticides from indoor residual spray in District Tapi (part of erstwhile Surat), Gujarat: A retrospective and prospective study

A study was undertaken in Tapi district (earlier part of Surat district) with the objectives: (a) to determine the persistence of resistance/susceptibility in time and space in *An. culicifacies* population to DDT, malathion and deltamethrin after their sequential withdrawal from indoor residual spray in the field; (b) to determine the impact of migration of the mosquito populations on the persistence of resistance or susceptibility

in the field (if foci of resistance/susceptibility exist with more than 15% variation between the areas); and (c) to elucidate biochemical/molecular mechanisms of resistance in *An. culicifacies* populations in the study area. Surveys were conducted in Tapi district and susceptibility tests in five villages, namely Kasav, Ringerkutch, Antapur, Palsava and Serula. In three villages namely, Kasav, Ringerkutch and Antapur, regular indoor residual spray operations were carried out in the past few years. In other two villages namely, Palsava and Serula insecticide spray operations could not be carried out in the past 2–3 years. The results showed that *An. culicifacies* population in both insecticides sprayed and unsprayed villages of Tapi district was resistant to DDT (4%), malathion (5%) and deltamethrin (0.05%). The difference in average mortalities recorded in sprayed and unsprayed villages was <15% in the case of three classes of insecticides tested and was found below the criterion considered for the study. Hence, the study areas were not found suitable for conducting field studies. The study has been concluded.

1.3.2 Monitoring of insecticide resistance of malaria vectors in India

The study was carried out in 13 states, including 7 north-eastern states comprising of about 156 districts. Based on the results of the survey carried out in the areas of the selected districts, the following observations are made.

Anopheles culicifacies, the major vector for malaria influencing the rural plains of the country was tested for susceptibility to insecticides, DDT, malathion and deltamethrin that are in use in vector control in the country, in different districts of the states of Andhra Pradesh, Chhattisgarh, Jharkhand, Madhya Pradesh, Odisha and West Bengal. The species was found resistant to DDT in all the above states and found resistant to malathion and deltamethrin in Andhra Pradesh and Chhattisgarh, while in other states it was mostly tolerant.

The susceptibility status of another important malaria vector, *An. fluviatilis*, prevalent in foothills and forested regions of the country was determined in Chhattisgarh, Jharkhand and Odisha states. This species was found resistant to DDT in

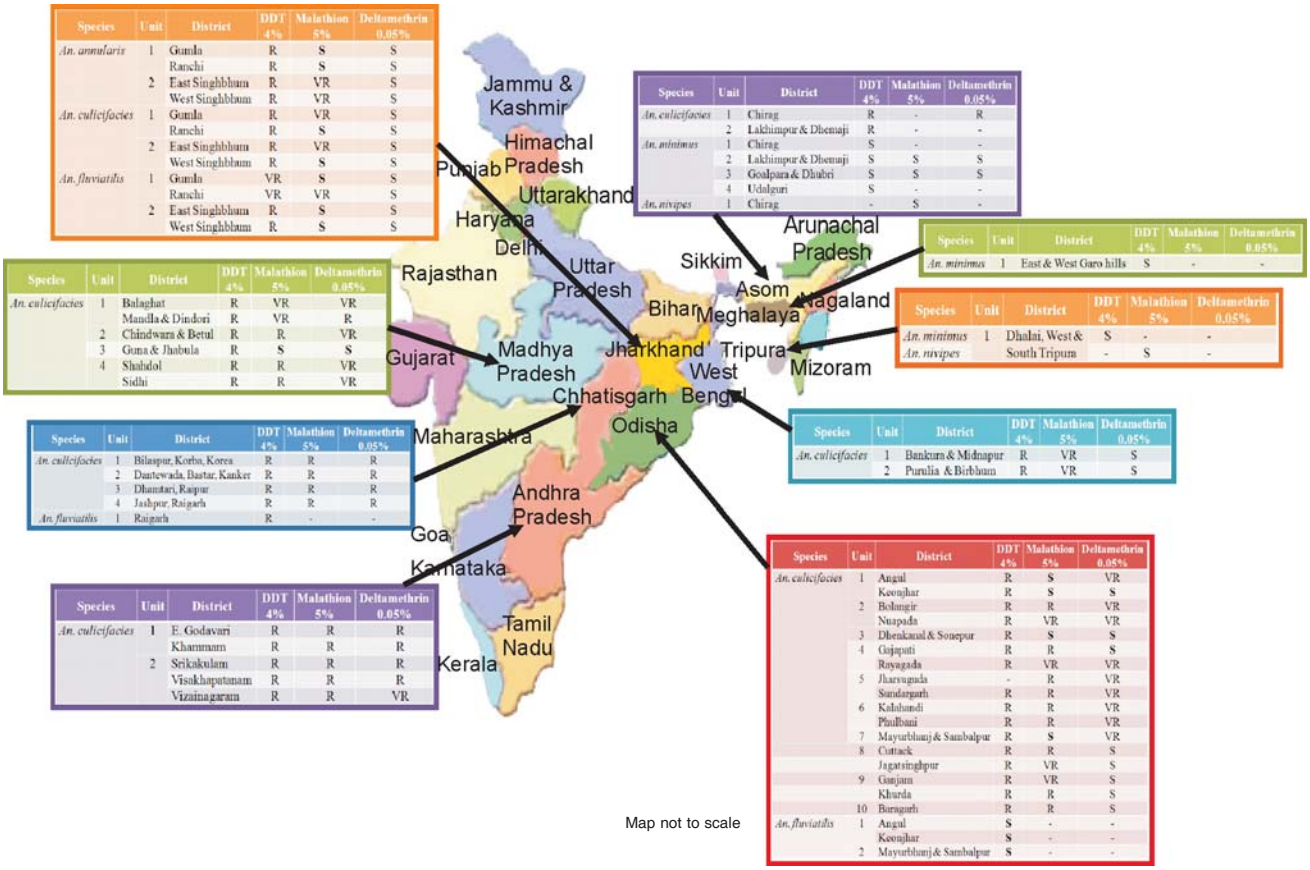


Fig. 20: Insecticide resistance status of anophelines in different states of India.

Chhattisgarh, tolerant/resistant in Jharkhand but was susceptible in Odisha. In Jharkhand, this species was mostly susceptible to malathion and deltamethrin.

Other anophelines, *An. annularis*, the secondary malaria vector in Jharkhand was found resistant to DDT, tolerant to malathion and susceptible to deltamethrin. *Anopheles minimus*, the major vector of malaria in the northeastern region was susceptible to DDT in the study areas of Asom, Meghalaya and Tripura. While in Asom, it was also susceptible to malathion and deltamethrin. *Anopheles nivipes*, another important vector in north-eastern region was found susceptible to malathion in Asom and Tripura. The studies in north-eastern states are not conclusive owing to prevalence of mosquitoes in very low densities, hence, work in some other north-eastern states could not be conducted. In some areas of Asom, during these studies *An. culicifacies* was encountered in very low densities and was found resistant to DDT as in other states (Fig. 20).

Thus, this study clearly brought out that in the mainland states there is a trend to develop pyrethroid-resistance in *An. culicifacies* in areas where pyrethroids are being used.

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

Initially, the diagnostic doses for deltamethrin using indigenous bottle (narrow mouth 200 ml capacity with bakelite screw cap) was determined (10 µg/bottle) on laboratory-reared *An. stephensi*. Further studies on validation with the diagnostic doses for determination of shelf life and storage condition of insecticide coated bottles have been done. Tests were carried out with bottles stored at different temperatures 4, 25, 30, 35, and 40°C. The bottles have shown efficacy up to 5 days against susceptible mosquitoes in continuous exposure with shelf life of 7 days stored between 25 and 40°C. Studies will be carried out to validate the results and to assess the feasibility for use of this method in the field conditions. Comparative evaluation will be done with the standard WHO susceptibility tests using insecticide-impregnated papers.

1.3.4 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 (WHO Project)

The project is part of a 5-country WHO Global Projects and was sanctioned in October 2012. The study is being undertaken in CHC Keshkal in Kondagaon district of Chhattisgarh state. The study area is comprised of 105 villages. The first year of the project 2012–13 includes baseline studies to generate data on various indicators to enable to design the study. Baseline studies include census of the villages, generate information to allocate the interventions, entomological and epidemiological data and other aspects relevant to the transmission of the disease. Various confounding factors that may influence the transmission of disease such as topographical features, socioeconomic and cultural practices in the community will also be considered during the design of the study to assess the impact. Combination interventions (IRS and LLINs) will be assigned to study clusters appropriately by randomization. The three-year intervention phase will be to assess the impact of the combination of interventions on transmission of malaria in the presence of multiple-insecticide-resistant malaria vectors, mainly *An. culicifacies*. Recruitment of staff has been done and training imparted on different entomological and parasitological techniques pertinent to the proposed study. Baseline data on insecticide susceptibility status of *An. culicifacies*, the principal malaria vector and epidemiological data in the study area are being collected. Standardization of molecular and biochemical techniques for characterization of resistance has been initiated. The study is in progress.

1.3.5 Establishment of WHO Collaborating Centre for Phase-I testing and evaluation of Public Health Pesticides

The World Health Organization Pesticide Evaluation Scheme (WHOPES) conducted three assessments in September 2008, November 2009 and May 2011 to assess the capacity of the insecticide and Insecticide Resistance Laboratory at National Institute of Malaria Research. Assessments were made on different aspects related to infrastructure facilities and through direct inspection, and review of the methodology and management of the laboratory investigations. WHO

Dr VM Katoch, Director General, ICMR inaugurating Insecticide Testing Laboratory at NIMR, New Delhi.

standard methods for Phase-I testing and evaluation of adulticides, larvicides and LNs have been standardized and are in regular use. Standard operating procedures and manual for the testing procedures was approved by the WHOPES. WHO has designated the Insecticide Testing Laboratory at NIMR as Collaborating Centre for 'Phase-I Testing and Evaluation of Public Health Pesticides' in December 2012 for a period of 4 years and designated Dr K. Raghavendra as Scientist Incharge of this facility. This facility will be the first of its kind in India and in the WHO Southeast Asia region and second such facility globally. The collaborating centre was formally inaugurated by Dr V.M. Katoch, Secretary (DHR, Govt. of India) and Director General, ICMR on 11 April 2013.

1.3.6 Molecular basis of knockdown resistance (*kdr*) in *Anopheles stephensi*

Whole voltage-gated sodium channel (VGSC) the target site of action for DDT and pyrethroids, of *An. stephensi* was sequenced.

The distribution of *kdr* alleles in India was mapped. It was noted that L1014F is found only in Raipur, whereas L1014S found only in northern India. No mutation was found in southern India.

Malaria control in India relies mainly on insecticide-based vector control. Currently, there are three insecticide groups being used in India, i.e. DDT, pyrethroids and malathion. DDT and pyrethroid group of insecticides are neurotoxins which act on the VGSC. One of the mechanisms of resistance against these two major insecticide groups is knockdown resistance (*kdr*) which is due to alteration in the VGSC which results in reduced sensitivity to these insecticides.

Sequencing of whole voltage-gated sodium channel transcript

Earlier we reported the presence of two *kdr* mutations L1014F and L1014S mutations in *An. stephensi*. In our attempt to find out any other mutations, our primary objective was to first characterize the whole VGSC of this vector. Total RNA was isolated from mosquito and cDNA was synthesized. The full VGSC transcript was amplified using two primers from UTR regions. Sequencing was done using primer-walking strategy. The sequences were aligned with genomic sequence of VGSC downloaded from VectorBase to delineate exon-intron boundaries. The whole transcript comprise of 6414 nucleotide bases (2138 amino acids).

Allelic frequencies of *kdr* alleles in different populations of *Anopheles stephensi*

Anopheles stephensi mosquitoes were collected from Alwar (Rajasthan), NCR (National capital region), Raipur (Chhattisgarh) and Mangalore and Mysore (Karnataka), Goa, and Chennai (Tamil Nadu). The mosquitoes were genotyped for the presence of L1014F and L1014S mutations using PCR assays developed. The results of genotyping of *An. stephensi* mosquitoes for *kdr* alleles (1014F and 1014S), distribution of different genotypes and allelic frequencies for seven Indian populations are provided in Table 8. Out of seven populations studied, *kdr* alleles were found in three populations, i.e. NCR, Alwar and Raipur. No *kdr* mutation was recorded in south Indian population, i.e. Goa, Mysore and Chennai and Mangalore. In Alwar and NCR, both the *kdr* alleles were present with preponderance of 1014S showing a high allelic frequency of 0.264 to 0.330. The frequency of

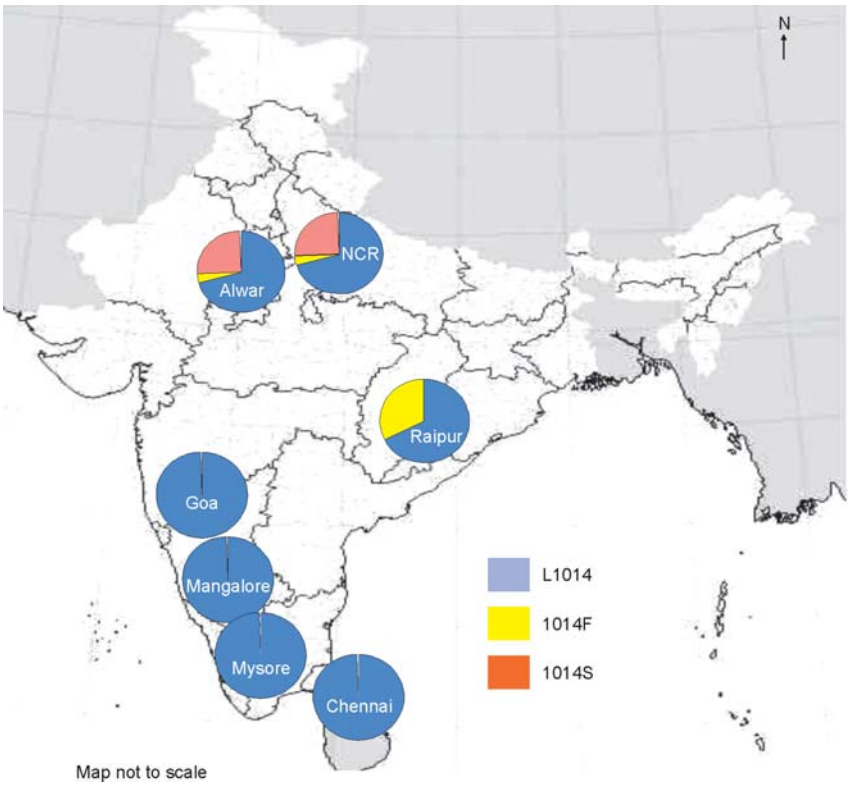


Fig. 21: Frequency distribution of different *kdr* alleles in different populations of *An. stephensi* in India.

1014F was too low, i.e. 0.033 to 0.06 as compared to 1014S. In Raipur, only one *kdr* mutation 1014F was recorded with a comparatively high frequency, i.e. 0.329.

The distribution and diagrammatic representation of allelic frequencies of different L1014-alleles (in pie diagram) are displayed in Fig. 21.

The expected and observed heterozygosity for 7 populations are provided in (Table 8) which didn't differ significantly ($p > 0.05$) in any population except NCR. Thus, the different genotypes in all the populations were as per expectation of the Hardy-Weinberg equilibrium.

1.4 Other Studies

1.4.1 Surveillance and control of *Aedes aegypti*, vector of dengue and chikungunya, using attracticide (oviposition pheromone in combination with insect growth regulator) at Delhi, Bengaluru and Kerala

The study was carried out in three places, viz.

Table 8. Allelic frequencies of 1014L and 1014S in different populations of *An. stephensi* in India

Locality	n	Genotypes						Allelic frequencies			HWE parametres		
		L/L	L/F	L/S	F/S	F/F	S/S	L1014	L1014F	L1014S	H_O	H_E	p
NCR	32	17 (0.53)	4 (0.12)	1 (0.03)	0	0	10 (0.31)	0.61	0.06	0.330	0.15625	0.52530	0
Alwar (Rajasthan)	217	133 (0.613)	11 (0.051)	87 (0.401)	6 (0.028)	0	22 (0.101)	0.703	0.033	0.264	0.4015	0.4360	0.4101
Raipur (Chhattisgarh)	44	17 (0.386)	25 (0.568)	0	0	2 (0.045)	0	0.670	0.329	0	0.5681	0.4469	0.0923
Mangalore (Karnataka)	129	129 (1)	0	0	0	0	0	1	0	0	0	0	1
Mysore (Karnataka)	33	33 (1)	0	0	0	0	0	1	0	0	0	0	1
Chennai	101	101 (1)	0	0	0	0	0	1	0	0	0	0	1
Goa	136	136 (1)	0	0	0	0	0	1	0	0	0	0	1

Delhi, Bengaluru (Karnataka) and Alappuzha (Kerala). The results of the study are given below:

Delhi

The study was initiated at Delhi in the month of April 2011 and completed in March 2012. A total of 945 ovitraps, 315 each in experimental, control and water control were placed in 315 houses of 5 localities, i.e. Akshardham temple, Lodhi Colony, Pandav Nagar, Ganesh Nagar and Sewa Nagar. Overall positivity in experimental and control ovitraps revealed that out of 147 found positive, 84 (57.14%) were positive in experimental, 47 (31.97%) in control and 16 (10.88%) in water ovitraps (Fig. 22). Egg collection data revealed that out of 6081 egg collected, 3877 (63.76%) eggs were collected from experimental ovitraps, 1584 (26.05%) eggs were collected from control and 620 (10.20%) were collected from water ovitraps (Fig. 23).

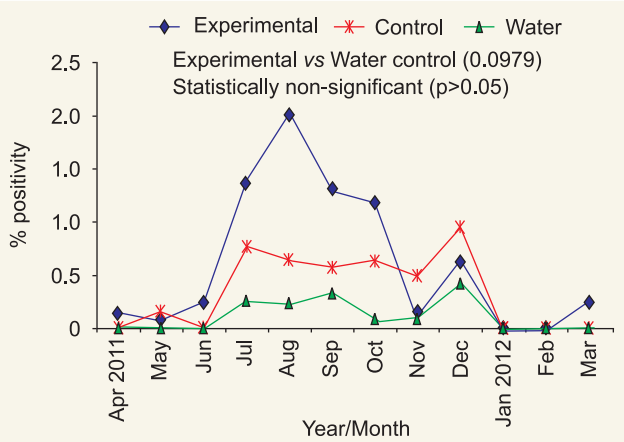


Fig. 22: Month-wise positivity of experimental, chemical and water control ovitraps in Delhi.

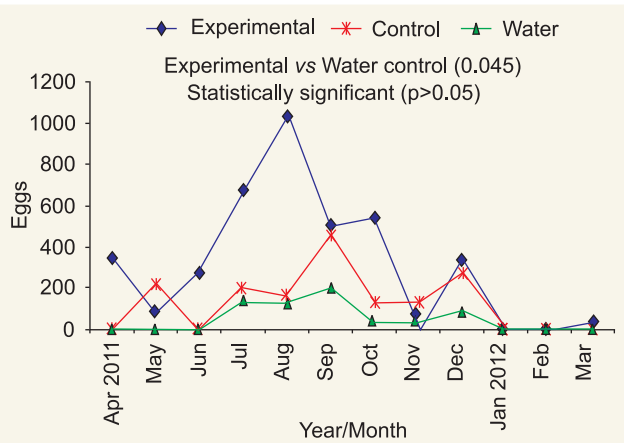


Fig. 23: Month-wise eggs collected from experimental, chemical and water control ovitraps in Delhi.

Alappuzha (Kerala)

The study was initiated at Alappuzha district of Kerala in the month of June 2011 and completed in May 2012. A total of 1200 ovitraps (400 each in experimental, control and water) were placed in 227 houses of 2 localities, i.e. Kadakkarapally and Vettackal. Overall positivity in experimental and control ovitraps revealed that a total of 8179 ovitraps were found positive, out of which 3154 (38.56%) were experimental, 2365 (28.91%) were control and 2660 (32.52%) were water ovitraps (Fig. 24). Egg collection data revealed that out of 169,446 eggs collected, 77,757 (45.89%) eggs were collected from experimental ovitraps, 39,466 (23.29%) eggs were collected from control and 52,223 (30.82%) were collected from water ovitraps (Fig. 25).

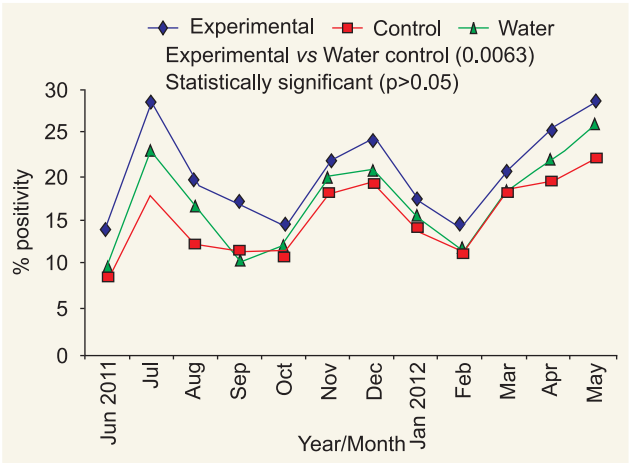


Fig. 24: Month-wise positivity of experimental, chemical and water control ovitraps in Kerala.

