1

Vector Biology and Control

1.1 Studies on Anopheline Species Complexes

1.1.1 The Culicifacies Complex

Bionomics and Distribution Pattern

Anopheles culicifacies populations from malaria endemic districts, namely Ranchi and Gumla (Jharkhand state) were examined for sibling species composition. In District Ranchi, cytological examination of the samples collected from villages under Primary Health Centres, Angada and Bharno revealed the prevalence of species B and C with predominance of the former, whereas in District Gumla, *An. culicifacies* species B and C were found in almost equal proportion in the villages surveyed. Similarly, in District Bastar (Chhattisgarh state) species B and C were sympatric with predominance of the latter (87.5%). In a longitudinal study in District Jabalpur (Madhya Pradesh), examination of *An. culicifacies* populations for the second successive year revealed the prevalence of species B, C and D in the study villages with predominance of species C (>70%). The established vector species (C & D) together constituted >90% of the total *An. culicifacies* population, whereas proportion of non-vector species B was low suggesting high malariogenic potential of the study area. In all above mentioned districts, *An. culicifacies* sibling species were found to be predominantly zoophagic as revealed by blood meal source analysis.

1.1.2 Fluviatilis and Minimus Complexes (Minimus Group)

Bionomics and Distribution Pattern

An. fluviatilis populations from malaria endemic districts, namely Damtari, Surguja and Korba (Chhattisgarh state) were analyzed for sibling species composition and host preference. In all these districts, only species T was prevalent and was found to be polymorphic for q^1 inversion (Table 1.1.1). In the study areas, this species was found resting predominantly in cattlesheds or mixed dwellings and was totally zoophagic, which indicates its limited role in malaria transmission in the above mentioned districts.

Table 1.1.1. *An. fluviatilis* sibling species composition in the study districts of Chhattisgarh state

Districts	Total identified	Sibling species						HBI
		S	T			U	V	
			$+q^1$	$+q^1/q^1$	q^1			
Dhamtari	34	0	1	7	26	0	0	0
Surguja	33	0	0	4	29	0	0	0
Korba	23	0	0	2	21	0	0	0

$+q^1$, $+q^1/q^1$ and q^1 — Chromosomal inversion genotypes; HBI— Human blood index.

Similarly, in Ranchi and Gumla districts, only *An. fluviatilis* species T was found prevalent in the study areas and was totally zoophagic. Species T in these districts was sympatric with *An. culicifacies* sibling species B and C.

Identification of *An. fluviatilis* Species 'T' as Malaria Vector

An. fluviatilis species 'T' is regarded as a non-vector due to its zoophagic nature. However, for the first time, it was found that *An. fluviatilis* which were malaria sporozite positive as determined by sporozoite enzyme linked immunosorbent assay (ELISA) are *An. fluviatilis* species T. We characterized four specimens of sporozoite positive *An. fluviatilis* from Jabalpur and two specimens from Ranchi by sequencing ITS2. It was found that their ITS2 sequences are homologous to *An. fluviatilis* T. This is the first record of *An. fluviatilis* T incriminated as a malaria vector.

Molecular Characterization of *An. fluviatilis* and *An. minimus* from Jalpaiguri

A total of 43 specimens of morphologically identified *An. minimus* and 125 specimens of *An. fluviatilis* were characterized for sibling species through polymerase chain reaction (PCR) assay and PCR-RFLP. All *An. minimus* samples were identified as *An. minimus* A (sensu stricto). Out of 125 *An. fluviatilis* examined, 118 were identified as species T, and rest seven were identified as *An. minimus* A. Later these were confirmed to be *An. minimus* A based on their ITS2 sequences. Some of the *An. fluviatilis* samples were also sequenced for ITS2 rDNA for confirmation of PCR results.

"Anopheles fluviatilis species T was incriminated as malaria vector for the first time which was earlier thought to be a non-vector"

1.1.3 The Annularis Complex

Identification of *An. annularis* Species 'A' as Malaria Vector

Five specimens of *An. annularis* from District Ranchi, Jharkhand, which were found to harbour *Plasmodium falciparum* sporozoites were identified as *An. annularis* species A based on ITS2 sequence analysis.

1.2 Vector-Parasite Interactions

1.2.1 Susceptibility of *Anopheles fluviatilis* Species 'T' and 'U' to *Plasmodium vivax*

Susceptibility of *An. fluviatilis* species T and U to *P. vivax* was ascertained by feeding the mosquitoes on *P. vivax*-infected blood samples through artificial membrane. The *An. stephensi* was used as positive control. The oocyst rates in *Anopheles fluviatilis* species T, U and *An. stephensi* were 42.37, 45.63 and 58.78%, respectively. The geometric mean number of oocysts were 17.70, 17.44 and 17.70, respectively. The oocyst rates and geometric mean number of oocysts in these species didn't differ significantly. The sporozoite rate in *An. fluviatilis* species T and U was 39.5 and 32.03%, respectively as against 54.95% in *An. stephensi*. Statistically there was no significant difference in sporozoite rates between species T and *An. stephensi* but significantly lower sporozoite rate was recorded in species U as compared to *An. stephensi* ($\chi^2 = 0.0340$, df = 1, $p < 0.01$).

The overall proportions of mosquitoes with oocyst count ranges of 0, 1–50, 51–100 and 101–200, for each species infected with *P. vivax* are shown in Fig. 1.2.1. On the basis of above finding, it may be concluded that *An. fluviatilis* species T and U are susceptible to *P. vivax*. Thus, it may be extrapolated that *An. fluviatilis* species T and U may act as vectors when they are in high density in an endemic area and/or in the absence of other vector species.

1.2.2 Phenoloxidase Activity in Different Members of the Culicifacies Complex

The members of Culicifacies Complex differ in their susceptibility to plasmodial sporogonic

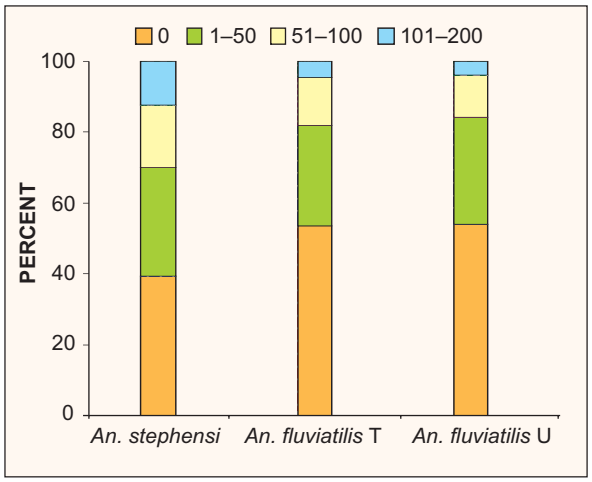

Fig. 1.2.1: Proportion of oocyst densities in species T and U of Fluviatilis Complex against *P. vivax*

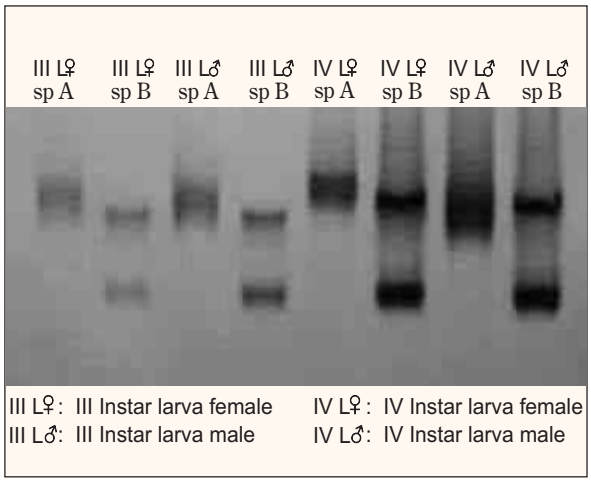

Fig. 1.2.2: Comparison of PO activity in III & IV instar male and female larvae of *An. culicifacies* species A and B

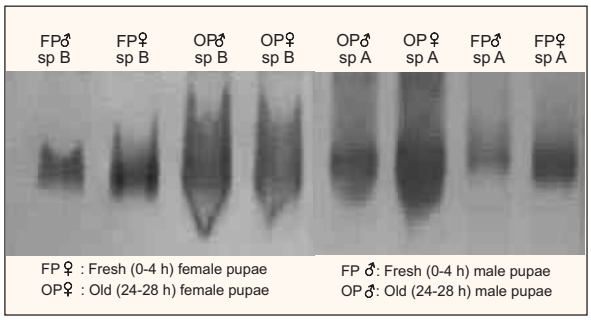

Fig. 1.2.3: Comparison of PO activity in fresh and old male and female pupae of *An. culicifacies* A and B

success. Encapsulation of invading parasite is one of the mechanisms by which sporogony is aborted in some strains of *An. culicifaces* B. The phenoloxidase (PO) is the prime enzyme (14.18.1.1) responsible for melanisation, which is secreted by fat bodies and/or hemocytes in its inactive form, the prophenoloxidase (PPO), and further activated by serine proteases. We studied the differential activity and isozymes of PO in the members of *An. culicifacies.* For the purpose, cyclic colonies of *An. culicifacies* sibling species A (Dehra strain) and species B (Haldwani strain) were used.

Qualitative Assay

Qualitative assay of enzyme phenoloxidase was done using PAGE to study the isozyme profile of both the sibling species. Tyrosine (mono-phenol compound) and DOPA (a di-phenol compound) were used as substrate to visualize the enzyme on the polyacrylamide gel for mono and di-phenoloxidase activity respectively. Third and fourth instar larvae of *An. culicifacies* B showed two bands of enzyme on substrate staining with more or less identical enzyme activity. Both the bands were apart by very little gap when resolved on 5% SDS PAGE, which inferred slight difference in their molecular weight. These bands may be marked as slow and fast isozymes of phenoloxidase enzyme (Fig. 1.2.2). Same isozyme pattern was observed in both the sexes of III and IV instar larvae. One broad and light band of PO was observed in fresh (0–4 h old) and old pupae (24–28 h old) of *An. culicifacies* B. This may be due to high amount of protein loaded per well because of less activity of PO in pupae as shown in Fig. 1.2.3.

On the other hand, *An. culicifacies* A showed only one darker and broad band of PO in III and IV instar male and female larvae (Fig. 1.2.2). The Rf (Resolution factor) value of isozyme present in *An. culicifacies* A is nearly equal to that of slow isozyme present in species B. So, it may be concluded that species B has two isozymes of PO— slow and fast, while species A has only one, i.e. slow. Similarly, slow isozymes of PO were also found in fresh (0-4 h) as well as old (24–28 h) male and female pupae of *An. culicifacies* A (Fig. 1.2.3). The isozymes of PO found in different develop-

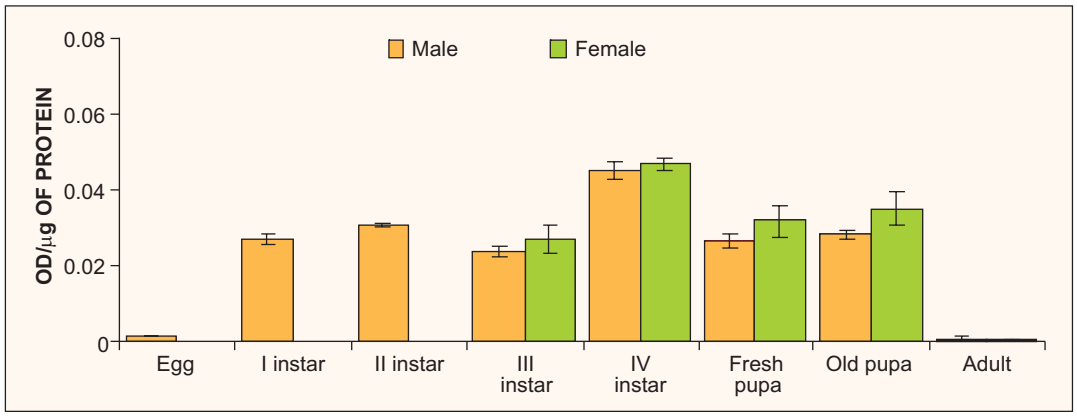

Fig 1.2.4: Quantitative assay of phenoloxidase activity in different developmental stages of male and female *An. culicifacies* A

mental stages of *An. culicifacies* species A and B showed activity for mono- as well as diphenoloxidase.

Quantitative Assay

Enzyme activity was measured in optical density (OD) per microgram of protein. Activity of PO was analyzed among different developmental stages of *An. culicifacies* species A. Increase in enzyme activity was observed with the development. First, second and third instar larvae showed more or less equal activity. On the other hand, IV instar larvae showed significantly higher enzyme activity when compared to III instar larvae ($p < 0.0001$, both for male and female). While, in the later stages of development a steep decrease in enzyme activity was observed in pupae (~1.5 fold) and 4–6 days old adult (~60 fold) (Fig. 1.2.4).

Activity of PO was also analyzed among different developmental stages of *An. culicifacies* B where a significant increase in PO activity was noticed among III and IV instar larvae ($p > 0.001$, both for male and female). A sudden decrease (~3 fold) was observed in pupal stage (Fig. 1.2.5). Rest of the developmental stages could not be analyzed because of non-availability of biological material due to contamination in the colony.

On the basis of above finding one may infer that the IV instar larvae showed highest enzyme activity as compared to other developmental stages in both the species and sexes. In comparison to males, females showed slightly high enzyme activity in all developmental stages. *An. culicifacies* species B showed high PO activity as compared to species A.

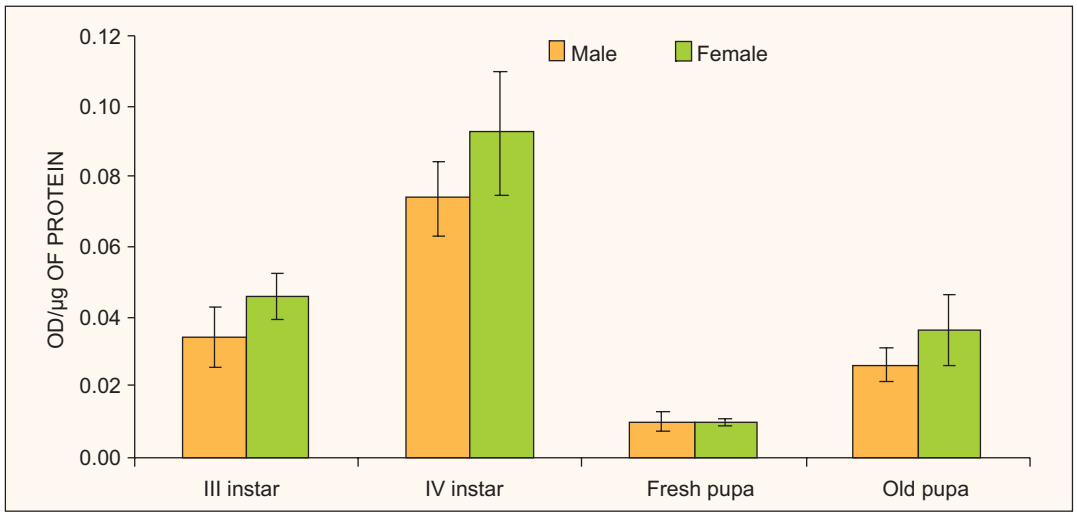

Fig. 1.2.5: Quantitative assay of phenoloxidase activity in different developmental stages of male and female *An. culicifacies* B

1.2.3 Nitric Oxide Synthase (NOS) in Malaria Vectors

Molecular Characterization of NOS in *An. stephensi*

Nitric oxide (NO) is a ubiquitous free radical produced by Nitric oxide synthase (NOS), that plays a protective role against the invading pathogen as well as a role of a biological messenger. In *An. stephensi*, one of the major malaria vectors in India, the NOS gene is encoded by 19 exons, spanning 33 kilobases with an open reading frame of 1247 amino acids. The induction of NO during *P. falciparum* and *P. berghei* infections in *An. stephensi* and the importance of promoter polymorphisms in altering gene expression levels prompted us to study the genetic diversity in NOS promoter by employing sequencing methods, as well as to measure the induction of NO synthesis on *P. vivax* infection in *An. stephensi* using Griess method. No variation was observed in the ~300 bp NOS promoter region of *An. stephensi*, analyzed from different regions of the Indian subcontinent, but a difference at four nucleotide positions was observed when compared to previously reported sequence by Luckhart *et al.* 1999.

A highly significant elevation in NO concentration was observed in *P. vivax* infected mosquitoes when compared to unfed ones (p = 0.0001) (Fig. 1.2.6). In addition, variation in NO levels was observed among unfed mosquitoes collected from different regions of India

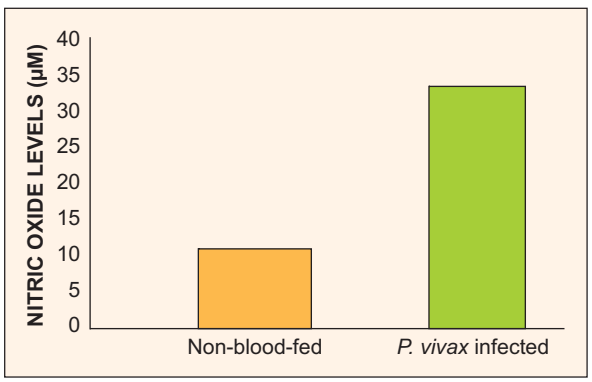

Fig. 1.2.6: A graphical representation of nitric oxide levels, measured using Griess method in *P. vivax* infected and non-blood-fed *An. stephensi* samples

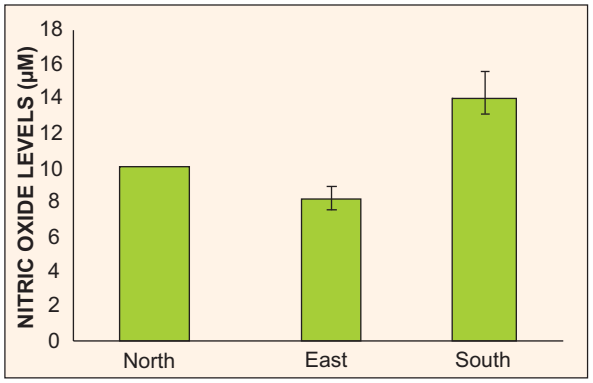

Fig. 1.2.7: A graphical representation of nitric oxide levels measured using Griess method in *An. stephensi* samples collected from North, East and South Zones of the Indian subcontinent

(Delhi, Punjab, Hardwar, Chennai and Kolkata). Moreover, NO concentration was found to be significantly different amongst unfed *An. stephensi* populations of North, East and South zones of India (North *vs* South, p = 0.0005; North *vs* East, p = 0.015; South *vs* East, p = 0.0006) (Fig. 1.2.7). A substantial increase in the NO levels was observed in *An. stephensi* as early as 2 h post-vivax infection up to two days and decline thereafter (Fig. 1.2.8). Taken together, from the above results, it can be concluded that the elevated levels of NO as early as 2 h post-blood meal could be a consequence of NOS induction in response to the parasite invasion of the midgut, suggesting that the parasite (asexual blood stages and gametocytes) itself is the prime target of NO mediated destruction. Moreover, the apt explanation for increased NO levels up to two days post-blood meal could be that NO may be responsible for maximum interference during this sexual phase of parasite development. The intrapopulation variation in NO concentration could be attributed to the variation at the gene level. Therefore, NO levels could be of biological importance, adversely affecting *P. vivax* development.

1.2.4 Biochemical Determination of Nitric Oxide Metabolites, Nitrate and Nitrite in *An. culicifacies* by HPLC

The diverse physiological and pathological role of nitric oxide in innate immune defenses against many intra and extracellular patho-

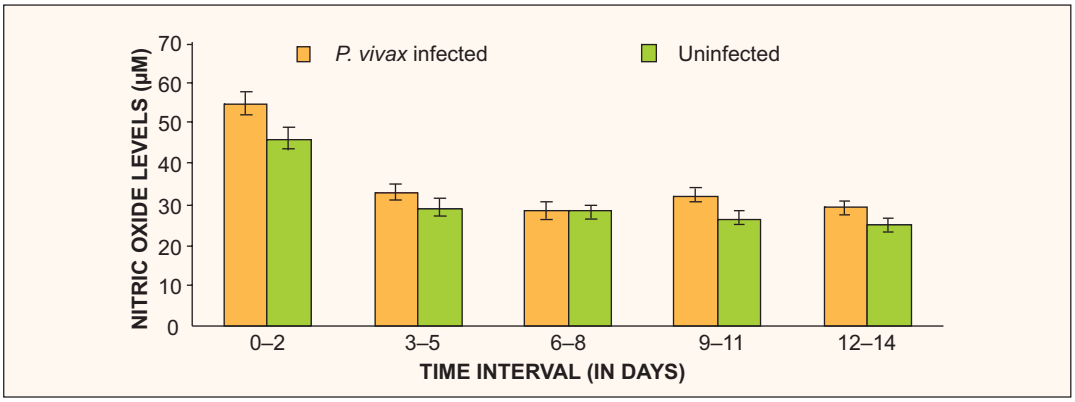

Fig. 1.2.8: A graphical representation of NO levels in *P. vivax* infected and uninfected blood-fed *An. stephensi* samples collected at regular intervals post-blood meal. NO levels were measured using Griess method

gens, have led to the development of various methods for determining nitric oxide (NO) synthesis. NO metabolites, nitrite (NO_2^-) and nitrate (NO_3^-) are produced by the action of an inducible *An. culicifacies* NO synthase (AcNOS) in mosquito midguts and may be central to antiparasitic arsenal of these mosquitoes.

While exploring a plausible mechanism of refractoriness based on nitric oxide synthase physiology among the sibling species of *An. culicifacies*, a sensitive, specific and cost-effective high performance liquid chromatography (HPLC) method was developed, which is not influenced by the presence of biogenic amines, for the determination of NO_2^- and NO_3^- from mosquito midguts and haemolymph. This method is based on extraction, efficiency, assay reproducibility and contaminant minimization. It entails de-proteinization by centrifugal ultra filtration through ultracel 3K filter and analysis by high performance anion exchange liquid chromatography (Sphereclone, 5µ SAX column) with UV detection at 214 nm. The lower detection limit of the assay procedure is 50 pmoles in all midgut and haemolymph samples. Retention times for NO_2^- and NO_3^- in standards and in midgut samples were 3.42 and 4.53 min respectively (Fig. 1.2.9). Assay linearity for standards ranged between 50 nM and 1 mM. Recoveries of NO_2^- and NO_3^- from spiked samples (1–100 µM) and from the extracted standards (1–100 µM) were calculated to be 100%. Intraassay and interassay variations and relative standard deviations (RSDs) for NO_2^- and NO_3^- in spiked and unspiked midgut samples were 5.7% or less (Tables 1.2.1 and 1.2.2). Increased levels NO_2^- and NO_3^- in haemolymph and midguts (Figs. 1.2.10 and 1.2.11) of *An. culicifacies* sibling species B in comparison to species A reflect towards a mechanism of refractoriness based on AcNOS physiology.

Table 1.2.1. Recoveries of NO_2^- and NO_3^+ in extracted KNO_2/KNO_3 standards (Mean ± SD, n = 8)

Concentration	Recovery	
	NO_2^-	NO_3^-
48 nM	90.7 ± 1.9	28.3 ± 3.4
97 nM	94.5 ± 6.2	54.8 ± 4.3
195 nM	94.7 ± 5.9	87.8 ± 5.1
390 nM	93.4 ± 4.0	80.2 ± 1.5
780 nM	93.6 ± 1.9	93.7 ± 4.5
1.56 µM	93.7 ± 2.9	87.9 ± 3.9
3.12 µM	91.8 ± 1.6	95.2 ± 2.9
6.25 µM	98.5 ± 2.4	98.5 ± 3.1
12.5 µM	97.1 ± 1.8	94.8 ± 1.9
25 µM	99.0 ± 5.4	100.5 ± 3.2
50 µM	100 ± 5.8	97.3 ± 2.9
100 µM	97.1 ± 2.4	99.2 ± 4.9

"HPLC was performed for the determination of NO_2 & NO_3 from mosquito mid guts and haemolymph"

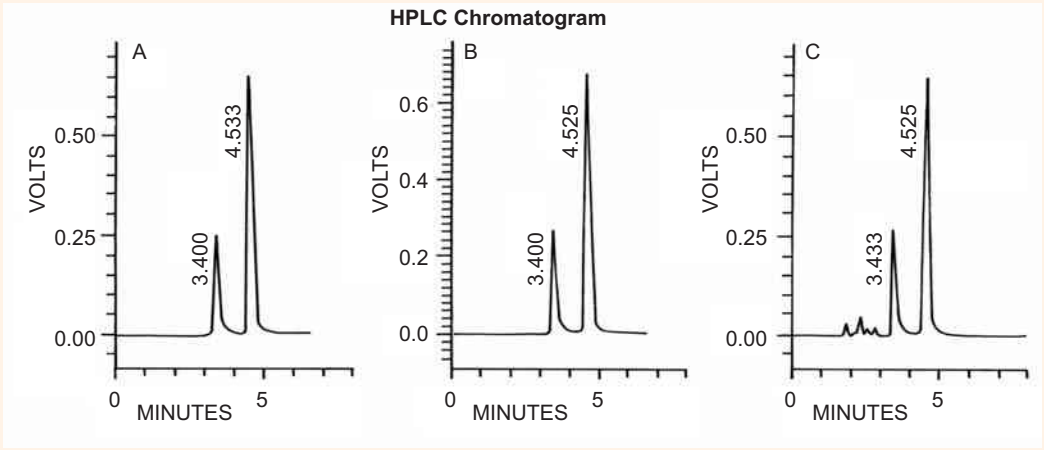

Fig.1.2.9: HPLC analysis of nitrite and nitrate. Chromatograms of an aqueous standard containing 25 µM nitrite and nitrate (A); washed and spiked *An. culicifacies* midguts obtained under control (B); and *An. culicifacies* haemolymph (C)

Table 1.2.2. Relative standard deviations (RSDs) and recoveries for NO_2^- and NO_3^- from mosquito mid-gut spiked* with 1–100 µM KNO_2/KNO_3 (mean ± SD, n=6)

Concentration (µm)	NO_2^-			NO_3^-		
	Intra assay RSD (%)	Inter assay RSD (%)	Recovery (%)	Intra assay RSD (%)	Inter assay RSD (%)	Recovery (%)
0	8.3	8.9	–	4.2	5.6	–
1	8.8	9.3	94.4 ± 4.4	8.7	9.9	98.9 ± 2.2
2.5	7.1	9.8	98.2 ± 6.6	7.2	8.5	98.5 ± 2.4
5	6.7	9.9	95.2 ± 2.9	3.9	5.9	97.1 ± 1.8
10	5.1	9.2	98.5 ± 3.1	3.1	2.9	99 ± 5.4
25	4.5	5.3	99.8 ± 1.9	2.2	3.0	100 ± 5.8
50	5.5	4.9	100.5 ± 3.2	2.9	2.6	94.5 ± 6.2
100	3.9	4.7	97.3 ± 2.9	1.2	4.1	94.7 ± 5.9

*Unspiked midgut samples, i.e. midgut samples to which exogenous NO_2^- and NO_3^- has not been added

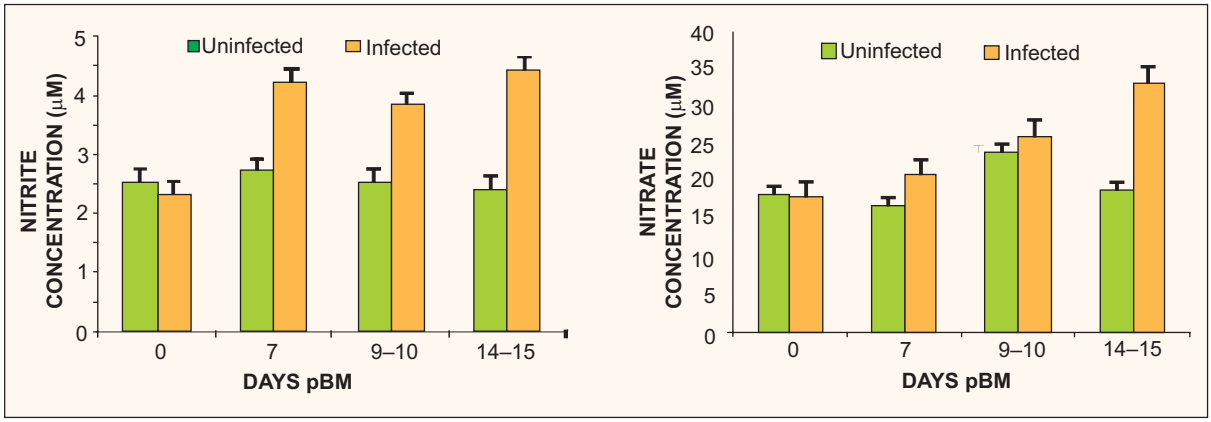

Fig. 1.2.10: Haemolymph nitrite/nitrate of blood-fed uninfected and blood-fed *P. vivax* infected *An. culicifaces* species B was determined at 7, 9–10 and 14–15 days pBM using a high performance anion liquid chromatography method. Means were analysed by using a paired *t*-test (α = 0.075); p-values are represented above the bars

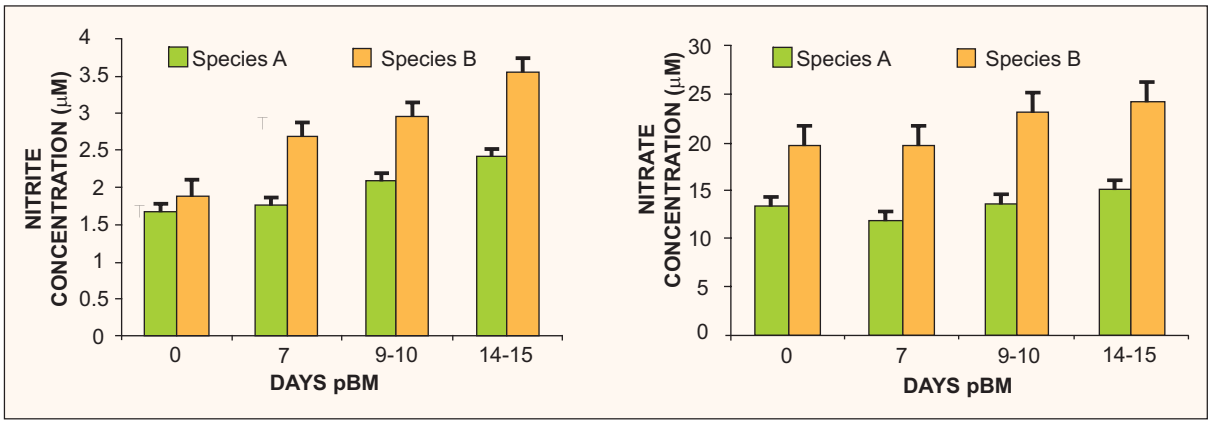

Fig. 1.2.11: Midgut nitrite/nitrate of blood fed *An. culicifaces* species A and species B was determined at 7, 9–10 and 14–15 days pBM using a high performance anion liquid chromatography method. Means were analysed by using a paired *t*-test (α= 0.075); p-values are represented above the bars

The procedure is suitable for the routine determination of nitrite and nitrate. It has proved a sensitive, accurate and reproducible method. The principal strength of this procedure is its simplicity. This anion HPLC method coupled with ultrafiltration to reduce protein and salt contaminants has not been used earlier to measure midgut and haemolymph nitrite and nitrate concentrations in mosquitoes. This method can be used for the detection, identification and quantitative measurement of all nitric oxide metabolites, namely nitrite and nitrate, thus, making it an effective tool for diagnostic purposes and useful for identifying the AcNOS gene products that may impart refractory phenotype that is associated with the immune response to malaria parasites. Such responses may be important for the vectorial capacity of the mosquito and understanding of parasite-vector interactions and mechanism of refractoriness. This procedure may also be suitable for routine determination of NO_2^- and NO_3^- in various other biological fluids/samples.

Different types of surfaces used for evaluation of chlorfenapyr indoor residual spraying

1.3 Vector Control

1.3.1 External Audit of DDT Use in Different States of India

The project was undertaken with a view to find out the rationale of DDT use in different states of India. Field visits were undertaken in 10 districts of five states (Assam, Uttar Pradesh, Rajasthan, Andhra Pradesh and Orissa) for evaluation of proper use of DDT as per norms and deficiencies, if any, for remedial measures. The advance plan, dosage, requirement of insecticide, receipt of insecticide, spray quality, coverage and acceptability of DDT were assessed through questionnaires, cone bioassays and insecticide susceptibility tests.

The vector species was found susceptible to DDT in Assam and Uttar Pradesh and to some extent in Rajasthan, while it was resistant to DDT in Andhra Pradesh and Orissa. The coverage of houses and rooms ranged from 12.5 to 80%. But the results of cone bioassay (to assess the quality and impact of spray on walls) were unsatisfactory as the mortality of vector species ranged from 5 to 50% only. However, in

Uttar Pradesh the results were found satisfactory (74–90% mortality after 27 days). It shows that DDT spray did not provide required residual impact except in two districts of Uttar Pradesh. It was found that strict supervision, data management and training of spray workers is still required for effective IRS. Pilferage of DDT was negligible. Suggestions for remedial measures have been given to the National Vector Borne Disease Control Programme.

1.3.2 Evaluation (Phase-I) of Chlorfenapyr (Pyrrole Insecticide) against Susceptible and Resistant Strains of Mosquito Species

Chlorfenapyr, a pyrrole group insecticide, is a pro-insecticide which acts by inhibiting the reaction of conversion of mitochondrial ADP to ATP. Chlorfenapyr 10% SC was sprayed on different substrates, namely mud, mud-coated with lime, wood, cement, cement-coated with distemper in doses ranging from 0.125 to 4%. *An. culicifacies*, *An. stephensi* and *Cx. quinquefasciatus* were exposed to the surfaces in the laboratory. Doses up to 1% on all the surfaces have resulted in decrease in efficacy within 2 to 3 weeks and bioassays with these doses were discontinued. Further, studies were carried out with 2, 3 and 4% corresponding to 400, 600, 800 mg/m^2 respectively impregnated surfaces. Cone bioassays were performed on the surfaces by exposing 3-day-old sugar-fed mosquitoes on different species, namely *An. culicifacies, An. stephensi, Cx. quinquefasciatus* for 30 min and percent mortality was recorded after 24 h holding period. Chlorfenapyr was found effective in causing mortality of mosquitoes. However, there was no mortality during the exposure period, but mortalities could be observed within 48 h of holding period. The determined diagnostic concentration of chlorfenapyr for assessing susceptibility in field mosquitoes was 5% with 2 h exposure and 48 h holding. Chlorfenapyr @ 400 mg/m^2 was found to be effective up to 28 weeks against *An. culicifacies*; up to 34 weeks against *An. stephensi* while against *Cx. quinquefasciatus* two surfaces, namely mud+ lime and wood have shown consistent results and with other surfaces variable persistence of effectiveness was observed. No cross-resistance with laboratory selected insecticide-resistant lines was noticed with chlorfenapyr. The result indicated that chlorfenapyr has a residual efficacy of six months and above. Hence, this insecticide can be used as an effective insecticide for residual spraying for vector control and insecticide resistance management which has a novel mode of action.

"Chlorfenapyr can be used as an effective insecticide for indoor residual spray against malaria vectors"

1.3.3 Bioefficacy Studies on Interceptor® Long-lasting Insecticidal Nets Impregnated with Alpha-cypermethrin

A one year laboratory study on the efficacy of Alpha-cypermethrin-treated LNs was undertaken. The study was carried out on cold wash-shade dried and cold wash-sunlight dried nets. The bioavailability was assesed by conducting WHO cone bioassays and ring net bioassays. In bioassays, 93% mortality on unwashed and 100% on washed nets up to 30 washes was registered. Slight variations observed in the mortalities were not significant for the over all bioefficacy of the net. However, with the sunlight dried nets, the effect on *Cx. quinquefasciatus* was relatively less as compared to *An. stephensi*. Tunnel tests for bioefficacy on washed nets are in progress.

1.3.4 Evaluation of ZeroFly®, an Insecticide Incorporated Plastic Sheeting against Mosquitoes with Particular Reference to Malaria Vectors

This study was initiated in the month of August 2006 in labour camps in Delhi and Noida. In both the localities Zerofly plastic sheets, incorporated with deltamethrin @ 265 mg

Table 1.3.1. Malaria incidence (active case detection) in Zerofly and control plastic sheets used in labour camps in Noida

Period	Labour Camps	BSE	Total (+)ve	*Pf*	SPR	SFR	PI
July 2006	E (Zerofly)	9	3	0	33.3	0	11.1
(Pre-intervention)	C (Untreated)	8	1	0	12.5	0	3.8
August 06 to July 07	E (Zerofly)	86	1	0	1.1	0	3.7
(Post-intervention)	C (Untreated)	112	12	7	16.9	6.2	73.0

a.i./m^2, were fixed at a distance of at least one km from the control localities where plastic sheets without insecticide (untreated) were fixed. In addition to two localities, the study was also carried out in RAC Police Camp in Delhi, where the company provided plastic sheets during the month of December 2006. Bioassay tests on Zerofly sheets with 3-minute exposure period resulted in 100% mortalities in *An. culicifacies* and *An. stephensi.* The effect of Zerofly sheets persisted at 100% mortality level against *An. culicifacies* even after one year of use under field conditions. Fortnightly monitoring of entomological parameters showed almost complete reduction in the indoor resting density of vector and non-vector mosquitoes in the labour camps provided with Zerofly plastic sheetings as compared with the camp provided with untreated plastic sheetings. In labour camp at a construction site in Noida, man hour density (MHD) of malaria vectors *An. culicifacies* and *An. stephensi* during post-intervention period was in the range of 0–5 in the experimental area as compared to 0–12 and 0–15.5 in the control area (Fig. 1.3.1) respectively.

