Annual Report
2010–11

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Contents

Preface v

Executive Summary vii

1. Vector Biology and Control 1
2. Parasite Biology 24
3. Epidemiology 39
4. Clinical Research 51
5. Highlights of the Research Activities under IDVC Project 57
6. Research Support Facilities 61
7. Inter-Institutional Collaboration 64
8. Human Resource Development 66
9. Research Papers Published 67
10. Other Activities 71
11. संस्थान में राजभाषा विकास संबंधी गतिविधियाँ 78
12. Committees of the Institute 80
13. Scientific Staff of the Institute 86

Preface

With great fervour and rectitude I present the Annual Report for the year 2010–11 of the National Institute of Malaria Research (NIMR). Providentially, my taking over of the Directorship of NIMR and the centenary year of our parent institute, Indian Council of Medical Research coincided with each other, and I feel privileged for this coincidence. If I look back to the path, NIMR has travelled so far, I feel happy of its progress in maintaining the reputation and fulfilling the commitment of its establishment, about four decades ago. While the basic research in the fields of vectors and parasites at NIMR are well-recognized, applied and operational research activities are well-adored among the malaria research communities world-over and policy makers of the country. The unique blend of cooperation between NIMR and the National Vector Borne Disease Control Programme (NVBDCP) has resulted in many policy decisions which have changed the face of malaria intervention strategies of the country over the years. Not surprising, these decisions were based on the results of research conducted by NIMR. The extension of artemisinin-based combination therapy (ACT) to treat *Plasmodium falciparum* malaria cases all over the country, introduction of ACT in pregnant women, strengthening the phase-out of oral artemisinin derivatives and introduction of long-lasting insecticidal nets for vector control are some of the exemplary of NIMR's contribution to the national programme.

The strength of NIMR rely on the diverse subject expertise of scientists and especially in its 10 field units placed in different eco-epidemiological settings of malaria in India. Apart from undertaking malaria intervention measures with the help of local health officials, these field units are the resources of biological material for cutting-edge laboratory-based research being undertaken at NIMR, New Delhi. Apart from active case malaria detection methods, passive case detection is also regularly conducted at Malaria Clinics established in each field unit and at NIMR, New Delhi. Adopting these practices, NIMR, in its last four decades of existence, has become remarkably successful in delivering technologies and strategies to the national programme for effective control and containment of malaria in India. Additionally, the translational research activities of NIMR entered into the project mode and about five patents have been granted or applied. Furthermore, NIMR conducts regular field trials for insecticides, antimalarials, etc. for malaria interventions, most notable of them are: (i) efficacy testing of long-lasting insecticidal nets for mosquito vector control; and (ii) the drug efficacy of Artemisinin-based Combination Therapies (ACTs) for the treatment of *P. falciparum* malaria. In this concern, the Malaria Parasite Bank of NIMR, which serves as the national repository of malaria parasites, was given long-term project status by ICMR this year. This will be of immense help for furthering research on several aspects of malaria parasites sampled from different endemic places of the country and maintained at NIMR.

Due to its active involvement and capacity to deliver basic, applied and operational research, NIMR has been recognized by highly reputed international organizations. The National Institutes of Health (NIH), USA has recently identified NIMR as one of the 10 International Centers of Excellences in Malaria Research (ICEMR) to study the complex malaria in India. This recognition comes with funding for seven years to undertake cutting-edge modern biological research on several aspects of malaria. Furthermore, NIMR is approved by WHOPES for designation of collaborating centre for Phase I testing and evaluation of public health pesticides, which is first of its kind, not only in India, but also in the entire south-east Asia region.

In order to disseminate knowledge on malaria generated from field and laboratory, NIMR had conducted several meetings, hands-on-trainings, workshops and discussions of international reputes. Some of these important meetings are: (i) Consultation on Standard Protocol Development for Estimating Malaria Disease Burden in Southeast Asia Region; and (ii) Global Exchange Lecture Course on Molecular and Evolutionary Genetics of Malaria, funded by the European Molecular Biology Organization (EMBO).

Furthermore, several training programmes on malariology to the health personnel working in the state health departments, municipal corporations, hospitals, medical colleges, etc. have also been conducted. The *Journal of Vector Borne Diseases* (JVBD) published by NIMR, which serves as an interface between researchers and policy makers through publication of research articles on all aspects of vector borne diseases has reached new heights of being the third best journal among the Indian biomedical journals, as ranked by SCImago.

The construction of the animal facility is in full swing and this state-of-the-art facility would be ready in few months. Considering a need to further strengthen malaria research in the country, we are now trying to increase our field laboratories and also add more medical colleges to our network apart from other scientific agencies. We are also part of different public–private partnerships and executed projects.

NIMR would not have been the same as I see today without the vibrant leadership of its previous Directors and lively participation of scientific staff. I am happy that I have been provided an impeccable base on which I will have to capitalize and move forward fulfilling the mandate of the NIMR. I sincerely acknowledge the help and guidance of the Director General of the Indian Council of Medical Research and the Secretary, Department of Health Research, Government of India and hope for his continuous patronage in future.

Neena Valecha
Director

Executive Summary

Vector Biology & Control

- Studies on distribution and biology of the members of the Fluviatilis-Minimus group in tribal areas of India were conducted in six districts of north-eastern India and also in four districts in southern part of India. The studies revealed prevalence of *Anopheles minimus sensu stricto* (Species A) in north-eastern states and *An. fluviatilis* species T was found only in Jalpaigudi district. In peninsular India, *An. fluviatilis* was predominant.
- Ecological succession studies in north-eastern states showed changing species composition in this region.
- Insecticide and insecticide resistance laboratory of NIMR has been approved by the WHOPES for establishing a collaborating centre for Phase I testing and evaluation of public health pesticides and the designation is in process.
- Extended field trials of PermaNet and Olyset Net, long-lasting insecticidal nets were undertaken in District Gautam Budh Nagar, Uttar Pradesh and the follow-up studies showed good performance of these nets in reducing the mosquito densities and interrupting malaria transmission in the villages where these nets were used.
- C-21 Attracticide was found effective in surveillance and control of dengue and chikungunya vector, *Aedes aegypti* in Delhi, Bengaluru and Alappuza, Kerala.
- Insecticide resistance monitoring in different parts of India showed that *An. culicifacies* was resistant to DDT and malathion in most parts of India and to synthetic pyrethroids in Chhattisgarh and Andhra Pradesh.
- Absence of cross resistance between DDT, malathion, deltamethrin and bendiocarb with chlorfenapyr was observed in *An. stephensi* and *An. culicifacies*. Chlorfenapyr could be a potential option for manangement of insecticide resistance.
- PCR-based methods have been developed for detecting *kdr* mutation in mosquitoes.
- Upregulation of AcNos (*Anopheles culicifacies* nitric oxide synthase) activity was found in refractory strain of *An. culicifacies* species A in comparison to susceptible strain in Real Time PCR assys at different days pBM.
- Bioinformatic studies on NADPH cytochrome P450 reductase gene evolution in Indian *An. minimus* showed that the population had experienced population bottle neck in the recent history and genetic drift has shaped variations in this insecticide resistant conferring gene.

Parasite Biology

- Characterization studies on Glucose-6-phosphate dehydrogenase enzyme deficiency and haemoglobin variants in tribal dominated malaria endemic villages of Sundargarh district, Odisha showed high prevalence of G-6-PD deficiency which warrants preliminary screening of the patients before administering malaria treatment.
- Studies on genetic variation in microsatellite marker flanking *pfmdr-1* gene and *pfcrt* gene showed that resistant pfcrt allelle may be under strong selection pressure and pfmdr-1 86Y allele may be under weak selection pressure.
- Mapping of *dhpr* and *dhps* genes in Indian isolates of *Plasmodium vivax* collected from different geographic areas revealed tandem repeat variation in these genes and frequency of *dhfr* genotypes varied significantly among

different geographical populations. Three distinct geographical clusters of wild (northern India), double mutant (southern India), and quadruple mutant (north-eastern India and island areas) genotypes were observed.

- Human leukocyte antigen studies in patients infected with either *P. vivax* or *P. falciparum* samples and healthy controls collected from different malaria endemic areas, namely Delhi, Rourkela and Ranchi revealed high diversity among the study areas.
- Genetic polymorphism in diagnostic antigen of *P. falciparum* histidine rich protein 2 & 3 among Indian isolates showed high polymorphism and only 68% of *P. falciparum* isolates were likely to be detected at densities <200 parasites/μl; which may provide an alternative explanation for variable sensitivity of rapid diagnostic kits in different areas.
- Sequence analysis of virulence genes of *P. vivax* collecetd from Delhi, Managlore, Goa and Rourkela showed high variability existing within and between the isolates and that they are randomly dispersed with no particular distribution pattern among the regions.
- The study on *P. vivax* aspartic protease plasmepsin V predicts a putative mechanism to demonstrate antigenic variations of more virulent *P. vivax* for correlating their effect in relation to serotypes in cultivable *Plasmodium* species for immune evasion.
- Evolutionary history studies of Indian *P. vivax* revealed that this species might be a part of the ancestral distribution range of this species.

Epidemiology & Clinical Research

- Impact of deforestation in Sonitpur and Nagaon districts of Asom showed invasion of new species in deforested villages, e.g. *An. culicifacies* in addition to *An. philippinensis/ nivipes, An. annularis, An. minimus;* whereas in forested villages, *An. culicifacies, An. nivipes* and *An. annularis* were collected in addition to *An. dirus* and *An. minimus*. Malaria data revealed more number of cases in deforested villages than the forested villages.
- Mapping of malaria receptivity in Angara PHC of Jharkhand state using GIS showed that malaria cases are reported more in high receptive areas than in the medium receptive areas. Identification of different levels of malaria receptivity will help to plan prioritised control.
- A framework for predicting malaria outbreaks in rural and urban areas in Gujarat, India is being developed using monthly epidemiological and meteorological data.
- Projected scenario of transmission windows of malaria and dengue by the year 2030, 2071, 2081, 2091 and 2100 were determined at national level as well as for some specific states like Delhi, Uttarakhand, Asom, Odisha and Rajasthan in terms of climate change.
- Health impact assessment of Indira Sagar Dam and resettlement and rehabilitation colonies in SSP reservoir impoundment areas in Narmada Valley in Madhya Pradesh was undertaken and mitigating measures were suggested after detailed studies. As a result of mitigating measures suggested by NIMR, vector borne diseases are under control in these areas.
- Detailed studies in SSP project command areas in Rajasthan were undertaken in 233 villages and mitigating measures were suggested to respective authorities for implementation.
- A Phase III double blind randomized multicentre trial comparing safety and efficacy of arterolane maleate and piperaquine phosphate vs Coartem in uncomplicated *P. falciparum* malaria patients showed that the arterolane + PQP had good efficacy in curing.
- Efficacy trial of two ACTs for the treatment of malaria in pregnancy is being undertaken in three hospitals. So far 66 patients were included and the study is in progress.
- Monitoring of the therapeutic efficacy of antimalarials in different parts of India showed that AS+SP is well-tolerated and is effective for the treatment of *P. falciparum* malaria. Chloroquine remains effective in the treatment of vivax malaria.
- Quality assurance of RDTs is being undertaken in India. The panel detection score was 91.9% while specificity was 100%.
- Pharmacovigilance of antimalarials in India is in progress and 74 adverse events were reported so far among the 2969 patients' follow up proformae received from different places in the country.

Other Activities

- NIMR has undertaken several collaborative projects with other Institutes in India and also in other countries.
- Repositories of mosquitoes and malaria parasites are being maintained.
- Human resource development activities continued this year.
- Forty-five research papers were published by NIMR scientists during the year 2010.
- *Journal of Vector Borne Diseases* published by NIMR stood at Third rank among Indian biomedical journals for the year 2010 as per SCImago journal rankings.
- NIMR organized informal consultation on "Standard protocol development for estimating malaria disease burden in SEA Region" and Global exchange lecture course on "Molecular and evolutionary genetics of malaria", besides training courses to various health departments.

❑

Vector Biology and Control

1

1.1 Vector Biology

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

The project work was extended to selected districts in north-eastern region and peninsular India to study the distribution pattern, biological characters and vectorial potential of the members of *Anopheles fluviatilis*/*An. minimus*/*An. culicifacies* Complexes. Six districts in north-eastern region, viz. Jalpaigudi (West Bengal); Nalbari, Kamrup, Chairang & Golaghat (Asom); Changlang (Arunachal Pradesh) and four districts of southern states, namely Vizianagaram, Visakhapatnam (Andhra Pradesh), Nilgiri (Tamil Nadu), and Wayanad (Kerala) were surveyed for the first time. In addition, repeat surveys were done in districts Keonjhar, Deogarh, Mayurbhanj (Odisha); Surguja, Dantewada, Bastar (Chhattisgarh); Gumla, Simdega, West Singbhum (Jharkhand) (Fig. 1).

In north-eastern region, the study areas in all the districts represented foothill forest ecotype. Analysis of vector mosquitoes collected revealed the prevalence of *An. minimus sensu stricto* (species A) in these districts, whereas *An. fluviatilis* species T was found only in Jalpaigudi district. The indoor resting collections of *An. minimus* were poor and majority of the specimens were collected by light-trap catches in human dwellings and mosquito landing collections on human baits (indoors) and *An. minimus* A was found to be highly anthropophagic. These observations indicate endophagic and exophilic behaviour of this species in study areas. In addition, *An. culicifacies* were collected in good numbers in Chairang and Darrang districts of Asom state which were primarily zoophagic and comprised species B and C. The change in behaviour and ecology of *An. minimus* A and expansion of distribution of the Culicifacies Complex and its probable role in malaria transmission in north-eastern states need further investigations.

In peninsular India, surveys were carried out in Districts Vizianagaram & Visakhapatnam (Andhra Pradesh), Nilgiri (Tamil Nadu) and Waynad (Kerala). Study areas were selected in foothill and hilly forested ecotypes of these districts. In Vizianagaram and Visakhapatnam districts, *An. fluviatilis* species S was predominant, highly anthropophagic and found resting in human dwellings. Species T was sympatric with species S

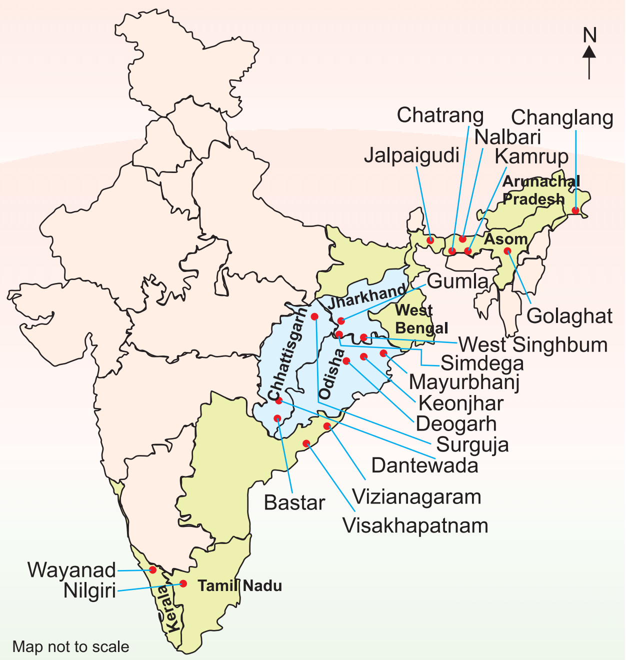


Fig. 1: Districts surveyed for studies on *An. fluviatilis* and *An. minimus* sibling species
- Districts in states with green colour are those where surveys were carried out for the first time.
- Districts in states with blue colour are those where repeat surveys were carried out during 2010-11.

in Visakhapatnam and was found to be primarily zoophagic. The density of *An. culicifacies* was very low in study areas and it comprised species B and C. Only species S of Fluviatilis Complex was incriminated in above mentioned districts for *Plasmodium falciparum* and *P. vivax* sporozoites. These observations strongly indicate that *An. fluviatilis* S is playing a major role in hilly and foothill forest areas of districts Vizianagaram and Visakhapatnam. In contrast, only species T was prevalent in forest areas of districts Nilgiri (Tamil Nadu) and Waynad (Kerala) where it was collected from cattlesheds and was found exclusively zoophagic.

Repeat surveys were carried out in selected districts of Odisha, Chhattisgarh and Jharkhand states for seasonal variations in the sibling species composition of vector mosquitoes. Highly anthropophagic *An. fluviatilis* S was found prevalent and predominant in Districts Keonjhar, Deogarh, Mayurbhanj (Odisha); and Dantewada, Bastar (Chhattisgarh). This species was incriminated again in Districts Keonjhar, Dantewada and Bastar which confirms it as primary principal vector in forest areas of above mentioned districts whereas only species T of Fluviatilis Complex was prevalent in geographically adjacent districts, viz. Surguja (Chhattisgarh), Gumla, Simdega, West Singbhum (Jharkhand). This species was found primarily zoophagic and polymorphic for q^1 inversion thus confirming our previous findings and *An. minimus* species A was recorded in very low numbers in the districts of Odisha. Therefore, no seasonal variation in the prevalence and sibling species composition of Fluviatilis/Minimus Complexes was observed in the study districts of above mentioned states. Further work under the project is in progress.

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

During the first year, two surveys were carried out by the NIMR team, first in the month of March-April 2010 and second in the month of August-October 2010 in two states, viz. Asom and Meghalaya. In these surveys, 7 districts in Asom (Lakhimpur, Nagaon, Sonitpur, Dibrugarh, Golaghat, Kamrup, Goalpara) and 2 districts in Meghalaya (East Khasi hill and East Garo hill) were covered by NIMR team and 4 districts of Arunachal

Fig. 2: Study areas of north-east covered by NIMR & RMRC teams.

Pradesh (Lohit, Upper Subansiri, West Kameng and East Siang) and 2 districts of Nagaland (Mokokchung and Kohima) were covered by RMRC Dibrugarh team (Fig. 2). In Asom, many ecological changes occurred— forest cover decreased to 1386 (thousand hectare) in 2006 as compared to 2114 (thousand hectare) in 1974. Irrigation channels also increased as a result, the net irrigated area reached to 756 (thousand hectare) in 2007 while it was 572 (thousand hectare) in 1976. Many dams got constructed. One major dam is under construction on the River Subansiri at the border of Arunachal Pradesh and Dhemaji districts.

In Meghalaya state, we found that the forest cover decreased from 740 (thousand hectare) in 1974 to 111 (thousand hectare) in 2003. The net irrigated area also increased from 48 (thousand hectare) in 1973–74 to 572 (thousand hectare) in 1975–76. Many industries got established here. The numbers of mining/industries were 2084 in 2005. The areas covered by tea gardens also increased in both Asom and Meghalaya. Influx of labour population from endemic areas at construction sites was recorded.

During the surveys in Asom and Meghalaya, both adult and immature mosquitoes were collected from different habitats by using the standard WHO techniques. The following different types of collections were carried out during the surveys: Indoor resting (morning collection); Indoor resting (evening collection); Landing collection; Space spray collection (total catch); Outdoor resting collection (total catch); and Larva collection & emergence (Figs. 3–8). Due to all these ecological changes we found out that many new species of mosquitoes appeared and some species got disappeared. In Asom, species which were found to be present in earlier records got disappeared in the survey done by NIMR in 2010. These species are: *An. aitkenii, An. annandalei, An. karwari, An. sundaicus, An. crawfordi, An. turkhudi, An. hyrcanus, An. sintoni, An. umbrosus*. *Anopheles crawfordi* (Das et *al* 2007), *An. jeyporiensis, An. hyrcanus*, *An. subpictus*, *An. splendidus*, *An. pallidus* (Dev et *al* 2004) and *An. ramsayi* (Sarkar et *al* 1990). Those species which were recorded first time in Asom state are: *An. theobaldi, An. nivipes, An. maculatus* var. *willmorei, An. balabacensis*, *An. aitkenii, An. culicifacies, An. umbrosus* (Vishwanathan 1941; Mortimer 1946 and Sen *et al* 1973), *An. nigerrimus, An. jamesii* and *An. sinensis*. In Meghalaya, the following mosquito species were found to be present in earlier data got disappeared in the survey done by NIMR 2010—*An. philippinensis*, *An. hyrcanus* (Das et *al* 1984), *An. tessellatus* (Rajgopal *et al* 1976), while species those recorded by our team in 2010 survey were: *An. subpictus, An. theobaldi, An. nivipes, An. gigas, An. culicifacies, An. varuna,* and *An. fluviatilis.*

Figs. 3–8: **3.** Indoor resting (morning collection), **4.** Indoor resting (evening collection), **5.** Landing collection, **6.** Space spray collection (Total catch), **7.** Outdoor resting collection (Light trap), and **8.** Larva collection & emergence.

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

A preliminary study was undertaken to investigate the sudden appearance of *An. fluviatilis* in high densities in Dadri PHC area, where this species was not observed during past three decades in various other studies undertaken in this area. Also there is no published report of the prevalence of *An. fluviatilis* from this area. The area in Dadri PHC in District G.B. Nagar, U.P. is located within a distance of about 40-45 km from Delhi and is accessible round the year. *Anopheles culicifacies* is the primary malaria vector species in this area, which breeds in irrigation channels, ponds, pools and rice-fields. Besides, *An. culicifacies*, *An. annularis* and *An. subpictus* are the major anopheline species prevalent in this area. In addition, some other anophelines, viz. *An. stephensi*, *An. pulcherrimus* and *An. nigerrimus* are also found sometimes in very low densities. During this study, regular (fortnightly) monitoring of the indoor resting mosquito density was made by hand catch method in six villages of the Dadri PHC. The study revealed the appearance of *An. fluviatilis* in high densities in the Dadri PHC area during November to December 2009 till July 2010 (Fig. 9). The species was found to be *An. fluviatilis* species T by cytotaxonomic and molecular diagnostic techniques. This species was found to be totally zoophagic as revealed by blood meal source analysis. Cytological examination of *An. culicifacies* populations from the same area revealed that species A & B are prevalent in the study villages with predominance of species A, which was found primarily zoophagic. 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. The prevalence of *An. fluviatilis* was not affected by seasonal changes, while the prevalence of other species was found to be influenced by seasonal changes. The appearance of *An. fluviatilis* was probably due to the presence of thick vegetation on the surface of slow moving water in the drain which later disappeared after removal of

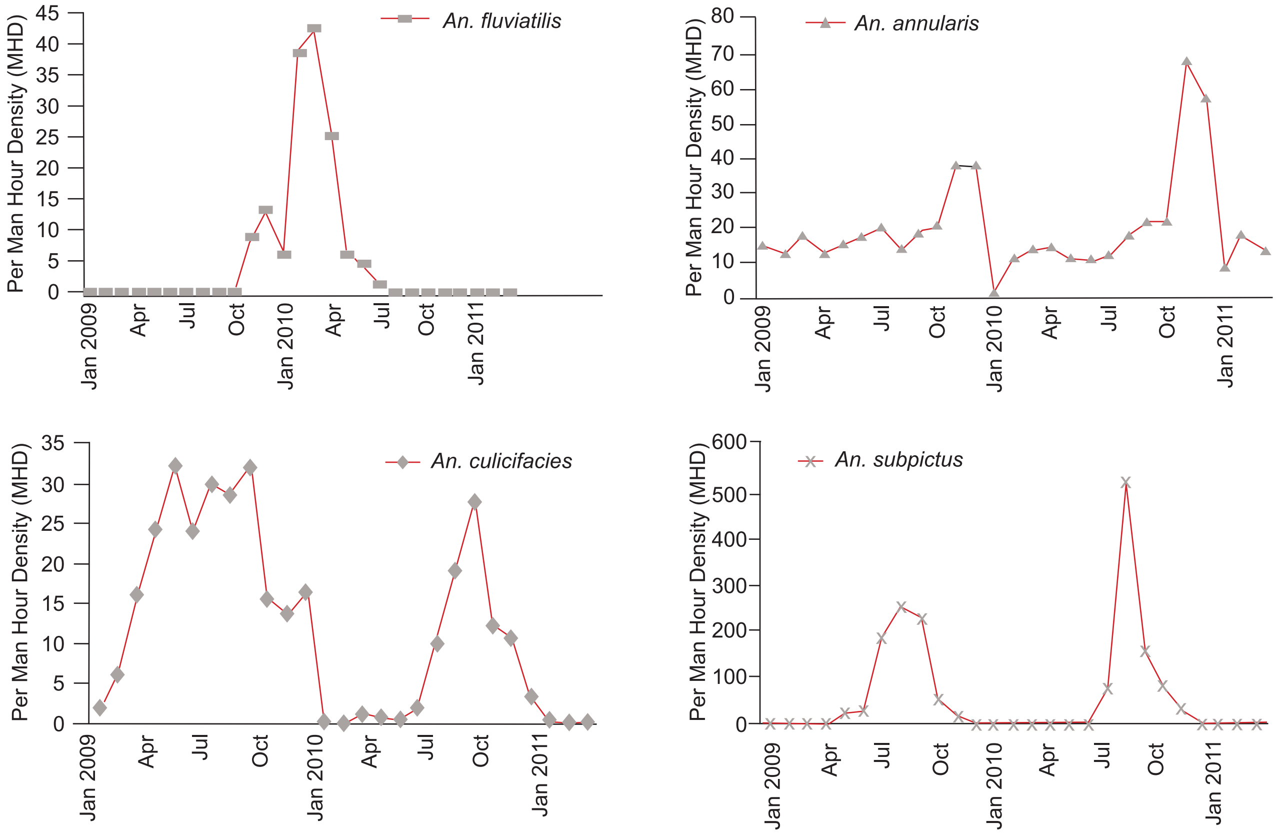


Fig. 9: Monthly data on indoor resting density of *Anopheles* mosquito species in Dadri PHC area during 2009 and 2010.

the vegetation cover on the surface of drain manually.

1.2 Vector Control

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

The second assessment of the facility was done in the new campus from 23–27 November 2009 by WHOPES team. Assessment was made on the progress in implementation of the recommendations of first capacity evaluation carried out in September 2008. Assessment was made on different aspects related to infrastructure facilities and through direct inspection, and review of the methodology and management of the laboratory investigations. The recommendations were made for certain modifications in techniques and provision of proper infrastructure in terms of equipments, space and personnel. Further work on the establishment of the facilities is in progress as per the recommendations made in the assessment report of the WHOPES. The final assessment of the capacity establishment of the laboratory facilities is due in May 2011.

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

Extended Phase III field evaluation of PermaNet® 2.0—a long-lasting insecticidal net (LLIN) factory treated with deltamethrin was undertaken after the initial trial period of one year to assess the long-term efficacy and durability of PermaNet® 2.0 against malaria vector *An. culicifacies* and its impact on malaria transmission in the endemic areas of Uttar Pradesh. PermaNet® 2.0, have been given full recommendation by WHOPES in 2009, subject to further evaluation at local levels in different countries. The trial was initiated in 2007 in three villages with population of 1187, 1165 and 1337 randomly selected for the distribution of PermaNet® 2.0 and controls with untreated net and no net, in Dadri PHC of District Gautam Budh Nagar, U.P.

Table 1. Efficacy of PermaNet® 2.0 against *An. culicifacies* after different intervals of use in field

(Cone bioassay)

Period	No. of nets checked	% knockdown after 1 h	% mortality after 24 h
May 2007	5	100	100
Apr 2010	20	76.5	85.9

Four replicates of 5 mosquitoes each were exposed for 3 min in cone bioassays on each net.

(Ring-net bioassay)

Period	Used/ unused net	Time for knock-down of 1st mosquito (min)	Time for knock-down of 6th mosquito (min)	Time for knock-down of 11th mosquito (min)
Aug–Oct 2007	Unused net	2.37	5.0	6.5
	Used net	3.3	5.4	7.2
Apr 2010	Unused net	3.0	5.0	7.5
	Used net	6.6	9.4	19.5

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

Table 2. Percentage of PermaNet® 2.0 net samples exceeding WHO efficacy criteria[1] in Cone bioassay tests using *An. culicifacies* after different intervals of use in the field

Period	No. of nets checked	No. of nets showing ≥ 95% knockdown in 1 h	No. of nets showing >80% mortality after 24 h
May 2007	5	5 (100)	5 (100)
Apr–May 2009	24	20 (83.3)	23 (95.8)
Apr–May 2010	20	6 (30)	17 (85)

[1] WHO criteria: Cone bioassay tests ^{3} 95% knockdown and/or ^{3} 80% mortality; Four replicates of 5 mosquitoes each were exposed for 3 min in cone bioassays on each net; Figures in parentheses indicate percentages.

The results of cone bioassays on community used Permanet® 2.0 in field conditions showed ≥ 80% mortality even after three years of use (Table 1). More than 80% nets that were checked after 3 years exceeded WHO efficacy criteria of ≥ 95% knockdown and/or >80% mortality (Table 2). The study revealed a reduction in the man hour density (MHD) and parity rate of *An. culicifacies* in the PermaNet® 2.0 village as compared to untreated net and no net areas (Fig. 10). The study also revealed a reduction in the prevalence of malaria (Parasite Index) in the PermaNet villages from 3.36 during pre-intervention period in May 2007 to 0 during the post-intervention period till October–November 2010 (Table 3). Survey on the assessment of durability and compliance rate of

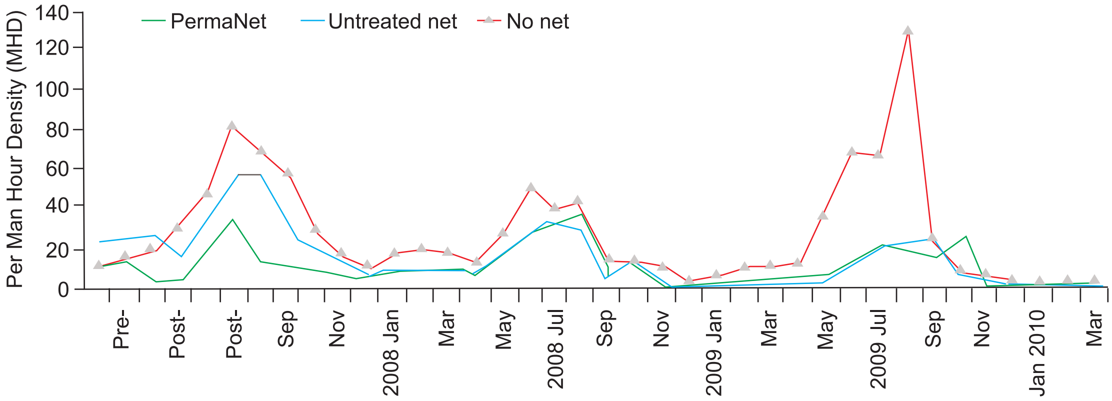


Fig. 10: Indoor resting per man hour density of *An. culicifacies* in the study villages with PermaNet 2.0, untreated net and no net in Dadri PHC, District Gautam Budh Nagar, U.P.

Table 3. Malaria prevalence in the population using PermaNet® 2.0, untreated nets and no nets during pre- and post-intervention phase as recorded through mass blood survey

Months/Year	Study arm	Population	B.S.	Total malaria positive cases	*Pf*	SPR	SFR	PI
May 2007	PermaNet	1187	300	4	1	1.33	0.33	3.36
Pre-intervention	Untreated net	1165	340	4	1	1.17	0.29	3.43
	No net	1337	358	5	1	1.39	0.28	3.73
Oct 2007	PermaNet	1187	210	0	0	0	0	0
Post-intervention	Untreated net	1165	208	2	0	0.96	0	1.72
	No net	1337	204	3	1	1.47	0.49	2.24
Oct–Nov 2009	PermaNet	1187	300	0	0	0	0	0
	Untreated net	1165	300	2	0	0.66	0	1.70
	No net	1337	300	1	0	0.33	0	0.74
Apr 2010	PermaNet	1187	300	0	0	0	0	0
	Untreated net	1165	300	0	0	0	0	0
	No net	1337	300	0	0	0	0	0

Table 4. Assessment of net usage and durability (Physical condition) of PermaNet® 2.0 in the field

Month	No. of nets distributed	No. of nets lost/ damaged/ torn out	No. of nets found intact or partially damaged	% in use
May 2007	1084	0	1084	100
Apr 2010	1084	290	787	72.6

PermaNet 2.0 were distributed to the villagers in May 2007.

PermaNet® 2.0 by the net users in the PermaNet® 2.0 village revealed >80 and 72.6% of the nets were in use even after 2 and 3 years of the distribution of PermaNet in the village (Table 4). These results indicate that PermaNet® 2.0 can be used up to three transmission seasons for effective control of malaria vector *An. culicifacies* and interruption of malaria transmission in this area.

1.2.3 Extended follow up study on the long-lasting efficacy of Olyset Net® against malaria vectors and incidence of malaria in a village of District Gautam Budh Nagar, Uttar Pradesh

The study was continued during 2010 in three villages, viz. Khandera (Olyset net village), Beel Akbarpur (untreated net village) and Anandpur (without-net village) in District Gautam Budh Nagar, U.P., beyond five years of trial period in July 2009.

The Olyset Nets were found highly effective even after five years of use as determined by cone bioassays with *An. culicifacies* collected from field (mortality >80%), but the efficacy of used Olyset nets collected randomly after six years was found to be significantly reduced (mortality<80%) (Fig. 11). About 80% of the Olyset net samples checked after 5 years, exceeded WHO efficacy criteria of ≥ 80% mortality, but after six years only 50% net samples showed ≥ 80% mortality in cone bioassays (Table 5).

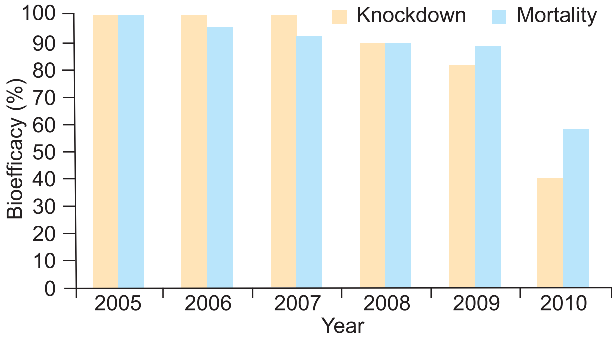


Fig. 11: Residual efficacy of Olyset Nets® against *An. culicifacies* after different period of use in field conditions in Dadri PHC, District Gautam Budh Nagar, U.P. Results of Cone bioassays.

Pooled month-wise entomological data showed a reduction in the indoor resting man hour density (MHD) of the major malaria vector *An. culicifacies* and other mosquito species in the Olyset net village, when compared with no net village during the post-intervention years during 2004–05 to 2008–2009 but no reduction was noticed in *An. culicifacies* and other anopheline spp. during 2009–10 (Table 6). Epidemiological data of three study villages revealed significant reduction in the incidence of malaria even after 6 years of use in the experimental village during post-intervention years, as compared to the untreated net village and no net village (Table 7).

Table 5. Percentage of Olyset Nets* qualifying WHO criteria[1] on cone bioassays against *An. culicifacies* at different time periods of use in field conditions

Year	Condition of Olyset net	No. of nets checked	No.(%) nets showing ≥ 95% knockdown in 1 h) after exposure for 3 min	No.(%) nets showing ≥ 80% mortality in 24 h) after exposure for 3 min	Percentage of Olyset nets® qualifying WHO criteria[1]
2004	Unused new Olyset net	11	11 (100)	11 (100)	100
2009	Used net	10	2 (20)	8 (80)	80
2010	Used net	12	0 (0)	6 (50)	50

[1]WHO criteria: Cone bioassay tests ≥ 95% knockdown and/ or ≥ 80% mortality; *Olyset nets were randomly collected from the villagers and used for bioassays and durability studies; Figures in parentheses indicate percentages.

Table 6. Man hour density (MHD) of *An. culicifacies* and other mosquitoes in the Olyset Net, untreated net and without net villages in Dadri PHC, District Gautam Budh Nagar, Uttar Pradesh

Year/Period (August-July)	Average man hour density								
	Olyset net			Untreated net			No net		
	An. culicifacies	All anophelines spp.	Total mosquitoes	*An. culicifacies*	All anophelines spp.	Total mosquitoes	*An. culicifacies*	All anophelines spp.	Total mosquitoes
Pre-intervention 2003–04	32.6	114.32	216.2	37.8	118.9	260.5	33.7	109.9	217.0
Post-intervention 2004–05	10.3 (73.9)	28.2 (73.8)	77.0 (68.9)	22.6	71.9	181.0	39.6	107.8	247.7
2008–09	12.91 (62.25)	129.2 (27.3)	179.2 (40.8)	31.54	188.2	321.0	34.2	177.8	303.1
2009–10	19.7 (Nil)	166.8 (Nil)	402.0 (18.3)	25.5	194.8	434.7	16.1	161.0	492.6

Figures in parantheses indicate percent reduction over control.

Table 7. Impact of Olyset Nets on malaria prevalence (Mass blood survey) in the study villages in Dadri PHC, District Gautam Budh Nagar, Uttar Pradesh

Period	Olyset net (Pop. 2000)				Untreated net (Pop.1800)				No net (Pop. 2000)			
	TBS	SPR	SFR	PI	TBS	SPR	SFR	PI	TBS	SPR	SFR	PI
Pre-intervention Apr 2004	240	3.3	0	4.0	220	3.2	0.4	3.8	240	2.9	0	3.5
Post-intervention Oct 2004	200	0	0	0	210	1.4	0.4	1.66	200	7.5	0	7.5
Oct 2010	308	0.64	0.32	1.0	300	2.0	1.0	3.3	306	2.9	0.98	4.5

Table 8. Physical status of Olyset Nets (distributed originally in 2004) after 6 years (July 2010) of use in Khandera village of Dadri PHC, Distt. Gautam Budh Nagar, U.P.

Parameters	Frequency
No. of houses checked	253
No of Olyset nets issued in 2004	1203
No. of nets available (in use)	969
Percentage of nets in use	80.5
No. of nets in good condition	348
No. of nets partially damaged	207
No. of torn out nets in use	414
Percentage of torn out nets in use	42.7

Torn out nets were replaced with new nets during July–August 2010.

More than 80% of the originally distributed Olyset nets were found in use even after six years during the survey in 2010. Of these, only 53.3% olyset nets were intact or partially damaged, while 42.7% nets in use were in torn out (Table 8).