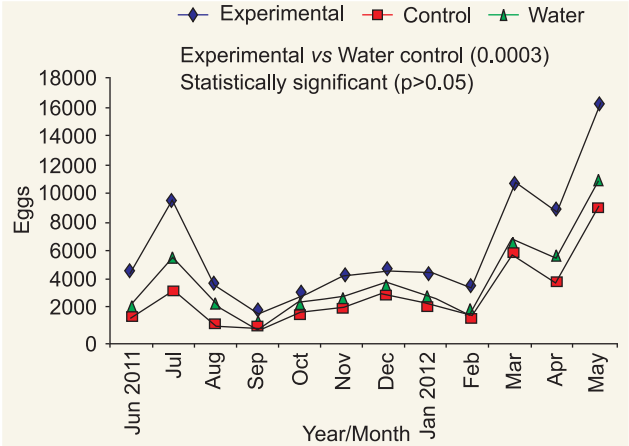


Fig. 25: Month-wise eggs collected from experimental, chemical and water control ovitraps in Kerala.

Bengaluru (Karnataka)

The study was carried out in two localities, viz. Modi Garden and Sanjay Gandhi Nagar in Bengaluru City in month of May 2011 and completed in June 2012. A total of 360 houses, i.e. 180 each in both the localities were selected for placement of 780 ovitraps (180 each in experimental and control, and 30 in water ovitraps). Month-wise positivity in experimental and control ovitraps revealed that a total of 761 ovitraps were found positive, out of which 414 (54.40%) were experimental, 339 (44.55%) were control were ovitraps and 8 (1.05%) were water ovitraps (Fig. 26). Egg collection data revealed that out of 27,659 eggs collected, 16,382 (59.23%) were collected from experimental, 11,130 (40.24%) were collected from control and 147 (0.53%) were collected from water ovitraps (Fig. 27).

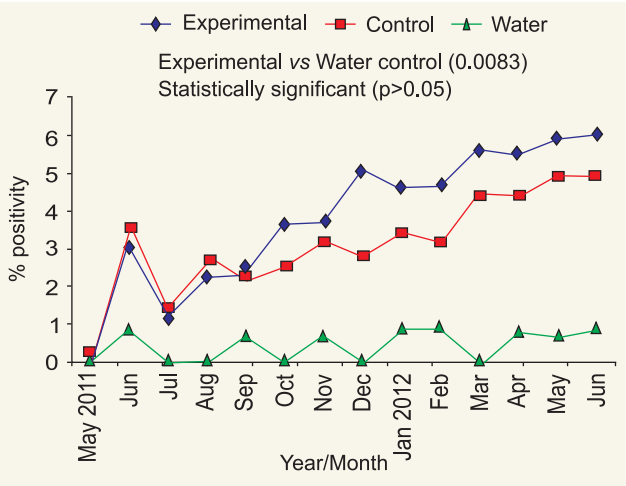


Fig. 26: Month-wise positivity of experimental, chemical and water control ovitraps in Bengaluru.

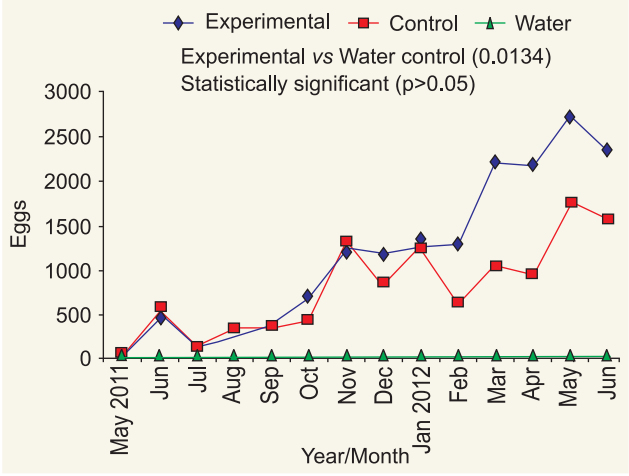


Fig. 27: Month-wise eggs collected from experimental, chemical and water control ovitraps in Bengaluru.

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

In consultation with MHO, MCD, west zone of Delhi was selected as pilot study site for the control of dengue and chikungunya by controlling the *Aedes* breeding in key containers in pre-monsoon season. West zone is the second largest zone in respect of population and consists of 36 wards and about 275 colonies. Number of dengue cases in west zone increased gradually from 2006 to 2011 and from 6th position in 2006 it occupied 2nd position in 2011 in terms of reported dengue cases.

Based on the last 5 years data on dengue cases, localities in west zone were prioritized and survey was started in 20 localities having consistent dengue cases in June. During the survey, *Aedes* breeding was removed from the key containers by using source reduction and by application of

Identification of key containers in pre-monsoon season.

Removal of *Aedes* breeding from the key containers found positive during mapping in collaboration with MCD by using source reduction and Temephos 1% granules.

Source reduction by MCD.

Recording of positive houses.

temephos 1% granules in collaboration with MCD. Positive houses were recorded and localities were re-surveyed at subsequent interval of 15 days and it was observed that container index of the surveyed localities decreased (CI 7.64 during June–July to 2.09 in November), whereas during the same period the time container index of other localities increased (CI 2.2 in January to 30.25 in September).

As per the NVBDCP records, till 23 November 2012, the number of dengue cases in the west zone were 105 and stood at the 8th position in Delhi. It is noteworthy to mention that till date no dengue case has been recorded from surveyed localities.

1.4.3 *Aedes* breeding survey in Delhi

On the request of the Municipal Corporation of Delhi (MCD), New Delhi Municipal Corporation (NDMC) and Delhi Administration, *Aedes* breeding surveys were carried out in Delhi. During 2012, surveys were carried out in about 85 localities of Delhi. Few localities where maximum positive breeding was recorded were: R.K. Puram, PushpVihar, Kalkaji, Khel Gaon, Naraina village, Bijwasan, Nangloi Dairy, Kapashera, Delhi Cantt., Shakur Basti, Gopi Nath Bazar, Palam Colony, Tughlakabad, Goyla Dairy, Masoodpur Dairy, Mahipalpur, Pandav Nagar, Karol Bagh, Delhi University, Buddha Garden, Samalakha, Rajokari, Vasant Kunj, Hari Nagar, Mehrauli, etc. The goverment offices, schools, colleges, nurseries, parks, picnic spots, police stations, bus depots, dispensaries, hospitals, hotels, stadiums, zoo etc. were also surveyed. From the survey, it was found that the breeding was more in the month of September container index (CI) was 25.6, followed by 20.82 in October and 9.86 in November.

The survey also revealed that the breeding was more in peridomestic containers (solid waste, coolers, mud pots, etc.) as compared to domestic containers (OHTs, ground tanks etc.). The results of positivity at different breeding sites in different localities of Delhi were analyzed and monthly locality-wise GIS maps were prepared. The maps of *Aedes* breeding were compared with dengue cases and the information were provided to NVBDCP/MCD/NDMC/Delhi Administration during the fortnightly meeting chaired by the Hon'ble Health Minister and the Secretary, Health and bimonthly meeting chaired by the Commissioner, MCD for necessary action. During 2012, NIMR was given the responsibility to undertake entomological surveillance activities in NDMC areas since NDMC was not having entomological surveillance capacity. Five training workshops were organised to impart training to about 150 participants.

❑

Training course to MCD Laboratory Technicians.

2

Parasite Biology

2.1 Nitric Oxide Synthase (NOS) Activity in Infected Red Blood Cells of *Plasmodium falciparum* : Implication of Aspartic Protease Inhibition

Establishment of parasitic infections is dependent on a delicate and constant interaction between host and parasite, specifically, interactions between the host immune system and molecules released by the parasite or located at the parasite surface. Parasitic organisms have evolved the ability to survive in such hostile environments by evading or neutralizing host defence systems. Aspartic proteases of human malarial parasites are thought to play key role in essential pathways of merozoite release, invasion and host-cell haemoglobin degradation during the intra-erythrocytic stages of their life cycle. The demonstration of the involvement of proteases and NO inhibitors of this activity could constitute a new approach for the treatment of malaria. This postulation can facilitate the use of NO donors and NO releasing drugs in designing of novel approaches for the treatment of malaria in humans.

We initiated our work with the isolation of different stages of *Plasmodium falciparum* culture; ring, trophozoite and schizont stages in culture. We measured aspartic protease and nitrate/ nitrite concentrations in all the three stages of parasite maturation. In other experiments, L-NAME (NO inhibitor) and D-NAME (Inactive isomer) and L-arginine (NO activator) was added (1 mg/ml) to the *P. falciparum* cultures. Different stages of parasite were isolated after these three treatments separately and aspartic protease activity was measured in all the samples. We have also carried out *In silico* docking analysis of aspartic protease 3D structure with NO donor by molecular modelling.

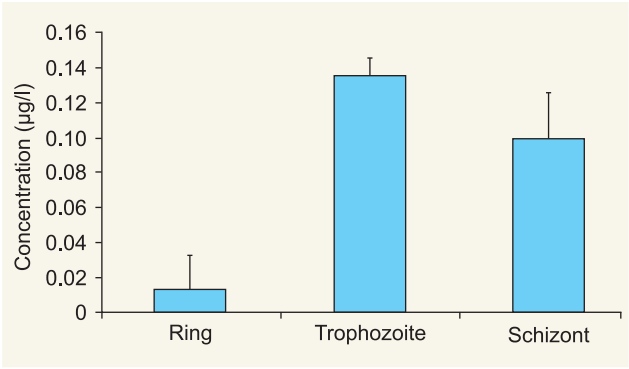


Fig. 1: Aspartic protease assay in ring, trophozoite and schizont stages of *P. falciparum* CQ sensitive strain.

The cytoplasmic proteases are largely involved with rupture and invasion and both these processes are inconspicuous in the ring stage. The trophozoite stage is marked by extensive metabolism and haemoglobin degradation, combined with cellular growth. Aspartic protease was, therefore, found to be higher in trophozoite stage as compared to the schizont stage of the parasite growth (Fig. 1).

In another experiments, we have analyzed the effect of NOS inhibitor (L-NAME), inactive isomer (D-NAME) and activator (L-arginine) on aspartic protease of *P. falciparum*. Our data indicate that increased aspartic protease activity in *Plasmodium* red cell lysates treated with L-NAME that leads to lowering of the nitric oxide concentration in both trophozoite and schizont stages. Lower aspartic protease activity was, however, found in red cell lysates treated with NOS activator which leads to increase in NO concentration (Figs. 2a & b).

During docking studies of aspartic protease enzyme, NO donor (S-nitrosoglutathione) showed central cavity of plasmepsin active site. Docking analysis revealed strong and clear interactions between side chain amino acids and amino acid residues of active pocket with nitric oxide donor molecule (S-nitrosoglutathione) (Fig. 3).

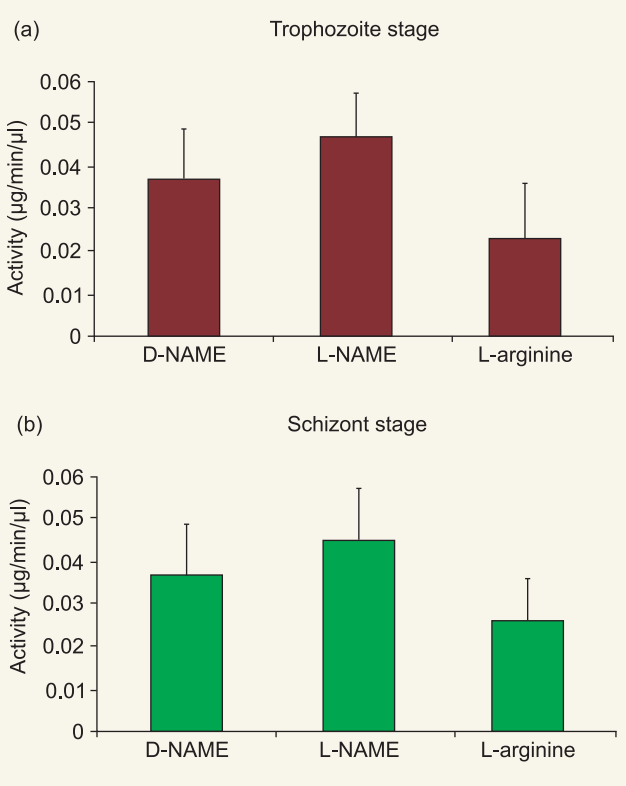


Fig. 2a & b: Effect of D-NAME, L-NAME and L-arginine on aspartic protease activity in: (a) trophozoite, and (b) schizont stages of *P. falciparum* CQ sensitive strain.

Our present results indicate that aspartic proteases are inhibited by NOS donors and activators. *In silico* studies showed inhibition of plasmespin by NO donor. Involvement of proteases and NO to inhibit this activity may constitute a new approach for the mechanism of killing of malaria parasite. The dose dependent effects of NO donor (S-nitroso-glutathione) on aspartic protease activity will be checked and same effect will also be evaluated by aspartic protease gene expression by real time PCR.

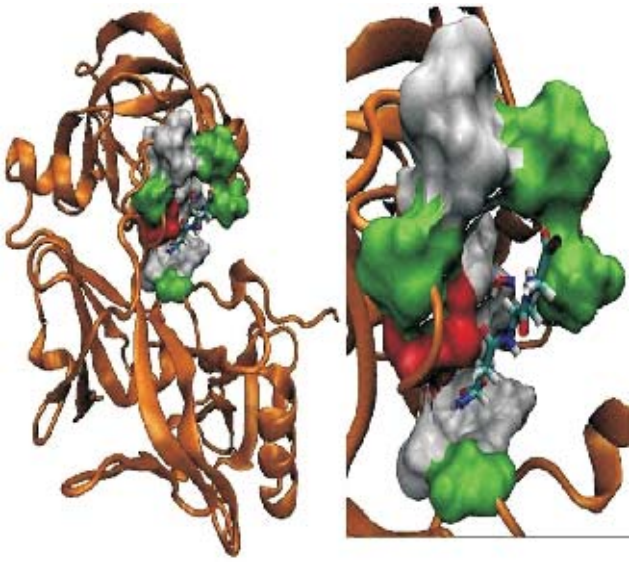


Fig. 3: Docking analysis of plasmepsin with S-nitroso-glutathione.

2.2 The Repertoire Diversity of the *Plasmodium falciparum stevor* Multigene Family in Complicated and Uncomplicated Malaria

The repertoire diversity of *P. falciparum stevor* multigene family is highly diverse in Indian isolates, however, the genetic repertoire from complicated cases was less diverse.

The deep vascular sequestration of parasitized erythrocytes is a central pathological event in falciparum malaria. Variant surface antigens are encoded mainly by three multi-copy gene families, namely *var*, *stevor* and *rifín*. *Var* is one of the most important families that plays a crucial role in antigenic variation and immune evasion. Clinical and epidemiological studies have shown that severe or complicated malaria is manifested in a limited number of patients. This indicates that a subset of these multigene families could be determinants in the manifestation of different malaria phenotypes. Recent studies have indicated the possible role of *stevor* (sub-telomeric variable open read), a multigene family, in erythrocyte invasion, antigenic variation and host cell modification of infected erythrocytes. In this study, we describe the repertoire and diversity of members of the *stevor* multigene family in patients with complicated and uncomplicated malaria in India.

Plasmodium falciparum complicated isolates (n = 8) from Odisha and uncomplicated isolates (n = 13) from Asom, Madhya Pradesh, Odisha, Gujarat, Tamil Nadu and Goa were collected. Members of the *stevor* multigene family were amplified using degenerate PCR primers. Amplified PCR products were cloned and a total of 35 clones per cloning experiment were sequenced. A maximum likelihood phylogeny was constructed in order to understand the genetic repertoire of members of the *stevor* multigene family in severe and non-severe isolates and extent of *stevor* repertoire in Indian isolates.

A range of 21–31 unique sequences was obtained out of 35 clones sequenced for each of the 21 isolates. Nucleotide diversity analysis shows extensive genetic polymorphism that supports the hyper-variability nature of *stevor* multigene family

in the field isolates. The repertoire and diversity of the *stevor* multigene family varied among all the four geographical regions of the Indian subcontinent. The phylogenetic tree analysis showed clustering of sequences from complicated isolates, and suggests that the *stevor* genetic repertoire is less diverse in comparison to uncomplicated isolates.

This study suggests an extensive genetic diversity of *stevor* in Indian *P. falciparum* isolates, however, the genetic repertoire from complicated cases was less diverse. The high degree of *stevor* diversity has important implications for the design of effective antimalarial control measures.

2.3. *Plasmodium vivax* Chloroquine Efficacy Studies Confirm Drug Susceptibility in Chennai, India

The objective of this study was to evaluate CQ sensitivity in a local *P. vivax* population in the urban city of Chennai, Tamil Nadu. We identified no detectable CQ resistance from the results of an *in vitro* CQ inhibition assay or an *in vivo* therapeutic efficacy study, nor any indication using molecular genetic methods that might indicate a pending CQ resistance sweep in the parasite population. Instead, valuable information was gained about the population-level genetic diversity of extent *P. vivax* and the rate of relapsing infections within this study site. Future studies will help resolve the relationship between the parasite genotype, phenotype, and disease outcome (Fig. 4).

Vivax malaria is endemic throughout India, with more than 645,000 cases reported in 2011 by the National Vector Borne Disease Control Programme (NVBDCP) (*http://www.nvbdcp.gov.in/Doc/malaria-situation-79 Nov12.pdf*). This neglected tropical disease constitutes approximately one-half of the total malaria burden in India and as much as 81% of the total burden in south-east Asia. Assessing the *P. vivax* burden in India is further compounded by the continual threat of an emerging chloroquine (CQ) resistant parasite population. *Plasmodium vivax* resistance to CQ is reported in some regions of south-east Asia and western Pacific for decades, while other regions of the world is less evident and represented in the literature mainly in the form of medical case reports.

Samples were collected from patients attending the Central Malaria Laboratory (CML) in George Town, Chennai, in the state of Tamil Nadu, south-east India. From each patient 3–4 ml of venous

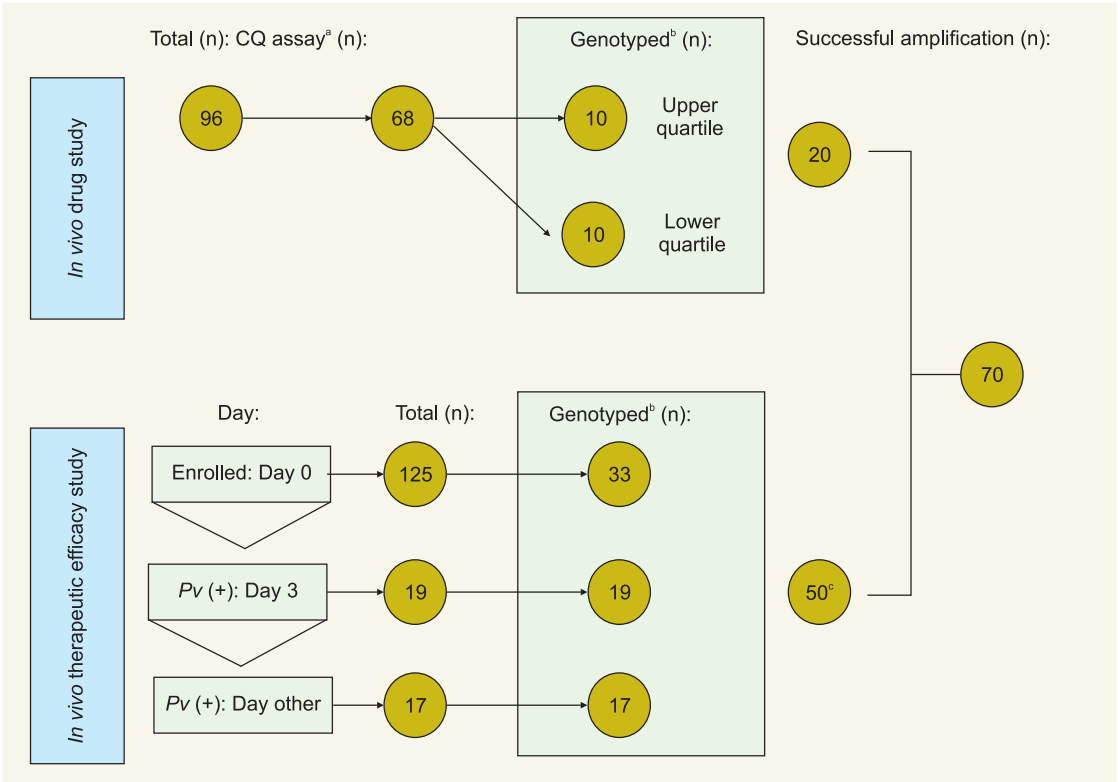


Fig. 4: Schematic design for *in vitro* drug study and *in vivo* therapeutic efficacy study ([a]Assay performed on all isolates, but reproducible for only 68; [b]geno-typed using eight genome-wide microsatellite marker, and sequencing of *Pvmdr 1*; [c]n = 50 due to unsuccessful amplification of nine Day-3 samples).