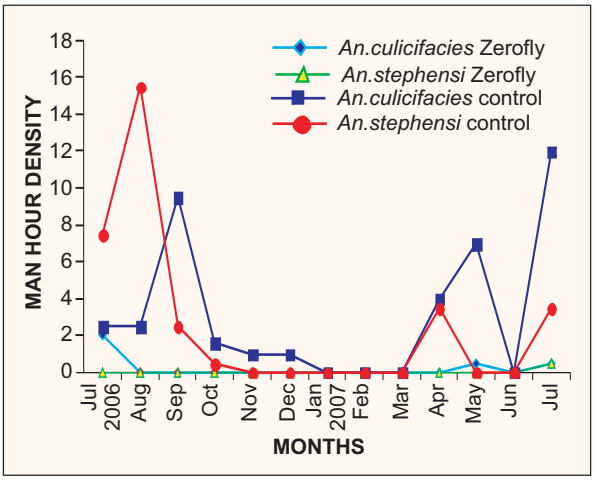

Fig. 1.3.1: Impact of Zerofly sheeting on indoor resting density of malaria vectors in labour camp at Noida

Similarly, in JJ cluster of agricultural labour in the Jamuna belt area in Delhi, MHD of malaria vectors *An. culicifacies* and *An. stephensi* ranged between 0 and 5 in the experimental area as compared to 0 and 13, and 0 and 15 in the control areas respectively. Parasite incidence (PI, cases per thousand population) at the construction site labour camp in Noida was 3.7 in the experimental area as compared to 73 in the control area (Table 1.3.1). Similarly, in the JJ cluster in Delhi the parasite incidence in the experimental and control areas was 42 and 62.7, respectively. In RAC Police Camp, intervention with Zerofly sheeting revealed reduction of culicine density in the experimental tents as compared to the control tents. Survey about the perception of the users about side-effects and benefits revealed a highly positive response in favour of the benefits of Zerofly sheetings and no adverse events were reported by the users.

1.3.5 Follow-up Study on the Long-lasting Efficacy of Olyset® Net against Malaria Vectors and Incidence of Malaria in a Village of District Gautam Budh Nagar, Uttar Pradesh

Follow up study on the long-lasting efficacy of Olyset nets was continued in the three villages, viz. Khandera (Olyset net), Beel (untreated net) and Anandpur (without net) in

Distt. Gautam Budh Nagar, U.P., beyond the initial trial period of one year. The cone bioassays with *An. culicifacies* carried out on Olyset net collected randomly from field after three years of use and with different number of washes revealed no significant difference in the efficacy of nets after different number of washes. However, the median knockdown time on fresh nets was 6.8 min as compared to 12-13.5 min on nets with different number of washes. This indicate that washing of net did not reduce the effect of Olyset net, but the overall knockdown time was increased after three years of use. Fortnightly monitoring of the man hour density (MHD) of mosquitoes and surveillance of malaria incidence was carried out. Data of MHD and malaria incidence of each fortnight in the three villages were pooled and recorded month wise. Results revealed a marked difference in the indoor resting density of mosquitoes, particularly the major malaria vector, *An. culicifacies* in the Olyset net village, when compared with untreated net and without net villages. Average MHD of this species in human dwellings with Olyset nets was 34.3 as compared to 37.8 and 33.7 in untreated net and without net villages during 2003–04 in the pre-intervention year. The average MHD of *An. culicifacies* in the Olyset net village declined from 10.3 to 8.3 during 2005–06 and 2006–07 but during 2007–08 the average MHD was 20 per man hour. The average MHD during 2004–05, 2005–06 and 2006–07 in untreated net village was 22.6, 21.1 and 26.5, respectively, whereas in the without net village it was 39.6, 24.6 and 27.7, respectively. The data clearly indicate the impact of Olyset nets in reducing the density of *An. culicifacies* during the first two years of post-intervention period, while the difference in the MHD during the third year was much less.

Epidemiological results from the three villages revealed considerable difference in the prevalence of malaria. Parasite incidence (cases per thousand population) in village with Olyset nets during 2003–04 (pre intervention year) was 39.5, which declined to 1.5, 0 and 2.5 during 2004–05, 2005–06 and 2006–07 respectively in the post-intervention years. The PI in the untreated net village during 2003–04 (pre-intervention) was 44 as compared to 6.1, 3.8 and 8.8 during 2004–05, 2005–06 and 2006–07 respectively. Whereas in the without net village, the PI during 2003–04 (pre-intervention) was 19 and during intervention years 2004–05, 2005–06, 2006–07 was 19.5, 11.5 and 10 respectively. Similar trend in *Pf* per thousand population in the three villages was also observed. Results indicate long-lasting impact of Olyset nets. Results also revealed in reduction of mosquito nuisance in the Olyset-net village. Follow up observation on the effect of Olyset net are still in progress.

"Usage of Olyset nets reduced the parasite incidence from 39.5 in pre-intervention to <2.5 in post-intervention"

1.3.6 Phase III Evaluation of PermaNet® 2.0 against Malaria Vectors and Disease Transmission

Permanet 2.0, a deltamethrin-treated LN have already undergone Phase II entomological evaluations to demonstrate bio-efficacy and wash-resistance in field against malaria vectors in three different areas in India. The present study (Phase III) field evaluation of Permanet 2.0 against malaria vectors and disease prevalence was initiated in the endemic areas of District Gautam Budh Nagar, in western Uttar Pradesh in April 2007 and in tribal areas of Orissa in August 2007, following uniform protocol of NIMR (ICMR).

Three villages with similar malaria endemicity, topography and mosquito prevalence in District Gautam Budh Nagar in Uttar Pradesh, where malaria is transmitted mainly by *An. culicifacies* and *An. stephensi* and three clusters of small villages in tribal areas in Sundargarh district, Orissa, where *An. fluviatilis* and *An.*

culicifacies, the major vectors of malaria have been selected for the Phase III evaluation of Permanet. Entomological and epidemiological parameters as per uniform protocol were monitored following standard procedures.

Results revealed that the MHD of *An. culicifacies* during pre-intervention period in Permanet, untreated net and without net villages ranged from 10–13, 24–27 and 8–12, respectively. With the commencement of intervention, there was sharp decline in density of *An. culicifacies* in June 2007, whereas the density in the untreated and without net control villages did not decline in June 2007. However, there was an increase in the resting density of *An. culicifacies* in all the villages during the monsoon and post-monsoon period of August to November 2007, but the build-up of *An. culicifacies* density was much higher in the control villages as compared to Permanet village. The parity rate of *An. culicifacies* was low in Permanet village as compared to untreated net and without net villages. The parity rate of *An. culicifacies* in June 2007 in the first month during post-intervention period in Permanet, untreated net and without net villages was 20, 66.6 and 60% respectively.

"Malaria incidence (cases/1000) in Permanet used village was (0.84) much less than the untreated net (5.19) and without net (13.46) villages"

Comparison of malaria incidence data showed that during pre-intervention period of April–May 2007, the PI in the Permanet villages was 2.5 and in the villages with untreated nets and without nets was 1.7 and 2.9 respectively. There was no significant difference ($p > 0.05$) in the malaria endemicity in the Permanet and control villages. During intervention phase, the malaria incidence in the Permanet net, untreated net and without net villages was 0.84, 5.19 and 13.46, respectively.

The compliance rate of the net usage in the Permanet and control villages was ascertained through random checking of houses and recording of people sleeping under mosquito nets. There was 85–99% compliance rate of net usage in the study population during different months. The community perceptions on adverse effects and collateral benefits of Permanet usage was assessed by conducting cross-sectional survey among the users (n = 394, M–220, F–194). Almost every respondent asserted that they are sleeping under the treated nets. There were minimal complaints of skin irritation (0.5%) and eye irritation (0.25%). However, these effects were only temporary, lasting for few hours of the first usage. Majority of the respondents enthusiastically reported that Permanets provided them relief not only from mosquitoes but also from other household pests such as headlice, bed-bugs, cockroaches, ants and houseflies.

1.3.7 Field Evaluation of Bacticide DT (Dispersible Tablets), a Formulation of *Bacillus thuringiensis* var. *israelensis* H-14, Strain 164 against Larvae of Mosquito Vectors

A multicentric trial of Bacticide DT formulation was carried out in urban areas to see persistence in domestic and peridomestic breeding containers, such as desert coolers, containers, water tanks which are potential mosquito breeding habitats of *Ae. aegypti* and *An. stephensi* and also against *Cx. quinquefasciatus*. The study was carried out in urban/periurban

Application of bacticide

areas of Raipur (Chhattisgarh), Hardwar (Uttarakhand) and Sonepat (Haryana) areas. The methodology was based on the common protocol developed by NIMR for evaluation of biolarvicide. The Bacticide DT (400 mg) was evaluated in natural breeding habitats of *Anopheles*, *Culex* and *Aedes* species. Application

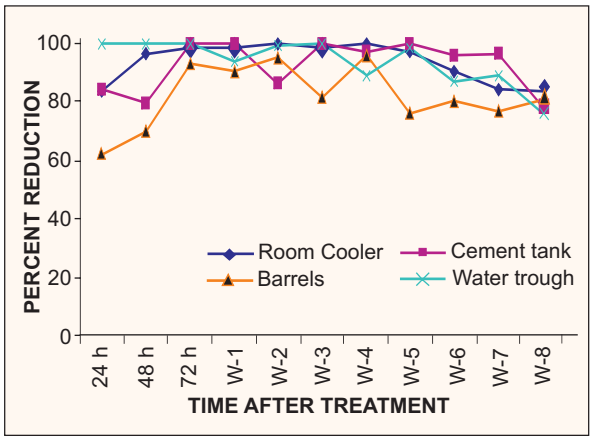

Fig. 1.3.2: Effect of Bacticide DT 400 mg on late instars of *Ae. aegypti* in different breeding habitats in Raipur

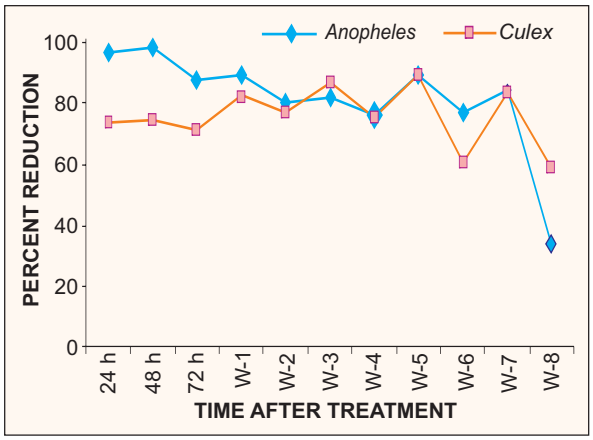

Fig. 1.3.3: Effect of Bacticide DT 400 mg on late instars of *Ae. stephensi* and *Cx. quinquefasciatus* in different breeding habitats in Sonepat

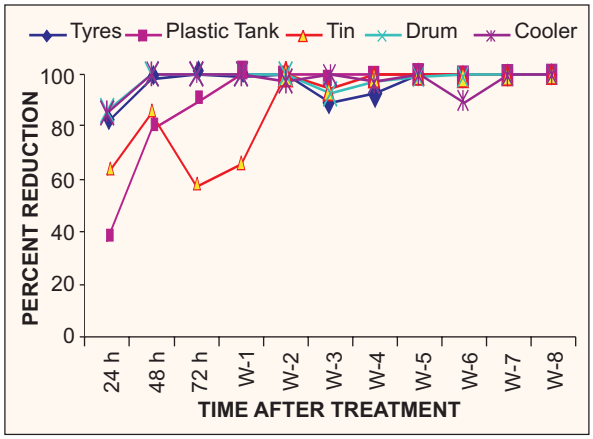

Fig. 1.3.4: Effect of Bacticide DT 400 mg on late instars of *Ae. aegypti* in different breeding habitats in Hardwar

of one dispersible tablet (400 mg)/m^2 of water surface or one tablet per container with <50 litre of water produced >80% reduction of late instars up to two weeks . In container habitats of *Anopheles* and *Aedes*, where water was >20 litre, the effect was up to four weeks (>80% reduction) (Figs. 1.3.2 to 1.3.4). Bacticide DT formulation was more effective against *Aedes* compared to *Anopheles* and *Culex* species.

1.3.8 Field Evaluation (Phase III) of Bacticide WP, a Formulation of *Bacillus thuringiensis* var. *israelensis* H-14, Strain 164 against Larvae of Mosquito Vectors

This study was carried out to evaluate the effectiveness of Bacticide WP formulation for control *of An. culicifacies, An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* in a locality as a multicentric trial (Table 1.3.2). Bacticide WP was evaluated at the dose of 200 mg/m^2 in

Table 1.3.2. Habitats and target species selected for the trial at various sites

Site	Trial habitats	Target species
Raipur	Cement tanks	*An. stephensi, An. subpictus* and *Cx. quinquefasciatus*
	Coolers	*Ae. aegypti, An. stephensi*
	Drains	*Cx. quinquefasciatus,* Anopheline species
	Pools	*An. subpictus, Cx. quinquefasciatus*
Sonepat	Cement tanks	*An. stephensi*
	Pools	*An. subpictus*
	Tanks and drains	*Cx. quinquefasciatus*
Mathura	Pits, Pools	*An. culicifacies*
	Drains	*Cx. quinquefasciatus,* Anopheline species

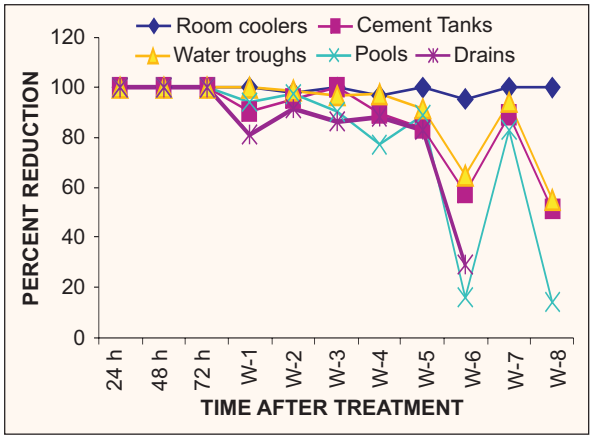

Fig. 1.3.5: Effect of Bacticide WP @ 200 mg/m² on late instars of anophelines in Raipur

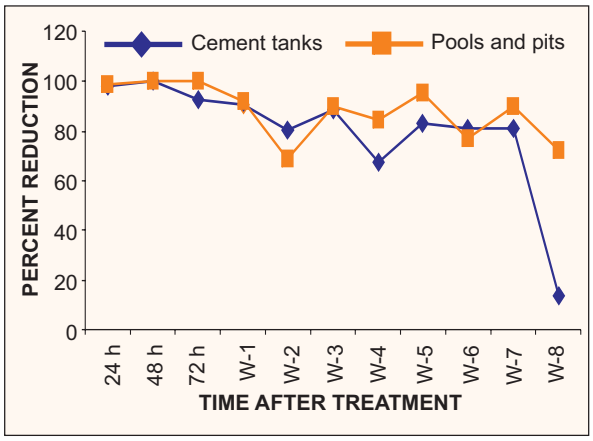

Fig. 1.3.6: Effect of Bacticide WP @ 200 mg/m² on late instars of anophelines in Sonepat

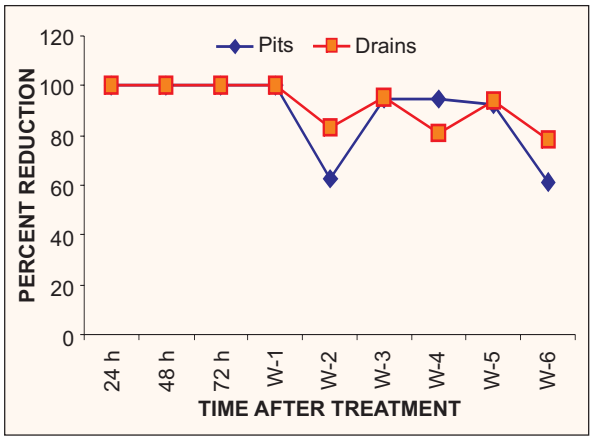

Fig. 1.3.7: Effect of Bacticide WP @ 200 mg/m² on late instars of anophelines in Mathura

natural breeding habitats against *An. stephensi*, *An. subpictus*, *Cx. quinquefasciatus* at Raipur and Sonepat. It was also evaluated against *Ae. aegypti* in Raipur. In Mathura, it was evaluated against *An. culicifacies* and also against *Cx. quinquefasciatus*.

Results showed within a week maximum of 100 percent reduction of late instar larvae of target species *An. stephensi* in coolers, cemented tanks, *An. subpictus* in pits and pools, cemented tanks, clean water drains and *Ae. aegypti* in coolers during post-treatment period. Bacticide WP was effective (>80% reduction) in clean water small habitats for two weeks. (Figs. 1.3.5 to 1.3.7). Against *Cx. quinquefasciatus* in surface drains with organic matter, the reduction was >80% for seven days and in small containers such as coolers, tanks the effect was (>80%) for two weeks. In general, the formulation was more effective against *Aedes* compared to *Anopheles* and *Culex*.

1.3.9 Multicentric Study on Efficacy Trial with Enhanced Dose of Fenthion 82.5% E.C. against Mosquitoes in Polluted Water

At present fenthion is used in polluted water under NVBDCP at a dose of 100 ml/ha. However, a recent study has shown ineffectiveness of fenthion @100 ml/ha against mosquitoes, which breed in polluted water. Since currently used dose of 100 ml/ha of Fenthion (E.C. 82.5%) per week was found to be ineffective to control larval breeding of *Cx. quinquefasciatus* in polluted water habitats, on the recommendation of NVBDCP expert group meeting held on 6 June 2007, a multicentric study was initiated by NIMR during August 2007–March 2008 to evaluate the efficacy of fenthion against *Culex* larvae using enhanced doses of 150 ml and 200 ml/ha in Delhi, Chennai and Raipur.

In Delhi, four different localities representing South, East, North and West Delhi areas having cesspools, which support *Cx. quinquefasciatus* breeding were selected. Fenthion has been in use in Delhi for the last 30 years and an additional area included in the study is from Noida having stagnant polluted drains with prolific breeding of *Culex* mosquitoes. In this area fenthion was never used in control programme.

Weekly application of enhanced dose (150 ml/ha) of fenthion for three weeks in cesspools in different areas of Delhi showed considerable variations in its efficacy. In South Delhi, >80% reduction in the density of III+IV instar larvae was observed on Day 16, i.e. after three applications. In East Delhi, >80% reduction was observed immediately after the spray on three occasions. In North Delhi, the reduction always remained below 71%. In West Delhi, >80% reduction was observed on Day 9 onwards.

Similar weekly application of fenthion (200 ml/ha) for three weeks in cesspools in South Delhi registered >80% reduction immediately after the spray on three occasions, but it declined to 43% on Day 7 and 64% on Day 14, however, after third application >80% reduction was observed up to Day 21. In North Delhi also, >80% reduction was observed immediately after the spray on three occasions, but the percentage reduction declined to 79% on Day 7, 72% on Day 14 and 61.5% on Day 21. In East and West Delhi areas, however, >80% reduction was observed up to three weeks.

In Noida, fenthion treatment at 150 ml/ha caused 73.8 to 90.7% reduction from Day 1 to Day 3, but it declined to 67% on Day 7. Further applications registered >80% reduction throughout next two weeks. Higher dose of 200 ml/ha was effective for the entire period of three weeks as the percentage reduction ranged from 88 to 97.

In Chennai, fenthion has been in use for the last 30 years, the selected areas for the fenthion trial included cesspools, stagnant polluted drains and moderately polluted unused wells from different parts of the city. Single application of 150 ml/ha of fenthion in drains caused <80% reduction up to one week, however, 200 ml/ha caused 71% reduction on Day 1, which increased to 89% on Day 3 but again it declined to 17% on Day 7. In cesspools, 150 ml/ha of fenthion produced 93–95% reduction on Day 2 and 3, but it reduced to 63% on Day 7. Similar treatment of cesspools with 200 ml/ha caused >80% reduction for the first three days then it declined to 73% on Day 7. Both the doses of fenthion, however, registered >80% control of *Culex* breeding for a period of one week in moderately polluted unused wells.

In Raipur, where fenthion was never used in urban malaria control, single spray of 150 ml/ha of fenthion in polluted drains registered >90% reduction up to 14 days. In another area having highly polluted drains, application of 200 ml/ha caused 98–100% reduction for one week. In pools, 150 ml/ha caused >90% reduction up to Day 3 and it declined to 79% on Day 7. However, at 200 ml/ha treatment in pools, >93% reduction was observed for a period of one week and >80% reduction up to three weeks. Application of both the doses of fenthion in moderately polluted cemented tanks, which supported *Anopheles* breeding, produced 100% mortality.

1.3.10 Multicentric Study on Evaluation of Mosquito Larvicide Temephos for Use in Polluted Water

Mosquito larvicides, viz. temephos and fenthion are used in vector control programme against mosquito vectors under urban malaria scheme in India. At present temephos is used in clean and potable water under NVBDCP due to its very low mammalian toxicity. National Programme Technical Advisory Committee of NVBDCP recommended a study on the efficacy of temephos in polluted water. In view of this recommendation, a multicentric study was initiated by NIMR to determine the effective doses of temephos for mosquito larval control in polluted water. The study was carried out at three urban field sites, namely Delhi, Raipur and Ranchi.

In Delhi, a treatment of temephos in highly polluted cesspools in Rohini showed only a dose of 200 g/ha could cause >80% reduction

in *Cx. quinquefasciatus* larval density for a period of one week. In Gautam Budh Nagar, 25 g/ha of temephos was effective in reducing larval density >80% for one week in clean water. With polluted cesspools 100 g/ha was effective for seven days, while 200 g/ha produced >80% reduction up to 14 days. However, treatment of polluted ain with 100 and 200 g/ha caused effective control for seven days.

In Raipur, breeding in choked drains was reduced to >80% with a dose of 50 and 100 g/ha and with 200 g/ha similar effect was for two weeks. In Ranchi, treatment of 100 and 200 g/ha produced >80% reduction for one week. pH of water in habitats of Delhi, Raipur, and Ranchi varied between 7.0 and 8.0. This range of pH did not influence the toxicity of temephos. Overall results from the multicentric trial indicate 200 g/ha of temephos 50% EC is effective for larval control for seven days in highly polluted water, while in moderate polluted water, the effect extended up to 14 days in Delhi and Raipur but not in Ranchi. Thus, 4 to 8 fold increase in temephos dosage in clean water by the national programme is required to effectively control *Cx. quinquefasciatus* breeding in polluted water.

Launching of the experiment on efficacy of attracticide in Delhi

1.4 Vector Surveillance

1.4.1 Application of Attracticide (Oviposition Pheromone in Combination with Insect Growth Regulator) for Surveillance and Control of Dengue and Chikungunya Mosquitoes

The experiment was initiated in Delhi in October 2007 and in Bengaluru in December 2007. In Delhi, about 6500 ovitraps were placed in five localities, viz. Mayur Kunj (Trilok Puri), Valmiki Colony (Panchkuian Road), Netaji Nagar, R.K. Puram and Railway Colony (Tughlakabad). In Bengaluru, about 6000 ovitraps were placed in three localities, viz. Ashok Nagar, Kanteerava Nagar and Sanjay Gandhi Nagar + Narayanpura. The experimental ovitraps contained 395 ml water treated with 5 mg C-21, IGR and solvent. Untreated ovitraps contained 400 ml water with solvent only. The experiments will also to be carried out in Kerala where baseline data hav been collected for selection of study sites.

Before starting the experiment, a meeting with community was organized at Delhi and Bengaluru to make them aware about this experiment taking place. Experiment at Delhi in R.K. Puram location was inaugurated by Mr. Deepak Gupta, IAS, Special Secretary, Ministry of Health & Family Welfare. Training to the newly appointed supervisors and surveillance workers was provided to carry out the experiment and to check the mosquito breeding. A surveillance worker was asked to check ovitraps and record the breeding in about 50

houses in a day. Thus, a surveillance worker covered about 250 houses in a week. The supervisors monitored the work of the surveillance workers, collected data from the field for processing on computers and provided IEC to the community regarding the experiment being carried out. The study is in progress.

1.5 Insecticide Resistance

1.5.1 Molecular Basis of kdr Resistance in *An. culicifacies* and Development of PCR-based Methods for kdr Genotyping

An. culicifacies, the most important malaria vector in India, is resistant to DDT and is developing resistance to pyrethroids—only alternative so far available for the impregnation of bednet. The presence of *kdr*-based resistance in vector is a threat to the success of the pyrethroid-impregnated bednet programme. The NIMR established the presence of *kdr* mutation in the field population of *An. culicifacies*, and developed PCR-based methods for *kdr* genotyping in field populations.

To identify the presence of *kdr*-based mechanism of knockdown resistance in *An. culicifacies*, we sequenced 1.4 kb span encompassing S4–S6 region of domain II of para type voltage gated sodium channel which revealed a single point mutation Leu-Phe at *kdr* locus in few specimens of DDT and pyrethoid-resistant *An. culicifacies*. We developed and tested three molecular methods for *kdr* genotyping, viz. Allele Specific PCR (ASPCR), amplification refractory mutation system (ARMS) and primer introduced restriction assay (PIRA) (Figs. 1.5.1–1.5.3). The results were validated following DNA sequencing of samples.

"Three high throughput molecular assays were developed for kdr genotyping in Anopheles culicifacies"

The presence of Leu-Phe mutation at *kdr* locus was demonstrated in some DDT and pyrethroid resistant *An. culicifacies* from Surat district of Gujarat, India. We didn't find Leu-Ser mutation at *kdr* locus, as reported in *An. gambiae*, and Leu-His mutation at position 29 of exon I of VGSC (upstream to the *kdr* locus) as has been reported in Iranian *An. culicifacies*

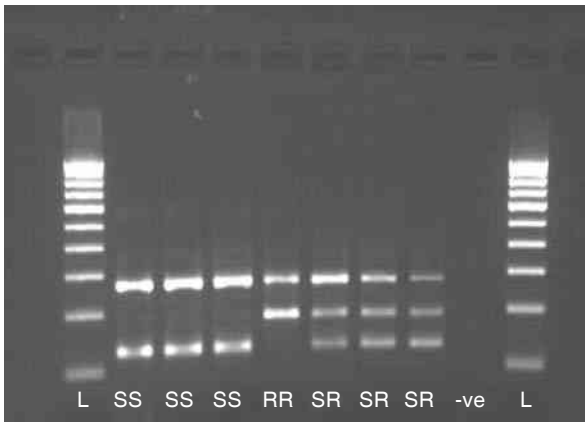

Fig. 1.5.1: Allele-specific PCR (ASPCR) assay for *kdr* genotyping

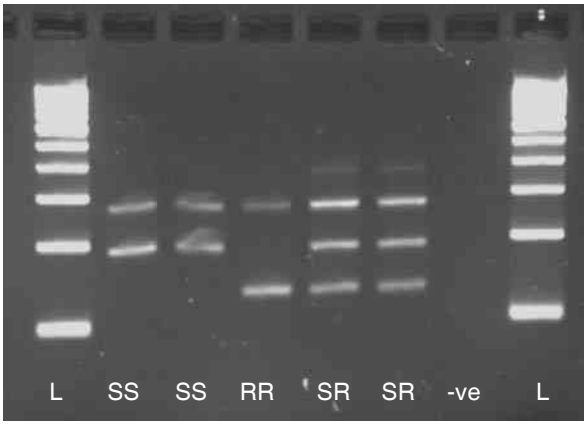

Fig. 1.5.2: Amplification refractory mutation system (ARMS) assay for *kdr* genotyping

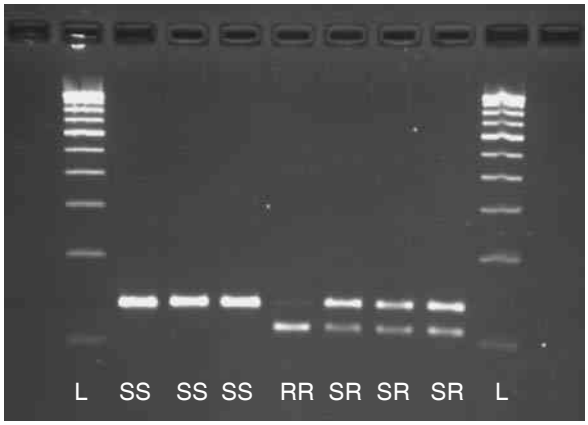

Fig. 1.5.3: Primer induced restriction assay (PIRA) for *kdr* genotyping

s.l. The genotyping of a DDT and pyrethroids-resistant *An. culicifacies* population from Surat, India comprising of species B and C was done using three PCR-based assays which revealed a low frequency of *kdr* allele mostly in heterozygous conditions. The reliability of three PCR-based assays was confirmed following sequencing all the samples genotyped as homozygous and heterozygous resistant, and some randomly selected homozygous susceptible samples. All the three PCR based assays were found to be specific and no descrepancy in result was noticed.

1.6 Evolutionary Genetics

1.6.1 Evolutionary Genetics of *Anopheles gambiae* X-chromosome

Understanding the genetic architecture of individual taxa of medical importance is the first step for designing disease preventive strategies. To understand the genetic details and evolutionary perspective of the model malaria vector, *An. gambiae* and to use the information in other species of local importance, we scanned the published X-chromosome sequence for detailed characterization and obtain evolutionary status of different genes. The telocentric X-chromosome contains 106 genes of known functions and 982 novel genes. We considered the known genes and first classified them based on size (in nucleotide base pairs) (Fig. 1.6.1). Majorities of both the known and novel genes are with introns. The known genes are strictly biased towards less number of introns; about half of the total known genes have only one or two introns (Fig. 1.6.2). The extreme sized (either long or short) genes were found to be most prevalent (58% short and 23% large). Statistically significant positive correlations between gene length and intron length as well as with intron number and intron length were obtained signifying the role of introns in contributing to the overall size of the known genes of X-chromosome in *An. gambiae*. We compared each individual gene of *An. gambiae* with 33 other taxa having whole genome sequence information. In general, the mosquito *Ae. aegypti* was found to be genetically closest and the yeast *Saccharomyces cerevisiae* as most distant taxa to *An. gambiae* (Fig. 1.6.3). Further, only about a quarter of the known genes of X-chromo-

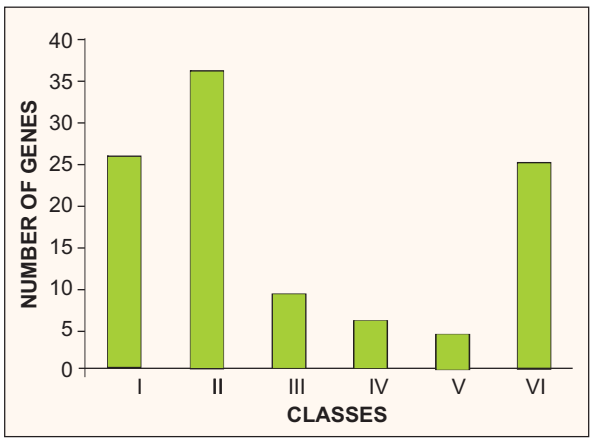

Fig. 1.6.1: Classification of known genes of *An. gambiae* X-chromosome based on size (nucleotide base pair)

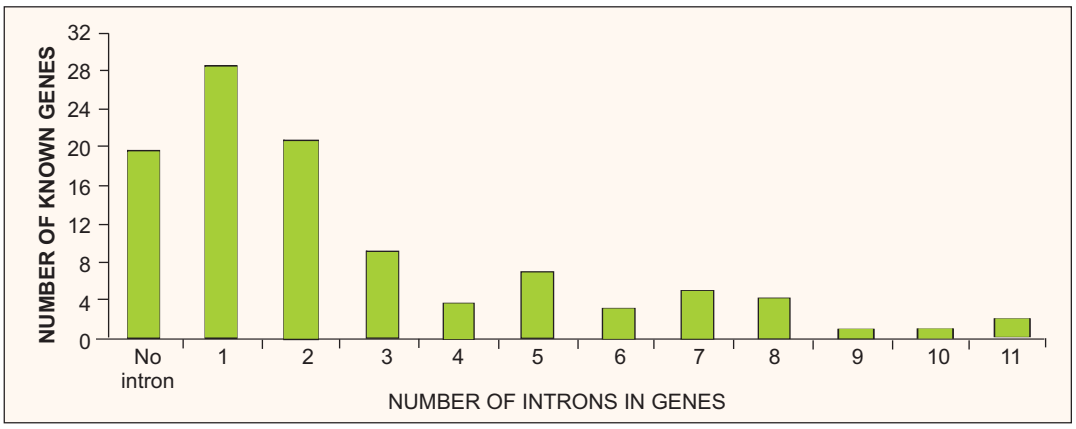

Fig. 1.6.2: Distribution of *An. gambiae* X-chromosome known genes according to the number of introns

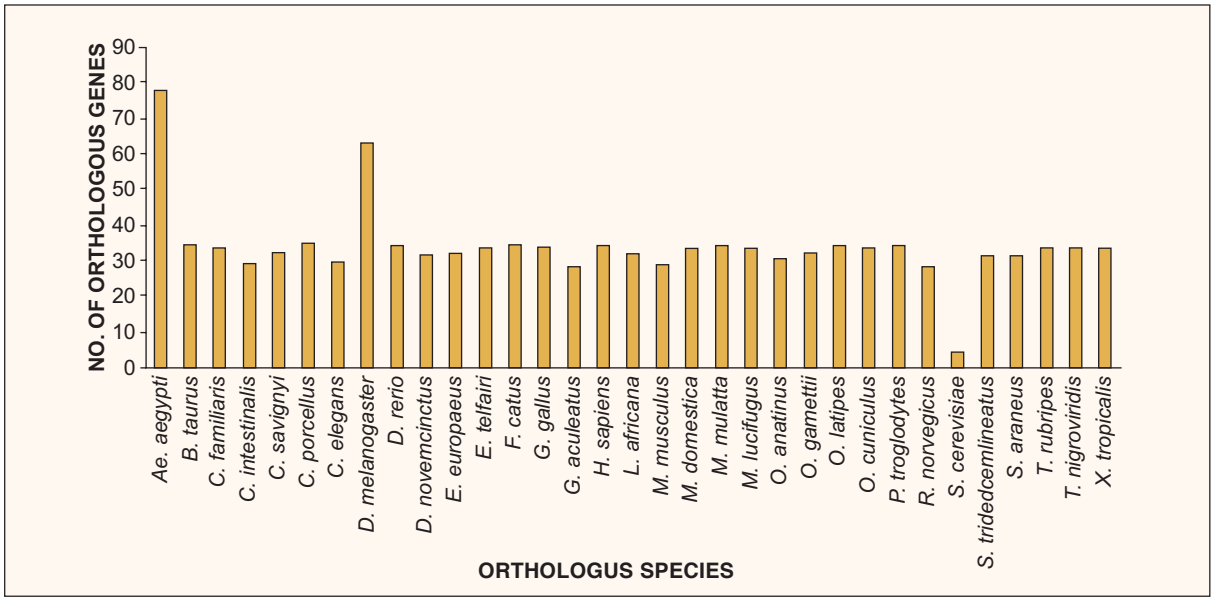

Fig. 1.6.3: Distribution of different taxa showing number of shared genes with *An. gambiae*

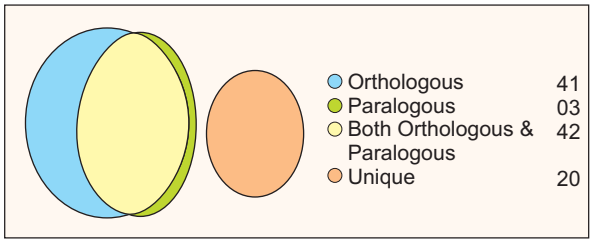

Fig. 1.6.4: Distribution of different gene types (based on homology prediction) in X-chromosome of *An. gambiae*

some were unique to *An. gambiae* and majorities have orthologs in different taxa (Fig. 1.6.4). A phylogenetic tree was constructed based on a single gene found to be highly orthologous across all the 34 taxa. Evolutionary relationships among 13 different taxa were inferred which corroborate the previous and present findings on genetic relation-

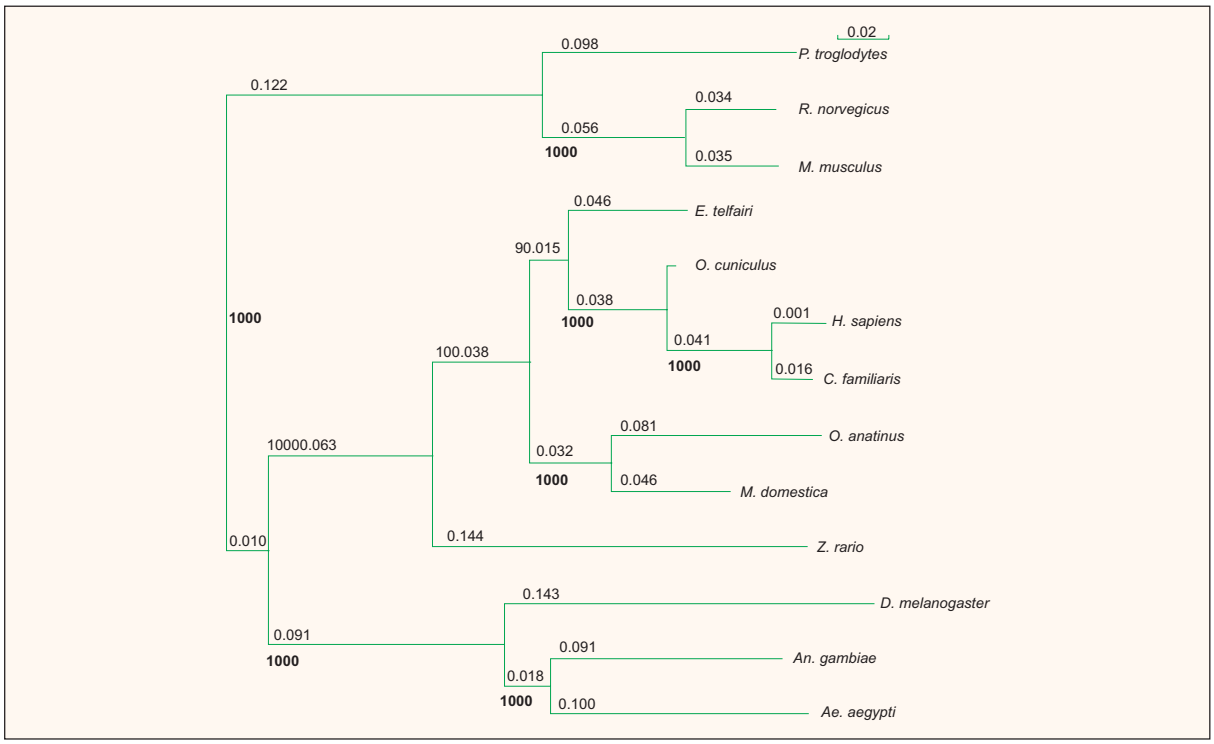

Fig. 1.6.5: Un-rooted neighbour-joining (NJ) phylogenetic tree with bootstrap values (in bold font) in 13 different taxa. The figures (in each horizontal line) indicate the lengths of each branch leading towards taxa

ships across various taxa (Fig. 1.6.5). The study not only provides fine-scale views to the genetic architecture of the X-chromosome of the model malaria vector of African importance, but also reveals several interesting features on evolutionary insights into genes and taxa of different taxonomic status. The information is of great importance, especially to the population geneticists, to understand genetic diversity and infer the respective roles of demography and natural selection in evolution of genes in different *Anopheles* species populations of local importance. ❑

"Comparative genetic studies of An. gambiae X-chromosome genes with other sequenced taxa provide evidence of genetic relatedness"

2

Parasite Biology

2.1 Human Malaria Parasites: Molecular Characterization

2.1.1 Genetic Structure of *Plasmodium vivax*

Plasmodium vivax genome sequence revealed abundant mini and microsatellites. Identification of polymorphic mini and microsatellite markers is crucial for studying the pattern of genetic diversity, population genetic structure and linkage disequilibrium of population for genotype-phenotype association mapping. To understand the population genetic structure of *P. vivax* among Indian sub continent, we used polymorphic mini and microsatellites.