1.2.4 Preliminary studies to assess the toxic effect of nanoparticles on laboratory strains of mosquito vector species

Failure of ongoing vector control methods and to achieve the desired level of success prompts intensive research and studies to ideally develop more advantageous and ecofriendly approaches of vector control. In this prospect, a study was attempted to assess the effectiveness of nanosilica of different nature hydrophobic, hydrophilic and lipophilic on the toxicity to aquatic stages and oviposition behaviour (cage simulation study) in three important laboratory reared species of human disease vectors, namely *An. stephensi* Liston, *Aedes aegypti* Linnaeus and *Culex quinquefasciatus* Say.

Results of the study indicated the toxicity of different types of nanosilica (112.5–900 ppm) on the mosquito species tested and was in the order hydrophobic > hydrophilic > lipophilic nanosilica in larval susceptibility tests. A dose-dependent effect of hydrophobic nanosilica was found on the mosquito species tested. The toxic effect of hydrophobic nanosilica on mosquito species was in the order *An. stephensi* > *Ae. aegypti* > *Cx. quinquefasciatus* (Fig. 12). Similarly, the toxic effect of hydrophilic nanosilica at 112.5 ppm on pupae of different mosquito species was in the order

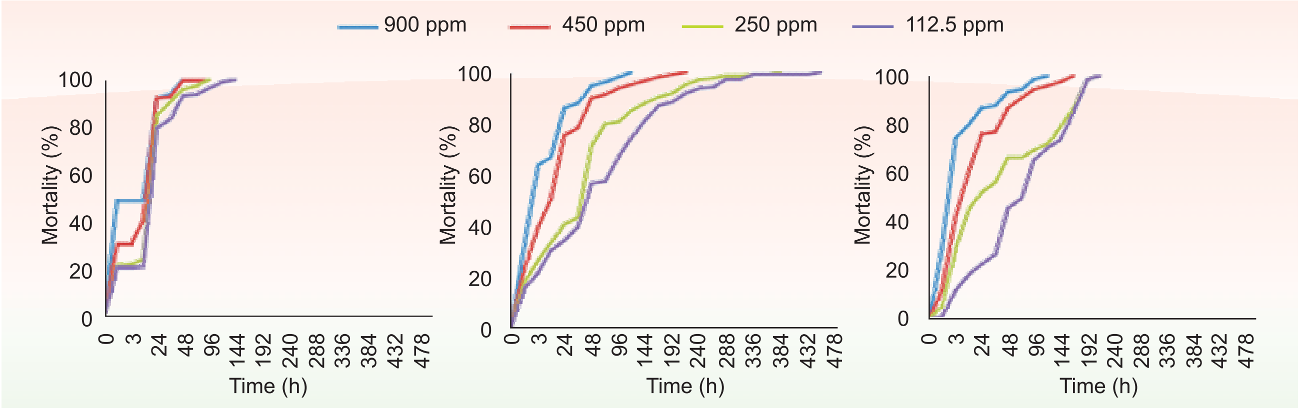


Fig. 12: Cumulative mortality of mosquito larvae after exposure to hydrophobic nanosilica at different time intervals. Data represent mean values of mortality of five replicates of 25 larvae each for each concentration.

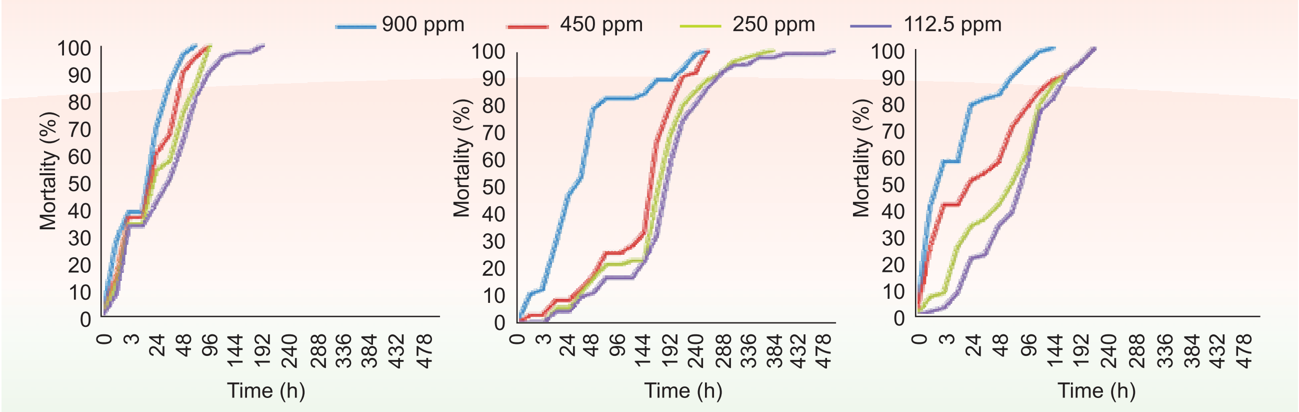


Fig. 13: Cumulative mortality of mosquito larvae after exposure to hydrophilic nanosilica at different time intervals. Data represent mean values of mortality of five replicates of 25 larvae each for each concentration.

An. stephensi > *Cx. quinquefasciatus* > *Ae. aegypti* (Fig. 13). Furthermore, ovi-deterrence activity of hydrophobic nanosilica at lower concentration of 56 ppm in *An. stephensi* and 112.5 ppm in *Ae. aegypti* and *Cx. quniquefasciatus* was found.

1.2.5 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

Dengue and chikungunya are upcoming major public health problems in India and control of breeding of vector *Ae. aegypti* is very difficult because of its breeding behaviour.

During the year 2008-09, the efficacy of C-21 attracticide developed by DRDE, Gwalior in combination with IGR compound was evaluated at Kerala, Bengaluru and Delhi, and the results were found very encouraging and statistically significant but there were some variations in efficacy of attracticide in different localities. To find out the parameters responsible for breeding behaviour this study was taken up.

Kerala

The study was initiated in the month of December 2009 at Alappuzha district of Kerala. A total of 746 ovitraps (373 each in experimental and control) were placed in 216 houses of 2 localities, i.e. Kadakkarapally and Vettackal. Overall positivity in experimental and control ovitraps revealed that a total of 9462 ovitraps were found positive, out of which 5171 (55%) were experimental and 4291 (45%) were control ovitraps. Figure 14 shows month-wise positivity in experimental and control ovitraps in Kerala from December 2009–December 2010.

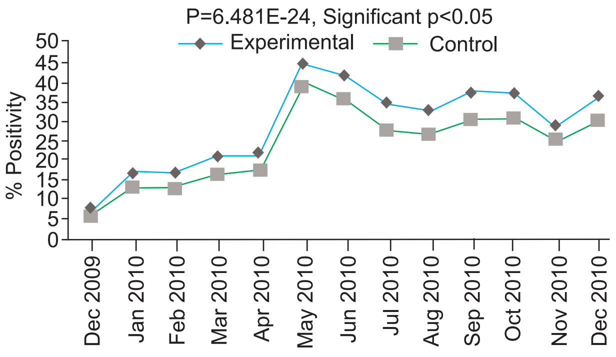


Fig. 14: Month-wise positivity of experimental and control ovitraps in Kerala.

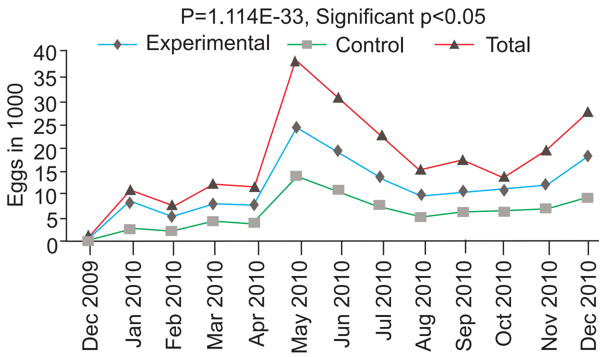


Fig. 15: Month-wise eggs collected from experimental and control ovitraps in Kerala.

Eggs collection data revealed that out of 228,207 eggs collected, 146,994 (64%) eggs collected from experimental ovitraps and 81,213 (36%) eggs were collected from control ovitraps. Figure 15 shows month-wise eggs collected from ovitraps in experimental and control ovitraps in Kerala from December 2009–December 2010.

Bengaluru

In Bengaluru City, three localties, viz. Modi Garden, Sanjay Gandhi Nagar and Venkateshpuram were selected for the study. A total of 541 houses, i.e. 269 in Modi Garden, 150 in Sanjay Gandhi Nagar and 122 in Venkateshpuram were selected for placement of 1082 ovitraps (541 each in experimental and control ovitraps). Month-wise positivity in experimental and control ovitraps revealed that a total of 1564 ovitraps were found positive, out of which 1013 (65%) were experimental and 551 (35%) were control ovitraps. Figure 16 shows month-wise positivity of ovitraps in experimental and control ovitraps in Bengaluru from February–December 2010.

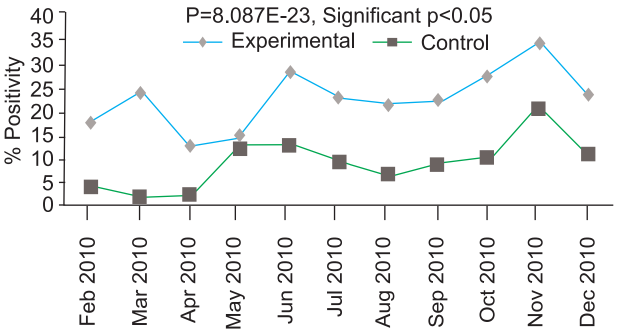


Fig. 16: Month-wise positivity of experimental and control ovitraps in Bengaluru.

Eggs collection data revealed that out of 33,314

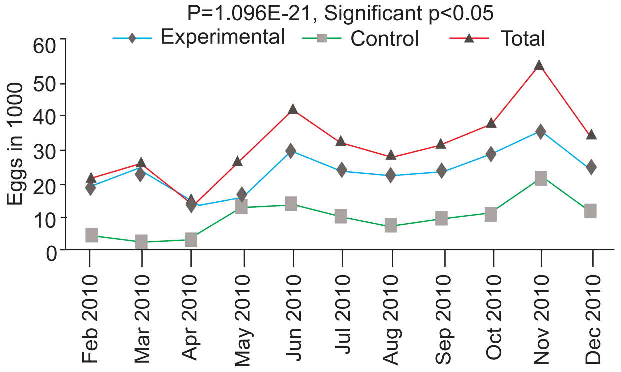


Fig. 17: Month-wise eggs collected from experimental and control ovitraps in Bengaluru.

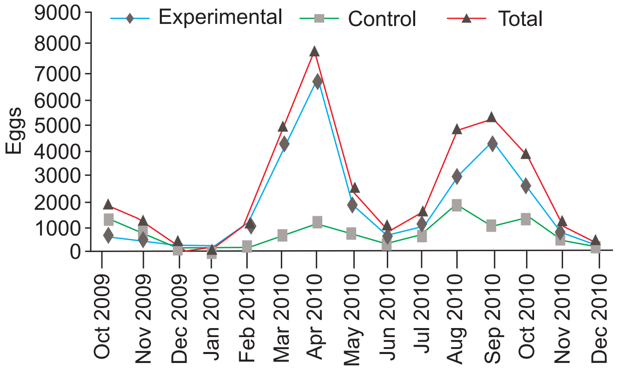


Fig. 19: Month-wise eggs collected from experimental and control ovitraps in Delhi.

eggs collected, 24,202 (73%) eggs collected from experimental ovitraps and 9112 (27%) eggs were collected from control ovitraps. Figure 17 shows month-wise eggs collected from experimental and control ovitraps in Bengaluru from February–December 2010.

Delhi

The study was initiated in the month of October 2009 at Delhi. A total of 480 ovitraps each experimental and control were placed in 60 houses of 4 localities, i.e. New Chitra Lane, Sewa Nagar, DCM Colony and Sarai Rohilla Railway Colony. Overall positivity in experimental and control ovitraps out of 893, was 436 (49%) and 457 (51%) respectively. Month-wise positivity of experimental and control ovitraps in Delhi from October 2009–December 2010 is shown in Fig. 18.

Eggs collection data revealed that out of 36,235 eggs collected, 26,549 (73%) eggs were collected from experimental ovitraps and 9686 (27%) eggs were collected from control ovitraps. Figure 19 shows month-wise eggs collected from experimental and control ovitraps in Delhi from October 2009–December 2010.

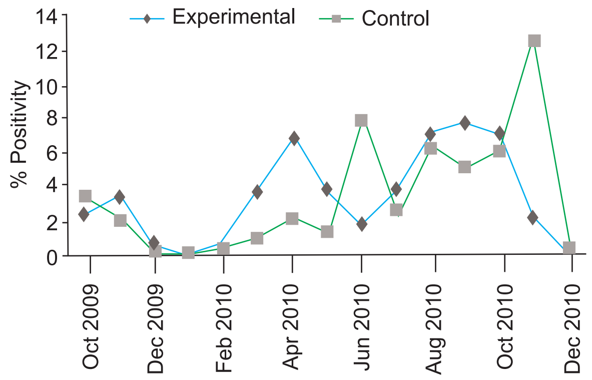


Fig. 18: Month-wise positivity of experimental and control ovitraps in Delhi.

July to November remains the peak season of *Aedes* breeding during which Commonwealth Games have taken place at Delhi. From the month of September 2010, the study was extended in 6 other localities adjacent to Commonwealth Games (CWG) village and venues, i.e. Lodhi Colony (near JLN Sports Complex), Pandav Nagar (near Games Village), Ganesh Nagar (near Games Village), Akshardham Temple premises (near Games Village), Govt. Qtrs. (near Talkatora Stadium) and Thyagraj Nagar (near Thyagraj Stadium). Table 9 shows locality-wise number of ovitraps placed in experimental and control houses at CWG sites.

Table 9. Number of experimental and control ovitraps

Locality	Ovitraps placed		
	No. of houses	Experimental	Control
Akshardham Temple	40	40	80
Govt. Qtrs, Lodhi Road complex	300	300	600
Pandav Nagar I	423	423	846
Pandav Nagar II	140	140	280
Govt. Qtrs, Talkatora Stadium	58	58	116
Tyagraj Nagar	67	67	134
Total	1028	1028	2056

Month-wise positivity in experimental and control ovitraps revealed that till December 2010, a total of 39 ovitraps were found positive, out of which 25 (64%) were experimental and 14 (36%) were control (Fig. 20).

Month-wise collection of eggs revealed that out of 1834 (23 *Aedes*) eggs collected till December

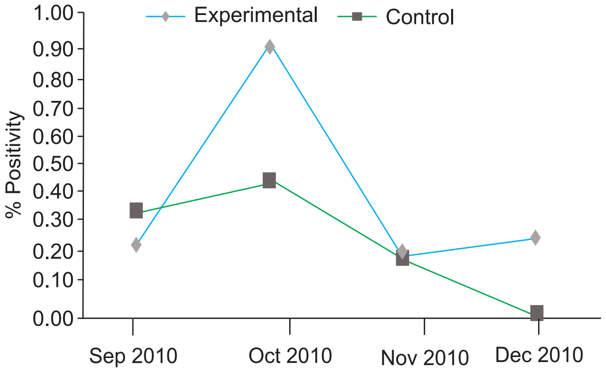


Fig. 20: Month-wise positivity of experimental and control ovitraps at CWG sites.

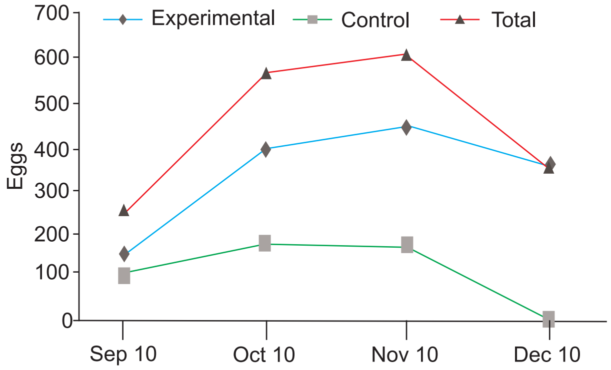


Fig. 21: Month-wise eggs collected from experimental and control ovitraps at CWG sites.

Fig. 22: Cross-checking and monitoring of work at Alappuzha and Bengaluru.

2010, 1389 (17 *Aedes,* 76%) eggs were collected from experimental and 445 (6 *Aedes,* 24%) eggs were collected from control ovitraps (Fig. 21). The progress of the work was cross-checked from time-to-time (Fig. 22).

The above results revealed that C-21 attracticide is working well for surveillance of *Ae. aegypti,* vector of dengue and chikungunya as the positivity of ovitraps and number of eggs were much higher in experimental as compared to control ovitraps and can be used as a management tool to control *Ae. aegypti*.

1.3 Insecticide resistance

1.3.1 Monitoring of insecticide resistance of malaria vectors in India

A project was sanctioned to assess the susceptibility status in the EMCP and GFATM implementation project areas by the NVBDCP. The study area constituted 13 states, including 7 NE-States consisting of 156 districts. The investigations were carried in the selected units involving a group of districts with homogeneity to ecotype, vector prevalence and other factors. In Year-1, studies were carried out in the states of Madhya Pradesh, Chhattisgarh, Andhra Pradesh, Odisha, Jharkhand, West Bengal, Asom, Mizoram and Meghalaya. Out of above nine states, studies were completed in four states, namely Madhya Pradesh, Chhattisgarh, Andhra Pradesh and West Bengal in Year-1. In Chhattisgarh, *An. culicifacies* was triple resistant to DDT, malathion and deltamethrin and in Andhra Pradesh, triple resistant to DDT, malathion and deltamethrin except in Vizianagaram where it showed verification required (VR) to deltamethrin. However, in Madhya Pradesh, *An. culicifacies* was variable resistant to insecticide in different districts. In West Bengal, vectors were resistant to DDT tolerant to malathion and susceptible to deltamethrin. Results have indicated that the vector species are mostly resistant to DDT and malathion, while in districts of Chhattisgarh and Andhra Pradesh the vectors were resistant to pyrethroids also.

1.3.2 Study to assess cross-resistance pattern against Chlorfenapyr in susceptible/resistant laboratory and field strains of mosquitoes

In Phase-I study, cross-resistance pattern to other insecticides was assessed and synergism/antagonism using piperonyl butoxide (PBO), studies with chlorfenapyr, a diagnostic dose of 5% with two hours exposure and 48 hours holding period was determined to discriminate the susceptible and resistant adult mosquito populations of different genera namely, *Aedes, Culex* and *Anopheles*. The molecule can be used for managing insecticide resistance in vectors because of novel mechanism of toxic action that is different from the mechanisms of the presently used neurotoxic insecticides. No cross-resistance between DDT, malathion, bendiocarb and deltamethrin resistance was observed with chlorfenapyr in laboratory-reared strains of *An. stephensi* and field-caught *An. culicifacies* (Table 10; Fig. 23). Studies demonstrated the antagonistic effect of PBO. However, cross-resistance to DDT, malathion, bendiocarb and deltamethrin was observed with chlorfenapyr in laboratory-reared and field-collected strains of *Cx. quinquefasciatus* and antagonism with PBO (Table 11; Fig. 24). The results have shown that chlorfenapyr can be a potential insecticide for the control of multiple insecticide resistant strains of *Cx. quinquefasciatus*. However, in countries where indoor residual spray (IRS) is

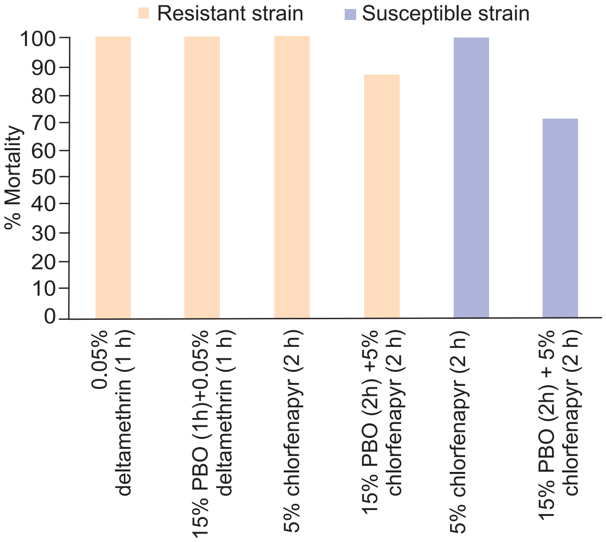


Fig. 23: Potentiation studies on susceptible (Sonepat strain) and resistant (Goa strain) of *An. stephensi*. There was no mortality in pyrethroid control replicates.

Table 10. Results of insecticide susceptibility tests on insecticide-susceptible (Sonepat and Nadiad) and insecticide-resistant strains (Goa) of *An. stephensi* and field-collected strains of *An. culicifacies* from Chhattisgarh and Gujarat states

Species	Insecticides					Control		
	DDT 4.0%	Malathion 5.0%	Bendiocarb 0.1%	Deltamethrin 0.05%	Chlorfenapyr 5.0%	OC	OP	PY
Susceptible strains								
An. stephensi (Sonepat)	98.3±2.3* (57)	100 (48)	100 (102)	100 (68)	100 (169)	0 (35)	0 (15)	4.7 (21)
An. stephensi (Nadiad)	95.9±2.8 (50)	98.0±2.7 (48)	100 (30)	100 (49)	100 (125)	0 (16)	0 (45)	0 (17)
Resistant strain								
An. stephensi (Goa)	10.3±5.1 (77)	26.2±5.9 (46)	ND	84.9±3.5 (47)	100 (116)	0 (15)	0 (16)	0 (15)
Field collected strain - Raipur, DDT-malathion-deltamethrin resistant								
An. culicifacies	4.2±2.1 (120)	73.3±3.9 (116)	80 (30)	78.2±2.5 (124)	100 (211)	0(48)	0 (50)	0 (18)
Field collected strain - Panchmahals, DDT-malathion-deltamethrin resistant								
An. culicifacies	6.4±2.6 (140)	30.1±3.2 (123)	ND	43.1±3.1 (130)	100 (60)	0 (21)	0 (19)	0 (20)
Field collected strain - Vadodara, DDT-malathion-deltamethrin resistant								
An. culicifacies	11.6±2.9 (120)	41.1±3.6 (124)	ND	59.2±3.4 (130)	100 (60)	0 (20)	0 (20)	0 (20)

*% mortality; ±: S.E.; Figures in parentheses indicate number of mosquitoes exposed; ND: Not done; OC: Organochlorine; OP: Organophosphate; PY: Pyrethroid.

Table 11. Results of insecticide susceptibility tests on laboratory-reared and field-collected insecticide resistant strains of *Cx. quinquefasciatus* from Chhattisgarh and Gujarat states

Species	Insecticides					Control		
	DDT 4.0%	Malathion 5.0%	Bendiocarb 0.1%	Deltamethrin 0.05%	Chlorfenapyr 5.0%	OC	OP	PY
Laboratory reared resistant strain								
Cx. quinquefasciatus (deltamethrin resistant)	14.3±2.3* (48)	4.4±1.7* (46)	89.5±5.7* (67)	43.8±2.6* (104)	96.4±1.9* (142)	0 (16)	0 (15)	4.7 (95)
Cx. quinquefasciatus (permethrin resistant)	11.87±1.9* (87)	3.7±1.6* (52)	32.1±2.8* (61)	68.4±2.8* (96)	70.8±3.7* (113)	0 (20)	0 (16)	0 (41)
Cx. quinquefasciatus (lambdacyhalothrin resistant)	55.7±2.0* (63)	9.5±3.1* (62)	85.2±5.7* (60)	43.5±2.1* (65)	78.0±3.4* (172)	0 (21)	0 (20)	0 (111)
Field collected strain-Raipur, DDT-malathion-bendiocarb-deltamethrin resistant strain								
Cx. quinquefasciatus	0 (53)	9.75±3.3* (51)	5.7±1.4* (120)	44.9±1.0* (49)	100 (117)	0(20)	0 (20)	0 (78)
Field collected strain-Kheda, DDT-malathion- bendiocarb-deltamethrin resistant strain								
Cx. quinquefasciatus	3.3±1.6* (60)	33.3±6.0* (60)	16.2±1.2* (80)	75.0±2.8 * (60)	100 (200)	0 (20)	0 (20)	0 (40)

*% mortality; ±: S.E.; Figures in parentheses indicate number of mosquitoes exposed; OC: Organochlorine; OP: Organophosphate; PY: Pyrethroid.

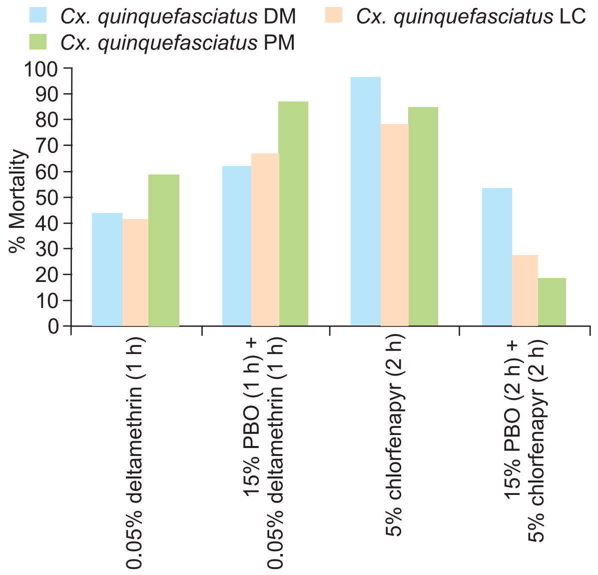


Fig. 24: Potentiation studies on insecticide resistant strains of *Cx. quinquefasciatus*. There was no mortality in pyrethroid control replicates. DM: Deltamethrin; LC: Lambda-cyhalothrin; PM: Permethrin.

not targeted for the control of this species, like in India, chlorfenapyr used in IRS for the control of malaria vectors in rural and peri-urban areas can additionally provide control of *Cx. quinquefasciatus* also.

1.3.3 Insecticide resistance status in *Anopheles culicifacies* in Gujarat state

Anopheles culicifacies populations showed resistance to DDT and malathion in the Districts Panchmahals, Vadodara and Kheda in studies in 2010. For deltamethrin field population showed resistance in Vadodara and Panchmahals but were susceptible in Kheda district indicating triple resistance in Vadodara and Panchmahals while double resistance in Kheda district (Fig. 25). Further supportive biochemical (enzyme assays) and molecular (*kdr* frequency) analyses are in progress.

1.3.4 Molecular characterization of the voltage-gated sodium channel of *Anopheles stephensi*

Knockdown resistance is one of the mechanisms of resistance against pyrethroid group of insecticides and DDT, both act on the voltage-gated sodium channel (VGSC) by modifying gating kinetics leading to paralysis and subsequent death of the insect. We amplified and sequenced the genomic DNA of *An. stephensi* spanning IIS4-S5 linker-to-IIS6 covering area where both *kdr* and *super-kdr* loci responsible for knockdown resistance are reported in other insects. There were two introns, where the first intron was located in IIS5-S6 linker (intron-1) and second intron in IIS6 segment (intron-2). The size of intron-1 was 995 bp whereas the size of intron-2 was highly variable due to the presence of highly polymorphic microsatellite marker of CT sequence repeats. The minimum and maximum numbers of CT repeats, which were identified in this study, were 6 and 26, respectively.

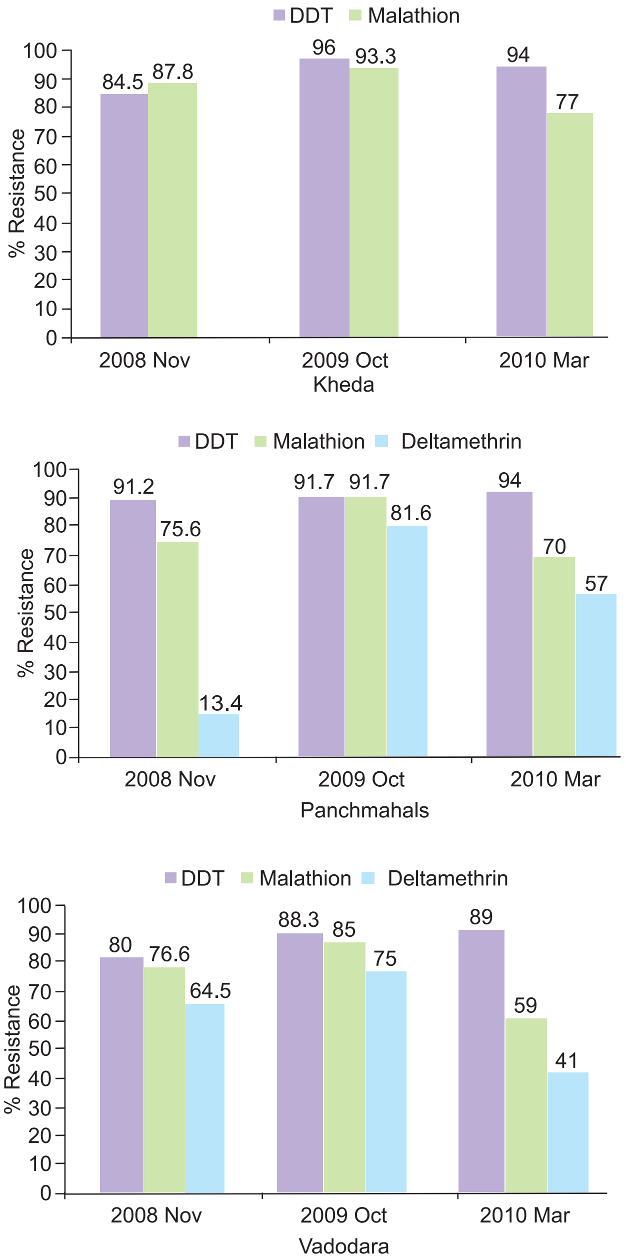


Fig. 25: Resistance status of *An. culicifacies* collected from Gujarat state.

1.3.5 Screening of populations for the detection of mutations in the VGSC

Based on the vgsc sequences, two PCRs were designed to amplify most of the exons leaving most part of intron-1. Using these PCRs two populations, viz. Alwar and Gurgaon were screened for detection of possible *kdr* mutations. Analysis of DNA sequences revealed the presence of two alternative non-synonymous point mutations in the IIS-6 transmembrane of VGSC both at residue Leu1014. These two mutations are due to c.3041T>C and c.3042A>T substitution leading to L1014S (TCA) or L1014F (TTT) amino acid mutations. No other non-synonymous mutation was found in other region sequenced. Several point mutations were also noticed but most of them were restricted to intron region only. Exons were highly conserved and no SNP was recorded in exon-1 whereas two synonymous SNPs were recorded in exon-2 at residues F968 and I987 both resulting from T>C substitutions.

1.3.6 Development of PCR assays for *kdr* detection

The presence of microsatellite markers in VGSC was noted downstream to *kdr* locus (72 bp apart) which is highly variable in pattern and size. We noted tandem repeat of 'CT' sequence ranging from 8–26 units in different individuals. Due to variation in sizes of microsatellite markers, the region downstream to microsatellite stretch is not suitable for primer design. It was anticipated that the amplicon sizes will vary in different mosquitoes with presence of two bands in case the subject is heterozygous for two microsatellite alleles of different lengths. We observed that two microsatellite alleles may differ by up to >40 bp. Therefore, we were unable to design classical Allele Specific PCR (ASPCR) or Amplification Refractory Mutation System (ARMS) for SNP detection, where allele-specific primers are designed in opposite directions. It was anticipated that the size of amplicon for a specific *kdr* allele containing microsatellite will be variable and produce two bands in case the sample is heterozygous for microsatellites of different lengths.

Two PCRs were designed for genotyping of the *kdr* alleles wherein three primers were used for each PCR—one universal forward primer St-F (Table 12) and two reverse allele-specific primers, all three located upstream to microsatellite region. Both the allele-specific primers designed for each PCR were from the same regions and direction (reverse), so a 26-bp tail was added to the 5′ end of one of the two allele-specific primers used in each PCR to differentiate two alleles by the size of amplicon. In the first PCR, hereafter called as PCR-F, the allele 1014F is discriminated from other alleles (wild and 1014S) whereas in the second PCR, hereafter called as PCR-L/S, 1014S and wild (L1014) alleles are discriminated. The allele-specific primers designed were: St-L/SR and St-PheR for PCR-F, and St-LeuR and St-SerR for PCR-L/S.

Table 12. List of primers designed for the identification of *kdr*-like mutations

Name of primer	Sequence (5′-3′)	Specificity
St-F (forward)	GAT TGT GTT CCG TGT GCT GT	Universal
St-L/SR (reverse)	GCG GGC AGG GCG GCG GGG GCG GGG CCC GAT CGG AAA GTA AGT TAC TTA CG**t** CT	Specific to L1014 and 1014S
St-PheR (reverse)	GAT CGG AAA GTA AGT TAC TTA CG**g** CA	Specific to 1014F only
St-LeuR (reverse)	GCG GGC AGG GCG GCG GGG GCG GGG CCC GAT CGG AAA GTA AGT TAC TTA CGA **g**TA	Specific to L1014 only
St-SerR (reverse)	CGA TCG GAA AGT AAG TTA CTT ACG A**t**T G	Specific to 1014S only

St-L/SR was designed specific to both L1014 and 1014S alleles, St-PheR to 1014F, St-LeuR to L1014 and St-SerR to 1014S allele. The sequences of primers diagrammatic representation of annealing specificity of each allele-specific primer to specific template DNA is shown in Fig. 26. A tail of 26 bp was incorporated in primer St-L/SR and St-LeuR (shown underlined in primer sequence) following Saavedra-Rodriguez *et al* 2007. To prevent non-specific annealing, an additional mismatch was incorporated on the 3rd base from the 3′ end in each of the allele-specific primers, which are shown in lower case in primer sequences. The expected amplicon sizes formed by allele-specific primers St-L/SR and St-PheR (with universal primer St-F) in PCR-F are 166 and 139 bp receptively. The expected size of amplicons in PCR-L/S with allele-specific primers St-LeuR and St-SerR are 166 and 140, respectively.

The optimized PCR conditions for PCR-F were as follows. The PCR was carried out in 15 µl reaction volume containing 0.50 µM of St-PheR, 0.25 µM of St-L/SR and 0.25 µM of St-F, 1X buffer, 1.5 mM of $MgCl_2$, 200 µM of each dNTP and 0.375 units of *Taq* DNA polymerase (AmpliTaq Gold, Applied Biosystems). The PCR thermal cycling conditions were: one cycle at 95°C for 5 min; followed by 35 cycles each at 95°C for 30S, 55°C for 30S and 72°C for 45S, and a final extension step at 72°C for 7 min. The amplified products were eletrophoresed on a 3.0% agarose gel containing ethidium bromide and visualized under UV illumination in gel documentation system. The presence of 139 bp product was scored as 1014F allele and a 166 bp that of the other alternative alleles (L1014/1014S). Presence of 139 bp and absence of 166 bp was scored as homozygous 1014F (Fig. 27).

The PCR conditions for PCR-L/S were similar to

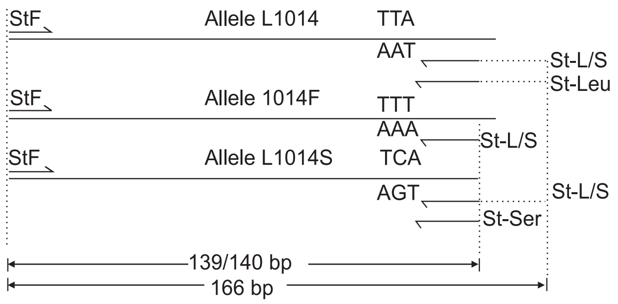


Fig. 26: Diagrammatic representation showing location of primers used in PCR developed for *kdr* genotyping, their annealing specificity with different *kdr* alleles and expected amplicon sizes. Horizontal solid lines represent DNA templates with different alleles, harpoons represent primers and dotted lines represent primer-tail.

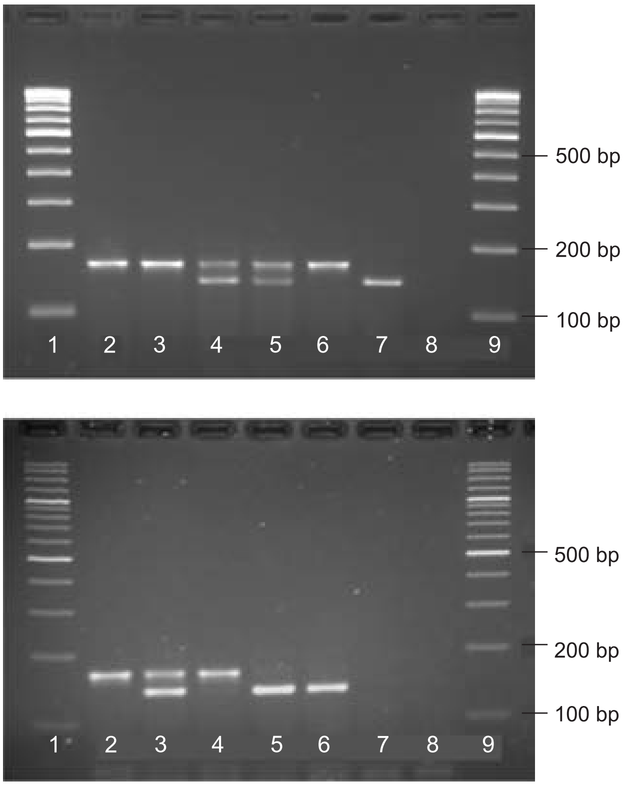


Fig. 27: Gel photographs showing result of PCR-F and PCR-L/S. Lanes 1 and 9: 100 bp DNA ladder; Lane 2: L/L; Lane 3: L/S; Lane 4: L/F; Lane 5: F/S; Lane 6: S/S; Lane 7: F/F (samples collected from Delhi, India); Lane 8: Negative control. The letters L, S and F stands for leucine, serine and phenylalanine, respectively.

PCR-F except for primers concentration, which was 0.50 µM for all the primers (St-F, St-L/SR and St-SerR). The presence of 166 bp PCR band was scored as wild allele (L1014) and 140 bp as 1014S allele. No band was expected for homozygous 1014F in this PCR; it is, therefore, not necessary to run PCR-L/S for samples scored as homozygous 1014F in first PCR, i.e. PCR-F.