Table 1. Genotyping results of 10 lower interquartile range (Group 1) and 10 upper interquartile range (Group 2) isolates identified from the chloroquine *in vitro* assay

Isolate classification	No. isolates tested by *in vitro* CQ test	IC_{50} range (ng/ml)	Microsatellite genotyping				*Pvmdr1* haplotypes	
			No. infections genotyped	No. multiclonal infections	No. clones	MOI	$M_{958}Y_{976}L_{1076}$	$M_{958}Y_{976}F_{1076}$
Group 1	51	8–11.9	10	4	14	1.40	8	1
Group 2	17	12–17.5	10	2	12	1.20	9	0
Total	68	8–17.5	20	6	26	1.30	17	1

blood, host white blood cells were removed by CF11 filtration, and the packed infected red blood cells divided as follows: 1 ml was cryopreserved in glycerolyte 57 solution (Baxter, Deerfield, IL), 200 µl was spotted onto filter paper, and 800 µl was used for the *in vitro* drug susceptibility assay as described. Briefly, drug plates were prepared by coating the wells with CQ di-phosphate (Sigma) dissolved in water, serially diluted from a maximum concentration of 514 ng/ml to a minimum of 8 ng/ml, dried in a non-humidified incubator overnight at 37°C, and stored in the dark at 4°C. Batches of plates were tested for efficacy of the drug by using two *P. falciparum* laboratory strains from the NIMR Parasite Bank, MRC2 (CQ sensitive) and RKL9 (CQ resistant). Approximately, 200 µl of a 2% haematocrit blood medium mixture consisting of McCoy's 5A media (Gibco) and 20% AB+human serum were added to each well, including control wells, and patient samples were tested in duplicate. The plates were incubated at 37°C in a candle jar for 36 h, a thick smear prepared for each of the wells, stained with Giemsa or JSB stain, and parasitaemia quantified by microscopy. The number of schizonts per 200 asexual stage parasites was determined for each slide and the result for each drug concentration normalized to the control well. Only healthy schizonts with six or more distinct chromatin dots were quantified. Data were then analysed using non-linear regression analysis (HN-NonLin v.1.1) (*http://www.meduniwien.ac.at/user/harald.noedl/malaria/download.html*), and the 50% inhibitory concentration (IC_{50}) obtained.

2.4 *In vitro* CQ Drug Assay and *in vivo* Therapeutic Study and Genetic Diversity and *Pvmdr1* Genotype of *in vitro* Samples

In all, 96 samples were collected from Chennai (2009–10). *In vitro* assay was performed for 36 h and 68 samples were analysed in duplicate sets and 20 samples were analysed for microsatellite genotyping along with *Pvmdr1* mutations (Table 1).

IC_{50} values showed that all isolates to be in the CQ susceptible range, which varied from 8 to 17.5 ng/ml (M = 9.8 ng/ml, IQR 25% = 8.5 ng/ml, 75% = 12 ng/ml) and were positively skewed (Fig. 5). To determine whether CQ resistance might be an emerging problem in Chennai, we identified isolates having a higher IC_{50} value for additional genetic analysis. We used the interquartile range to group isolates into either Group 1 susceptible (n = 51, IQR <75% = 8 to 11.9) or Group 2 reduced susceptibility (n = 17, IQR <75% = 12 to 17.5), and selected a total of 20 isolates (10 for each group) for further analysis. The mean number of alleles per locus for Group 1 isolates was 5.89 (range = 4 to 9, σ = 1.55) and for Group 2 isolates it was 6.5 (range = 3 to 8, σ = 1.93), and did not differ significantly (p = 0.38, Fisher's exact test).

Next, a 604 bp *Pvmdr1* fragment capturing three non-synonymous mutations ($T_{958}M$, $Y_{976}F$, and $F_{1076}L$) was amplified and sequenced to examine the distribution of specific SNPs that have been associated in other studies with *P. vivax* CQ resistance (Fig. 6).

Successful genotyping of 18 isolates for *Pvmdr1* revealed the mutant $M_{958}Y_{976}L_{1076}$ as the dominant haplotype (n = 17, 94.4%) and $M_{958}Y_{976}F_{1076}$ as the minor haplotype (n = 1, 5.6%). The majority

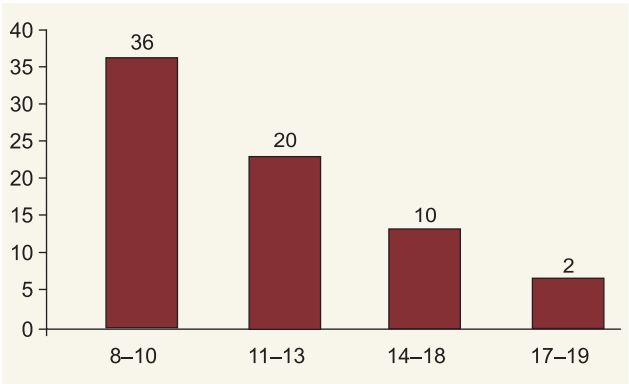


Fig. 5: Frequency distribution of IC_{50} value.

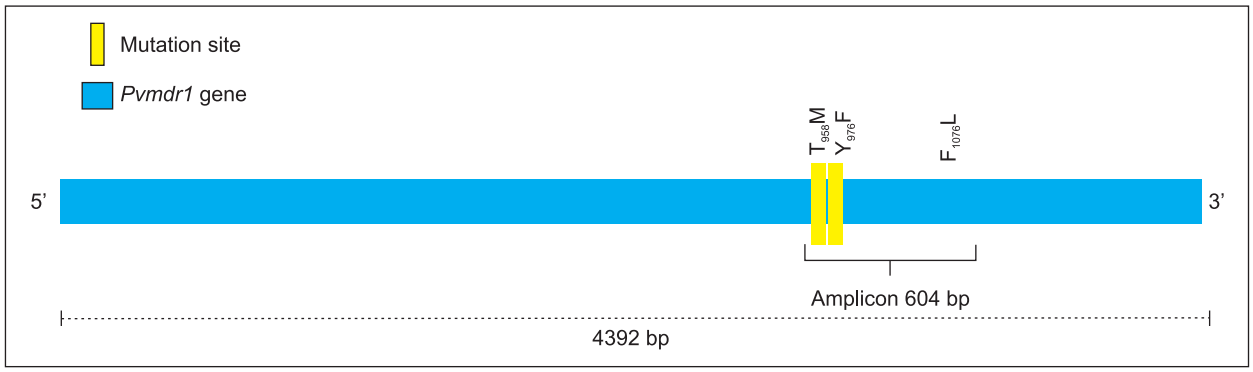


Fig. 6: Schematic representation of point mutation in *P. vivax mdr1* gene.

of isolates contained the $F_{1076}L$ mutation, which is highly prevalent across the globe and often found in regions with CQ-sensitive *P. vivax*. The mutant $Y_{976}F$, which has previously been associated with drug resistance, was not detected in any of the Indian isolates tested. The two limiting factors in this genotyping study, sample size and a lack of variability within the IC_{50} values for each group, may have influenced statistical power, but provided a preview into CQ susceptibility in India.

In the same time, the *in vivo* efficacy study corroborated *P. vivax* sensitivity to CQ in Chennai (Table 2). Of the 125 patients enrolled, 107 completed follow-up and were microscopically negative for both asexual and sexual parasites by Day 7. In all, 17 subjects remained microscopically negative until a time point after Day 28, called Day other, which ranged from 44 to 148 days after the initial enrolment (Day 0). Isolates from Day 0 and on any other day were genotyped to determine relatedness between the infecting clones in an attempt to differentiate between recrudescence, relapse, reinfection, and new infection. Of the 17 paired-infections, eight were found to be either entirely new infections or a relapse from an infection prior to Day 0; these eight infections shared ≤50% of the same alleles. The remaining nine paired-infections were classified as a relapse from Day 0, sharing >50% of the same alleles. Recrudescence was excluded as a possible explanation for these highly related recurrent infections due to the rapid rate of parasite clearance after CQ treatment and the lengthy time-to-recurrence. Again, fragment sequencing of *Pvmdr1* revealed that the mutant $M_{958}Y_{976}L_{1076}$ was the dominant haplotype, with no statistical correlation with any variable.

CQ resistance results from an *in vitro* CQ inhibition assay or an *in vivo* therapeutic efficacy study, or any indication using molecular genetic methods might indicate a pending CQ resistance sweep in the parasite population. Instead, valuable information were gained about the population-level genetic diversity of extant *P. vivax* and the rate of relapsing infections within this study site. Future studies will help resolve the relationship between the parasite genotype, phenotype, and disease outcome.

2.5 Population Genetics of Indian Chloroquine Resistant *Plasmodium falciparum* Isolates

The variable malaria epidemiology in India can support a strong population stratification of *P. falciparum*, which further can affect the spread of drug resistance. Efforts in prevention of malignant malaria caused by *P. falciparum* are severely

Table 2. Genotyping results of isolates from the chloroquine *in vivo* efficacy study

Day of collection	No. of subjects enrolled	No. of isolates selected for genotyping	Microsatellite genotyping			*Pvmdr1* haplotypes[a,b]	
			No. of multiclonal infections	No. of clones	MOI	$M_{958}Y_{976}$-L_{1076}	$M_{958}Y_{976}$-F_{1076}
Day 0	125	33	5	38	1.15	33 (1)	0
Day3[a]	19	–	–	–	–	–	–
Day other	17	17	3[c]	21	1.24	14 (0.933)	1 (0.067)
Total	125	50	8	59	1.18	47 (0.979)	1 (0.021)

– = Not detected; [a]GenBank KC818349–KC8184192; [b]The *P. vivax* Salvador 1- reference strain, which is chloroquine sensitive, *Pvmdr1* haplotype is $T_{958}Y_{976}F_{1076}$; [c]Of the three multiclonal infections, two had two alleles at more than one locus (double infection), and one had three alleles at more than one locus (triple infection).

hampered by acquisition of parasite resistance to most effective antimalarial drug, i.e. chloroquine. It necessitates the evaluation of spread of chloroquine resistance in any malaria endemic area. India reflects highly variable malaria epidemiology and also shares porous international borders with malaria endemic south-east Asian countries having multi-drug resistant malaria. The malaria epidemiology in India is believed to be affected by two major factors, namely high genetic diversity and evolving drug resistance in *P. falciparum*. How transmission intensity of malaria can influence the genetic structure of chloroquine resistant *P. falciparum* population in India is unknown.

Single clone infection was screened by length polymorphism in single-copy genes merozoite surface protein-1 (*msp-1*) and *msp-2*. Nuclear microsatellites developed for *P. falciparum* including three putatively neutral microsatellite loci from chromosome 5, 11, 13 and seven microsatellites loci flanking *pfcrt* gene, putatively under hitchhiking effect due to chloroquine selection were used for understanding the population genetics of Indian chloroquine resistant *P. falciparum* isolates. The genetic diversity within and among the *P. falciparum* isolates was analysed with respect to their prevalence and chloroquine resistance was observed at 13 different locations in India. The genetic diversity was analysed in terms of expected heterozygosity ($He = [n/(n-1)][1-\Sigma pi^2]$) at each polymorphic microsatellite loci. STRUCTURE 2.3.1 software was used to test how microsatellite haplotypes clustered according to the geographic origins of isolates. This provides about the genetic admixture in parasite population. The degree of genetic differentiation between different parasite populations was analyzed with F-statistics in software Arlequin. Isolation by distance was tested by Mantel test in Arlequin, to understand any genetic differentiation according to distance between different areas.

The analysis of length polymorphism in single-

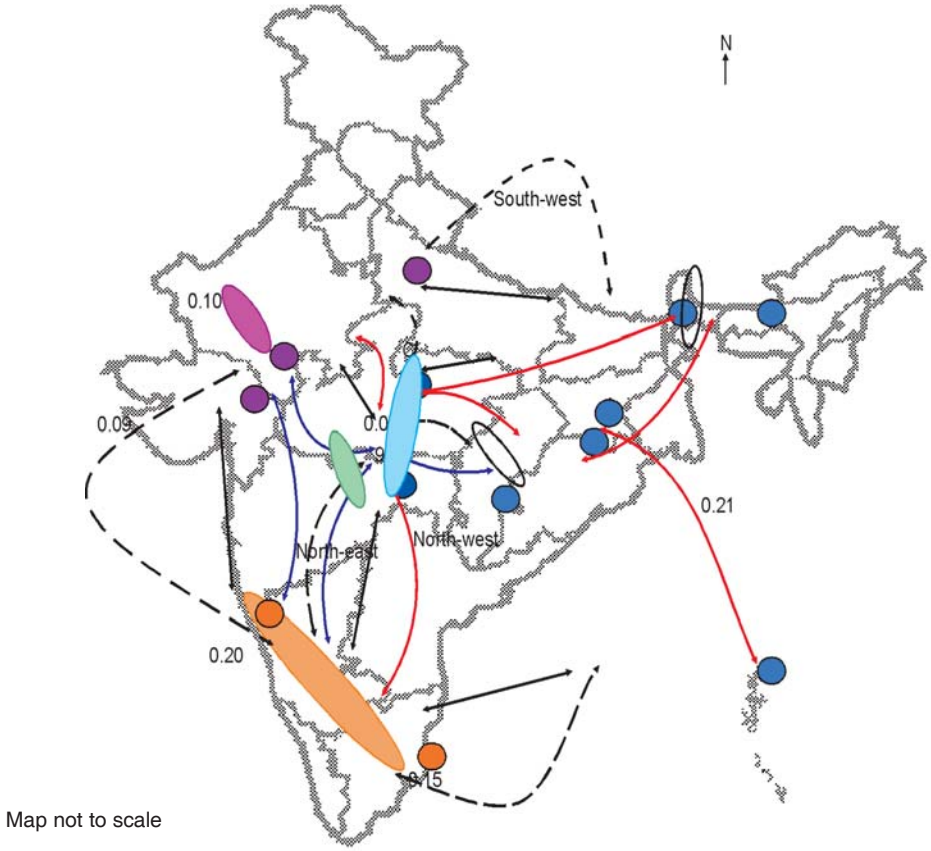


Fig. 7: The diagram illustrates the gene flow of chloroquine resistant parasite in India. The black double arrow show the significant pairwise F_{ST} (genetic differentiation) at *pfcrt*-flanking loci among four groups of chloroquine resistant parasite population outlined in green (north-east Island), orange (south-west), pink (north-west) and blue (central). The red arrows show the probable gene flow of various mutant *pfcrt* genes in India. The blue arrows show another probable route of gene flow in mutant SVMNT haplotype of Indian *P. falciparum*. The black dot double arrows show the significant pairwise F_{ST} (genetic differentiation) at genome wide neutral loci among the four groups of chloroquine resistant parasite population.

copy genes of merozoite surface protein-1 (*msp-1*) and *msp-2*, results into 213 single-clone infections. A further round of genotyping at three genome-wide microsatellite loci (chromosome 5, 11, 13) identified 25 additional multi-clone infections. Of the 188 single-clone infections identified, 169 isolates successfully amplified for all seven microsatellite loci flanking (–24 kb to +106 kb) the *pfcrt* gene (Fig. 7). A genetic hitchhiking was observed in five (–24 kb to +22 kb) of seven microsatellites flanking the gene responsible for chloroquine resistance. The genetic admixture analysis and F-statistics detected genetically distinct groups in accordance to transmission intensity of different locations and the probable use of chloroquine. A large genetic break between the chloroquine resistant parasites of North-east-East-Island group and South-west group at both genome wide neutral loci (F_{ST} = 0.25, $p < 0.001$) and *pfcrt*-flanking (–24 kb to +22 kb) loci (F_{ST} = 0.20, $p < 0.001$) (Fig. 7) suggests a long period of isolation or a possibility of different origin among them. A pattern of significant isolation by distance was observed in low transmission areas (r = 0.49, p = 0.003, n = 83, Mantel test).

An unanticipated pattern of spread of hitchhiking suggests genetic structure in Indian *P. falciparum* populations. Overall, the study suggests that transmission intensity can be an efficient driver for genetic differentiation at both neutral and adaptive loci across the geographic scale of India.

2.6 Pharmacogenomic Profiling of Indians Based on Single Nucleotide Polymorphisms (SNPs) at the *N*-acetyl Transferase 2 (*NAT2*) Gene

The *N*-acetyl transferase (NAT) enzymes are responsible for the metabolism of a wide variety of exogenous compounds (including different drugs) by catalyzing the transfer of an acetyl group from acetyl-CoA to the amine nitrogen atom of aromatic amines and hydrazines, and are mainly represented by two enzymes, NAT1 and NAT2. While the NAT1 enzyme has limited substrate specificity (p-aminobenzoic acid), NAT2 metabolizes a wide variety of therapeutic drugs, that is, dapsone, sulfadoxine, isoniazid, procainamide and hydralazine as well as exogenous chemicals present in the diet. The NAT1 and NAT2 enzymes are encoded by two different genes *NAT1* and *NAT2*, respectively; both the genes are present in the short arm of sub-metacentric human chromosome 8. Owing to the genetic basis of the NAT2 enzyme, inter-individual variations among human populations and the ability to acetylate drugs and exogenous compounds have been widely established. The variations on acetylation profile have been assigned to different alleles of the *NAT2* gene based on the presence of SNPs in exon 2 of the *NAT2* gene. An individual can thus be classified as either a fast (rapid) acetylator, slow acetylator or intermediate acetylator depending on the presence of a specific type of *NAT2* allele. Thus, in order to characterize acetylation profiles of Indians, where data are poorly available, we sequenced the 873 bp *NAT2* coding region in 250 Indians, representing different parts of India including three tribes. Altogether, 35 *NAT2* alleles forming two acetylator phenotypes (distributed almost in equal proportion in India) were found (Fig. 8); while the alleles determining slow acetylators were highly differentiated, the fast acetylator alleles were less in number but highly frequent. Interestingly, distribution of two different acetylation phenotypes correlated well with historical dietary pattern in India (Fig. 9). The neighbour-joining phylogenetic tree based on *NAT2* gene polymorphisms in Indians reflects the observed patterns on dietary habits too (Fig. 10).

While the majority of the north and west Indians are slow acetylators, the central, east, north-east

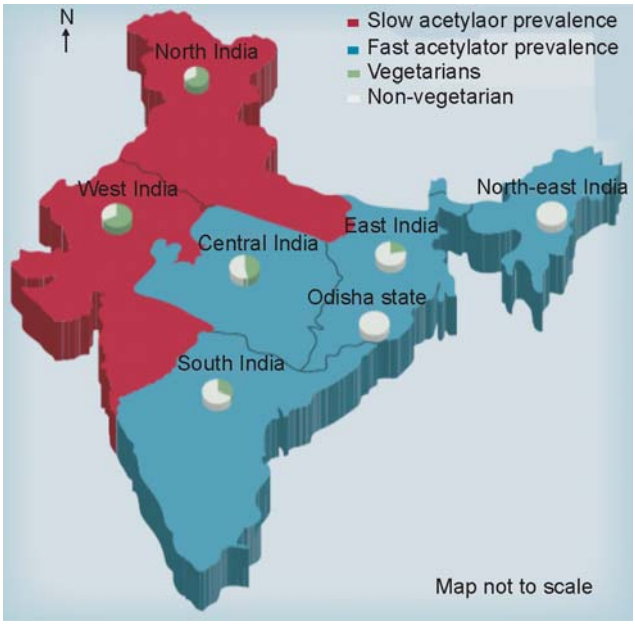


Fig. 8: Map of India depicting regions majorly dominated by the slow and fast acetylation phenotypes.

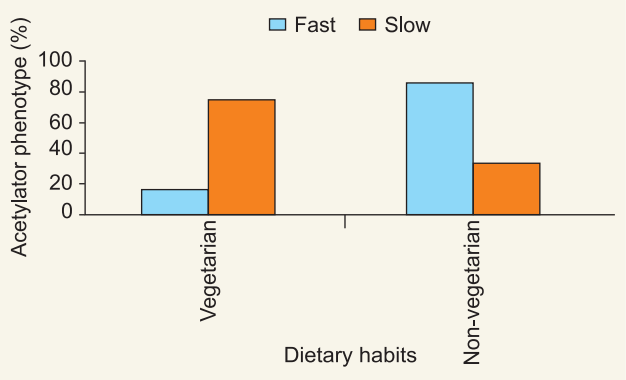


Fig. 9. Frequency of Indians with different acetylation phenotypes consuming vegetarian and non-vegetarian diets.

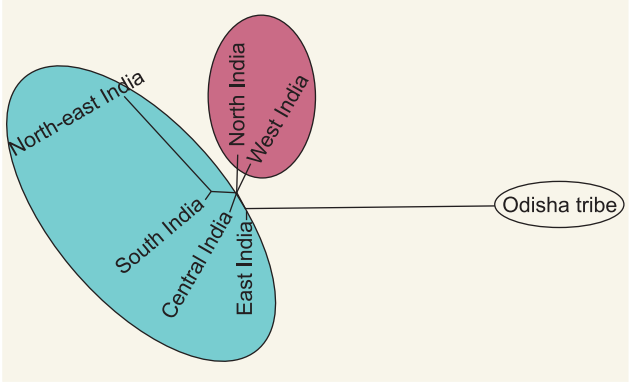


Fig. 10: Genetic inter-relationships among different Indian zones as inferred from the neighbour-joining phylogenetic tree.

and south Indians are fast acetylators. The pie charts showing the proportion of individuals consuming vegetarian to non-vegetarian diets are also shown in the respective zones.

It should be noted that approximately 80% of the individuals categorized as fast acetylators are non-vegetarians, whereas approximately 65% of the slow acetylators are vegetarians.

The cluster including the north and west India depicts slow acetylators (Fig. 8) and the cluster including north-east, south, central and east India depicts fast acetylators (Fig. 10).