Minisatellites for *P. vivax*

In this pioneer study, Indian *P. vivax* populations have been characterized using ten minisatellites which were identified in the laboratory. Single clone *P. vivax* isolates from five geographically separated regions, namely Delhi, Nadiad, Panna, Chennai and Sonapur were analyzed using multi-locus minisatellite markers. Amplified PCR fragments were run on high resolution metaphor agarose for allele sizing (Fig. 2.1.1). Extensive genetic polymorphism was observed among the five *P. vivax* populations (Fig. 2.1.2). Number of alleles observed per locus was 11–22 alleles, except one locus that showed six alleles. Analysis revealed average heterozygosity per locus was similar or slightly higher (*He* = 0.815–0.915) than the average heterozygosity revealed by the microsatellite marker. Magnitude of the genetic diversity and the diversity pattern was similar among the five geographical regions of India. Phylogenetic tree based on Nie genetic distance revealed that isolates from different populations cluster together suggesting *P. vivax* isolates circulating in five widely separated geographical regions possess same genetic structure (Fig. 2.1.3). High degree of allele sharing observed among the five geographical regions indicates high de-

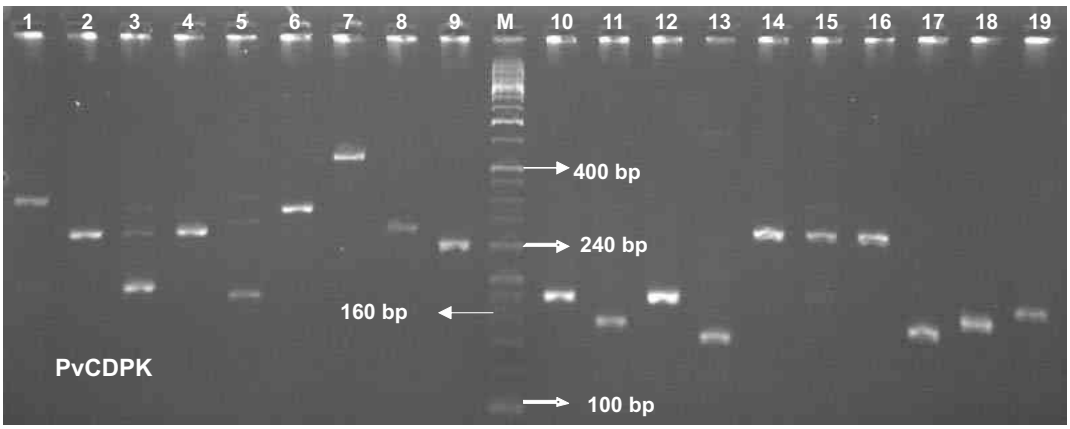

Fig. 2.1.1: Gel images of minisatellite variations in Indian *P. vivax* isolates. Amplicons were visualised in 3% high resolving Metophore agarose gel for allele sizing. M represents 20 bp DNA ladder

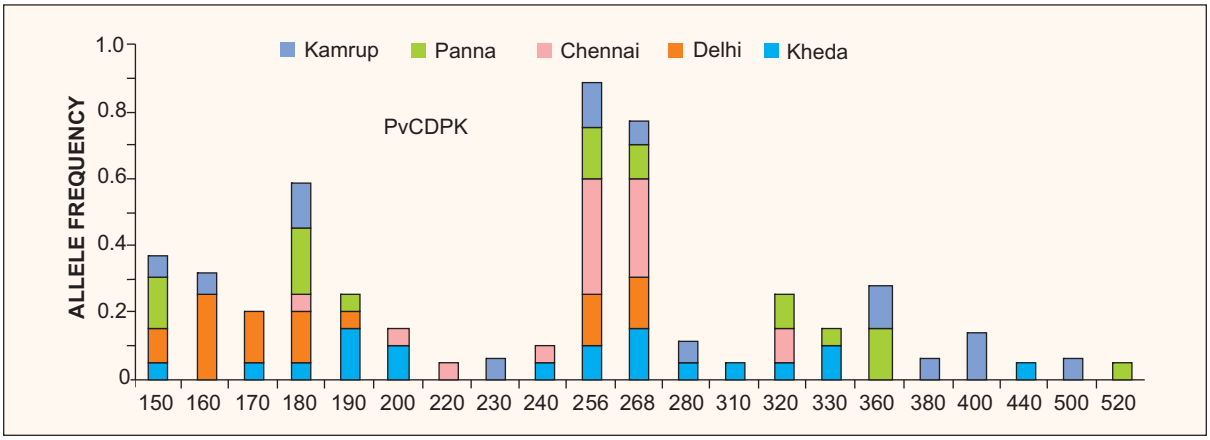

Fig. 2.1.2: Extent of genetic diversity and allele sharing in *P. vivax* isolates from five different populations at minisatellite locus

gree of gene flow among the widely separated geographical regions of India. The study suggests that minisatellites have potential resolving power for genetic diversity.

Microsatellite Markers

Microsatellite markers were identified by scanning *P. vivax* genome sequence. About 2–3 nucleotide repeat sequences with at least 12–15 copy number (repeat number) were selected for the study. Eight microsatellites out of ten studied were observed to be highly polymorphic. Number of alleles observed per locus was 6–13 and heterozygosity among the study populations varied between 0.65 and 0.90. High degree of allele sharing observed

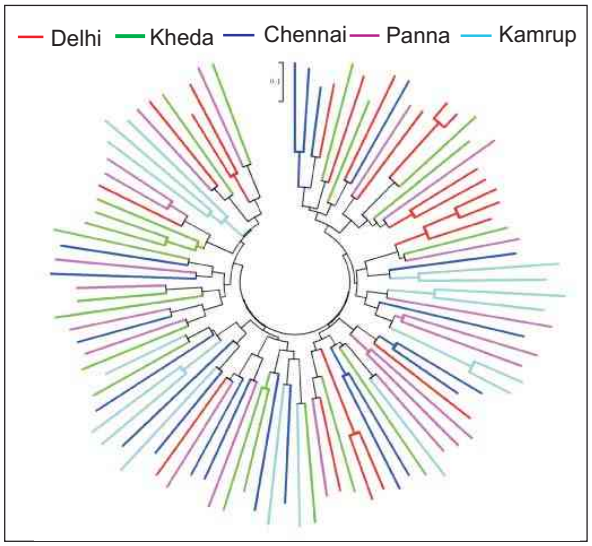

Fig. 2.1.3: Neighbour-Joining phylogenetic tree showing genetic relationship among Indian *P. vivax* isolates using ten minisatellites

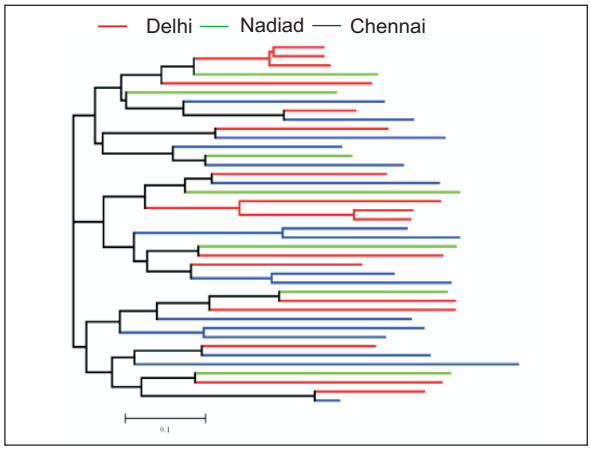

Fig. 2.1.4: Neighbour-Joining phylogenetic tree derived from genetic distance scored at eight microsatellites showing genetic relationship among Indian *P. vivax* isolates. Line colours in the phylogenetic tree represent geographical origins of isolates. Blue, red, and green coloured lines represent Chennai, Delhi and Nadiad population isolates respectively

among the isolates from three geographical regions namely, Delhi, Nadiad and Chennai indicates high degree of gene flow among the widely separated geographical regions of India. Phylogenetic analysis reflected that *P. vivax* isolates circulating in different geographical regions cluster together, suggestive of similar genetic structure among different geographical regions of India (Fig. 2.1.4).

2.1.2 Antigenic Repertoires of Vaccine Candidates

Antigenic diversity in the natural parasite populations is the major obstacle in the development and success of effective antimalarial control measures. Antigenic repertoires of

human malaria parasites have been widely studied. However, day-by-day new antigenic variants are being reported from different parts of the globe.

Antigenic repertoires of *P. vivax* vaccine candidates were investigated in five widely separated geographical regions (Delhi, Panna, Kheda, Chennai and Kamrup) of India to understand the local antigenic repertoires. Sequence analysis of five vaccine candidates from asexual [*Circumsporozoite Protein (CSP), Apical Membrane Antigen-1 (AMA-1)* and *Duffy Binding Protein-II (DBP-II)*] and sexual stages (*Pv25S* and *Pv28S*) revealed differential levels of antigenic repertoires. The asexual stage revealed high antigenic repertoires in comparison to the sexual stage. Extensive non-synonymous and synonymous nucleotide substitutions were found and the overall number of non-synonymous substitutions exceed over the synonymous substitutions suggesting signature of diversifying selection at vaccine candidates. Extensive non-synonymous substitutions found in the vaccine candidates in comparison to the housekeeping genes explained that high antigenic variation among antigenic genes is the adaptive mechanism of parasite to evade the host immune response. Substantial amount of local antigenic repertoires for each candidate in all the five regions have been uncovered. The antigenic repertoires of five vaccine candidates for all the regions were very high, however, each region showed a fraction of specific antigenic repertoires. The overall observed SNPs in five vaccine candidates revealed that a good number of antigenic repertoires are shared among different regions, however, signature of region-specific antigenic repertoire was also observed (Fig. 2.1.5).

"Local antigenic variations are very crucial in the understanding of total antigenic repertoires in a country like India and in turn, planning effective vaccine based control measures"

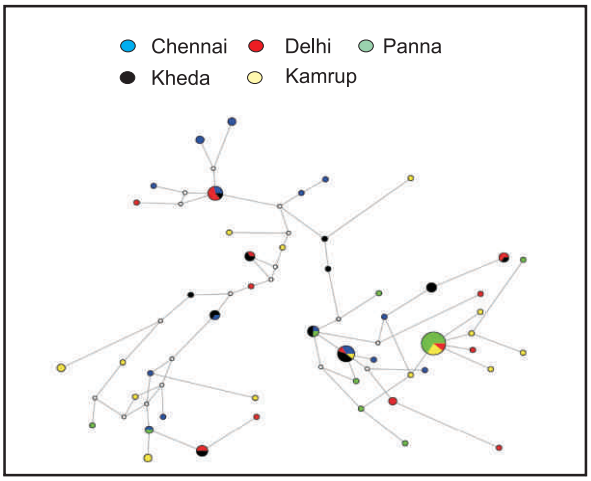

Fig. 2.1.5: Region-specific haplotypes and shared haplotypes of *P. vivax* as well as phylogenetic relationship between *AMA-1* haplotypes. Each circle represents different haplotype, colour of the circle represents geographical origin of isolates and different colours in a circle represent haplotypes shared between respective regions, while white circle represents missing haplotypes. Haplotypes are connected with the mutational events; length of connecting line is proportional to the number of mutational events and size of the circle is proportional to the frequency of haplotypes

2.1.3 Distribution and Genetic Relatedness of Two Sub-populations (Subtypes) in Indian *Plasmodium vivax*

P. vivax has been categorized into two distinct lineages, the 'New World' and 'Old World', distinguishable by gene conversion in the SSU rRNA S-type and mutations in an open reading frame (ORF 470) in the apicoplast genome. The distribution of the two subtypes of *P. vivax* (Old and New world) based on S-type 18S SSUrRNA was studied in field isolates from different locations in India. A total of 354 *P. vivax* field isolates collected from nine different geographical regions of India including coastal, mainland, forest and island regions were analyzed for length polymorphism in S-type SSUrRNA gene.

P. vivax S-type SSUrRNA genotyping for Old world and New world isolates was carried out by one step touch down PCR assay. Two 18S SSUrRNA S-type were confirmed by sequenc-

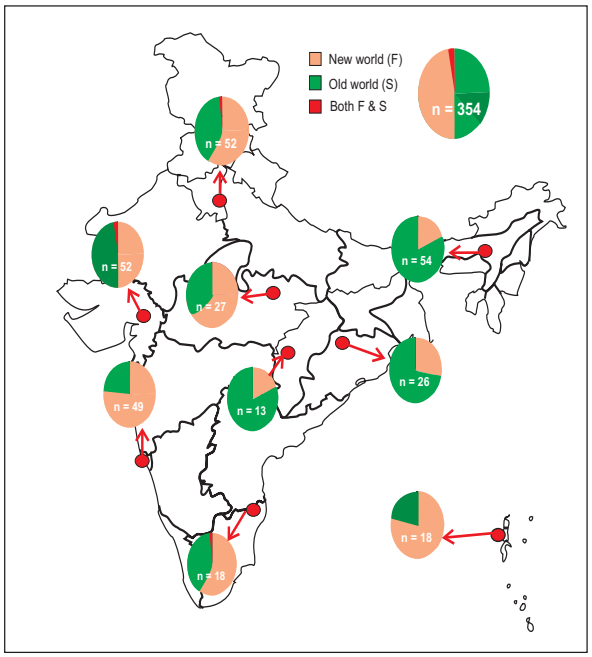

Fig. 2.1.6: Distribution of two *P. vivax* SSUrRNA subtypes in India

ing. Dimorphic nature of SSUrRNA S type gene was observed among the isolates. Based on the fragment size, a slow moving (S) 480 bp fragment (Old world or type I) or fast moving (F) 454 bp (New world or type II) were designated. Distribution of both types of S-type 18S SSUrRNA was nearly equal among the study isolates, however, their proportions varied among isolates of different regions (Fig. 2.1.6). In Delhi, both 'Old' and 'New world' isolates were in equal proportions, while Nadiad and Chennai isolates showed 55 and 58% 'New world' isolates respectively. Similarly, Panna and Goa isolates were predominantly of 'New world' type. Isolates of Car Nicobar also showed higher proportion of 'New world' (80%) isolates. On the other hand, isolates of Sonapur, Rourkela and Raipur were dominated by 'Old world' type and their proportions were about 80%.

"Both Old and New world P. vivax subtypes are equally prevalent all over India"

To understand the genetic structure and relatedness of two sub-types (Old and New world), multi-locus genotyping was initiated. We used highly polymorphism marker, MSP-3α to identify single clonal isolates using PCR-RFLP method. Analyses revealed about 20% of the total isolates are multi-clonal and the rest are single clones. One hundred single-clone isolates, comprised of 50 'Old world' and 50 'New world' types were selected from the pooled 354 *P. vivax* isolates for further molecular characterization of point mutations in *dhfr* and *dhps* genes, known to be responsible for the pyrimethamine and sulphadoxine resistance respectively. In all, 100 isolates of successful amplification for *Pvdhfr* and *Pvdhps* genes were obtained. Amplified PCR products were purified with the commercially available gel extraction kit and sequenced. DNA sequences were edited and aligned with reference sequence (wild type) for both the genes to identify mutant isolates.

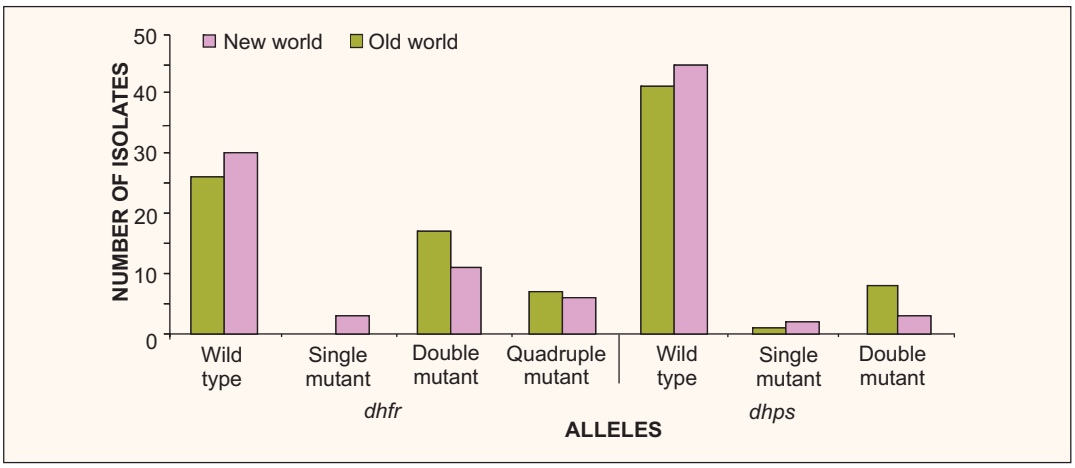

Fig. 2.1.7: Genotype wise distribution of dhfr/dhps alleles among 'Old' and 'New world' sub-populations of *P. vivax*

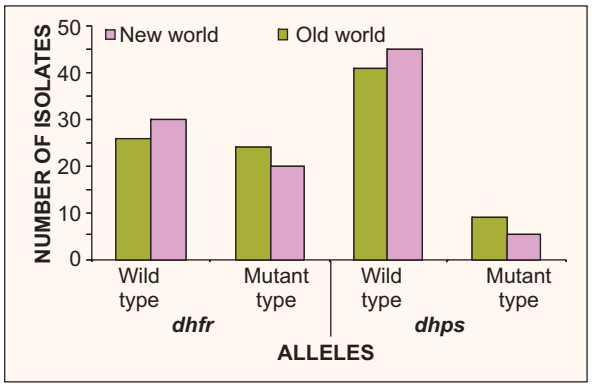

Fig. 2.1.8: Distribution of dhfr/dhps mutant genotypes among 'Old' and 'New world' sub-populations of *P. vivax*

Sequences obtained were submitted to GenBank vide accession numbers EU145878-EU145947 for *dhfr* and EU149665-EU149764 for *dhps*.

In the *Pvdhfr* gene, single, double and quadruple mutants were observed alongwith wild type genotype. In *Pvdhps* gene, limited point mutations (single and double mutants) were observed. Proportion of mutant *dhfr* alleles were more in Old world types (48%) compared to New world types (40%). Similar scenario was observed for mutant *dhps* alleles, where mutant alleles were more in 'Old world' isolates (18%) compared to 'New world' isolates (10%). Proportions of wild and mutant alleles at both *dhfr* and *dhps* loci in 'Old world' and 'New world' isolates are given in Figs. 2.1.7 and 2.1.8. Similarly, proportions of mutant alleles conferring drug resistance (double mutant) and higher level of resistance (quadruple mutant) were more in 'Old world' isolates compared to 'New world' isolates, at both *dhfr* and *dhps* loci but these differences were not statistically significant ($\chi^2 = 1.98$, df = 3, p = 0.577).

"Proportion of mutant alleles (dhfr and dhps) conferring SP resistance was higher in Old world isolates than New world isolates, though differences were not statistically significant"

2.1.4 Molecular Determination of Chloroquine Resistance in Indian *P. falciparum* Isolates

Widespread use of chloroquine (CQ) for the past few decades has led to chloroquine resistant parasites and emergence of clinical failure of chloroquine treatment. Previous studies on the *Pfcrt* (*Plasmodium falciparum* chloroquine resistance transporter) gene, that is responsible for the chloroquine resistance, revealed a heterogeneous situation of chloroquine resistance in Indian *P. falciparum* isolates. These studies analyzed the randomly collected samples and were not supported with clinical assessment of chloroquine efficacy, however, their results indicate *Pfcrt* gene as an attractive target for studying the epidemiological and molecular aspects of drug resistance. About 200 malaria patients enrolled for the chloroquine (CQ) therapeutic efficacy studies and 68 uncomplicated malaria patients from different geographical regions were assessed for genetic basis of chloroquine resistance using molecular markers. The status of point mutation responsible for CQ resistance was assessed by PCR amplification of *Pfcrt* gene. Three types of amino acid haplotypes, encoding 72aa–76aa (amino acid) of Pfcrt protein, namely SVMNT, CVIET and CVMNK were observed. We observed the prevalence of SVMNT haplotype in both clinically sensitive (70%) and resistant (74%) isolates. The wild type CVMNK was found only in

"SVMNT (mutant) haplotype was observed in both clinically sensitive (70%) and resistant (74%) isolates, however, the wild type CVMNK was found only in clinically sensitive cases (20%). Prevalence of SVMNT haplotype is observed in all geographical regions irrespective of endemicity of malaria"

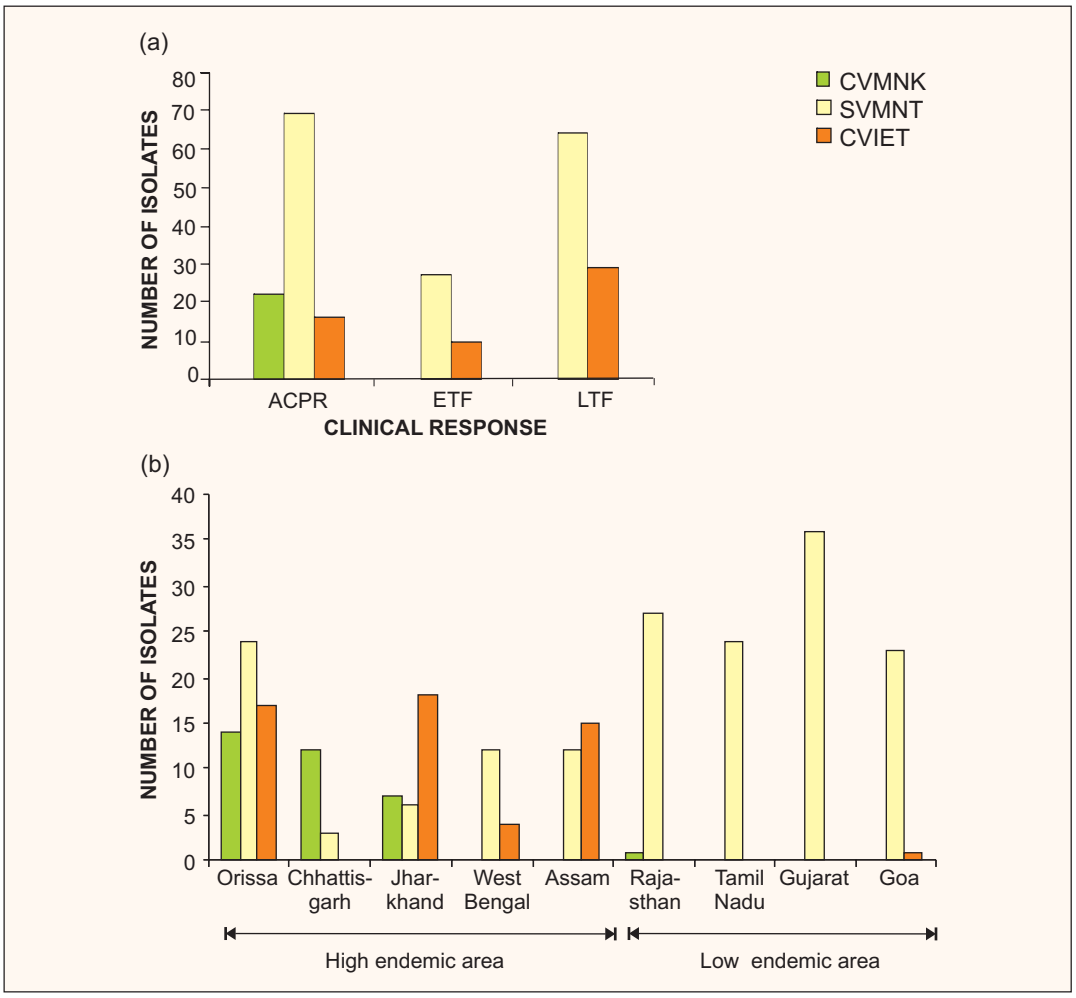

Fig. 2.1.9: Distribution of Pfcrt haplotypes among field isolates. (a) Prevalence of haplotypes in chloroquine-treated malaria cases and (b) Prevalence of haplotypes in high and low malaria endemic areas. ACPR— adequate clinical and parasitological response; ETF— early treatment failure; and LTF—late treatment failure

clinically sensitive cases (20%) (Fig. 2.1.9a). Prevalence of SVMNT haplotype was observed in all geographical regions irrespective of endemicity of malaria. Proportion of SVMNT was nearly 100% in low endemic areas, while in high endemic areas all the three haplotypes were observed (Fig. 2.1.9b).

2.1.5 Characterisation of the *P. falciparum* Strains Prevalent in Northeastern States

Objectives of this study were: (i) to identify drug resistant cases using therapeutic efficacy protocol in *P. falciparum* and to validate using molecular markers; (ii) to generate data on parasitic diversity using microsatellite markers, anchored primer amplification of DNA (APAD) and resistant markers for chloroquine (CQ) and sulfadoxine-pyrimethamine (SP); and (iii) to correlate clinical and parasitological data with genotypic data.

Molecular Characteristics of Drug Resistance Associated Mutations in *Plasmodium falciparum* from Northeastern India

The therapeutic efficacies of commonly used antimalarials were ascertained for the treatment of uncomplicated *P. falciparum* malaria patients who were enrolled for the follow-up of *in vivo* antimalarial response according to WHO protocol with regular clinical and parasitological assessment.

Blood samples were collected from two sites; (i) PHC Kumarikata, District Nalbari (Indo-

Bhutan border area), Assam (KNA); (ii) CHC Dalu, District Tura (Indo-Bangladesh border area), Meghalaya (DTM). Patients reported with fever were clinically examined. Both thick and thin blood smears were checked by microscopy for the presence of *P. falciparum*. Four to five drops of blood were spotted onto sterile filter paper (Whatman No. 3) strips in triplicate for molecular studies. Finger-prick blood samples (59 of KNA and 55 of DTM) were taken from each patient before treatment. Post-treatment sample was also taken as and when the patients reported with parasitaemia during the 28-day follow-up period. DNA from blood spots was extracted with Qiagen DNA mini kit according to manufacturer's protocol.

Parasitized blood before and after treatment was analyzed by PCR assay for variants in the target genes for *pfcrt*, *dhfr* and *dhps*. The nested mutation-specific PCR methods were used to determine the prevalence of *pfcrt* allele Lys-76 and Thr-76 (K76T), *dhfr* polymorphism at 16, 51, 59, 108 and 164 and *dhps* polymorphism at 436, 437, 540, 581 and 613 codon sites, respectively.

The unequal distribution of genotypes was observed in two areas. We found some of the isolates had mixed genotypes of both wild and mutant (Fig. 2.1.10). Majority of the isolates had Thr_{76} mutation in *pfcrt*. The mutant genotypes Ile_{51}, Arg_{59} and Asn_{108} of *dhfr* and Gly_{437} and Glu_{540} of *dhps* were significantly associated with the recrudescent parasites.

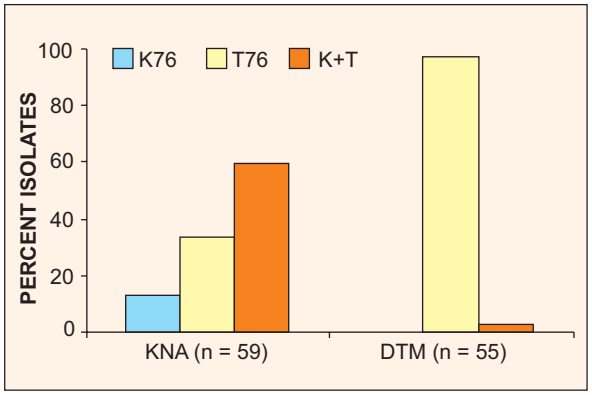

Fig. 2.1.10: Prevalence of wild, mutant and mixed genotypes of *Pfcrt* (KNA—Kumarikata, Nalbari district, Assam; DTM—Duke, Tura district, Meghalaya)

Analysis of Recrudescent Infection

Analysis of recrudescence in *P. falciparum* infection was done by genotyping of paired *P. falciparum* samples. Three paired *P. falciparum* samples collected on Day 0 and on the day of reappearance of parasitaemia were analyzed to distinguish between recrudescence and new infection. Two genetic markers, MSP-1 and MSP-2 were used for the genotyping by studying length variations in repeat nucleotide sequence regions. The genotyping of recrudescence infections using three surface protein markers, MSP-1, MSP-2 and GLURP revealed that of five chloroquine failure cases, only three had same genotype suggestive of true drug failure (Fig. 2.1.11). Similarly, for ACT, of six failure cases, four had different genotypes suggestive of new infections. In 2007, studies based in Dalu CHC of West Garo Hill district of Meghalaya along Indo-Bangla border population groups revealed that ACT was seemingly effective for the treatment of *P. falciparum* cases. Of 54 cases, 51 (94%) were ACPR, and only 3 (6%) were late clinical failures (LCF).

"Majority of the isolates from north-eastern states had Thr_{76} mutation in pfcrt. The mutation of Ile_{51}, Arg_{59} and Asn_{108} of dhfr and Gly_{437} and Glu_{540} of dhps were significantly associated with the recrudescent parasites"

Genetic Diversity Study

Genetic diversity study in *P. falciparum* isolates was done with MSP-1, MSP-2, GLURP and microsatellite markers. Ninety-six *P. falciparum* isolates collected from Kumari Kata District Nalbari, were analyzed with GLURP and the results revealed polymorphic nature of the field isolates and a total of 14 alleles were found. Proportion of multi-clone isolates were less compared to Dalu isolates. Microsatellite analysis using two loci (ARA2 and TAA60)

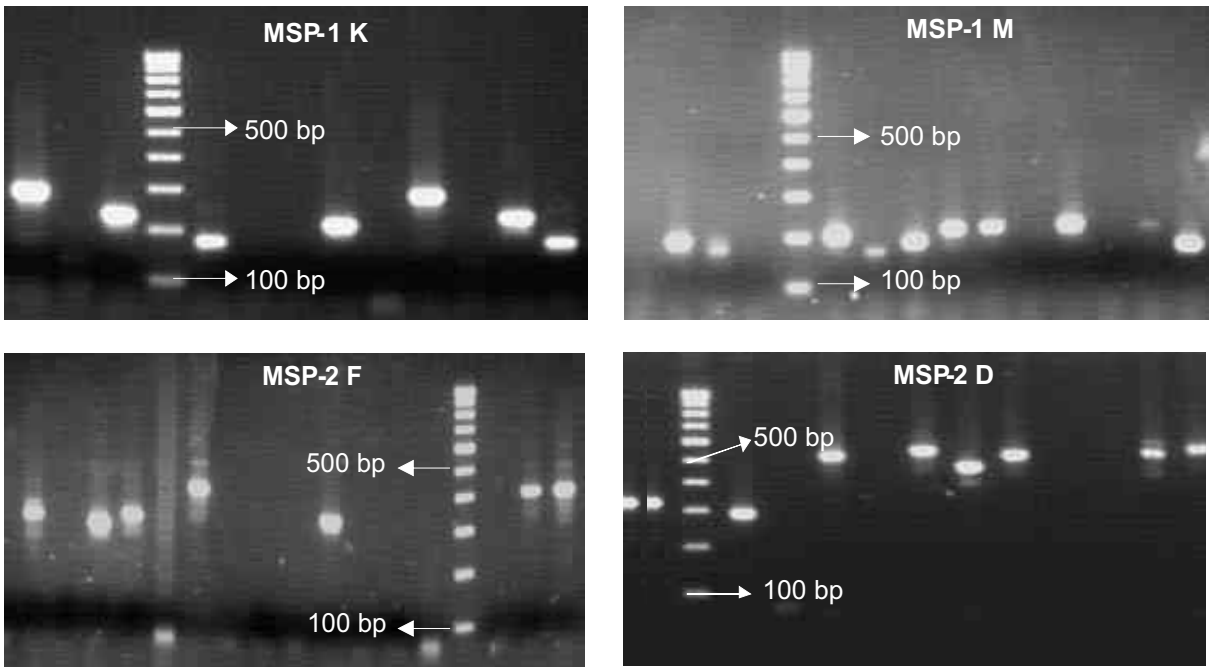

Fig. 2.1.11: Gel image showing variations in *MSP-1* and *MSP-2* gene allelic families in the *Plasmodium falciparum* field isolates

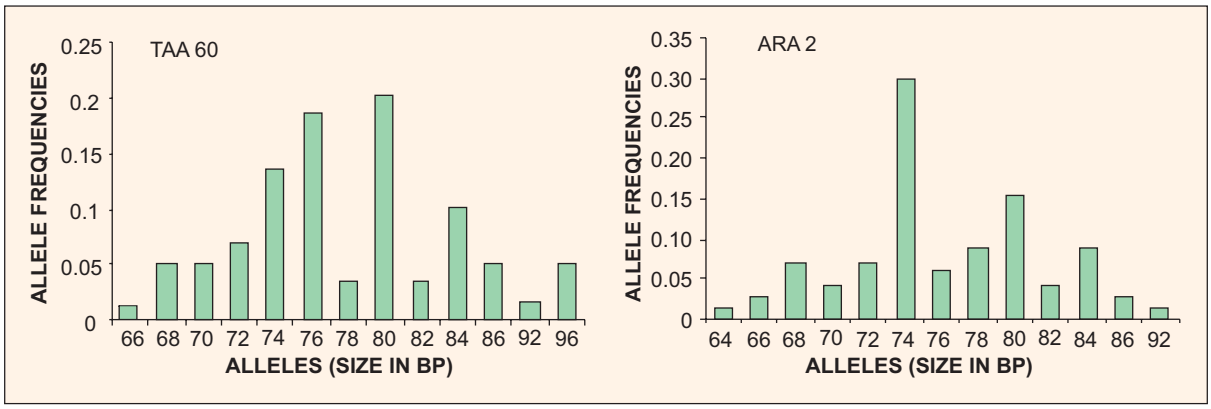

Fig. 2.1.12: Allelic frequencies of two microsatellite loci TAA60 and ARA 2 alleles in the population

revealed both loci were highly polymorphic in the field isolates. Allele per locus was 13 and heterozygosity at both loci were 0.86 (ARA2) and 0.89 (TAA60). Allele frequencies per locus per allele were variable in the population (Fig. 2.1.12). This high degree of genetic diversity at both loci in the field isolates of *P. falciparum* suggests that microsatellite could be a potential genetic marker for the recrudescence infection study.

Forty-eight *P. falciparum* isolates from Dalu collected during 2007 were analyzed for *MSP-1* and *MSP-2* genes to assess the genetic diversity. The results revealed that the *P. falciparum* isolates from Dulu were highly polymorphic and diverse. Proportion of multi-clone infections (48.83%) was very high. *MSP-1* gene has three allelic families, namely K, RO33 and MAD, of them only RO33 was monomorphic, whereas K and MAD were having wide allele range in the population. Similarly, *MSP-2* gene has two allelic families 3D7 and FC27 and both were polymorphic in the population. The distribution of allelic families in the MSP-1 and MSP-2 varied in the population (Fig. 2.1.13). Studies on microsatellite markers are in progress for more number of loci to resolve the genetic diversity and their pattern in the populations.

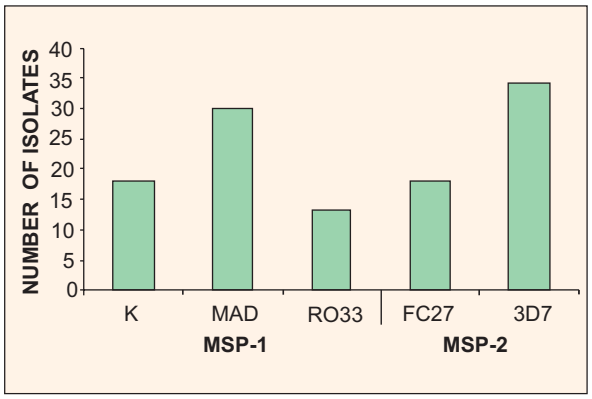

Fig. 2.1.13: Distribution of MSP-1 and MSP-2 allelic families in the population

Data generated on clinical efficacy of drugs will be validated by molecular markers as well as prevalence of drug resistance related markers among the field isolates, thus, generating accurate data on problem of drug resistance in the area.