1.3.7 Studies on insecticide resistance using bioinformatics

Studies with bioinformatic approaches carried out during this period include: Analysis of exon-intron organization in P450 supergene family of *An. gambiae*, and *Culex*. Three major supergene families are reported to contribute development of insecticide resistance; namely monooxygenases (cytochrome P450s), glutathione-S- transferases, and carboxyl esterases. Study was done on cytochrome P450 supergene family to understand the exon, intron organization with available data on public domain through neofunctionalization and deciphering the functional role of members of gene family through conserved exon-intron organization.

The glutathione-S-transferases (GSTs) are phase II class of detoxification enzymes that are responsible for insecticide resistance mechanisms. The *Cx. quinquefasciatus* GST superfamily genome sequence was analyzed by utilizing the public domain. In total, 35 cytosolic and 5 microsomal putatively active GSTs were retrieved, classified, and annotated. The study revealed the presence of three unclassified GSTs. Of 35 cytosolic GSTs, 65% contributed by insect specific Delta–Epsilon classes. The studies on intron gain and intron loss events revealed that the Delta GSTs have encountered a higher number of loss and gains during their evolution. Finally, the comparative genomic analysis has shown the GST supergene family evolution in insects.

1.4 Host-parasite interactions

1.4.1 Study of immune response in Indian malarial vectors in response to *Plasmodium falciparum* infection

The immunity of the *Anopheles* mosquito is highly developed and is a potential obstacle towards development of malaria parasite. However, malaria parasite has developed means to circumvent the vector defence. The study is aimed to identify the immune genes that are triggered during various stages of *P. falciparum* development

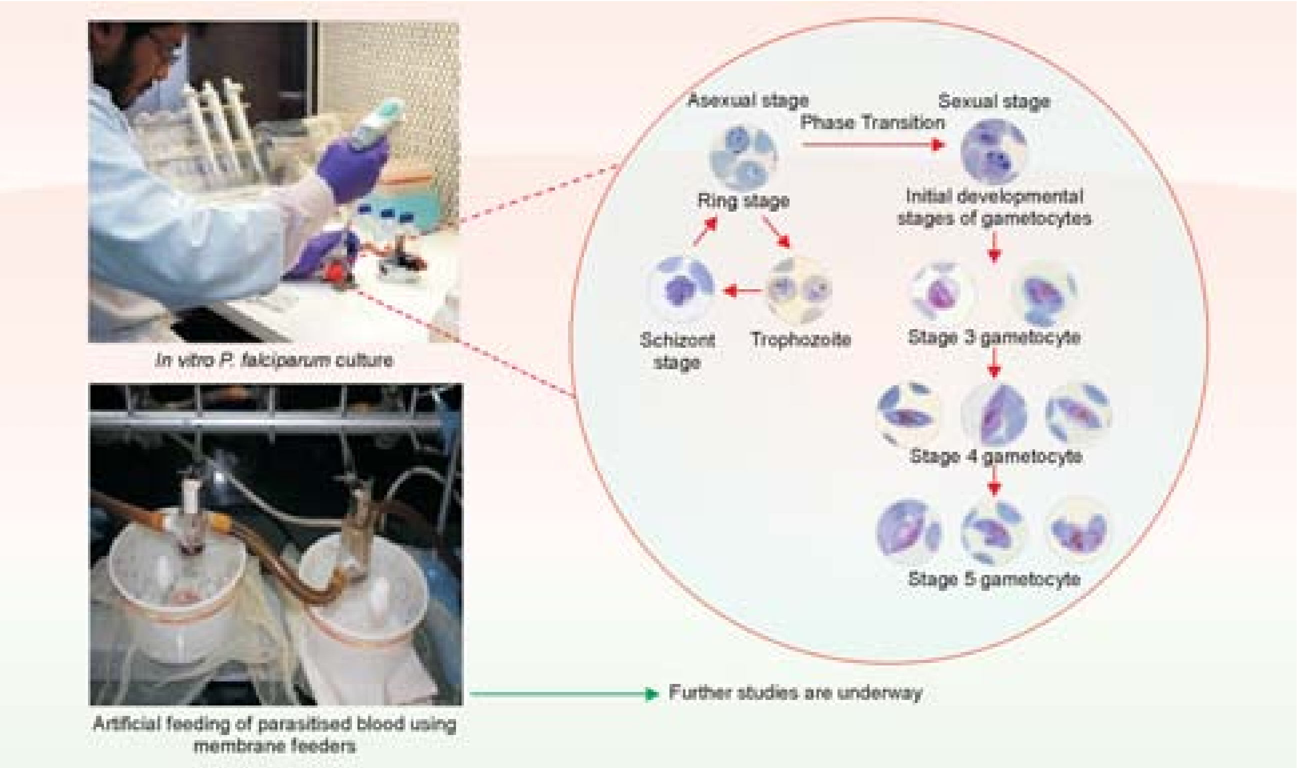


Fig. 28: Schematic diagram showing the process of study of immune response in mosquitoes to *P. falciparum* infection.

in two important Indian malaria vectors; namely *An. culicifacies* and *An. stephensi*. The wild mosquitoes, collected from their resting places were colonized in the insectary after which they were fed with blood containing *in vitro* cultured *P. falciparum*. The mosquitoes were collected at different time intervals of parasite development and the immunity related genes expressed at different time points were identified by the process of subtractive hybridization. The expression of the immune related genes were then analyzed by real time PCR with gene specific primers. Till now we have successfully cultured gametocytes producing strains of *P. falciparum* in our laboratory which has been fed to the laboratory reared mosquito using artificial membrane feeders (Fig. 28). Further studies are underway.

1.4.2 Characterization of symbiotic gut flora in Indian malarial vectors

Microorganisms are important components of the ecological system and during the course of evolution they occupy the niches created by insects. The gut micro biota represent all aspects of microbial relationships like pathogenic, mutualism and symbiotic associations. Insect gut bacteria also protects the insect gut from colonization by an insect pathogen, like in case of *Aedes* it protects to a certain limit the infection of dengue virus. In *An. gambiae* mosquito, a resident gut bacteria *Enterobacter* sp. renders the mosquito resistant to malaria parasite *P. falciparum*. Due to increasing insecticide resistance in mosquito, there is a need of some alternative methods for vector control. Paratransgenesis is the method in which gut bacteria of mosquito targeted against the malaria parasite by transgenic method.

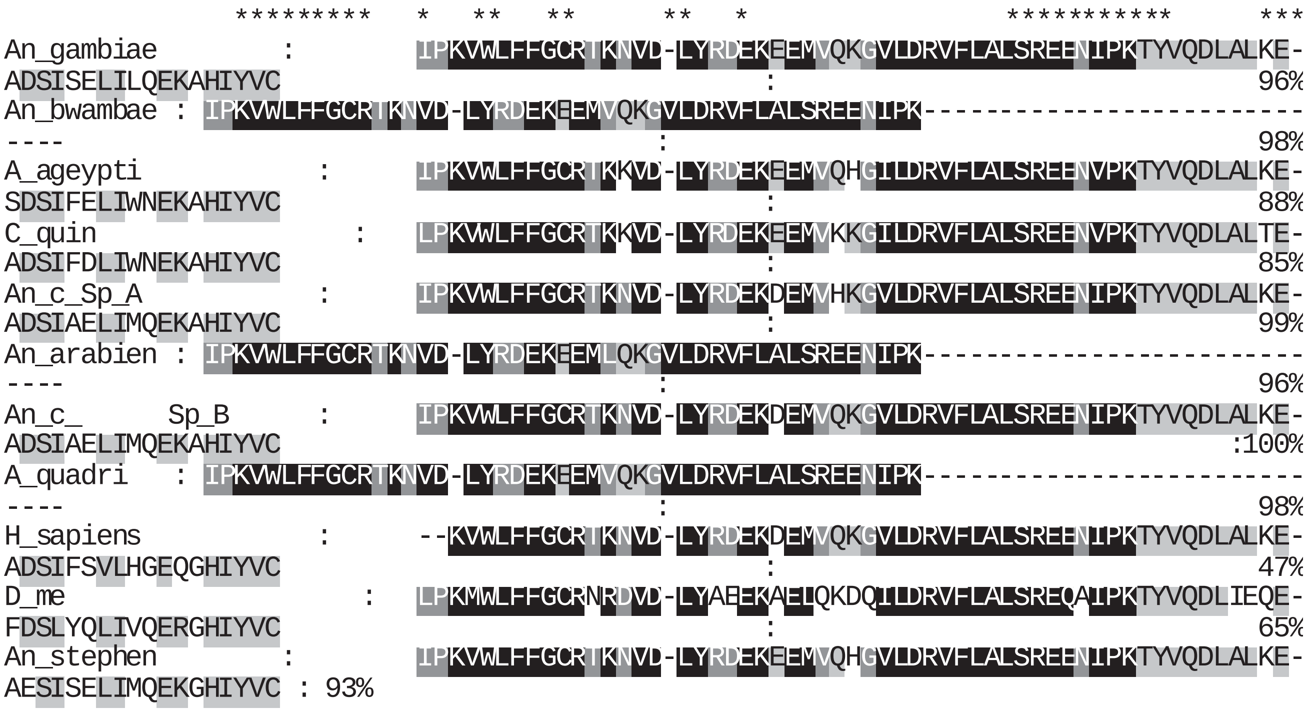
Fig. 29: (a) shows the *Anopheles* mosquito life cycle; and (b) shows gut biota of different developmental stages of *An. stephensi (A/s)* mosquito. Individual guts were plated on LB Agar plate of pH 7.0 and plates were incubated at 37°C for 24 h.

Till date, all of the culturable bacteria from the laboratory reared cyclic colony of *An. stephensi* mosquito at different developmental stages (eggs, larvae, pupae and adults) have been done. Establishment of pure culture from all developmental stages and identification through colony PCR is underway. Preliminary analysis revealed differences in the micro biota of larva, pupa and adult stages, although no significant difference was observed between female and male mosquito gut biota (Figs. 29a & b).

1.4.3 Transcriptional upregulation of nitric oxide synthase in *Anopheles culicifacies* species A and species B by Real Time PCR at different pBM

Recognition of transcriptionally upregulated genes that may inhibit the parasite at specific stages might offer new hope towards the fight of the disease. In this study, we report homology analysis and real time expression profiling of a *Plasmodium*-responsive nitric oxide synthase gene of sensitive and refractory *An. culicifacies* following infected blood feeding at various time intervals namely; 1, 3, and 7 days.

Genomic DNA was prepared from the mid-guts of both *An. culicifacies* species A and B. PCR assay was carried out and amplification of 300 base pairs against Exon 17 and 18 was observed by using primer sequences 5' ACATCAAGACGGAAATGGTTG 3' and 5' ACAGACGTAGATGTGGGCCTT 3'. The sequence homology to other reported NOS was confirmed by BLAST homology analysis (Fig. 30a). Homologous sequence (99%) of NOS in both *An. culicifacies* species A and species B have been obtained and sequence submitted to GenBank (JN591374 and JN591375 respectively). A phylogenetic tree (Fig. 30b) was constructed on the basis of alignment of the partial AcNOS amino acid sequence and the corresponding homologous



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                  *********   *   **   **      **   *               ***********      ***
An_gambiae        :        IPKVWLFFGCRTKNVD-LYRDEKEEMVQKGVLDRVFLALSREENIPKTYVQDLALKE-
ADSISELILQEKAHIYVC                                   :                             96%
An_bwambae : IPKVWLFFGCRTKNVD-LYRDEKEEMVQKGVLDRVFLALSREENIPK------------------------
----                                          :                                    98%
A_ageypti             :        IPKVWLFFGCRTKKVD-LYRDEKEEMVQHGILDRVFLALSREENVPKTYVQDLALKE-
SDSIFELIWNEKAHIYVC                                   :                             88%
C_quin                  :    LPKVWLFFGCRTKKVD-LYRDEKEEMVKKGILDRVFLALSREENVPKTYVQDLALTE-
ADSIFDLIWNEKAHIYVC                                   :                             85%
An_c_Sp_A             :        IPKVWLFFGCRTKNVD-LYRDEKDEMVHKGVLDRVFLALSREENIPKTYVQDLALKE-
ADSIAELIMQEKAHIYVC                                   :                             99%
An_arabien : IPKVWLFFGCRTKNVD-LYRDEKEEMLQKGVLDRVFLALSREENIPK------------------------
----                                          :                                    96%
An_c_     Sp_B        :        IPKVWLFFGCRTKNVD-LYRDEKDEMVQKGVLDRVFLALSREENIPKTYVQDLALKE-
ADSIAELIMQEKAHIYVC                                                               :100%
A_quadri   : IPKVWLFFGCRTKNVD-LYRDEKEEMVQKGVLDRVFLALSREENIPK------------------------
----                                          :                                    98%
H_sapiens             :        --KVWLFFGCRTKNVD-LYRDEKDEMVQKGVLDRVFLALSREENIPKTYVQDLALKE-
ADSIFSVLHGEQGHIYVC                                   :                             47%
D_me                     :    LPKMWLFFGCRNRDVD-LYAEEKAELQKDQILDRVFLALSREQAIPKTYVQDLIEQE-
FDSLYQLIVQERGHIYVC                                   :                             65%
An_stephen          :        IPKVWLFFGCRTKNVD-LYRDEKEEMVQHGVLDRVFLALSREENIPKTYVQDLALKE-
AESISELIMQEKGHIYVC : 93%
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Fig. 30a: Clustal alignment of AcNOS with known homologous NOS sequences of other insects and vertebrates.

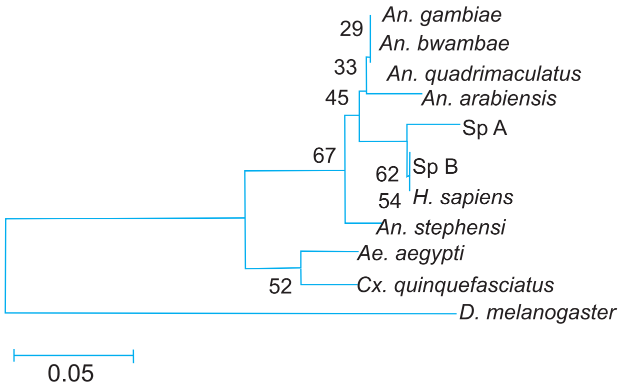

Fig. 30b: Phylogenetic bootstrap consensus tree-based on amino acid sequence alignment using Neighbour-Joining method.

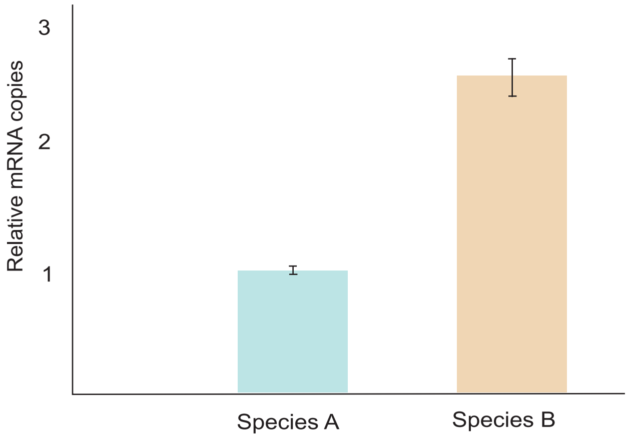

Fig. 31: Basal level (0 day) expression pattern of NOS in mid gut of *An. culicifacies* sp A and sp B (n = 25–30). Error bars representing standard deviation from three independent experiments are shown.

regions of several invertebrate and vertebrate NOS.

Real time RT-PCR was performed using SYBR Green RT-PCR kit (Roche Diagnostics, USA) and Light Cycler 480 system (Roche Diagnostics, USA) to measure relative transcript levels of AcNOS. cDNA of both the species was reverse-transcribed from 500 ng total RNA using oligo (dT) primer and transcriptor reverse transcriptase (Roche), following the manufacturer's instructions. The NOS primer forward sequence of Exon 17 and 18 region as above indicated and Normalizer gene S7 RNA polymerase having forward primer sequence 5′ GGTGT-TCGGTTCCAAGGTGA 3′ and reverse primer sequence 5′ GGTGGTCTGCTGGTTCTTATCC 3′.

We have found approximately 2.5 fold higher expression in refractory species B at basal level than species A (Fig. 31). We have found approximately 3.5 fold and 4 fold higher expression in blood fed uninfected species B as compared to basal level on Day 1 and Day 3 pBM respectively (Fig. 32a and 32b). On Day 7 pBM the level of expression was much higher in species B of about 7 fold expression in comparison to species A (Fig. 32c).

In three independent trials, NOS induction in refractory *P. vivax* infected mosquitoes showed a mean high expression about 4.8–5 fold than

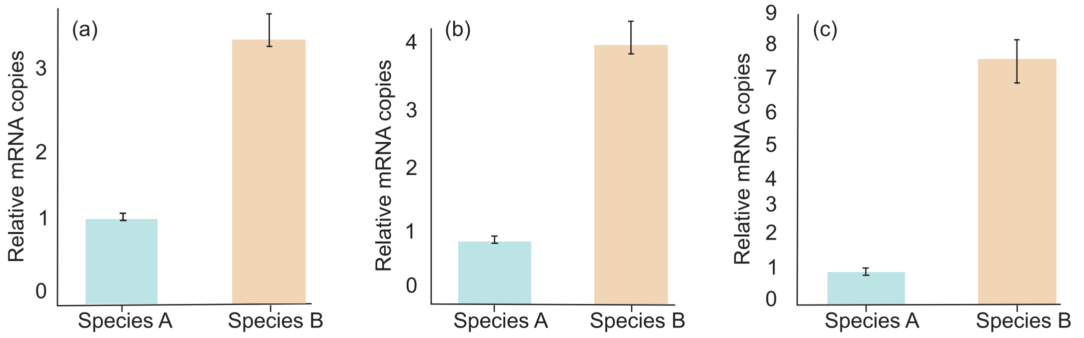

Fig. 32: Expression of NOS in mid gut of uninfected *An. culicifacies* sp A and sp B (n = 25–30) at various days pBM: (a) 1 day pBM; (b) 3 day pBM; and (c) 7 day pBM. Expression of target genes is normalized to the reference housekeeping gene S7 RNA polymerase. Representative data (mean ± S.D.) from three independent experiments are shown.

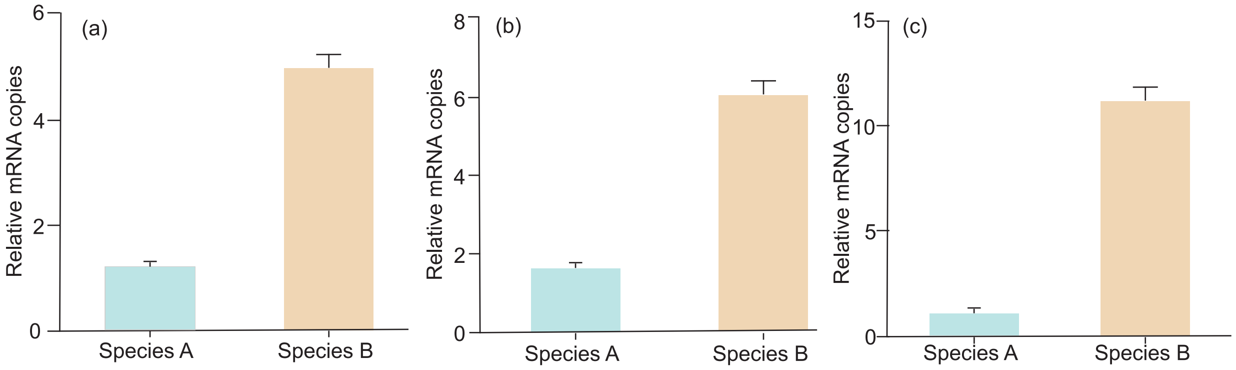

Fig. 33: Relative transcript abundance of AcNOS in *P. vivax* blood infected susceptible (species A) and refractory strain (species B) (n = 25–30): (a) 1 day pBM; (b) 3 day pBM; and (c) 7 day pBM. Gene transcript quantity was measured by relative RT-PCR using the internal standard S7 RNA polymerase gene. Error bars representing standard deviations from three independent experiments are shown.

susceptible mosquitoes on Day 1 pBM (Fig. 33a) ($p < 0.05$). This upregulation of AcNOS was higher on Day 3 pBM with 6 fold in refractory infected mosquitoes ($p = 0.009$) (Fig. 33b). In the refractory species on Day 7 pBM the expression levels of NOS were increased to nearly 10–11 fold (*t*-test; 0.0125 $p < 0.05$) (Fig. 33c) that was similar as previously.

1.4.4 MS-based proteomic approach to the identification of salivary gland proteins from the malaria vector *Anopheles stephensi* : 2D electrophoresis

The salivary gland proteins are relevant for malaria research since the *Plasmodium* sporozoites invade the salivary glands and are injected with the saliva into vertebrate hosts during blood feeding. Main objective of this study is to identify and characterize the salivary gland proteomes from *An. stephensi* and functional annotation of salivary gland proteomes through a detailed bioinformatics analysis and data analysis by MS.

Two samples of *An. stephensi* (sensitive species) and *An. stephensi* (resistant species) have been taken for 2 D gel electrophoresis to differentiate between the known and unknown (novel) proteins. Samples of salivary gland supernatant, corresponding to 50 or 120 mg of protein, were used for 2-D gel analysis. Samples were treated using a ReadyPrep 2-D Cleanup kit (Bio-Rad) to improve 2-D gel profiles. The pellet recovered after the last centrifugation step was dissolved in 15 mM NaCl, 0.5% SDS (final concentration) and 2% Triton X-100 (final concentration). The sample was heated at 95^{0}C for 3 min, flash-frozen in liquid nitrogen and lyophilized. The lyophilized material was dissolved in 2-DE sample buffer (7 M urea, 2 M thiourea, 4% CHAPS, 150 mM DTT, and 2% ampholytes). Salivary gland samples (30 ml) were loaded onto IEF 18-cm gels containing ampholines of pH ranging from 4 to 8 (Bio-Rad), and run for 20000 Vh. The second dimension was carried out on 12.5% acrylamide 22 cm slab gels. There were different protein spots in both *An. stephensi* sensitive (Fig. 34a) and resistant species (Fig. 34b) by 2D electrophoresis method.

Now, the total set of spots is being analyzed and by MS which will show the different proteins produced during electrophoresis. These observations will serve as a basis for future work to determine the possible role of novel proteins in the *Anopheles* sensitive and resistant species. Studies are in progress.

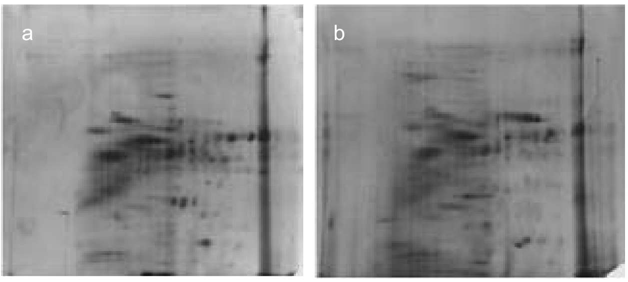

Fig. 34: 2 D electrophoresis analysis of salivary gland extract from (a) *An. stephensi* sensitive species; and (b) *An. stephensi* resistant species. Salivary gland extracts were purified by ReadyPrep.

1.5 Vector evolutionary genomics

1.5.1 NADPH cytochrome P450 reductase (CPR) gene evolution in Indian *Anopheles minimus*

Development of insecticide resistance (IR) in mosquito vectors is a primary huddle to malaria control programme. Since IR has genetic basis, and genes constantly evolve with response to environment for adaptation to organisms, it is important to know the evolutionary pattern of the genes conferring IR in malaria vectors. To this respect, *An. minimus* is a major malaria vector of the south-east Asia and north-east India, still susceptible to insecticides in the field, and thus it is of interest to know if natural selection or drift has shaped variation in this gene. For this, the whole genome sequence information of *An. gambiae* was used and sequenced a ~569 bp DNA segment (Fig. 35) (both coding and

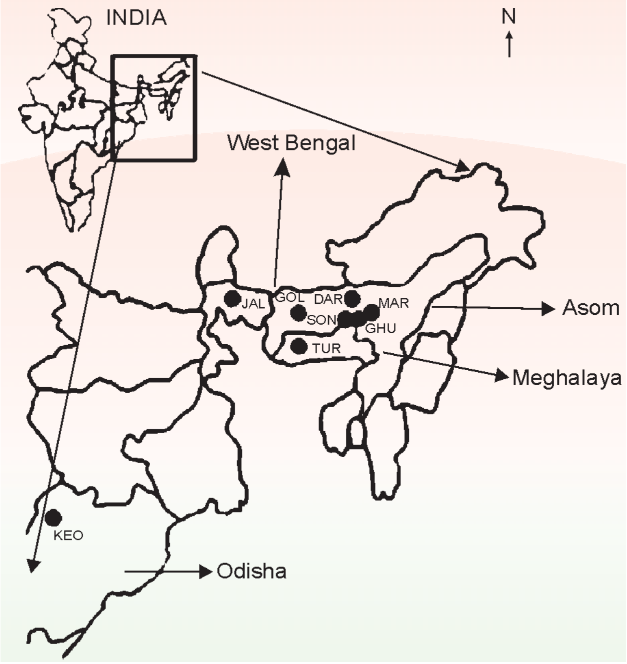

Fig. 36: Map of India indicating location of *An. minimus* sample collection sites in India. The name of the population samples have been abbreviated as follows: DAR: Darrang, JAL: Jalpaiguri; GOL: Goalpara; MAR: Marigaon; SON: Sonapur; GHU: Ghuli; TUR: Tura; KEO: Keonjhar.

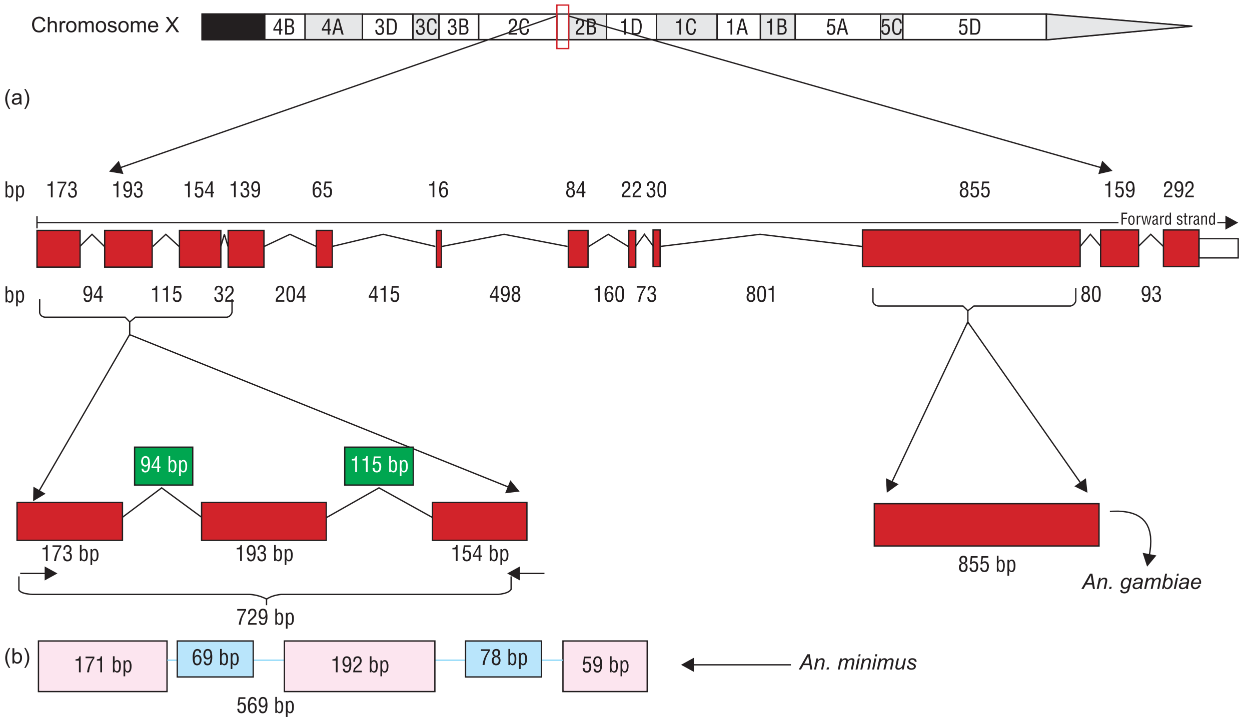

Fig. 35: (a) Location and characteristic details of the CPR gene in the X-chromosome of *An. gambiae*; and (b) Portion of the gene homologous to the sequenced portion of *An. minimus*. The name of the NADPH Cytochrome P450 reductase gene has been abbreviated as CPR gene.

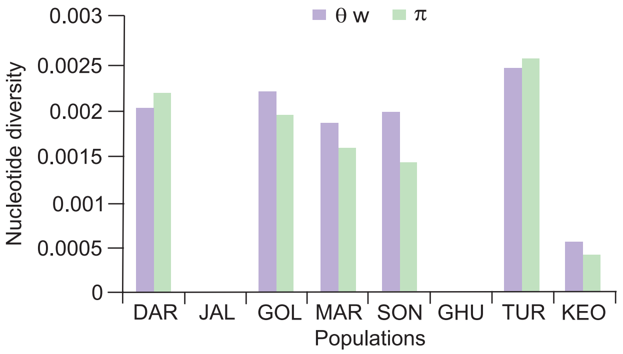

Fig. 37: Two measures of nucleotide diversity; black- Q_W and grey- π for each population sample across the eight Indian population samples of *An. minimus*. The name of the population samples have been abbreviated as follows: DAR: Darrang, JAL: Jalpaiguri; GOL: Goalpara; MAR: Marigaon; SON: Sonapur; GHU: Ghuli; TUR: Tura; KEO: Keonjhar.

non-coding elements) of the NADPH cytochrome P450 reductase (CPR) gene in 102 individuals of *An. minimus* collected in eight locations in India (Fig. 36) and inferred evolutionary history of this gene segment based on genetic diversity data. *Anopheles minimus* mosquitoes from two populations were found to be completely monomorphic; in six samples only four SNPs could be detected. Nucleotide diversity was fairly low in this gene segment (Fig. 37). We have also amplified and sequenced the homologous DNA segments of this gene segment in two closely related species of *An. minimus*, *An. fluviatilis* and *An. stephensi* and homologous gene sequences of other mosquito vectors of the family Culicidae (*An. gambiae*, *Ae.*

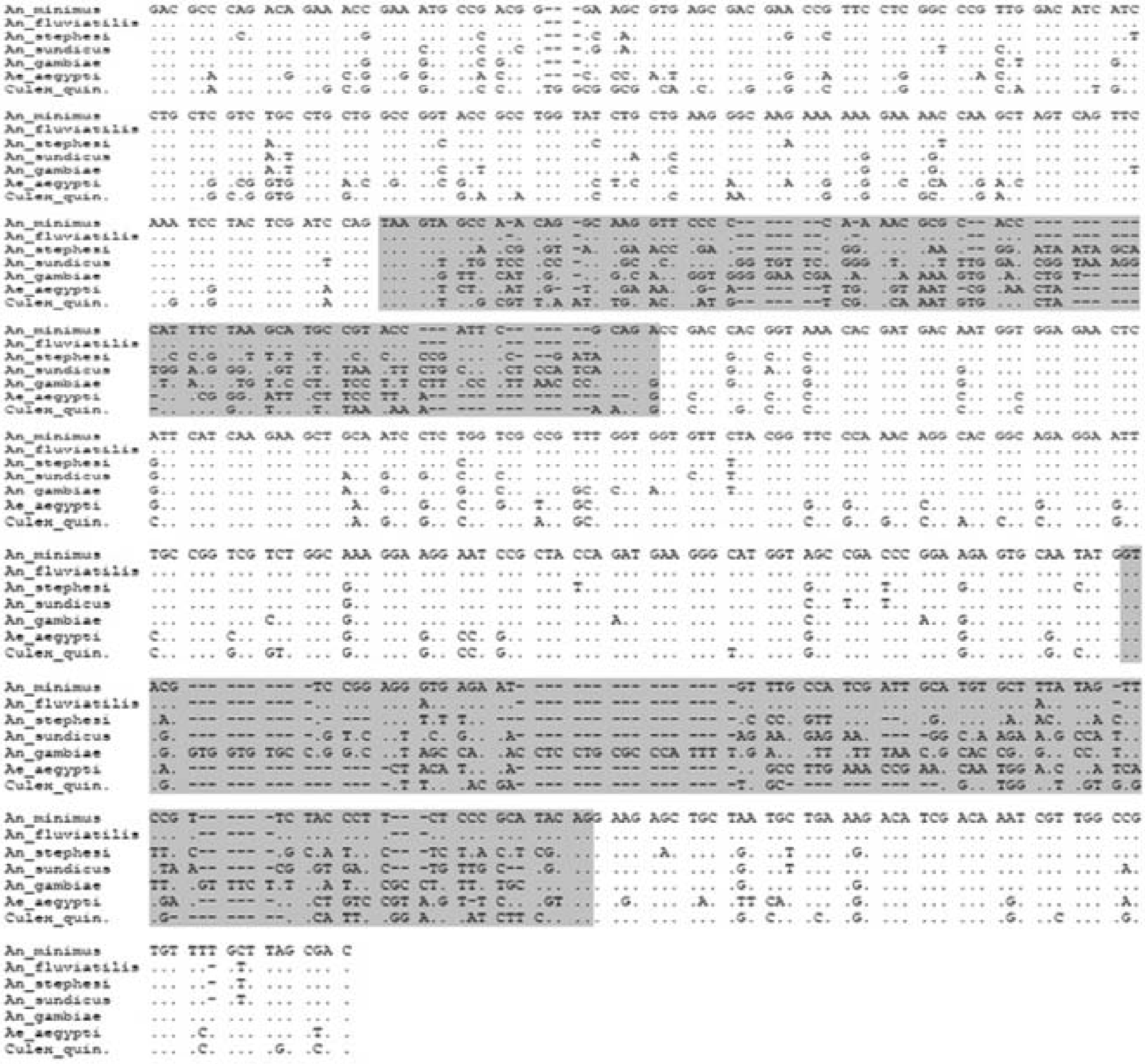

Fig. 38: Inter-specific DNA sequence alignment of the CPR gene segment among members of the Culicidae family. The grey coloured portion of the alignment represents introns and the rest are all exons of *An. minimus*.

aegypti and *Cx. quinquefasciatus)* were retrieved from the Ensemble web database for interspecific analysis (Fig. 38). Several statistical tests of neutrality and natural selection have been conducted (Fig. 39) and no significant evidence of natural selection in any population samples for this gene segment could be attained. The data were further analyzed to infer population structure (Fig. 40) and demography of this species in India. It was apparent that the data follow the isolation-by-distance model of population structure and majority of the samples have experienced population bottleneck in the recent history (Fig. 41). The population genetic study confirmed that genetic drift has shaped variations in this IR-conferring gene in Indian *An. minimus*.

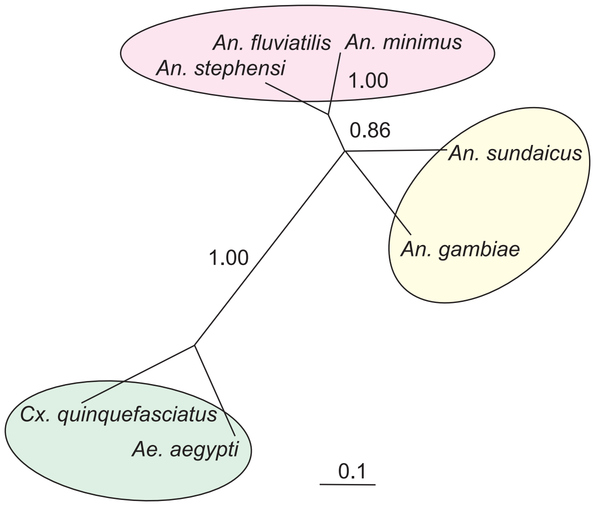

Fig. 39: Inter-specific phylogenetic tree among the members of the Culicidae family based on the CPR gene segment. Numbers on branches are the posterior probabilities of clades.

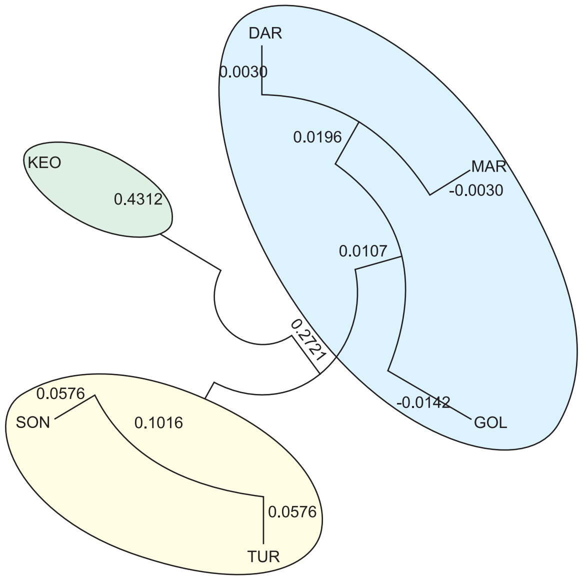

Fig. 40: The names of the population samples have been abbreviated as follows: DAR: Darrang, JAL: Jalpaiguri; GOL: Goalpara; MAR: Marigaon; SON: Sonapur; GHU: Ghuli; TUR: Tura; KEO: Keonjhar. The phylogenetic tree showing three clades of the genetic interrelationships among six different Indian populations of *An. minimus*. Blue colour represents one clade which consists of the population samples from Darrang, Marigaon and Goalpara, Yellow colour represents another clade which consists of samples from Sonapur and Tura, and Green colour represents entirely separate clade of one population sample from Keonjhar in the phylogenetic tree. Numbers on branches represent the branch length of the clades.