2.7 Whole Mitochondrial Genome Sequence of an Indian *Plasmodium falciparum* Field Isolate

Mitochondrial genome sequence served as an ideal marker to understand evolutionary history of many model and non-model organisms and has been a marker of choice for reconstructing historical patterns of population demography and phylogenetic studies. Mitochondrial genome sequences of malaria parasites have served as potential markers for inferring evolutionary history

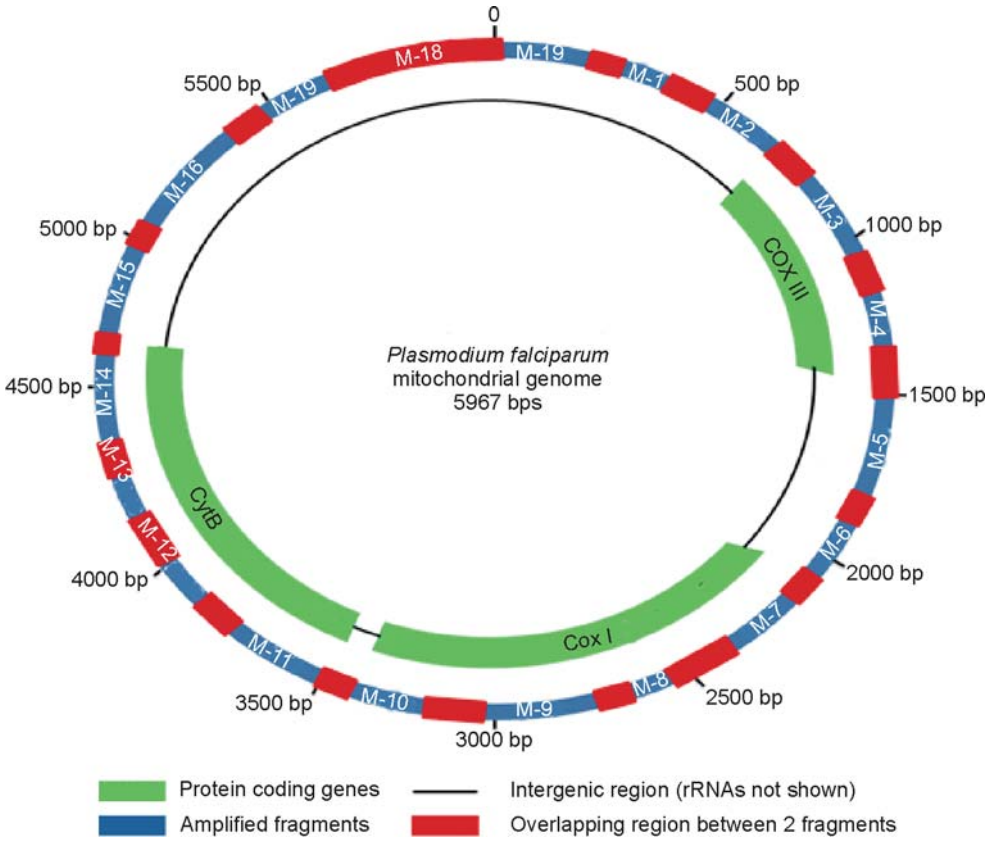


Fig. 11: Schematic overview of the ~6 kb *mt* genome and the location of primers to amplify the whole *mt* genome of Indian *P. falciparum*.

of the *Plasmodium* genus. Moreover, in malaria parasites, *mt* genome is of particular relevance, due to: (i) its small size (~6 kb); (ii) haploid in nature; and (iii) the presence of three protein-coding genes, cytochrome c oxidase I (*cox I*), cytochrome c oxidase III (*cox III*) and cytochrome b (*cyt b*). All these three genes are essential for a range for cellular processes, like membrane potential maintenance, heme and coenzyme Q biosynthesis, and oxidative phosphorylation. Most importantly, the *cyt b* gene of *P. falciparum* mitochondria is a potential target for an antimalarial drug, Atovaquone. Since, Atovaquone is not used in India, it is of interest that how the *cyt b* gene has evolved in Indian *P. falciparum*. Whole mitochondria genome sequence and related analyses have already been conducted but Indian mitochondrial genome variation data have not yet been utilized. In this study, we have designed 19 novel PCR and sequencing primers (Fig. 11) and have sequenced the whole mitochondrial genome of a single *P. falciparum* field isolate collected from Bilaspur (Madhya Pradesh) using these novel primers and compared with the 3D7 reference sequence and one previously reported Indian sequence. While the two Indian sequences were highly divergent from each other, the presently sequenced isolate was highly similar to the reference 3D7 strain.

2.8 Analyses of Genetic Variations at Microsatellite loci Present in-and-around the *Pfcrt* Gene in Indian *Plasmodium falciparum*

The evolution and spread of chloroquine resistant (CQR) malaria parasite *P. falciparum* have posed great threat in malaria intervention across the globe. The occurrence of K76T mutation in the *P. falciparum* chloroquine receptor transporter (*Pfcrt*) gene has been widely attributed to CQR with four neighbouring mutations providing compensatory fitness benefit to the parasite survival. Understanding evolutionary patterns of *Pfcrt* gene is of great relevance not only for devising new malaria control measures but also could serve as a model to understand evolution and spread of other human drug-resistant pathogens. Several studies, mainly based on differential patterns of diversities at the microsatellite loci placed in-and-around the *Pfcrt* gene have indicated the role of positive natural selection under the 'hitchhiking' model. However, the studies were limited to limited number of microsatellite loci present inside the *Pfcrt* gene.

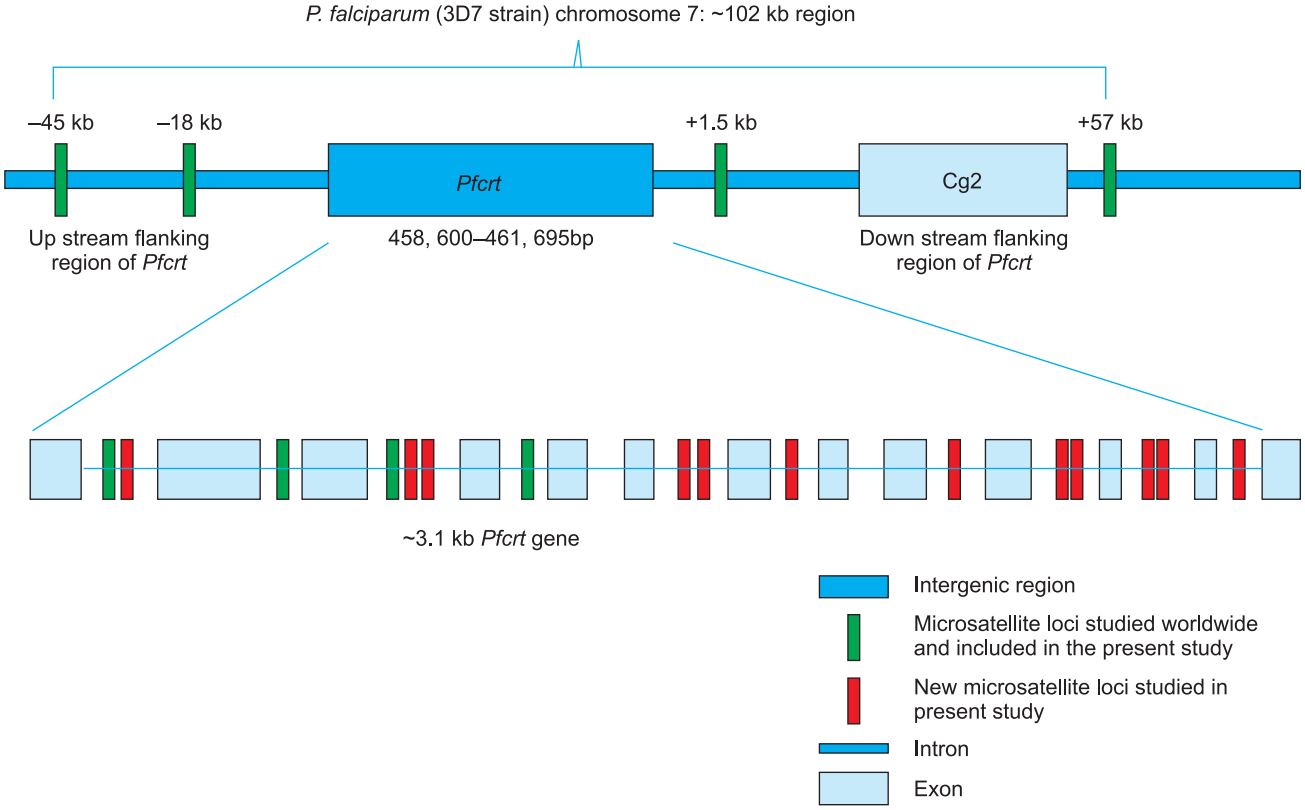


Fig. 12: Chromosomal location and other details of the 20 microsatellite loci (16 inside and the four flanking the *Pfcrt* gene).

Moreover, comparatively higher level of diversities in microsatellite loci present inside the *Pfcrt* gene than the loci flanking the *Pfcrt* gene are hallmarks of Indian *P. falciparum*, presenting contrasting evolutionary models to global isolates. With a view to infer evolutionary patterns of the *Pfcrt* gene in Indian *P. falciparum*, we have adopted a sampling scheme of two populations of cultured (one CQ sensitive and one CQ resistant) and two field population samples (48 isolates in total) and utilized 20 polymorphic microsatellite loci (16 located inside the *Pfcrt* gene and four in the two flanking regions, Fig. 12). Microsatellite variations across the chromosome were also measured for each of the four populations. However, no significant differences in the level of diversity could be observed among populations (Fig. 13). In general, the two field populations displayed higher microsatellite allelic diversity than the two cultured population samples. We analyzed the data employing different population genetic and evolutionary approaches to conclude that genetic drift rather than natural selection could better explain the observed data on the diversity of microsatellite alleles present in-and-around the *Pfcrt* gene in Indian *P. falciparum*.

2.9 Immuno-modulatory Role of Mesenchymal Stem Cell (MSC) in Pathogenesis of Malaria Infection

Malaria caused by *Plasmodium* species claims 500 million clinical episode with approximately 2–3 million deaths annually worldwide (WHO). Malaria parasite escapes the immune response before transforming to the blood stage of infection. It is well-documented that acquired immunity mounted by host limits the clinical impact of infection and provides protection against malaria. During malaria infection inflammatory cells accumulated in secondary lymphoid organs. However, cellular composition of these cells after malaria infection has not been fully characterized. Here we reported that recruitment of MSCs during infection and infusion of these cells into naïve mice was able to confer host resistance against malaria infection. MSCs augmented interlukin (IL)-6 productions, whereas suppressed IL-10 production in recipient animals. Previous studies have indicated that natural killer T-cells (NKT) infiltrate the spleen of mice with malaria, which further assists in the migration of other cell types into the infected organ. These studies further showed that the levels of NKT-cell accumulation are directly proportional to the parasite load in the organ. Therefore, we examined the content of NKT cells in the spleens of infected animals that had been infused with MSCs or control cells. As expected, there was a dramatic accumulation of $CD4^+TCR\alpha\beta^{int}DX5^+$ cells in infected animals, which was reduced in animals that received $Sca\text{-}1^+$ cells (Fig. 14).

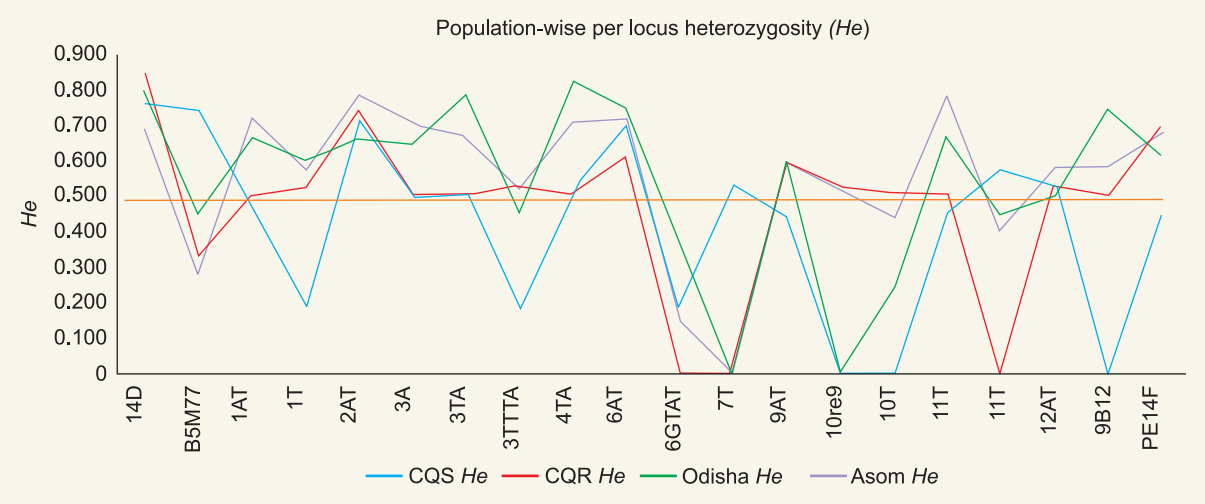


Fig. 13: Patterns of genetic diversity at 20 different microsatellite loci in four different populations of Indian *P. falciparum*.

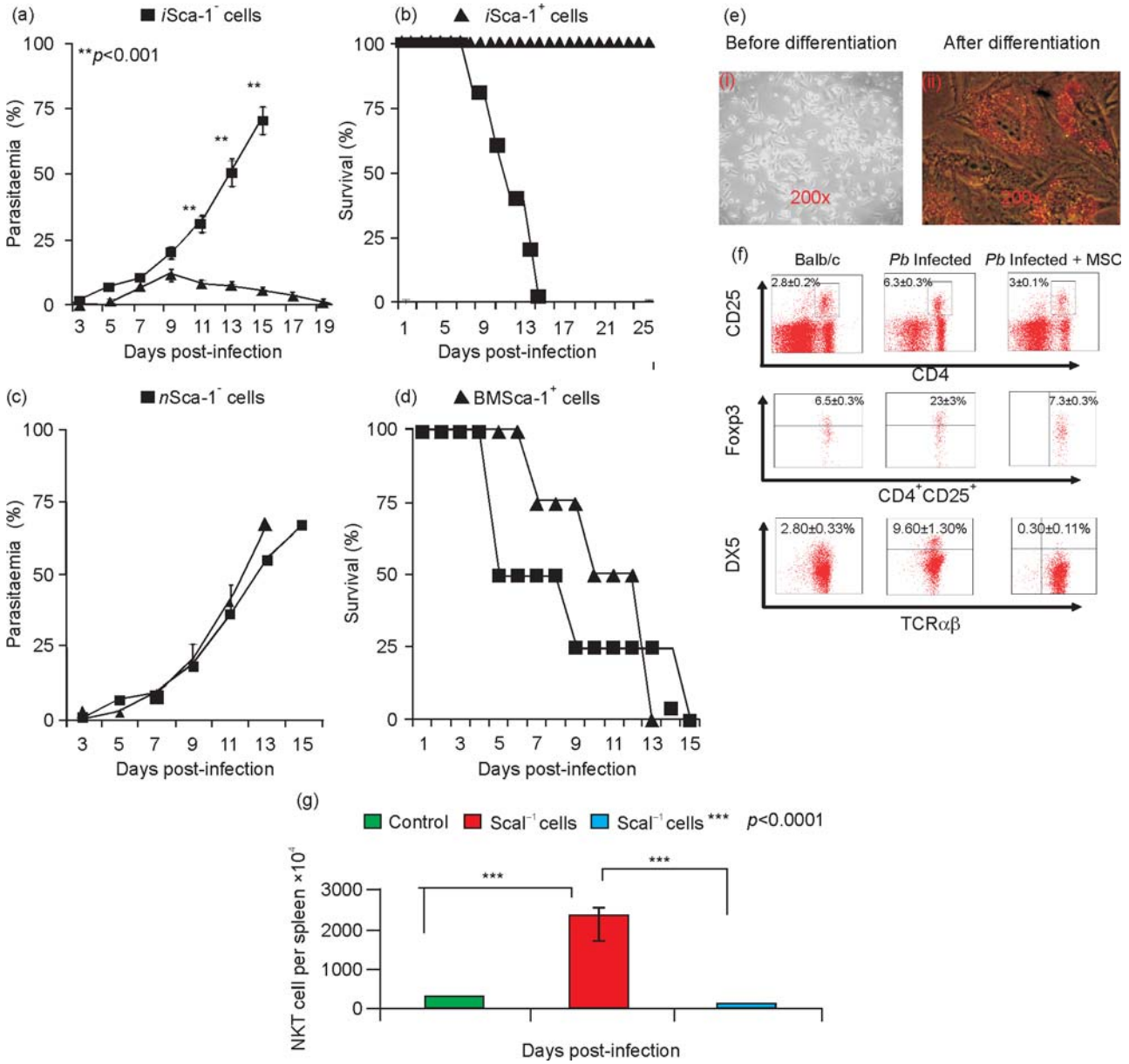


Fig. 14: Balb/c mice were infected with 5×10^5 *P. berghei* (*Pb*)-parasitized erythrocytes via intra-peritoneal injection and Sca-1$^+$ or Sca-1$^-$ cells (5×10^6/mouse) were injected into *Pb*-infected mice intravenously. (a) Balb/c mice infected with *Pb* parasites were divided into two groups and one group received lineage-differentiated depleted cells (triangular symbols) while the other group of mice received cells depleted of Sca-1$^+$ cells (square symbols). Blood collected from tails was used to prepare smears to determine parasitic load. Statistical significance was determined by unpaired two tailed student t-test. *p<0.05, **p<0.001; (b) Survival of mice that received Sca-1$^+$ cells *vs* control cells (a & b). Data are shown as mean ± SD of 20 mice pooled from five independent experiments consisting of four mice in each group; (c) Two groups of Balb/c mice were infected with *Pb* parasites. One group of mice received bone marrow derived MSCs from wild type mice BM Sca-1$^+$ cells (triangular symbols) while the second group of mice received Sca-1$^-$ cells from uninfected mice (square symbols) and parasitic loads were determined in thin blood smears. Data are shown as mean ± SD of 12 mice pooled from three independent experiments; (d) Survival of mice that received normal BMSca-1$^+$cells *vs* Sca1$^-$ cells; (e) Splenocytes were cultured in adipocyte differentiating medium and stained with oil red-O after full differentiation— (i) Spindle-like morphology of cells before differentiation is shown; and (ii) Cells after adipocyte differentiation and stained with oil red-O; (f) Treg cells were analysed by flow cytometry staining with antibodies against CD4 and CD25 (top). Treg cells (gated on the CD4$^+$CD25$^+$ population) were also identified by staining for Foxp3 (middle). The percentage of NKT cells after staining with DX5 antibody (CD49b) in spleen with infusion of recipient mice with either Sca-1$^+$or Sca-1$^-$ cells and following infection with malaria parasites was also determined (bottom). Data shown are compiled from 3 independent experiments with a total of 12 mice in each group; and (g) Total number of NKT cells in control mice infused with Sca-1$^-$ cells or Sca-1$^+$ cells. Data are shown as mean SD of 12 mice pooled from three independent experiments. Statistical significance was determined by unpaired two tailed *t*-test. ***p<0.0001.

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3

Epidemiology

3.1 Environmental Epidemiological Studies

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

The study initiated in 2009 in selected field sites of Uttarakhand, Asom and Mizoram, was continued for generation of data on entomological, parasitological and climatic aspects. A field site was established in Jharkhand state and also at Ranchi and Ramgarh sites for the detailed study. In Mizoram, which is reporting a very low malaria incidence, field work is being undertaken in alternate months.

In Asom field site, parasite prevalence was found to be more than those of other study sites. *Anopheles minimus,* the known vector of malaria was found in Bokajan on few occasions only. However, *An. culicifacies* which is a vector of malaria in plains, was also encountered from Bokajan field site.

As input to mathematical model (Basically adapted from MacDonald Model, 1957) for malaria transmission in Indian conditions, laboratory experiments are being undertaken on different stages of larvae and adults of *An. stephensi* by putting them in environmental chamber for determining the development/survival rate at different temperatures (15 to 35°C). The survival time of adults of *An. stephensi* exposed for 30 to 180 min at 40 to 45°C revealed that with one hour exposure at 40°C, 100% mortality was observed. Further, combination with temperature and relaive humidity are being studied. As an input for mathematical model, sporozoite rate was also determined at different field sites. In Ranchi, a total of 805 and 1004 *An. culicifacies* and *An. fluviatilis* were collected, respectively and one each *An. fluviatilis* in February and *An. culicifaceis* in April were found positive for *Pf.* In Bhimtal, a total of 1004 *An. culicifacies* and *An. fluviatilis* were collected from January to July and sporozoite positivity was found in May and June in different villages and also in Nishola village, where malaria has not been reported.

As the day-time temperature affects the development of aquatic stages of mosquitoes and night-time temperature affects the resting of adult mosquito, devices have been installed to record water bodies' temperature at two hourly intervals. The efforts are being made to create simulated conditions for natural breeding and emergence of mosquitoes.

3.1.2 Microstratification of malaria in problematic districts of Rajasthan for development of strategic action plan for control

The study is in second year of its continuation. Field surveys were undertaken during August–September 2012. Breeding habitats supporting proliferation of mosquitoes were underground tanks and cemented tanks for *An. stephensi* and ponds for *An. culicifacies*. The results of fever survey indicated the need for categorization of high/low endemicity based on the present study using ecological and epidemiological approach.

The satellite data of selected study area were procured and analysis was done for study villages under Barmer district. Ecological map generated through satellite images revealed that village settlements were poorly detected in LISS III resolution (23.8 m) and further analysis with LISS IV and Cartosat-2 is in progress.