2.1.6 Genotyping of *Plasmodium falciparum* parasites using Anchored Poly-A and Poly-T oligonucleotides from Northeastern India

Malaria parasites provide an excellent system to study the genomic effects of strong selection in a recombining eukaryote. The genetic structure of *P. falciparum* shows deletion and rearrangement of chromosome segments, expansion or shortening in the number of repetitive elements, point mutations and size polymorphism. These changes seem to be one of the important mechanisms to help the parasites in evasion of host immunity for their survival. A couple of markers have been developed for genotyping of parasites; anchored primer amplification of DNA (APAD) is one of them. This is a recently developed method, which readily displays genetic difference in A-T rich DNA sequences.

Blood samples of *P. falciparum* patients collected from two sites: (i) PHC Kumarikata, District Nalbari (Indo-Bhutan border area), Assam (KNA); and (ii) CHC Dalu, District Tura (Indo-Bangladesh border area), Meghalaya (DTM) were analysed by APAD. DNA extraction was performed with Qiagen DNA mini kit according to manufacturer's protocol. APAD was performed by using different sets of Poly-A and

"The APAD method could be a useful tool for parasite genotyping"

Poly-T primers with di- to hexa-nucleotide anchored at 3'end. Out of these primers, some were giving smear or no bands, so were excluded from the study and some selected primers giving discrete bands were used for further study. Mostly di- and tri-nucleotide anchored primers produced smears or no bands. Most of the penta– and hexa-nucleotide anchored primers produced discrete bands.

Following primers produced discrete bands:

(1) A_{14}GCATCG
(2) A_{14}GGTTCC
(3) A_{14}CGACGA
(4) A_{14}CATGCC
(5) A_{15}GTGTA
(6) T_{14}CGACGA
(7) T_{14} GCAGCA
(8) T_{15} GTCTA

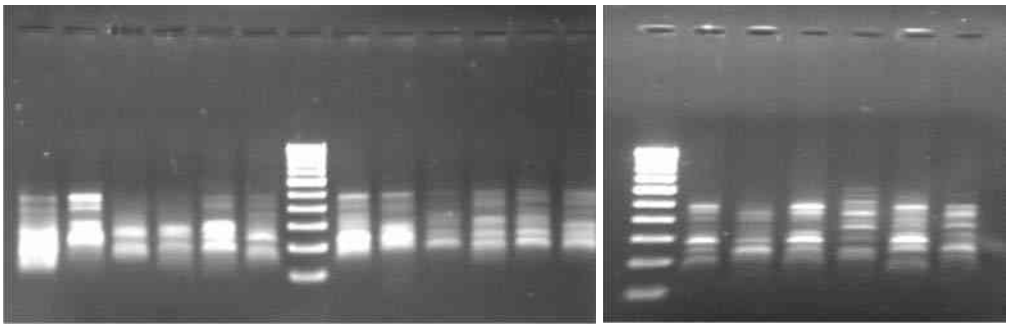

Fig. 2.1.14: Different isolates of *P. falciparum* showing variations by the primer A_{14}GCATCG by APAD; M=100 bp

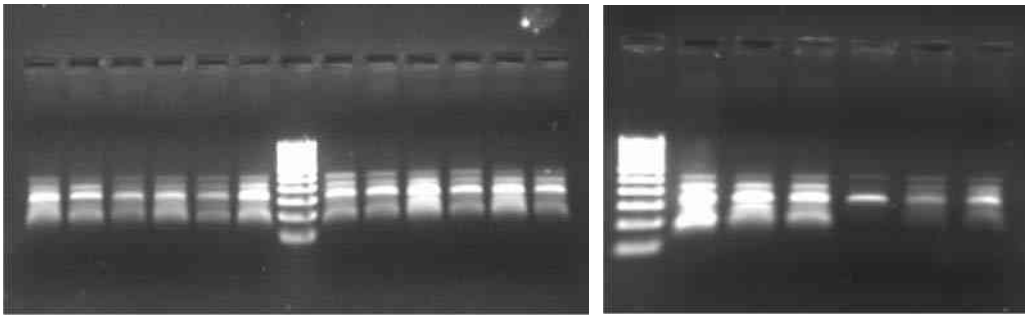

Fig. 2.1.15: Different isolates of *P. falciparum* showing variations by the primer A_{14}GGTTCC by APAD; M=100 bp

Twenty isolates have been studied for genetic diversity study. Some primers gave multiple and distinct bands with all the isolates and some show slight differences in the pattern of bands. Differences in the patterns of bands with the same primer in *P. falciparum* parasites revealed diversity among the isolates obtained from different patients (Figs. 2.1.14 and 2.1.15).

This method can be very useful for identifying polymorphism that can distinguish closely related parasite isolates. This method is very useful for rapid parasite typing because large number of loci in the genome can be checked quickly. This method also provides a potentially powerful tool for developing genetic markers from different parasite species.

2.2 Parasite Immunology

2.2.1 Naturally Acquired Immune Responses to Stage-specific *P. falciparum* and *P. vivax* Antigens in a Population of Central India

The study has been initiated with objectives: (i) to characterize immune responses to stage-specific *P. falciparum* and *P. vivax* antigens in children and adults naturally exposed to malaria; (ii) to study the development and maintenance of immune responses in different age groups with emphasis of infants, their older siblings and mothers, including identification of epitopes that correlate with protection; (iii) to determine the role of stage-specific antigens in the development and maintenance of natural immunity to malaria; and (iv) to evaluate the immune mechanisms, those are involved in pathogenesis of malaria, especially anaemia, cerebral malaria and placental malaria. The study was conducted in three populations. They are: (i) infants, children and adults from the community; (ii) pregnant women from the community; and (iii) Hospitalised patients with severe malaria. Peripheral blood, placental and cord blood at delivery were taken for determining the antibodies against species and stage-specific antigens by enzyme immunoassay. The antibody levels were quantified using known antimalarial antibody (positive controls) and this allowed us to estimate antibody levels in O.D. values. Sera from non-endemic healthy subjects were taken as negative control (Figs. 2.2.1 and 2.2.2). Cellular response was determined in peripheral blood mononuclear cells by lymphocyte transformation test in the presence of *P. falciparum* and *P. vivax* antigens and cytokines (IL-4 and IFN-γ) level was estimated in activated T-lymphocytes culture supernatant by sandwich ELISA. The peripheral blood mononuclear cells (PBMC) were isolated from venous blood of subjects who have past experience of malaria and the proliferative responses against *P. falciparum* and *P. vivax* antigens (5 each) were determined in individual set of PBMCs. Only a subset of adults and older children above 14 years were participated in this study. In general, the study subjects responded to T-cell epitopes. The mean stimulation index (SI) was not significantly different among positive responders. The SI of >2 was taken as cut-off to determine the positive responders. The *in vitro* stimulation of T-cells

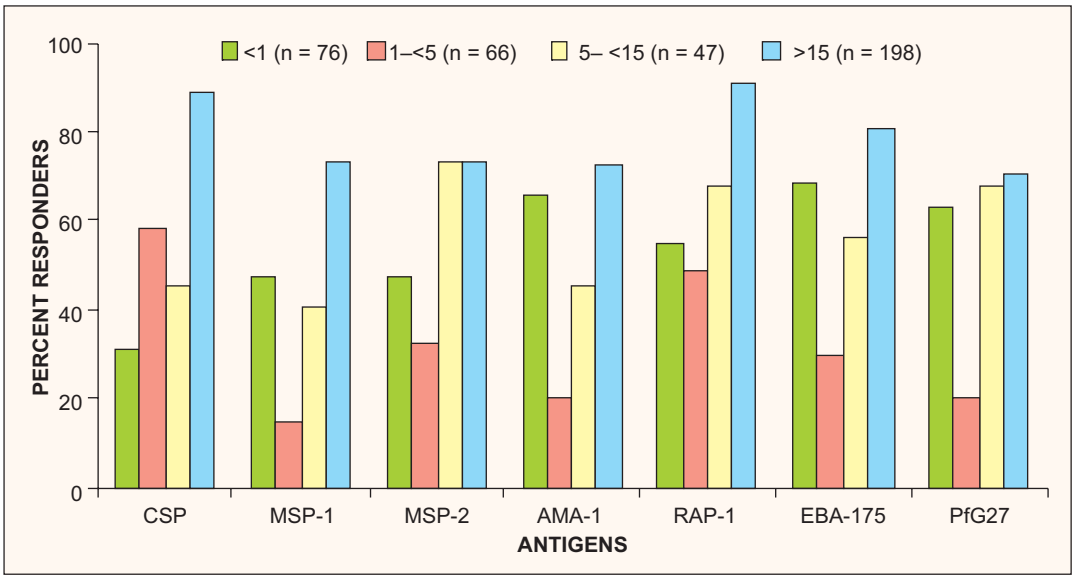

Fig. 2.2.1: Responder frequency to *Plasmodium falciparum* antigens

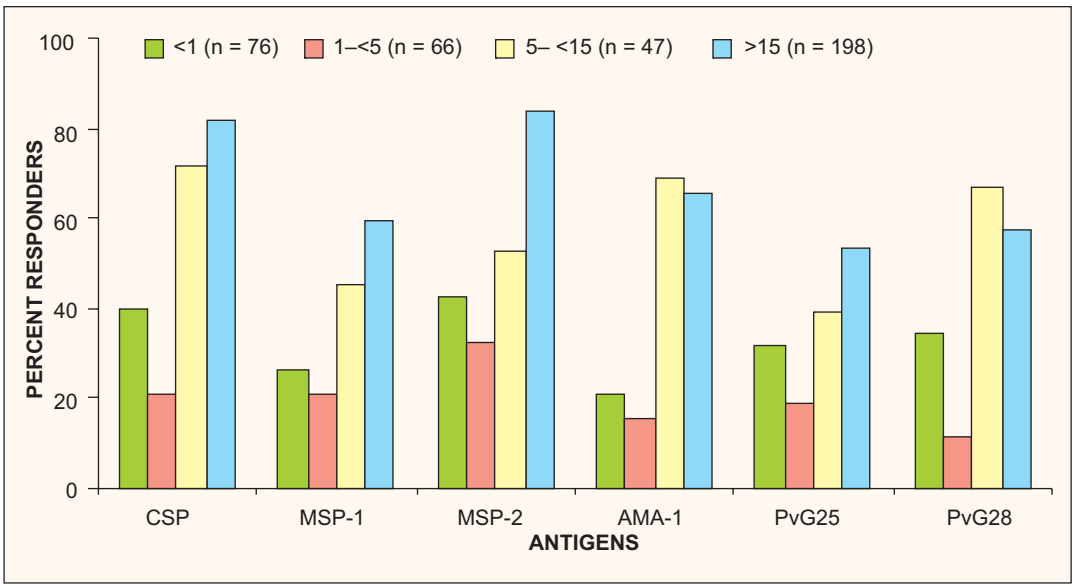

Fig. 2.2.2: Responder frequency to *Plasmodium vivax* antigens

from malaria-exposed donors results in the production of IL-4 and IFN-γ in concordance with the serum concentrations of antibodies specific for the antigens used for lymphocyte stimulation (Figs. 2.2.3 and 2.2.4). Peripheral blood from healthy cohort participants and *P. falciparum* malaria patients were taken for the estimation of cytokines (IL-4, IL-10, IP-10, IFN-γ and TNF-α) in plasma using commercially developed two-site ELISA assay kits.

Study needs to identify the antigen-specific antibody responses in the serum of pregnant women who do not suffer from the placental malaria, which may suggest that these antibodies are important for protecting the mother against infection. Antibodies produced by adults (mothers) were correlated with protection. Therefore, it is important to determine if infants mount antibody responses to species and stage-specific antigens and the association between infant's antibodies with protection. An important component of this study is to understand the role of immunologic factors in the pathogenesis of severe malaria. The current understanding of

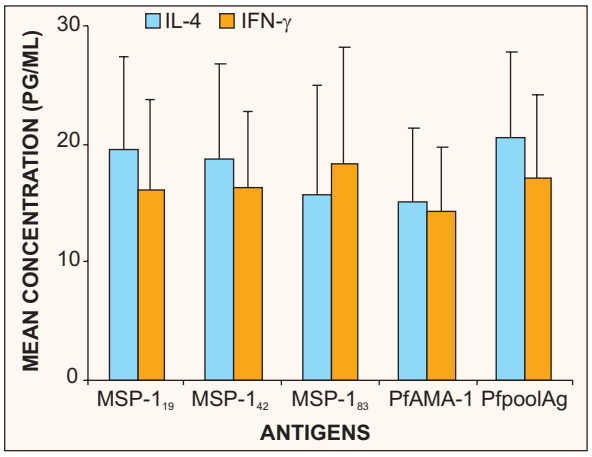

Fig. 2.2.3: Cytokine level in lymphocyte culture supernatant with *Plasmodium falciparum* antigens

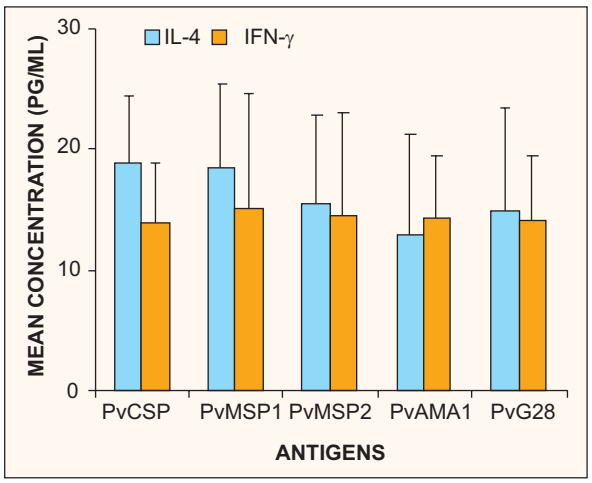

Fig. 2.2.4: Cytokine level in lymphocyte culture supernatant with *Plasmodium vivax* antigens

the immunology of severe malaria is mostly based on African studies. However, Indian populations face a different epidemiologic setting due to differences in the malaria transmission and occurence of both *P. falciparum* and *P. vivax*.

As proposed in the project, data on all above parameters would be generated to fulfill the objectives in more number of samples for determining the role of stage-specific antigens in the development and maintenance of immune responses in different age groups with emphasis on infants and mothers. Cellular immune responses and cytokine profiles in both *P. falciparum* and *P. vivax* patients would also be determined by a hospital-based survey.

2.2.2 Parasite Growth Inhibition and ELISA IgG Responses by Antibody Dependent Cellular Inhibition (ADCI) Assay

Batches of high antibody titer sera from clinically immune subjects were tested for their effect in growth of *P. falciparum in vitro* by antibody dependent cellular inhibition (ADCI) assay in the presence of peripheral blood monocytes. The isolation of monocytes was done from peripheral whole blood of healthy adults, who had no past malaria history. A well-adapted, stable culture line of *P. falciparum* was used for determining the effect of various sera in the parasite growth. The average rate of multiplication of this strain was 8–10 fold after 96 h. Sera of clinically immune individuals (n = 20) with high antibody titer were taken for the assay. They were tested at 1 : 10 dilution for their effect on the parasite growth in the absence of monocytes by growth inhibition assay or in the presence of monocytes by ADCI assay in an established *P. falciparum* culture line. Culture synchronized at ring stage was added in 96-well flat bottom tissue culture plates at 5% hematocrit with 1% parasitaemia. Assay was done for 48 h at 37 °C by Candle-jar technique. Growth of the parasite (development and multiplication) was monitored microscopically in thin smears counting number of ring and early trophozoite stages. The sera showed growth inhibition at a range of 33–67%, but same sera showed enhancement of 49–82% in the presence of monocytes. The control serum pool also

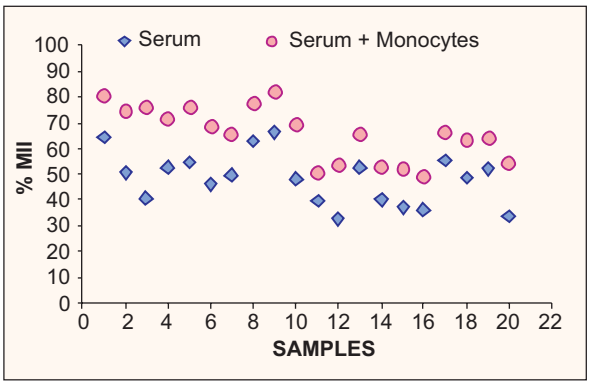

Fig. 2.2.5: Merozoite invasion inhibition (MII) in the absence or presence of monocytes

showed an average of 12.3% inhibition in the presence of monocytes. Therefore, test serum showing monocyte-mediated inhibition above 15% (Mean ± 2 SD; 12.3 ± 2.7) was considered significant. All 20 sera, which showed ability to promote monocyte-mediated cytotoxicity to *P. falciparum* had substantial level of antigen-specific IgG (Fig. 2.2.5). However, there was no significant correlation between the presence of antibodies and merozoite invasion inhibition.

The biological function of antibodies detected by ELISA was assayed in *P. falciparum* culture for their ability to inhibit the parasite growth. All 20 clinically immune donors had antibodies to merozoite stage antigens substantially. However, all of them could not cause significant inhibition of parasite multiplication. The addition of monocytes to cultures containing these antibodies affected parasite multiplication to some extent with all the sera. It is to be expected that sera containing cytophilic or opsonic antibodies mediate in phagocytosis of free merozoites causing inhibition in merozoite invasion of new RBC. The lack of significance of correlations between serum-dependent monocytes-mediated cytotoxicity and antibodies may indicate the failure of the sera to recognize appropriate antigens on the surface of infected erythrocytes. It was observed that most of the sera, despite the presence of high antibodies to blood stage antigens, could not substantially control parasite growth alone. Antibody-dependent monocytes-mediated antimerozoite activity has been suggested as one of the important *in vivo* antiparasite mechanisms. To monitor such mechanisms, appropriate assays would be essential to provide feed back in the vaccine development.

2.2.3 Purification and Characterization of Monoclonal Antibodies against Erythrocytic Stages of *Plasmodium vivax*

This is in continuation of the earlier work related to characterization of monoclonal antibodies. Of the 27 hybridomas, 15 lines were revived. They were grown *in vitro*. These clones were taken for antibody production and characterization. Supernatant of each culture line was tested by ELISA for antibody against *P. vivax*, *P. falciparum* and human-NRBC lysate. Fifteen *P. vivax* antibody positive clones were characterized by immunoglobulin isotyping by ELISA. Of the 15 hybridomas, 13 produced IgG type of antibody, one showed both IgG and IgM responses and one produced IgM type antibody. Of the 13 IgG producing hybridomas, 11 showed stable growth and by subclass analysis these were found to be IgG1 type (Fig. 2.2.6). Culture supernatant from 11 growing hybridomas was tested for

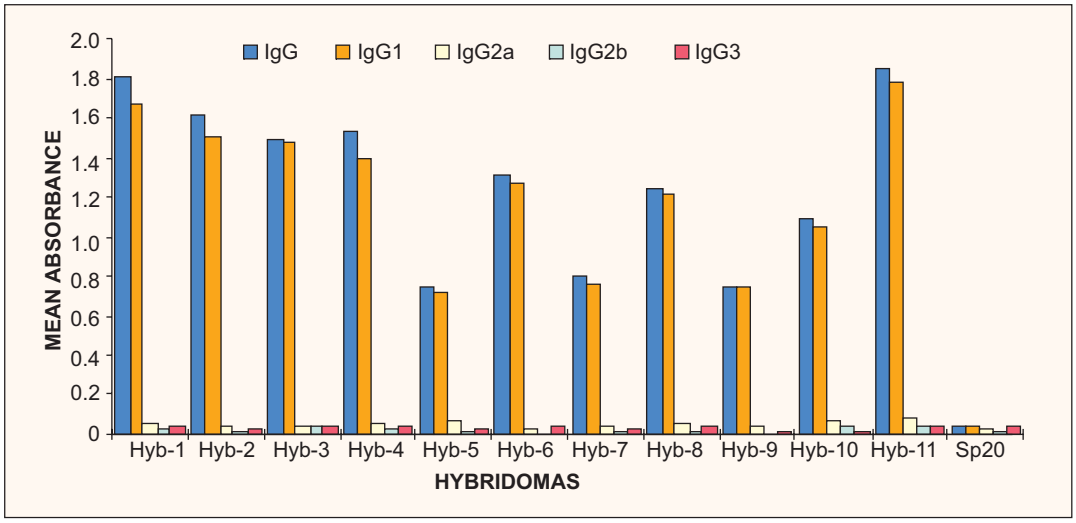

Fig. 2.2.6: Immunoglobulin subclass analysis of hybridomas

their reactivity with *P. vivax* erythrocytic stages by IFA test. All 11 MAbs reacted with blood stages of *P. vivax* with varying intensity. Of the 11 monoclonals, 8 were tested on dot-blots with patients' blood samples. The *P. vivax* crude antigen (PvC) was subjected to SDS-PAGE to check the band patterns on the gel. Proteins from the unstained SDS-PAGE slab gel were transferred electrophoretically to polyvinylidene difluoride (PVDF) membrane. They were used to develop Western blot by reacting with six MAbs. On Western blot, each MAb reacted with proteins of *P. vivax* lysate. Seroreactivity of 6 MAbs was also determined against PvC antigen by competitive binding assay in an indirect ELISA. The addition of second MAb showed a rise in seroreactivity as observed in percent increase in antibody binding. Dot ELISA showed specific reaction of monoclonal antibodies with *P. vivax* infected patients' blood.

"Humoral responses to the erythrocytic stage antigens were acquired in an age-dependent manner during natural course of infection, and these sera containing antigen-specific antibodies mediated in parasite phagocytosis"

2.2.4 Circulatory Cytokines in Clinically Active *Plasmodium vivax* Infection

Malaria parasites have evolved to acquire diverse immune evasion mechanisms that evoke poor immune responses and allow infection to individuals previously exposed. The malarial parasite, *P. vivax* is sensitive to inflammatory response, and thus, the circulating cytokine evaluation has attracted interest in the field of diagnosis, therapeutics and monitoring in view of malarial and clinical complications. Our knowledge of the cytokine profile and imbalance in cytokine network in clinical severity, pathogenesis, protection and susceptibility of vivax malaria is very limited. Thus, to establish its relevance to active clinical vivax malaria as well as the clinical response of pro- and anti-inflammatory cytokine in severity, pathogenesis and diagnosis of disease, we investigated the impact of the content of TNF-α, IL-2, IL-6, IL-8, IFN-γ, IL-10 and IL-12 in serum of adults with an emphasis of clinically defined heterogeneous groups. These cytokines were evaluated because of their biological relevance in inflammatory and immunomodulatory responses in order to obtain a global measure of the patients' actual reactivity towards *Plasmodium* challenge. We also studied the association and specific involvement in terms of clinically heteroge-

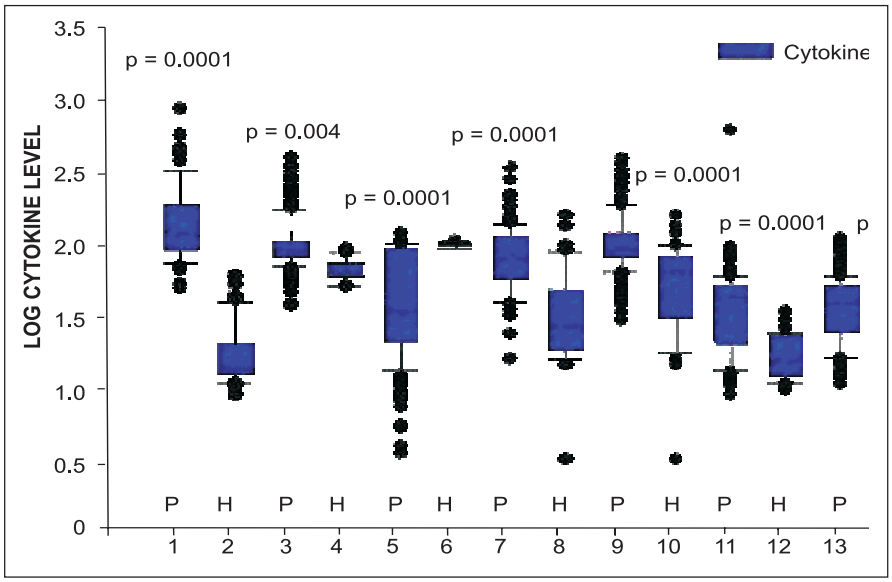

Fig. 2.2.7: Differences in serum cytokine levels of TNF-α (1, 2), IL-6 (3,4), IL-10 (5,6), IFN-γ (7,8), IL-8 (9,10), IL-12 (11,12) and IL-2 (13,14) (in pg/ml) in clinical vivax malaria as compared to healthy subjects. Horizontal bars indicate cytokine wise medians and SE for each cytokine is denoted by error bars; P— Patients; H— Healthy subjects

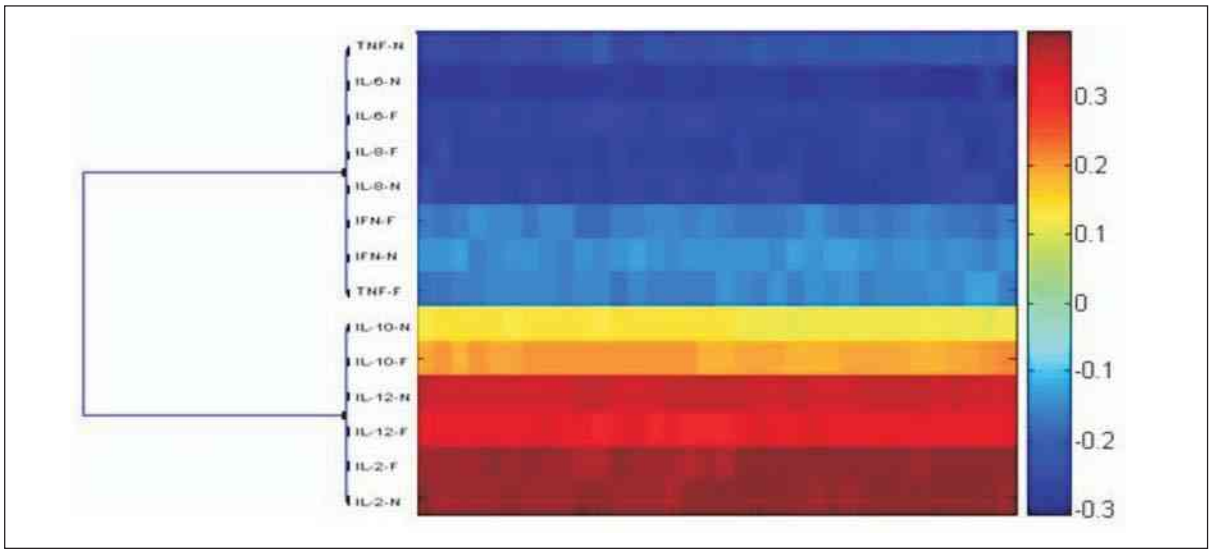

Fig. 2.2.8: Two-way coupled cluster analysis. Each cell in the 2-dimensional graph indicates the measure of a single cytokine in one sample with standardized levels indicated by colour according to the scale on the right. Sample clustering resulting from the algorithm applied is shown at the left side of the graph as a vertical dendrogram, with an indication of the group to which each individual sample belongs. Major clusters discriminated the clinical groups exactly. Cytokine clustering is depicted analogously in the vertical order to the left of the graph. TNF—Tumor necrosis factor; IL—Interleukin; IFN—Interferon

neous groups/clusters between serum cytokine network/profile and clinical parameters like temperature, weight and age.

An overall significant elevation of serum TNF-α, IL-6, IFN-γ, IL-2, IL-8 and IL-12 content ($p < 0.05$), whereas highly significant depletion of IL-10 content ($p = 0.0001$) was observed in vivax patients (Fig. 2.2.7). Two-way coupled cluster analysis revealed two clusters of cytokines relevant to clinical subgroups stratified according to patient's cytokine level, with (P-F) and without fever (P-NF). The first cluster, composed of TNF-α, IL-6, IL-8 and IFN-γ, revealed predominance of pro-inflammatory cytokines, with elevated levels of all, except TNF-α. Whereas the second cluster, composed of IL-10, IL-12 and IL-2, displayed anti-inflammatory response and immunomodulatory cytokines, with elevated levels of all cytokines, except IL-10. Moreover, in both the cluster groups the levels were found to be statistically significant ($p < 0.05$) in all cytokine groups during infection (Fig. 2.2.8). Furthermore, a considerable qualitative and quantitative inter-individual variability in the Th1 and Th2 type cytokines was found and a statistically significant difference ($p < 0.05$) with an initial dominance of Th1 response (Figs. 2.2.9 and 2.2.10a).

"The sequential and prospective characterization of cytokine levels during infection with vivax malaria could provide additional insight into their pathogenic and protective role in the disease and might ultimately suggest therapeutic interventions"

A significant upsurged ratio of pro-inflammatory response compared to anti-inflammatory ($p = 0.001$) response was observed with the onset of vivax infection and this eventual imbalance is a vital determinant of pathological conditions, host defense, hemopoiesis and inflammation (Fig. 2.2.10b). In addition, our findings suggest that variability in the circulating level of immunomodulatory cytokine ratio is of biological significance and may play important roles in host defense mechanisms against vivax infection by enhancing cell-mediated immunity and stimulating the protective immunological cascade (Fig. 2.2.10c).

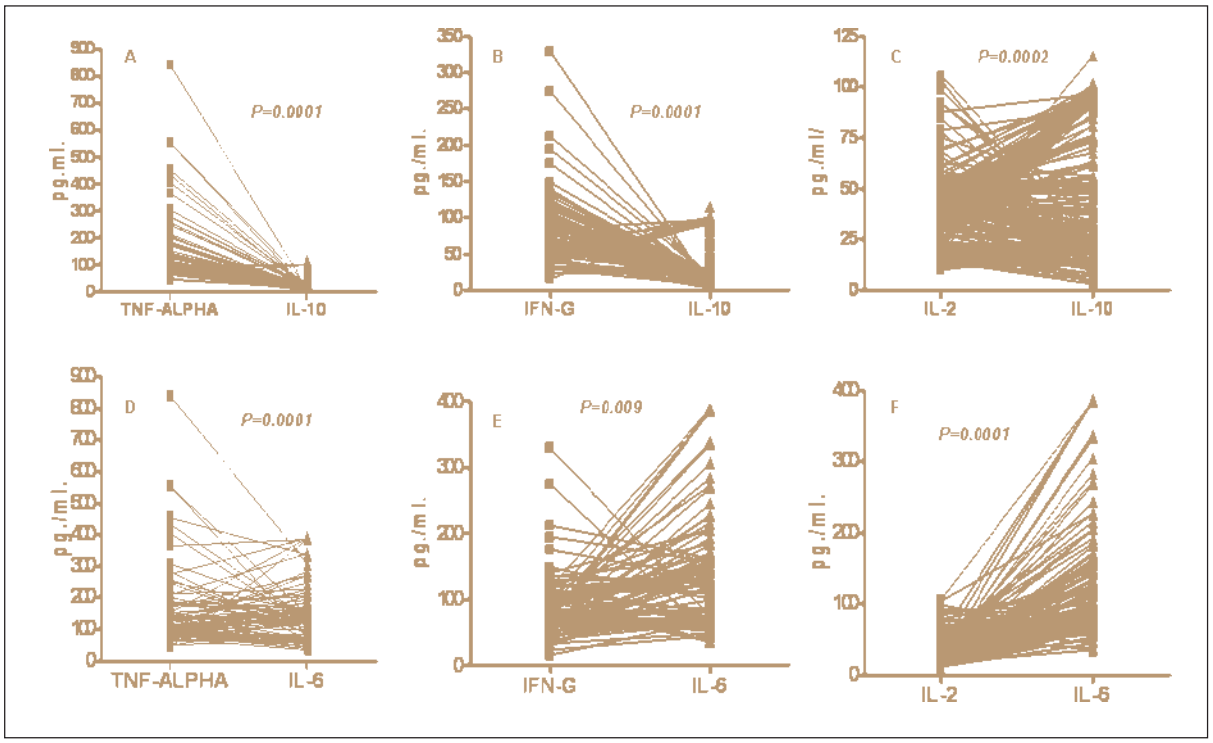

Fig. 2.2.9: Relationship between the individual values of Th1-type cytokine (TNF-α, IFN-γ and IL-2) *vs* Th2-type cytokine (IL-10 and IL-6) of *P. vivax* exposed individuals. The differences between the Th1-type cytokine *vs*. Th2-type cytokine were statistically significant (p <0.05)

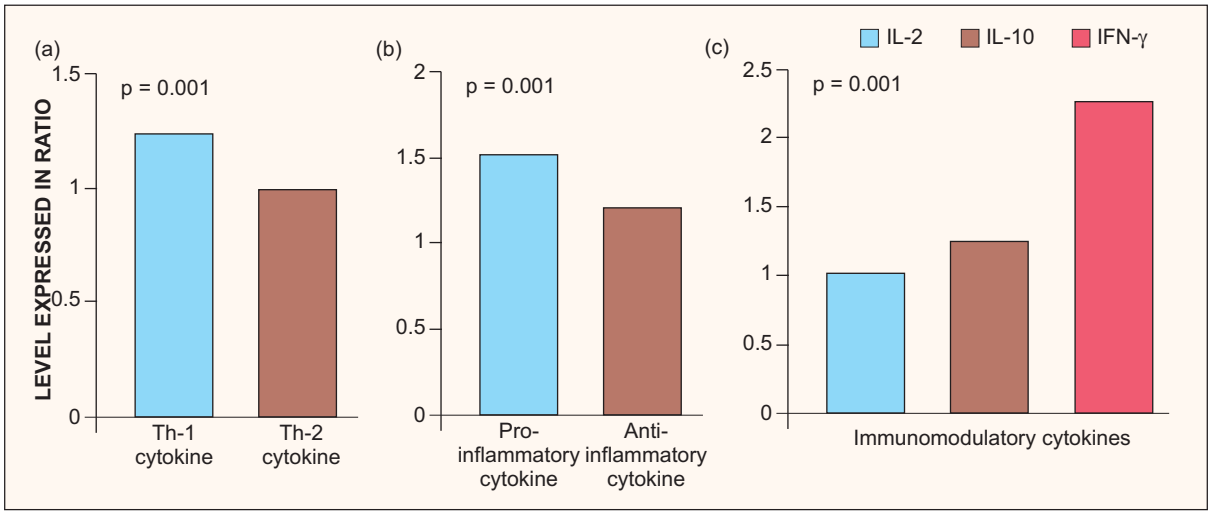

Fig. 2.2.10: (a) Level of Th1 cytokines (TNF-α, IL-2 and IFN-γ) and Th2 cytokines (IL-10 and IL-6) cytokines in vivax infected patients. The differences between the ratios of two groups were statistically significant; (b) Level of pro-inflammatory (TNF-α and IL-6) and anti-inflammatory (IL-10 and IL-12) cytokines in vivax infected patients. The differences between the ratios of two groups were statistically significant; and (c) Level of immunomodulatory cytokines (IL-2, IL-10 and IFN-γ) in vivax infected patients

In view of the present findings of cytokine profile and other clinical parameters of vivax infection, we suggest that pro-inflammatory responses are associated with rapid control of parasite growth at the cost of developing clinical symptoms, suggesting a profound consequence of initial cellular response on disease outcome and may be considered a reliable immunological marker, a promising rational for diagnostic potential and immuno-therapeutic interventions in clinical vivax malaria.