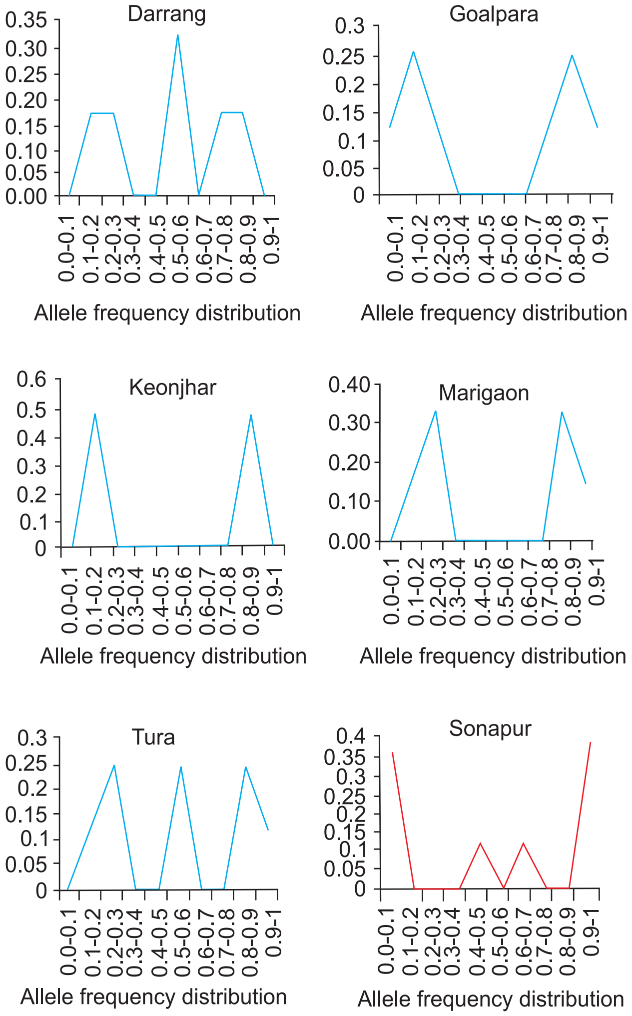

Fig. 41: Allele frequency distribution in six population samples of *An. minimus* which discriminates (graph line Blue) bottle-necked populations from (graph line Red) constant-sized populations.

1.6 Other Studies

1.6.1 Surveillance of Dengue vector, *Aedes aegypti* before Commonwealth Games 2010

As per the directions of the Directorate of

National Vector Borne Disease Control Programme (NVBDCP), the surveillance of *Ae. aegypti* had been carried out in coordination with New Delhi Municipal Council (NDMC) as a precautionary measure against dengue, which is one of the major vector borne diseases reported from Delhi in the past, namely Ward No. 188 of Sangam Vihar, and Ward No. 190 of Chittaranjan Park. During the Commonwealth Games surveys were conducted for 20 days initiating from 29th September 2010 till 15th October 2010 in nearby habitations where Commonwealth Games were conducted.

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❑

Parasite Biology

2

2.1 Characterization of malaria parasites

2.1.1 Frequency and characterization of glucose-6-phosphate dehydrogenase deficiency and haemoglobin variants in malaria endemic Sundargarh district of Odisha

Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency and sickle-cell haemoglobinopathy are common in populations living in malaria endemic areas. It has been proposed that high frequency of deficient alleles arise because it confers a selective advantage against malaria. A study was conducted to assess the prevalence of G-6-PD deficiency and haemoglobin variants in malaria endemic Sundargarh district of Odisha and to characterize the G-6-PD deficient and Hb variant samples at molecular level. G-6-PD phenotype was assessed by fluorescent spot test procedure. G-6-PD genotype of three common Indian forms (G-6-PD Odisha, G-6-PD mediterranean, and 1311T/C) were determined. Haemoglobin (Hb) electromorphs were typed using cellulose acetate membrane electrophoresis III (CAM III; Shandon Scientific Company, UK) and stained with Ponseau-S. G-6-PD deficiency studies in India indicate that deficiencies reported vary from complete absence to approximately 27%. In our study, 9.3% of the population was found to be G-6-PD deficient and the gene frequency of G-6-PD deficiency was observed to be 0.093 (Table 1). Molecular characterization revealed the presence of all the three types of G-6-PD variants studied, namely G-6-PD Odisha, G-6-PD Mediterranean and 1311T/C showing different prevalence rates (Fig. 1) with highest prevalence observed for G-6-PD Odisha. Studies among Indian populations showed that HbS allele is found with a frequency ranging from complete absence to 0.41 with an average frequency of 0.031. This study observed that 6.9% of the population is having haemoglobinopathy and a gene frequency of 0.36 (HbS) of this disorder was observed. A high proportion of heterozygote genotype, HbAS (6.61%) was observed which revealed that the study area has a high prevalence of this haemoglobinopathy. Approximately, 93% were of AA type and the proportion of AS and SS is 6.61 and 0.32% respectively. Molecular analysis by PCR-RFLP confirmed the presence of these (AS and SS) mutant variants. The study clearly indicates that malaria is a serious health issue in this particular region and G-6-PD deficiency and haemoglobin variants are disorders occurring at a relatively high frequency in this tribal dominated malaria endemic region (Table 2). Treatment of patients with malaria

Table 1. Distribution of G-6-PD deficiency and haemoglobin variants in the study population

Blood system phenotype	% Phenotype frequency	Gene frequency
G-6-PD deficient	9.3	Gd^d = 0.093
Non- deficient	90.6	Gd^D = 0.906
HbAA	93.06	HbA = 0.96
HbAS	6.61	HbS = 0.36
HbSS	0.32	-

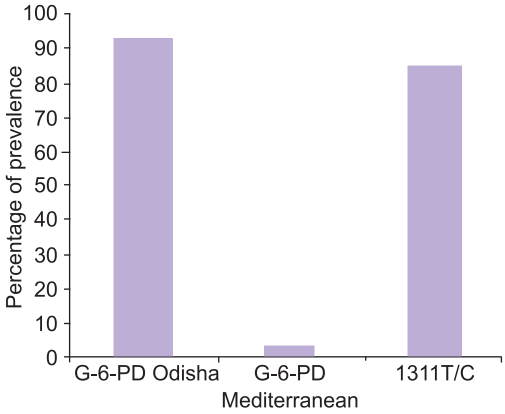

Fig. 1: Prevalence of different G-6-PD variants in the study population.

Table 2. Details of oligonucleotides and conditions for PCR-RFLP in the analysis of G-6-PD alleles

G-6-PD variants	Primer (5′→3′)	Restriction enzyme	Allele size in normal	Allele size in variants
G-6-PD Odisha	CAGCCACTTCTAACCACACACCT CCGAAGTTGGCCATGCTGGG	*Hae*III	107, 75, 66, 48, 45, 11	123, 107, 66, 45, 11
G-6-PD Mediterranean	ACTCCCCGAAGAGGGGTFTCAAGG CCAGCCTCCCAGGAGAGAGGAAG	*Mbo*II	377, 119	277, 119, 100
1311T/C	TGTTCFTCAACCCCGAGGAGT AAGACGTCCAGGATGAGGTGATC	*Bcl*I	203	180, 23

having G-6-PD deficiency is a serious issue because certain antimalarial drugs like primaquine cause haemolytic disorders. So, on large scale mass surveys are necessary and these should be carried out in malaria endemic areas before giving treatment because of the possibility of the occurrence of these disorders.

2.1.2 Genetic variation in microsatellite marker flanking *pfmdr-1* gene

The *pfcrt* K76T mutant allele is the most reliable molecular marker for chloroquine resistant *P. falciparum* isolate. But, point mutations and copy number variation of another transporter gene of the parasite, named *pfmdr*-1 at chromosome 5, contribute to parasite's susceptibility to various antimalarial drugs used in Artemisinin-based combination therapy (ACT) and considerably play a modulatory role in chloroquine resistance. As CQ is being replaced by newer artemisinin-based combination therapy such as artemether-lumefantrine and *pfmdr-1* being a major modulator of resistance to these drugs, we require understanding of the regulation of genetic variation at *pfmdr-1*. The current efforts to understand the evolution of the parasite genome under changing drug pressure revealed a reduction in allele diversity around *pfcrt* gene in CQR falciparum in India. It becomes essential to study the genetic variation around *pfmdr-1* to generate the baseline of selection pressure in this part of the parasite genome before introduction of ACT programme in India. Thus, we studied the evolutionary dynamics of *pfmdr-1* locus in 213 *P. falciparum* isolates collected from 13 field sites during 2002 to 2006. PCR amplification of microsatellite loci 5-956456 (–1.8Kb), 5-957861 (–400bp), 5-963445 (+700Kb) and 9-966096 (+4.3Kb) (extends ~10 Kb flanking *pfmdr-1*) were performed with semi-nested strategy. The Arlequin 3.11 package was used to compute the locus by locus diversity in 167 single allele infected isolates.

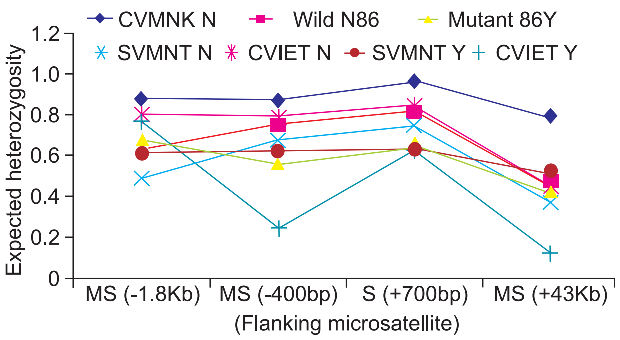

Fig. 2: Genetic variation around *pfmdr-1* gene and *pfcrt* gene in comparison with *pfcrt* + *pfmdr-1* flanking microsatellites.

The genetic diversity measured at locus by locus in terms of expected heterozygosity (H_e) is shown in Fig. 2. In an earlier study, we observed high genetic diversity in MS loci flanking CQS *pfcrt* gene and reduced genetic diversity in MS loci flanking CQR *pfcrt* gene. But in this study we observed the genetic diversity is relatively high in both CQS and CQR *pfmdr-1* alleles. It was strikingly different pattern of variation with CQR *pfcrt* loci exhibiting reduced variation and CQR *pfmdr-1* loci exhibiting high variation. This marked difference between the two candidates of chloroquine resistance suggests a different mechanism of evolutionary dynamics of both genes under drug pressure in India. We found high genetic diversity at all the above microsatellite loci in isolates which have CQS alleles for both *pfcrt* and *pfmdr-1* genes. But a mild reduction in genetic diversity was observed at CQR allele and CQS allele for *pfmdr-1* when compared to isolates having CQS alleles for both *pfcrt* and *pfmdr-1* genes. That means, possibly the strength of CQ selection is different for both the genes. Here, in this study we understood that resistant pfcrt allele may be under strong selection pressure, whereas pfmdr-1 86Y allele may be under weak selection pressure. The reason for above observation may lie in the functional values of the protein, as pfcrt is considered to be the primary transporter for CQ and pfmdr-1 is considered to be the primary transporter for mefloquine and variety of other antimalarials used in ACT programme.

2.1.3 Mapping of *Plasmodium vivax* anti-folate drug resistance in India

Due to the emergence and spread of drug resistant strains of human *Plasmodium* species, monitoring the efficacy of drug by follow up study and drug resistance related point mutations in the concerned genes are now essential steps specially to design and administer an effective antimalarial drug policy. Sulfadoxine and pyrimethamine are antifolate drugs that show synergistic antimalarial effect. Point mutations in *dihydrofolate reductase* (*dhfr*) and *dihydropteorate synthatase* (*dhps*) cause antifolate drug resistance phenotype in human malaria parasites. This study presents pattern of point mutations in *dhfr*/*dhps* genes in the Indian isolates of *P. vivax*. *Pvdhfr* and *pvdhps* genes were PCR amplified and sequenced. Sequence analysis revealed single (S58R), double (S58R/S117N) and quadruple (F57L/S58R/T61M/S117T) point mutations at *dhfr* and single (A383G) to double (A383G/A553G) mutations at *dhps* in *P. vivax* field isolates. Both, *dhfr* and *dhps* genes revealed tandem repeat variations in field isolates and the tandem repeat variants were designated as Type 1–4 for *dhfr* and Type A–H for *dhps* (Figs. 3 & 4) and *dhps* revealed very low mutation frequency (14%) compared to *dhfr* (64.78%). We observed few new mutations (synonymous and non-synonymous) at *dhfr*. Comparative analysis revealed a progressive increase in frequency of quadruple mutant genotype (χ^2 = 68.8, $p \leq 0.001$) within five years in a north-eastern state (Kamrup, Asom). Frequency of mutant *dhfr* genotypes varied significantly among different geographical regions and three distinct geographical clusters of wild (northern India), double mutant (southern India), and quadruple mutant (north-eastern and island regions of India) genotypes were observed in the Indian subcontinent (Fig. 5). Study suggests that *P. vivax* may be susceptible to SP in India except Andaman and north-eastern states. The geographical clustering of *dhfr* mutant genotypes suggest the distinct geographical regions of sensitive and resistant phenotypes and, therefore, would be highly useful for designing and administering national anti-malarial drug policy.

```
256 QGGGDNTSGGDNTHGGDNTHGGDNADKLQT Type 1
256 QGGGDNTSGGDNTHG------GDNADKLQT Type 2
256 QGGGDNT------------HGGDNADKLQT Type 3
256 QGGGDNT------------SGGDNADKLQT Type 4
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Fig 3: Tandem repeat variation in *pvdhfr*.

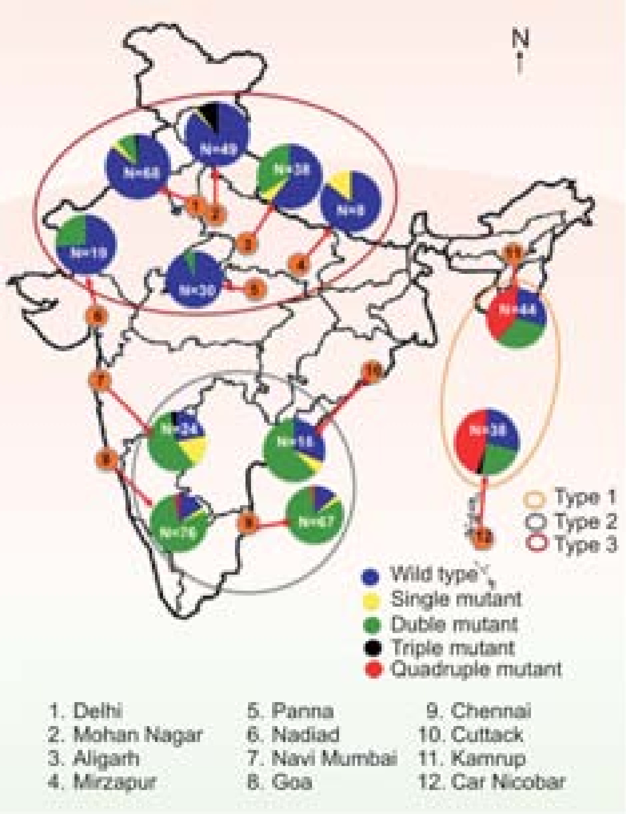


Fig. 5: Frequencies of *Pvdhfr* genotypes and their geographical clustering in the Indian subcontinent.