It appears that uneven land surface in and around villages got filled with rain water which is not detectable owing to cloud cover in monsoon. It

warrants digital terrain modelling of vulnerable villages within 1.5 km radius of settlements.

3.1.3 Developing a framework for predicting malaria outbreaks in rural and urban areas of Gujarat, India

The study is in third year of its tenure. So far three tools for early warning of malaria have been developed using cumulative rainfall, vegetation index and sea surface temperature from tropical south Atlantic Ocean. Of various models developed for early warning of malaria, rainfall was found best even at taluka level followed by NDVI. The analysis at taluka level revealed that the critical month showing highest correlation with exacerbation of cases varied from June to August.

In view of report of prevalence of mixed infections of malaria, i.e. *P. vivax* and *P. falciparum* from some parts of India, it was planned to determine the prevalence of mixed infection as an input in mathematical model. Of 61 samples collected from Ahmedabad City, 25 were *P. vivax*, three *P. falciparum* and one mixed infection of *Pv* and *Pf*.

Framework of web-based early warning is being developed with inputs from experts of Michigan University.

3.1.4 Impact of climate variability and urbanization on water storage practices and vector-borne disease incidence: Developing an understanding for risk prediction and response using Delhi, India as context

The objective of the study was to characterize the role of water storage and supply infrastructure across the urbanization gradient with respect to vector-borne disease epidemiology and to identify how storage practices and climate affect mosquito habitats for disease transmission. A transect of Delhi consisting of highly urbanized location, moderate and rural type was selected for undertaking entomological survey for larval indices of *Aedes* mosquitoes, household survey to elicit the information on socioeconomic aspects of inhabitants and observations on ecological/environmental aspects of households. After procuring the list of inhabitants, 60 households from each category of transect were selected randomly and surveyed for larval density, observational and socioeconomic aspects. The obtained data are being analysed.

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

The study was carried out to determine the role of *An. subpictus* as a vector of malaria both under natural and experimental conditions in a changing climatic scenario. Maps depicting distribution of temperature and relative humidity in India during the year 1990 were prepared using PRECIS data of A1B scenario for establishing the correlation of meteorological parameters with distribution of *An. subpictus*. Distribution map of *An. subpictus* was also prepared based on the available published records. The analysis of meteorological and epidemiological data showed that in South 24 Parganas (West Bengal), malaria cases increased with onset of monsoon season. During this period the temperature remained 28–30°C and relative humidity was >80%. In Ramanathapuram district (Tamil Nadu), malaria cases were recorded throughout the year but the peak was at the temper-ature between 29 and 31°C and relative humidity >70%.

A field visit was also undertaken during the malaria transmission period (August–September) in South 24 Parganas and Ramanathapuram districts for the collection of adult *An. subpictus* mosquitoes. A total of 27 *An. subpictus* mosquitoes were collected in South 24 Parganas and 3 in Ramanathapuram districts. ELISA test showed that none of the mosquitoes was found positive for *Plasmodium* parasite. Further the study is in progress.

3.2 HIA Studies

3.2.1 Health impact assessment of Narmada Basin dams and resettlement and rehabilitation colonies in Madhya Pradesh

Health impact assessment was started in 2010 in Narmada Basin area of Madhya Pradesh. The study centres were established in Jabalpur, Bhopal and Narmada Nagar for routine survey and laboratory work in the affected area of Narmada Basin. Routine surveys for entomological, parasitological and microbiological indicators are being carried out as per the protocol.

Jabalpur Unit

In 2012, Jabalpur Unit surveyed 145 villages of 7 districts under 9 projects in Narmada Basin area. Per man hour density of malaria vector, *An. culicifacies* and *An. fluviatilis* was 1–182 and 1–26,

respectively. Highest density was recorded at Bargi and Upper Bhudhner projects area.

In a cross-sectional survey, 598 blood slides were examined from the villages by NIMR team. Out of these, 28 cases malaria (18 *Pv* and 10 *Pf*) were detected in surveyed areas of Narmada Basin in 2012. Simultaneously, rapid diagnostic kits were also used for diagnosis of the symptomatic patients.

IEC activities were also organized in the villages to spread the knowledge amongst the people regarding prevention and cure of vector and water borne-diseases with live demonstration of larvae/pupae and mosquitoes, health education and by pamphlet distribution. The team counselled villagers for reduction of breeding source by emptying drums and pots, utensils, tyres, etc. The objective was to coordinate with community to participate in the control and reduction of vector breeding sites in-and-around their houses.

In all, 24 drinking water samples were collected from different project areas and all were found positive for *Salmonella typhimurium, S. enteritidis, Citrobacter freundii, Vibrio cholera* and *V. para-haemolyticus* using HiWater™ Test kit (HiMedia). No water sample was found to be safe for drinking purpose.

For each survey, possible mitigation measures have been suggested to the NVDA and the reports of the conditions of malaria cases were also intimated to the respective health authorities.

Bhopal

Health impact assessment of 11 projects in Narmada Basin area covered 262 villages in 7 districts of Madhya Pradesh. MHD of *An. culicifacies* was highest (49) in Chinki project area and lowest (9) in Tawa project area in 2012. A total of 2198 blood smears were prepared, and 24 cases of malaria (13 *Pv* and 11 *Pf*) were detected in surveyed area of Narmada Basin in 2012. In all, 34 water samples were examined and 9 were found safe and 25 were unsafe for drinking. Slogans on

Photographs showing: (a) water stagnation in canal, (b) stagnation at gates, (c) breeding sites at religious places, and (d) broken margins of canal.

walls were written and two health camps were organized with the help of State Health practitioners for awareness in Narmada Basin area. IEC Activities were undertaken in all the surveyed villages.

The concerned health institutions were being informed for necessary action regarding malaria cases and unsafe water. The State Health Department is taking necessary steps under various States and National Health Programmes to control the said diseases in the impact area.

Narmada Nagar

Narmada Nagar covers 9 dams along with the Punasa Lift Irrigation Project which has latter been added to ISP. The dams covered under Narmada Nagar are ISP, Omkareshwar, Maheshwar, SSP Reservoir, Upper Beda, Lower Goi, Man, Jobat Dam and Sukta Dam. The Unit surveyed 219 villages of 6 districts under 9 dam projects in Narmada Basin area for the year 2012.

Per man hour density of malaria vector, *An. culicifacies* and *An. fluviatilis* was 0–92 and 0–34, respectively. *Anopheles stephensi* was found in the range of 0–54 and *Aedes aegypti* was found in the range of 0–26. In the cross-sectional survey, 3480 blood slides were examined from the villages. In all, 131 *Pf* and 121 *Pv* cases were found positive.

A total of 34 drinking water samples were tested and all were found positive for *Salmonella typhimurium, S. enteritidis, Citrobacter freundii, Vibrio cholera* and *V. parahaemolyticus* using HiWater™ Test kit (HiMedia).

In total, 255 Anganwadi Centres, 95 CHCs, 30 PHCs, 159 Govt. Primary Schools and 87 Govt. Higher Secondary Schools were covered for IEC activities to generate awareness among communities and to increase their participation towards eliminating vector breeding sites in their surroundings.

Engineering problems were found in dam seepage, vegetation and stones covered with moss in the seepage of almost all the dams under study, while damaged canals, blockage with vegetation and rocks, seepage from broken margins were common problems found in most of the under construction canals. Heavy vegetation in downstream was also found to be a major problem as it creates pools and puddles favourable for vector breeding. Other domestic problems were mosquito breeding in cemented tanks, domestic containers like drums, buckets, pots, and plastic utensils. Absence of drainage system, swamps and water logging near hand pumps and wells, and in gutters surrounding the village-houses were also found to be adding to the complexity of disease dynamics.

3.2.2 Studies on health impact assessment of Sardar Sarovar Project in command areas of Rajasthan

Narmada Canal Project (NCP) is a multidisciplinary project whereby Rajasthan receives its share of water from Sardar Sarovar Dam through the Narmada Main Canal (NMC) of Sardar Sarovar Project (SSP). The Project envisages providing irrigation water to part of Sanchore tehsil (129 villages) of Jalore district and part of Gudhamalani and Chauhtan tehsils (104 villages) of Barmer district through 74 km long main Narmada Canal and 1403 km long distribution system network. NCP will also provide drinking water facility to over 1336 villages and 3 towns within its command areas in Rajasthan. Out of these 233 irrigated villages, 87 villages will be served by flow distributaries from the main canal and 146 by lift (Sanchore lift, Bhadrai lift and Panoriya lift) distributaries. A project on health impact assessment (HIA) of SSP in both the districts was initiated by NIMR in November 2010 funded by the Government of Rajasthan.

Two surveys were carried out during 2012 in 34 villages within the command areas of NMC including 2 control villages (in non-NMC area). The breeding of *An. culicifacies* was found in minors, sub-minors, distributaries, PHD points, lift pumping stations, outlets and NMC excess water escape-sites with heavy breeding in diggies, sump-wells built on minors, sub-minors and distributaries. Due to erroneous designing, the continued presence of water throughout the year in these diggies, sump-wells, lift pumping stations and outlets is the prime reason for the breeding of this vector species. Besides *An. culicifacies*, *An. stephensi* as well as *Ae. aegypti* mosquitoes were seen-established in these concrete cemented sites.

Potability of water (HiWater™ Test kit) was examined in 14 villages from command areas. The bacterial contamination, viz. *Salmonella typhimurium*, *S. enteritidis* and *Citrobacter freundii* was found in 4 villages which were informed to the concerned PHCs.

As part of the mitigation measures, the release of insectivorous fishes was recommended in diggies, sump-wells, outlets and excess escape water sites (channels) to check the breeding of

Breeding of mosquitoes in different habitats.

mosquito larvae to prevent VBDs like malaria, chikungunya and dengue. Fishes have been released in some NMC network system. The recommendations have also been given to apply expanded polystyrene beads to check larval breeding specifically in diggies, sump-wells and outlets. The plastic sheets have been recommended in the canal margins near Sisawa water seepage area to stop seepage. Recommendations for removing unwanted soil and weeds regularly from the main canal, minors as well as sub-minors have also been given involving local people/farmers associations (PIM system) with the help of technical engineering inputs to avoid unnecessary blockage in the path of water and supporting breeding of mosquitoes.

3.3 GIS-based studies

3.3.1 Deforestation and its impact on malaria epidemiology in districts of Asom: A Remote Sensing and GIS-based study

The study was undertaken in Sonitpur and Nagaon districts of Asom. Four field surveys were conducted during the months of August–September 2009 (monsoon), November–December 2009 (winter), March–April 2010 (pre-monsoon), and October 2011 (post-monsoon). Entomological and epidemiological data were collected from identified deforested and forested PHCs of two districts. The study recorded *An. culicifacies* from deforested areas and for the first time it was incriminated as vector from Dhekiajuli PHC of Sonitpur. The study identified >80% of *An. culicifacies* resting indoors, therefore, IRS has been recommended to control the situation.

3.3.2 Mapping of spatio-temporal trends of malaria (1995 – 2008) to identify endemic- and epidemic-prone areas for decision support in malaria control in India

The work under the project has been completed and the process of printing of the monograph comprising nearly 466 plates is in progress.

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4

Clinical Research

4.1 Pharmacovigilance of Antimalarials in India

The project attempts at evaluating the safety of antimalarials by both the passive and active pharmacovigilance. The District Malaria Officers from the states of Asom, 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.

Till date, 4602 filled in AER forms have been received. These include 1966 forms filled by the medical officers and information of 2636 patients participating in the therapeutic efficacy study. Total 3200 forms have been analysed till date (Table 1). A total of 87 adverse events have been reported in the form of nausea, vomiting, giddiness, gastritis, etc. The study shows that the antimalarials being used in the national programme are safe.

4.2 Quality Assurance of Malaria Rapid Diagnostic Tests in India

To ensure the quality of malaria RDTs used by NVBDCP, this project was started in the year 2009. The essential components of the project are pre-dispatch and post-dispatch quality assurance of malaria RDTs procured by the NVBDCP. Pre-dispatch quality check was performed in 60 batches, of which 59 were found to be acceptable. For post-dispatch quality assurance, RDTs are being picked up at random by District Malaria Officers

Table 1. Forms received for different antimalaria along with adverse events

S.No.	Drug	No. of forms	Adverse events	No. of events
1.	Chloroquine + Primaquine	1423	Loss of appetite	3
			Nausea	21
			Vomiting	20
			Giddiness	5
			Pain in abdomen	2
			Loss of appetite	3
2.	Artesunate + Sulphadoxine-Pyrimethamine	2683	Headache	13
			Vomiting	3
			Jaundice	1
			Nausea	1
			Stomatitis	1
			Gastritis	1
3.	Other ACT	1510	–	–
4.	Artesunate alone	5	Nausea	1
			Vomiting	1
5.	Artesunate + Doxycycline	5	Nausea	3
			Vomiting	2
6.	ACT + Chloroquine	93	–	–
7.	Quinine	7	Gastritis	4
			Itching	3
8.	Non-antimalarial treatment	61	–	–
9.	Amodiaquine	5		
10.	Miscellaneous	169	–	–
11.	Total	4602	Total	87

Table 2. Results of RDT testing

S.No.	State	No. of RDTs tested	Results (Satisfactory/Total tested)			Satisfactory results
			2000/µl	200/µl	Negative	
1.	Nagaland	360	104/104	193/205	50/50	347
2.	Manipur	156	44/46	83/90	19/19	146
3.	Mizoram	319	92/95	160/180	44/44	296
4.	Meghalaya	170	47/50	90/96	24/24	161
5.	Asom	216	62/64	115/126	26/26	203
6.	Madhya Pradesh	632	182/189	330/370	66/66	578
7.	Arunachal Pradesh	128	38/38	69/72	15/15	122
8.	Odisha	326	97/103	168/184	38/38	303
9.	Maharashtra	52	16/16	29/29	7/7	52
10.	Andhra Pradesh	7	2/2	4/4	1/1	7
11.	Jharkhand	95	30/30	56/56	9/9	95
12.	Chhattisgarh	70	20/20	40/40	10/10	70
13.	Gujarat	847	260/260	509/520	67/67	836
	Total	3378	994/1017	1846/1972	376/376	3216

from PHCs, sub-centres and ASHAs every quarter. One hundred and eighty three districts of 199 study districts in 13 states are participating in the study and sending RDTs to NIMR for quality assurance. So far, 4126 RDTs from all over India were received, of which 3378 have been tested for their quality by positive panels of parasitaemia of 2000 and 200/µl and negative panels. Overall panel detection score was 95.2% with a specificity of 100% (Table 2).

4.3 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 the 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 multicentre 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. Till date, 6508 pregnant women were enrolled in this cohort and 210 pregnant women with malaria have been enrolled in the trial. In all, 105 patients received AS + MQ treatment and remaining 105 received AS + SP. A total of 157 enrolled patients have successfully given birth to babies. There were 21 serious adverse events during the study. None of the severe adverse event was deemed to be related to the study drugs.

4.4 Monitoring the Therapeutic Efficacy of Antimalarial Medicines in India

To combat the problem of antimalarial drug resistance and to update and rationalize treatment policies, the National Institute of Malaria Research (NIMR) in collaboration with National Vector Borne Disease Control Programme (NVBDCP) has been conducting therapeutic efficacy studies of antimalarial medicines at 15 sites each year during last four years (2009–12). These studies have been used to monitor the efficacy of artemisinin-based combination therapy (ACT; AS + SP) in *P. falciparum* and chloroquine (CQ) in *P. vivax* in the country. In addition to clinical efficacy, the molecular markers of drug resistance of partner drug sulphadoxine-pyrimethamine have also been monitored.

A total of 894 patients were enrolled during 4th year (2012–13) at 14 sites. Out of these, 736

patients of *P. falciparum* and 158 patients of *P. vivax* completed follow-up up to Day 42. The chloroquine efficacy in *P. vivax* malaria was 100% at Ahmedabad (Gujarat) and Kolkata (West Bengal) while the efficacy of ACT (AS+SP) in *P. falciparum* ranged from 76.3–100% at 12 sites. There is high proportion of treatment failure at selected study sites during the reported period.

The residual levels of antimalarials were measured on Day 0 in *P. falciparum* malaria patients enrolled in therapeutic efficacy studies carried out during 2010–12 at Bilaspur (Chhattisgarh), Betul (Madhya Pradesh) and Simdega (Jharkhand). Residual levels of antimalarials, namely sulphadoxine (19.8%) and chloroquine (9.9%) were observed in enrolled patients (n=172). Also, the preliminary results showed inverse-correlation between parasite density and residual levels of sulphadoxine and chloroquine in Day 0 samples of malaria patients.

Molecular studies to detect partner drug resistance were undertaken in 20% samples. These include point mutations in DHFR and DHPS genes, which could be completed at 7 study sites. Preliminary results showed the presence of 42.1% *dhfr* double mutation followed by single (30.5%), triple (18.9%) and quadruple mutation (1.1%) with 7.4% wild type pattern. DHPS triple mutation was observed in 32.6%, followed by wild type pattern (31.5%), double (18.5%), quadruple (12%) and single mutation (5.4%).

A multicentric trial to detect *in vivo* resistance of *P. falciparum* to artesunate in patients with uncomplicated malaria was initiated during 2011–12 at Nagrakata block in Jalpaiguri district, West Bengal. However, due to stringent inclusion criteria and low transmission of malaria in the selected study site, only one patient could be enrolled. The study has been abandoned. The recent data from therapeutic efficacy studies conducted during 2012–13 showed declining efficacy of the prescribed ACT (AS+SP) *in P. falciparum* malaria at selected study sites (Fig. 1). Thus, to detect resistance to artemisinin which has been reported from Thai-Cambodia border, delayed parasite clearance time (PCT) will be monitored at selected endemic sites in the country. PCT has been used as a marker for monitoring artemisinin resistance as no other molecular marker is reported to be associated with it as yet. Also, the threat of artemisinin resistance is pronounced in India as resistance to partner drug SP is already reported

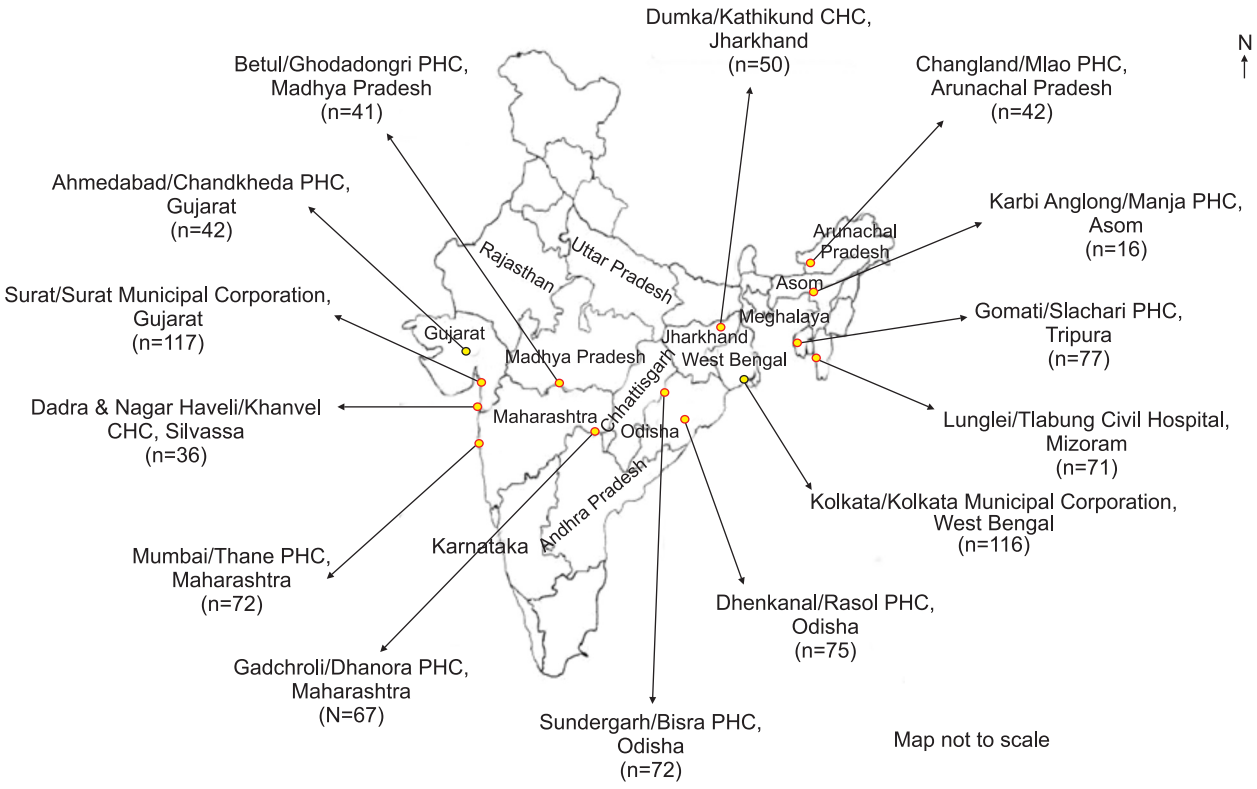

Fig. 1: Study sites under therapeutic efficacy conducted during 2012–13.

from India. The results will be helpful in understanding the actual data on PCT at selected sites and understanding the molecular basis of artemisinin resistance, if any. Chloroquine in *P. vivax* remains highly effective and safe.