2.3 Bioinformatics

2.3.1 Comparative Evolutionary Genomics of *Plasmodium falciparum* and *P. vivax*

Complex and rapidly evolving behaviours of the two human malaria parasites, *P. falciparum* and *P. vivax* have always been mysterious to the evolutionary biologists as the former is the most virulent and the latter is most prevalent malaria parasite species across the globe. With the availability of whole genome sequence data, it is now feasible to pinpoint genomic similarities and differences between the parasites with comparative evolutionary genetic approaches and thus, define new measures for malaria control. We herewith utilized available genome information of these two species and compared functional genes of *P. falciparum* with partially-assembled whole genome sequences of *P. vivax*. Four different kinds of genes are present in the entire genome of *P. falciparum*. The distribution of these genes are shown in Fig. 2.3.1. About 82% of total functional genes of *P. falciparum* were found to be conserved in *P. vivax* and rest 18% to be unique to *P. falciparum*. Although both types of genes were distributed across all 14 chromosomes of *P. falciparum*, the distribution was slightly biased towards two separate chromosomes for each category (Fig. 2.3.2). About a half of the conserved genes was intron-less, whereas almost all unique genes have introns. However, number of introns was comparatively higher (usually >2) in the intron-possessing conserved genes than in the unique genes (mostly <2) (Fig. 2.3.3). Statistically significant positive correlations between total intron length and gene lengths were detected in 11 chromosomes for unique genes, whereas only in three chromosomes for conserved genes. Three most conserved genes (Actin, Elongation factor alpha 1 and Ribosomal protein L 10 putative) between *P. falciparum* and *P. vivax* were found to be highly conserved in four other species of *Plasmodium* (except Actin gene in *P. chabaudi*) and were

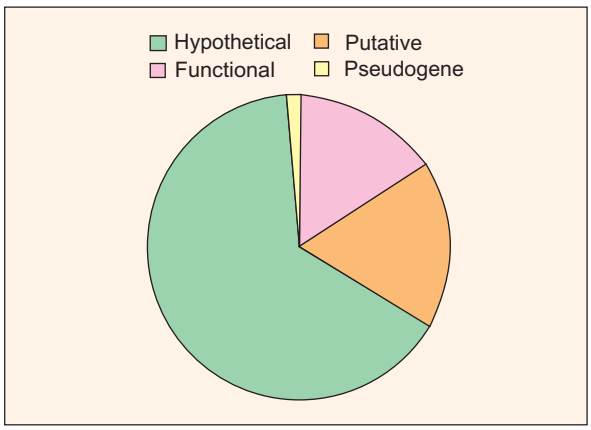

Fig. 2.3.1: Total genes in *P. falciparum* genome. Note that the putative and functional categories (together considered as functional) of genes have been utilized in the present study

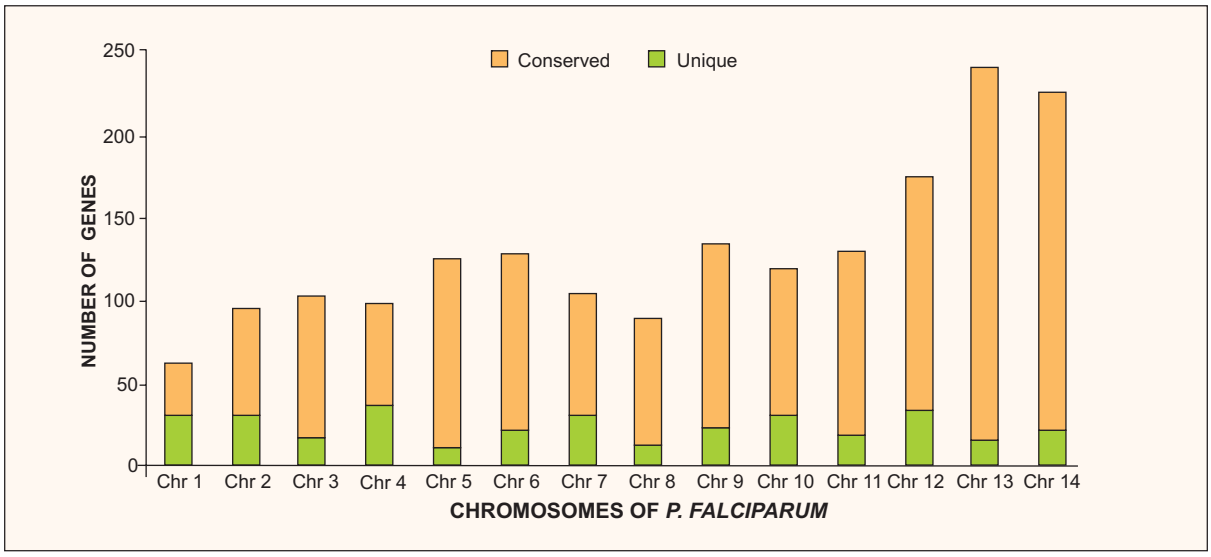

Fig. 2.3.2: Different proportions of unique and conserved genes in chromosomes of *P. falciparum*

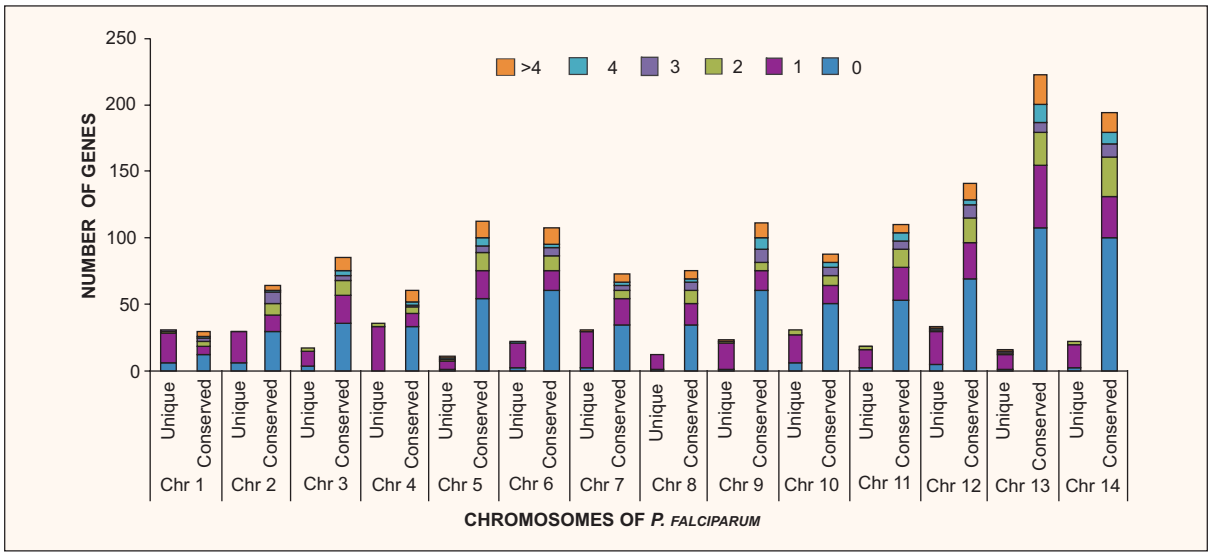

Fig. 2.3.3: Distribution of genes with different intron numbers in conserved and unique genes in chromosomes of *P. falciparum*

mostly intron-less. Phylogenetic trees were constructed separately for each of the three genes (Fig. 2.3.4); in two genes (Actin and Elongation factor alpha 1) (Fig. 2.3.4 a, b) different *Plasmodium* species were placed in almost similar positions, whereas Ribosomal protein L 10 putative show different relationships between *Plasmodium* species (Fig. 2.3.4c). Three unique gene families in three *Plasmodium* species (*P. falciparum*, *P. vivax* and *P. knowlesi*) were studied in detail for total intron length and correlations between intron lengths and gene lengths, which corroborate findings on the overall patterns of whole unique genes of *P. falciparum*. The results are discussed in terms of chromosome and intron evolution in *Plasmodium* in general, relevance of introns in differential functions of *P. falciparum* genes and genetic similarities and differences between *P. falciparum* and *P. vivax* and its implications in malaria, in particular.

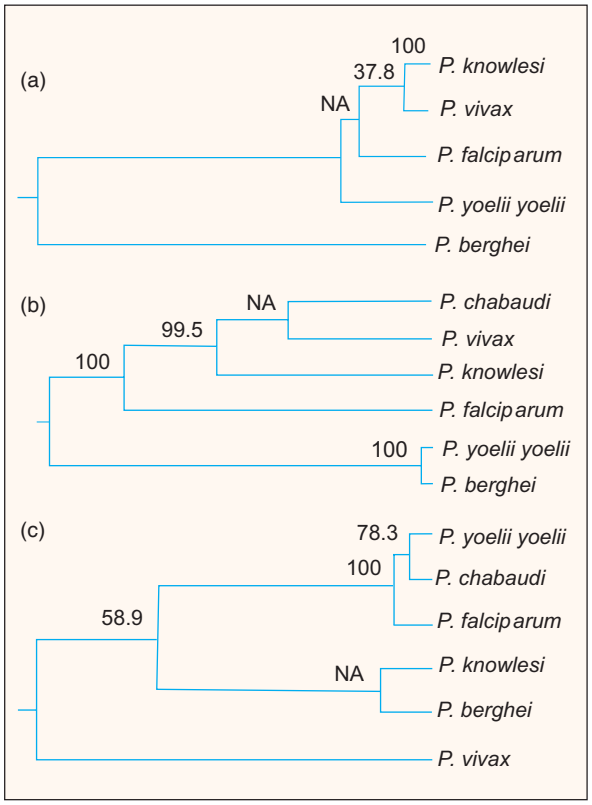

Fig. 2.3.4: (a) Phylogenetic status based on actin; (b) Phylogenetic status based on elongation factor alpha 1; and (c) Phylogenetic relationship based on ribosomal protein L10 putative

2.3.2 Genetic Characterization and Evolutionary Analysis of Human CD36 Gene

Understanding evolutionary genetic details of immune system genes responsible for infectious diseases is of prime importance concerning disease pathogenecity. Considering malaria as a devastating disease in the world including India, detail evolutionary understanding on human immune system gene is essential. The primary aim of this study is to initiate work on such genes. To start with, we have considered the human CD36 gene that is responsible in malaria pathogenesis. DNA sequences of the human CD36 gene present in chromo-

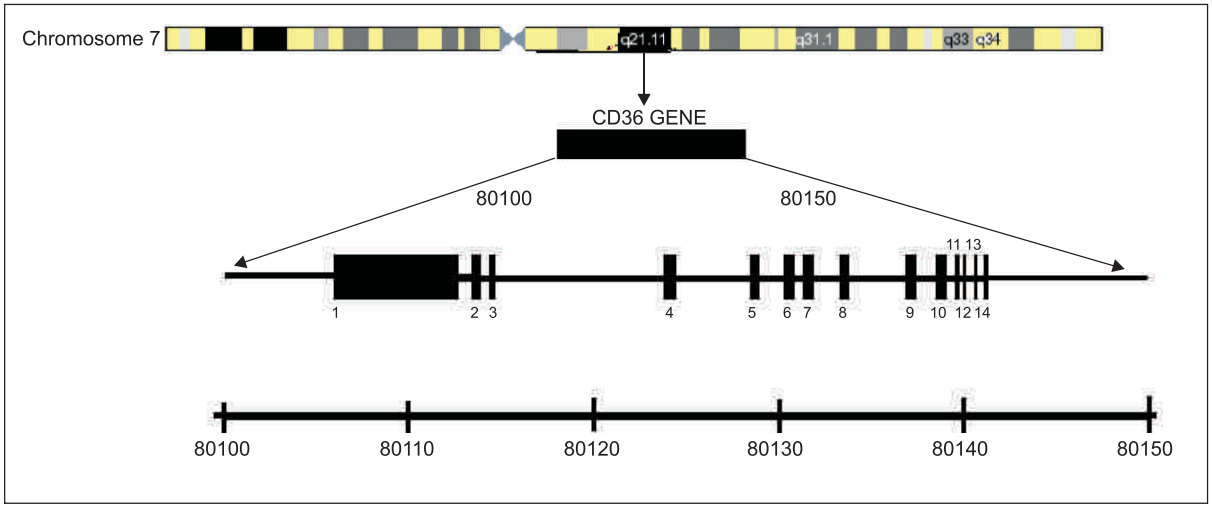

Fig. 2.3.5: Location of human CD36 gene at the locus 7q21.11 on the chromosome 7 showing 14 exons (excluding the untranslated region) (Figure not in scale)

some 7 (Fig. 2.3.5) was retrieved from public domain and fine-scale details were characterized (Fig. 2.3.6). Both comparative and evolutionary analyses were performed with sequences from six other taxa, namely *Mus musculus*, *Rattus norvegicus*, *Pan troglodytes*, *Macaca mulatta*, *Canis familiaris* and *Gallus gallus*, where CD36 homologs are present. Different statistical analyses were also performed. We detected differential distribution in the lengths of exons and introns in CD36 gene across seven taxa (Fig. 2.3.7). The cpG islands were also found to be distributed unevenly across the gene and taxa. We constructed neighbour-joining tree and observed that the chimpanzee and human are diverged at the CD36 gene relatively recently. The chicken, *Gallus gallus* was found to be diverged from rest of the taxa sig-

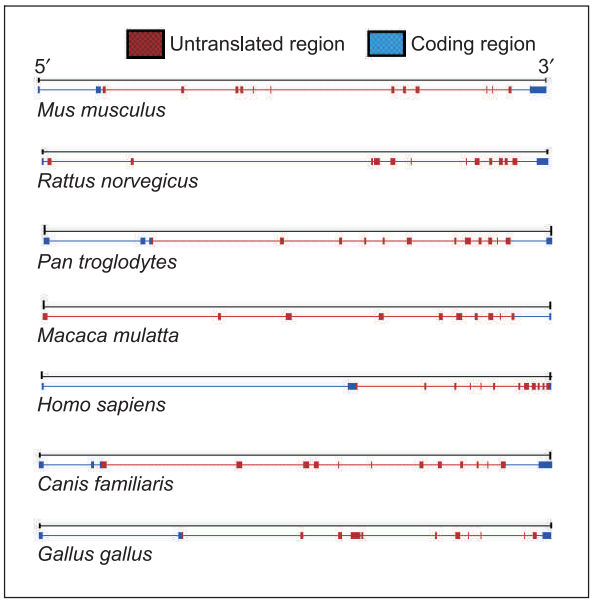

Fig. 2.3.6: Detailed characterization of CD36 gene in different taxa showing the position of coding (exons) and non-coding (introns) (Figure not in scale)

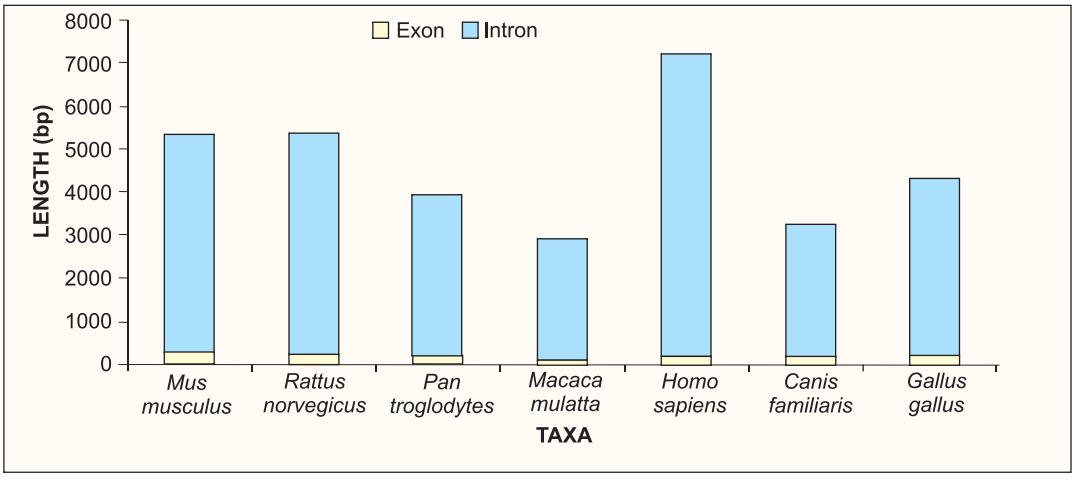

Fig. 2.3.7: Relative composition of exon and intron in CD36 gene among different taxa

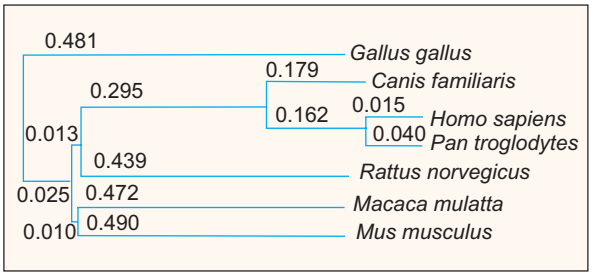

Fig. 2.3.8: Phylogenetic status of different taxa at CD36 gene. Values depict length of each branch leading to a single taxon

nificantly (Fig. 2.3.8). Gene copy number variation was observed across different taxa (Fig. 2.3.9). Comparative genomic study of a human immune-system gene was conducted for the first time in CD36 gene, which revealed relationships among different taxa at the evolutionary level. The information can be used for future study in this gene at the molecular level, especially the level of genetic diversity in malaria endemic zones and correlate it further with malaria pathogenecity.

"Cluster analysis of cytokines revealed that balance of inflammation mediates host defence against vivax infection and differential cytokine responses correlate with clinical outcomes and triggers clinical immunity during active clinical malaria"

2.3.3 *In silico* Genetic Characterization and Evolutionary Inference of TNF-α

TNF-α is an important human cytokine that imparts dualism in malaria pathogenecity. At high dosages TNF-α is believed to exhibit pathogenecity against cerebral malaria and at lower dosages TNF-α is protective against severe human malaria. In order to understand the human TNF-α gene closely and to ascertain evolutionary aspect of its dualistic nature on malaria pathogenecity, we first characterized this gene in detail in six different mammalian taxa. The avian taxa, *Gallus gallus* were

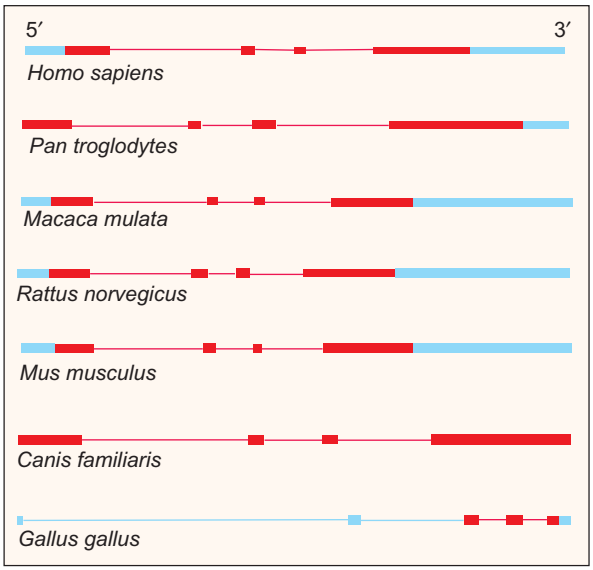

Fig. 2.3.10: Fine-scale characterization of TNF-α gene among six mammalian taxa with coding exons (red) and untranslated region (UTR) or non-coding exons (blue). For *G. gallus* information on the TNF-β has been provided. The length of non-coding exons, coding exons and introns are not in scale.

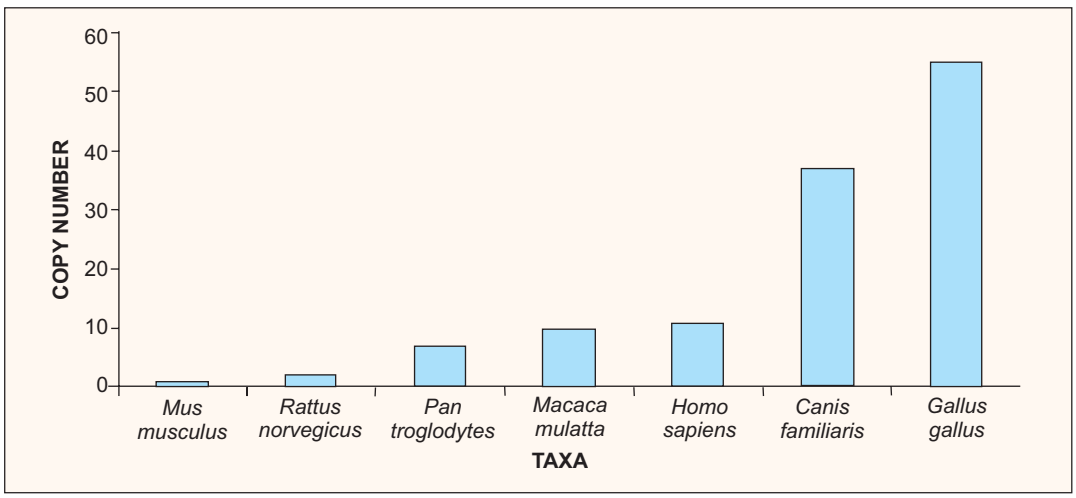

Fig.2.3.9: Copy numbers of CD36 gene in different taxa

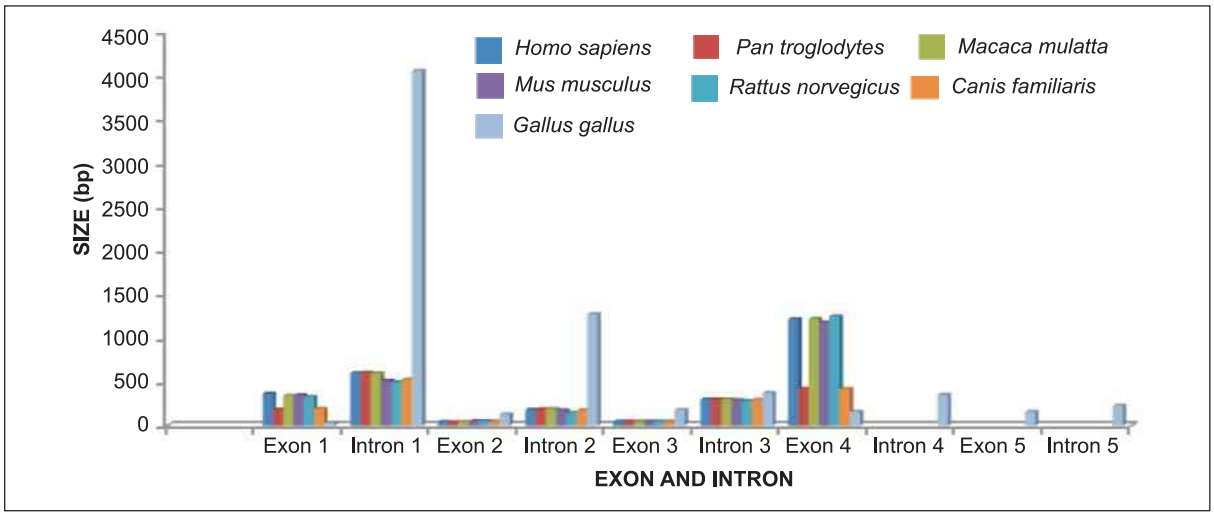

Fig. 2.3.11: Size distribution of exons and introns across all the seven taxa

included in the present study, as TNF-α is not present in birds, therefore, a tandemly placed duplicate of TNF-α (LT-α or TNF-α) was included in this study (Fig. 2.3.10). Comparative study was performed on nucleotide length variation, intron and exon size and number variation, differential compositions of coding to the non-coding bases etc. to look for similarities/dissimilarities at the TNF-α gene across all seven taxa (Fig. 2.3.11 & 2.3.12). The

"Genetic characterisation of human TNF-α gene and comparison among six mammalian taxa ascertain evolutionary aspects and dualistic nature in malaria pathogenecity"

"Comparative study of P. falciparum functional genes with other Plasmodium species signifies chromosome and intron evolution"

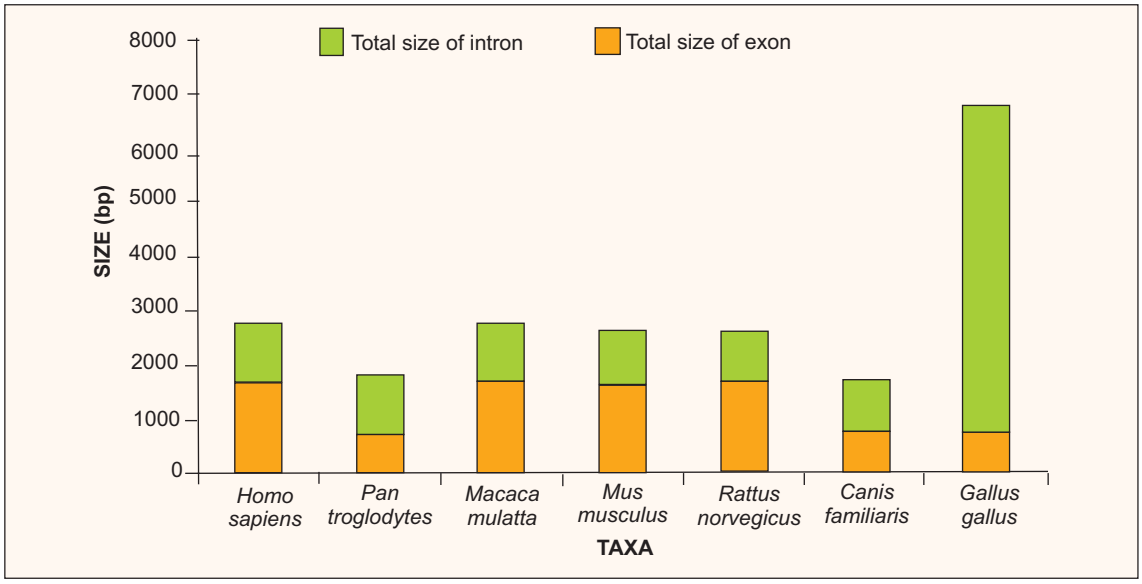

Fig. 2.3.12: Proportion of total coding and non-coding nucleotide in TNF-α gene across different taxa

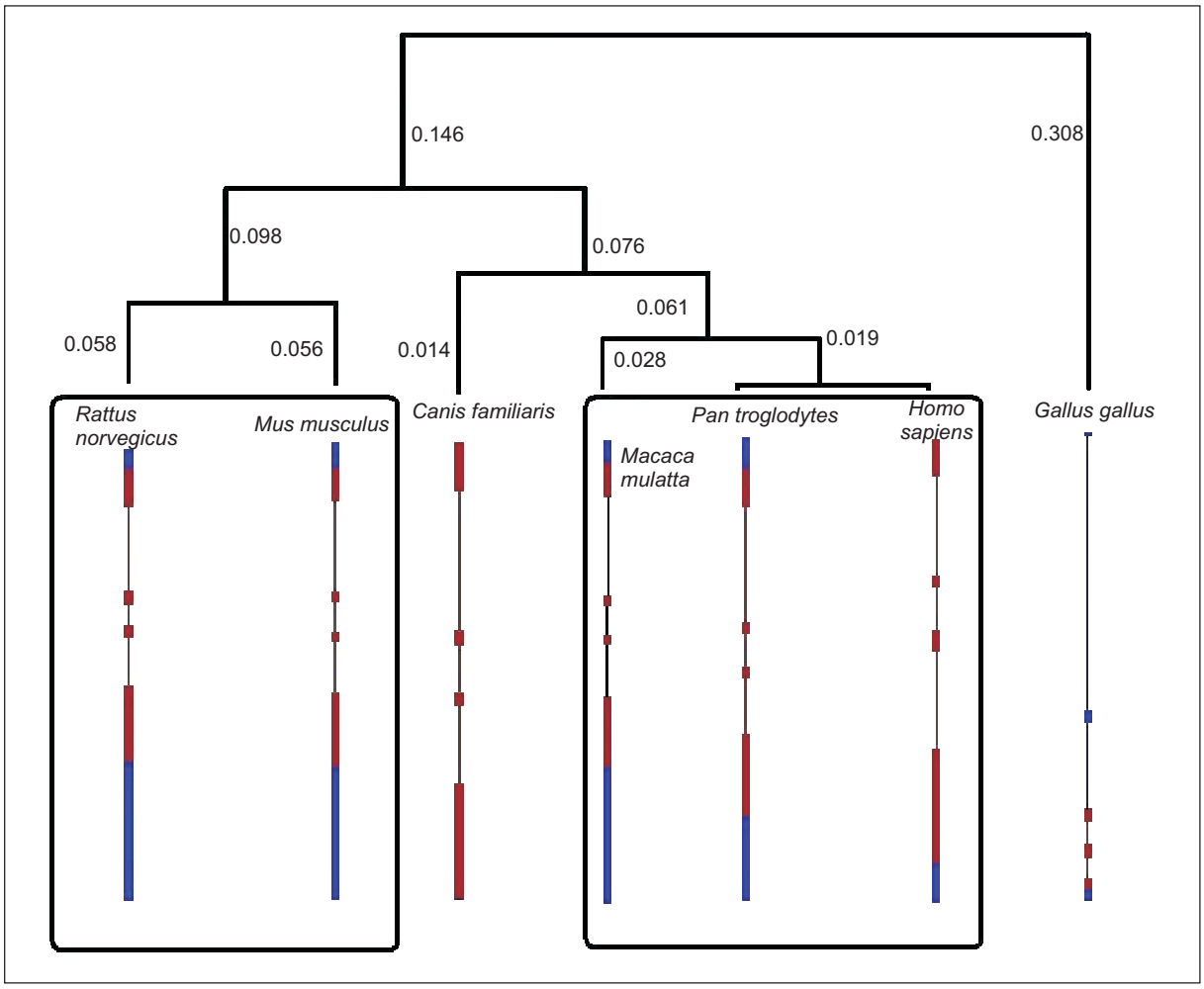

Fig. 2.3.13: Taxonomic position of different taxa in an unrooted Neighbour-Joining tree at the TNF-α gene. The branch length values are shown at each branch leading to taxa. In case of *G. gallus*, the TNF- β gene has been considered, as the TNF-α gene is not present in the birds

phylogenetic study revealed the pattern found in other genes, as human, chimpanzee and rhesus monkey were placed in a single clade and rat and mouse in another, with the *G. gallus* in a clearly separate branch (Fig. 2.3.13). We further focused on these three taxa and aligned the amino acid sequences and found fewer differences between human and chimpanjee but great differences were observed in rhesus monkey from the other two taxa. Further, comparison of coding and non-coding nucleotide length variations and coding to non-coding nucleotide ratio between TNF-α and TNF- β among these three mammalian taxa provided a first-hand indication on the role of TNF-α gene, not its duplicate TNF-β in dualistic nature of TNF-α in malaria pathogenecity. ❑

3

Epidemiology

3.1 Remote Sensing and Geographic Information Systems

3.1.1 Regional Level Mapping of Malaria Vectors using RS and GIS in North-eastern States in India to Develop Strategic Plan for Malaria Control

We analysed the IRS-IDLISS-III satellite image datasets of two districts of Assam, Sonitpur and Nagaon. Unsupervised image classification of the images taking 100 cluster classes was done, and finally a base layer with four classes, i.e. land use/land cover (LULC) classes of semi-evergreen; moist deciduous; shrubs and grassland; and non-forest area were generated (Fig. 3.1.1). Water bodies, tea gardens and settlements were extracted out from FCC images of each district, and were mosaiced with the base layer for final LULC map generation. Area under different classes was calculated. Sonitpur is mainly occupied by moist deciduous forests followed by tea gardens, shrubs and grassland. Semi-evergreen forests were found to occupy very less area.

"Significant correlation was observed between presence of tea garden and annual parasite index"

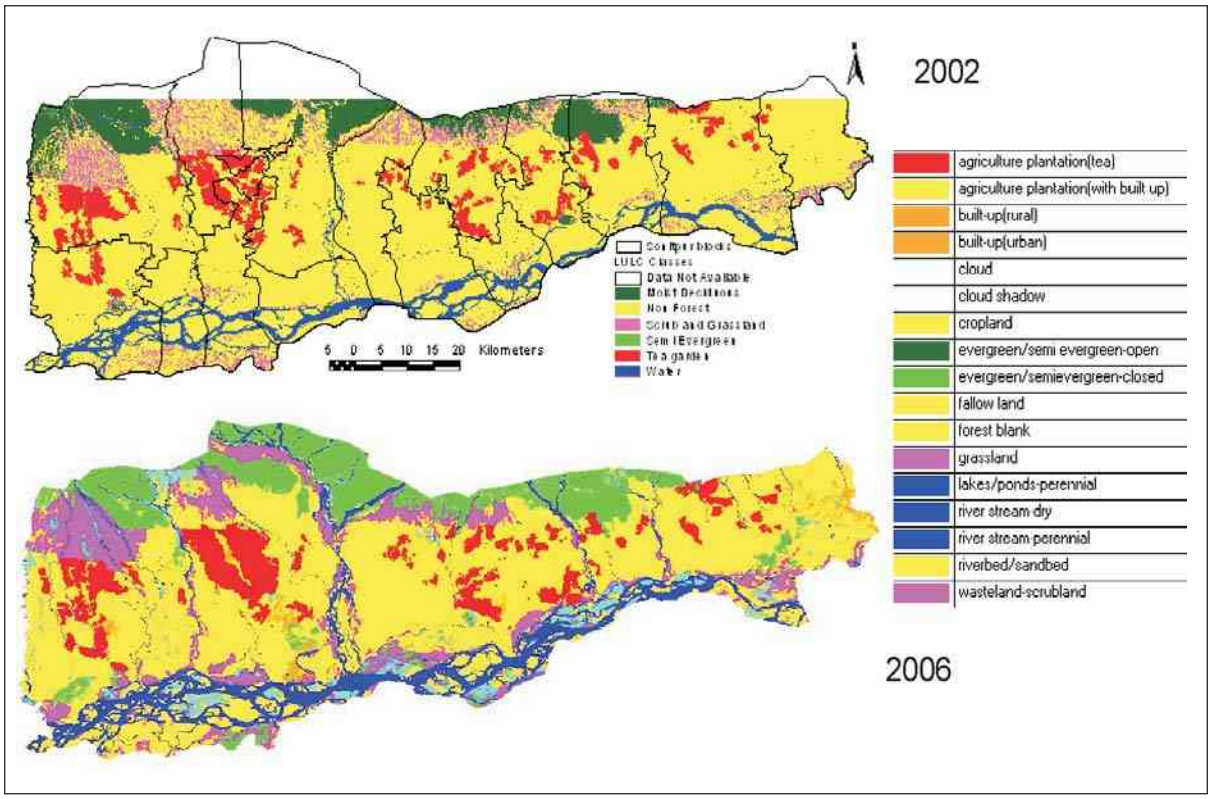

Fig. 3.1.1: Classified land use/land cover images of Sonitpur district, Assam

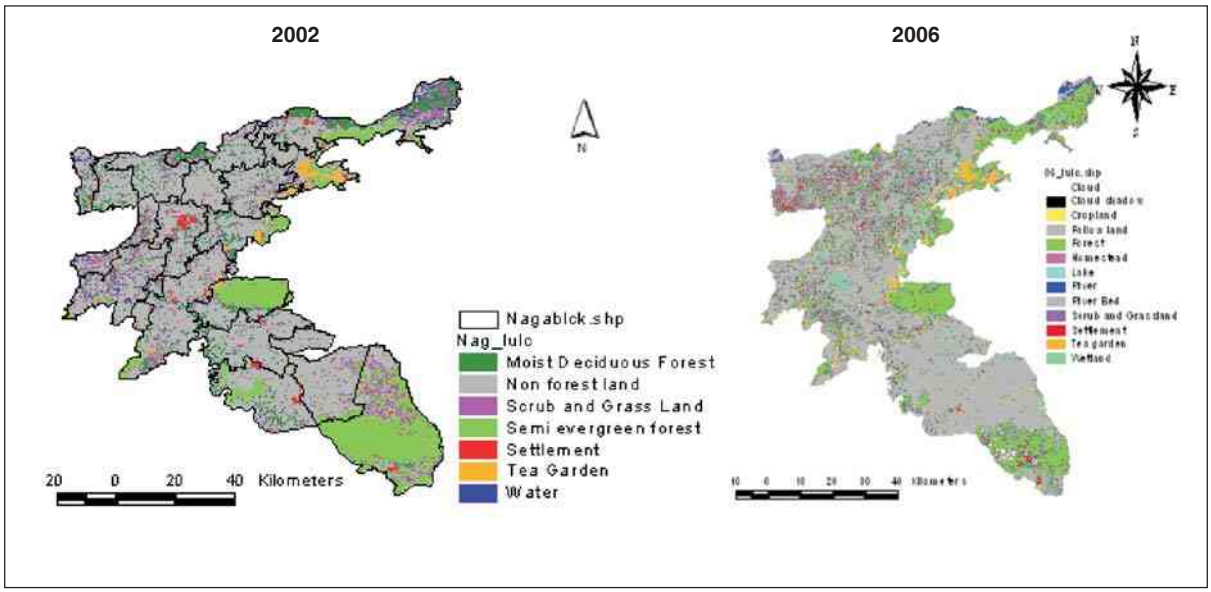

Fig. 3.1.2: Classified land use/land cover images of Nagaon, Assam

In Nagaon, area under different classes, as estimated by satellite images of the year 2002 and 2006 was compared. It was found that nearly 6% area was deforested and there was an increase in the area under tea gardens and settlements (Fig. 3.1.2).

"The presence of An. minimus in bordering areas was predicted using GIS"

Field surveys were carried out using Gramin Handheld GPS instrument for ground truth data (Fig. 3.1. 3). Daily survey track route and way points were recorded along with the landuse/land cover information. Track points and way points were overlaid on the classified satellite image for validation of land use/land cover classes and the errors were rectified accordingly. Parasitological and entomological data were also collected during the surveys.

Fig. 3.1.3: Field surveys in Nagaon, Assam

Malaria data for the two districts were obtained from District Malaria Office and PHCs, various epidemiological indices, such as API, SPR, SFR, etc. mapped for GIS were analysed. A significant correlation was found between the presence of tea gardens and API. The study is in progress.

3.1.2 Micro Level Mapping of Malaria Vectors using GIS in Bordering Districts of Assam and Arunachal Pradesh in India to assist Malaria Control

Digital datasets of IRS-1D LISS-III satellite image of 2002, provided by DRL, Tezpur, were used in this study. Unsupervised image classification was done, a base layer with four LULC classes of semi-evergreen; moist deciduous; scrubs and grassland and non-forest area was generated (Fig 3.1.4). Water bodies and tea gardens extracted out from the satellite images were mosaic with the base layer for final LULC map generation. Land classification

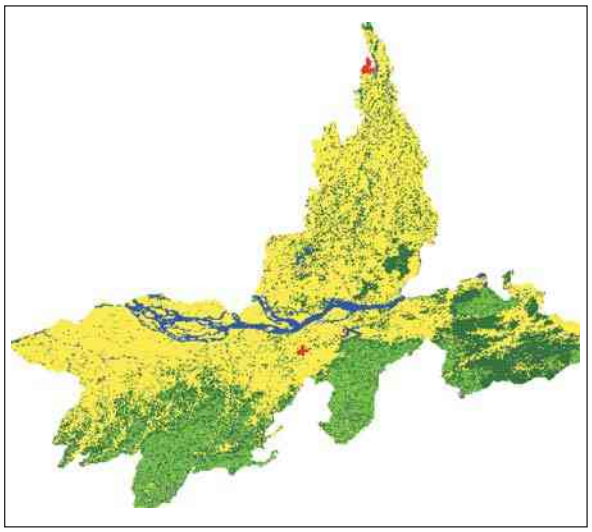

Fig 3.1.4: Classified image of Kamrup, Assam

shows about 7.5% area, having moist deciduous forest and tea garden; semi-evergreen forest is found to be the least.