```
754 GEAKLTNGEGKLTNGEAKLTNGEGKLTNGEAKLTNGEGKLTNGDAKLTNGDSKLTNG Type A
754 GEAKLTNGEGKLTNGEAKLTNGEGKLTNG---------------------DSKLTNG Type B
754 GEAKLTNGEGKLTNGEAKLTNGEGKLTN--------------GDAKLTNGDSKLTNG Type C
754 GEAKLTNGEGKLTNGEAKLTNGEGKLTNGEAKLTNGEGKLTN-------GDSKLTNG Type D
754 GEAKLTNGEGKLTNG-----------------------------------DSKLTNG Type E
754 GEAKLTNGEGKLTNGEAKLTN-------GEAKLTNGEGKLTNGDAKLTNGDSKLTNG Type F
754 GEAKLTNGEGKLTNGE-------GKLTNGEAKLTNGEGKLTN-------GDSKLTNG Type G
754 GEAKLTNGEGKLTNGEGKLTNGEGKLTNGEAKLTNG--------------DSKLTNG Type H
```

Fig 4: Tandem repeat variation in *pvdhps*.

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

Genes encoding the HLA proteins are among the most diverse in the human genome and evidences suggest that HLA molecules are considered to play a crucial role in the defence of the host against malaria infection. It has recently been suggested that some of this protection may have an immune basis and that interactions between susceptibility to *P. vivax* and *P. falciparum* may be relevant in populations where both are prevalent. We have used molecular methods to determine the frequencies of human leukocyte antigen (HLA)-A, -B and -C alleles in patients infected with either *Pf* or *Pv* as well as normal healthy unrelated individuals from different parts of India using polymerase chain reaction.

Out of 182 blood samples (both infected and control), a total of 81 samples from Delhi (*Pf* = 20, *Pv* = 20 and healthy controls (HC = 41), 41 samples from Ranchi (*Pf* = 21 and HC = 20) and 60 samples from Rourkela (*Pf* = 20, *Pv* = 20 and HC = 20) have been collected and processed for DNA extraction. Forty-eight alleles for HLA-B locus, 24 alleles for HLA DRB locus and 8 alleles for HLA DQB1 locus have been analyzed in both infected as well as in controls by using DNA-SSP polymerase chain reaction.

Various histograms showing percentage frequency distribution and comparison of variants of HLA B, DR and DQ loci in malaria patients as well as in healthy controls from Delhi (Figs. 6 & 7), Ranchi (Fig. 8) and Rourkela (Figs. 9 & 10) are developed.

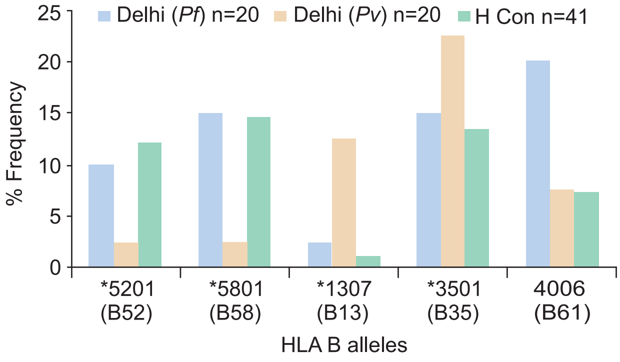


Fig. 6: Histogram showing percentage of frequency of different alleles of class I (HLA B) in *Pf* and *Pv* infected patients and controls of Delhi.

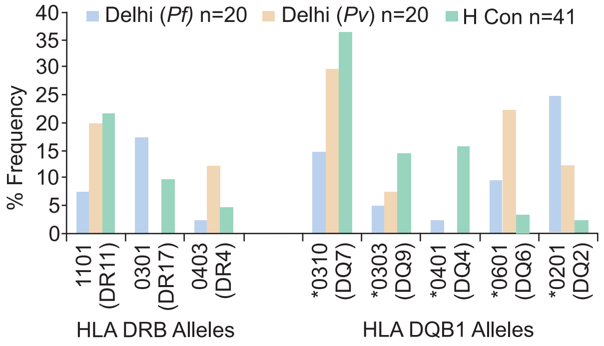


Fig. 7: Histogram showing percentage of frequency of different alleles of class II (HLA DR & DQ) in *Pf* and *Pv* infected patients and controls of Delhi.

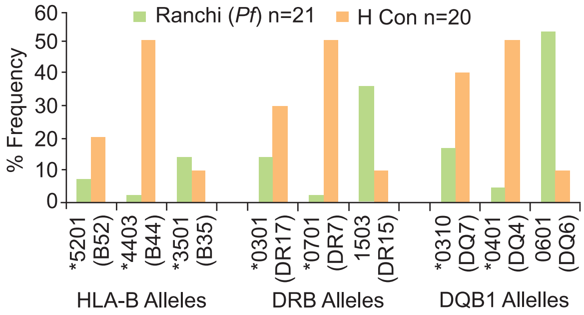


Fig. 8: Histogram showing percentage of frequency of different alleles of both class I (HLA-B) and class II (HLA DR & DQ) in *Pf* infected patients and controls of Ranchi.

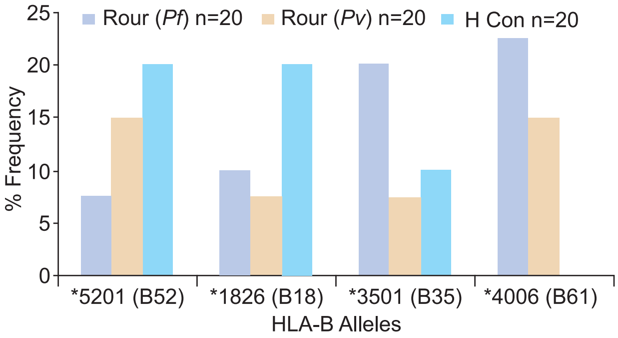


Fig. 9: Histogram showing percentage of frequency of different alleles of class I (HLA B) in *Pf* and *Pv* infected patients and controls of Rourkela.

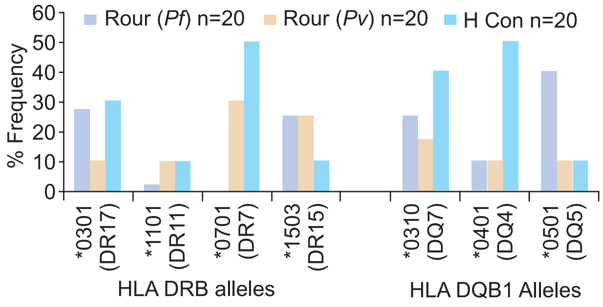


Fig. 10: Histogram showing percentage of frequency of different alleles of class II (HLA DR & DQ) in *Pf* and *Pv* infected patients and controls of Rourkela.

Observations

Class-I	Delhi		Ranchi	Rourkela	
HLA B alleles	*Pf*	*Pv*	*Pf*	*Pf*	*Pv*
Protective	NF	B58	B44	B52	B52
Susceptible	B40	B13	B35	B61	B61
Class-II					
HLA DRB alleles					
Protective	DR11(5)	DR17(3)	DR7	DR7	DR17(3)
Susceptible	DR17(3)	DR4	DR15(2)	DR15(2)	DR15(2)
HLA DQB1 alleles					
Protective	DQ7	DQ4	DQ4	DQ4	DQ4
Susceptible	DQ2	DQ6	DQ6	DQ5	NF

At **HLA B** locus, protective alleles B58 for Delhi (*Pv*), B44 for Ranchi (*Pf*) and B52 for Rourkela (*Pv* & *Pf*) and diagnostic alleles as B40 (61) and B13 for Delhi (*Pf* & *Pv*), B35 for Ranchi (*Pf*) and B40(61) for Rourkela (*Pf* & *Pv)* were identified. **At HLA DRB** locus, DR5 for Delhi (*Pf*) and DR17 for Delhi and Rourkela (*Pv*), DR7 for Ranchi & Rourkela (*Pf*) as protective alleles and DR17, DR4 for Delhi (*Pf* & *Pv)* and DR15 for Ranchi (*Pf*) and Rourkela (*Pf* & *Pv)* as diagnostic alleles were observed. At **DQB1** locus, DQ7 for Delhi (*Pf*) and DQ4 alleles for Ranchi (*Pf*) and Rourkela (*Pf* & *Pv)* were associated with protection from malaria whereas DQ2 & DQ6 for Delhi (*Pf* & *Pv*), DQ6 & DQ5 for Ranchi and Rourkela (*Pf*) were diagnosed as susceptible alleles. Some common HLA alleles in samples infected either with *Pf* or *Pv* were found in our study, e.g. B40(61) common to both Delhi & Rourkela (*Pf*); DR7 to Ranchi and Rourkela (*Pf*); DR17(3) found in both Rourkela and Delhi (*Pv*); DR15(2) common to Ranchi & Rourkela (*Pf*); DQ4 found in Ranchi & Rourkela (*Pf*) and also Rourkela and Delhi (*Pv*) associated either with protection or susceptibility suggesting existence of close relationship among them. The overall data indicated that the relative importance of different HLA alleles may vary in different populations studied from *Pf* prevalent endemic regions (Rourkela and Ranchi) and *Pv* prevalent region (Delhi). HLA diversity in malaria pathogenesis and protection will provide comprehensive and base line data about the genetic and immunological status of the population studied from endemic regions, which subsequently help in new vaccine designing and vaccine trial site development.

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

The role of TLRs 2, 4 and 9 in combating the malaria parasite, *Plasmodium* has been elucidated recently. Moreover, for the development of new treatment strategies TLR polymorphism studies can be crucial in generating data for understanding the genetic make up of the exposed population.

In addition to the previous TLR polymorphic data collected from Car Nicobar, 22 samples from Ranchi and 26 samples from Rourkela were also analyzed for TLR 2 at residue positions Arg677Trp and Arg753Gln, TLR4 at residue positions Asp299Gly and Thr399Ile and TLR9 at nucleotide positions –1486 (T>C)and –1237 (T>C). Studies revealed the existence of only wild genotypes in TLR2 at both residue positions Arg677Trp (G>A) and Arg753Gln (G>A) in samples from all the three regions Car Nicobar, Rourkela and Ranchi (Fig. 11).

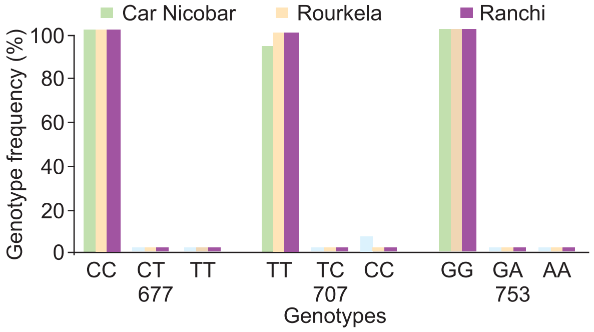


Fig. 11: A graphical representation of the genotype frequency of TLR 2 at residue positions Arg677Trp (C>T), Phe707Phe (T>C) and Arg753Gln (G>A) in malaria patients from Car Nicobar, Rourkela and Ranchi.

In TLR 4, position Asp299Gly (A>G) was found to be polymorphic in samples from Rourkela and Ranchi but only wild type genotype was observed in samples from Car Nicobar, whereas position Thr399Ile (C>T) was found to be polymorphic in samples from all the three regions, Car Nicobar, Rourkela and Ranchi (Fig. 12).

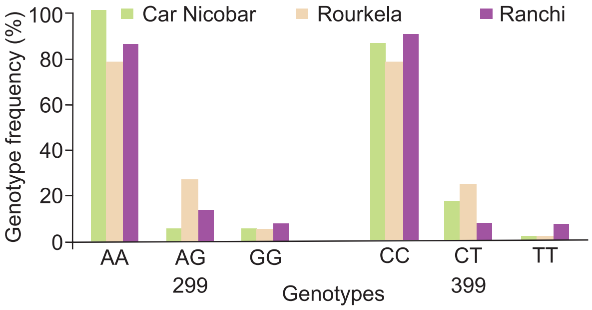


Fig. 12: A graphical representation of the genotype frequency of TLR 4 at residue positions Asp299Gly (A>G) and Thr399Ile (C>T) in malaria patients from Car Nicobar, Rourkela and Ranchi.

In TLR 9, at nucleotide positions –1237 (T>C) and –1486 (T>C) though a high frequency of the wild type genotype was observed in samples from all the three regions but the mutant genotype was observed only in samples from Car Nicobar and Rourkela at nucleotide position –1237 and –1486 respectively (Fig. 13).

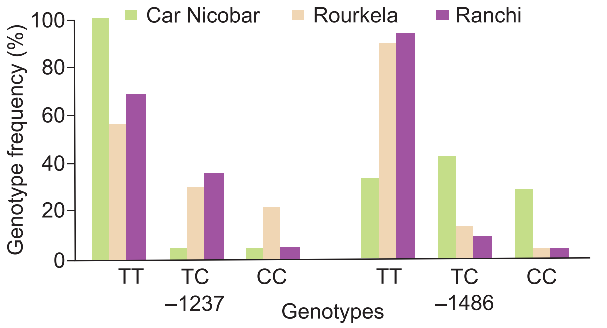


Fig. 13: A graphical representation of the genotype frequency of TLR 9 at nucleotide positions –1237 (T>C), and –1486 (T>C) in malaria patients from Car Nicobar, Rourkela and Ranchi.

A complete picture of the genotype frequency of TLR 2, 4 and 9 in the Indian population can only be concluded on the completion of analysis of data collected from other endemic regions of the country and the significance of the presence of the mutant allele in a population can be correlated to malaria once the analysis of blood samples from healthy subjects is done.

2.1.6 Genetic polymorphism in diagnostic antigen of *Plasmodium falciparum*, histidine rich protein 2 & 3 (PfHRP-2 & PfHRP-3) among Indian isolates and their possible impact on Rapid Diagnostic Test

Most of the rapid diagnostic tests are based on the detection of *P. falciparum* histidine-rich protein (PfHRP) 2, but reports from field tests have questioned their sensitivity and reliability. Many factors may affect the performance of malaria RDTs but one of the important factors is genetic variability of the antigens detected by the antibody component of the RDT. We assessed the genetic variability of *PfHRP-2* and *PfHRP-3* genes of *P. falciparum* isolates from different malaria endemic regions of the country and their possible effect on performance of RDTs.

A total of 130 *P. falciparum* isolates were collected during September 2009 to December 2010 from different epidemiological strata of India. Genomic DNA was extracted and analyzed for genetic variations by polymerase chain reaction (PCR). Molecular weight of PCR products was analyzed by gel documentation system.

Extensive variations were observed in the minimum detection limit by RDT as well as the molecular weight of *PfHRP-2* and *PfHRP-3* genes both within and between regions. PCR amplification for *PfHRP-2* was seen in 98/130 (75.38%) samples while for *PfHRP-3* in 53/130 (40.76%) samples. Both genes showed variable forms among these clinical isolates as well as in cultured lines MRC2 and RKL9 from Malaria Parasite Bank as indicated by the presence of different PCR products (Figs. 14 & 15).

Ten different PCR products, ranging from 669 to 1000 bp were observed for *PfHRP-2* and nine different PCR products, ranging from 477 to 832 bp for *PfHRP-3*. These different PCR products of

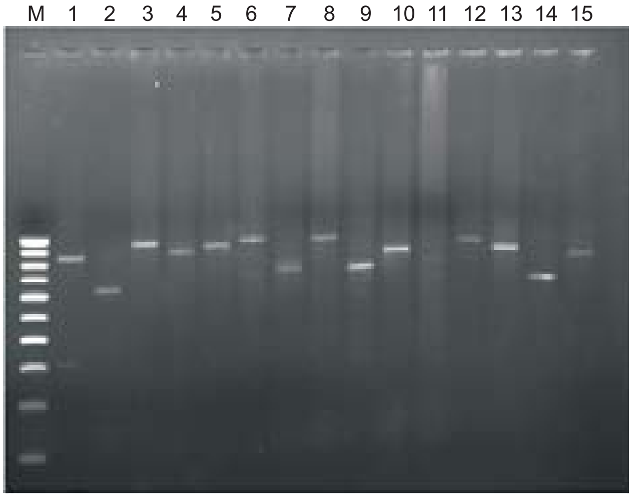


Fig. 14: Size variation in the PCR products of *PfHRP-2* gene among the Indian field isolates of *P. falciparum*.

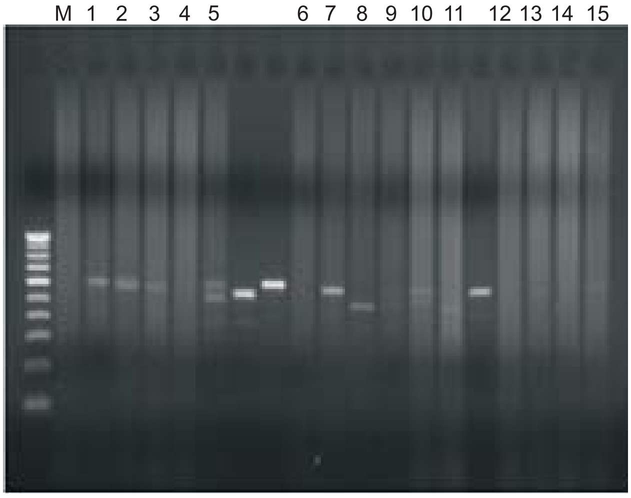


Fig. 15: Size variation in the PCR products of *PfHRP-3* gene among the Indian field isolates of *P. falciparum*.

Table 3. Allelic types of PfHRP-2 and PfHRP-3 on the basis of molecular weight of PCR products

Allelic types	*PfHRP-2* gene (n = 108)		Allelic types	*PfHRP-3* gene (n = 63)	
	Molecular weight (bp)	Frequency (%)		Molecular weight (bp)	Frequency (%)
Type 1	1000	6 (5.55)	Type 1	832.3	13 (20.63)
Type 2	984.4	15 (13.88)	Type 2	770.8	7 (11.11)
Type 3	942.8	12 (11.11)	Type 3	708.3	6 (9.52)
Type 4	928.5	11 (10.18)	Type 4	687.5	7 (11.11)
Type 5	885.7	7 (6.48)	Type 5	645.8	4 (6.34)
Type 6	857.1	5 (4.62)	Type 6	616.2	3 (4.76)
Type 7	839.2	8 (7.40)	Type 7	583.3	11 (17.46)
Type 8	785.7	20 (18.51)	Type 8	533.3	9 (14.28)
Type 9	735.5	15 (13.88)	Type 9	477.1	3 (4.76)
Type 10	669.5	9 (8.33)			

These findings may provide an alternative explanation for the variable sensitivity in the field tests of malaria RDTs that is not due to quality of RDTs.

both the genes were assigned numbers that represented the allele types.

It was found that only 68.3% of *P. falciparum* isolates in different malaria endemic regions were likely to be detected at densities ≤ 200 parasites/μl (Table 3). Although two isolates of Chhattisgarh, i.e. CB18 & CB21 were tested and detected by two RDTs, and have been excluded from the analysis because those were slide positive but RDT negative so there may be the case of *PfHRP-2* gene deletion, which needs further investigations. Further analysis for the sequence variations in these genes by sequencing is in progress.

These findings may provide an alternative explanation for the variable sensitivity in the field tests of malaria RDTs that is not due to quality of RDTs.

Note: The detection limit for sensitive isolates was ≤ 200 parasitized erythrocytes/μl and that for non-sensitive isolates was ≥ 200 parasitized erythrocytes/μl. Two samples CB-18 and CB-21 were not amplified.

Fig. 16: *Plasmodium* infection during malaria causes splenomegaly due to infiltration of inflammatory cells. Spleen from infected mice become 4–5 fold large in size compared to uninfected mice.

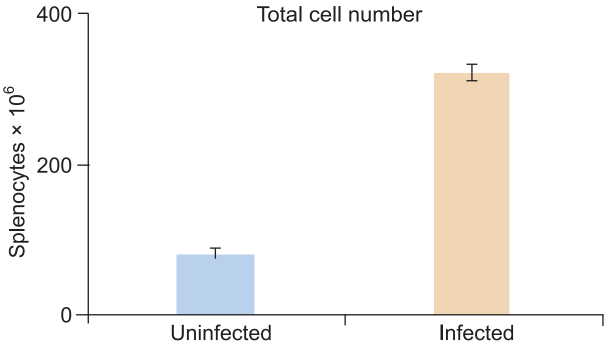


Fig. 17: Total numbers of splenocytes in infected compared to wild type controls.

2.1.7 Role of mesenchymal stem cells during malaria infection

Plasmodium infection during malaria causes splenomegaly due to infiltration of inflammatory cells (Fig. 16). Total numbers of splenocytes become 3–4 fold higher as compared to wild type controls (Fig. 17). However, cellular composition of these cells after malaria infection has not been fully characterized. Our preliminary data suggest that though there is increase in T helper cells like $CD4^+$, $CD8^+$ T cells, antigen presenting cells like $CD11b^+$, $Cd11c^+$, $CD19^+$ as well as Treg cells but there was dramatic increase in Sca-1^+ (Stem cell antigen) cells in the spleen (Fig. 18). It has been reported that some activated T and B cells also express Sac-1 on their surface. Therefore, further phenotypic characterization of these cells from infected splenocytes reveal that these Sca-1 positive

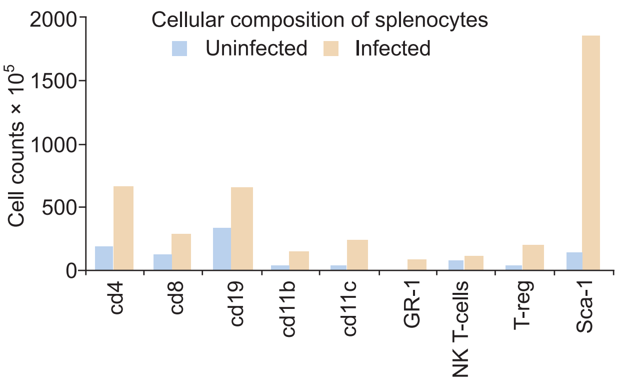


Fig. 18: Different types of cell populations (higher in infected than uninfected mice).

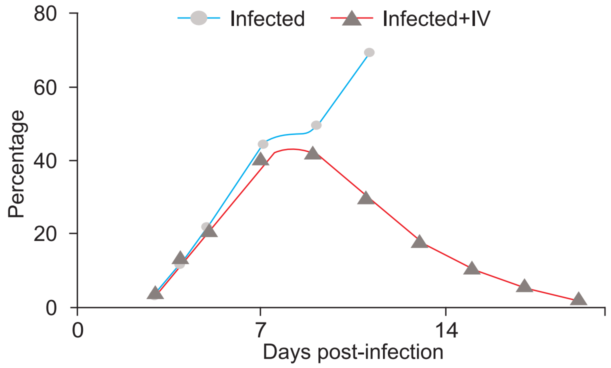


Fig. 20: Percentage of parasite becomes down after adoptive transfer of Sca-1 positive cells in infected Balb/c mice.

cells are positive for CD44$^+$, CD29$^+$ surface markers but were negative for other surface markers like Flk-1, CD34, CD11b and CD11c cells suggesting non-conventional stem cells are infiltrated to the site during malaria infection (Fig. 19).

Further studies were conducted to examine the role of these Sca-1 positive cells in immunoregulation during malaria infection. These Sca-1$^+$ cells were isolated by negative depletion of lineage differentiated cells using magnetic beads and then adoptively transferred to syngeneic mice along with parasite infected RBCs (Fig. 20). Some recent reports suggest that Sca-1 positive stem cells are immunosuppressive in nature. But surprisingly, adoptive transfer of these cells was able to immunoregulate the immune response and help in the survival of recipient mice (Fig. 21). There was >50% increase in the survival rate of mice. The load of parasites was lower compared to mice without receiving the Sca-1 positive cells. The findings of this study suggest that these Sca-1 positive cells may have effect in the modulation of cytokine profile required to exert protection against parasite infection.

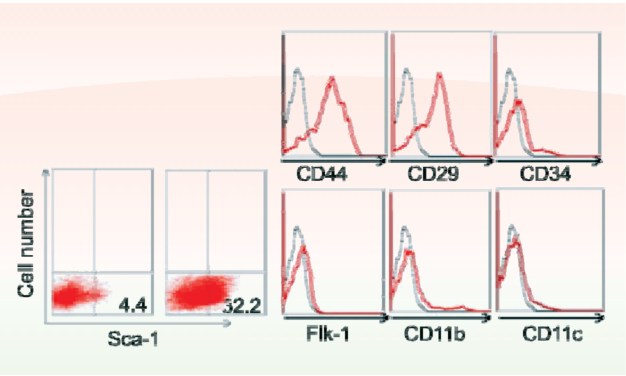


Fig. 19: After FACS staining number of Sca-1 positive cells higher in infected compared to uninfected mice and phenotypic characterization of Sca-1 positive cells in infected mice.

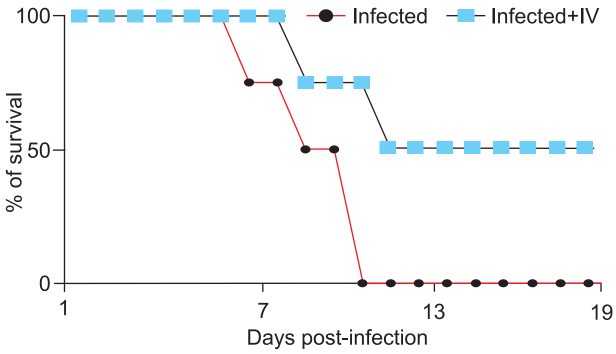


Fig. 21: Survival rate after adoptive transfer of Sca-1 positive cells in infected mice, adoptive transfer of these cells was able to immuno-regulate the immune response and helping in the survival of recipient mice.

2.1.8 Sequence analysis of *vir* genes in Indian *Plasmodium vivax*

In the *Plasmodium* species most chromosomes contain multigene families coding for variant surface antigens (VSAs) on their telomeric and subtelomeric regions. Variant surface antigens present on the surface of parasitized erythrocytes facilitate many *Plasmodium* species to escape the host immune system during infection. *Plasmodium vivax* genome also contains a multigene superfamily *vir* (variant interspersed repeats), present in the subtelomeric region (Fig. 22).

In the present study, we tried to investigate the existing diversity of *vir* genes in Indian isolates of *P. vivax*. For this study, the blood samples were collected from malaria patients from four different regions of India, i.e. Delhi, Mangalore, Goa and Rourkela. Preliminary diagnosis for malaria was carried out by Rapid Diagnostic test (RDT) and

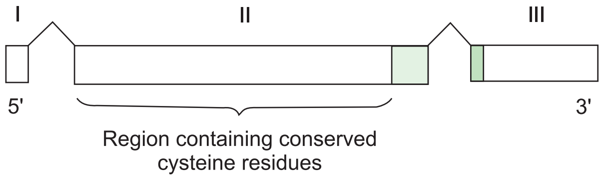


Fig. 22: Illustration showing three exon organization of the *P. vivax vir* genes. The second longer exon contains the transmembrane domain. The figure modified from del Portillo *et al* (2001).

microscopy. Genomic DNA from the positive samples was extracted from the filter paper blood spots which were analyzed for mixed infection by nested PCR method. Fifteen samples from Mangalore showed the presence of mixed infection of *P. falciparum* and *P. vivax* whereas others were *P. vivax* infections. Our work was carried out on four *vir* genes belonging to subfamilies I, C, E and B. Due to the size of the genes spanning from 974 to 2548 bp, two sets of primers were designed for each gene by Primer 3 software. Only samples with single *P. vivax* infection were amplified with *vir* specific primers. The PCR products with positive amplification for *vir* genes were purified and sequenced. The sequences were edited and aligned using Clustal W. Single nucleotide polymorphisms (SNPs) were identified and validated using MEGA 4.0 software. After aligning the sequences of the *vir* genes in different *P. vivax* isolates, various synonymous and non-synonymous SNPs were observed when compared to Sal-I reference strain. The analysis of the four *vir* genes showed high variability existing within and between the isolates and that they are randomly dispersed with no particular distribution pattern among the regions from where the samples were collected.

2.1.9 Identification of virulence gene family in primate malaria parasites

Pathogenesis in malaria parasites is regulated via virulence gene family that has wide range of orthologs in rodent, primate, and human malaria parasites. The virulence gene family encoded proteins are involved in antigenic variation, which help the parasite to escape host immune response. Majority of the primate malaria parasites are closely related to *Plasmodium vivax* and their infection to human in *in vitro* condition suggests that in near future several of the simian parasites could become human malaria parasites as like *P. knowlesi*. Therefore, identification of virulence gene family among primate malaria parasites would provide insights of the evolution of virulence and pathogenesis among primate malaria parasites. This study identifies *vir* gene family orthologs in *P. simium, P. simiovale, P. cynomolgi* and *P. fieldi* species using previously reported degenerate PCR primers of *P. vivax*. Virulence gene sub family (*vir-D*) was successfully amplified from primate malaria parasites (Fig. 23) followed by cloning and sequencing of 30 clones per amplification. Each sequence was subjected to BLAST at NCBI and PlasmoDB for confirmation that these sequences belong to parasite genome and have high identity with *vir* gene family. Sequence analysis revealed substantial number of *vir-D* subfamily orthologs in all four *Plasmodium* species (Fig. 24) and showed 68–84% identity. Further, a Neighbour-Joining phylogenetic tree was reconstructed to infer genetic identity with known subfamily (*vir-D*) that suggests virulence gene family is shared among *Plasmodium* species infecting to humans and primates. Further, comparison of virulence gene family sequences of primate malaria parasites with the sequenced genomes would provide rationale to understand the evolution of virulence and role of virulence gene family in shaping disease pathogenesis.

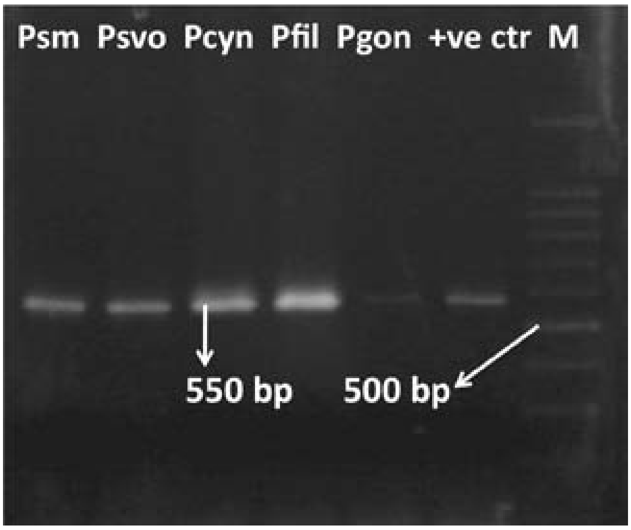


Fig. 23: Degenerate PCR amplification of *vir* ortholog gene family from primate malaria parasites. Psm: *Plasmodium simium*; Psvo: *P. simiovale*; Pcyn: *P. cynomolgi*; Pfil: *P. fieldi*; Pgon: *P. gonderi*; M: 100 bp DNA ladder/marker.

2.1.10 Molecular characterization of aspartic protease gene of *Plasmodium vivax*

Plasmodium vivax is the most predominant form of human malaria in south-east Asia and India. Plasmepsin V is an integral *Plasmodium* ER

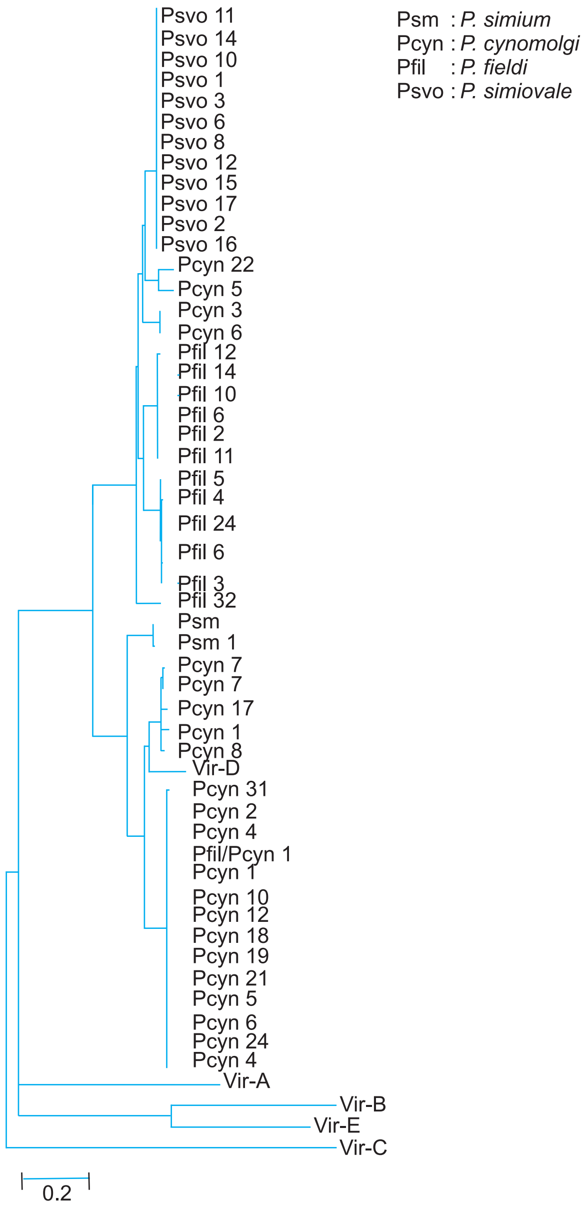


Fig. 24: N-J Phylogenetic cluster of *Vir-D* sub family orthologs in primate parasites.

membrane protease involved in the recognition and processing of the conserved (PEXEL) motif for export of pathogenicity-related proteins/antigens for parasite viability. To investigate whether *P. vivax* plasmepsin V (*PvPM-V*) gene had also diverged in binding capacity with PEXEL motifs and to test if binding could be predicted by structural modeling, we generated *in silico* bioinformatic protocols for cleavage site antigenic variation processing to facilitate antimalarial drug development. We have attempted to understand the molecular nature and viability of *P. vivax* plasmepsin V in terms of sequence analysis, *in silico* bioinformatic protocols and structural modeling predictions based on docking studies with PEXEL motif. We demonstrated that *PvPM-V(Ind)* is highly conserved gene among all the Indian isolates although it has an imperfect duplication insertion type of mutation in comparison to *P. vivax* Sal-1 isolate (Fig. 25).

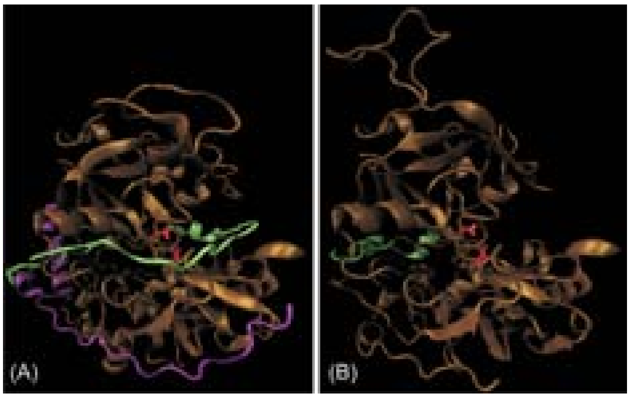


Fig. 25: Structural representations of model of Plasmepsin-V (A) and (B) displaying structural changes post cleavage of C-terminal transmembrane domain (Purple). The prodomain peptide (lime) frees active site (Red aspartyl amino acid residue side chains).

Our extensive *in silico* analysis on variation in antigenic binding clearly shows significant effect of these mutations on substrate binding with data mined PEXEL sequences and on binding of known inhibitor Lopinavir. Pepstatin A failed to exhibit any binding *in silico* with both PvPM & PvPM-V(Ind). The predicted variation in the docking score and interacting amino acids of PvPMV Sal-1 (wild type) and PvPM-V-Ind (mutant) proteins with both PEXEL

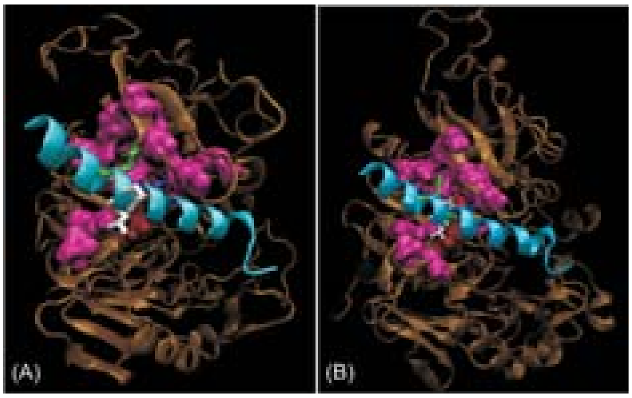


Fig. 26: Structural representations of model: (A) PvPMV Sal-1 (wild type); and (B): PvPMV-Ind (mutant). Displaying docked PEXEL motif (sky Blue helix) with the active site showing different pockets of interaction with different PEXEL amino acid side chains. Deepest pockets for first (Green) and last (Blue) AA. While low number of interacting AA (white) suggest more ambiguity allowed at the PEXEL member. Active aspartyl residues (Red) clearly interact with the backbone of docked peptide at the point of cleavage.

and Lopinavir supports that these mutations may result in the modification of the virulence of PM-V (Fig. 26). Our study predicts a putative mechanism to demonstrate antigenic variations of more virulent *P. vivax* for correlating their effect in relation to serotypes in cultivable *Plasmodium* species for immune evasion. Our functional prediction data to identify antigenic variations processing activity and also understanding of this consensus architecture of PEXEL side chains can be used to design novel inhibitor/pharmacophores specific to *P. vivax*.

2.2 Parasite evolutionary genomics

2.2.1 Evolutionary history of Indian *Plasmodium vivax*

Recent developments and utilization of putatively neutral DNA fragments have revolutionized the approach of deducing evolutionary history of species populations in several model and non-model organisms. Human pathogens, due to simple genome organization and short generation time, can rapidly adapt and spread through human movements, thus, understanding the evolutionary history should be the first step to devise disease control measures. The human malaria parasite, *P. vivax* is globally widespread causing high malaria morbidity. Evolutionary history of *P. vivax* is still unclear due to inconclusive inferences from population genetic analyses using different types of markers with different evolutionary potential. We herewith have utilized the recently developed multilocus putatively neutral DNA fragments placed in ~133 Kb chromosomal region in the 13th

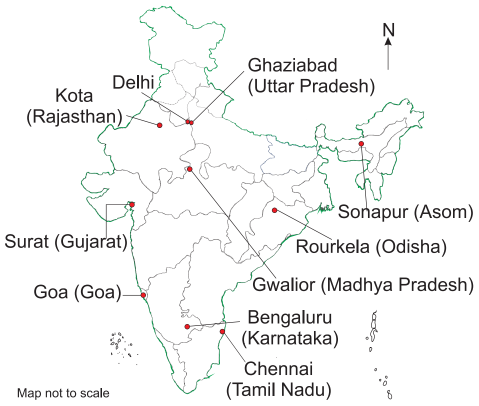


Fig. 27: Map of India indicating *P. vivax* sample collection sites.

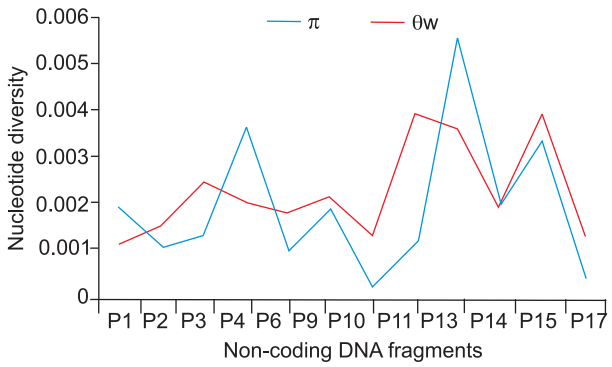


Fig. 28: Pattern of variation in nucleotide diversity (pooled samples) across the ~133 kb genetic region of Indian *P. vivax* in introns (Red) and intergenic regions (Blue).

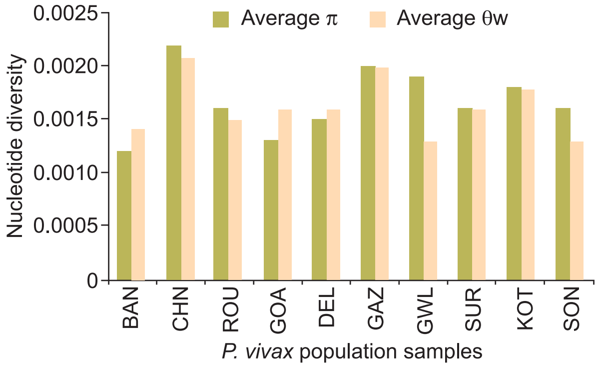


Fig. 29: Pattern of variation in nucleotide diversity (averaged over all the 12 loci) in 10 population samples of Indian *P. vivax*. BAN–Bengaluru; CHN–Chennai; ROU–Rourkela; GOA–Goa; DEL–New Delhi; GAZ–Ghaziabad; GWL–Gwalior; SUR–Surat; KOT–Kota; SON–Sonapur.

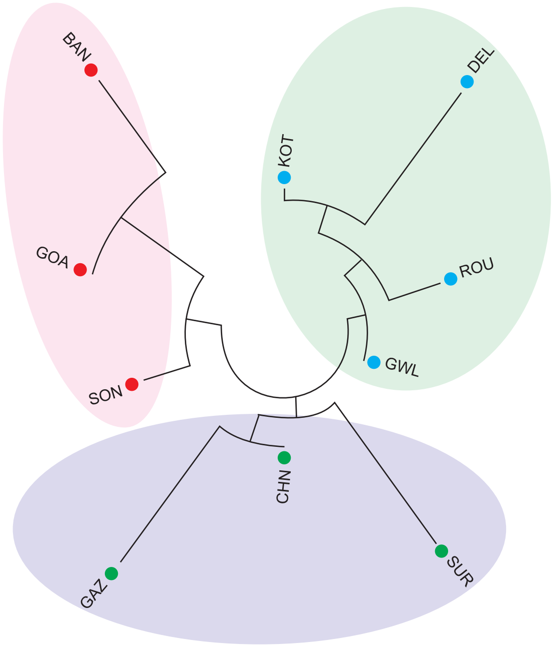


Fig. 30: Neighbour-Joining population phylogenetic tree based on pair-wise F_{ST} values between population samples of Indian *P. vivax*. BAN–Bengaluru; CHN–Chennai; ROU–Rourkela; GOA–Goa; DEL–New Delhi; GAZ–Ghaziabad; GWL–Gwalior; SUR–Surat; KOT–Kota; SON–Sonapur.

chromosome in *P. vivax* to score SNPs in 126 *P. vivax* isolates collected from 10 different places in India (Fig. 27). Indian *P. vivax* bears high nucleotide diversity in each DNA fragments of all the 10 population samples (Figs. 28 and 29) but moderate amount of genetic differentiation between different geographical regions. Such differentiations, however, do not correlate with either the geographic location of population samples or endemicity of *P. vivax* malaria. This fact was reflected from analyses of population structure by different methods using different algorithms (NJ population tree, STRUCTURE and PCoA; Figs. 30, 31 and 32). Furthermore, analyses of past demographic events indicate reduction of population size in individual population samples, but when isolates from all the 10 samples were analyzed as a single population, demographic

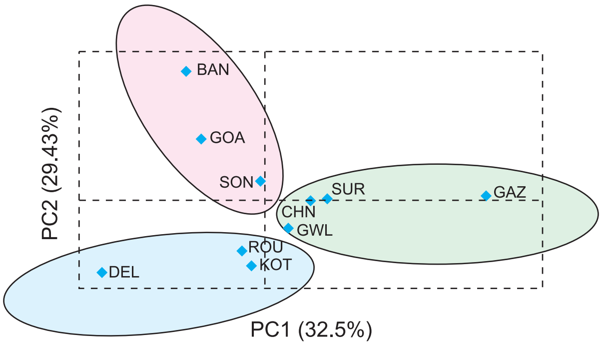
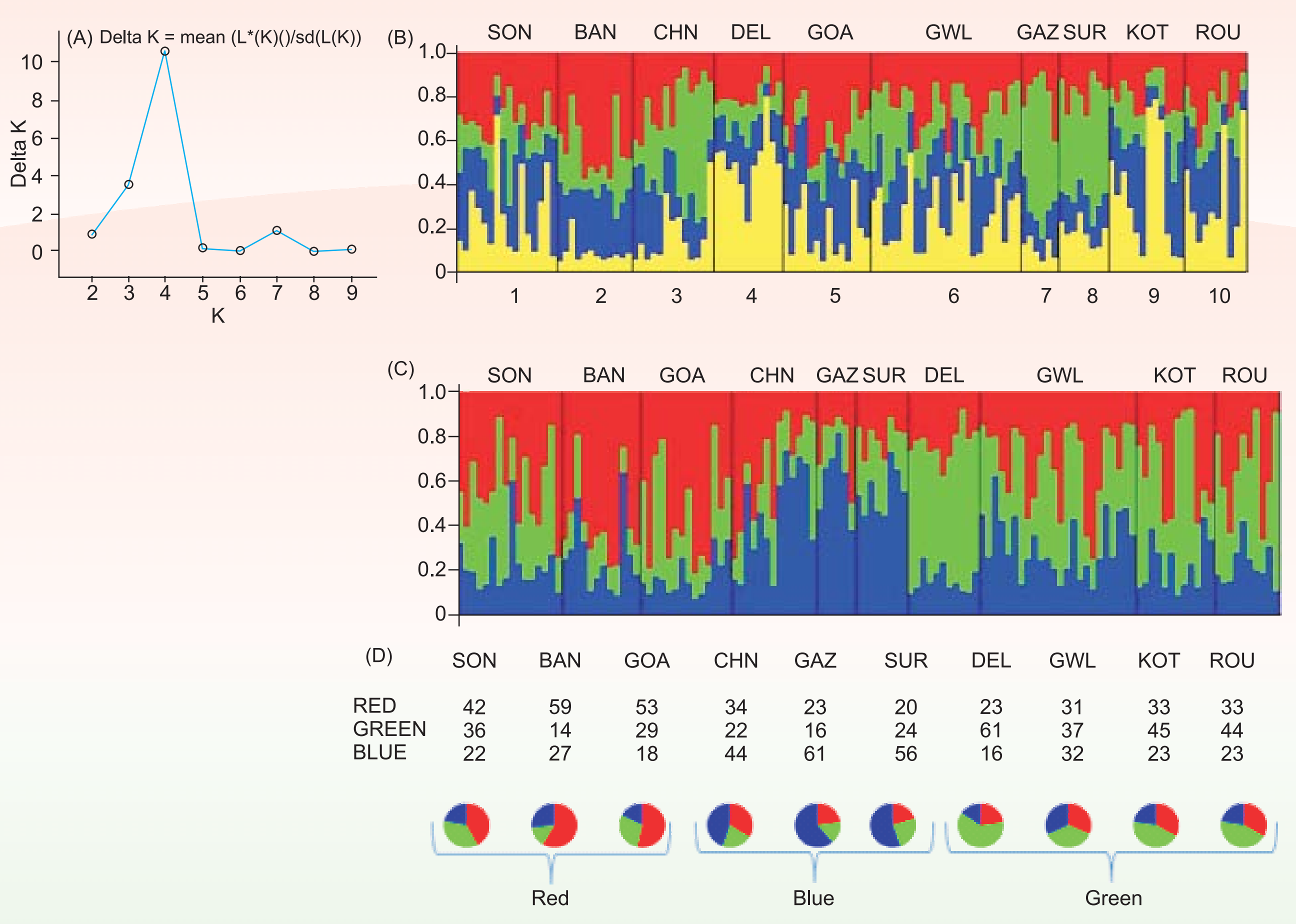


Fig. 32: Principal Coordinate Analysis (PCoA) using genetic distance between the population samples. X and Y-axes represent 32.5 and 29.43% genetic variability between population samples, respectively. BAN–Bengaluru; CHN–Chennai; ROU–Rourkela; GOA–Goa; DEL–New Delhi; GAZ–Ghaziabad; GWL–Gwalior; SUR–Surat; KOT–Kota; SON–Sonapur.



	SON	BAN	GOA	CHN	GAZ	SUR	DEL	GWL	KOT	ROU
RED	42	59	53	34	23	20	23	31	33	33
GREEN	36	14	29	22	16	24	61	37	45	44
BLUE	22	27	18	44	61	56	16	32	23	23

Fig. 31: Inference of population structure in Indian *P. vivax* following STRUCTURE analysis: (A) The graph of K and Ä K value showing highest peak for K at 4 and second highest at K=3; (B) Proportion of genetic ancestry (Y-axis) in each population sample (partitioned by black solid lines) at K=4; (C) Proportion of genetic ancestry (Y-axis) in each population sample (partitioned by black solid lines) at K=3; (D) Contribution of each genetic ancestry (in %) to each population sample. Note that population samples can be grouped into three groups based on the highest contribution of each ancestry in a population sample (see the pie chart). BAN–Bengaluru; CHN–Chennai; ROU–Rourkela; GOA–Goa; DEL–New Delhi; GAZ–Ghaziabad; GWL–Gwalior; SUR–Surat; KOT–Kota; SON–Sonapur.

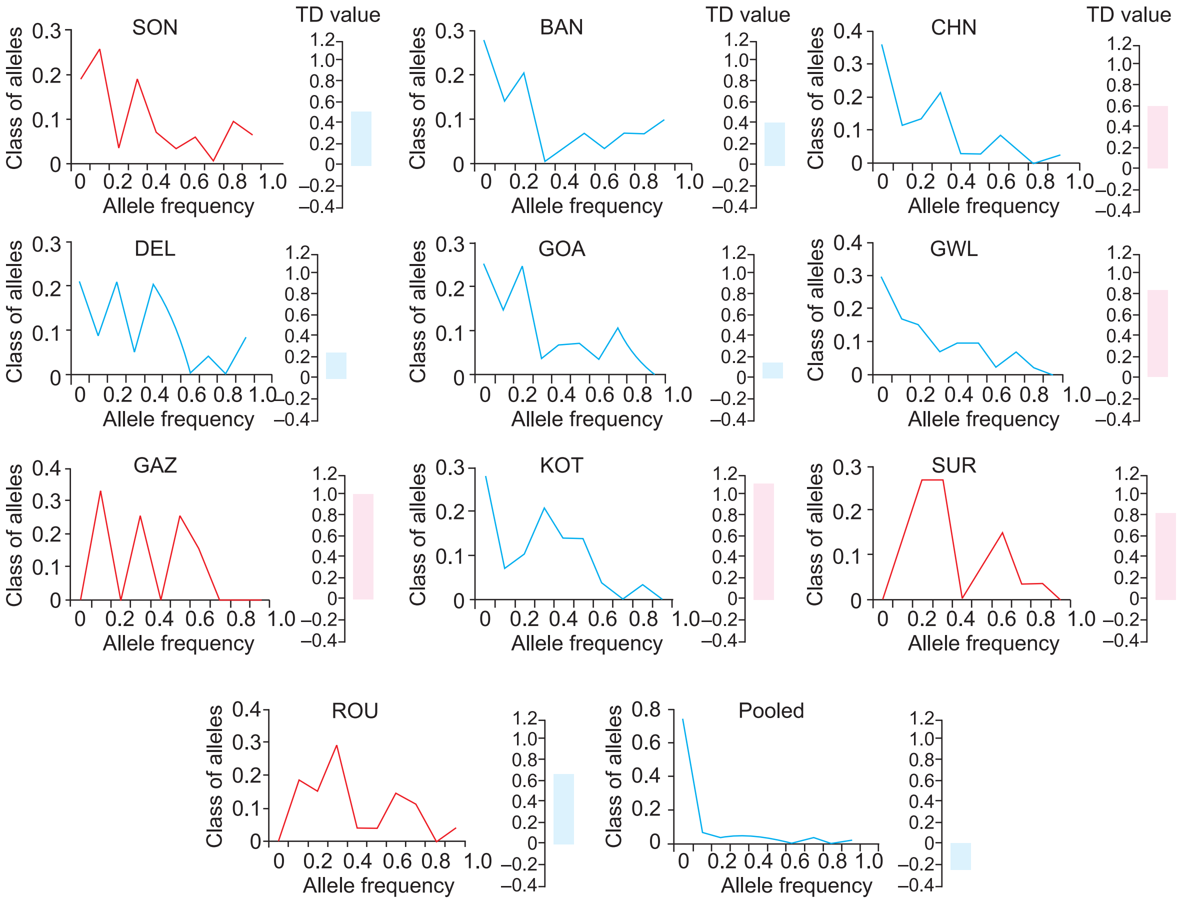


Fig. 33: Statistical tests of demographic model-fitting in 10 population samples and the pooled samples of Indian *P. vivax*. The line graphs [Red: mode shift (evidence for population bottleneck); Blue: L-shaped (evidence for demographic equilibrium)] show allele frequency distribution curves and the bars in right show Tajima's D-values (Blue bars: insignificantly deviated from demographic equilibrium model; Red bars: statistically significant deviations from demographic equilibrium model). BAN–Bengaluru; CHN–Chennai; ROU–Rourkela; GOA–Goa; DEL–New Delhi; GAZ–Ghaziabad; GWL–Gwalior; SUR–Surat; KOT–Kota; SON–Sonapur.

equilibrium model was observed (Fig. 33). All these observations clearly indicate that Indian *P. vivax* might be a part of the ancestral distribution range of this species.

2.2.2 Evolutionary genomics of malaria susceptibility in Indians

It has now been well-documented that the type (coding, non-coding) and location (nuclear, mitochondrial etc.) of genetic markers heavily influence evolutionary inferences; realistic assumptions can be drawn if multiple putatively neutral DNA fragments spread across the genome presenting single nucleotide polymorphisms (SNPs) are used. Human evolutionary histories have majorly been inferred from genes from mitochondria and Y-chromosome. Although SNPs have been utilized, genetic markers designated as "putatively neutral markers" have not yet been used for human evolutionary inferences. In order to

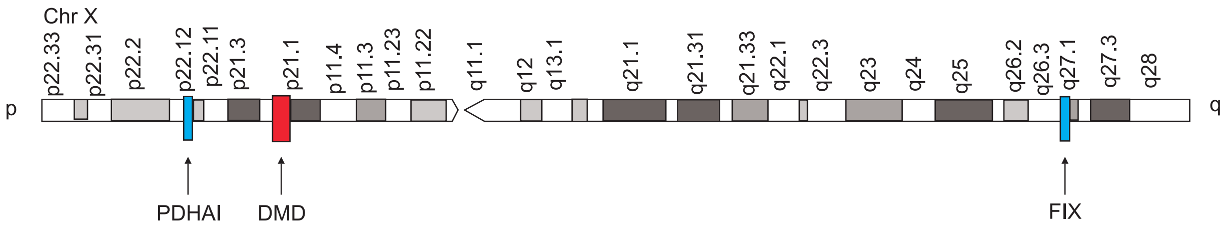


Fig. 34: The human X-chromosome and location of three genes.

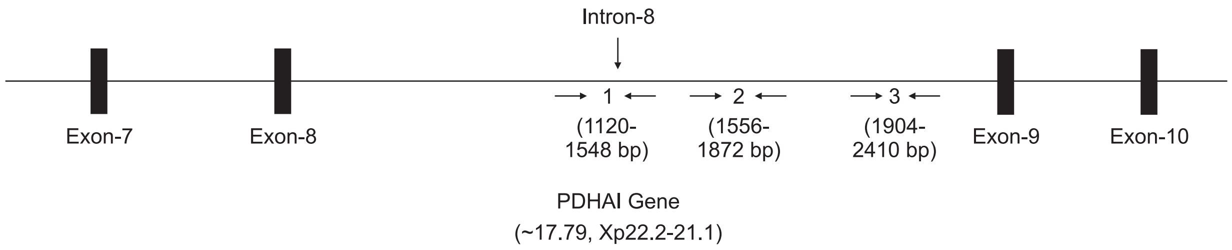


Fig. 35: Location of three fragments in PDHA1 (pyruvate dehydrogenase) gene.

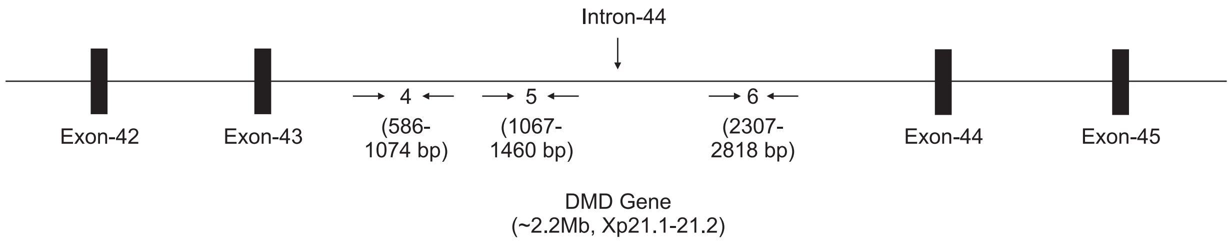


Fig. 36: Location of three fragments in DMD (Duchenne muscular dystrophy) gene.

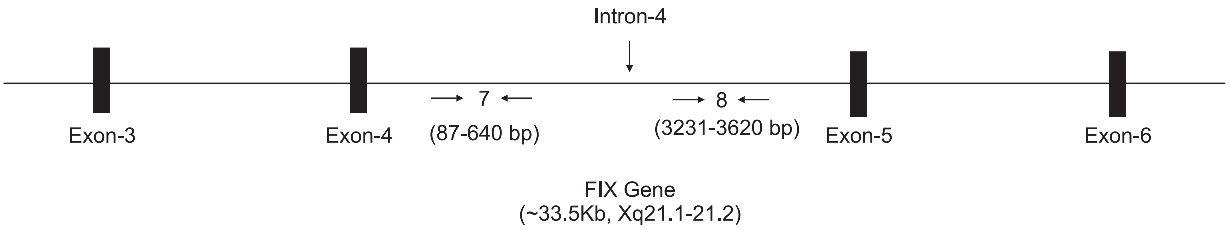


Fig. 37: Location of two fragments in Factor IX (FIX) gene.

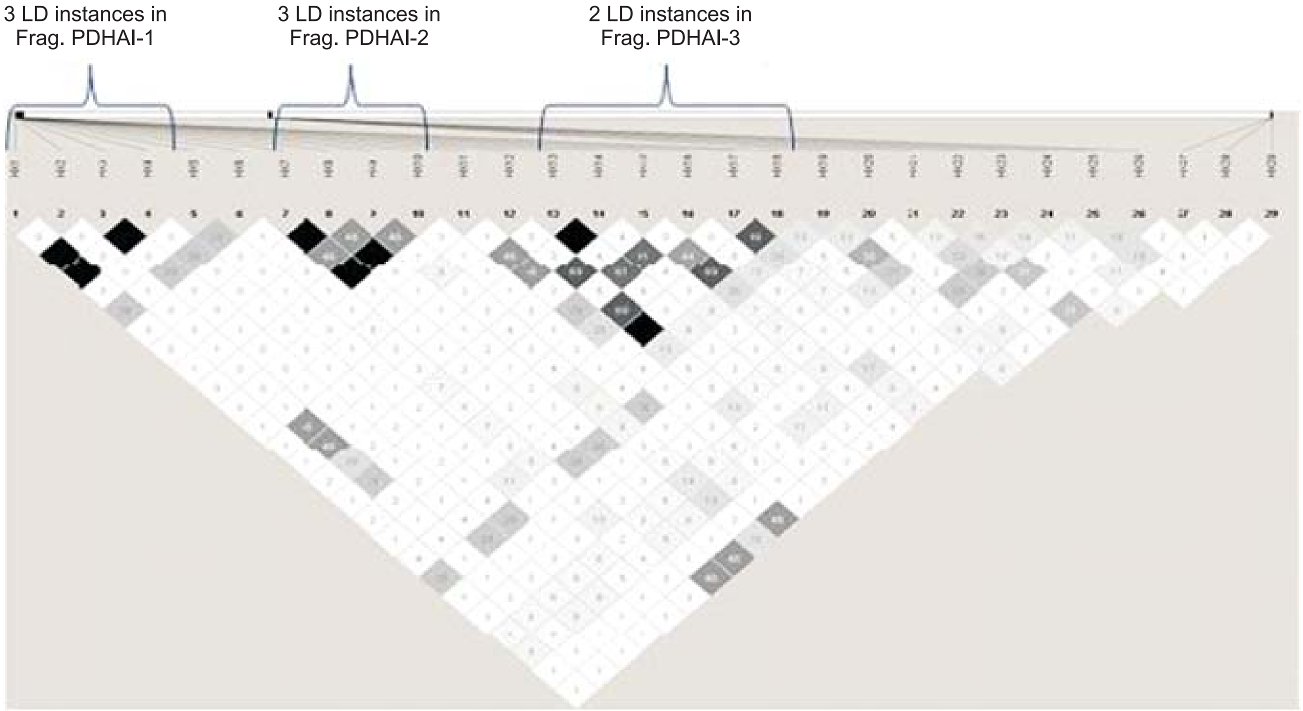


Fig. 38: LD (r^2) plot between 29 SNPs in western Indian population sample. Black colour squares indicate significant LD (r^2=1) and white colour squares indicate non-significant LD (r^2=0).

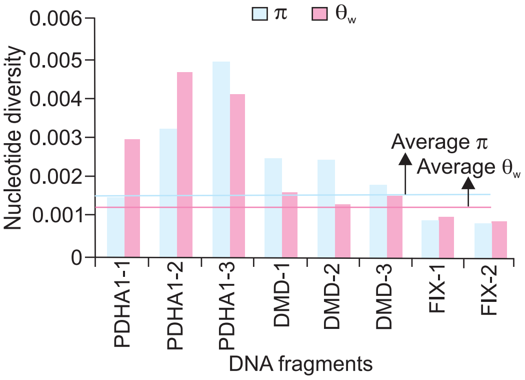


Fig. 39: Patterns of variation in nucleotide diversity across the eight DNA fragments in western Indian population sample.

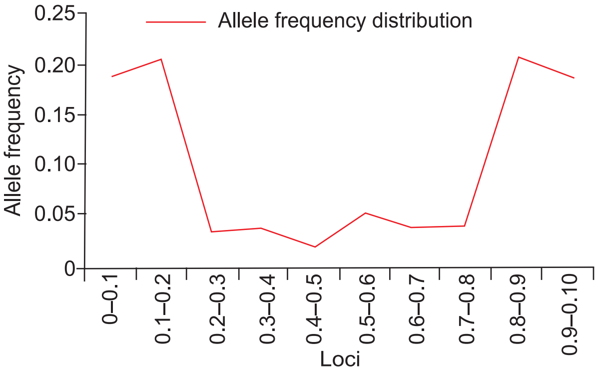


Fig. 40: Results from the "BOTTLENECK" analyses, showing the mode-shift curve, indicating the signature of recent bottleneck in western Indian population sample.

develop such markers on human, we utilized the human genome information and isolated eight DNA fragments located in introns of three genes (Fig. 34); Pyruvate dehydrogenase E1α subunit (Fig. 35); Duchenne muscular dystrophy (Fig. 36); and Factor IX (Fig. 37) of the X-chromosome. Multilocus linkage disequilibrium and single locus linkage disequilibrium analysis further confirms that all the eight fragments evolve independently among each other (Fig. 38). PCR amplification and DNA sequencing in blood samples collected from 16 male individuals from western India confirm polymorphic status of all the fragments for SNPs (Fig. 39). Moreover, several tests of neutrality ascertain that all the eight fragments evolve putatively neutrally (Fig. 40). Utilizing the sequence data we estimated nucleotide diversity and demographic parameters of this Indian population sample. All the eight DNA fragments thus seem to bear the characteristics for being considered as "putatively neutral genetic markers" and could be utilized for inference of human population and demographic histories. Such baseline information could be helpful in disentangling the effects of demography from natural selection in genes of functional importance (disease susceptibility, drug metabolization etc.) in different human populations.

❑

Epidemiology

3

3.1 GIS-based epidemiological studies

3.1.1 Deforestation and its impact on malaria epidemiology in districts of Asom: A remote sensing and GIS-based study

The study is being carried out in Sonitpur and Nagaon districts of Asom. After comparing remote sensing imageries of 1999 and 2008, a high rate of deforestation was observed in Dhekiajuli PHC of Sonitpur district. IRS-P6/LISS IV imageries of Dhekiajuli PHC for 2008 were procured, processed and classified as given in Fig. 1. Field validation trip was undertaken during November–December 2009. Socioeconomic and other attribute data were also generated from this PHC. Land use land cover (LULC) information was recorded and the validation of classes was done for the classified satellite imageries and accordingly necessary corrections were made. As identified earlier, Behali was taken as forested PHC from Sonitpur district while Lanka and Jakhlabandha were taken as deforested and forested PHCs respectively from Nagaon district.

The field validation confirmed that the deforested land in Dhekiajuli PHC was being primarily used for agriculture and human settlement purposes (Fig. 2). Socioeconomic data were also collected from this PHC and 40 proformae were filled up and 68% of the population was migratory Bodo tribe from the nearby districts who have settled in these deforested areas. Literacy rate was found low and occupation was mostly agriculture/labour with income <₹ 2000.00 p.m. Kuchcha houses with thatched and tin sheets were observed.