4.5 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

Currently available data on the epidemiology of malaria in pregnancy (MiP), the efficacy, safety and cost-effectiveness of potential interventions to control MiP in the country are inadequate to recommend evidence-based policies. The study was initiated to generate information on the effect of intermittent screening and treatment (IST) on the risk of placental malaria. Also, the incidence of clinical malaria in pregnancy and the effect of IST on clinical malaria during pregnancy will also be studied. Screening for malaria is done during routine antenatal care visits, using rapid diagnostic tests (RDTs).

The study was launched on 30 April 2012 at Jharkhand by Dr V.M. Katoch, Secretary/DHR and DG, ICMR and Shri Vidhya Sagar, Secretary, Health, Govt. of Jharkhand. Patient recruitment has been initiated in 4 blocks in two study districts, namely Simdega and Gumla. Till date, a total of 1619 pregnant women have been enrolled in two study arms, namely passive case detection (PCD) and intermittent screening and treatment (IST) group. Enrolment of 1619 patients has been completed in 56 sub-centres. Out of 1619 enrolled patients in two arms, 780 patients are enrolled in IST arm. A total of 594 deliveries have taken place till date.

MIP launch meeting at RIMS, Ranchi.

4.6 Deletions of *HRP2* and *HRP3* antigens among Indian *Plasmodium falciparum* isolates

The study suggests the presence of *Pfhrp2*/*Pfhrp3* deletions in Indian *P. falciparum* isolates which may be the possible factor behind misdiagnosis/false negative malaria rapid diagnostic tests. A total of 100 samples positive for *P. falciparum* by microscopy from six different malaria endemic regions of country were analysed. Their parasite densities ranged from 1800 to 54,448 parasites/µl. All the samples except two from Chhattisgarh (CB18 and CB21) were found positive for *P. falciparum* by both the RDTs. Parasite densities of CB18 and CB21 were 47,136 and 6952 parasites/µl, respectively. All the three single copy genes (*Pfmsp1, Pfmsp2* and *Pfglurp*) were amplified and confirmed the intactness of sample DNA. When *Pfhrp2* and *Pfhrp3* genes were characterized, eight expected PCR products were observed in all samples except two (CB18 and CB21). The samples CB18 and CB21 did not yield the expected PCR products of exon 2 of *Pfhrp2*/*Pfhrp3* and flanking upstream and downstream genes (Table 3).

The reference strain 3D7 yielded all the eight expected products of *Pfhrp2* and *Pfhrp3*. Reference strain Dd2 lacked *Pfhrp2* and both flanking genes, but *Pfhrp3* and flanking genes were present. The quantification of HRP2 protein in patient's serum/plasma samples were performed by ELISA and compared with standard curve prepared by using recombinant HRP2 antigen (Table 4). Serological test also confirmed the same as that of molecular analysis. The study suggests presence of *P. falciparum* isolates lacking *Pfhrp2* and *Pfhrp3* genes.

Table 3. Enrolment status at study sites

Characteristics	Ranchi	Rourkela	Jamshedpur
No. of pregnant women in cohort	1708	3072	1588
Number of eligible patients	104	73	64
Number of enrolled patients	88	64	58
Number of patients completed the study	78	56	52
Number of SAE	8	7	6
Adverse reaction	0	0	0
Re-infection	0	0	1

Table 4. RDTs and PCR results of *Pfhrp2*, *Pfhrp3* and their flanking gene in reference culture lines and selected *P. falciparum* field isolates

Lines/Isolates	Parasitaemia p/µl	RDTs	*Pfmsp1*	*Pfmsp2*	*Pfglurp*	Upstream *Pfhrp2*	*Pfhrp2* Exon 1–2	*Pfhrp2* Exon 2	Downstream *Pfhrp2*	Upstream *Pfhrp3*	*Pfhrp3* Exon 1–2	*Pfhrp3* Exon 2	Downstream *Pfhrp3*
3D7	NA	NA	+	+	+	+	+	+	+	+	+	+	+
Dd2	NA	NA	+	+	+	–	–	–	–	+	+	+	+
CB18	47.136	–	+	+	+	–	–	–	–	–	–	–	–
CB21	6.952	–	+	+	+	–	–	–	–	–	–	–	–

Note: Only suspected *P. falciparum* isolates out of 100 have been shown here, rest of isolates were positive throughout; (+)ve—Positive result; (–)ve—Negative result.

4.7 A Randomized Controlled Trial of Artesunate + Sulfadoxine-Pyrimethamine (AS+SP) *vs* Artesunate+Sulfadoxine-Pyrimethamine + Primaquine (AS+SP+PQ) for decreasing Malaria Transmission in India

Table 5. Enrolment by arm

Arm	N	Mean Hb (g/dl) D0	Mean Hb (g/dl) D7	Gametocytaemia D0 (%)
ASP	38	10.77	10.16	18.4
ASP + PQ D0	38	10.35	9.84	21
ASP + PQ D2	37	10.38	10.02	18.9

The study compares the safety and efficacy of Artesunate + SP and Artesunate + SP + PQ (Day 0 or Day 2) for the treatment of *P. falciparum* gametocytaemia. The Artemisinin combination therapies (ACTs) are now the first-line treatment in most of the countries including India. A key benefit of ACTs is their activity against immature gametocytes and subsequent reduction of transmission. It is unclear whether the addition of another agent to eliminate mature gametocytes will be advantageous. Before the switch to ACT, the use of primaquine for gametocidal effect was part of the national drug policy in India. A single dose of primaquine is inexpensive and effective against all the stages of gametocytes. Thus, the key operational question now is whether primaquine should be added to the artemisinin combination treatments for the treatment of falciparum malaria to reduce further transmissibility of the treated infection.

This is a randomized open labelled clinical trial. Cases of malaria were detected by passive surveillance in Ranchi and received artesunate plus sulfadoxine-pyrimethamine in a standard 3-day course (4 mg/kg each day + 25/1.25 mg/kg stat). They were randomized to receive a single dose of either primaquine or no drug (0.75 mg base/kg; usual adult dose 45 mg). Within the patients selected to receive primaquine, they were randomized to receive primaquine on D0 or D2. Thus, the allocation ratio for no PQ, D0 PQ, and D2 PQ is 2:1:1. The patients were tested for haemoglobin, parasite counts, G6PD, and patients

Table 6. Study sites

District	Balangir		Dhenkanal		Angul		Kandhamal	
Type	Low		Medium		High		Hyper	
Status	Control	Inverv	Control	Inverv	Control	Interv	Control	Interv
Block	Saintala	Puintala	Bhuban	Hindol	Chhendi	Athama	Khajuripa	Nuagaon
ABER	14	14	13	12	17	20	34	18
SPR	1	2	1	4	6	8	9	16
Pf%	37	56	70	58	89	96	99	99
API	2	2	2	5	10	17	29	30
Cases	246	261	186	908	1489	1938	1556	1683
Pop (1000s)	117	110	104	174	167	121	53	56

were examinted weekly for a total of 28 days according to WHO protocol. The enrolment is complete with 113 patients and the data are being analysed (Table 5).

4.8 Comprehensive Malaria Case Management Pilot Programme, Odisha

A case management system for malaria is essential for endemic areas as a mean of 'treating the sick' to reduce the duration of sickness and prevent the disease progressing to severe malaria. However, beyond this purpose, early termination of infections serves, particularly in areas of unstable malaria with low to moderate intensities of transmission, as an important means of curtailing the size of the infectious reservoir. Thus, comprehensive case management system will contribute significantly to transmission reduction, particularly, if attention is paid to anti-gameto-cytocidal medication as a complement to curative treatment.

This is a 3-year project in partnership with the State Govt. The CCM project staff of 22 employees have been recruited and trained. Baseline surveys including facility, household, and vector work is completed. A data management system and patient cards were developed. Training of trainers from four districts was conducted and implementation is ongoing (Table 6).

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5

Highlights of Research Activities under IDVC Project

5.1 Bengaluru (Karnataka)

- Two larvivorous fish, *Poecilia reticulata* and *Gambusia affinis* are being used in malaria control in Karnataka. The study has been extended to the northern districts of Karnataka.
- Therapeutic efficacy of ACT against *Plasmodium falciparum* in Gadchiroli district showed good response.
- Clinical trial of Arterolane showed satisfactory response in *Plasmodium vivax* patients in Mangalore city.
- The extended phase of C-21 trial for *Ae. aegypti* indicated a better option for its control.
- Plant extract from *Rutacae* family is being analyzed for its antimosquito properties.
- *In vitro* gametocytogenesis of *P. falciparum* has been successful. Proteome analysis of gametocytes of chloroquine resistant and sensitive strains is underway.
- Field trial on Lambdacyhalothrin 10CS has been initiated in Karnataka.
- Malaria research training programme has been initiated. Clinical blood samples are being collected.

5.2 Chennai (Tamil Nadu)

- Field evaluation on the application of Attracticide (oviposition pheromone in combination with IGR) for surveillance and control of dengue and chikungunya mosquitoes was undertaken in Alappuzha district, Kerala.
- Phase-III trial to evaluate Biocide (*Bti* AS VCRC B17) was carried out in Chennai and 25 weeks of post-treatment survey for a period of six months was completed.
- A multicentric study on the impact of new control tools such as ACT, RDT and LLIN on changing patterns of malaria was initiated and 48 PHCs in different districts of Tamil Nadu were selected for the study.
- Plant and soil samples from mangrove ecosystem were screened to test the larvicidal, ovicidal, ovipositional and repellent properties of plant crude extracts and also isolated the bacterial and fungal strains from soil samples to test the potency and efficacy for larvicidal properties.
- Preliminary study was undertaken to detect the presence of *Wolbachia* in the mosquito species present in nature.
- Under Centre for the Study of Complex Malaria in India (CSCMi), studies on vector bionomics, eco-epidemiology of malaria including cross-sectional study on the socio-demographic profile of the households; mapping of malaria cases; and differential diagnostic study to compare the various diagnostic methods were carried out.

5.3 Guwahati (Asom)

- Malaria-risk maps are being developed for northeastern state of Tripura to strengthen the malaria control programme.
- 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 are in progress.
- Study on the therapeutic efficacy of ACT (AS + SP) was undertaken along Indo-Bangladesh border in Mizoram. Based on extended follow-up study, treatment failures have been recorded in Lunglei district of Mizoram.
- Controlled study of possible adverse effects of DDT on human reproductive health is being

undertaken with special reference to lactation and pregnancy outcome.

- With climate change and associated disease transmission profile, "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" is presently in progress. Systematic sampling of mosquito species revealed that populations of *Anopheles minimus* are diminishing and the disease transmission trends are declining across the north-eastern states.
- To understand the bionomics of disease vectors in the changing ecological context, study on distribution and biological characteristics of the members of the Fluviatilis-Minimus group was undertaken in high-risk districts of Asom. Populations of *An. minimus* and *An. culicifacies* were characterized by genetic and molecular assays for targeting focused interventions for effective disease control.
- Other activities included technical inputs to strengthen the malaria control activities specific to north-eastern region, viz. health education and capacity building measures, mass propagation and distribution of larvivorous fish (Guppy and *Gambusia*) in town areas, and in providing technical expertise on long-lasting insecticidal nets.

5.4 Hardwar (Uttarakhand)

- A plant-based immersion oil as a substitute of synthetic immersion oil is being developed.
- DDT residue in human milk, human blood and adipose tissue from DDT sprayed areas (5 states) and non-sprayed areas (2 states) have been compared.
- DDT residues in human blood and human milk were determined in women before pregnancy and after pregnancy to correlate lactation and pregnancy outcome.
- A project on remote sensing has been undertaken to prepare cost-effective model for malaria control based on the results extracted through Remote Sensing and Geographic Information System.
- Studies on evaluation of NetProtect LLIN impregnated with deltamethrin are under monitoring for second year.
- Entomological investigations have been carried out to establish vector of J.E. in District Saharanpur, Uttar Pradesh.
- Evaluation of 2% granular formulation of Diflurobenzuron against larvae of mosquitoes has been completed.
- Epidemiological investigations of malaria in District Saharanpur were carried out.
- Antiplasmodial activity of some synthetic compounds received from Jamia Hamdard University was carried out against *P. falciparum* isolates.
- Consultancy services to various industries for control of malaria is being provided.

5.5 Jabalpur (Madhya Pradesh)

- The evaluation of long-lasting insecticidal nets in Madhya Pradesh was carried out in CHC Kundam of Jabalpur.
- 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.
- Malaria outbreak investigation was carried out in Anooppur, Sidhi and Singrauli districts of Madhya Pradesh.
- Cone bioassay tests were carried out in 9 districts in which decreased efficacy of Alpha-cypermethrin was observed.
- Three training workshops for Medical Officers of various districts of Madhya Pradesh on malaria and other vector-borne diseases were organized during the year.

5.6 Nadiad (Gujarat)

- Studies were continued on the health impact assessment of development project (Sardar Sarovar Project) on vector-borne diseases in Gujarat.
- Centre for Study of Complex Malaria in India (CSCMi), a collaborative project of Indian and US-based scientists was initiated in Gujarat in November 2012. Nadiad town has been included in the study to determine how different *P. falciparum* and *P. vivax* transmission ratios impact malaria burden.
- Therapeutic efficacy of chloroquine in *P. vivax* patients was conducted in Ahmedabad city.

- Studies on developing a framework for predicting malaria outbreaks in rural and urban areas of Gujarat and Rajasthan were continued.
- WHOPES sponsored small-scale field testing and evaluation to compare residual efficacy of Pirimiphos-methyl CS (Actellic 30% CS) with Pirimiphos-methyl 50% EC by indoor residual spraying on different local indoor surfaces was conducted.
- Effectiveness and persistence of *Bacillus thuringiensis* (*Bti* AS VCRCB 17), an Indian strain was evaluated in Kheda, Gujarat as per the directives of the ICMR Expert Committee on *Bti* technology.
- Technical support was provided to the State Vector-Borne Diseases Control Programme in terms of malaria diagnosis and treatment, assessment of malaria situation and control programme in Ahmedabad and entomological surveys in dengue-affected areas of Gandhi Nagar district.

5.7 Panaji (Goa)

- A comprehensive proteomic analysis of *An. stephensi,* a vector of malaria was carried out to characterize proteins expressed in diverse tissues and developmental stages.
- Studies on characterization of dengue/DHF, chikungunya and yellow fever vector *Ae. aegypti* proteome were initiated.
- Larvicidal activity of methanol and chloroform extracts of dried leaves of IC_Goa plant was evaluated against urban malaria vector *An. stephensi*, filariasis vector *Cx. quinquefasciatus*, dengue and chikungunya vector *Ae. aegypti*.
- The efficacy of VectoBac GR (*Bacillus thuringiensis israelensis* strain AM65-52), was evaluated as a larvicide using WHO guidelines for testing larvicides.
- Vector surveillance was carried out in seven malaria high risk areas of Goa in support of malaria elimination project.
- Epidemiological surveillance was carried out to study malaria problem in Mumbai in coordination with the Municipal Corporation of Greater Mumbai and State Directorate of Health Services.
- A collaborative project with University of Washington and funded by NIH was initiated to study epidemiology and malaria evolution in south Asia.
- Conducted quality assessment of LLINs procured by NVBDCP-DHS by periodical bioassays.

5.8 Raipur (Chhattisgarh)

- Evaluation of PermaNet® 3.0, a combination LLIN against malaria vectors and for its impact on malaria incidence in selected villages of Kanker district was initiated.
- Evaluation of indoor residual spraying of chlorfenapyr SC 240 g a.i./l, a novel insecticide belonging to pyrrole group was undertaken at 150 and 250 mg a.i./m^2 in comparison to deltamethrin WG (250 g a.i./kg) at 25 mg/m^2 in Kondagaon district.
- A Phase-III evaluation of a new candidate long-lasting insecticidal net DawaPlus 2.0 was initiated for its bio-efficacy, durability and community acceptability.
- A field site has been established at Kondagaon district in southern Chhattisgarh to study the impact of insecticide resistance in malaria vectors on the effectiveness of combination of indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs).
- Therapeutic efficacy of ACT is being monitored in various districts of Chhattisgarh in uncomplicated *P. falciparum* malaria positive cases.

5.9 Ranchi (Jharkhand)

- The survey for the species composition of anopheline mosquitoes was undertaken in Latehar and West Singhbhum districts.
- Studies were conducted on the sibling species composition of malaria vector, *An. culicifacies, An. fluviatilis* and *An. annularis* in Gumla, Simdega and West Singhbhum districts.
- Studies were continued on field evaluation of NetProtect LLIN (impregnated with deltamethrin) against malaria vectors and its impact on malaria.
- Therapeutic efficacy of ACT (AS+SP) was investigated at Kathikund CHC, Dumka district.
- Filariasis survey was carried out among the

tribes of Santhal, Munda and Oraon in PHC Jasidih, Deoghar district.

- Assessment of mass drug administration for filariasis control was carried out in Godda and Deoghar districts and evaluation was carried out according to the guidelines of the NVBDCP.

5.10 Rourkela (Odisha)

- WHOPES Phase-III evaluation to compare insecticidal efficacy and community acceptance of long-lasting insecticidal net Duranet® with conventional insecticide-treated nets was completed.
- Study was continued on Phase-II/III randomised clinical trial on the efficacy and safety of artesunate + sulphadoxine-pyrimethamine and artesunate + mefloquine to treat uncomplicated *falciparum* malaria in pregnancy.
- Comprehensive malaria case management programme was initiated in four districts of Odisha representing low, medium, high and hyper-endemic malaria transmission.
- Study on the assessment of treatment seeking behaviour, LLIN usage and IRS acceptance by the tribal communities was carried out in Keonjhar district of Odisha.
- A multicentric study on the impact of new control tools and changing patterns of malaria was initiated in a tribal district of Odisha.
- Centre for the Study of Complex Malaria is operational with the funding from NIH to understand the eco-epidemiology of malaria in Odisha.
- Technical support was provided to NVBDCP in identifying the *Aedes* breeding in the affected districts so that targeted interventions may be launched during the outbreak.

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Research Support Facilities

6.1 Animal House Facility

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

6.2 Repository of Biological Materials

6.2.1 Mosquito species

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

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

Mosquito species	Strain/Origin	Mitotic/Karyotype/ Y-chromosome	Sibling species
Anopheles stephensi	Delhi		
An. stephensi	Punjab		
An. stephensi	Haryana		
An. stephensi	Masoodpur (Delhi)		
An. stephensi	Manesar (Haryana)		
An. stephensi	Alwar (Rajasthan)		
An. stephensi	Raipur (Chhattisgarh)		
An. stephensi	Gurgaon (Haryana)		
An. stephensi	Kolkata (West Bengal)		
An. stephensi	Goa		
Anopheles culicifacies	Burari (Delhi)	Sub-metacentric	A
An. culicifacies	Rameswaram (Tamil Nadu)	Sub-metacentric	A
An. culicifacies	Dehra (Himachal Pardesh)	Sub-metacentric	A
An. culicifacies	Dadri (Uttar Pradesh)	Sub-metacentric	A
An. culicifacies	Beel Akbarpur (Dadri)	Sub-metacentric	A
An. culicifacies	Manki (Uttar Pradesh)	Sub-metacentric	A
An. culicifacies	Raipur (Chhattisgarh)	Sub-metacentric	B
Anopheles fluviatilis	Rourkela (Odisha)		T
An. fluviatilis	Haldwani (Uttarakhand)		T
An. fluviatilis	Nangla Nain Sukh (Dadri)		T
Culex quinquefasciatus	B.S.S.S.		
Cx. quinquefasciatus	Jamshedpur (Jharkhand)		
Cx. quinquefasciatus	Manesar (Haryana)		
Cx. quinquefasciatus	Mewat (Haryana)		
Aedes aegypti	Delhi		
Mutant Lines			
An. stephensi	Black larvae with white eye		
Cx. quinquefasciatus	Red eye		

6.2.2 Malaria Parasite Bank (MPB)

Malaria Parasite Bank is supporting to a large number of organizations working on various aspects of malaria and is functioning as a National Resource facility. Biological materials including non-human and human plasmodia preserved/ maintained in Malaria Parasite Bank (MPB) were supplied to various research organizations.

Collection of biological materials

Till now, a total of 1309 isolates of human malaria parasites *P. falciparum, P. vivax* and *P. malariae* were collected (Table 2) and cryopreserved in Malaria Parasite Bank. A total of 56 isolates including 11 *P. falciparum* and 45 *P. vivax* were collected from different geographical regions of India during 2012–13. All these parasites are cryopreserved in liquid nitrogen in the centralized facility of MPB.