Field Survey

The survey was carried out between April and May 2007 in District Sonitpur, Assam. Due to rains, heavy breeding was observed in the road side pits. The team carried out the entomological (hand catch, night collection) and parasitological (active blood smear) data collection (Fig. 3.1.5). During hand catch, malaria vector mosquitoes, viz. *An. culicifacies*, *An. fluviatilis* and *An. annularis* were found along with *Aedes* mosquitoes. The temperature recorded was between 22.8 and 32.7°C, and humidity ranged between 65 and 75%. Seven PHCs were surveyed in May 2007, out of seven PHCs as per records highest malaria and deaths were observed in North Jammu-guri followed by Charali, no death was recorded in Bihaguri and Balipara. Highest *Pf*% about 70 was observed in Dhekiajuli followed by Behili PHC. About 588 mosquitoes were collected and among them 75 (13%) were gravid and 158 (26.5%) were semi-gravid. Out of 106 slides collected, five were found positive out of which four were *Pf*.

3.1.3 Application of GIS to Map Distribution of Malaria Vectors and to Develop Disease Surveillance System in Jodhpur Cantonment Area

The study was conducted in Gandhinagar Cantonment area in Gujarat (Fig. 3.1.6). Adult mosquitoes and larvae were collected from Sectors 1–5 and civil area (Sector 6). Highest indoor per man hour density was observed in

Fig. 3.1.6: Gujarat state showing the study site

Fig. 3.1 5: Entomological and epidemiological surveys in Sonitpur, Assam

Sector 4, followed by Sectors 1 and 5. No anopheline mosquito was found in Sectors 2 and 3. *Culex* per man hour density was highest in Sector 1, followed by Sector 4. *Culex* mosquitoes were collected from all the sectors. In outdoor collections, no anopheline mosquito was collected. A few number of *Culex* was also collected. Larval density was found highest in Sector 4, followed by in Sector 5.

GIS Model for Gandhinagar

Entomological data, viz. adult indoor and outdoor density of *Anopheles* and *Culex*, and larval density was attached to the sectors on the digitized maps of Gandhinagar. A click at these sectors retrieves information related to that particular sector (Figs. 3.1.7 to 3.1.9). Greatest advantage on GIS model is that once the basic infrastructure is ready, any information can be updated or attached to the maps for quick retrieval and fast dissemination, and decision support in malaria control.

"The GIS model can be attached to maps can be useful for quick retrieval and fast dissemination & decision support in malaria control"

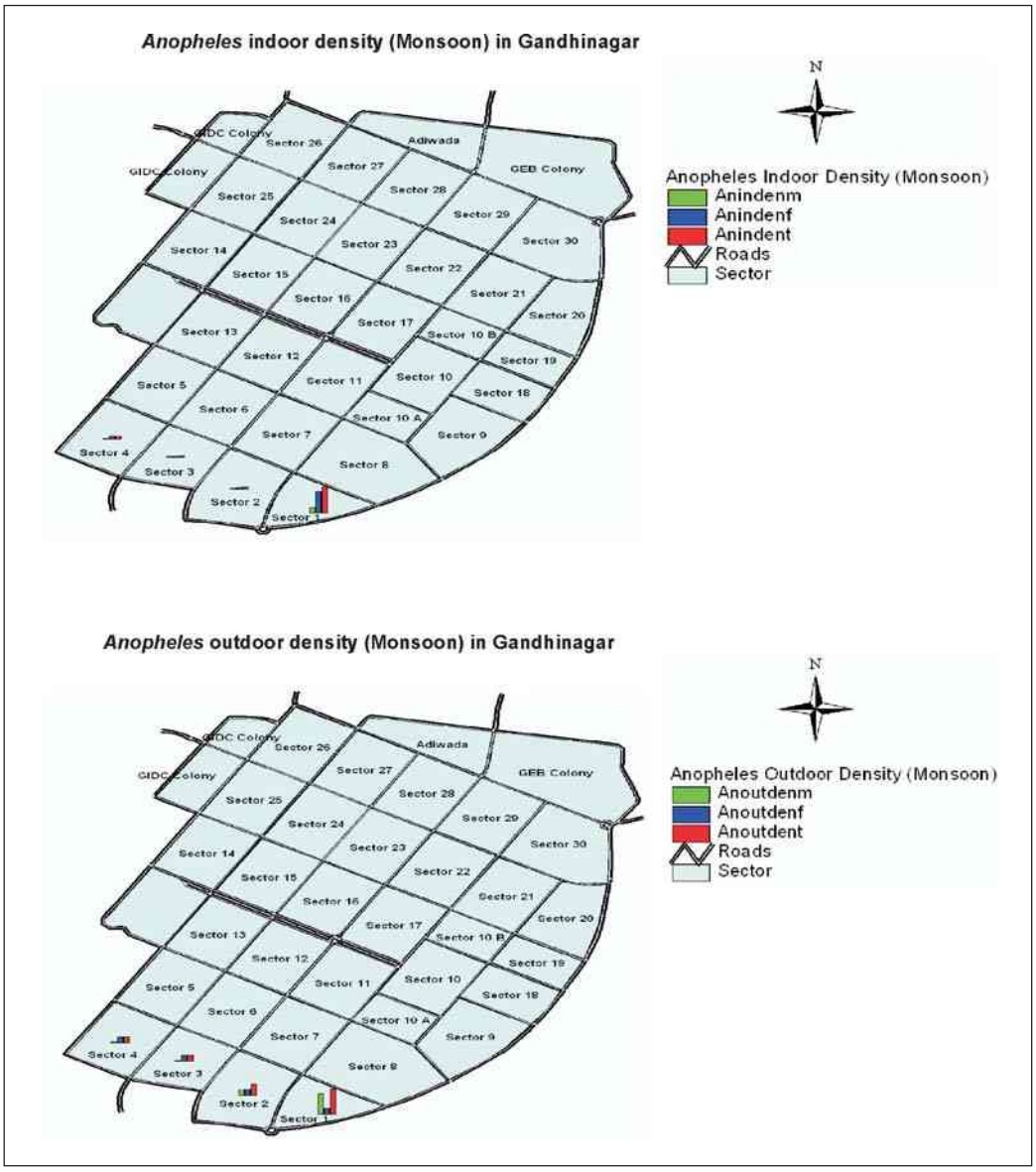

Fig. 3.1.7: Density of *Anopheles* mosquitoes in indoor and outdoor in Gandhinagar (2007)

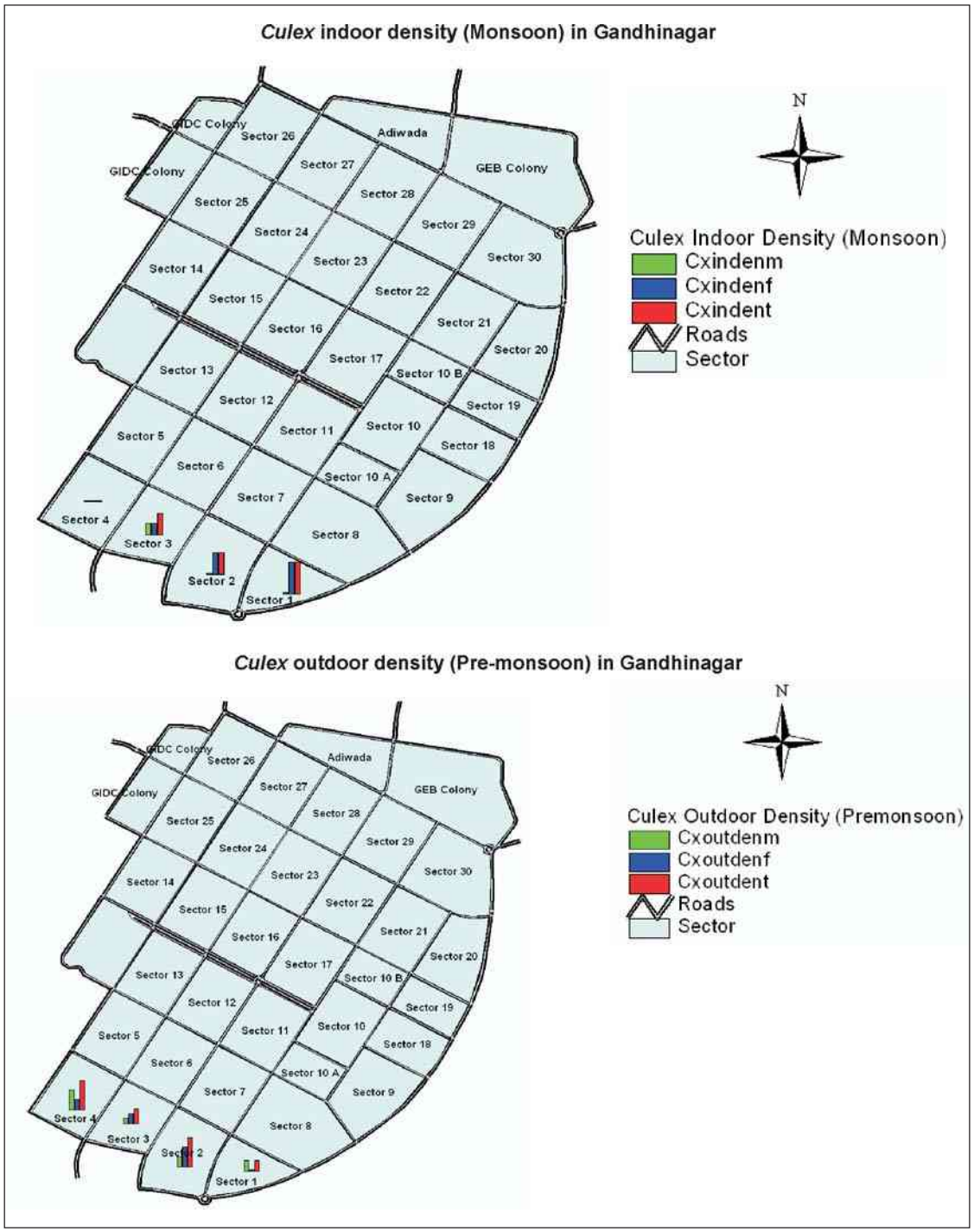

Fig. 3.1.8: Density of *Culex* mosquitoes in indoor and outdoor habitats in Gandhinagar (2007)

3.1.4 Retrospective Study on Chikungunya Outbreak in India

A retrospective study on chikungunya outbreak in India was initiated during 2007 in five states, viz. Delhi, Madhya Pradesh, Orissa, Maharashtra and Kerala (Fig. 3.1.10). Seven questionnaires, namely household survey-Q1A; information of all household members - Q1B; knowledge, attitude, belief, practice regarding chikungunya fever prevention & control -Q1C; patient inventory-Q1D; mortality in household-Q1E; health facility survey-Q2; and stakeholder interview-Q3 were filled up from urban and rural areas of each state except Delhi from where only urban areas were taken (Fig. 3.1.11).

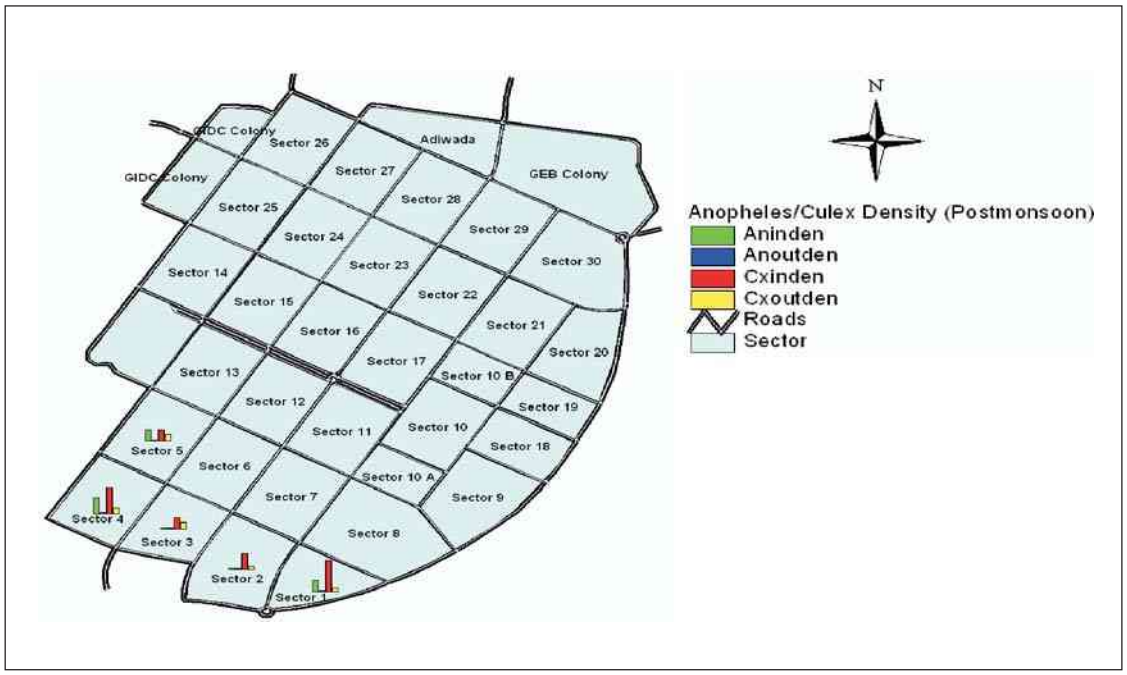

Fig. 3.1.9: *Anopheles* and *Culex* post-monsoon density in Gandhinagar (2007)

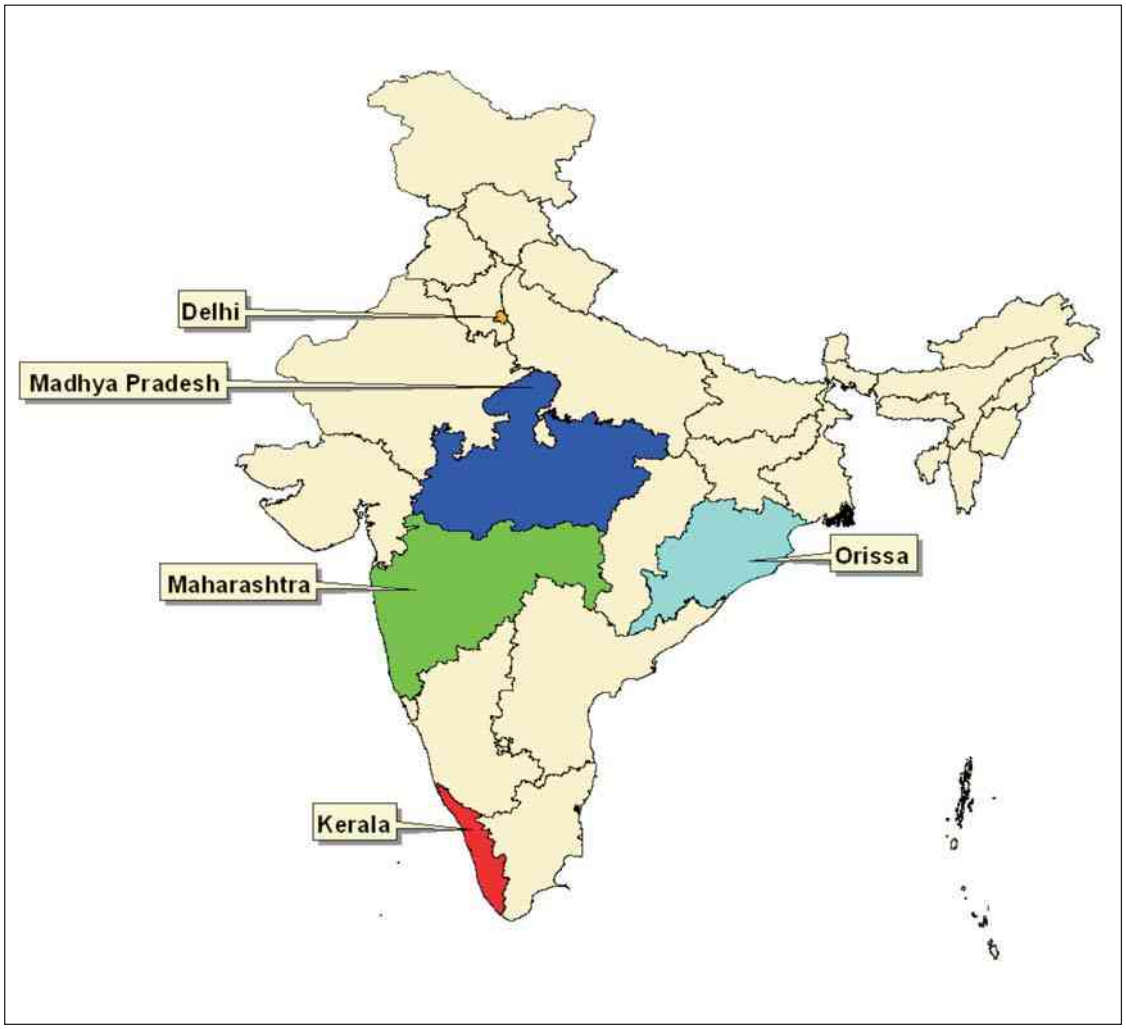

Fig. 3.1.10: Study sites selected for the retrospective study on chikungunya outbreak in India

Fig. 3.1.11: Filling up of the questionnaire

The highest incidence districts identified were: Sundargarh in Orissa, Latur in Maharashtra, Betul in M.P., Alappuzha in Kerala, MGF Zone and Dilshad colony in New Delhi. The lowest incidence districts identified were Ganjam in Orissa, Ratnagiri in Maharashtra, Katni in M.P., Kannur in Kerala and Sadar Paharganj, Najafgarh zone and Dwarka in New Delhi. Thus, a total of five states, 10 districts, 20 sub-centres, 20 urban wards and 2000 households each from urban and rural areas were covered. All filled up questionnaires from different states were analysed at NIMR.

Orissa appeared as the most ignorant state as far as knowledge, attitude, belief and practices for chikungunya fever prevention and control was concerned. Loss of man days/school absenteeism per attack was mostly recorded as 5–10 days and the symptoms were mainly recorded as fever, headache and bodyache. Many patients told the duration of treatment as 5–10 days and the expenditure on treatment and food was mostly ≤Rs. 500 and ≤Rs. 250. Besides, the study revealed that the facilities for chikungunya case management did not exist in any of the surveyed hospitals of Orissa.

Maharashtra appeared as the second most ignorant state regarding knowledge, attitude, belief and practice for chikungunya fever prevention and control. The average loss of man days/school absenteeism was more than 15 days. Symptoms were mainly recorded as fever and bodyache, and most of the patients took treatment for more than 15 days. In some families of Maharashtra, many members suffered from the disease simultaneously and on an average the expenditure on treatment was high. Chikungunya case management facility was provided by all the health facilities surveyed during this study.

"Orissa appeared to be the most ignorant state as regards the knowledge, attitude, belief and practices for chikungunya fever prevention & control"

In Madhya Pradesh, most of the houses were *Kuchcha* type especially in rural areas (Fig. 3.1.12). In some high incidence areas, air coolers were found. General sanitary conditions around most of the urban houses of the highest incidence area were found good. Water storage containers mostly used were cement tanks, metal tanks, overhead tanks and buckets (Fig. 3.1.13). Re-

Fig. 3.1.12: *Kuchcha* houses in Madhya Pradesh

Fig. 3.1.13: Breeding sources in Madhya Pradesh

garding other water collection in the houses/ surroundings, mostly water for animals and pet bowls figured out. As far as emptying/ drying of all water containers was concerned; some residents said that they did on weekly basis while others said that they did once in a while. Loss of man days/school absenteeism was mostly recorded as 5–10 days. Symptoms were mainly recorded as fever and arthralgia. The duration of treatment recorded was >15 days in most of the cases. Treatment expenditure was mostly ≤Rs. 500 and expenditure on food was ≤Rs. 250. Majority of the patients in Madhya Pradesh didn't receive any information of treatment from local hospital and private agencies/doctors. Chikungunya case management facility was provided by all the health facilities surveyed in Madhya Pradesh.

In Delhi, most of the houses were *Pucca* types and had air coolers. General sanitary conditions around most of the houses were found good. Water storage period in some areas was for 3–6 and >6 days. The key containers in Delhi were overhead tanks, underground tanks, ground level tanks and buckets (Fig. 3.1.14). In the highest incidence areas, emptying/drying of water containers was mostly done on weekly basis. Migration has been featured out as a major problem in Delhi. Loss of man days/school absenteeism was mostly recorded as 1–5 days in the highest incidence areas. Symptoms mostly recorded were fever and headache. Duration of treatment in the highest incidence urban area was 1–5 days. Treatment expenditure was mostly ≤Rs. 500 and on food ≤Rs. 250. Chikungunya case management facility was not provided in the MCD Hospital surveyed during the study.

In the urban areas of the highest incidence district of Kerala, more people were found residing in *Kuchcha* houses. In the urban areas of the highest incidence district, very few

Fig. 3.1.14: Mosquito breeding sources in Delhi

Fig. 3.1.15: Water collections supporting mosquito breeding in Kerala

households had air coolers, but in the low incidence urban areas under the lowest incidence district, all the houses had air coolers. Water storage containers mostly used were: overhead tanks, plastic drums and buckets. Regarding other water collection in the houses/surroundings were mostly troughs for drinking and coconut shells figured out (Fig. 3.1.15). Most of the respondents from Kerala knew answers to the questions related to knowledge, attitude, belief and practice for Chikungunya fever prevention and control. Loss of man days/ school absenteeism was mostly recorded as >15 days. Symptoms were mainly recorded as fever and bodyache. Duration of treatment in the highest incidence urban area was >15 days; while in rural areas it varied from 5–10 to 10–15 days. Treatment expenditure was mostly ≤Rs. 500 and on food ≤ Rs. 250. Majority of the patients in Kerala received information for treatment from local hospitals and other sources. Chikungunya case management facility was provided by all the health facilities surveyed in the highest incidence district.

3.1.5 Studies on the Epidemiology of Urban Malaria in Mega, Medium and Small Cities of India

With the objective to study magnitude of the problem of urban malaria in mega, medium and small cities of the country, to measure the level of malaria transmission in selected cities and to develop a cost-effective control strategy in Integrated Vector Management (IVM) mode, a study was initiated in three cities, namely Ajmer, Rajasthan (small city), Visakhapatnam, Andhra Pradesh (medium city) and Delhi (mega city).

Major problem for malaria in all the three urban areas, viz. Delhi, Visakhapatnam and Ajmer was found to be the migration of population. In Delhi, most of the migration is due to labours congregations from endemic areas for constructions of housing societies, malls, roads, etc. In Visakhapatnam, migration is due to ports and industries and Ajmer being a religious places for both Hindus and Muslims attracts many tourist visitors.

Most of the malaria cases were recorded by private hospitals. In Delhi, during 2006–07 (till September), MCD recorded 364 cases, whereas from 19 laboratories about 300 cases were recorded. In Ajmer, during 2006–07 (till June) Municipal Corporation recorded 33 cases whereas a total of 120 cases were recorded from six laboratories. The Municipal Corporation, Visakhapatnam recorded 1610 cases during 2006–07 (till June) whereas, five laboratories recorded 1351 cases (Fig. 3.1.16).

A survey done by the National Institute of Malaria Research revealed paradigms having highest malaria cases. In Delhi, it was mainly low income group followed by industrial labour; in Ajmer, it was recreation centre (Urs

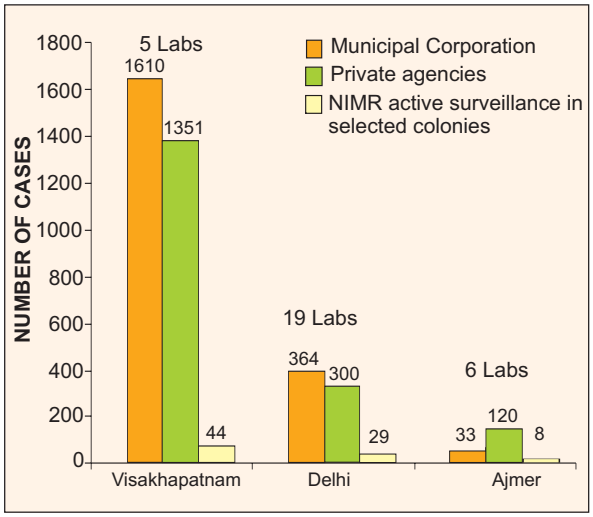

Fig. 3.1.16: Malaria burden in mega, medium and small urban cities (2006–07)

Mela Haz); and in Visakhapatnam, it was low income group followed by transport. There are around 2000 registered hospitals, labs, clinics in Delhi, 54 in Visakhapatnam and 14 in Ajmer. Therefore, the actual number of cases

> *"The actual number of malaria cases could be much higher than recorded by Municipal Corporation in urban areas"*

could be much higher than recorded by Municipal Corporation. The transmission period in Delhi was from July to November, in Ajmer from August to October, whereas in Visakhapatnam it is from June–July and October–November. GIS mapping revealed that in the year 2006, malaria was recorded from 13 out of 14 zones of Delhi (Fig. 3.1.17). *An. stephensi* and *An. culicifacies* were recorded from Delhi and Ajmer but only *An. stephensi* was recorded from Visakhapatnam.

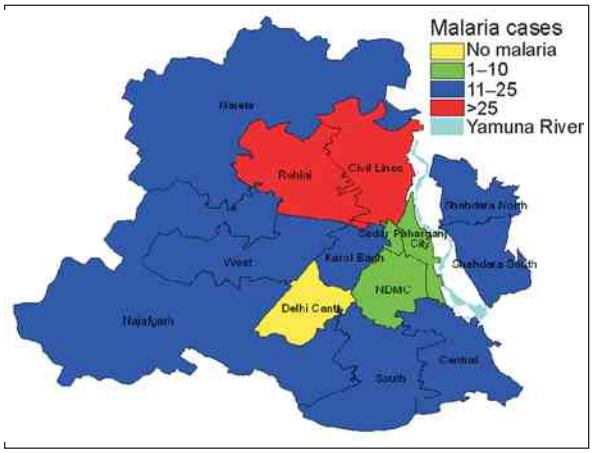

Fig. 3.1.17: GIS mapping of malaria cases during 2006 in Delhi

In Delhi, the man hour density of *An. stephensi* was highest in July, in Ajmer, it is in November and July, whereas in Visakhapatnam it is in September. In Delhi, the man hour density of *An. culicifacies* was highest in June to Au-

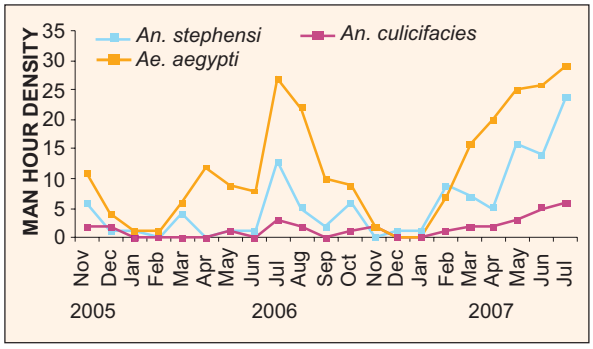

Fig. 3.1.18: Man hour density of *An. stephensi*, *An. culicifacies* and *Ae. aegypti* in Delhi (November 2005 to July 2007)

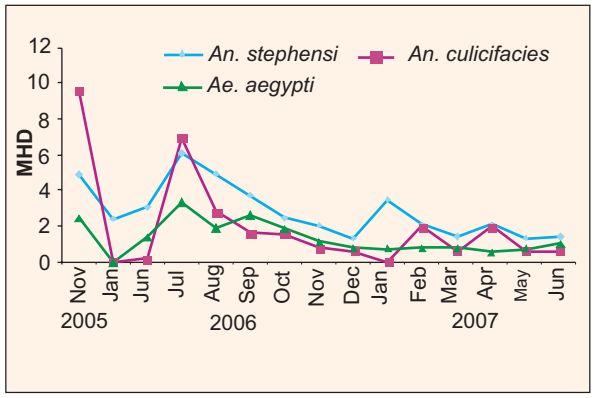

Fig. 3.1.19: Man hour density of mosquitoes in Ajmer (November 2005– June 07)

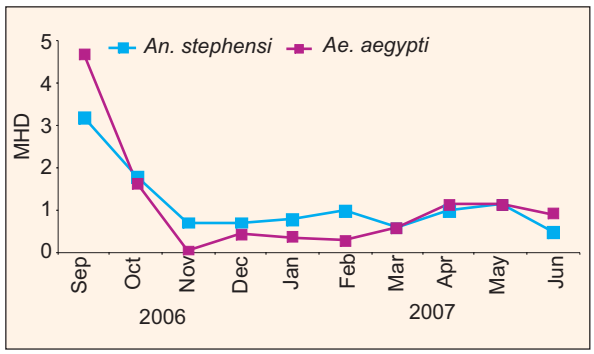

Fig. 3.1.20: Man hour density of *An. stephensi* and *Ae. aegypti* in Visakhapatnam (2006–07)

gust, whereas in Ajmer in July to September (Figs. 3.1.18 to 3.1.20).

Peak biting time of *An. stephensi* on human/animal bait was 2200 to 2400 hrs in Delhi; 0200 to 0400 hrs in Ajmer; and 2000 to 2200 hrs & 0100–0300 hrs in Visakhapatnam. The key containers supporting breeding of vectors of malaria, dengue and chikungunya are overhead tanks, tyre dumps and underground tanks in Delhi; ground cement tanks and tankas in Ajmer; and ground cemented tanks, tyre dumps and OHTs in Visakhapatnam (Fig. 3.1.21).

Fig. 3.1.21: Mosquito breeding habitats in Delhi, Ajmer and Visakhapatnam

Very high breeding of *Ae. aegypti* was recorded from all three areas. It is noteworthy to mention that *Ae. aegypti* has not been recorded for more than one decade in Ajmer. From all the three areas, suspected and confirmed chikungunya cases were recorded in 2006.

The health seeking behaviour survey showed that the community in all the three areas has limited knowledge about the source of vector breeding. Nobody knows about the insecticide-treated bednets. Most of the community (70–100%) is using coils and mats as mosquito repellents.

3.1.6 Identification of Epidemiological Risk Factors of Malaria for Development of Strategic Action Plan for Malaria Control in Problematic Districts of Karnataka

This ICMR sponsored project was continued to identify epidemiological and ecological risk factors of malaria in canal irrigated areas of Upper Krishna Project (UKP) area, and to validate the detection of landscape features in southern Karnataka. Based on village-wise data of past three years, villages from highest and lowest malaria endemic districts, i.e. Gulberga, Bijapur, Raichur and Bagalkot were selected for detailed survey (Fig. 3.1. 22). Field surveys were carried out for point prevalence of malaria during peak (November), and low peak (April), breeding habitats, man hour density of adult malaria vectors for mosquitogenic potential in and around each village. The area had rivers, irrigation channels, drains and borrow pits as breeding habitats. Satellite images of IRS P6 LISS IV MX were also procured for UKP area (Fig. 3.1.23).

To validate the relationship between Remote Sensing derived landscape features and malaria endemicity in Tumkur and Chitradurga

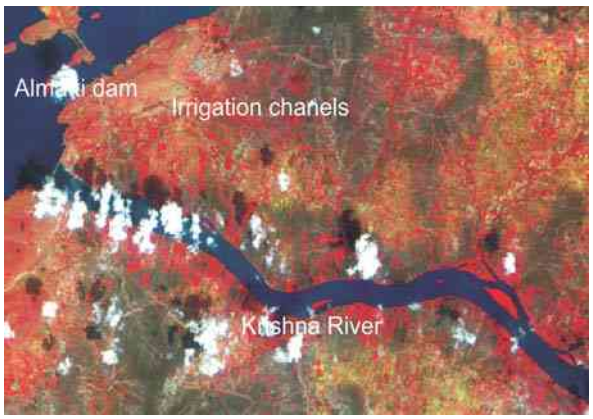

Fig. 3.1.23: False colour composite image of Almatti Dam area (Source: IRS P6 LISS IV, 15 October 2006).

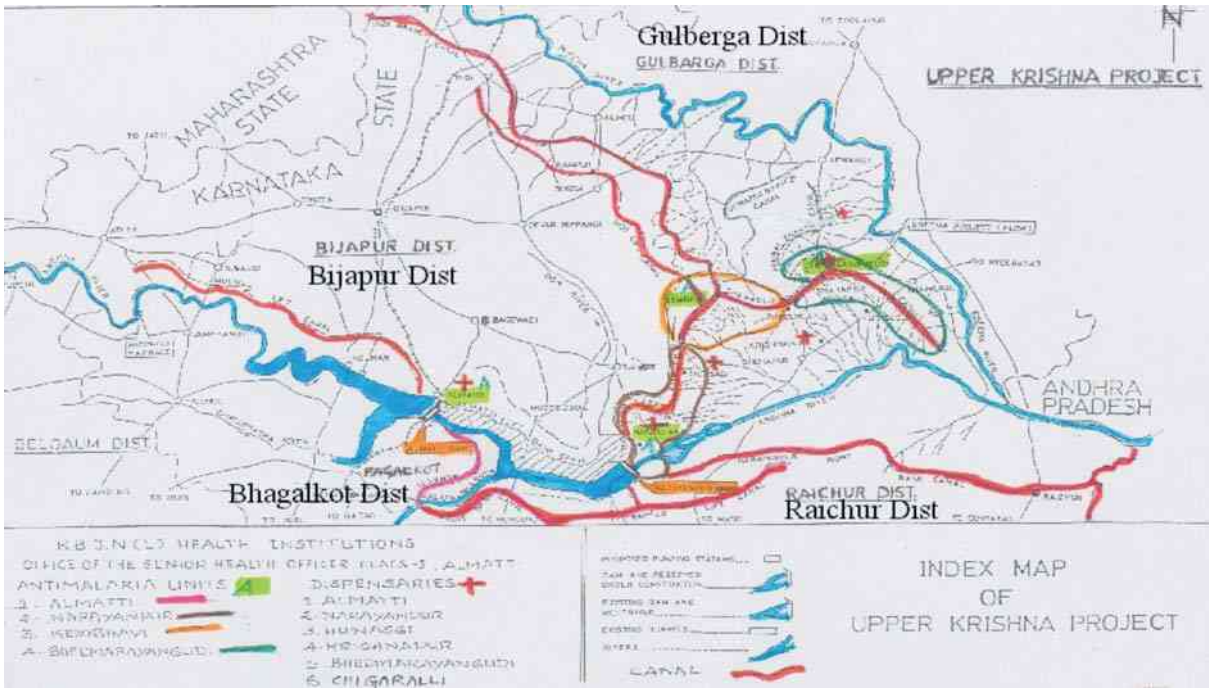

Fig. 3.1.22: Upper Krishna Project area in Karnataka

Fig. 3.1.24: Photographs of Upper Krishna Project area showing mosquito breeding habitats

districts, Ranganathpura and Yelladakere PHCs under Hiriyur taluka were selected from Chitradurga district, while Kallembela, Tarur and Taverakere PHCs under Sira Taluka were selected from Tumkur district. Ground truth of ecological features identified from FCC was undertaken in selected villages of Ranganathpura and Kallembela PHCs. All the villages under Kallembela and Taverakere PHCs were listed. FCC based on merged satellite data of LISS III and PAN sensors was generated, and statistics of landscape features in respect of four villages was generated.

"Irrigation channels, inhabitation near channels and labour congregation were found to be the eco-epidemiological factors responsible for high malaria incidence in project areas"

Eco-epidemiolological risk factors were found to be introduction of irrigation channels in hitherto water scarce area, vicinity of human settlements near channels/seepage drains, local migration and settlement of rehabilitated colonies. Rivers, seepage drains, irrigation channels, borrow pits, ditches, cement tanks and rice-fields were found as the main breeding habitats of anopheline mosquitoes in ground truth surveys (Fig. 3.1.24). River, irrigation channels, borrow pits, seepage drains and rice-fields were identifiable in satellite images, while cement tanks and small ditches could not. Highest larval density was found in borrow pits and seepage drains. Man hour density of *An. culicifacies* in low endemic villages was up to 0.5 and up to 24 in high endemic villages.

3.1.7 Identification of Malaria Risk Factors in Different Ecosystems of Assam using Remote Sensing

It is an ongoing project with an objective to identify environmental and ecological risk factors of malaria in forested and plain ecosys-

tems of Assam. Field visits were undertaken in selected Primary Health Centres of Sonitpur and Kamrup districts. One PHC with highest risk and another with lowest risk of malaria were selected from each district. Monthly retrospective epidemiological data of malaria were collected from six districts of Assam. Meteorological data of these districts were also collected. Entomological, parasitological and ecological data were generated in 16 villages.

The transmission windows of malaria were identified with respect to Kamrup and Tezpur districts based on minimum temperature and relative humidity (RH) required for ensuing transmission of *P. falciparum* malaria (18°C temperature and 55% RH) and seasonal occurrence of cases.

"High risk malarious villages were located near foothills with problem of accessibility in Assam"

Ground truth survey revealed that the high risk malarious villages were located near foothills with problem of accessibility. The major breeding habitats were ponds, streams, ricefields, etc. Satellite data of LISS III sensor with 23.5 m resolution did not help in delineation of mosquitogenic landscape features at village level. Data of higher resolution were not available for the peak transmission season, i.e. May/June.

In both Kamrup and Sonitpur districts, the peak incidence of *P. falciparum* reaches in June/July, while the lowest peak is in December to February. Based on minimum required temperature and RH, transmission windows are supposed to remain open for 10–11 months. The density of *An. minimus* ranged from six to nine per man hour in high risk villages. Surprisingly, in some of the low risk villages the MHD of vector species was also found up to eight. Malaria endemicity was high in Kamrup district as compared to Sonitpur district which is basically due to difference in topography. Parasite incidence in Kamrup and Sonitpur districts was 44.3 and 8.4 in November 2006, 17.3 and 4.8 in July 2007, and 7 and 4.4 in November 2007, respectively.

Preliminary analysis indicates that the delineation of mosquitogenic/malariogenic conditions at village level would be difficult by satellite images with 23.5 m resolution in selected areas of Assam.