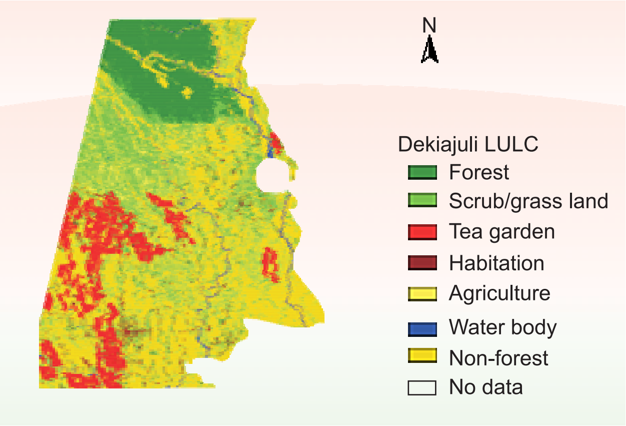


Fig. 1: Classified LULC of Dhekiajuli PHC in Sonitpur district.

A major development seen in the deforested areas of both the districts was the development of sub-stream network from the main streams for irrigation purpose. Also in forested villages of both the districts, some degree of deforestation was observed for agricultural purposes (mainly paddy cultivation). About 2–3 years back, these villages were situated inside the deep forest, now these are 1½ km away from it. Here also, sub-stream network from main streams was developed for irrigation purpose.

Two surveys were undertaken during November–December 2009 (winter) and March–April 2010 (pre-monsoon) to collect entomological and epidemiological data (Figs. 3–5). Entomological data collection from deforested and forested areas included indoor resting mosquito collection, total catch, outdoor collection, whole night mosquito landing collection, and larval collection. Parasitological data collection included active fever surveys in the area and data from the state health department of Asom.

Deforested areas

Comparison of collected entomological data in deforested areas of Sonitpur and Nagaon districts revealed collection of *Anopheles culicifacies, An. philippinensis/An. nivipes, An. annularis* and *An. minimus* species. Streams and pits appeared as the major breeding sites in deforested areas of Sonitpur district. Ponds, river, rice-fields, pits and streams

Fig. 2: Deforested areas, type of houses and labour settlement in Dhekiajuli PHC of Sonitpur district.

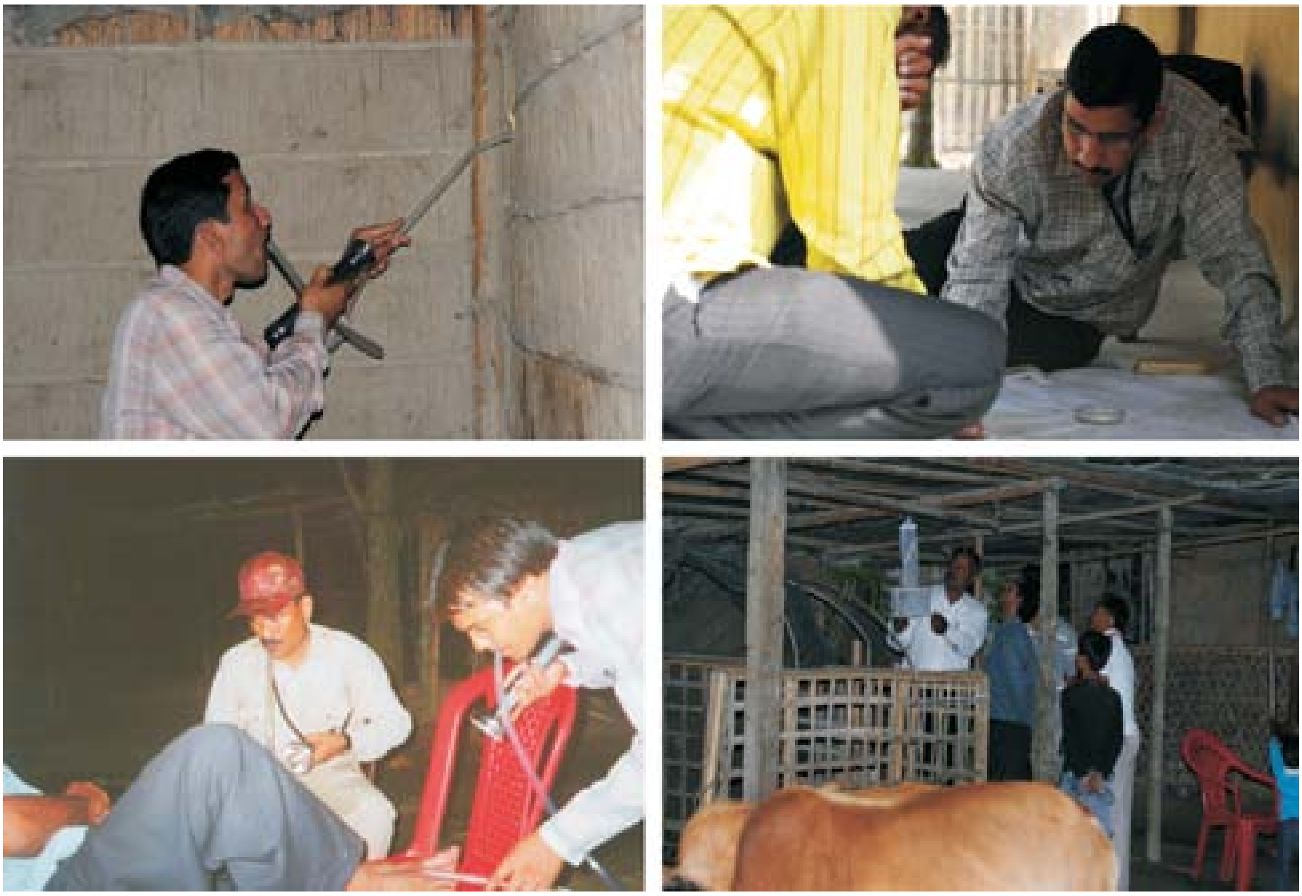


Fig. 3: Mosquito collections from deforested and forested areas of Sonitpur and Nagaon districts.

appeared as the major breeding sites in deforested areas of Nagaon district.

Forested areas

Comparison of collected entomological data in forested areas of Sonitpur and Nagaon districts revealed the collection of *An. culicifacies*, *An. nivipes* and *An. annularis* in addition to *An. dirus* and *An. minimus*. River, ponds, pits and streams appeared as the major breeding sites in forested areas of Sonitpur district. Streams, rice-fields and pits appeared as the major breeding sites in forested areas of Nagaon district.

In the surveyed forested villages of Sonitpur, use of insecticide-treated nets (ITNs) and long-lasting insecticidal nets (LLINs) was recorded and in Nagaon, the use of LLINs was recorded. This probably knocked down the forest species, namely

Fig. 4: Larval breeding sites in deforested and forested areas of Sonitpur and Nagaon districts.

Fig. 5: Active fever survey in deforested and forested areas of Sonitpur and Nagaon districts.

An. minimus and *An. dirus* which are anthropophagic in nature and for this reason only a few *An. minimus* and *An. dirus* were collected from these villages. Also as the forested villages are gradually being deforested and paddy cultivation is coming up with the development of irrigation network (channels), presence of *An. culicifacies, An. nivipes* and *An. annularis* in addition to *An. minimus* and *An. dirus* is being noticed.

Impact of deforestation on malaria epidemiology

During active fever survey in winter season in deforested Dhekiajuli PHC, 32 blood slides were examined, out of which 22 were found positive for *Plasmodium falciparum (Pf)*. During pre-monsoon season, out of 50, two slides were found positive for *Pf* in the same PHC while four slides were found positive for *Pf* in deforested PHC of Nagaon during active fever survey done in winter season. State collected malaria data for 2009 revealed more number of cases in deforested PHCs in comparison to forested PHCs in Sonitpur and Nagaon districts of Asom.

3.1.2 Mapping malaria receptivity in tribal areas of District Ranchi, Jharkhand using Remote Sensing and Geographical Information System

The study was carried out in Angara PHC of Ranchi district of Jharkhand state. Using ARCGIS 9.3, Digital Elevation Model (DEM) of the PHC was constructed using contour map (Fig. 6). The habitation map was overlaid over DEM (Fig. 7). It was found that population was settled up to an altitude of 620 m. These areas up to 620 m of altitude were termed as risky for malaria and above 620 m as risk-free. It is important to note that human hosts are required for malaria transmission to take place besides *Plasmodium* parasite and *Anopheles* mosquito vector.

Other thematic layers, namely forest, streams, water bodies and agricultural practices were overlaid on DEM and eight villages, namely Rupru, Getalsud, Chatra, Lapung, Angara, Pertol, Bisa and Childagsoso having combinations of different parameters representing all ecotypes were selected (Fig. 8). It is worth mentioning that Rupru, Getalsud, Chatra, Lapung and Angara had streams besides other water bodies. In other three villages, namely Pertol, Bisa and Childagsoso, no streams were found. Selected villages were further screened through Quick bird remote sensing imageries for identification of micro level breeding sites like small ponds, pits and pools of seepage from Getalsud reservoir which could not be identified through LISS III imageries.

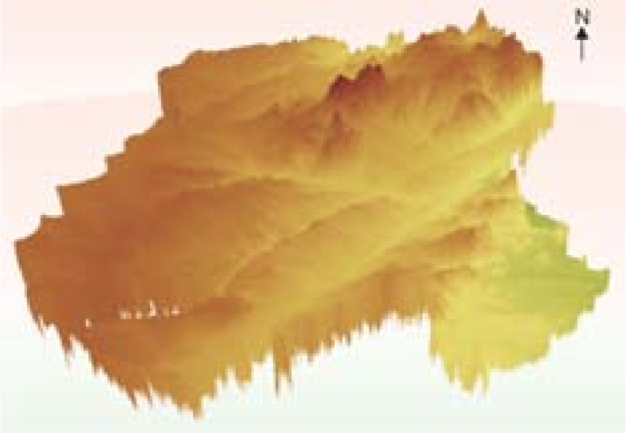


Fig. 6: Digital Elevation Model (DEM) of Angara PHC, Ranchi district, Jharkhand.

Field surveys were undertaken in Angara PHC during May–June 2010 where 200 questionnaires were filled up in order to collect socioeconomic and other attribute data and heads of the households were mainly interviewed. The data were processed and analyzed using standard software package.

Total catches in the selected villages were done to identify the species resting inside the houses and attached covered cattle sheds. A total of 1174 specimens of *An. culicifacies*, *An. annularis* and *An. subpictus* were collected from 32 houses and 16 cattle sheds of eight villages. Streams, seepage from reservoir, ponds, rice-fields and rice-field channels, pits and wells were the breeding sites where anopheline larvae were collected following

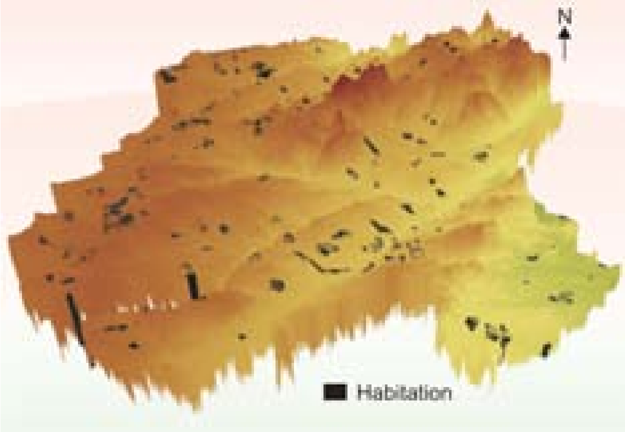


Fig. 7: Overlaying of Digital Elevation Model (DEM) and habitation (Brown colour) in Angara PHC, Ranchi district, Jharkhand.

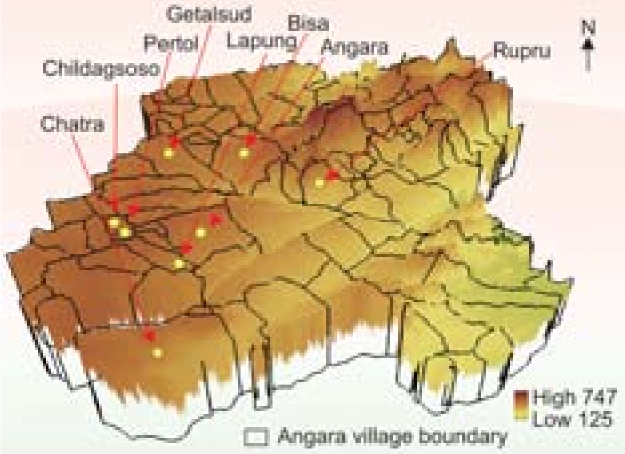


Fig. 8: Selected study villages in Angara PHC, Ranchi district, Jharkhand.

WHO standard technique. Maximum anopheline larvae were collected from streams and seepage water from reservoir.

Active fever surveys to collect parasitological data were also conducted. Also village-wise malaria parasitological data for 2009 were collected from the state health department of Ranchi district.

From Rupru, Angara, Lapung, Getalsud and Chatra villages more number of *An. culicifacies* were collected in comparison to *An. annularis*. In these villages, breeding was found mainly in streams and seepage water from reservoir. In other three villages, namely Pertol, Bisa and Childagsoso, more *An. annularis* in comparison to *An. culiicifacies* were collected. In these villages, as there was no stream, breeding was found mainly in wells and ponds. It is worth mentioning that *An. culicifacies* is the primary vector while *An. annularis* is the supporting vector. In villages with/without streams, difference in collected vector species from households and cattle sheds was found statistically significant (*p*-value <0.05).

Analysis of socioeconomic data revealed that nearly 90% of the population were labourers and farmers with monthly income of ₹ 2000–3000 and 80% of the houses in the area were poorly constructed, made of mud walls and floors with thatched roofs (Fig. 9). None of the 200 respondents knew about malaria and its breeding sites. In Angara PHC, 43% of the respondents from Rupru, Angara, Lapung, Getalsud and Chatra villages used ITNs whereas in other three villages, namely Pertol, Bisa and Childagsoso, ITN coverage was very low as only 10% used the same. ITNs were distributed to the tribals by the local health department. Use of repellents or traditional methods to protect from mosquito bites was not recorded from any of the villages.

In GIS-identified risk areas, 'High' and 'Medium' receptive areas were demarcated based on entomological findings. High receptivity was marked in villages with streams, seepage water from reservoir and medium receptivity in villages not having streams but with wells and ponds. Areas above 620 m of altitude were identified free from malaria risk (low receptive areas). Identified risk factors in Angara PHC were: (1) streams, seepage water from reservoir, rice-field channels, rice-fields, pits, ponds, and wells where breeding was found; (2) 90% of the settlements within 500 m of streams and seepage water from reservoir; (3) more than 50% tribal population and low literacy rate; (4) abundance of poorly constructed houses made of mud walls and floors with thatched roofs; (5) limited use of ITNs; (6) no use of repellents/traditional methods to protect from mosquito bites; and (7) complete ignorance regarding malaria and its breeding sites.

Buffer zone of 500 m was constructed around streams and seepage water from reservoir which were the potential breeding sites of primary vector *An. culicifacies* and habitation map was overlaid. Using geo-processing tools of GIS, it was worked out that 90% of the habitation was located around 500 m of streams and seepage water in Angara PHC. A distance of 500 m around streams and seepage water is suggested as cut-off to define primary risk areas under major threat.

Fig. 9: Type of population settlement and houses in Angara PHC, Ranchi, Jharkhand.

Validation of GIS-identified receptivity was done using malaria epidemiology data collected from state health department and generated through active fever surveys. More number of malaria positive cases was found in villages having high receptivity than villages with medium receptivity.

Identification of different levels of malaria receptivity will help to plan priority control in the PHC. From 2008, World Bank assisted malaria control project became operational in Ranchi and the emphasis is on the introduction of LLINs. The identification of primary risk areas is useful for planning distribution of LLINs to achieve useful results. Every year two rounds of indoor residual spraying (IRS) using insecticide are done following blanket coverage. World Bank assisted malaria control recommended stratified approach in the district. Identification of primary risk area will serve as focus area for cost-effective control.

3.2 Environment epidemiological studies

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

The project aims at determining the transmission windows of malaria and dengue in terms of climate and socioeconomic parameters, GIS-based outputs indicating the extent of disease spread under current and based on climate change, land use and socioeconomic conditions and formulation of adaptation framework. Monthly temperature, RH and rainfall (January 1961 to December 1990) extracted from PRECIS (Providing Regional Climate for Impact Studies) were used as baseline. Projected scenario (A2 scenario) for 2071, 2081, 2091 and 2100) of PRECIS were used. Transmission windows (TWs) of malaria were determined using lower and upper thresholds of temperature (T) and 55–90% relative humidity (RH). TWs were determined for dengue also. Details of projected scenario in respect of India as well as for Asom, Odisha, Rajasthan, Uttarakhand and Delhi states were generated.

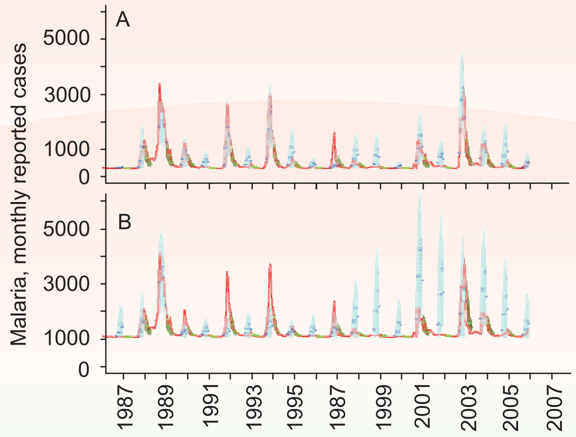
In 3–9 months TW open categories, appreciable increase in the months of TWs is expected leading towards stable malaria. In baseline, 128 pixels show no transmission which may reduce to 90 pixels by 2091. Baseline TWs in 10–12 months (546) are likely to be reduced to 322 by the year 2091. Results are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

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

For socioeconomic status in vulnerable areas of the five states selected for detailed analysis of socioeconomic conditions to arrive at possible adaptation measures, field visits were undertaken in Jodhpur (Rajasthan) and Sambalpur (Odisha) for eliciting information on KABP of the communities about malaria and existing health facilities/system. TWs using A1 B scenario by the year 2030 showed opening of windows of transmission in a few foci in Uttarakhand and Jammu & Kashmir while in north-eastern states the intensity has been projected to increase from 7–9 months to 10–12 months.

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

Initial analyses were focused on three districts of Gujarat, namely Kutch, Banaskantha, and Kheda-Anand (Fig. 10), and the time series of monthly rainfall and positive *P. falciparum* from 1986 to 2002/2006. One district of Rajasthan, Barmer, has also been included for comparison purposes. Initial correlative analyses revealed significant associations between rainfall during the monsoon season and malaria during the epidemic season that follows, particularly in more arid districts. In Kutch



Accumulated rainfall from May to August (Blue dots) could predict malaria cases in September to December (Green dots) using VSEIRS model (Laneri *et al* PLoS Com. Biol. 2010).

Fig. 10: Prediction for the epidemics of malaria in Kutch (India).

district, rainfall and malaria cases cumulated during the respective season. Similar patterns emerge if one considers specific months and specific lags. These associations are also evident in the frequency domain, that is in the spectra of frequencies present in rainfall and malaria. Dominant cycles present in the data using wavelet spectra were also determined. In Barmer, malaria data exhibit variability at a period of approximately 2 and 4 years; similar dominant periods are present in the rainfall anomalies and importantly, the timing of these cycles correspond to that of the malaria cases. This illustrates similar patterns of variability in rainfall and malaria, consistent with an important role of rainfall as a driver of epidemics.

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

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

3.2.3 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

The study is being undertaken in selected districts of Uttarakhand, Asom and Mizoram states to generate data on biophysical, climatic and socioeconomical determinants of malaria to understand the current transmission windows and ecological risk factors of malaria for development of transfer functions and simulation models; to evaluate and strengthen current adaptation measures for control of malaria; to develop projections of potential impact of climate change on seasonal transmission of malaria; and finally to develop a framework for adaptation measures. Keeping in view the additional institutional measures, technological interventions required to combat the adverse impacts of climate change and mainstream climate change adaptation concerns through capacity building of various categories of the state health personnel vis-a-vis climate change (Fig. 11).

Fig. 11: Field sites for generation of evidence-based data on malaria vis-a-vis climatic parameters.

Three sites at Bhimtal, Kolasib and Bokajan have been identified and two districts in each state have been selected, i.e. Nainital and Almora (Uttarakhand), Kolasib and Aizwal West (Mizoram) and Karbi Anglong (Asom). Project field units for continuous monitoring of temperature, rainfall, relative humidity, entomological and parasitological parameters have been set up. Three sites in each district at varying altitude have been identified for generation of entomological and parasitological data. Study is going on.

3.3 HIA studies

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

The change in environment affects the population on a large scale, and creates threat to the people. An effective action through preventive, curative and promotional health services are therefore essential. Central Water Commission (CWC) in its “Guidelines for Sustainable Water Resources Development and Management, 1992”, has made it mandatory to carry out Health Impact

Assessment, so that preventive actions based on environmental and engineering methods, can be taken up at the planning, construction and operational phases to reduce the disease burden in the water development projects. A retrospective study entitled 'Health impact assessment on Indira Sagar Dam and resettlement and rehabilitation colonies (RR) in Sardar Sarover Project (SSP)' reservoir was, therefore, initiated in January 2004. Project has been funded by the Narmada Valley Development Authority (NVDA) Bhopal. In India, this HIA project is the first longitudinal project which is operational for more than 5 years and is a remarkable foresightedness of NVDA.

Seven districts, viz. Khandwa and Dewas (Indira Sagar Project (ISP) and Omkareshwar Project (OSP), Khargone and Harda (ISP), Badwani, Dhar and Jhabua (SSP) consisting of 32 villages, 18 rehabilitation and resettlement (RR) centres, 5 command area villages and 6 labour colonies were taken up to initiate the study.

Breeding sites created due to dam construction were surveyed for larval breeding and species-specific breeding sites identified for all the disease vectors to suggest simple curative measures to control vector-borne diseases.

Entomological and epidemiological situation of all vector-borne diseases (VBDs), i.e. malaria, dengue, JE and filariasis were monitored to evaluate impact of construction of ISP and OSP, and their RR colonies including that of SSP, canals in command area and also impact of implementation of suggested mitigating measures.

GIS mapping of all the seven districts was done to identify problematic villages in the study area. Digital maps of villages were prepared and attached with attribute and malaria data. The data on various entomological and parasitological parameters which are being collected through periodic surveys are regularly put into GIS-based framework to review the impact of the construction of dams and implementation of mitigating measures in space and time.

Impact of dam construction on VBDs was observed in all the villages, RR colonies and labour colonies surveyed, the vector density was reported high (>300 per man hour) till 2005 which reduced gradually. In 2005, out of total 299 slides collected in the survey, 216 were found positive for *Pv* and 83 for *Pf*. In the year 2010, till October, only one positive case has been found. *Anopheles culicifacies* was found resistant to DDT and susceptible to synthetic pyrethroids. However, it may be pointed out that as per data of the state no malaria case was reported from the study area before the construction of Indira Sagar Dam.

In the ISP, SSP and RR colonies seepage of the reservoir, pits and pools of down streams, new canals, curing tanks etc were identified as preferred breeding sites for vector mosquitoes.

Mitigating measures

From October 2005, after completing each survey, meetings were held with Vice-chairman and other officials of NVDA, State Health Department and Narmada Hydroelectric Development Corporation Ltd (NHDC). Survey highlights and situation-specific mitigating measures, i.e. engineering, epidemiological and entomological to control the vector-borne diseases were suggested.

Workshops, Training, Health Camps etc.

Two workshops were also organized for all the stakeholders at NVDA, Bhopal to brief the progress of the work done and to provide training to CMOs and DMOs and make them aware of the vector species, disease dynamics and mitigating measures. Training was also provided to engineers, to highlight simple constructional defects which promote water stagnation supporting mosquito breeding and suggestions on simple engineering techniques to rectify those were explained, e.g. digging should always be horizontal in RR centres and during construction of the roads (problem pointed at RR Centres, Bagarda and Sarallaya), curing tanks should be demolished at the completion of work or should be properly covered to make mosquito breeding proof. RR Centres should always be away from the permanent breeding sites (minimum 1.6 km) not like Pipalkota and Jamkota close to ponds, Ganesh Nagar close to stream, and irrigation wells in the field areas should be covered hermitically to make mosquito breeding proof.

Besides this, health camps were organized to teach villagers to take simple steps to control mosquito breeding in and around their houses. Community was also involved in source reduction.

Implementation of mitigating measures

The following NIMR suggested mitigating measures were carried out by NVDA, State Health Department and Narmada Hydroelectric Development Corporation Ltd (NHDC):

In villages, RR Centre and Narmada Nagar

- Deweeding in canals and repair of rocky and broken margins.
- Canalization for pools, pits and seepage water.
- Cleaning and oiling of drains on weekly basis.
- Source reduction by filling or leveling of river-bed pools, pits, etc.
- Lining of plastic sheets in canal beds to stop seepages.
- Release of larvivorus fishes in tanks, ponds and wells.
- Radical treatment to all the *Pf* cases.
- Focal spray in the *Pf* incidence villages.
- Spray of pyrethroids in Narmada Nagar and DDT in problematic villages/RR centres.
- Source reduction of domestic breeding sites, viz. storage tanks, coolers, earthen pots, etc.
- IEC activities and community participation should be conducted.

Dam site

- Fogging in power house.
- Construction of mosquito proof houses.
- Radical treatment to all the *Pf* cases.

Impact of mitigating measures

Results of prompt interventions were apparent both in vector density of all vector-borne diseases and disease cases. Vector density was reduced drastically from 2004 to 2010 (Fig. 12).

Percent positivity of *Ae. aegypti* in Narmada Nagar including all types of breeding sites of *Aedes*, viz. OHT, ground tank, tyre, cooler, mud pot, drum, container, etc. (2004–January 2010) shown in Fig. 13, revealed drastic reduction in percent positivity in *Aedes* breeding.

The impact of interventions could be seen in malaria cases. Figure 14 is the compilation of the data collected from 2004–10. Initially in 2004 and 2005, number of malaria cases were high but after implementation of suggested mitigation measures there was a remarkable reduction in the disease prevalence.

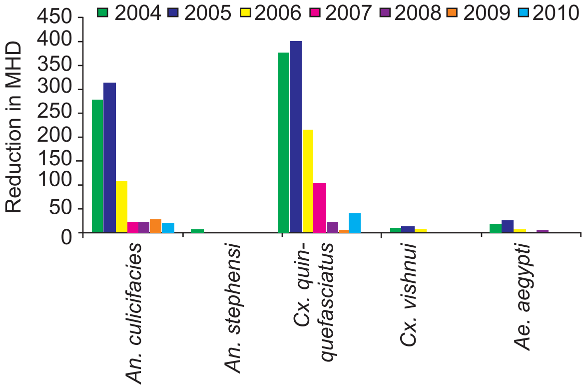
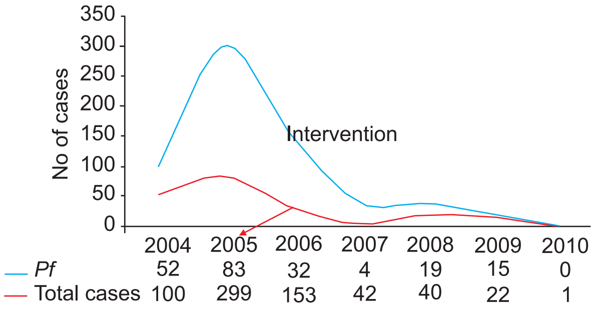


Fig. 12: Reduction in vector density of malaria, filaria, Japanese encephalitis, dengue and chikungunya from 2004–July 2010.

Fig. 13: Reduction in percent positivity of *Ae. aegypti* in Narmada Nagar from 2004–July 2010 (Intervention started in 2005).



	2004	2005	2006	2007	2008	2009	2010
— *Pf*	52	83	32	4	19	15	0
— Total cases	100	299	153	42	40	22	1

Fig. 14: Reduction in malaria cases as a result of implementation of mitigating measures from 2004–July 2010.

NVDA has extended the funding of the project till 2014 to carry out the HIA study for the new project entitled Health Impact Assessment of Narmada Basin Dams and RR Colonies in Madhya Pradesh. It would be progressively covering all the 30 major dam areas in Narmada Valley Development Project and as per schedule the study will be continued till December 2014. A Memorandum of Understanding was signed and the project kicked off on 8 April 2010. As per MoU three Study Centres, one each at Jabalpur, Bhopal and Narmada Nagar, District Khandwa have been opened.

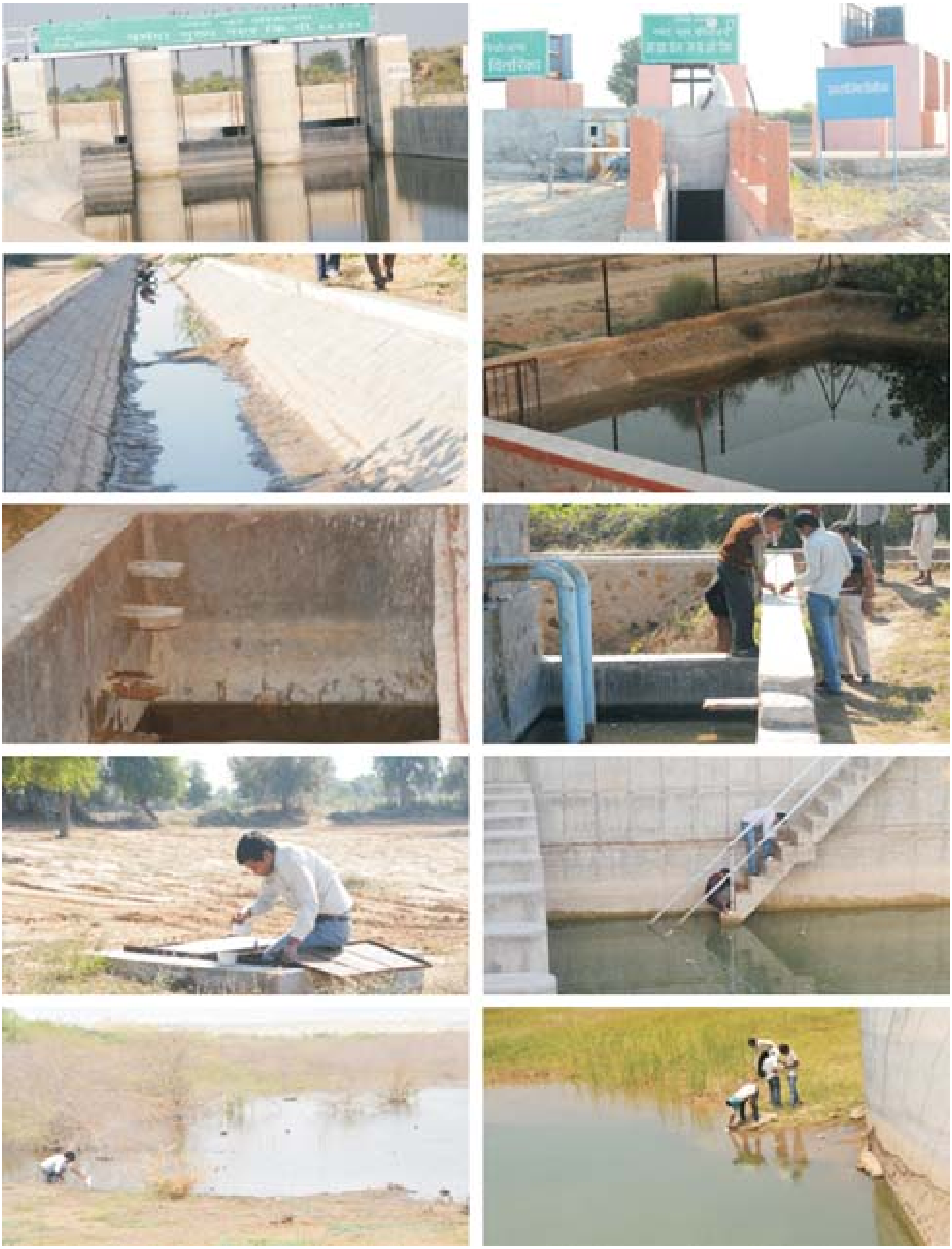


Fig. 15: Preferred breeding sources of mosquito vectors at the Dam site.

The Jabalpur Unit for the HIA Studies of Narmada Basin was inaugurated in December 2010. It was the first unit to be inaugurated in the presence of Prof. R.C. Mahajan (SAC Chairman), Mr Ansari (Member, Forest & Environment) and Dr Neeru Singh (Director, RMRCT, Jabalpur) and other senior members of NIMR, Delhi. The unit is operational in its full swing and the work is in progress.

The Bhopal unit is the second in the series after Jabalpur which was inaugurated on 6 April 2011 by the Hon'ble Minister of NVDA, Sri K.L. Agarwal in the presence of Shri O.P. Rawat, V.C. & ACS, NVDA, Dr J.K. Jain, SMS Health, and senior scientists from National Institute of Malaria Research, Delhi. Dr B.N. Nagpal, Principal Investigator, Dr Aruna Srivastava, Coordinator, and Dr M.C. Sharma, Co-investigator, under Health Impact Assessment Project along with other dignitaries from NVDA were also present. The study unit at Bhopal would be covering Kolar, Tawa, Barna, Morand, Handia, Ganjal, Dudhi and Sitarewa dam areas. The study was initiated with Kolar Dam area. Kolar dam project was completed in 1989 and is located on Kolar River, a tributary to Narmada River, near Lawakhari, Sehore district, Madhya Pradesh. This project aims to provide irrigation to 35,040 ha^2 of land in Budhni and Narsulaganj, Madhya Pradesh and water supply to the tune of 37 Mgd to Bhopal town. Water supply to Bhopal town is contemplated by lifting water from the reservoir. In the downstream 23 km river path, near Jholiapur village a barrage is constructed on the river. From Kolar barrage two canals, one on the right bank (24.72 km) and the other on left bank (29.5 km) have been constructed to cover the irrigation area 13,840 ha^2 and 21,200 ha^2 respectively. The four villages due to submergence have been rehabilitated in Kamalkhera, Gular Chhapri, Jeevantal and Abidabad villages.

The Narmada Nagar unit is the third in the series after Jabalpur and Bhopal which was inaugurated on 6 April 2011 by Mr K.M. Singh, Chief Executive Director, NHDC in the presence of Mr Rajan Narang, Ms Jayshree Gupta, Dr S. Bhattacharjee, Dr J.K. Jain, SMS Health, and senior scientists from National Institute of Malaria Research, Delhi. Dr B.N. Nagpal, Principal Investigator, Dr Aruna Srivastava, Coordinator, and Dr M.C. Sharma, Co-Investigator, under HIA Project along with other dignitaries from Narmada Nagar.

The staff appointed in all the three centres is trained and the data are being generated for entomology and epidemiology of the vector-borne diseases in the proposed areas.

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

Sardar Sarovar Project aims to provide irrigation to 233 villages and drinking water to 1107 villages including two towns through canal in Jalore and Barmer districts of Rajasthan. A project on Health Impact Assessment of Sardar Sarovar Project in both the districts was initiated in November 2010 and funded by the Government of Rajasthan.

First survey (November 2010–11) was carried out in 22 villages situated at distributaries, sub-distributaries, minors, sub-minors, PHD points of canal including two control villages in Jalore and Barmer districts of Rajasthan (Fig.15). It revealed that diggies, sump wells, outlets, pumping stations discharge of escape water points, i.e. 1000 hectare land point near Meghwa villages, 100 hectare land point near Bhimguda distributary and Keriya water storage point were the main breeding sources of malaria and dengue/chikungunya vectors. Due to present designing of diggies, sump wells, pumping stations and outlets, *An. culicifacies*, *An. stephensi* and *Ae. aegypti* have been established which is also evidenced from epidemiological data (2005–10).

There was a trend of increase in humidity and decrease in temperature due to discharge of canal water in large areas, creating environmental favourable conditions (humidity >55%; temperature 24–30°C) for the transmission of malaria, dengue and chikungunya. Our survey also justifies the same as MHD of *An. culicifacies* and *An. stephensi* (malaria vector), *Ae. aegypti* (Dengue/Chikungunya vectors) and *Cx. quinquefasciatus* (Filaria vector) was higher in the villages located in the vicinity of NMC as compared to control villages (Table 1).

Microbiological testing of drinking water using HiWater^{TM} Test Kit from five villages showed the presence of bacterial contamination, i.e. *Salmonella typhimurium*, *S. enteritidis*, *Citrobacter freundii*. The results were given to concerned PHCs for immediate action.

Action was taken immediately to remove the breeding with the help of community participation as well as the state health department. Health

Table 1. Man per hour density (MHD) of vector of malaria, filaria, dengue and chikungunya

MHD	Malaria		Dengue/Chikungunya	Filaria
	An. culicifacies	*An. stephensi*	*Ae. aegypti*	*Cx. quinquefasciatus*
Villages in the vicinity of NMC	11.7	6.6	2.4	26.44
Control villages	1.5	1.25	1.0	11.25

education was given to the community about malaria and dengue/chikungunya vectors breeding and their control. Concerned public health departments and engineers were informed about the problems and mitigation measures were suggested for the control of breeding in diggies, sump wells, outlets and escape water points for the control of vector-borne diseases as indicated in Table 2.

❑

Table 2. Mitigation measures suggested for the problematic sites

Problematic sites	Mitigation measures
Diggies	Release of larvivorus fishes
Sump wells	Expanded polystyrene (EPS) beads
Outlets	Expanded polystyrene (EPS) beads
Escape water points	Release of larvivorus fishes

Clinical Research

4

4.1 Malaria Clinic

A total of 2660 fever cases attended the Malaria Clinic at Dwarka, New Delhi, either directly or referred from hospitals for diagnosis and treatment of malaria during April 2010 to March 2011. In all, 134 patients were found positive for malaria. Out of these, 104 were diagnosed as *P. vivax* and 27 as *P. falciparum* and 3 as mixed infections.

4.2 Clinical Trials

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

This Phase III, double-blind, randomized, parallel-group, multicentre trial was carried out in patients with acute uncomplicated *P. falciparum* malaria during November 2009 to December 2010. Patients were randomly assigned to one of the two treatment groups; either FDC of arterolane maleate + PQP or Coartem®. A total of 6 doses were administered over 3 days. Each randomized patient was administered a combination of active and/or placebo for a total amount of five tablets in a single dose. A total of 327 patients were recruited in this study. This included 202 patients enrolled at NIMR sites at Mahadevi Birla Hospital, Ranchi, Tata Main Hospital, Jamshedpur, Ispat General Hospital, Rourkela, Community Welfare Society Hospital, Rourkela and Wenlock Hospital, Mangalore. The study provided 280 evaluable (PCR corrected) patients. One patient was lost to follow up.

Patients' participation in the study was at least for 42 (±2) days following the first dose of study medication. Patients were hospitalized for at least 3 days (Days 0, 1 and 2). The patients were advised to return to the study site for follow up visits on Days 7 (±1), 14 (±1), 21 (±2), 28 (±2), 35 (±2) and 42 (±2). If adverse events reported during the study remained unresolved by Day 42, patients were followed until resolution of the event or determination that no further medical management was deemed necessary.

There were no early treatment failures in both the groups. Late clinical failure and late parasitological failures were 13 out of 217 in patients treated with arterolane + PQP and 7 out of 109 in patients treated with Coartem®. Arterolane + PQP was found to be non-inferior to Coartem® considering uncorrected and corrected ACPR on Day 28.