Man power development

During the reporting period 20 scientists/ students/researchers completed their training in the MPB including one foreign scientist for one year and five students completed their M.Sc. (Biotechnology/Microbiology) dissertation work for

Table 2. Human malaria parasites collected in Malaria Parasite Bank (1992–2013)

Parasite species	Collection sites		1992–2011	2012	2013	Total
	States	Districts				
Plamodium falciparum	Andhra Pradesh		12	—	—	12
	Asom		27	—	—	27
	Chhattisgarh		54	—	17	71
	Delhi		199	—	—	199
	Gujarat	Kheda	21	—	—	21
	Haryana		25	—	—	25
	Jharkhand	Ranchi	37	—	22	59
	Karnataka		29	—	1	30
	Madhya Pradesh		17	—	—	17
	Maharashtra	Gadchiroli	—	10	—	10
	Meghalaya		18	—	—	18
	Mizoram		6	—	—	6
	Odisha		106	—	—	106
	Goa		18	—	—	18
	Rajasthan		109	—	—	109
	Tamil Nadu		29	—	—	29
	Uttar Pradesh	Ghaziabad	160	1	—	161
	Uttarakhand		3	—	—	3
	West Bengal		19	—	—	19
	Total *P. falciparum*		889	11	40	940
P. vivax	Delhi		41	—	—	41
	Uttar Pradesh		54	—	—	54
	Odisha		22	—	—	22
	Madhya Pradesh		10	—	—	10
	Chhahttisgarh	Kanker	2	—	2	4
	West Bengal		1	—	—	1
	Asom		2	—	—	2
	Tamil Nadu		39	—	—	39
	Goa		23	—	—	23
	Karnataka		52	—	14	66
	Rajasthan	Jaisalmer	51	23	—	74
	Gujarat	Kheda	6	11	—	17
	Uttarakhand	Nainital	—	7	—	7
	Maharashtra	Gadchiroli	—	4	—	4
	Total *P. vivax*		303	45	16	364
P. malariae	Odisha		4	—	—	4
	Delhi		1	—	—	1
	Total *P. malariae*		5	—	—	5
	Total isolates		1197	56	56	1309

four months in the MPB laboratory. As part of manpower development, scientists/Ph.D. students/ researchers were trained in identification and *in vitro* cultivation of malaria parasites, *P. falciparum;* screening of antiplasmodial properties of medicinal plant extracts/handling of animals and maintenance of non-human malaria parasites *in vivo*, etc. A total of 210 students/scientists including 36 foreign scientists were trained in Malaria Parasite Bank to till date.

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 1A7 (anti-*P. vivax* sporozoite antibody secreting cells).

Supply of biological materials

During the reporting period 139 samples/vials/ positive sera/plasma has been supplied to different organizations. Biological materials including human and non-human malaria parasites and plasma/sera from malaria-infected human beings and animals were supplied to 75 Universities/Institutes/organisations to till date. A total of 1350 samples/vials were supplied to various major Research Institutions and Universities in all over India.

Resource generation

For resource generation, the MPB has been charging a very nominal amount for the supply of biological materials. Till now ₹ 4,07,000 have been collected on this account. A total of ₹ 19,000 has been generated during the year 2012–13.

Training facilities available at 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* and other species of *Plasmodium*
- *In vitro* cultivation of pre-erythrocytic stages of *P. vivax*
- *In vitro* testing for sensitivity of *P. falciparum* isolates to antimalarials
- *In vitro* and *in vivo* screening of medicinal plant extracts for its antiplasmodial properties.

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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 the Government of Odisha and Medicine 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. 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 the National Vector Borne Disease Control Programme (NVBDCP).
5. Monitoring the therapeutic efficacy of antimalarial medicines in India in collaboration with NVBDCP.
6. Pharmacovigilance of antimalarial 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. The project "Evidence-based assesment 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" is being undertaken in collaboration with IIT Delhi particularly for development of mathematical model of malaria transmission.
9. The project on Micro stratification of malaria in problematic districts of Rajasthan is being undertaken in collaboration with the Government of Rajasthan.
10. The project on Developing a framework for ealy warning of malaria was undertaken in collaboration with Michigan University (U.S.A.) and the state governments of Rajasthan and Gujarat.
11. Studies on Health impact assessment of Sardar Sarovar project in command area of Rajasthan in collaboration with Narmada Valley Development Authority (NVDA)/Govt. of Rajasthan.
12. Health impact assessment of Narmada Basin Dams and resettlement & rehabilitation colonies in Madhya Pradesh in collaboration with NVDA, Bhopal
13. Spatio-epidemiological analysis of dengue in New Delhi (*Aedes*) in collaboration with Institut Pasteur, Paris, France.
14. Finding the 'Gatekeeper residues' and engineering "substrate specificity" in collaboration with JNU, New Delhi, funded by ICMR.
15. Centre for the Study of Complex Malaria in India in collaboration with New York University and Pennsylvania State University, USA, funded by NIH.
16. Establishing immunological correlates against malaria vaccine candidates using functional bioassays and proteomic deciphering of host parasite interactions in collaboration with I.I.I. Medicine, 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, Denmark and Expression Biotechnologies, Horshlm, Denmark.

17. Comprehensive case management malaria pilot programme in Odisha, in collaboration with Stephan Duparc, Penny Grewal Daumereie Jaya Banerji, Medicine for Malaria Venture.
18. Phase-II baseline household survey in World Bank Project Districts of Gujarat, Maharashtra, Karnataka and West Bengal in collaboration with NVBDCP.
19. Assessment of the effectiveness of intensive intervention measures on malaria control programme in tribal district, Balaghat, Madhya Pradesh in collaboration with RMRCT, Jabalpur.
20. To determine the effectiveness of intensive intervention measures on malaria prevalence in tribal district, Dindori, Madhya Pradesh in collaboration with RMRCT, Jabalpur.
21. Bionomics of malaria vectors and their sibling species, and to establish their role in malaria transmission in Chhattisgarh in collaboration with RMRCT, Jabalpur.
22. Clinical and molecular surveillance for monitoring the emerging resistance to antimalarial drugs in *Plasmodium falciparum* in central India, in collaboration with RMRCT, Jabalpur.
23. Malaria control in highly malarious district Balaghat by mass blood surveys and drug administration in collaboration with RMRCT, Jabalpur.
24. Development of plant-based immersion oil for microscopy in collaboration with Forest Research Institute, Dehradun.
25. Synthesis, pka determination and *in vivo* toxicity of a new promising antimalarial 6-methoxy-5,8-di-(4-amino-1-methyl-butyl-amino)-quinoline in collaboration with Jamia Hamdard University, Delhi and National Institute of Pharmaceutical Research, Punjab.
26. Develpoment of botanical insecticide formulation of essential oils extracted from *Lantana camara* and *Valeriana jatamansii* and *Psoralea corylifolia* for the control of mosquitoes in collaboration with Defence Research Laboratory, Tezpur (Asom).

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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 9 students are working for their Ph.D. degree under the supervision of NIMR scientists.

8.2 Ph.D. Awardees

1. Ripu Daman Sood submitted Ph.D. Thesis to Indira Gandhi National Open University (IGNOU) and provisional degree has also been awarded.
2. Bhavna Gupta was awarded Ph.D. by Jiwaji University, Gwalior.
3. Jyotsana Dixit was awarded Ph.D. by Jiwaji University, Gwalior.
4. Gauri Awasthi was awarded Ph.D. by Jiwaji University, Gwalior.
5. Anita Chittoria was awarded Ph.D. by Jiwaji University, Gwalior.
6. Sonam Vijay submitted Ph.D. Thesis to Jiwaji University, Gwalior.
7. Manmeet Rawat submitted Ph.D. Thesis to Jiwaji University, Gwalior.

8.3 M.Sc. Projects

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

8.4 Training Courses Organized

NIMR has conducted regular training programmes as under:

- Collection, cryopreservation, revival and transportation of malaria parasite isolates/ strains
- *In vitro* cultivation of erythrocytic stages of *P. falciparum*
- Short-term cultivation of *P. vivax* and other species of *Plasmodium*
- *In vitro* cultivation of exo-erythrocytic stages of *P.* vivax
- *In vitro* testing for sensitivity of *P. falciparum* isolates to antimalarials
- *In vitro* and *in vivo* screening of medicinal plants for antiplasmodial properties
- Microscopic diagnosis of malaria parasites and cytological identification of sibling species of mosquitoes
- Field-oriented training on mosquito collection, preservation, dissection, etc.

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9

Research Papers Published
(January–December 2012)

1. Anvikar AR, Sharma B, Shahi BH, Tyagi PK, Bose TK, Sharma SK, Srivastava P, Srivastava B, Kiechel JR, Dash AP, Valecha N. Artesunate-amodiaquine fixed dose combination for the treatment of *Plasmodium falciparum* malaria in India. *Malar J* 2012; *11:* 97.
2. Anvikar AR, Sharma B, Sharma SK, Ghosh SK, Bhatt RM, Kumar A, Mohanty SS, Pillai CR, Dash AP, Valecha N. *In vitro* assessment of drug resistance in *Plasmodium falciparum* in five states of India: *Indian J Med Res* 2012; *135:* 494–9.
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10

Other Activities

10.1 Information, Education and Communication

10.1.1 Awareness about mosquito borne diseases

School children from various schools of Delhi visited the Institute. They were taken around to different laboratories and interaction with concerned scientists was held. Students were briefed about prevention from mosquito borne diseases through popular talks and with aid of slides and video shows. Pamphlets were distributed. Books were given for schools' libraries to be used by teachers and other students.

10.1.2 Household survey for NVBDCP

Preparations, such as finalization of 10 types of tools (questionnaires) and translation of English questionnaires into other regional languages was undertaken. Subcentre and village-wise data from states were collected for random sampling of the villages for forthcoming household survey to be initiated in July 2013.

10.1.3 Documentation cell

In Documentation cell, the following information were collected and compiled.

1. Detailed information regarding all research projects undertaken by NIMR such as Principal Investigator, Co-PI, Co-I, grant of budget, funding agency, date of start, date of completion, subject, status and reasons for extension of projects etc. were collected and enlisted for the period from 1981–2013.
2. Up to date lists of intramural and extramural projects undertaken by NIMR were prepared up to 2013.
3. All the project files of approved research proposals undertaken by NIMR for the period of 2005–13 were compiled.
4. A list of research publications published in national and international journals was updated.
5. Soft copies of published research papers by the NIMR scientists for the year 2012 were compiled.

10.1.4 Photography

In the photography section, photography work was carried out on various occasions/meetings/trainings/workshops/field surveys/functions held at NIMR and ICMR. Photography coverage of meeting on Malaria in southeast Asia: Perspectives, progress and partnerships, organised by MMV and NIMR at New Delhi, Hindi week celebrations at NIMR, New Delhi, induction training programme for VBD consultants organized by NIMR and NVBDCP, and workshop on Comprehensive case management pilot in Odisha protocol development was also undertaken.

Still photography

In the still photography section following photography work was carried out during various meetings/workshops/functions/scientific visits, etc:

- "Comprehensive case management pilot in Odisha" workshop held in May 2012
- Medical students visited NIMR in May 2012
- Photography of spleen gel for Department of Immunology Laboratory
- Hindi week celebrated in September 2012
- Induction training programme for VBD consultants during June to August 2012
- Malaria in southeast Asia perspectives and partnerships MMV 12th state holders meetings held at Radisson Blue Hotel, New Delhi in November 2012
- World Bank project training on Malaria microscopy and molecular studies held in October 2012

- Workshop of Medical Doctors for sanitation of doctors for their participation in Prevention and control of vector borne diseases held in November 2012
- RAC and SAC meetings held from 20–22 December 2012.

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

Anvikar Anup

1. Launch meeting of the project 'Safe and effective interventions for prevention of malaria in pregnancy' in Ranchi on 30 April 2012.
2. Delivered talk on Malaria treatment in India for Medical Officers of Brihad Mumbai Municipal Corporation, Mumbai from 17–18 May 2012.
3. Delivered a lecture on Monitoring drug safety in public health programme in the Certificate Programme on International Public Health at Empower School of Health, Delhi on 16 August 2012.
4. Attended workshop of the International Centres of Excellence for Malaria Research in Goa from 22–24 August 2012.
5. Delivered invited lecture on Challenges in diagnosis and treatment of malaria in the 18th Maharashtra Chapter Conference of IAMM in Mumbai on 7 September 2012.
6. ASEAN-India S & T Senior Officials meeting and IX ASEAN-India working group on Science and Technology meeting in New Delhi on 11 September 2012.
7. Attended 'G6PD testing and malaria advisory workshop' in Bangkok, Thailand from 4–5 October 2012.
8. Delivered invited lecture on Clinical and molecular insights into antimalarial drug resistance at the CME on Genomes in parasitic diseases under the Tropacon 2012 at Sri Aurobindo Institute of Medical Sciences, Indore on 12 October 2012.
9. Attended round table meeting on Accelerating easy translational development of malaria vaccine through challenge studies conducted in India in New Delhi from 22–23 October 2012.
10. Attended conference on Malaria in southeast Asia: Perspectives, progress and partnerships organized by MMV and NIMR in New Delhi from 6–8 November 2012.
11. Attended 61st Annual meeting of the American Society of Tropical Medicine and Hygiene in Atlanta, U.S.A. from 11–15 November 2012.
12. Delivered a talk on Challenges in malaria diagnosis at the training programme on 'Genomic technologies in malaria research' at NIMR on 19 November 2012.
13. Attended WHO Regional workshop on Quality of malaria microscopy at Bureau for Vector Borne Disease Training Centre, Saraburi, Thailand from 26–28 November 2012.
14. Delivered series of talks on malaria in the 'Training of Trainers' programme at Government Medical College, Surat on 19 December 2012.
15. Launched a meeting of the project "Çomprehensive case management of malaria" in Bhubaneswar on 19 January 2013.
16. Delivered a talk on Antimalarial chemotherapy workshop on Prevention and control of vector borne diseases at MCD, Delhi on 20 February 2013.
17. Delivered a talk on Antimalarial drug resistance in a 'Training of Trainers' programme at King George's Medical College on 27 February 2013.
18. Delivered series of talks on malaria in a 'Training of Trainers' programme at SCB Medical College, Cuttack on 9 March 2013.
19. Delivered series of talks on malaria in a 'Training of Trainers' programme at Agartala Government Medical College on 20 March 2013.

Das Aparup

A laboratory course on 'Application of genomic technologies in malaria research' was organized in the institute from 18 November to 1 December 2012 primarily focusing on providing hands-on training to participants on all aspects of molecular genetics, DNA sequencing and related bioinformatic analyses of DNA sequence data using malaria as a model. Fifteen mid-career scientists working on malaria or other vector borne diseases

Director NIMR (Dr Neena Valecha) addressing the participants

Inaugural ceremony of the workshop in progress

Participants collecting mosquito larvae from the field

The organizer (Dr Aparup Das) and the participants of the workshop

Dr Pawan Malhotra (ICGEM, New Delhi) delivering a lecture at the workshop

The Director NIMR (Dr Neena Valecha) distributing certificates to the participants

models from all over Indian universities and institutes attended the course. The course was mainly focused on providing hands-on training on Molecular genetics, genomics and bioinformatics with field trips and lectures from eminent scientists from India. The workshop was funded by the Department of Biotechnology (DBT) and the Indian Council of Medical Research (ICMR), New Delhi.

Dhiman RC

1. Participated as invited speaker in a symposium on 'Biomedical research in

Dr Arun Sharma, Scientist F, NIMR felicitating the Director NIMR

Dr Jyoti Das, Scientist D, NIMR felicitating Dr Hemlata Balaram of the JNCASR, Bengaluru

Participants collecting mosquito samples

A section of the participants of the workshop

medical institutions' at RMRC, Bhubaneswar on 1 April 2012.

2. Participated in a training programme conducted by NCDC and delivered lecture on 'Climate change and health emphasis on vector borne diseases' on 21 May 2012.
3. Participated in a meeting of the Indian Network of Climate Change Assessment (INCCA) Advisory Committee for Implementation of Science Programme at Paryavaran Bhawan, New Delhi on 18 July 2012.
4. Represented Ministry of Health (Nominated by the Secretary, DHR & DG, ICMR) with Indian delegation to World Meteorological Organization's Congress on Global framework for climate services held in Geneva from 26–27 October 2012.
5. Visited Ann Arbor, Michigan University, USA for discussion on Malaria prediction tool and its implementation for public health use related with HMSC approved collaborative project from 12–16 November 2012.
6. Participated as resource person in Training of Trainers programme sponsored by NIH & FW, organised by Medical College, Bellary from 6–7 December 2012.
7. Participated as resource person in Training of Trainers programme sponsored by NIH & FW, organised by SCB Medical College, Cuttack from 7–8 January 2013.
8. Delivered an invited lecture on 'Climate change and health' in a workshop organized by the Centre for Climate Change and Environment and the National Institute of Administrative Research, Lal Bahadur Shastri National Academy of Administration at IIC, New Delhi on 18 March 2013.
9. Participated as resource person in Training of Trainers programme sponsored by NIH&FW in Kohima, Nagaland from 20–21 March 2013.
10. Serving as one of the national coordinators for Climate change and health network constituted by DST and participated in meetings at IIT, New Delhi.

Dixit Rajnikant

Attended meeting of the Task Force project on 'Biology and bionomics of vectors', under Vector Science Forum of ICMR held on 2 November 2012.

Mishra Neelima

1. Attended Implementation Review World Bank Mission at Directorate of NVBDCP on 20 July 2012.
2. Attended review meeting of ongoing projects on Malaria at RMRC, Bhubaneswar on 25 July 2012.
3. Attended meeting on Tribal Health Forum at RMRC, Bhubaneswar from 8–9 August 2012.
4. Attended meeting of Inter-country consultation on Networking for malaria control/elimination in SEA region (WHO) in Paro, Bhutan from 14–16 August 2012.
5. Attended meeting on *Plasmodium* bioinformatics exercises under ICEMR in Goa from 22–24 August 2012.
6. Attended meeting with CARITAS India under Project Steering Committee at the Directorate of NVBDCP on 25 September 2012.
7. Attended workshop on Clinical research informatics and redcap in Chennai from 3–5 October 2012.
8. Poster presented on "Clinical and molecular insights into antimalarial drug resistance" in the VI National Conference of the Indian Academy of Tropical Parasitology and Department of Microbiology, Sri Aurbindo Medical College & P.G. Institute, Indore from 11–14 October 2012.
9. Attended meeting of IAEC (Institutional Animal Ethics Committee) of the National Institute of Malaria Research (NIMR), New Delhi on 6 November 2012.
10. Poster presented on "Nation-wide sentinel site system for monitoring antimalarial drug resistance in India" in ASTMH (The American Society of Tropical Medicine & Hygiene), Atlanta, Georgia, USA from 11–15 November 2012.
11. Attended meeting of Data Safety Monitoring Board (DSMB) under "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" at RIMS, Ranchi on 22 November 2012.
12. Attended ICMR workshop on Statistical concepts for clinical research professionals at National AIDS Research Institute (NARI), from 4–5 December 2012.
13. Invited talk on Current status of efficacy of ACT being used in national programme (i.e. Artesunate + Sulphadoxine) and way forward in Expert Group on Chemotherapy at Directorate of National Vector Borne Disease Control Programme, Delhi on 24 December 2012.

Pandey KC

Invited speaker in a national conference-cum-workshop on "Search for antimalarial: Mechanism based approach" organized by JNU and ICGEB in New Delhi from 27–30 April 2012.

Raghavendra K

1. Temporary Advisor of 15th WHOPES working group meeting, held in Geneva, Switzerland from 18–22 June 2012.
2. Attended the meeting on Standard operating procedure and common protocol for evaluating insecticides at VCRC, Puducherry from 30–31 January, and 3–4 October 2012.
3. Temporary Advisor in consultation workshop on Drafting guidelines for LLINs as space spray, at WHO (HQ), Geneva, Switzerland from 22–26 October 2012.
4. Delivered invited lecture on "Future of malaria vector control strategies" in an international conference—Global meet of biologists 2012 and Satellite conference vector control and management: Present status and future strategies held at IICT, Hyderabad from 26–28 December 2012.
5. Participated as country PI, India project "Implications of insecticide resistance (IIR) project" in a National coordinators meeting, held in Cotonou, Benin from 14–16 January 2013.
6. Attended 8th Annual meeting of Roll Back Malaria (RBM) partnership Working Group on Malaria Vector Control (VCWG) as a member and presented a poster on "A novel insecticide molecule for management of insecticide resistance in major malaria vectors in India", held in Geneva from 28–30 January 2013.

Member of committees

1. Member of Expert Committee on Insecticides for use in vector control by NVBDCP.
2. Member Secretary of the Translational Research Cell of NIMR.
3. Member of the Expert Committee on Clearance of *Bti* Technology, ICMR.
4. Member of the Technical Committee for Specifications, Directorate General of Health Services, Government of India.
5. Member of the ICMR sub-committee for revision of common protocol for evaluating insecticides.

Sharma Arun

1. Participated as faculty member in an induction training programme for district VBD consultants organized by NIMR, New Delhi in collaboration with NVBDCP from 25 June–3 August 2012.
2. Participated as faculty member in laboratory training course on Application of genomic technologies in malaria research held at NIMR from 18 November–1 December 2012.

Singh OP

1. Presented "The repertoire diversity of the *Plasmodium falciparum stevor* multigene family in complicated and uncomplicated malaria in India: Challenges in malaria research", organized by Biomed Central at University of Basel, Switzerland from 10–13 October 2012.

Sreehari U

1. Attended acquired training on Indoor residual spraying imparted by Dr Graham Mathews through WHOPES, Geneva at NIMR Field Unit, Nadiad from 10–14 September 2012.
2. Attended Global meet of biologists in Hyderabad from 26–28 December 2012 and presented a paper entitled, "Insecticidal efficacy of neonicotinoids against insecticide-susceptible and resistant mosquitoes".