4

Clinical Research

The treatment of malaria has been a challenge in south-east Asia since 1950s when the first case of chloroquine resistance in *P. falciparum* was detected in Thailand. The rapid change of treatment policy in some countries to new drugs led to multi-drug resistance, and few other countries including India continued to use chloroquine due to various technical and operational reasons. However, in the present scenario of the increased donor support and strong recommendations of the World Health Organization (WHO) for use of Artemisinin-based combination therapy (ACT), movement to ban production, marketing of artemisinin monotherapy and raising the desired cure rate level from 75 to 90%, it has become a necessity for all countries to change their treatment policies.

In India, ACT (Artesunate + Sulphadoxine- Pyrimethamine) has been recommended in more than 160 districts in 2008. Therefore, the efficacy of the new regimen was evaluated using WHO protocol, at different sites in India. In addition, the efficacy of fixed dose combination registered in India (Artemether + Lumefantrine) was also evaluated.

"ACT is highly effective in the treatment of uncomplicated falciparum malaria (cure rate 95–100%)"

It is well-known that compliance is better with fixed dose combinations than blister packs. Therefore, to facilitate the introduction of new fixed dose ACTs, Phase II and III clinical trials were also conducted in association with hospitals in endemic states. New linkages have been developed, for example with Tata Main Hospital in Jamshedpur, Jharkhand.

4.1 Assessment of Therapeutic Efficacy of Antimalarial Drugs against Uncomplicated *Plasmodium falciparum* Malaria

Antimalarial drug resistance is a challenge in the treatment of falciparum malaria. It is, therefore, necessary to evaluate the efficacy of first and second line antimalarial drugs at several sites, so as to help adopt alternative strategies for treatment as per need. The present studies were conducted to evaluate efficacy of ACT used/marketed in India with following objectives: (i) to assess the therapeutic efficacy of combination therapy in uncomplicated *P. falciparum* malaria in endemic districts in Assam, Jharkhand and Orissa; and (ii) to validate the *in vivo* drug resistance data using molecular markers.

The study was conduced according to WHO protocol for therapeutic efficacy. This was one arm prospective evaluation of parasitological and clinical response to directly observed treatment for uncomplicated malaria. All patients reporting to local clinics at study sites with complaint of fever were examined for prevalence of parasites in blood smear. Peripheral smear was examined, and those positive cases for *P. falciparum* were enrolled and given treatment. They were observed for 30 min and if they vomited during this period, full dose was repeated.

Re-assessment was done on Day 0 and on Days 1, 2, 3 and 7 after enrolment, and then weekly up to 28 days. The dosing response was classified as adequate clinical and parasitological cure (ACPR), early treatment failure (ETF) and late treatment failure (LTF). Rescue medication was given to treatment failures. Genotyping of blood spots on Day 0 and on the day of recrudescence by MSP-1, MSP-2 and GLURP. Nested PCR with family-specific assay was done to differentiate new infection from recrudescence. The study was conducted at Garden Hospital, Amchong Tea Estate, PHC Sonapur, District Kamrup, Assam; Angara PHC at Ranchi and Jaldega PHC at District Simdega, Jharkhand; and Keonjhar Town and PHC Banspal of District Keonjhar, Orissa (Fig. 4.1.1).

Therapeutic efficacy of Artemether- Lumefantrine (AL) was tested at two sites, namely Garden Hospital, Amchong Tea Estate, PHC Sonapur, District Kamrup, Assam and Keonjhar Town, District Keonjhar, Orissa. Therapeutic efficacy of Artesunate+ Sulphadoxine-pyrimethamine (AS+SP) was tested at three sites, namely Angara PHC, District Ranchi, Jaldega PHC, District Simdega, Jharkhand and PHC, Banspal of District Keonjhar, Orissa.

4.1.1 Assam

A total of 53 patients were enrolled for the study with Artemether and lumefantrine (AL) combination therapy from Sonapur PHC, District Kamrup. The cumulative success rate (ACPR) by survival analysis was 100% (Table 4.1.1). The drug combination was well-toler-

Table 4.1.1. Therapeutic efficacy of AL in District Kamrup, Assam

Response	Number	Prevalence
ETF	0	0
LCF	0	0
LPF	0	0
ACPR	53	1
Total analysis	53	
Withdrawn	0	
Lost to follow-up	0	0
Total	53	

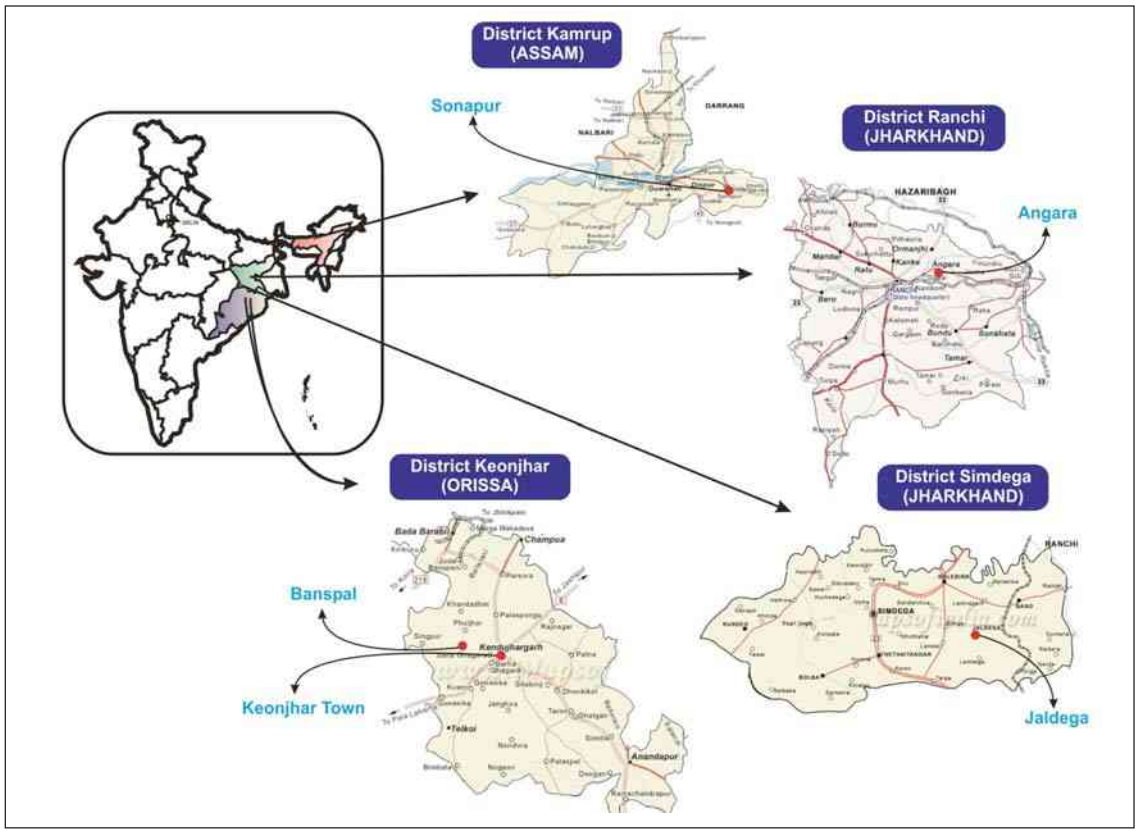

Fig. 4.1.1: Map showing study sites of therapeutic efficacy studies

ated. No adverse effects were reported with AL. Parasite clearance was also rapid.

4.1.2 Jharkhand

In PHC Angara, District Ranchi, two patients were late treatment failures, but were classified as re-infection after PCR leading to corrected cure rate of 100% (Table 4.1.2). At PHC Jaldega, District Simdega, there was no early or late treatment failure indicating that AS+SP is an effective ACT (Table 4.1.3).

Table 4.1.2. Therapeutic efficacy of AS + SP at District Ranchi, Jharkhand

Response	Number	Prevalence
PCR Uncorrected Data		
ETF	0	0
LCF	0	0
LPF	2	0.039
ACPR	49	0.961
Total analysis	51	
Withdrawn	2	
Lost to follow-up	0	0.038
Total	53	
PCR Corrected Data		
ETF	0	0
LCF	0	0
LPF	0	0
ACPR	49	1
Total analysis	49	
Withdrawn	4	
Lost to follow-up	0	0.075
Total	53	

Table 4.1.3. Therapeutic efficacy of AS + SP at District Simdega, Jharkhand

Response	Number	Prevalence
ETF	0	0
LCF	0	0
LPF	0	0
ACPR	45	1
Total analysis	45	
Withdrawn	0	
Lost to follow-up	0	0
Total	45	

4.1.3 Orissa

A total of 71 patients were enrolled at each site in PHC Banspal and Keonjhar Town in Orissa. The cumulative success rate by survival analysis was 100% with AS+SP in PHC Banspal. At Keonjhar there were two failures with AL, one on Day 7 and the other on Day 28. The latter turned out to be re-infection after PCR analysis. Therefore, the corrected cure rate was 98.6%. It can be concluded that AS+SP and Artemether- Lumefantrine combinations are highly effective in the treatment of falciparum malaria in India.

"AS+SP and Artemether-Lumefantrine combinations are highly effective in the treatment of falciparum malaria in India"

4.2 Clinical Trials

4.2.1 Multi-centre, Open-label, Randomized Clinical Study of Efficacy and Tolerability of the Fixed-dose Artesunate/Amodiaquine (AS/AQ) Combination Therapy and Amodiaquine (AQ) Monotherapy for the Treatment of Uncomplicated *P. falciparum* Malaria in India

In view of development of resistance to current antimalarials, WHO recommends use of Artemisinin-based combination therapy for the treatment of falciparum malaria. At present, only one ACT (Artemether-Lumefantrine–Coartem®) is available in India as a fixed dose drug in which both compounds are co-administered. Drug for Neglected Diseases *initiative* (DND*i*) in association with UNICEF-UNDP-World Bank-WHO (TDR) is developing a new fixed-dose combination of artesunate and amodiaquine that will allow a simple treatment of three days, with a single daily administration of two tablets.

An open-label, randomized clinical study was conducted at two sites in India to evaluate the efficacy and safety of this combination in India. A total of 300 subjects were randomized

(2:1) to Group A (fixed-dose combination of AS/AQ tablets), and Group B (Amodiaquine). The subjects recruited were those presenting with uncomplicated falciparum malaria defined by *P. falciparum* mono-infection with fever >37.5ºC and parasitaemia of 1000–100,000 asexual parasites/µl. The dose administration in both groups was according to age criteria and two formulations of AS/AQ containing 25 mg/67.5 mg and 100 mg/270 mg were used for children and adults respectively. The eligible subjects were recruited for the study after they signed the Institutional Ethical Committee (IEC) approved informed consent document. The treatment period was for three days with follow-up of 25 days. The safety reporting was done through the entire conductance of the study. The clinical study was conducted according to the local regulations including Schedule Y, ICMR guidelines, Indian Good Clinical Practice (GCP) and the standards conforming to the ICH and GCP.

The two participating centres screened 327 patients, out of them 27 were screen failures and 300 were randomized into the study. The intention-to-treat (ITT) population at both the sites was 298 and the per-protocol (PP) population was 292.

The cure rate based on ACPR (before PCR correction) was 92.4% in Group A (AS/AQ) and 83% in Group B (AQ). The difference seen between the two groups was statistically significant (p = 0.0144). The cure rate based on PCR genotyping (after PCR correction) was 97.5% in Group A (AS/AQ) and 88.3% in Group B (AQ).

"Cure rate of AS/AQ is according to WHO recommendation for ACT and AQ has desired efficacy of partner drug"

The study demonstrates that cure rate of AS/AQ is according to WHO recommendation for ACT and AQ has desired efficacy of partner drug. The study provides evidence for a fixed dose combination of AS+AQ which is easy to administer, cost-effective, well-tolerated and a short duration regimen. The study provides evidence that AS/AQ combination can be an option for implementation of this combination as an effective and safe drug.

4.2.2 A Phase III, Randomized, Non-inferiority Trial to Assess the Efficacy and Safety of Dihydro-artemisinin + Piperaquine (Artekin) in Comparison with Artesunate + Mefloquine in Patients Affected by Acute, Uncomplicated *Plasmodium falciparum* Malaria

Artekin™ (Dihydroartemisinin + Piperaquine) is a second generation of Artemisinin-based combination therapy (ACT) with similar efficacy to that of Artemether + Lumefantrine or Artesunate + Mefloquine, but with a simpler dosing scheme that will aid better compliance. A new phase III trial using Artekin produced according to good manufacture practice (GMP) and European regulatory standards was conducted in Thailand, India and Laos.

The study was conducted at three sites in India, namely Goa, Mangalore and Guwahati. A total of 1150 patients were recruited at all the sites in India and other countries. In order to ensure concealment of treatment allocation and avoid other biases, the randomization was under blind conditions and the treatment allocation was concealed until the final recruitment of the patients. The primary end point was the PCR-corrected adequate clinical and parasitological response (PCR corrected ACPR) on Day 63.

Males and females aged ≥18 years having microscopically confirmed monoinfection of *P. falciparum* (asexual forms parasitaemia ≥1000/µl – ≤100,000/µl or mixed infection), history of fever or presence of fever (temperature ≥37.5°C) were included. Dihydroartemisinin+ Piperaquine tablets containing

40 mg of Dihydroartemisinin and 320 mg of Piperaquine for adult patients were administered for three days and on Day 63 cure rate of 98% was observed, and parasite clearance time ranged between 1 and 3 days. The drug was well-tolerated. It was concluded that Artekin is non-inferior to standard treatment of Artesunate + Mefloquine.

4.2.3 A Phase II, Randomized, Open-label, Multi-centre 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 *Plasmodium falciparum* Malaria

RBx 11160, (Arterolane) a new peroxide, is a synthetic trioxolane that is easy to synthesize, inexpensive, achiral and orally rapid acting with high antimalarial activity. It produces antimalarial action by reductive activation of haem, released as a result of haemoglobin digestion and irreversible redox reaction produces carbon-centered free radicals, leading to alkylation of haem and proteins (enzymes). Preclinical and phase I human studies have confirmed safety of the drug. The Phase II studies with Arterolane alone and in combination with Piperaquine have shown excellent efficacy against *P. falciparum*. A Phase II clinical trial was then carried out to assess the antimalarial efficacy of Arterolane (RBx 11160) maleate and Piperaquine phosphate co-administration and Coartem in patients with acute uncomplicated *P. falciparum* malaria at Rourkela, Ranchi and Jamshedpur. The collaborating hospitals were Ispat General Hospital, Rourkela, Mahadevi Birla Hospital, Ranchi and Tata Main Hospital, Jamshedpur. Primary objective of the study was to estimate the Day 28 PCR corrected adequate clinical and parasitological response (ACPR) of three dose regimen of arterolane (RBx 11160) maleate 150 mg and piperaquine phosphate 750 mg co-administration and six dose regimen of Coartem in patients with acute uncomplicated *P. falciparum* malaria. In all, 200 patients were enrolled in the study including from India and results are encouraging and comparable to standard treatment with ACT.

"Pyramax was found as effective as chloroquine in the treatment of vivax malaria"

4.2.4 A Phase III Comparative, Open-label, Randomized, Multi-centre, Clinical Study to Assess the Safety and Efficacy of Fixed dose Formulation Oral Pyronaridine Artesunate (180 : 60 mg Tablet) versus Mefloquine (250 mg Tablet) plus Artesunate (100 mg Tablet) in Children and Adult Patients with Acute Uncomplicated *Plasmodium vivax* Malaria

Pyramax is a combination of Artesunate and Pyronaridine which has shown to be effective for the treatment of malaria in phase II trials. The present phase III study was designed as a multi-centre, randomized, comparative, parallel group study of the efficacy and safety of a three day regimen of the fixed combination of pyronaridine artesunate (180 : 60 mg tablets) versus chloroquine in vivax malaria. Patients between 3 and 60 years with minimum parasitaemia of 250 parasites/µl were enrolled and followed for 28 days after the first study drug administration. The primary efficacy end point was at 28 days. A total of 456 patients (228 in each group) were enrolled at all sites including Thailand, Cambodia, Indonesia and India. The cure rates were as good as standard treatment with chloroquine. The parasite clearance was faster than that of chloroquine. It can be concluded that Pyramax is as effective as chloroquine in the treatment of vivax malaria.

4.3 Operational Research on Drug use Practice and Pre-packaged Blister Pack Drugs

Therapeutic efficacy studies were conducted in Jharkhand state and high CQ resistance in

Workshop on operational research on drug use practice

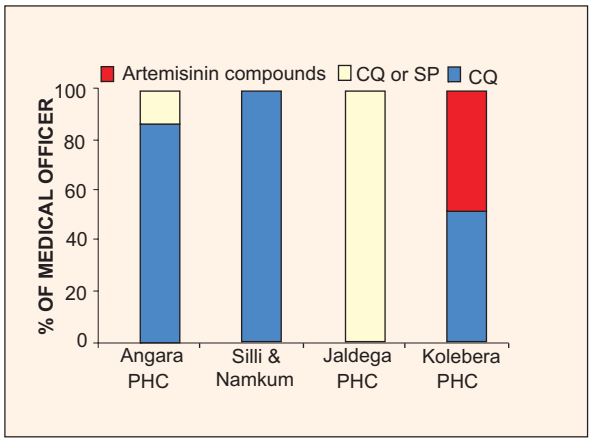

Fig. 4.3.1: Drugs prescribed by medical officers for the treatment of uncomplicated falciparum malaria

P. falciparum in study areas was observed. On this basis, the NVBDCP recommended change of drug policy in the affected PHCs of three districts to ACT (Artesunate and Sulphadoxine + Pyrimethamine). Blister packs for the radical treatment of adult patients (15 yrs and above) have been introduced in the national programme to improve acceptance of antimalarial drugs and compliance of the full course. A study was undertaken in Jharkhand state to document information on drug use practice and compliance of blister packs. The selected districts were Simdega and Ranchi. In Ranchi district, Angara PHC (Changed drug policy) and Silli and Namkum PHCs (No change in drug policy) were included. In Simdega district, Jaldega PHC (Changed drug policy) and Kolebera PHC (No change in drug policy) were included.

The first objective of the study was to evaluate drug-use practice with emphasis on districts with change in drug policy in Jharkhand state. Secondly, to study the knowledge and skills of paramedical personnel in the use of blister packs (including ACT), its acceptance by paramedical personnel and in the community; and to study the compliance for blister pack and the serious adverse events, if any, with the usage of blister packs. Pre-tested questionnaire for observations on diagnosis and treatment of malaria was developed. At all sites, in addition to verification of the prescribing patterns, the records and investigations were retrospectively analysed.

In the studied PHCs, all Medical Officers (100%) used blood slide examination to diagnose malaria and were aware of rapid diagnostic kits (RDKs). The reporting of malaria cases to higher authorities was found to be 100%. However, awareness about the training course on malaria is low. Treatment of *P. vivax* malaria in all the studied PHCs is with chloroquine (100%). Despite drug policy change, the drugs prescribed for *P. falciparum* malaria were irrational (Fig. 4.3.1). The awareness about drug dose and duration is inadequate at sub-centre level. Availability of RDK at sub-centre is scanty.

In Jharkhand, recently all the DDCs are converted to FTDs. Majority of the staff at FTDs are not well qualified and are unaware of the drug policy. The supply of RDKs to sub-centres located at remote places is inadequate. The overall knowledge about new drug policy and treatment guidelines was insufficient. Medical Officers should be made aware of treatment guidelines through various means.

4.4 *In vitro* Sensitivity of Indian *Plasmodium falciparum* Strains to Antimalarial Agents

This is a cross-sectional sample survey to estimate the *in vitro* drug resistance of *P. falci-*

Table 4.4.1: Success rate of culture according to origin

Origin	No. attempted	No. successful	Percent
Orissa	43	28	65.1
Jharkhand	38	24	63.2
Other states	22	9	40.9
Parasite Bank	19	7	36.8
Total	122	68	55.7

parum to antimalarials. The study is being conducted at various field units of NIMR at Rourkela, Guwahati, Ranchi, Bengaluru, Goa and Raipur.

Subjects having *P. falciparum* monoinfection with a parasitaemia of 1000 to 80000 per µl blood and not having a history of antimalarial consumption were recruited for the study. Sensitivity was carried out for dihydroartemisinin, chloroquine, amodiaquine and mefloquine by using the WHO microtest mark III. The mean inhibitory concentrations (IC_{50}) of individual samples for each drug were determined by non-linear regression analysis.

Table 4.4.2: *In vitro* susceptibility of *P. falciparum* to four drugs

Drug	IC_{50} mean (nM/l)	IC_{99} mean (nM/l)	Resistance threshold (nM/l)
Chloroquine	20.8	154.48	>160[a]
Monodesethyl amodiaquine	4.04	27.60	>80 [a]
Dihydroartemisinin	2.59	5.26	>10.5[b]
Mefloquine	24.90	56.15	>640 [a]

a— WHO, Threshold refers to IC_{99}; b—Restrepo-Pineda *et al*, Threshold refers to IC_{50}.

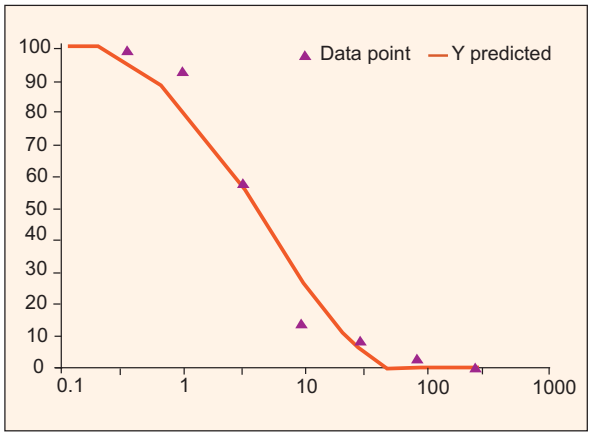

Fig. 4.4.1: Response of a *P. falciparum* strain to chloroquine

For this, HNnonLin version V1.1 software (downloaded from www.malaria.farch.net) model was used.

In all, 122 samples have been studied from four different sites, namely Jharkhand, Orissa, Karnataka and Goa (Table 4.4.1). The male : female ratio was 1 : 2.6. The average parasite count in the patients subjected to *in vitro* testing was 17540/µl blood. In addition, 19 isolates have also been studied from the malaria parasite bank of NIMR. The proportions of successful assays were 59.2 and 36.8% in fresh and cultured isolates respectively.

The geometric mean IC_{50} were 20.8 nmol/l, 4.04 nmol/l, 2.59 nmol/l and 24.9 nmol/l, for chloroquine, monodesethyl amodiaquine, dihydroartemisinin and mefloquine respectively (Table 4.4.2). Figure 4.4.1 shows a representative graph illusrating the response of a *P. falciparum* strain to chloroquine. The level of resistance in chloroquine was 50% (30 isolates), while that of in amodiaquine was 25% (15). Of these 15 isolates, seven were also re-

Table 4.4.3: Number of drug resistant isolates/total isolates in different places according to origin

	Chloroquine (%)	Amodiaquine (%)	Artemisinin (%)	Mefloquine (%)
Orissa	20/28 (71.4)	9/28 (32.1)	1/28 (3.6)	0/28 (0)
Jharkhand	6/18 (33.3)	4/17 (23.5)	0/17 (0)	0/17 (0)
Others	4/14 (28.6)	2/15 (13.3)	0/15 (0)	0/15 (0)
Total	30/60 (50)	15/60 (25)	1/60 (1.66)	0/60 (0)

sistant to chloroquine. One isolate was resistant to dihydroartemisinin. The isolate also showed resistance to chloroquine as well as amodiaquine. No isolate showed resistance to mefloquine.

"Very high resistance to chloroquine (71%) was found in Orissa followed by Jharkhand (33.3%) among the study states"

Table 4.4.3 shows the drug sensitivity pattern according to the origin. Very high resistance to chloroquine was seen in Orissa, followed by Jharkhand and other states. It is important to note that there is a change in the drug policy in both Jharkhand and Orissa for the treatment of malaria; while there is no change in the policy in the other studied states. The sole case of artemisinin resistance was detected in Orissa. ❑

5

Highlights of Research Activities under IDVC Project

5.1 Bengaluru (Karnataka)

- A Phase III, randomized, non-inferiority trial, to assess the efficacy and safety of Dihydro-artemisinin + piperaquine (DHA+ PPQ, Artekin) in comparison with Artesunate + Mefloquine (AS+MQ) in 54 patients affected by acute, uncomplicated *Plasmodium falciparum* malaria was successfully carried out in Mangalore. Clinical trial on Artesunate + Pyronaridine against uncomplicated *P. falciparum* and *P. vivax* was initiated. The drug is found to be very effective. Project on HPR2 and pLDH-based diagnosis for malarial parasites has been initiated.

- Alpha-cypermethrin treated (@ 0.2 g/m^2) long- lasting insecticidal nets were found effective against *Anopheles stephensi* in Mangalore City. No adverse effects on the bednet users were observed. A Project on *Aedes* control using C21 attracticide has been initiated in Bengaluru City.

- Molecular studies on therapeutic efficacy revealed that the prevalence of chloroquine resistance which can be correctly estimated by the PfCRT T76 genotype resistance index as against genotype failure index in Africa.

- Larvivorous fishes which have proved very effective for malaria control are being implemented in Karnataka through NRHM and *Panchayat Raj* Institution.

- As support to the national programme, malaria control monitoring was carried out in three high *Pf* incidence districts and a malaria outbreak investigation was carried out in Raichur district. DDT was found totally ineffective against *An. culicifacies* in two districts of Andhra Pradesh. Besides the above, this field unit is supporting the State Health Department on capacity building and vector control activities.

5.2 Chennai (Tamil Nadu)

- Work on environmental, social and behavioural risk factors related to persistent malaria transmission was carried out in Chennai.

- Phase III trials of Lambda-cyhalothrin 10% CS as indoor residual spray were undertaken in Thangachimadam PHC of Rameswaram Island, Ramanathapuram district and subsequent assessment indicated similar effectiveness of Lambda-cyhalothrin in both CS and WP formulations. Field efficacy trial of enhanced doses of Fenthion (82.5% EC) was carried out in moderately polluted unused wells, polluted drains and cess pools in Chennai against the immature stages of *Cx. quinquefasciatus*.

- Entomological investigations undertaken in the suspected chikungunya outbreak districts of Kerala revealed high breteau and pupal indices.

- Technical support was provided to various centres/institutes, and health education/ training programmes on malaria were undertaken. Malaria clinic continued to cater to the needs of general public by providing early diagnosis and prompt treatment.

5.3 Hardwar (Uttarakhand)

- NIMR, Field Unit, Hardwar is working on industrial malaria control since 1986 and successfully controlled malaria in Bharat Heavy Electricals Limited (BHEL), Hardwar, Indian Drugs and Pharmaceuticals Limited (IDPL), Rishikesh and Indian Oil Corporation (IOC), Mathura.

- High performance liquid chromatography (HPLC) method has been developed for simultaneous determination of curcumin and piperine in plasma.

- Work on the search of new molecules with antimalarial and insecticidal properties is in progress. Impact of insecticides on the environment and monitoring of the levels of antimalarials in malaria cases as per the mandate of the field unit are underway.

- Field evaluation of Aza neem as larvicide supported by BMR, Pune and field evaluation of Bacticide WP (wettable powder) and Bacticide DT (dispersible tablets) formulation of *Bacillus thuringiensis* var. *israelensis* H-14, strain 164 against larvae of mosquito vectors were also carried out.

- Consultancy services on control of mosquitoes/malaria to NTPC, Rhindnagar and Ordnance Factory, Kanpur were provided.

5.4 Jabalpur (Madhya Pradesh)

- Under the project on 'Preparation of a field site for Malaria Vaccine trial in and around Jabalpur', genetic polymorphism in the vaccine candidate antigen genes (MSP-1, MSP-2, MSP-3, TRAP, RAP-1, CSP, EBA-175& AMA-1) and drug resistance genes (*pfcrt*, *pfdhfr* and *pfdhps*) were studied. Altogether, 348 new pregnant women were included in the community cohort (Population 98,632). IgG transfer from mother to infants revealed that in blood smear MP negative placenta, maternal side has higher levels of IgG antibodies than cord side against all *Pf* and *Pv* stage-specific synthetic peptides.

- Work on assessment of burden of malaria in pregnancy in Jharkhand revealed that peripheral parasitaemia was significantly more common in pregnant women in the semi-urban and rural anti natal clinics (ANCs) ($p < 0.001$) and in primigravid and secundigravid relative to multigravid ($p = 0.0042$).

- Under the study on 'Burden of malaria in pregnancy in Chhattisgarh, India', a total of 857 women were enrolled in delivery unit, of which 33 were positive for malaria (21 *Pf* and 12 *Pv*). Rapid assessment of the burden of malaria in pregnancy in Madhya Pradesh revealed that 26.5% samples were positive for malaria parasite by PCR, while only 11.4% were positive by microscopy.

- Facilities for diagnostic PCR, vector incrimination by ELISA, identification of sibling species complex and sequencing of genes were established.

- Support was provided to the national programme by undertaking monitoring of NVBDCP micro action plan to control *Pf* malaria in five districts of Madhya Pradesh. Two training workshops on malaria and other vector borne diseases for Medical Officers of various districts of Madhya Pradesh were organized in January and February 2008 at IDVC Field Unit, Jabalpur.

5.5 Nadiad (Gujarat)

- The major research studies conducted during the reporting periods were: randomized controlled trial of an indigenous fish, *Aphanius dispar* for malaria control in Gujarat; assessment of the impact of Sardar Sarovar Narmada water resources development project (SSP) on communicable diseases with particular reference to mosquito-borne diseases; developing a framework for predicting malaria outbreaks in rural and urban Gujarat; assessment of malaria treatment practices in public and private health sectors; and monitoring of susceptibility status of *An. culicifacies* to malathion and pyrethroids in Gujarat.

- Work on the assessment of mosquitogenic potential created by the development of Ahmedabad-Vadodara Express Highway and mosquito breeding in relation to aquatic vegetation and some physico-chemical factors in central Gujarat was initiated.

- Technical support/consultancy to the national programme on routine surveillance in District Kheda; monitoring of chikungunya and dengue vectors in Bhavnagar; epidemiological investigation on high malaria risk in brick kiln hutment in District Bharuch; situation analysis and assessment of malaria in districts with high incidence of *P. falciparum*; and cross- checking of blood smears and training in malaria to the state health officials was provided.

5.6 Panaji (Goa)

- Estimation of malaria burden in Jharkhand state of India conducted in six districts revealed that highest malaria was found in irrigation paradigm followed by mining, border, forest and plains in the rural paradigms. Expectedly, malaria in urban areas was far less compared to rural areas in all the three urban paradigms.

- Characterisation and bioassays of eight *Bacillus* strains pathogenic to mosquito larvae isolated from sediment soil of mangrove vegetation from Goa showed that *Bacillus* sp KSD-2, 3, 4 & 7 were more effective against *Cx. quinquefasciatus* than *An. stephensi*.

- Situational analysis of chikungunya outbreak in Kerala in 2007 revealed that there was enormous breeding of *Aedes albopictus* (predominant species) and *Ae. aegypti* (restricted to Kozhikode and Kasargod districts) involving multiple sites. Retrospective study on outbreak of chikungunya in Maharashtra state revealed that there was great potential for *Aedes* breeding in both Latur and Ratnagiri districts.

- Evaluation of HRP-II based detection system for *P. falciparum* antigen test by latex agglutination developed by Defence Research and Development Establishment (DRDE), Gwalior revealed that the serum of the *Pf* positive patients showed agglutination with the reagent while with *Pv* did not.

- In Phase III randomised, non-inferiority trials to assess the efficacy and safety of Dihydroartemisinin plus Piperaquine (DHA+ PPQ, Artiken) in comparison with Artesunate plus mefloquine in patients affected by acute uncomplicated falciparum malaria, 28 patients were enrolled which were being followed up for 63 days. Assessment of efficacy, safety and population-pharmakokinetics of the fixed-dose combination of Artesunate-Mefloquine in the treatment of acute uncomplicated *P. falciparum* malaria is also being undertaken.

- Technical support was provided to NVBDCP on training of Medical Officers, MPHWs, Health Supervisors and Insect Collectors; special cross-checking of blood smears of PHCs, assessment of preparedness of Navi Mumbai Municipal Corporation for World Bank assistance and out-

break investigation of chikungunya in Kerala and Maharashtra states.

5.7 Raipur (Chhattisgarh)

- Field evaluation of long-lasting insecticidal nets (LNs) impregnated with alpha-cypermethrin (Interceptor®) against vector mosquitoes was carried out in malaria endemic villages of Amoda CHC in District Kanker.

- Two formulations of Bacticide (*Bacillus thuringiensis* var. *israelensis*) wettable powder (WP) and dispersible tablet (DT) were tested against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* larvae in various breeding habitats in urban areas.

- A field evaluation of organophosphate larvicide fenthion 82.5% EC was undertaken against the mosquito larvae in polluted waters at two enhanced dosages, viz. 150 and 200 ml/ha.

- Therapeutic efficacy of chloroquine was monitored against uncomplicated *P. falciparum* malaria in Antagarh CHC of District Kanker.

- Technical support to the national programme was provided by monitoring of malaria control activities in seven high *Pf* endemic districts of Chhattisgarh; entomological survey for dengue vectors in Bhilai; monitoring of insecticide resistance in malaria vectors in various parts of the state; facilitating the cross-checking of blood slides (for malaria and filariasis parasites) received from various districts and organizing training programmes for laboratory technicians and MBBS students.

5.8 Ranchi (Jharkhand)

- Studies on mosquito fauna, biology of mosquito vectors, cytogenetic studies, insecticide resistance, incrimination of malaria vectors, and survey for fish fauna were undertaken. *An. culicifacies, An. fluviatilis* and *An. annularis* were incriminated as vectors of malaria by sporozoite detection by ELISA. Insecticide susceptibility status of anophelines revealed development of resistance in *An. culicifacies* and *An. annularis.*

- Survey for prevalence of filariasis undertaken in Ranchi and Bokaro elicited microfilaria rate up to 6.9% in the age group ranging from 6 to 60 years. Investigations of outbreaks in Silli and Bundu PHCs of Ranchi brought the inadequacy of surveillance and intervention measures.

- Hatcheries of Gambusia and guppy fishes were established in Ranchi district.

- Malaria and filaria clinic of the unit provided early diagnosis to 1298 and 131 patients respectively. Of 1298 fever cases, *P. falciparum* was found in 68 (*Pf* % 14.8).

- As support to the national programme, monitoring of programme implementation was undertaken in Dumka, East Singhbhum, West Singhbhum, and Simdega districts; IRS activities against Kala-azar vector were supervised in Araria district of Bihar; 40 group meetings were organized in Bundu and Silli PHCs of Ranchi district; two health education camps were organized in TB Sanitorium, Ranchi in which 140 Anganwadi workers and 800 tribals attended the exhibition for health education.

5.9 Rourkela (Orissa)

- Development of a field site for malaria vaccine trial was continued and the project was evaluated by external experts from Barcelona Centre for International Health Research (CRESIB), Spain and Manhiça Health Research Centre (CISM) Mozambique.

- Under evaluation of new tools for vector control, studies were undertaken on field evaluation of long-lasting insecticidal net

(LN) impregnated with alpha-cypermethrin (Interceptor®); Evaluation of ZeroFly® –an insecticide incorporated plastic sheeting against mosquitoes; and field evaluation of long-lasting PermaNets impregnated with deltamethrin. There was a significant reduction of 57 and 76% in malaria incidence in Interceptor net used area as compared to untreated net and without net area respectively. In ZeroFly plastic sheeting area, there was a significant reduction of 86.7, 100 and 84% in the entry rate of *An. culicifacies, An. fluviatilis* and other anopheline species respectively.

- Assessment of therapeutic efficacy of sulphadoxine-pyrimethamine + artesunate and Coartem (Artemether+Lumifantrine) in uncomplicated *P. falciparum* in Keonjhar rural and urban areas and a phase II, randomised, open label, multicentre study to assess the antimalarial efficacy and safety of arterolane (RBx 11160) maleate and piperaquine phosphate coadministration and Coartem® in patients with acute uncomplicated *P. falciparum* malaria.

- As a support to NVBDCP, surveys were undertaken in Sundargarh and Mayurbhanj districts for evaluation of judicious use of DDT spray; monitoring of IRS quality in Sundargarh and Keonjhar districts and malaria monitoring in seven high *Pf* endemic districts, namely Jharsuguda, Bargarh, Angul, Nuapada, Sambalpur, Phulbani and Rayagada were undertaken.

- Malaria clinic provided early diagnosis and prompt treatment to 13,878 fever cases examined till January 2008 of which 886 cases were found positive for malaria (*Pv* 292, *Pf* 567, *Pm* 1 and Mix 26).

5.10 Sonapur (Assam)

- Studies were continued on field evaluation of long-lasting insecticidal nets (LNs) impregnated with alpha-cypermethrin (Interceptor®) against malaria transmitting mosquitoes in Assam; characterization of *P. falciparum* strains prevalent in north-eastern states; and therapeutic efficacy studies in selected sentinel sites in north-eastern states of India.

- New projects have been initiated on follow-up efficacy of Olyset nets against malaria vectors and incidence of malaria and bio-monitoring of organochlorine residue in human populations from Assam.

- Technical support was provided to the national programme in strengthening malaria control activities specific to north-eastern region; in-depth review of the malaria control activities in high-risk districts of Assam; evaluation of DDT susceptibility of vectors of malaria; health education and capacity building measures; mass propagation and distribution of larvivorous fishes (guppy) in town areas; and malaria outbreak investigations in affected districts of Assam.

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6

Research Support Facilities

6.1 Animal House Facility

NIMR has an animal facility which maintains laboratory mice, rabbits as per CPCSE guidelines. Laboratory mice are used for screening the antimalarials, host parasite relationship and maintainance of rodent plasmodia. There is an experienced veterinarian looking after the same. Experiments are performed with the approval of the scientific advisory committee and the animal ethics committee of the institute.

6.2 Repository of Biological Material

6.2.1 Mosquito Species

The details of mosquitoes maintained in the NIMR insectory are furnished in Table 6.2.1.