There was no death reported during the course of the study. There were three serious adverse events, all of these were reported receiving Coartem®. Out of these serious adverse events, pneumonia and cellulitis of lower limbs were considered to be not related to the treatment; and one Wenkebach's phenomenon (6:5 AV conduction) was considered to be probably related to the treatment. Some adverse events like nausea, vomiting, diarrhea, headache and prolonged QTc were more commonly observed with patients treated with arterolane + PQP. Arterolane + PQP effectively cures *P. falciparum* malaria and attains acceptable level of cure up to Day 28.

4.2.2 Effective and safe treatment for malaria in pregnancy in India: A randomized controlled trial

Artesuante + Sulphadoxine-Pyrimethamine (AS + SP) is the first line of treatment for *P. falciparum* malaria in India. The combination has also been recommended for treatment of malaria in pregnancy in second and third trimesters. This study was planned to assess the efficacy of Artesunate + Mefloquine compared to Artesunate + Sulphadoxine-Pyrimethamine for the treatment of *P. falciparum* malaria in pregnancy.

It is a multi-centre randomized open-label clinical trial of AS + SP and AS + MQ. Inclusion criteria include pregnant women of all parities in 2nd and 3rd trimester having *P. falciparum* parasitaemia (monoinfection). Sample size is 250 eligible women per arm (total 500). The primary endpoint is adequate clinical and parasitological response corrected for new infections by PCR by Day 63 post-treatment. The study is being carried out at three sites: (1) Mahadevi Birla Hospital, Ranchi; (2) Tata Hospital, Jamshedpur; and (3) Ispat General Hospital, Rourkela.

Cases of malaria in pregnancy are detected by active surveillance of a cohort of pregnant women. The entry criteria for the cohort is pregnant women residing within 25 km radius of the study hospitals. Cohort is visited fortnightly and screened for malaria infection by a rapid diagnostic test, if they have a history of fever within 48 hours. Until July 2011, there were 3140 pregnant women in this cohort. In addition, testing for malaria by RDT, blood slides and filter paper samples were also collected once a month from all women enrolled in the cohort to know asymptomatic malaria parasitaemia.

Till date, 66 patients have been enrolled in the trial, where 33 patients received AS + MQ treatment and other 33 received AS + SP. Among them 36 enrolled patients have successfully given birth to babies. There were 7 severe adverse events during the study. None of the severe adverse events was deemed to be related to the study drugs (Table 1).

Table 1. Enrolment status at study sites

	Ranchi	Rourkela	Jamshedpur
Number of eligible patients	20	26	33
Number of enrolled patients	17	19	30
Number of patients completed the study	12	7	17
Number of SAE	0	4	3
Adverse reaction	0	0	0
Re-infection	0	0	1

4.2.3 Monitoring therapeutic efficacy of antimalarial medicines in India

This study was continued for the second year and in all 12 study sites were completed during 2010–11 (Fig. 1). The aim of the study is to monitor therapeutic efficacy of antimalarials including combination regimens in *P. falciparum* and *P. vivax* malaria and to develop capacity in the states for drug efficacy evaluation. All patients reporting to local clinic or in the field area with fever were examined for malaria parasites in blood smear. The temperature, body weight and other demographic information were recorded. Peripheral smear was examined and the patients positive for *P. falciparum* or *P. vivax* were enrolled. Informed consent was obtained and case record form (CRF) was completed for each patient. WHO (2009) protocol was followed for inclusion and exclusion criteria.

The studies conducted during the year 2010–11 have shown 100% efficacy of chloroquine for *P. vivax* in Gulbarga, Karnataka while the efficacy of ACT (AS + SP) for *P. falciparum* ranged from 96.3–100% (PCR corrected) at 11 sites.

Molecular genotyping (MSP2/MSP1/GLURP) was done in paired samples of treatment failure cases. Out of 11 study sites for the efficacy of ACT (AS + SP) in *P. falciparum*, a total of 10 treatment failure cases were reported. Out of these cases, eight true treatment failure cases showed recrudescence through MSP2/MSP1/GLURP genotyping (Fig. 2). Two cases were withdrawn as one was *Pf* reinfection and the other remained PCR unclassified. These true treatment failure cases are from five sites, namely Angul (Odisha) (n = 1), Kolkata (W. Bengal) (n = 1), Surat (Gujarat) (n = 3), Mumbai (Maharashtra) (n = 1) and Betul (Madhya Pradesh) (n = 2).

To monitor the drug resistance pattern in the samples for chloroquine resistance, molecular marker (*pfcrt*) was done for the samples obtained on Day 0. Samples were randomly selected from 11 sites, namely Angul (Odisha) (n = 12), Kolkata (W. Bengal) (n = 16), Bilaspur (Chhattisgarh) (n = 14), Visakhapatnam (Andhra Pradesh) (n = 13), Surat (Gujarat) (n = 18), Dumka & Latehar (Jharkhand) (n=12) & (n = 14), Mumbai (Maharashtra) (n = 17), Pratapgarh (Rajasthan) (n = 14), West Garo Hills (Meghalaya) (n = 5) and Betul (Madhya Pradesh) (n = 14). Out of the total, 149 samples analyzed, 140 (93.95%) showed K76T mutations, 1 (0.67%) sample showed mixed type of response, whereas 4 (2.68%) samples were sensitive and 4 (2.68%) samples were not amplified (NA) (Figs. 3 & 4).

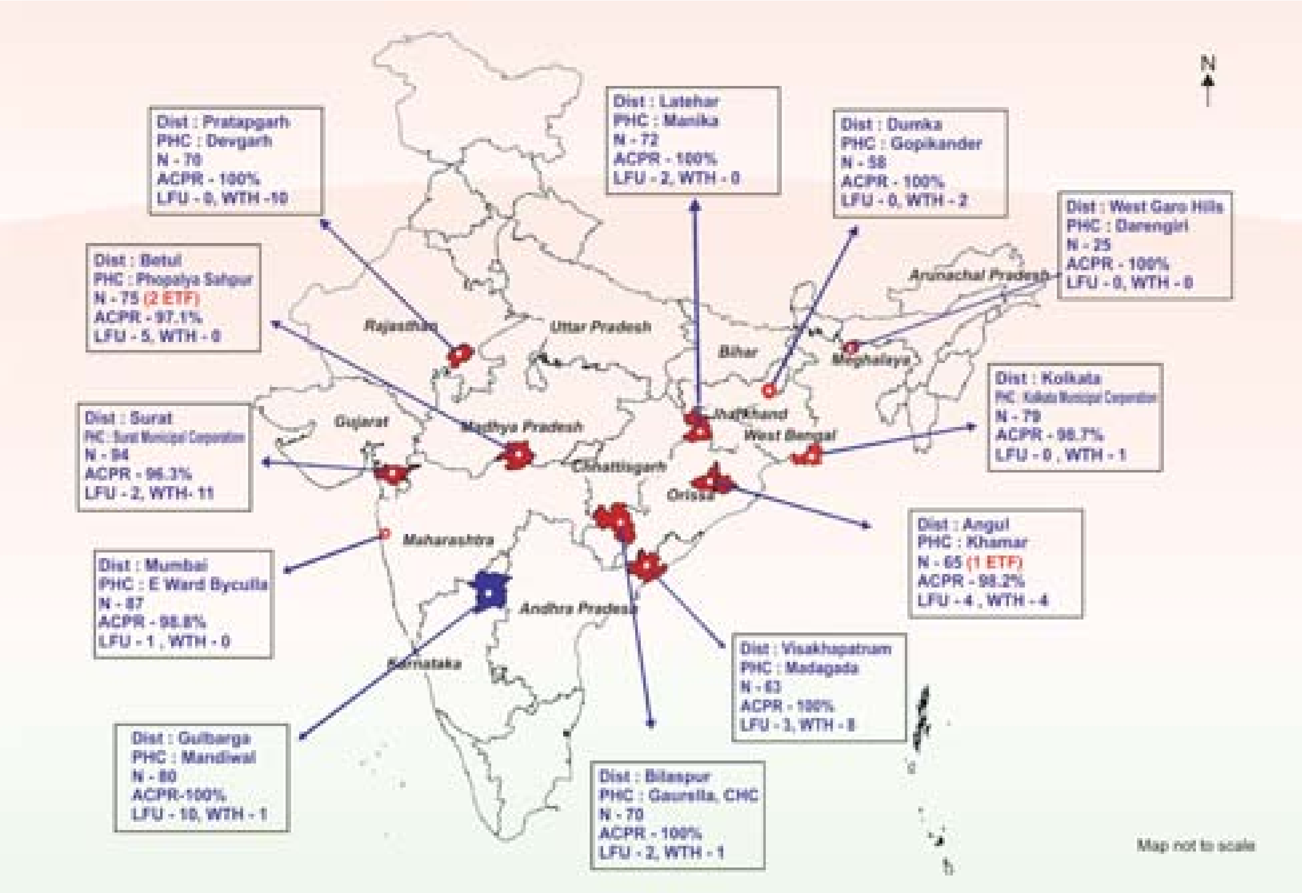


Fig. 1: Study sites selected for therapeutic efficacy of AS+SP.

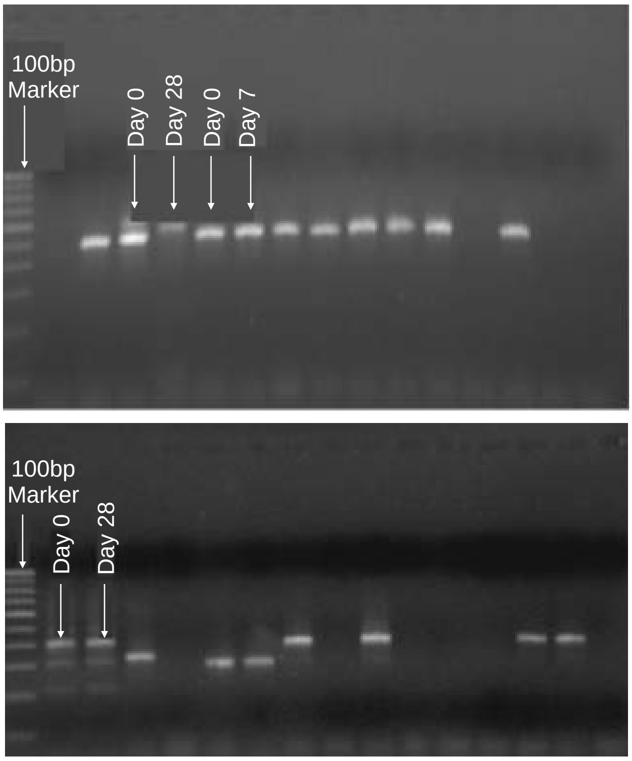


Fig. 2: Genotyping of paired samples showing reinfection and recrudescence.

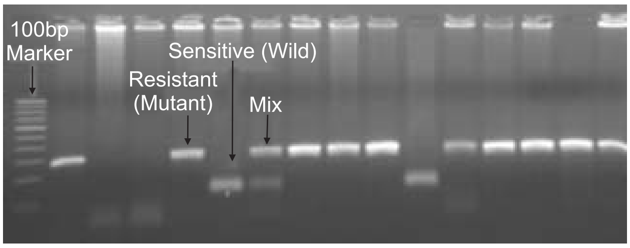


Fig. 3: Chloroquine resistance analysis (wild, mutant and mix type).

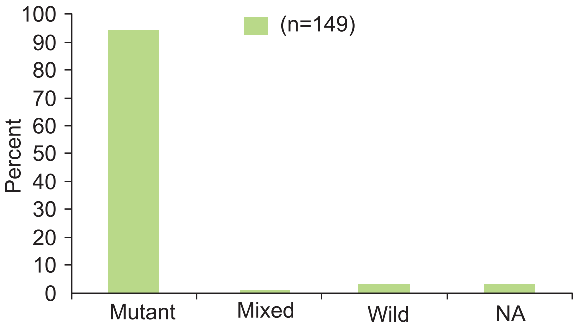


Fig. 4: Analysis of *pfcrt* samples from different sites.

To monitor the drug resistance pattern in the samples for SP resistance, molecular markers (*dhfr* and *dhps*) were analyzed for the samples obtained on Day 0. A total of 149 samples (randomly selected from each site) have been analyzed from 11 different sites, viz. Angul (Odisha), Kolkata (W. Bengal), Bilaspur (Chhattisgarh), Visakhapatnam (Andhra Pradesh), Surat (Gujarat) Dumka & Latehar (Jharkhand), Mumbai (Maharashtra), Pratapgarh (Rajasthan), West Garo Hills (Meghalaya) and Betul (Madhya Pradesh). Out of the total 149 samples, 137 could be amplified by PCR and remaining 12 (8.1%) samples were not amplified. In most of the cases, *dhfr* double mutations (74.5%) were prevalent (Fig. 5). Single *dhfr* (7.4%) and triple (2.7%) mutations have also been observed in some of the samples. Wild type *dhfr* (7.4%) pattern was also observed in some cases (Fig. 6). However, a total of 149 cases were analyzed from all the 11 different sites for *dhps* mutation pattern. Cent percent wild type pattern was observed in all the analyzed cases.

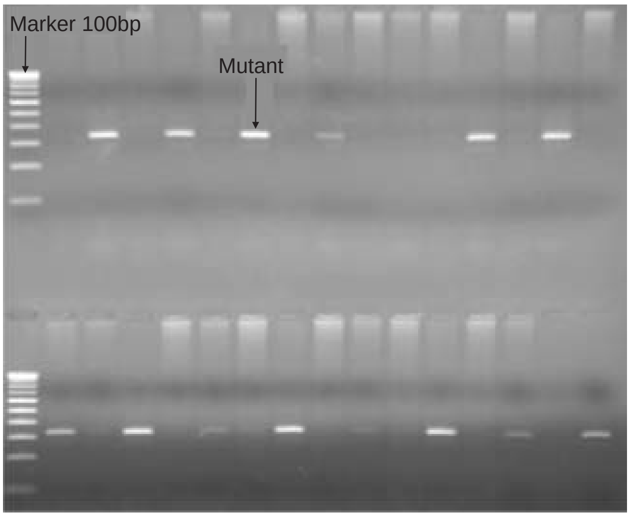


Fig. 5: *Dhfr* codon 108 mutant genotype from Kolkata (West Bengal).

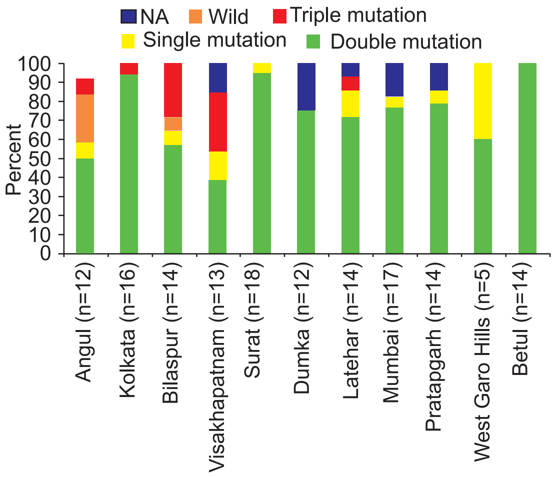


Fig. 6: Analysis of *dhfr* mutations from different sites.

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

4.2.4 Quality assurance of rapid diagnostic kits

NIMR being National Referral Laboratory for quality assurance of laboratory diagnosis of malaria and NVBDCP being a nodal agency, the regional and state referral laboratories were identified. Major components of the quality assurance of RDTs for malaria included preparation of quality control (QC) panels, pre-dispatch QC and post-dispatch QC, external quality assurance scheme (EQAS) and internal QC. Staff working at NIMR was trained in the preparation of panels with samples at the NIMR field unit, Rourkela, Odisha. Patient with >20,000 parasites/µl was selected as donor. Parasitized blood was diluted with O positive blood group or AB positive fresh frozen plasma (FFP) to attain a low positive panel of 200 parasites/µl and a high positive panel of 2000 parasites/µl. In all, 16 panels in appropriate quantity (200 aliquots per panel) were prepared and panel preparation was done every quarter since shelf life of panel is 180 days.

Testing of RDT

Pre-dispatch QC (National Level): This was achieved by lot-testing of the kits. From each RDT lot, 50 RDTs were drawn and tested using positive and negative control for immediate QC. In all, 30 batches were tested and 29 were found to be acceptable.

Quality assurance of RDTs used by health workers at the periphery: RDT samples were drawn from the representative Primary Health Centres/Sub-centres/ASHAs and tested for their quality. The District Malaria Officers (DMOs) collected RDT samples from their districts and used to send the same to the Referral Laboratory every quarter.

Table 2. Results of RDT testing

S.No.	State	No. of RDTs tested	Results satisfactory/Total tested			Satisfactory results
			2000 p/µl	200 p/µl	Negative	
1.	Nagaland	275	80/80	147/158	38/38	265
2.	Manipur	74	20/22	37/42	10/10	67
3.	Mizoram	305	86/90	152/173	42/42	280
4.	Meghalaya	140	41/42	73/78	20/20	134
5.	Asom	113	32/34	55/66	13/13	100
6.	Madhya Pradesh	377	106/113	187/221	43/43	336
7.	Arunanchal Pradesh	89	27/27	49/52	10/10	86
8.	Odisha	188	56/62	86/102	24/24	166
9.	Maharashtra	12	4/4	6/6	2/2	12
	Total	1573	452/474	792/898	202/202	1446

Results: Correct results/total tested

Progress

Kits procured by the NVBDCP through United Nations Office for Project Services (UNOPs) India were received by NIMR for evaluation. Till date 30 batches have been evaluated and 29 were found to be acceptable. The post-dispatch quality assurance is also going on. Every quater, District Malaria Officers (DMOs) are picking up seven RDTs at random from different PHCs, Sub-centres, ASHAs. Out of 199 districts, trainings were conducted for 179 districts which were attended by the District Programme Officers of 138 districts and 97 districts have been sending RDTs to NIMR from all over India. So far, 1960 RDTs from all over India have been received by NIMR. The RDTs received from the field were tested for their quality by standard panels.

Out of 1960 RDTs received, 1573 have been tested so far. The panel detection score was 91.9%, while specificity was 100% (Table 2).

4.2.5 Pharmacovigilance of antimalarial medicines in India

The project Pharmacovigilance for antimalarial medicines in India has been funded by the World Bank through NVBDCP with the objective "Assessment of benefit, harm, effectiveness and risk of ACTs in the treatment of malaria". The project was initiated in the month of June 2009 along with collaborating institutes. All India Institute of Medical

Table 3. Adverse events reported for different antimalarials

S.No.	Drug	No. of forms	Adverse event	No. of events
1.	Chloroquine + Primaquine	938*	Loss of appetite	3
			Nausea	18
			Vomiting	17
			Giddiness	5
			Pain in abdomen	2
2.	Artesunate + Sulphadoxine-Pyrimethamine	1798*	Headache	13
			Vomiting	1
			Jaundice	1
			Stomatitis	1
			Gastritis	1
3.	Other ACT	13	_	_
4.	Artesunate alone	5	_	_
5.	Artesunate + Doxycycline	5	Nausea	3
			Vomiting	2
6.	ACT + Chloroquine	72	_	_
7.	Quinine	7	Gastritis	4
			Itching	3
8.	Non-antimalarial treatment	61	_	_
9.	Miscellaneous	70	_	_
	Total	2969	Total	74

*Include 76 forms from chloroquine and 1548 forms from AS + SP therapeutic efficacy studies.

Sciences (AIIMS), New Delhi and the National Vector Borne Disease Control Programme (NVBDCP). Adverse drug reaction form was developed in consultation with AIIMS and NVBDCP. The sample size of patients in this cohort study was finalized to be 10,000. Sensitization meeting of State Programme Officers was held at NVBDCP on 22 December 2009 and attended by the representatives of WHO, AIIMS, NVBDCP, NCDC and 26 State Programme Officers.

Trainings of the District Malaria Officers were organized at respective state capitals and DMOs from 12 states— Asom, Meghalaya, Arunachal Pradesh, Nagaland, Jharkhand, Odisha, Gujarat, Madhya Pradesh, Chhattisgarh, Manipur, Mizoram and Karnataka participated in the trainings.

Till date about 2969 filled in AER forms have been received (Table 3). These include 1360 forms filled in by the medical officers and information of 1624 patients participating in the therapeutic efficacy studies, while 136 forms were found incomplete. A total of 74 adverse events have been reported in the form of nausea, vomiting, giddiness and gastritis. The study is in progress.

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Highlights of Research Activities under IDVC Project

5

5.1 Bengaluru (Karnataka)

- The social and cover experiments with the mosquito fish (*Gambusia affinis*) indicated that both the sexes were equally capable of consuming IV instar larvae of *Anopheles* and *Culex* and more so with *Anopheles*.
- Indigenous production of monoclonal antibodies of *Pf*HRP-2 and pLDH have been successfully completed and rapid diagnostic kits have been produced.
- Clinical trial of Arterelone is underway and all 88 *P. falciparum* patients who received the treatment responded satisfactorily up to 42-day follow-up in Mangalore City.
- Biodart-M, a liquid formulation of *Bti* was found effective against *An. stephensi* when the pH was <10 in Mangalore City.
- The second phase of C-21 trial for *Ae. aegypti* surveillance and control in Bengaluru City indicated a better option for *Aedes* control.
- All the 69 *P. vivax* cases showed adequate response to chloroquine in Gulberga district.
- Extraction of *Ruta sp* is underway for further analysis of anti-mosquito and anti-malarial properties.
- Malaria is under control in all the larvivorous fish project areas undertaken in 1992 onwards. Efforts have been made to implement in the northern districts of Karnataka.

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 besides, phase III trial to evaluate Novaluron 10% EC (Mosquiron), an insect growth regulator for mosquito vector control in urban settings.
- Therapeutic efficacy of chloroquine for the treatment of *P. vivax* malaria and evaluation of Rapid Diagnostic Kit - SD Bioline Malaria Ag *Pf*/PAN were also carried out in Chennai.
- Screening of plant extracts for anti-mosquito activities; purification and identification of active compounds from the selected plants for vector mosquitoes have been undertaken.
- Effect of Kitazin and Hostathion on the larvivorous potential of *Oryzias carnaticus;* foraging behaviour and larvivorous potential of *Aplocheilus parvus* (Raj, 1916), endemic to south-eastern India were evaluated.
- Technical support was provided to various Institutes/Govt. agencies and collaborative research studies were also undertaken with NIMR, Delhi.
- Malaria Clinic continued to function catering to the needs of the public by providing early diagnosis and prompt treatment.

5.3 Guwahati (Asom)

The major research projects included field evaluation of alternative technologies for vector control including:

- Follow-up field evaluation and extended follow-up investigations of long-lasting insecticidal nets (LLINs) impregnated with pyrethroids against malaria transmitting mosquitoes in Asom, and associated disease transmission.
- Monitoring of insecticide resistance against disease vectors in north-eastern states.
- Bio-monitoring of organochlorine residues in human populations and their correlates with food intake.
- Ecological succession of anophelines and

other mosquitoes in north-eastern states of India.

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

5.4 Hardwar (Uttarakhand)

The following activities were undertaken during the reporting period by the field unit:

- Insecticidal and genotoxic activity of *Psoralea corylifolia* against *Cx. quinquefasciatus*.
- Organochlorine residues in soil, water, whole blood and major local food products from low and high malaria endemic areas of Asom.
- Antimalarial properties of some plants from Garhwal region of north west Himalaya.
- *In vitro* antimalarial properties of some synthetic compounds.
- Determination of lumefantrine and its metabolite desbutyl-lumefantrine in plasma from patients infected with *P. falciparum* malaria by LC/MS/MS.
- Sensitive and specific LC/MS/MS assay for the simultaneous determination of chlorproguanil, dapsone and their metabolites in human plasma.
- Phase III evaluation of Pyriproxyfen (Sumilarv.0.5 G) against mosquito vectors.
- Monitoring of insecticide resistance of malaria vectors in West Bengal.
- Entomological investigation of dengue vector in Uttarakhand.
- Epidemiological investigation of malaria in District Saharanpur, Uttar Pradesh.
- Field evaluation of long-lasting insecticidal nets (LLINs) impregnated with alpha-cypermethrin (DuraNet) against malaria vectors in Uttar Pradesh.
- Evaluation of Net Protect LLIN (impregnated with deltamethrin) against malaria vector in District Saharanpur of Uttar Pradesh.
- Studies on the transmission dynamics of encephalitis in District Saharanpur of Uttar Pradesh: An action plan for the prevention and control.
- Epidemiological investigation of malaria in NTPC, Rihand Nagar
- Industrial malaria control at BHEL, Hardwar and IOC, Mathura
- Consultancy provided to control malaria at NTPC, Rihand Nagar, Distt. Sonbhadra, and NTPC Unchahar, Distt. Raibareilly, Uttar Pradesh.

5.5 Jabalpur (Madhya Pradesh)

- The evaluation of long-lasting insecticidal nets in Madhya Pradesh was carried out in CHC Kundam of Jabalpur for the first time. The pre-intervention studies showed high malaria incidence in selected bed net villages. The nets were distributed in 8 villages. Post intervention activities are in progress.
- 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 Govt. of Madhya Pradesh which resulted in the decreasing trend of malaria and mosquito prevalence during the post-intervention period as compared to the pre-intervention period.
- On the request of Govt. of Madhya Pradesh, two training workshops for Medical Officers and eight for malaria workers of various districts of Madhya Pradesh on malaria and other vector borne diseases were organized during the year.

5.6 Nadiad (Gujarat)

The following studies were undertaken by the Nadiad field unit during the reporting period:

- Phase III evaluation to compare insecticidal efficacy and community acceptance of long-lasting insecticidal nets with conventional insecticide treated nets in India.

- Health Impact Assessment of development project: Impact of Sardar Sarovar Project on Vector borne diseases in Gujarat.
- Multi-centre phase-III evaluation of the effectiveness of Novaluron 10% EC (Mosquiron), an insect growth regulator, for mosquito vector control in urban settings.
- Developing a framework for predicting malaria outbreaks in rural and urban areas of Gujarat and Rajasthan, India.
- Assessment of preparedness for mass drug administration for elimination of lymphatic filariasis in District Rajkot.
- Independent assessment of malaria situation and control measures in five districts of Gujarat.
- Diagnostic and treatment services were provided at the Malaria Clinic.

5.7 Panaji (Goa)

The following studies were undertaken by the Panaji field unit during the reporting period.

- Estimation of malaria morbidity burden in India
- Investigation of malaria outbreak in Mumbai and recommendation for control.
- Monitoring of insecticide resistance of mosquito vectors in Odisha.
- Multi-centre Phase II and III evaluation of the effectiveness of Novaluron 10% EC (Mosquiron), an insect growth regulator, for mosquito vector control in urban settings.
- Screening for larvicidal effect of plant extracts (Code *PL-COG*) against vector mosquito species *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*.
- Efficacy of aqueous extracts of various plant parts such as root, leaf, petiole, flower of plant IcG against vector mosquito species *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*.
- Efficacy of methanolic extracts of leaf and callus of the plant, Pl-DmG against vectors *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*.
- Characterization and efficacy of mosquito pathogenic bacteria from mangrove and paddy fields in Goa, India.
- Characterization of newly found strains of *Bacillus subtilis* active against *Anopheles* and development of formulation of mosquitocidal insecticide using *Lysinibacillus* sp. and *Bacillus subtilis*.

5.8 Raipur (Chhattisgarh)

- Carried out various activities under the WHOPES supervised Phase III evaluation of Interceptor long lasting insecticidal nets (LLIN) being undertaken in 7 study villages in district Kanker since 2008.
- Evaluated DuraNet, a LLIN incorporated with alpha-cypermethrin, against malaria vectors and its impact on malaria incidence in several villages of CHC Pendra in Bilaspur district.
- Monitored insecticide resistance of malaria vectors in selected areas of Chhattisgarh.
- Studies were undertaken on distribution and biological characteristics of the members of Fluviatilis-Minimus groups for effective vector control strategies in tribal areas of Chhattisgarh.
- Monitored the therapeutic efficacy of anti-malarial medicines in PHC Basti in District Bilaspur.
- Undertook the evaluation of malaria rapid diagnostic kits, ParaHit and EzDx against malaria microscopy.
- Provided technical support to NVBDCP in monitoring of malaria control activities, mass drug administration for LF control and malaria epidemic investigation.
- Provided training support for Laboratory Technicians, District VBD Consultants, students of Medical and Homeopathic Colleges, examination of malaria/filaria blood slides, cross-checking of blood slides and running Malaria Clinic at the Field Unit for societal benefits.

5.9 Ranchi (Jharkhand)

- Mosqutio fauna survey was undertaken with particular reference to anopheline fauna in Jharkhand state.
- Breeding habitats of *An. fluviatilis, An. culicifacies, An. annularis* and *An. splendidus* were mapped in selected areas of Ranchi district.
- Studies on the sibling species composition of *An. culicifacies, An. fluviatilis* and *An. annu-*

laris species were undertaken.

- Insecticide susceptibility status of *An. culicifacies*, *An. fluviatilis* and *An. annularis* was monitored in Ranchi , Gumla and West Singhbhum districts of Jharkhand state.
- Field evaluation of Net Protect LLIN (impregnated with deltamethrin) against malaria vectors and its impact on malaria incidence was undertaken in Jharkhand.
- Short-term comparative field evaluation of deltamethrin impregnated woven flat yarn of polyethylene zero vector (Durable Lining) to assess bio-efficacy, impact on disease transmission and acceptability to the community and IRS in village(s) of Jharkhand.
- Filariasis survey in Ranchi, Garhwa and Gumla districts of Jharkhand state.
- Monitoring the therapeutic efficacy of ACT, (Artesunate + Pyrimethamine & Sulphadoxine) against uncomplicated *P. falciparum* malaria in tribal area of Ranchi district.
- Diagnostic and treatment services were provided to malaria and filarial patients attending the clinic at field unit.

5.10 Rourkela (Odisha)

- Studies on development of field site for malaria vaccine trial were continued.
- WHOPES phase III evaluation (household randomized trial) to compare insecticidal efficacy and community acceptance of long-lasting insecticidal net (DuraNet®) with conventional insecticide treated nets in India is under progress in study villages under Bisra PHC in Sundargarh district.
- Studies were completed on extended evaluation of the bio-efficacy of field distributed deltamethrin treated long-lasting insecticidal nets (PermaNet® 2.0) against malaria vectors in Odisha.
- Extended evaluation of Olyset long-lasting insecticidal nets (LNs) were undertaken.
- Monitoring of insecticide resistance in malaria vectors was undertaken in four districts of Odisha.
- Studies were completed to assess epidemiological impact of rotation of insecticide for indoor residual spraying in malaria endemic area of Sundargarh district, Odisha.
- Study was conducted on the therapeutic efficacy of Artesunate + Sulfapyrimethamine (ACT) in uncomplicated *P. falciparum* patients in Khammar PHC of Angul district, Odisha.
- A GCP trial is being conducted on phase III, randomized, open label, multicentre study to assess the antimalarial efficacy and safety of arterolane (RBx 11160) maleate and piperaquine phosphate co-administration and Coartem® in patients with acute uncomplicated *P. falciparum* malaria.
- Phase II, multicentric, open label clinical trial of arterolane maleate + piperaquine phosphate in paediatric patients with uncomplicated *P. falciparum* malaria is in progress.
- Studies were initiated on phase II/III randomised clinical trial on the efficacy & safety of artesunate + sulphadoxine pyrimethamine and artesunate + mefloquine to treat uncomplicated *falciparum* malaria in pregnancy.
- Evaluation of malaria rapid diagnostic kits (ParaHIT and EzDx) against microscopy was completed.
- Technical support was provided to the NVBDCP in training manpower, disease outbreak investigations and also in the areas of capacity building on entomological aspects and focused vector control planning.

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Research Support Facilities

6

6.1 Animal house facility

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

6.2 Repository of biological material

6.2.1 Mosquito species

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

Table 1. Details of mosquito species maintained at NIMR Insectary

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

6.2.2 Malaria Parasite Bank

Parasite Bank is the National Repository which is supporting a large number of organizations working on various aspects of malaria. Biological material including non-human and human plasmodia preserved/maintained in the Malaria Parasite Bank are supplied to various research organizations. A total of 1076 isolates of human malaria parasites, viz. *Plasmodium falciparum, P. vivax* and *P. malariae* are cryopreserved in the Bank. During the current year, 116 field isolates including 75 *P. vivax* and 41 *P. falciparum* were collected and cryopreserved in liquid nitrogen. Details of the human and non-human isolates present in the repository are listed in Tables 2 and 3. Since the year 1993, a total of 287 *P. falciparum* samples from different regions were tested for the sensitivity to chloroquine and 187 (65.16%) were found to be resistant to chloroquine (Table 4).

As part of manpower development, scientists/ students/researchers are trained in identification and *in vitro* cultivation of *P. falciparum;* screening of anti-plasmodial properties of medicinal plant extracts/ handling of animals and maintenance of non-human malaria parasites *in vivo*. A total of 167 students/ scientists including 37 foreign scientists were trained in the Malaria Parasite Bank. Several M.Sc. Biotechnology/Microbiology students have completed their dissertation work in the laboratory.

Cell lines available at MPB

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

Table 2. Human malaria parasites collected in the Malaria Parasite Bank (1992–2011)

Parasite species	Collection sites		1992–2004	2005	2006	2007	2008	2009	2010	2011	Total
	States	Districts									
Plamodium falciparum	Andhra Pradesh	1. Vishakhapatnam	12	—	—	—	—	—	—		12
	Asom	1. Sonapur	20	—	—	—	—	—	—		27
		2. Tezpur	6	—	—	—					
		3. Nalbari		—	1						
	Chhattisgarh	1. Jagdalpur	14	—	—	—	—	—	—		40
		2. Bilaspur	—	—	26	—					
	Delhi	1. Delhi	191	—	—	2	5	—	1		199
	Gujarat	1. Anand	4	—	—	—	—	—	—		11
		2. Kheda	7	—	—	—					
	Haryana	1. Gurgaon	25	—	—	—	—	—	—		25
	Jharkhand	1. Ranchi	—	—	—	—	—	—	13		13
	Karnataka	1. Mangalore	—	—	14	—	14	1	—		29
	Madhya Pradesh	1. Mandla/Jabalpur	14	—	—	—	—	—	—		17
		2. Jabalpur							3		
	Meghalaya	1. Tura	—	—	18	—	—				18
	Mizoram	1. Kolasib	—	—	—	6	—		—		6
	Odisha	1. Rayagada	29	—	—	—	—	—			
		2. Sundargarh	42	—	—	—	—	—	—		106
		3. Rourkela	—	—	—	—	13	5	—		
		4. Rourkela	—						17		
	Goa	1. Panaji	—	—	—	—	18		—		18
	Rajasthan	1. Alwar	25	—	—	—	—	—	—		
		2. Bharatpur	35	—	—	—	—	—	—		
		3. Jaisalmer	38	—	—	—	—	—	—		101
		4. Jaisalmer (Pokhran)	—	—	—	—	—	—	3		
	Tamil Nadu	1. Chennai	—	4	—	—	5	—-	—		29
		2. Ramanathapuram	1	—	—	19	—				
	Uttar Pradesh	1. Baharaich	22	—	—	—	—	—			
		2. Gautam Budh Ngr.	37	—	—	—	—	—			
		3. Ghaziabad	17	—	—	—	—	—			141
		4. Allahabad	60	—	—	—	—	—			
		5. Ghaziabad, Modi Ngr	—	—				—	5		
	Uttarakhand	1. Haldwani	—	—	—	—	—	3	—		3
	West Bengal	1. Kolkata	18	—	—	—	—		—		19
		2. Midnapur	1	—	—	—					
	Total *P. falciparum*		618	4	59	27	55	9	42		814
P. vivax	Delhi	1 Delhi	20	—	—	—	18	—	—	1	39
	Uttar Pradesh	1. Shankargarh	4	—	—	—	—		—		
		2. Mirzapur	11	—	—	—	—		—		
		3. Baharaich	2	—	—	—	—		—		29
		4. Gautam Budh Ngr.	2	—	—	—	—		—		
		5. Ghaziabad (M. Ngr)	—	—	—	—	—	—-	10		
	Odisha	1. Rourkela	4	—	—	—	8	2	4		22
		2. Bissam Cuttak	4	—	—	—	—		—		
	Madhya Pradesh	1. Jagdalpur	3	—	—	—	—	—	—		10
		2. Jabalpur	—	—	—			—	7		
	West Bengal	2. Kolkata	1	—	—	—	—		—		1
	Asom	1. Sonapur	2	—	—	—	—		—		2
	Tamil Nadu	1. Ramanathapuram	—	—	—	9	—		—		39
		2. Chennai	—	—	9	—	7	—	14		
	Goa	1. Panaji	—	—	—	—	23		—		23
	Karnataka	1. Mangalore	—	6	—	—	32	14	—		52
	Rajasthan	1. Jaisalmer (Ramgarh)	—	—	—	—	—	—	15		40
		2. Jaisalmer (Pokhran)		—	—			—	25		
	Total *P. vivax*		53	6	9	9	88	16	75	1	257
P. malariae	Odisha	1. Sundergarh	2	—	—	—	—		—		4
		2. Rayagarha	2	—	—	—	—				
	Delhi	1. Delhi	1	—	—	—	—		—		1
	Total *P. malariae*		5	—	—	—	—		—		5
	Total Isolates		676	10	68	36	143	25	116	1	1076

Table 3. Total non-human malaria parasites preserved in Malaria Parasite Bank

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

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

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

Adapted isolates susceptible to chloroquine	54
Adapted isolates resistant to chloroquine	52
NF-54, an infective gametocytes producing strain of *P. falciparum*	1
3D 7A : a clone of NF-54	1
A-4 : a clone with binding property to CD36	1
Dd2: a clone which can invade trypsin-treated erythrocytes	1
Field isolates which can invade trypsin-treated erythrocytes	3
Field isolates which can invade neuraminidase-treated but not trypsin-treated erythrocytes	3
Field isolates which can invade normal erythrocytes but not in neuraminidase or in trypsin-treated erythrocytes	3
Field isolates which can invade both in neuraminidase-treated and in trypsin-treated erythrocytes	5
Field isolates which can form rosettes	3
Field isolate which can bind to CSA	1
Field isolates which can bind to CD36	9
Field isolates which can bind to ICAM-1	2
Isolates with isoenzyme profile of GPI, GDH, ADA & LDH markers	22
Isolates with MSP-1, MSP-2 and GLURP markers	40

Resource Generation

For resource generation, there are parasite bank charges for the biological materials supplied. The funds collected on this account is deposited in the Centre's fund and till now ₹ 3,81,000 have been collected. For the year 2010-11, ₹ 26,000 were collected for the supply of biological material.

6.3 Library

The Institute has one of the best libraries in the country in the field of malaria having at present more than 7550 books, 4300 bound journals, 3700 reprints, 18 video cassettes, 27 audio cassettes, 20 microfilms, 24 theses and 106 national and international reports. About 34 journals are being subscribed besides 8 journals which are received on complimentary and exchange basis. The Library is the supporting centre for researchers of 10 fields units of NIMR located in different parts of the country.

The library collection mainly focuses on literature related to malaria and other vector borne diseases like dengue, chikungunya, Japanese encephalitis, Kala-azar.

Library provides information services to the scientists, research scholars and outside visitors and also foreign delegates. Library provides other necessary services such as current awareness service, paper clipping, citation search, reprographic and reference services.