Valecha Neena

1. Meeting to launch the project 'Safe and effective interventions for the prevention of malaria in pregnancy in India' in Ranchi on 30 April 2012.
2. Attended New Drugs Advisory Committee meeting at CDSCO Office, New Delhi on 25 May 2012.
3. Delivered a talk on Malaria treatment in children in the Indian Academy of Pediatrics symposium held at BLK Memorial Hospital, New Delhi on 21 June 2012.
4. Delivered a talk on 'Possible collaboration between NIMR & London School of Hygiene & Tropical Medicine at LSHTM, London on 2 July 2012.
5. Attended WWARN Board meeting as a member of SAC at Oxford from 4–6 July 2012.
6. Attended a meeting of the ICMR forum on Tribal health research at the National Institute of Pathology, New Delhi on 12 July 2012.
7. Attended meeting of the ICMR forum on Tribal health research at RMRC, Bhubaneswar from 8–9 August 2012.
8. Intercountry consultation on Networking for malaria control/elimination in southeast Asia region in Paro, Bhutan from 14–16 August 2012.
9. Attended meeting on "Malaria policy advisory committee" meeting of WHO as Temporary Advisor held in Geneva, Switzerland from 11–13 September 2012.
10. Attended Access and delivery advisory committee meeting of MMV as co-chair of the committee held in Geneva, Switzerland from 2–3 October 2012.
11. Attended "Expert scientific advisory committee" meeting as a member in Amsterdam, Netherlands from 16–18 October 2012.
12. Attended round table meeting on "Accelerating early translational development of malaria vaccines through challenge studies conducted in India" in New Delhi on 22 October 2012.
13. Attended IX Joint Annual Conference of Indian Society for Malaria and Other Communicable Diseases and Indian Association of Epidemiologists in New Delhi on 2 November 2012.
14. Delivered a talk on Preventing the emergence of artemisinin resistance in India: What should be done? in the meeting on "Informal consultation with National Malaria Programme Managers to strengthen malaria control and elimination" by WHO/SEARO from 5–6 November 2012.
15. Delivered a talk on the 'Role of research in improving malaria control' in "Malaria in

southeast Asia: Perspectives, progress and partnership" at MMV's in XII Stakeholders meeting organized by MMV and NIMR in New Delhi from 6–8 November 2012.

16. Delivered a talk on Malaria treatment in India in the meeting 'Genomic technologies in malaria research' at NIMR, New Delhi on 19 November 2012.
17. Delivered guest lecture on Malaria in India: Challenges and prospects in the Global meet of Biologists in Hyderabad from 26–28 December 2012.
18. Attended review meeting of the project 'Tracking resistance to artemisinin collaboration' at Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand from 10–11 January 2013.
19. Launch meeting of the project 'Comprehensive case management pilot programme in Odisha' in Bhubaneswar on 18 January 2013.
20. Attended as special invitee of Technical Advisory Committee meeting of the NVBDCP in New Delhi on 30 January 2013 and prescribed states of drug resistance in NE.
21. Delivered a talk on 'Drug resistance and its implications to malaria control/elimination with special reference to India' in an Informal Intercountry consultation to intensify malaria control towards elimination at WHO/SEARO, New Delhi on 19 February 2013.
22. Delivered a lecture on Malaria treatment: Past, present and future in an International symposium on "CTDDR-2013; Drug development for Orphan/neglected diseases" at Central Drug Research Institute, Lucknow on 28 February 2013.
23. Delivered a talk on Malaria treatment in India: Challenges and opportunities in the national symposium on "Fight against malaria: Prospects and perspectives", at Maharishi Dayanand University, Rohtak on 9 March 2013.
24. Attended meeting as Temporary Advisor of the Malaria Policy Advisory Committee of World Health Organization, Geneva, Switzerland from 10–15 March 2013.

By Ph.D. Students

1. Mr Manmeet Rawat presented his research work as a poster presentation at Host parasite interactions biology, conference on "Evolved antigen processing plasmepsin-V of *Plasmodium vivax* in emerging infections; suggesting a novel virulence mechanism" organized by Gordon Research at Salve Regina University in Newport RI, United States in June 2012 (Travel award).
2. Ms Sonam Vijay presented her research work as a poster presentation at malaria conference on Nitric oxide elements inhibit aspartic protease activity in *Plasmodium*: Mechanism of parasite killing" organized by Keystone Symposia in New Orleans, USA in January 2013 (Global travel award).

10.3 Awards & Prizes

Dr Aparup Das

Received "Established Scientist Award 2013" from Scientific Planet Society, Dehradun.

Dr K Raghavendra

Felicitated and awarded by Zoological Society of India for contribution in the cause of science in the field of Zoology and Life Sciences in the Global meet of Biologists, Osmania University, Hyderabad on 26 December 2012.

Dr Neena Valecha

1. Awarded Bela Devi oration on Current perspectives on diagnosis and treatment of malaria conferred by East Delhi Physicians Association at their annual CME in New Delhi on 16 December 2012.
2. Received Professor B.K. Das award in recognition of outstanding achievement in biology as Biologist conferred by Zoological Society of India at the Global Meet of Biologists in Hyderabad December 2012.

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11

संस्थान में राजभाषा विकास संबंधी गतिविधियाँ

संस्थान में वर्ष 2012–13 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 न केवल तिमाही बैठकें, बल्कि *मलेरिया पत्रिका* (हिंदी) का प्रकाशन किया गया वरन् इसके साथ ही राजभाषा विभाग द्वारा लागू विभिन्न प्रोत्साहन योजनाएं भी कार्यान्वित की गईं जिनके पुरस्कारों का वितरण हिन्दी सप्ताह के पुरस्कार वितरण समारोह में किया गया। इस महत्वपूर्ण समारोह में संस्थान की निदेशक महोदया द्वारा वर्ष 2012–13 के दौरान राजभाषा के प्रयोग को और अधिक बढावा देने के उद्देश्य से एक नई प्रोत्साहन योजना 'अधिक शब्द सीमा' की पुरस्कार राशि में वृद्धि करते हुए लागू की गई जिसके पुरस्कारों का वितरण अगले वर्ष हिन्दी सप्ताह के दौरान किया जाएगा। यही नहीं राजभाषा के प्रति कर्मचारियों में रूचि जागृत करने के उद्देश्य से संस्थान के मुख्य प्रवेश स्थल पर प्रतिदिन एक नया अंग्रेजी–हिन्दी शब्द एवं एक सुविचार लिखने का नवीन एवं सराहनीय प्रयास किया गया जिससे प्रतिदिन कर्मचारियों में भारतीय संस्कृति से जोड़ने वाले नैतिक विचार एवं शब्दों के हिन्दी रूपांतरण को पढ़ने की जिज्ञासा बनी रहती है जोकि राजभाषा के प्रति रूचि जागृत करने में सहायक है।

इसके साथ ही प्रतिवर्ष की भांति इस वर्ष भी हिन्दी सप्ताह पूर्ण उत्साह के साथ मनाया गया जिसमें जहाँ एक ओर प्रशासन वर्ग के अधिकारियों एवं कर्मचारियों हेतु पूर्णकालिक हिन्दी कार्यशाला का आयोजन किया गया था जिसका उद्घाटन डॉ. नीना वलेचा एवं संचालन डॉ. आर.सी. धीमान द्वारा किया गया। वहीं दूसरी ओर महत्वपूर्ण गतिविधि पुरस्कार वितरण समारोह का आयोजन था किन्तु इसी के साथ निबंध प्रतियोगिता, वाद–विवाद (कर्मचारी वर्ग), वाद–विवाद (अधिकारी वर्ग), एवं टिप्पण–प्रारूपण प्रतियोगिताओं का भी आयोजन किया गया।

हिन्दी कार्यशाला का संचालन करते डॉ. आर.सी. धीमान


हिन्दी कार्यशाला में व्याख्यान देते व्याख्याता

टिप्पण–प्रारूपण प्रतियोगिता का प्रथम पुरस्कार लेते श्री के.सी. सेहरा

दिनांक 21 सितम्बर 2012 को आयोजित पुरस्कार वितरण समारोह का संचालन हिंदी अधिकारी डॉ. वंदना शर्मा द्वारा किया गया। इस समारोह में डॉ. (प्रो.) प्रतिमा मित्तल, प्रसव एवं स्त्री रोग विज्ञान विभाग, वर्धमान महावीर मेडिकल कॉलेज एवं सफदरजंग अस्पताल और डॉ. मुकेश कुमार, वैज्ञानिक 'ई' एवं प्रमुख, अंतर्राष्ट्रीय स्वास्थ्य प्रभाग, भारतीय आयुर्विज्ञान अनुसंधान परिषद, नई दिल्ली को मुख्य अतिथि के रूप में आमंत्रित किया गया था। इस समारोह का आरंभ मुख्य अतिथि और संस्थान की निदेशक महोदया डॉ. नीना वलेचा के स्वागत के साथ किया गया। इसके बाद संस्थान की निदेशक महोदया ने अपने संबोधन में संस्थान में सरकारी कामकाज में हिंदी के बढ़ते हुए प्रयोग की सराहना की। तत्पश्चात् डॉ. मुकेश कुमार ने सभा को संबोधित करते हुए उच्च अधिकारियों को हिंदी में काम करके अपने अधीनस्थ कर्मचारियों हेतु प्रेरणा का स्रोत बनने के लिए प्रोत्साहित किया। पुरस्कार वितरण के पश्चात् मुख्य अतिथि डॉ. (प्रो.) प्रतिमा मित्तल ने सभा को संबोधित करते हुए संस्थान के सभी अधिकारियों एवं कर्मचारियों द्वारा पुरुस्कार वितरण समारोह में अत्यंत उत्साह के साथ भाग लेने पर हर्ष व्यक्त किया और इसके साथ ही सभी को रोजमर्रा के सरकारी कामकाज में हिन्दी का प्रयोग करने हेतु प्रेरित किया।

पुरस्कार वितरण समारोह में संबोधित करती निदेशक महोदया

पुरस्कार वितरण समारोह में संबोधित करती मुख्य अतिथि डॉ. प्रतिमा मित्तल

अंततः कार्यक्रम का विधिवत् समापन डॉ. रमेश धीमान, वैज्ञानिक 'एफ' के धन्यवाद ज्ञापन द्वारा हुआ।

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12

Committees of the Institute

12.1 Scientific Advisory Committee

Dr VM Katoch
Secretary, Department of Health Research &
Director General
Indian Council of Medical Research
V. Ramalingaswami Bhawan
Ansari Nagar, New Delhi–110 029

Chairman

Dr Shiv Lal
Former Special DG (PH) &
Former Director, NCDC
Chairperson, SAC, NIMR
Programme Coordinator-cum-Adviser
JE/AES, NVBDCP
22, Sham Nath Marg
Delhi–110 054

Members

Dr LS Chauhan
Director
National Centre for Disease Control
22, Sham Nath Marg
Delhi–110 054

Prof. MKK Pillai
Retired Professor of Zoology
(University of Delhi)
162, Abhinav Apartments
B-12, Vasundhara Enclave
Delhi–110 096

Dr Nilima A Kshirsagar
Dean
ESIC-PGIMSR
Mahatma Gandhi Memorial Hospital
Dr S.S. Rao Road, Parel
Mumbai–400 012

Dr AC Dhariwal
Director
National Vector Borne Disease Control Programme
22, Sham Nath Marg
Delhi–110 054

Dr B Ravindran
Director
Institute of Life Sciences
Nalco Square
Bhubaneswar–751 023 (Odisha)

Dr Dhanpat Kochar
C-54, Sadulganj
Near Medical College
Bikaner–334 003 (Rajasthan)

Dr PL Joshi
Former Director, NVBDCP &
Former Senior Consultant, NIHFW
Independent WHO Consultant
Pocket-B, Sector-13, House No. 580
Metro View Apartments, Dwarka
New Delhi–110 075

Dr Dileep N. Deobagkar
Honorary Professor
Department of Bioinformatics
University of Pune
Pune–411 007

Dr Arvind Pandey
Director
National Institute of Medical Statistics
Ansari Nagar
New Delhi–110 029

Dr P Jambulingam
Director
Vector Control Research Centre
Medical Complex
Indira Nagar
Puducherry–605 006

Dr GS Sonal
Additional Director
National Vector Borne Disease Control Programme
22, Sham Nath Marg
Delhi–110 054

Dr Rashmi Arora
Scientist 'G' & Head (ECD)
Indian Council of Medical Research
V. Ramalingaswami Bhawan, Ansari Nagar
New Delhi–110 029

Special Invitee
Prof AP Dash
Former Regional Adviser (VBN), WHO
112, Milano Mahagun Mansion II
Plot 1/4, Vaibhav Khand, Indrapuram
Ghaziabad–201 014 (U.P.)

Member Secretary
Dr Neena Valecha
Director
National Institute of Malaria Research
Sector 8, Dwarka
New Delhi–110 077

12.2 Research Advisory Committees

12.2.1 Vector Biology and Control

Chairman
Prof. MKK Pillai
Retired Professor of Zoology
(University of Delhi)
162, Abhinav Apartments
B-12, Vasundhara Enclave
Delhi–110 096

Members
Dr P Jambulingam
Director
Vector Control Research Centre
Medical Complex, Indira Nagar
Puducherry–605 006

Dr Sarala K Subbarao
Emeritus Medical Scientist
Indian Council of Medical Research
V. Ramalingaswami Bhawan
Ansari Nagar
New Delhi–110 029

Dr RS Sharma
Head
Department of Medical Entomology
National Centre for Disease Control
22, Sham Nath Marg
Delhi–110 054

Member Secretary
Dr Neena Valecha
Director
National Institute of Malaria Research
Sector 8, Dwarka
New Delhi–110 077

12.2.2 Parasite Biology

Chairman
Dr B Ravindran
Director
Institute of Life Sciences
Nalco Square
Chandrasekharpur
Bhubaneswar–751 023 (Odisha)

Members
Dr Shobhona Sharma
Professor
Department of Biological Sciences
Tata Institute of Fundamental Research
Homi Bhabha Road, Colaba
Mumbai–400 005

Dr Shashi Khare
Additional Director (Speciality Microbiology)
National Centre for Disease Control
22, Sham Nath Marg
Delhi–110 054

Dr Chetan Chitnis
Staff Research Scientist
International Centre for Genetic Engineering
and Biotechnology
Aruna Asaf Ali Marg
New Delhi–110 067

Member Secretary
Dr Neena Valecha
Director
National Institute of Malaria Research
Sector 8, Dwarka
New Delhi–110 077

12.2.3 Epidemiology and Clinical Research

Chairman
Dr PL Joshi
Former Director, NVBDCP &
Former Senior Consultant, NIHFW
Independent WHO Consultant
Pocket-B, Sector-13, House No. 580
Metro View Apartments, Dwarka
New Delhi–110 075

Members
Dr GS Sonal
Additional Director
National Vector Borne Disease Control Programme
22, Sham Nath Marg
Delhi–110 054

Dr Chhemendra Sharma
Scientist E-II
Radio and Atmospheric Science Division
National Physical Laboratory
Dr KS Krishnan Marg
New Delhi–110 012

Dr Sanjib Mohanty
Joint Director
Ispat General Hospital
Rourkela Steel Plant, Sector 19
Rourkela–769 005 (Odisha)

Dr Rashmi Arora
Scientist 'G' & Head (ECD)
Indian Council of Medical Research
V. Ramalingaswami Bhawan, Ansari Nagar
New Delhi–110 029

Member Secretary
Dr Neena Valecha
Director
National Institute of Malaria Research
Sector-8, Dwarka
New Delhi–110 077

12.3 Research Advisory Committee of IDVC Project

Chairman
Dr PL Joshi
Former Director, NVBDCP &
Senior Consultant, NIHFW
Independent WHO Consultant
Pocket-B, Sector-13, House No. 580
Metro View Apartments, Dwarka
New Delhi–110 075

Members
Prof. MKK Pillai
Retired Professor of Zoology
(University of Delhi)
162, Abhinav Apartments
B-12, Vasundhara Enclave
Delhi–110 096

Dr Dileep N. Deobagkar
Honorary Professor
Department of Bioinformatics
University of Pune
Pune–411 007

Dr AC Dhariwal
Director
National Vector Borne Disease Control Programme
22, Sham Nath Marg
Delhi–110 054

Dr P Jambulingam
Director
Vector Control Research Centre
Medical Complex, Indira Nagar
Puducherry–605 006

Dr BK Das
Professor
Department of Medicine
SCB Medical College
Cuttack–753 003

Dr Sanjay M Mehendale
Director
National Institute of Epidemiology
2nd Main Road, Tamil Nadu Housing Board,
Ayapakkam, Chennai–600 077

Dr Rashmi Arora
Scientist 'G' and Head (ECD)
Indian Council of Medical Research
V. Ramalingaswami Bhawan
Ansari Nagar
New Delhi–110 029

Member Secretary

Dr Neena Valecha
Director
National Institute of Malaria Research
Sector-8, Dwarka
New Delhi–110 077

12.4 Building Advisory Committee

Chairman

Dr Shiv Lal
Programme Coordinator-cum-Adviser
JE/AES, NVBDCP
22, Sham Nath Marg
Delhi–110 054

Members

Dr Pradeep Das
Scientist 'G' & Director
Rajendra Memorial Research Institute of Medical Sciences, Agam Kuan
Patna–800 007

Dr RC Sharma
Consultant, ICMR
190, Anupam Apartments
M.B. Road
New Delhi–110 068

Dr UD Gupta
Scientist 'F'
National JALMA Institute for Leprosy and other Microbacterial Diseases
P.B. No. 101, Tajganj
Agra–282 001

Dr Arvind Rai
Joint Director
National Centre for Disease Control
Directorate General of Health Services
22, Sham Nath Marg
Delhi–110 054

Convenor

Dr Neena Valecha
Scientist 'G' and Director
National Institute of Malaria Research
Sector-8, Dwarka
New Delhi–110 077

12.5 Ethics Committee

Chairman

Prof. KD Tripathi
Former Professor & Head
Department of Pharmacology
Maulana Azad Medical College
New Delhi–110 002

Members

Prof. MKK Pillai
Retired Professor of Zoology
(University of Delhi)
162, Abhinav Apartments
B-12, Vasundhara Enclave
Delhi–110 096

Dr Dinesh Srivastava
Consultant
Department of Medicine
Dr. Ram Manohar Lohia Hospital
New Delhi–110 001

Prof. Ramesh Kumar
Retired Professor
(All India Institute of Medical Sciences)
B-601, Rishi Apartments
Alaknanda
New Delhi–110 019

Dr (Mrs) Sunita Bhatia
Department of Paediatrics
Kasturba Gandhi Hospital
Daryaganj
New Delhi–110 002

Dr BS Nagi
Council for Social Development
53, Lodhi Estate
New Delhi–110 003

Mr Raju Dudani
Advocate
Patiala House Court
New Delhi–110 001

Mr Maheswar Singh
Senior Programme Officer
39, Basement, Sant Nagar
East of Kailash
New Delhi–110 065

Member Secretary

Dr Neena Valecha
Director
National Institute of Malaria Research
Sector-8, Dwarka
New Delhi–110 077

12.6 Animal Ethics Committee

Chairman

Dr SK Sharma
Scientist 'F'
National Institute of Malaria Research
Sector-8, Dwarka
New Delhi–110 077

CPCSEA Main Nominee

Dr PK Yadav
Senior Veterinary Officer
Laboratory Animal Facility
All India Institute of Medical Sciences
Ansari Nagar
New Delhi–110 029

CPCSEA Link Nominee

Dr Vijay Pal Singh
Institute of Genomics & Integrative Biology
Mall Road
Delhi–110 007

CPCSEA Socially Aware Member

Sh. CB Jarodia
138, DDA Flats
Pocket-II, Dwarka
New Delhi

CPCSEA Scientist

Prof. HS Rehan
Head
Department of Pharmacology
Lady Hardinge Medical College
New Delhi–110 001

Member-Expert Veterinarian

Dr DN Sharma
F-75, Sector-20
Noida (U.P.)

Members

Dr Nutan Nanda
Scientist 'F'
National Institute of Malaria Research
Sector-8, Dwarka
New Delhi–110 077

Dr Neelima Mishra
Scientist 'E'
National Institute of Malaria Research
Sector-8, Dwarka
New Delhi–110 077

Member Secretary/Scientist (Veterinarian)

Dr PK Atul
Scientist 'D'
National Institute of Malaria Research
Sector-8, Dwarka
New Delhi–110 077

12.7 Annual Report Committee

Chairperson

Dr RC Dhiman
Scientist 'G'
National Institute of Malaria Research
Sector-8, Dwarka
New Delhi–110 077

Member

Dr SK Sharma
Scientist 'F'
National Institute of Malaria Research
Sector-8, Dwarka
New Delhi–110 077

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Scientific Staff of the Institute

Scientist G and Director
Dr Neena Valecha

Scientist G
Dr RC Dhiman
Dr VK Dua

Scientist F
Dr RM Bhatt
Dr Vas Dev
Dr SK Ghosh
Dr Ashwani Kumar
Dr MS Malhotra
Dr BN Nagpal
Dr Nutan Nanda
Dr K Raghavendra
Dr Arun Sharma
Dr SK Sharma
Dr OP Singh

Scientist E
Dr Anup Anvikar
Dr Aparup Das
Dr AK Mishra
Dr Neelima Mishra
Dr PK Mittal
Mrs Rekha Saxena
Dr MC Sharma
Dr MM Shukla
Dr Ranvir Singh
Dr HC Srivastava

Scientist D
Dr PK Atul
Dr Jyoti Das
Dr MK Das

Scientist C
Dr Alex Eapen
Dr VP Singh
Dr Vineeta Singh

Scientist B
Dr Ram Das
Mr Bhagirath Lal
Dr U Sreehari

Scientist G
Dr T Adak (Re-employed)

IDVC Project Staff

Senior Research Scientists
Dr Hemanth Kumar
Dr PK Tyagi

Research Scientists
Dr SK Chand
Dr GDP Dutta
Dr Ashish Gupta
Dr S Haq
Dr AK Kulshrestha
Dr Raj Kumar
Dr K Padhan
Dr SN Sharma
Dr SP Singh
Dr SN Tiwari

Names are listed in alphabetical order. Staff position as on 31 March 2013.