NIMR Library has been participating in resource sharing works like Union Catalogue of Biomedical Journals developed by the National Informatics Centre-ICMR and a member of Developing Library Networks (DELNET) to fulfil the users need for information. The general house keeping activities are automated using Libsys software and a dedicated server is developed with compatibility for multilingual records, i.e. English, Hindi. The documents are classified and database is updated regularly. The books are all barcoded for automation of issue/return and issue of barcoded library membership card has been done.

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

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Inter-Institutional Collaboration

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

1. The National Institute of Health (NIH), USA has recently recognized NIMR as one of the Centres of Excellence in malaria research to study the complex malaria in India. This recognition comes with funding for seven years to undertake cutting-edge modern biological research on several aspects of malaria, e.g. malaria epidemiology, malaria transmission dynamics, mosquito vector ecology, early-warning for drug resistance by population genetic studies, malaria parasite genomics, etc. In collaboration with scientists from the New York University, and Penn State University, USA, three field units of NIMR, Rourkela, Chennai and Nadiad will actively participate in the research programmes to fulfil the goal of the Centre of Excellence funded by the NIH to NIMR.
2. Evaluation of therapeutic efficacy of antimalarials in collaboration with the NVBDCP, Delhi and funded by the World Bank.
3. Pharmacovigilance of antimalarials in India in collaboration with AIIMS, New Delhi and NVBDCP, and funded by the World Bank.
4. Clinical trials of antimalarial agents in collaboration with Medical Colleges, Guwahati and Goa; Wenlock Hospital, Mangalore; Tata Main Hospital, Jamshedpur; Mahadevi Birla Hospital, Ranchi; Ispat General Hospital, Rourkela; Community Welfare Hospital, Rourkela and funded by agencies like Medicines for Malaria Venture, Geneva, Drugs for Neglected Diseases *initiative* (DND*i*), Geneva and Ranbaxy.
5. Quality Assurance of rapid diagnostic kits for malaria in India, in collaboration with NVBDCP, Delhi and funded by the World Bank.
6. Phase II/III clinical trials of ACT to treat uncomplicated *P. falciparum* malaria in pregnancy in collaboration with London School of Hygiene and Tropical Medicine and funded by MiP Consortium.
7. Phase II clinical trial of Arterolane maleate and piperaquine phosphate in collaboration with Ranbaxy Laboratories Limited, Gurgaon; Tata Main Hospital, Jamshedpur; Ispat General Hospital, Rourkela; Wenlock Hospital, Mangalore; Community Welfare Society Hospital, Rourkela.
8. 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 and funded by London School of Hygiene and Tropical Medicine, London.
9. A multicentric trial to detect *in vivo* resistance of *Plasmodium falciparum* to artesunate in patients with uncomplicated malaria and funded by the Department for International Development.
10. Application of attracticide (oviposition pheromone in combination with insect growth regulator) for surveillance and control of chikungunya and dengue mosquitoes in collaboration with Defence Research and Development Establishment (DRDE), Gwalior, Madhya Pradesh; Municipal Corporation of Delhi and NVBDCP, Delhi.

11 Micro level mapping of malaria vectors using GIS in bordering districts of Asom and Arunachal Pradesh to assist malaria control in collaboration with DRL, Tezpur, Asom.

12. Primary screening of medical plants from north-eastern states of India for their anti-malarial activity in collaboration with DRL, Tezpur, Asom.
13. Health impact assessment of Indira Sagar Dam and resettlement colonies in SSP Reservoir impoundment areas in Narmada Valley in Madhya Pradesh in collaboration with National Institute of Virology, Pune; National Institute of Cholera and Enteric Diseases, Kolkata; and Narmada Valley Development Corporation.
14. Development of site for malaria vaccine trial at Sundargarh district, Odisha in collaboration with International Centre for Genetic Engineering and Biotechnology, New Delhi and the State Government of Odisha.
15. Preparation of a field site for malaria vaccine trial in and around Jabalpur funded by ICMR task force and Center for Disease Control and Prevention (CDC), Atlanta, USA.
16. Developing a framework for predicting malaria outbreaks in rural and urban areas of Gujarat and Rajasthan in India in collaboration with Michigan University, Princeton University, London School of Hygiene and Tropical Medicine, London, BISAG, Gandhinagar, Govt. of Rajasthan and Gujarat, funded by Michigan University, U.S.A.
17. Differential diagnosis of malaria using HRP2 and pLDH based dignotic kits, in collaboration with M/s Bhat Biotech (P) Ltd., Bengaluru.
18. Capacity strengthening of laboratory facilities for phase I testing and evaluation of Public Health Pesticides in collaboration with WHOPES, Geneva.
19. Exotic fish and the biological control of malaria in a biodiversity hot spot in collaboration with Gatty Marine Laboratory, St. Andrew's University, Scotland, UK.
20. Screening and evaluation of selected members of Rutaceae from southern India for anti-mosquito and antimalarial activities in collaboration with Institute of Wood Science and Technology.

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Human Resource Development

8.1 Ph.D. Programme

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

8.2 Ph.D. Awardee

Mahesh B. Kaliwal was awarded Ph.D. degree from Goa University, Goa, on Bioecology of *Culex quinquefasciatus*, the principal vector of *Lymphatic filariasis* in Goa.

8.3 M.Sc. Projects

This year, more than 25 students of M.Sc. in Life Sciences/Biotechnology/Bioinformatics successfully completed their projects/dissertations under the supervision of NIMR scientists.

8.4 Trainings Imparted

NIMR conducts regular training programmes which are as under:

- Collection, cryopreservation, revival and transportation of malaria parasite isolates/ strains.
- *In vitro* cultivation of erythrocytic stages of *P. falciparum*.
- 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.
- NIMR has conducted series of training programmes for microscopists, district malaria officers, entomologists, VBD consultants, technicians on various aspects of malaria.

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January to December 2010

9

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Other Activities

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10.1 National Science Day Celebration

Open House/Health camps were organized at NIMR for the students of Queens Valley Public School, Dwarka, New Delhi for a week during February 2011. Popular lectures/speeches on basic knowledge and prevention from mosquito bites were delivered by the scientists every day. Various life stages of mosquitoes like eggs, larvae, pupae and adults of medically important vector mosquito species were demonstrated. Charts showing life cycle of malaria parasites (*Plasmodium falciparum* and *P. vivax)* were displayed. The causative agents of malaria, viz. *Plasmodium falciparum* and *P. vivax* were demonstrated under microscope to the children. Exhibitions in Hindi as well as English, highlighting several aspects of mosquito borne diseases prevention and control were held. Scientists interacted with the students and discussed various aspects of Vector Borne Diseases, and easy methods of prevention and control. Relevant videos were shown. The students were also involved in the question sessions with scientists/faculty and encouraged to clear their doubts.

10.2 Video Recording

Video Recording work was carried out on occasions of various meetings/workshops/ functions and field work activities held at NIMR or other places. Special effects were incorporated in video films produced by the NIMR.

10.3 Distribution of Video CDs

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

10.4 Photography

In the photography section, following photography work was carried out on various occasions/meetings/trainings/workshops/field surveys/functions held at NIMR and other places.

1. Foundation stone-laying ceremony of animal facility at NIMR, Dwarka.
2. Training courses for Epidemiologists, Medical Officers, District Health Officers, Additional District Health Officers, Chief District Health Officers, and Regional Chief Health Officers of Gujarat, were covered.
3. Photographs of *Hindi Karyashala*, *Hindi Diwas*, Group-D training, E.C.R. Division and Lectures delivered by Dr Neena Valecha to IHMR students were taken.
4. Photography coverage of "WHO Informal Consultation on Standard Protocol Development for Estimating Malaria Disease Burden in South-east Asia (SEA) Region, WHO, SEARO was done.

In addition, the Photographs of Nobel Prize winner Prof. Peter Agre and other distinguished visitors, malaria patients and also the mosquito breeding sites in Dwarka, New Delhi, European Molecular Biology Organization Lecture Course "Molecular and Evolutionary Genetics in Malaria"; National Science Day celebrations at NIMR, RAC/SAC meetings at NIMR were taken.

10.5 Documentation Cell

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

1. Research projects undertaken by NIMR from the year 1981 to 2010 were tabulated and updated.
2. Reprints of published research papers by NIMR scientists for the year 2010 were collected.
3. Contents of *Journal of Vector Borne Diseases* and *Malaria Patrika* were updated and compiled.
4. Published research papers pertaining to the drug resistance in malaria in India were collected to prepare data bank for anti-malarials against which resistance have been reported in various Indian states.

10.6 Publications

The Publication Division of the NIMR has been publishing scientific quarterly, *Journal of Vector Borne Deseases* regularly. The journal stood at third position among the Indian Biomedical journals for the year 2010 as per the rankings of SCImago journal ranking powered by Scopus database (*www.scimagojr.com*). Published four issues of *Malaria Patrika* in Hindi during the year and two issues of *Plasmodium* Newsletter both in Hindi and English. Besides regular periodicals of the Institute also published multi-coloured annual reports of the Institute as well as IDVC project, and bilingual compendium of HIA on Narmad Valley.

10.7 Workshops/Meetings/Training courses organized

10.7.1 Informal Consultation on Standard Protocol Development for Estimating Malaria Disease Burden in SEA Region

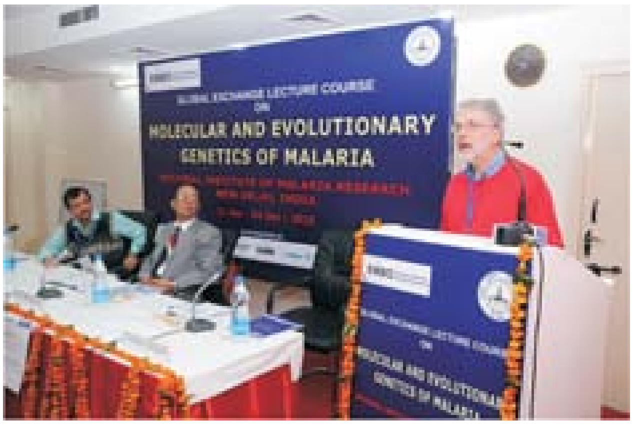
NIMR organized a WHO-ICMR Informal Consultation on Standard Protocol Development for Estimating Malaria Disease Burden in SEA Region from 11 to 13 August 2010. Scientists from NVBDCP, various NIMR field units, WHO, Government of India and renowned statisticians participated in the consultation

Participants of the Informal Consultation on Protocol Development meeting. Dr V.M. Katoch, Secretary, Department of Health Research, Ministry of Health and Family Welfare and Director General, Indian Council of Medical Research inaugurated the consultation. The deliberations of the consultation led to the consensus on common methodology for estimating malaria disease burden in the member countries of the region.

10.7.2 Global Exchange Lecture Course on "Molecular and Evolutionary Genetics of Malaria"

The European Molecular Biology Organization (EMBO), Heidelberg, Germany has funded to conduct a Global Exchange Lecture Course on "Molecular and Evolutionary Genetics of Malaria" at NIMR, New Delhi. The course was conducted at NIMR from 21 November to 4 December 2010. About 40 Ph.D/Post-doctoral students and junior faculties from various reputed Universities and Institutes of India participated in the Lecture Course. More than 20 eminent scientists from India and abroad, working on various aspects on malaria and evolutionary genetics have delivered lectures on their field of research. The course was inaugurated by the Director General, ICMR and the Secretary, Department of Health Research, Government of India, Dr V.M. Katoch on 22nd November 2010. Dr V.K. Dua, Director Incharge of NIMR and Prof. Wolfgang Stephan, from University of Munich, Germany also graced the occasion. Prof. R.C. Mahajan, Chairman of the NIMR Scientific Advisory Committee also graced the occasion on the fifth day of the Lecture Course and inspired the young participants on the importance of advanced research on malaria for developing opposite control measures. The course structure covered lectures, practicals and field visits for malaria sample collections. All the participants also presented their research work in the form of poster displays. The valedictory function was graced by Prof. Aditya P. Dash (former Director of NIMR), Regional Advisor of Vector Borne Diseases of the World Health Organization of the Southeast Asia Region. Dr V.K. Dua, Prof. Christian Doerig (Lussane, Switzerland) and Dr Estella Poloni (Geneva, Switzerland) also graced the occasion.

Dr V.M. Katoch inaugurating the EMBO lecture course at NIMR. Present in the chair are Dr V.K. Dua (Left) and Prof. W. Stephen (Right).



Dr W. Stephen addressing the participants

Participants of the EMBO lecture course

10.7.3 Training Courses Conducted at NIMR

The Institute continued its work on human resource development and carried out training and orientation programmes for about 50 CMOs, DMOs and other health authorities of Gujarat and of Delhi. Training course on vector borne diseases in the Health Department of Municipal Corporation of Delhi was also conducted for newly appointed epidemiologists and entomologists. Training course for state, regional and district level health officers was also organized to refresh their knowledge on the malaria control measures. Another training


Lectures of subject experts


Demonstration of mosquito species identification


Demonstration of total catch


Identification of larvae samples on site

programme for the spray squad of MCD was also organized in the month of September to train them on techniques introduced to control the vectors of malaria, dengue and chikungunya during the Common Wealth Games. An Induction Training for District VBD Consultants to discuss various dynamics of vector control and to discuss new ways for control strategies was also organized from November to December 2010. In this training 23 participants had undergone the orientation programme. Apart from the lectures by the subject experts, epidemiological, entomological and industrial malaria field training was also given to the participants in the foothills of Shivalik ranges.

10.8 Awards and honours received

1. Dr Jyoti Das received DBT-Crest (Cutting-edge Research Enhancement and Scientific Training by the Department of Biotechnology, Govt. of India) award for her distinguished contributions in the field of Immunology.

10.9 Foundation stone-laying ceremony of Test Research Laboratory

The foundation stone-laying ceremony of Test Research Laboratory (Animal House Facility) was held on 6th April 2010. Dr V. M. Katoch, Secretary Department of Health Research and Director General, Indian Council of Medical Research was the Chief Guest. Prof. R.C. Mahajan, Chairman, Scientific Advisory Committee, NIMR, Dr S. Pattanayak, Chairman, Research Advisory Committee (Epidemiology), Prof. A.P. Dash, Regional Advisor, WHO-SEARO, Sh. Sanjiv Datta, Financial Advisor, ICMR were the Guests of Honour. Dr V.K. Dua, Director Incharge, NIMR briefed the guests about the facilities at the proposed Animal House. He told that this would be a state-of-the art facility with all the modern equipments and will have segregated clean and dirty areas and that CPCSEA norms have been taken care of while designing the facility. Guests laid the foundation stone of the building. Dr Katoch, in his address urged the scientists to carry out

Dr Katoch addressing the audience. Also present, Sh. Datta, Prof. Dash, Dr Pattanayak, Prof. Mahajan and Dr Dua

multidisciplinary studies and said that the expectations will now increase from NIMR since it is now well-equipped and this new facility will add to it. Sh. Datta assured to provide financial support to the new facility as well as NIMR.

10.10 Conferences/Symposia/Meetings attended and lectures delivered

Atul PK

1. National symposium on new paradigms in laboratory animal science in an era of advanced bio-medical research at IVRI, Izatnagar, India.
2. International Conference (4th) on the Challenges ahead, at Mathura, Uttar Pradesh, India.

Anvikar Anup

1. 3rd DND*i* Partners' Meeting in collaboration with ICMR, New Delhi on 3 December 2010.
2. WHO-ICMR Informal consultation on Standard protocol development for estimating malaria disease burden in SEA region at New Delhi from 11–13 August 2010.
3. Investigators' Meeting of the project entitled, "Safe and effective treatment of malaria in pregnancy in India" at Ranchi on 8 September 2010.
4. 59th Annual Meeting of the American Society of Tropical Medicine and Hygiene at Atlanta, USA from 3–7 November 2010.
5. Training on Good clinical practices at Ranchi in September 2010.
6. Workshop on Operationalizing pharmacovigilance programme of India at AIIMS, New Delhi from 24–25 November 2010.

Guests laying the foundation stone

Das Aparup

1. Attended, delivered an oral presentation and co-chaired a session in the "Infectious disease genomics and global health" at the Wellcome Trust Centre, Hinxton, Cambridge, UK in September 2010.
2. Attended, delivered an oral presentation and co-chaired a session in the 10th International conference on "Molecular epidemiology and evolutionary genetics of infectious diseases", held at Amsterdam, Netherlands in November 2010.

Dhiman RC

1. Visited Dhaka (Bangladesh) as WHO Temporary Advisor for SEARO-WHO from 19–21 October 2010.
2. Visited Geneva (Switzerland) for participation in Global Earth Observation (GEO) meeting on health and environment from 29–31 March 2011.
3. Attended meetings of Country Coordinating Mechanism-India under Global Fund in the MoH&FW, New Delhi.
4. Delivered invited lecture at MD University, Rohtak on Global climate change and health on 14 October 2010.
5. Delivered invited lecture on Climate change and health at RMRCT, Jabalpur on 23 March 2011.
6. Participated as invited speaker by organizers of 4th DITAN International conference on infectious diseases, at Beijing (China) from 15–17 July 2010.
7. Visited Dhaka (Bangladesh) to deliver invited lecture in 2nd International conference on Climate change and NTDs at Dhaka

(Bangladesh) from 29–30 September 2010.

Mishra Neelima

1. Attended workshop on Malaria treatment and drug resistance monitoring in SEA countries at Bali, Indonesia from 15–17 September 2010.

Nagpal BN

1. Delivered lecture and demonstration on "GIS in vector borne disease control programme" delivered to public health professional from NCDC under MPH programme from 19–20 April 2010.
2. Delivered a lecture on "Pre-Test" at training course for EMOs, DHOs, ADHOs, CDHOs, and Regional Chief Health Officers of Gujarat on Prevention and Control of Vector Borne Diseases organized by NIMR and H&MS Gujarat on 21 June 2010.
3. Delivered lecture entitled, "Mosquitoes and its breeding sites" to Resident Welfare Association of NDMC on 14 August 2010.
4. Delivered series of lectures training course EMOs, DHOs, ADHOs, CDHOs, and Regional Chief Health Officers of Gujarat on Prevention and Control of Vector Borne Diseases organized by NIMR and H&MS Gujarat from 21 June to 17 July 2010.
5. Delivered lecture entitled, "Mosquitoes and its breeding sites" to Resident Doctors and MBBS students of Lady Hardinge Hospital on 17 August 2010.
6. Delivered lecture entitled, "Use of GIS in decision support" in workshop "Geographical information system (GIS) and public health: Practice of good mapping" organized by National Institute of Epidemiology at Chennai from 13–15 September 2010 and from 31 January 2011 to 2 February 2011.
7. Delivered lecture entitled, "Current trends in anopheline biodiversity" in an International symposium on Recent advances in ecology and management of vectors and vector borne diseases organized by Defence Research and Development Establishment (DRDE), Gwalior from 1–3 December 2010.
8. Attended and delivered lecture in a workshop on Community participation for prevention and control of vector borne diseases by RWAs/ NGOs organized by Public Health Department on 19 February 2011.
9. Lecture delivered to Sanitary Inspectors along with the Surveillance Workers & Anti Malaria Jamadars organized by Health Department, NDMC on 18 February 2011.
10. Delivered a series of lectures at Induction training programme for District VBD Consultants jointly organized by Public Health Foundation of India (PHFI), National Institute of Malaria Research (NIMR), National Centre for Disease Control (NCDC) and National Vector Borne Disease Control Programme (NVBDCP) from 18 November 2010 to 21 January 2011.
11. Demonstration and presentation in field visit to Raipur, Chhattisgarh" in an Induction training programme for District VBD Consultants organized by Public Health Foundation of India (PHFI), National Institute of Malaria Research (NIMR), National Centre for Disease Control (NCDC) and National Vector Borne Disease Control Programme (NVBDCP) from 17– 21 January 2011.
12. Attended meeting to participate in Implications of insecticide resistance (IIR) held at WHO-SEARO, New Delhi from 1–2 March 2011.
13. Attended and delivered lecture on "*Aedes aegypti* behaviour" in Informal expert consultation on yellow fever threat organized by WHO at Goa from 23–25 March 2011.
14. Attended meeting of Expert Committee on Estimation of Malaria Deaths at NIMR on 30 March 2011.

Saxena Rekha

1. Attended ESRI India nationwide seminar on New GIS technology trends at Delhi on 12 November 2010.
2. Attended conference on 'Remote sensing and GIS for environment management' organized by Jamia Millia Islamia on 10 August 2010 and presented a poster on "Deforestation and its impact on malaria epidemiology in districts of Assam: A remote sensing and GIS based study".

Singh Vineeta

1. Undergone training on Malaria pathogen for a period of 4 days from 5–8 June 2010 at New York University Langone Medical Centre, New York, USA.

2. Delivered lecture on "Genetic diversity in *P. falciparum* and *P. vivax*" in a Symposium on "Malaria: An update" at J.N. Medical College, A.M.U., Aligarh on 18 December 2010.

Valecha Neena

1. Lecture on "Pharmacovigilance and quality assurance at training workshop for district malaria officers of Madhya Pradesh", at Jabalpur from 10–11 April 2010.
2. Lecture on "Malaria treatment: Past, present and future" at National Conference on Medical Biotechnology Vision 2020 at Maharshi Dayanand University, Rohtak, Haryana on 17 April 2010.
3. Lectures on Chemotherapy of malaria with special reference to drug resistance and its management at Training course for VBD Officers from Gujarat at New Delhi on 22 June 2010.
4. Lecture on Diagnosis and management of DF/DHF & DSS in reorientation training programme for physicians and paediatricians of Sentinel Surveillance Hospitals at NCDC, Delhi on 27 July 2010.
5. Symposium entitled "Challenges and successes of the FACT Project through innovative partnerships for the development of Artesunate combination therapies for malaria" in the 59th Annual meeting of the American Society of Tropical Medicine and Hygiene (ASTMH) at Atlanta, USA from 3–7 November 2010.
6. Lecture on "Malaria treatment in India: Journey from chloroquine to Artemisinin" at International scientific meeting on recent developments in malaria research at ICGEB, New Delhi on 2 December 2010.
7. Lecture on "Fixed dose ACTs for malaria" at 3rd DND*i* Partners meeting in collaboration with Indian Council of Medical Research, at Constitutional Club, New Delhi on 3 December 2010.
8. Lecture on "Pharmacovigilance in public health programmes" at Drug Information Association meeting at Mumbai from 4–5 February 2011.
9. Attended 3rd meeting of the Scientific Advisory Committee on Antimalarial policy and access (MPR) at WHO, Geneva, Switzerland from 31 May to 1 June 2010.
10. Organized "Informal consultative meeting on Standard protocol development for estimating malaria disease burden in SEA region" at WHO-SEARO from 11–13 August 2010.
11. Attended workshop on Malaria treatment policy and drug resistance monitoring in SEA countries at Bali, Indonesia from 15–17 September 2010.
12. Co-chaired MMV's access & delivery advisory committee meeting (ADAC) at Geneva, Switzerland from 5–7 October 2010.

By Ph.D. students

1. Ms. Gauri Awasthi, ICMR-SRF of EGB Laboratory attended and presented a poster on "Infectious disease genomics and global health" at the Wellcome Trust Centre, Hinxton, Cambridge, UK in September 2010.
2. Ms. Jyotsana Dixit, ICMR-SRF, EGB Laboratory attended and presented a poster in the 10th International conference on "Molecular epidemiology and evolutionary genetics of infectious diseases", held at Amsterdam, Netherlands in November 2010.

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11

संस्थान में राजभाषा अधिनियम के अनुपालन की दिशा में वर्ष 2010–11 में भी राजभाषा हिन्दी के प्रगामी प्रयोग के उद्देश्य से राजभाषा अधिनियम 1963 की धारा 3(3) के अंतर्गत आने वाले दस्तावेजों और संस्थान में प्रयुक्त प्रपत्रों का अनुवाद संबंधी कार्य समय–समय पर किया गया। इसके साथ ही राजभाषा स्थिति की समीक्षा हेतु तिमाही बैठकें आयोजित की गईं। यही नहीं संस्थान में राजभाषा विभाग की 'हिन्दी में अधिकाधिक कार्य' किए जाने हेतु प्रोत्साहन योजना को भी वर्ष 2010–11 में लागू किया गया। यहाँ यह भी उल्लेखनीय है कि विज्ञान को हिन्दी से जोड़ने की दिशा में प्रति वर्ष संस्थान द्वारा *मलेरिया पत्रिका* (त्रैमासिक) एवं *प्लाज़्मोडियम* न्यूज़ लैटर (अर्द्धवार्षिक) प्रकाशित किया जाता है। इसके साथ ही प्रति वर्ष की भाँति इस वर्ष भी संस्थान में हिंदी सप्ताह पूर्ण उत्साह के साथ मनाया गया। हिन्दी सप्ताह के दौरान प्रशासन वर्ग के कर्मचारियों के लिए जहाँ पूर्णकालिक हिन्दी कार्यशाला का आयोजन किया गया था वहीं दूसरी ओर विज्ञानीय वर्ग के लिए एक संगोष्ठी का आयोजन किया गया था। इस पूर्णकालिक कार्यशाला में तीन व्याख्याताओं को आमंत्रित किया गया था। इसके साथ ही, निबंध प्रतियोगिता, टिप्पण–प्रारूपण प्रतियोगिता, वाद–विवाद प्रतियोगिता (कर्मचारी वर्ग) वाद–विवाद प्रतियोगिता (अधिकारी वर्ग) का आयोजन किया गया जिसमें संस्थान के प्रशासनिक एवं विज्ञानीय अधिकारियों एवं कर्मचारियों ने भाग लिया।

इस सप्ताह के दौरान उल्लेखित गतिविधियों के अलावा दिनांक 17 सितम्बर 2010 को एक और गतिविधि के रूप में कवि सम्मेलन एवं पुरस्कार वितरण समारोह का आयोजन किया गया जिसका संचालन हिन्दी अधिकारी

हिन्दी कार्यशाला

वैज्ञानिक संगोष्ठी में भाग लेते वैज्ञानिक

वाद-विवाद प्रतियोगिता (अधिकारी वर्ग) में सम्बोधित करते निदेशक प्रभारी

कवि संगोष्ठी का संचालन करती हुई हिन्दी अधिकारी

डॉ. वंदना शर्मा द्वारा किया गया। इस कवि सम्मेलन में तीन प्रतिष्ठित कवियों श्री बाबा कानपुरी, श्री अली हसन मकरेडिया एवं श्री अशोक शर्मा को आमंत्रित किया गया था। संबंधित कार्यक्रम के अंतर्गत अतिथि कवियों के काव्य का आनंद उठाने के साथ-साथ पुरस्कारों का भी वितरण किया गया अर्थात् सर्वप्रथम सप्ताह के दौरान आयोजित प्रतियोगिताओं के पुरस्कारों की घोषणा की गई। इसके अंतर्गत सबसे पहले वाद-विवाद (कर्मचारी) प्रतियोगिता के पुरस्कारों की घोषणा डॉ. नूतन नंदा वैज्ञानिक 'ई' द्वारा की गई और निबंध प्रतियोगिता के पुरस्कारों की घोषणा श्री एन.के. भल्ला, प्रशासनिक अधिकारी द्वारा की गई। इन पुरस्कारों की घोषणा के पश्चात् जाने-माने कवि बाबा कानपुरी ने अपनी कविताओं का पाठ कर सभी को मंत्रमुग्ध कर दिया।

इसके साथ ही टिप्पण-प्रारूपण प्रतियोगिता एवं वाद-विवाद (अधिकारी वर्ग) प्रतियोगिता की घोषणा डॉ. बी.एन. नागपाल वैज्ञानिक 'ई' द्वारा की गई। इन पुरस्कारों को देने के साथ ही कवि संगोष्ठी में आंमत्रित किए गए दूसरे कवि श्री अली हसन मकरोडिया ने राष्ट्र प्रेम के साथ गीता, रामायण एवं पुराणों पर आधारित अपनी कविताओं से सभी को सम्मोहित कर दिया। तत्पश्चात् हिन्दी में अधिकाधिक कार्य करने हेतु लागू वर्ष 2010-11 की प्रोत्साहन योजना के पुरस्कारों की घोषणा डॉ. चन्द्र प्रकाश बत्रा, वैज्ञानिक 'ई' द्वारा की गई। संबंधित पुरस्कार संस्थान के निदेशक प्रभारी महोदय के कर-कमलों द्वारा प्रदान किए गए। इन पुरस्कारों की घोषणा के पश्चात् प्रख्यात राष्ट्र कवि श्री अशोक शर्मा ने अपने काव्य पाठ में हंसी के रंग भरकर सभी को आनंदित कर दिया। अंतत: संस्थान के प्रशासन प्रभारी एवं वैज्ञानिक 'ई' डॉ. बी.एन. नागपाल ने पखवाड़े के दौरान आयोजित गतिविधियों का सफलतापूर्वक संचालन करने हेतु सभी संचालकों को धन्यवाद ज्ञापित करने के साथ ही समग्र कार्यक्रम के आयोजन में संस्थान के निदेशक प्रभारी महोदय व संस्थान की हिन्दी अधिकारी के योगदान की सराहना करते हुए उन्हें हार्दिक धन्यवाद ज्ञापित किया।

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Committees of the Institute

12

12.1 Scientific Advisory Committee

Dr VM Katoch
Secretary, Department of Health Research &
Director General
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Chairman
Prof. RC Mahajan
S.N. Bose INSA Research Professor &
Emeritus Professor
Postgraduate Institute of Medical Education and Research (PGIMER)
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Members
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Director
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Aruna Asaf Ali Marg
New Delhi–110 067

Poster Session during SAC

SAC in progress

Dr S Pattanayak
Former Advisor, WHO-SEARO and
Former Director, NMEP
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Prof. MKK Pillai
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Dr AC Dhariwal
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Dr Rashmi Arora
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Member Secretary
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Director Incharge
National Institute of Malaria Research
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12.2 Research Advisory Committees

12.2.1 Vector Biology & Control

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Member Secretary
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Director Incharge
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RAC, Vector Biology & Control in progress

12.2.2 Parasite Biology & Immunology

Chairman

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RAC, Parasite Biology in progress

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12.3 Research Advisory Committee of IDVC Project

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12.4 Building Advisory Committee

Chairman
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Professor of Eminence
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Convenor
Director
National Institute of Malaria Research
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12.5 Human Ethics Committee

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Dr Neena Valecha
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12.6 Animal Ethics Committee

Chairman

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CPSEA Nominee

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13

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

Scientists 'E'
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Dr RM Bhatt
Dr Sukla Biswas (Retired on 30 Nov 2010)
Dr Vas Dev
Dr Ashwani Kumar
Dr PK Mittal
Dr BN Nagpal
Dr Nutan Nanda
Dr K Raghavendra
Dr AM Reetha (Retired on 1 Dec 2010)
Dr MC Sharma
Dr SK Sharma
Dr MM Shukla
Mr OP Singh
Dr HC Srivastava

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Dr Anup R Anvikar
Dr PK Atul
Dr Aparup Das
Dr Jyoti Das
Dr AK Mishra
Dr Neelima Mishra
Mrs Rekha Saxena

Scientists 'C'
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Dr Alex Eapen
Dr Ruchi Singh
Dr VP Singh

Scientists 'B'
Mr Bhagirath Lal
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Research Scientists
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Dr Raj Kumar
Dr K Padhan
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Dr SP Singh
Dr SN Tiwari

Names are listed in alphabetical order by surname; Staff position as on 31 March 2011.