6.2.2 Parasite Species

Parasite Bank is supporting a large number of organizations working on various aspects of malaria. Biological materials including non-human and human plasmodia preserved/ maintained in Malaria Parasite Bank were supplied to various research organisations. During the reporting period a total of 38 isolates were collected from different parts of the country. Out of these collected isolates, 27 were *P. falciparum* and nine were *P. vivax* isolates. Details of human and non-human malaria parasite isolates collected are shown in Tables 6.2.2 and 6.2.3. Screening of medicinal plant extracts/fractions for their anti-plasmodial activity against CQ sensitive and resistant *P. falciparum* isolates is a routine activity of malaria parasite bank.

Cell lines available at Malaria Parasite Bank

- Hepatoma cell line: Hep G2 A16 used in the *in vitro* cultivation of exo-erythrocytic stage malaria parasites

Table 6.2.1. Details of mosquito species maintained at NIMR Insectary

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

Table 6.2.2. Human Malaria Parasites preserved in the Parasite Bank

Parasite species	Collection sites		No. of isolates collected/years of collection			Total
	State	District	1992–2004	2005–06	2007–08	
P. falciparum	Andhra Pradesh	Visakhapatnam	12	—	—	
	Assam	Sonapur	20	—	—	
		Tezpur	6	—		
		Nalbari	—	1	—	
	Chhattisgarh	Jagdalpur	14	—	—	
		Bilaspur	—	26	—	
	Delhi		191	—	2	
	Gujarat	Anand	4	—	—	
		Kheda	7	—	—	
	Haryana	Gurgaon	25	—	—	
	Karnataka	Mangalore	0	14	—	
	Madhya Pradesh	Mandla/Jabalpur	14	—	—	
	Meghalaya	Tura	0	18	—	
	Mizoram	Kolasib	—	—	6	
	Orissa	Rayagada	29	—	—	
		Sundargarh	42	—	—	
	Rajasthan	Alwar	25	—	—	
		Bharatpur	35	—	—	
		Jaisalmer	38	—	—	
	Tamil Nadu	Chennai	0	4	—	
		Ramanathapuram	1	—	19	
	Uttar Pradesh	Baharaich	22	—	—	
		Gautam Budh Nagar	37	—	—	
		Ghaziabad	17	—	—	
		Allahabad	60	—	—	
	West Bengal	Kolkata	18	—	—	
		Midnapur	1	—	—	
	Total		618	63	27	708
P. vivax	Karnataka		0	6	—	6
	Delhi, Uttar Pradesh, Orissa		53	—	—	53
	Tamil Nadu		0	9	9	18
	Total		53	15	9	77
P. malariae	Orissa		4	—	—	4
	Delhi		1	—	—	1
	Total		5	—	—	5
Total collection of *P. falciparum* isolates			708			
Total collection of *P. vivax* isolates			77			
Total collection of *P. malariae* isolates			5			
Total human malaria parasites			790			

- 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 6.2.3. Non-human m alaria parasites preserved in the Parasite Bank

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

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

6.3 Library

The Institute has one of the best libraries in the country in the field of malaria having more than 6955 books, 4283 bound journals, 3673 reprints, 18 video cassettes, 27 audio cassettes, 20 microfilms, 19 theses and 106 national and international reports. About 52 journals (39 Foreign and 13 National) are being subscribed besides 8 journals which are received on complimentary and exchange basis. About 201 new books had been added during this financial year. The library renders its services not only to the scientists/research scholars of the Institute but also to various national and international universities and organisations. Library has also been serving its users through DELNET. Other services such as information retrival, citation index, internet facility, inter library loan facility, reprographic services, etc. are also being provided to the users. J Gate and JCCC@ICMR & Proquest Medical Database are being provided to the NIMR scientists in Delhi and its IDVC field units located in different parts of India. In the process of modernisation, application of barcode system is in progress. Library provides abstracts, references, CAS and SDI services, Med-line CD search, etc. to its users.

96-capillary automated DNA sequencer0

6.4 Central Instrumentation Facility

The Central instrumentation facility of NIMR has all modern equipments including 96-capillary DNA sequencer, LCMS/MS, Real Time PCR, HPLC, Flow cytometer, etc. In addition NIMR has also has a liquid nitrogen plant. ❑

7

Inter-Institutional Collaboration

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

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

and Ranbaxy.

15. 'Primary screening of medical plants from north-eastern states of India for their antimalarial activity' in collaboration with DRL, Tezpur, Assam.
16. 'Screening of chloroquine sensitivity status of *P. falciparum* parasites from western border areas of India' in collaboration with DRDE, Gwalior, Madhya Pradesh.
17. 'Molecular characterisation of nitric oxide synthase in *An. culicifacies*: relevance for refractory mechanism' in collaboration with Institute for Cytology and Preventive Oncology, Noida, Uttar Pradesh.
18. 'Health impact assessment of Indira Sagar Dam and resettlement colonies in SSP Reservoir impoundment areas in Narmada Valley in Madhya Pradesh' in collaboration with National Institute of Virology, Pune, National Institute of Cholera and Enteric Diseases, Kolkata, and Narmada Valley Corporation.
19. 'Characterisation of *P. falciparum* strains prevalent in north-eastern states' in collaboration with Regional Medical Research Centre, Dibrugarh, Assam.
20. 'Screening of antimalarial activity of synthetic compounds in *P. falciparum* culture lines' in collaboration with Department of Chemistry, University of Delhi, Delhi and Indian Institute of Chemical Technology, Hyderabad.
21. 'Development of site for malaria vaccine trial at Sundargarh district, Orissa' in collaboration with International Centre for Genetic Engineering and Biotechnology, New Delhi and State Government of Orissa.
22. 'Preparation of a field site for malaria vaccine trial in and around Jabalpur' funded by ICMR task force and Center for Disease Control and Prevention (CDC), Atlanta, USA.
23. 'Assessing the burden of malaria in pregnancy in India (Chhattisgarh)' in collaboration with Boston University School of Public Health (BUSPH) funded by ICMR and NIH, Washington.
24. 'Rapid assessment of burden of malaria in pregnancy in Madhya Pradesh, India' in collaboration with CDC, Atlanta, USA, Liverpool School of Medicine, UK, funded by USAID, New Delhi.
25. 'Assessing the burden of malaria in pregnancy in east India (Jharkhand)' in collaboration with Boston University School of Public Health, funded by USAID, Washington.
26. 'Monitoring micro action plan to control *P. falciparum*' in collaboration with NVBDCP.

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9

Research Papers Published (2007)

1. Alam MT, Bora H, Bharti PK, Saifi MA, Das MK, Dev V, Kumar A, Singh N, Dash AP, Das B, Wajihullah, Sharma YD. Similar trends of pyrimethamine resistance-associated mutations in *Plasmodium vivax* and *P. falciparum*. *Antimicrob Agents Chemother* 2007; *51:* 857–63.
2. Alam MT, Das MK, Dev V, Ansari MA, Sharma YD. Identification of two cryptic species in the *Anopheles* (*Cellia*) *annularis* complex using ribosomal DNA PCR-RFLP. *Parasitol Res* 2007; *100:* 943–8.
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4. Bajaj R, Mohanty S, Dash AP, Das A. Fine-scale genetic characterization of *Plasmodium falciparum* chromosome 7 encompassing the antigenic var and the drug-resistant Pfcrt genes. *J Genet* 2007; *87*.
5. Biswas S, Seth RK, Tyagi PK, Sharma SK, Dash AP. Naturally acquired immunity and reduced susceptibility to falciparum malaria in two subpopulations of endemic eastern India. *Scand J Immunol* 2007; *67:* 177–84.
6. Biswas Sukla, Seth Ratanesh K, Sharma Geeta, Dash AP. A longitudinal investigation of *Plasmodium falciparum* malaria in children in northern India. *Scand J Infect Dis* 2007; *6:* 1–8.
7. Das A, Bajaj R, Mohanty S, Swain V. Genetic diversity and evolutionary history of *Plasmodium falciparum* and *P. vivax*. *Curr Sci* 2007; *92:* 1516–24.
8. Das A, Dash AP. Evolutionary paradigm of chloroquine-resistant malaria in India. *Trends Parasitol* 2007; *23:* 132–5.
9. Dash AP, Adak T, Raghavendra K, Singh OP. The biology and control of malaria-vectors in India. *Curr Sci* 2007; *92:* 1571–8.
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11. Dev V, Dash AP. Rainfall and malaria transmission in north-eastern India. *Ann Trop Med Parasitol* 2007; *101:* 457–9.
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15. Garg S, Alam MT, Das MK, Dev V, Kumar A, Dash AP, Sharma YD. Sequence diversity and natural selection at domain of the apical membrane antigen 1 among

Indian *Plasmodium falciparum* populations. *Malar J* 2007; *6:* 154.

16. Ghosh SK, Dash AP. Larvivorous fish against malaria vectors: a new outlook. *Trans R Soc Trop Med Hyg* 2007; *101:* 1063–4.
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10

Other Activities

10.1 Information, Education and Communication

10.1.1 National Science Day Celebration

The National Science Day was celebrated in Gyan Bharti School, Shankar Road, New Delhi in February 2008. Information on prevention and control of mosquito breeding and mosquito borne diseases was imparted to the senior secondary school students. Students took active part in debates and discussions. In addition to live demonstration of various stages of vector mosquitoes, larvivorous fish, expanded polystyrene (EPS) beads, biocides and rapid diagnostic kits were also demonstrated. Blood slides positive for malaria parasites were shown in compound microscope.

Video-films showing preventive and intervention methods of mosquito control were shown. The students were involved in discussions and debates after these activities. The exhibition in Hindi language was appreciated by students and teachers. NIMR publications, including books, charts and pamphlets were given to the school library.

10.1.2 Distribution of Video CDs

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

10.1.3 Publications in National Language

NIMR published three papers in national language:

1. Malhotra MS. *Malaria Niyantran mein Kathinayian. Malaria Patrika* 2007; *15*(1): 5–7.
2. Malhotra MS, Tyagi P. *Dengue Niyantran mein Jansamanya ki Bhumika. Malaria Patrika* 2007; *15*(3): 5–9.
3. Tyagi P, Malhotra MS. *Malaria Niyantaran: Ek Bahu-aayami Upkram. ICMR Bull* 2007; *21*(10–12): 37–46.

Demonstration of mosquito larvae to school children

Dr MS Malhotra, NIMR delivering the lecture on malaria and its control

10.2 Conferences/Workshops and Training Courses Organized

10.2.1 IX International Symposium on Vectors and Vector Borne Diseases

NIMR organised the IX International Symposium on Vectors and Vector Borne Diseases at Puri under the auspices of the National Academy of Vector Borne Diseases from 15–17 February 2008. More than 500 delegates across the globe attended the same. Sh. Sanatan Bisi, Hon'ble Minister of State for Health and Family Welfare, Govt. of Orissa presided over the function. Sh. Debasis Nayak, Hon'ble Minister of State, Information & Public Relations, Sports and Youth Services was the Guest of Honour. Dr S Habayab, WHO-Representative to India, Dr Altaf Lal, Health Attache, US Embassy, India, Dr VP Sharma, former Director, NIMR and Dr GPS Dhillon, Director, NVBDCP were also present to grace the inaugural function. There were various scientific sessions on vector biology and control, parasite biology, molecular biology, epidemiology, drug discovery, vaccines, etc. There were about 100 oral presentations and more than 200 posters focussing different areas of malaria research.

The National Academy of Vector Borne Diseases conferred several awards in recognition of outstanding research contributions in the field of vector borne diseases. Dr Sukla Biswas (2004) and Dr Hema Joshi (2007), NIMR received award for their contributions to Molecular Biology. Dr SK Sharma, NIMR, received award for outstanding contributions in the field of vector biology. The award for contribution in clinical aspects was given to Dr Ekta Gupta of Maulana Azad Medical College, New Delhi.

The Bayer Environmental Science Award was given to Dr DT Mourya, NIV, Pune. The Biotech International Life-time achievement award was given to late Dr MA Ansari, former Director, RMRCT, Jabalpur for his contributions in the field of vector borne diseases. Dr Arun Sharma, NIMR, won the best poster award in the senior category. Under junior category awards were won by Mr Manoj Chug, MD University, Rohtak (First), Ms Gauri Awasthi, NIMR (Second), Ms Sharmila Pahwa and Mr Prashant Mallick, NIMR, Mr Manas Sarkar, DRL, Tezpur, Ms Arkeja Kumar, DRDE,

Inaugural function (from left to right Dr VP Sharma, Dr Altaf Lal, Sh. Debasis Nayak, Sh. Sanatan Bisi, Dr S Habayab, Prof. AP Dash and Dr GPS Dhillon

Prof. G Padmanabhan delivering the key note address

Valedictory function (on the dias from left Dr Nutan Nanda, Prof AP Dash, Dr Altaf Lal, Dr JP Narain, Dr S Pattanayak and Dr VP Sharma

Gwalior and Mr LC Mishra, University of Delhi (Third).

10.2.2 International Workshop on Insecticide Resistance

An International workshop on 'Insecticide resistance' focussing on insecticide resistance in malaria vectors, management of resistance by new insecticides and other tools was held at Puri on 14 February 2008. More than 50 delegates participated in the workshop.

Prof. AP Dash welcoming the delegates

10.2.3 Brainstorming Meeting on Malaria

The National Institute of Malaria Research organized a brainstorming meeting on malaria from 12–13 November 2007 at Bhubaneswar, Orissa in collaboration with WHO/SEARO . Twenty-seven participants attended this meeting. There were representatives from the National Institute of Malaria Research, Indian Council of Medical Research, National Vector Borne Disease Control Programme, State Health authorities including the Principal Secretary, Health, Regional Medical Research Centre, Bhubanewar, and WHO staff from GMPHQ, SEARO and WRO India. The group made practical recommendations for control of malaria situation in the state.

10.2.4 Other Workshops Organized

1. NIMR Field Unit, Rourkela organized a workshop on "Environmental management of vector borne diseases" on 10 August 2007 in collaboration with Public Health Department of Rourkela Steel Plant.
2. NIMR Field Unit, Jabalpur organized an International workshop on "Molecular epidemiology and immunology of malaria and other vector borne diseases" in collaboration with Regional Medical Research Centre for Tribals, Jabalpur from 16 to 19 October 2007.

10.2.5 Training Courses and Meetings

1. NIMR Field Unit, Rourkela organized an awareness training programme on 9 October 2007 for 12 volunteers of a local NGO on vector borne diseases.
2. NIMR Field Unit, Jabalpur organized a meeting on "Preparation of a field site for malaria vaccine trial in and around Jabalpur" from 8 to 9 September 2007.
3. Lecture/demonstration of Rapid diagnosis of malaria for MD Community Medicine students of the Armed Forces Medical College, Pune was organized on 21 November 2007.
4. Three training courses for Laboratory Technicians of MCD were organized by NIMR in collaboration with NVBDCP and MCD, Delhi, from 19-23 November, 3–7 December and 10–14 December.
5. Organized a one day seminar on "Nanotechnology: current status and future trends in applied medicine" on 22 May 2007.
6. Mr Md. Tauqeer Alam and Ms Sheena Garg, Ph.D students in Department of Biotechnology, AIIMS were trained on fine structural analysis of human malaria parasites. As a member of Doctoral Committee attended periodic meetings to review their research work.
7. Training Programme in Vector control methods (Course 20/57 WHO FTP) for three In-Country WHO fellows (15–26 October 2007).
8. Training provided to Ms. Sujata Rohilla, the student of B.Tech (Biotechnology), 4th year, of Sardar Vallabh Bhai Patel University of Agriculture and Technology,

Modipuram, Meerut from 7 June to 6 July 2007.

10.3 Conferences/Workshops/Important Meetings Attended

Dash AP

1. Attended a meeting to discuss activities under ICMR-HGF joint programme for Indo-German Science Centre for Infectious Diseases at Delhi from 3–4 April 2007.
2. Attended WHO National workshop on Human Resources on Health at Ministry of Health and Family Welfare on 5 April 2007.
3. Addressed the participants at the workshop on Burden of malaria in pregnancy in India at NIMR, FU, Raipur on 10 April 2007.
4. Delivered plenary lecture on 'Are we losing battle ?' in the National Congress on Parasitic Diseases at Bengaluru from 13–15 April 2007.
5. Attended National workshop on Micro array Technology at Institute of Pathology, New Delhi on 6 April 2007.
6. Attended Scientific Advisory Committee meeting of DMRC, Jodhpur from 19–20 April 2007.
7. Attended meeting of State Governments on Chikungunya at Vigyan Bhawan New Delhi on 21 June 2007.
8. Attended Country coordination mechanism meeting under Global Forum for AIDS, tuberculosis and malaria as an expert member, at Nirman Bhawan, New Delhi on 29 June 2007.
9. Attended the workshop on Therapeutic efficacy study at Guwahati, Assam from 6–8 July 2007.
10. Attended International workshop on Clinical trials methodologies, good clinical practices at India International Centre, New Delhi on 13 July 2007.
11. Attended meeting to discuss Climate change on health under the Chairmanship of Principal Secretary, Advisor to the Prime Minister at New Delhi on 6 August 2007.
12. Visited the Vector Control Research Unit, Universiti Sains Malaysia for discussion on WHOPES testing and preparation of insecticide impregnated papers from 12-14 August 2007.
13. Attended 1st Conference on Medical Arthopodology at CRME, Madurai on 31 August 2007.
14. Attended a meeting on preparation of field site for trial of vaccine in and around Jabalpur at RMRCT, Jabalpur from 8–10 September 2007.
15. Attended ICMR Project review committee on Malaria, filariasis and leishmaniasis at ICMR, New Delhi on 4 October 2007.
16. Attended Indo-German workshop on Genetic susceptibility under ICMR-HGF at Braunschweig, Germany from 30 September to 2 October 2007.
17. Attended a meeting to initiate a clinical trial of new drug (Arterolane + Piperaquine) at Tata Main Hospital, Jamshedpur on 8 October 2007.
18. Attended International workshop on Molecular epidemiology and immunology of malaria and other vector borne diseases and chaired a session at RMRCT, Jabalpur.
19. Delivered oration lecture at VII Joint Conference of ISMOCD and IAE, India poised investment in public health for health security and quality of life at Jodhpur on 26 October 2007.
20. Attended the India Conference 2007 on 'Innovations and Technologies for India's Public Health System' from 1–2 November 2007.
21. Chaired a scientific panel on 'Adapting and adopting best practices for interventions at the India Conference 2007 on 'Innovations and Technologies for India's Public Health System' on 2 November 2007.
22. Delivered an invited lecture on Inte-

grated surveillance and control of vector borne diseases at the India Conference 2007 on 'Innovations and Technologies for India's Public Health System' on 2 November 2007.

23. Attended the ICMR/WHO-SEARO Brainstorming meeting on malaria situation in Orissa at Bhubaneswar from 12–13 November 2007.
24. Attended the 'Scientific Advisory Committee' meeting of the Centre for Research in Medical Entomology, Madurai from 11–12 January 2008.
25. Dash AP chaired a scientific session in the II International conference on 'Trends in cellular and molecular biology' at JNU, New Delhi from 5–7 January 2008.
26. Attended a meeting to discuss the results and publications of the 'DHA-Piperaquine (Artekin) trial' at Bangkok, Thailand on 5 February 2008.
27. Delivered the 'Foundation Day Oration' at Institute of Life Sciences, Bhubaneswar on 11 February 2008.
28. Attended meeting on 'Ongoing collaboration activities between NIMR and CDC' at CDC, Atlanta from 2 to 5 March 2008.
29. Dash AP attended the 'Coordination meeting on the Impact of vector control interventions in areas where vectors are resistant to insecticides' at Geneva from 25–29 March 2008.
30. Attended the 'II Scientific Advisory Committee (SAC)' meeting at Geneva from 31 March to 4 April 2008.

Atul PK

1. Participated in the National Science Day Celebrations on 27 February 2008 at Vidhya Bhavan, Shanker Road, New Delhi and delivered a talk on the subject "The Earth".
2. Attended II National seminar on "Sowa-Rigpa, the science of healing (Amchi system of medicine) and delivered speech on New avenues of research & cc development and the recognition of Sowa-Rigpa system at New Delhi from 30–31 March 2008.

Batra CP

1. Participated in VII joint conference of ISMOCD and IAE at DMRC, Jodhpur from 27–29 October 2007 and was co-chairman in Vector Biology and Control session.
2. Participated in WHO conference at Bhubaneswar on 22 November 2007.

Biswas Sukla

1. Attended the Review meeting to present the progress of work and to discuss on Future action in the collaborative project entitled "Preparation of field site for malaria vaccine trial in and around Jabalpur" at NIMR, Field Unit Jabalpur from 8–11 September 2007.
2. Participated in the XIX National Congress of Parasitology and International Symposium on Parasitic Diseases of Animals and Man (Focal theme: emerging perspectives in parasitology research), held at Visakhapatnam, Andhra Pradesh from 26–28 October 2007; sponsored by Indian Society for Parasitology; organised by Department of Zoology, Andhra University as an Invited Speaker. Delivered talk on "Immune responses to defined *Plasmodium falciparum* antigens and disease susceptibility in two subpopulations of northern India".
3. Attended the meeting to discuss the Progress and future action in the collaborative project entitled "Characterisation of the *P. falciparum* strains prevalent in northeastern states", an Extramural, ICMR funded project at NIMR, Sonapur, Assam and Aizawl, Mizoram from 3–7 December 2007.

Dhiman RC

1. Participated in Centenary celebrations of Royal Society of Tropical Medicine and presented paper "Monitoring of post tsunamic malariogenic conditions in Car

Nicobar (India) using satellite remote sensing in poster session at London from 13–15 September 2007.
2. Review and preparedness of various implementing agencies in NCT Delhi for prevention and control of dengue/DHF and chikungunya on 10 August 2007.
3. Participated in National workshop on Climate change and its impact on health organised by WHO and NEERI at Lonavala from 26–27 November 2007.
4. Participated in IV International Conference on Environmental Education at Ahmedabad on 25 November 2007 and delivered a lecture.
5. Participated in Tropacon conference at Madras Medical College, Chennai and delivered lecture on New ventures in malaria control on 6 October 2007.
6. Delivered lecture on Climate change induced health complexities and risks at National Institute of Disaster Management, Delhi on 28 November 2007.
7. Delivered lecture at LII National Conference of Indian Public Health Association on 7 March 2008 at MAMC, Delhi.

Mishra Neelima

1. Meeting on Operational research on drug use practice at NIMR, Delhi on 20 March 2007.
2. Workshop on Operational research on drug use practice and pre-packaged blister pack drugs at Ranchi, Jharkhand on 16 July 2007.

Mittal PK

1. Conducted a two-weeks Training programme in Vector control methods (Course 20/57 WHO FTP) for three In-country WHO fellows from 15–26 October 2007.
2. Participated as faculty and resource person for the Training course for insect collectors of MCD organised by (NIMR) in collaboration with MCD Health Department, Delhi in March 2008.
3. Participated in the workshop on Role of pesticide application technology in pest management, organised by Institute of Pesticide Formulation Technology from 24–26 July 2008.
4. Participated in the VII joint annual conference of Indian Society for Malaria and Other Communicable Diseases (ISMOCD) & Indian Association of Epidemiologists (IAE) and presented a paper on "Insecticide incorporated plastic sheeting for control of malaria vectors and transmission of disease in temporary shelters in Delhi" held at DMRC, Jodhpur from 27–29 October 2007.

Nanda Nutan

1. Delivered a lecture on "Fine structural analysis of growth and differentiation of successive stages in the life cycle of human malaria parasites" in XXIX Annual conference on electron microscopy and allied fields held at University of Delhi from 26–28 November 2007.
2. Attended "Ist International conference on medical arthropodology" and presented a paper titled 'Molecular evidence that the morphologically identified *Anopheles fluviatilis* from Assam (India) are in fact hypermelanic form of *Anopheles minimus*' at Madurai from 1–2 September 2007.

Raghavendra K

1. Nominated to attend the local project advisory committee (LPAC) meeting on study on status of Nanotechnology in Indian industry and Academia, organized by National Foundation of Indian Engineers, New Delhi on 11 April 2007 and 5 March 2008.
2. Attended the meeting of Technical review committee to short list GFATM round VII proposal from 30 May to 1 June 2007 at NVBDCP, Delhi.
3. Attended the ministers review meeting on Dengue control at Delhi on 10 July 2007.
4. Attended National workshop on Inte-

grated mosquito management and future directives" held at Osmania University, Hyderabad from 8–9 January 2007.
5. Attended XIX National Congress of Parsitology-2007 and International symposium on Parasitic Diseases of Animal and Man at Visakhapatnam from 26–28 October 2007.
6. Attended "National workshop on Functional Genomics and Evolutionary Biology" held at Institute of Life Sciences, Bhubaneshwar, Orissa from 21–23 November 2007.
7. Attended training programme "Perspectives and current trends in bioinformatics" held at Centre for Cellular and Molecular Biology, Hyderabad, Andhra Pradesh from 12–18 September.

Singh Ruchi

1. Delivered a talk on "The role of women in science and career opportunities for women in science" at Nagpur University and moderated the panel discussion under UNESCO L`OREAL For Women in Science programme.

Valecha Neena

1. Attended a meeting on "Review meeting on falciparum malaria in high endemic districts" at Vigyan Bhawan, New Delhi on 11 April 2007.
2. Guest lecture on "Rational use of drugs: malaria" organized by Family Planning Association India at FPAI Bhawan, New Delhi on 19 April 2007.
3. Meeting on Brainstorming session for malaria under golden triangle partnership (GTP) scheme on 11 May 2007.
4. Organising secretary "Brainstorming meeting on malaria in Orissa" at Bhubaneswar from 12–13 November 2007.
5. Meeting on Estimation of malaria disease burden in India at New Delhi from 21–23 November 2007.
6. JNC Biomedical Sciences meeting "Antimalarial resistance: need to look for new options for chemotherapy" at Rajanukunte, Bengaluru from 29 –30 November 2007.
7. Regional meeting on Private sector partnerships in the fight against HIV/AIDS, tuberculosis and malaria at New Delhi from 13–14 December 2007.
8. Meeting of India-Country Coordinator Mechanism (India-CCM) for the Global fund to fight AIDS, tuberculosis and malaria (GFATM) at Nirman Bhawan, New Delhi on 17 December 2007
9. Meeting on Participation in the World Antimalarial Resistance Network (WARN) meeting at Oxford, U.K. from 3–6 January 2008.
10. Meeting of "Core Group on chemotherapy of malaria" at NVBDCP on 8 January 2008.
11. Meeting on "Artekin Phase III trial in Asia" at Bangkok on 5 February 2008.
12. Meeting of Expert Group on Chemotherapy of malaria at Directorate of NVBDCP, Delhi on 25 February 2008.
13. Meeting "India CCM retreat" to discuss participation, representation and selection of stakeholders for CCM at New Delhi on 5 March 2008.
14. Meeting on "IDVC SAC" at Goa from 14–15 March 2008.
15. Member of selection committee for the post of Consultants and supporting staff under the World Bank/GFATM supported Project at Directorate of NVBDCP from 25–26 March 2008.

10.4 New Ranking for *Journal of Vector Borne Diseases*

The *Journal of Vector Borne Diseases* (JVBD) published by the National Institute of Malaria Research stood at 10th rank in country-wise ranking of Biomedical Journals in India as per the latest rankings provided by SCImago Journal Rank (SJR). The SJR of JVBD is 0.081. The journal publishes original research articles, reviews, case reports, short research reports in all aspects of vector borne diseases. The articles published in JVBD can be openly ac-

cessed through www.mrcindia.org/journal. This journal has been included in Directory of Open Access Journals (DOAJ) and is indexed by all major abstracting agencies.

10.5 Construction of the Research Block

The job of construction of NIMR building was awarded to M/s Rajasthan State Road Development & Construction Corporation Limited under the supervision of M/s Gherzi Eastern Ltd. The civil and utility works (electrical installations, plumbing, fire-fighting, HVAC etc.) have been completed. The construction of boundary wall of NIMR campus and the roads have been completed. The external developmental works like laying of sewer lines, construction of recharge wells land-scaping etc. are near completion. The permanent electricity and water connections would be acquired shortly from BSES and Delhi Jal Board respectively. Apart from laboratories and administration wing the research block also houses few animal rooms and has provision for few guest rooms till separate buildings come up for these facilities. The newly built research block was inaugurated by Prof. NK Ganguly, the then Director General, Indian Council of Medical Research on 8 November 2007. Dr. SK Bhattacharya, Additional DG, ICMR and Mr. Sanjeev Dutta, Financial Advisor were also present on that occasion. The process of procuring laboratory and office furniture has been initiated. Administrative and technical formalities are being completed to invite tenders from reputed companies. NIMR is likely to shift to its new building by mid-2008.

10.6 Construction of Animal House & Auditorium

Animal house and Auditorium would be constructed at NIMR complex in the second phase. For these a series of meetings were held between NIMR officials and representatives of M/s HSCC (India) Ltd. and the layout plans for these buildings were finalised. The estimated cost for these buildings was forwarded to ICMR and the administrative approval and expenditure sanction of the Council have been obtained. The work on this project would commence after the completion of the administrative and technical formalities.

10.7 NIMR Website

The Institute's website www.mrcindia.org contains information on research activities being carried out by NIMR, publications of the Institute, research activities of the field units of NIMR, audio-visual unit activities and profiles of scientists, etc. The documents and books published by the Institute are available on this website. A separate webpage is being maintained for Institute's english periodical, the *Journal of Vector Borne Diseases*. The website contains information on guidelines for contributors, subscribers and contact information of editorial office, etc.

10.8 संस्थान में राजभाषा विकास संबंधी गतिविधियाँ

संस्थान राजभाषा अधिनियम के अनुपालन की दिशा में वर्ष दर वर्ष प्रगति की ओर अग्रसर हो रहा है इसका प्रमाण है कि वर्ष 2007–08 में एक ओर जहाँ राजभाषा अधिनियम के अनुपालन के उद्देश्य से संस्थान में पत्राचार, प्रशासन संबंधी कार्य आदि में राजभाषा स्थिति की समीक्षा हेतु तिमाही बैठकें आयोजित की गईं, प्रोत्साहन योजना लागू की गई। वहीं दूसरी ओर विज्ञान दिवस मनाए जाने के साथ ही हिन्दी पखवाड़े के अवसर पर विभिन्न गतिविधियाँ आयोजित की गई जो कि वर्ष की मुख्य गतिविधियाँ रहीं। उल्लेखनीय है कि इस वर्ष संस्थान में हिन्दी पखवाड़ा दिनांक 14 से 25 सितम्बर 2007 तक पूरे हर्षोल्लास के साथ मनाया गया। हिन्दी पखवाड़ा के अवसर पर हिन्दी कार्यशाला, वैज्ञानिक संगोष्ठी, श्रुतलेख प्रतियोगिता, टिप्पण–प्रारूपण प्रतियोगिता, निबन्ध प्रतियोगिता, कर्मचारियों और अधिकारियों के लिए पृथक–पृथक वाद–विवाद प्रतियोगिताओं का आयोजन किया गया। संबंधित प्रतियोगिताओं का आयोजन संस्थान के निदेशक प्रो. ए.पी. दाश के निर्देशन में संस्थान की

हिन्दी अधिकारी एवं राजभाषा कार्यान्वयन समिति के विभिन्न सदस्यों द्वारा किया गया।

इस पखवाड़े का आरंभ दिनांक 14 सितम्बर 2007 को निबन्ध प्रतियोगिता के साथ हुआ, जिसका संचालन डॉ. चन्द्र प्रकाश बत्रा, सहायक निदेशक द्वारा किया गया। इस प्रतियोगिता में संस्थान के 30 अधिकारियों एवं कर्मचारियों ने उत्साहपूर्वक भाग लिया। प्रतियोगिता का विषय था— ''खाद्य पदार्थों में बढ़ती मिलावट के स्वास्थ्य पर दुष्प्रभाव'' अथवा ''मच्छर द्वारा फैलने वाली बीमारियाँ और उनकी रोकथाम''। संबंधित पखवाड़े की दूसरी गतिविधि टिप्पण एवं प्रारूपण प्रतियोगिता का संचालन दिनांक 17 सितम्बर 2007 को संस्थान के सहायक अनुसंधान अधिकारी श्री आर.एन. यादव द्वारा किया गया। इस वर्ष इन प्रतियोगिताओं में दिनांक 18 सितम्बर 2007 को श्रुतलेख प्रतियोगिता का आयोजन भी किया गया, जिसका संचालन डॉ. अरूण शर्मा, उपनिदेशक द्वारा किया गया। संबंधित पखवाड़े के अन्तर्गत दिनांक 19 सितम्बर 2007 को पूर्वान्ह् में संस्थान के वरिष्ठ प्रशासन अधिकारी श्री जय प्रकाश वर्मा के संचालन में पूर्णकालिक कार्यशाला का आयोजन किया गया। उक्त कार्यशाला संस्थान के प्रशासनिक वर्ग के कर्मचारियों के लिए आयोजित की गई थी, जिसमें संस्थान के निदेशक महोदय ने भी भाग लिया था। इस कार्यशाला के प्रथम चरण में मुख्य व्याख्याता के रूप में श्री रमेश चन्द्र जोशी, मानद निदेशक, केन्द्रीय सचिवालय हिन्दी परिषद को आमंत्रित किया गया था।

सर्वप्रथम उपस्थित माननीय मुख्य अतिथि और संस्थान के निदेशक को पुष्प भेंट कर विधिवत् स्वागत किया गया। इसके उपरान्त कार्यालय के सभी अनुभाग अधिकारियों को प्रशासन संबंधी कार्य को राजभाषा हिन्दी में करने हेतु प्रेरित करने के उद्देश्य से पुस्तकें एवं चार्ट निदेशक महोदय के कर-कमलों द्वारा वितरित किए गए। तत्पश्चात् हिन्दी कार्यशाला के संचालक द्वारा श्री जोशी का परिचय देते हुए उन्हें व्याख्यान हेतु आमंत्रित किया गया। श्री जोशी ने अपने व्याख्यान में "राजभाषा में काम करना आसान, फिर मुश्किल क्यों ?" विषय पर जानकारी देते हुए सभी को प्रेरणा, प्रोत्साहन, सद्भावना की नीति को अपनाते हुए राजभाषा हिन्दी का प्रयोग करने हेतु प्रेरित किया।

कार्यशाला के द्वितीय चरण का आरंभ अपरान्ह् 12 बजे हुआ, जिसमें श्री अशोक सचदेवा, उपनिदेशक, वित्त मंत्रालय (राजभाषा) को आमंत्रित किया गया। उन्होंने अपने व्याख्यान में अत्यंत ही रूचिपूर्ण ढंग से पत्राचार के विभिन्न रूपों जैसे टिप्पणी, आदेश, पत्र, अर्धशासकीय पत्र, ज्ञापन, परिपत्र आदि पर विस्तारपूर्वक जानकारी देते हुए इनके लिखने के सही ढंग पर प्रकाश डाला। चूंकि यह कार्यशाला पूर्णकालिक थी इसलिए भोजनावकाश के बाद अपरान्ह् 3 बजे कार्यशाला को पुनः आरंभ किया गया, जिसमें श्री दिनेश चन्द्र त्रिपाठी, वरिष्ठ हिन्दी अधिकारी, भारतीय आयुर्विज्ञान अनुसंधान परिषद को आमंत्रित किया गया था। श्री त्रिपाठी के व्याख्यान का विषय था— ''राजभाषा नीति''। उन्होंने बहुत रोचक ढंग से केन्द्र सरकार के कर्मचारियों द्वारा राजभाषा अधि नियम का अनुपालन करने के नैतिक उत्तरदायित्व पर जोर देते हुए राजभाषा विभाग के वार्षिक लक्ष्यों पर विस्तृत जानकारी दी।

दिनांक 20 सितम्बर 2007 को हिन्दी पखवाड़े की पाँचवी गतिविधि वैज्ञानिक संगोष्ठी का आयोजन किया गया, जिसका संचालन डॉ. रमेश चन्द धीमान, उपनिदेशक (प्रवरण कोटि) द्वारा किया गया था। संबंधित संगोष्ठी में मुख्य अतिथि के रूप में राष्ट्रीय विज्ञान, प्रौद्योगिकी एवं विकासात्मक अध्ययन (निस्टाड) के निदेशक, डॉ. पार्थसारथी बनर्जी, को आमंत्रित किया गया था। संगोष्ठी का विषय था- ''जलवायु परिवर्तन एवं स्वास्थ्य''। सबसे पहले माननीय अतिथि डॉ. बनर्जी का विधिवत् स्वागत करते हुए संगोष्ठी का प्रारंभ डॉ. धीमान द्वारा उक्त विषय पर स्लाइड शो से किया गया। तत्पश्चात् उपस्थित वैज्ञानिकों ने विषय के विभिन्न पहलुओं पर अपने विचार प्रकट किए। संगोष्ठी का अन्त करते हुए संचालक द्वारा बनर्जी को अपने विचार प्रस्तुत करने हेतु आमंत्रित किया गया। डॉ. बनर्जी ने विकसित देशों का उदाहरण देते हुए वैज्ञानिक उन्नति हेतु दैनिक विज्ञानीय चर्चा को अपनी राजभाषा हिन्दी में करने पर बल दिया। उन्होंने जलवायु परिवर्तन संबंधी नवीन जानकारी देते हुए भविष्य में वैज्ञानिकों को इस संबंध में और अधिक सजग रहने को कहा।

इसी क्रम में चलते हुए दिनांक 21 सितम्बर 2007 को अपरान्ह् 3 बजे कर्मचारी वर्ग के लिए वाद-विवाद

हिन्दी कार्यशाला

प्रतियोगिता का आयोजन किया गया, जिसका सफलतापूर्वक संचालन संस्थान के सहायक निदेशक डॉ. नूतन नन्दा ने किया। संबंधित प्रतियोगिता में निर्णायक के रूप में श्री शंभुनाथ सिंह, प्रशिक्षक, दैनिक जागरण एवं श्री नेत्र सिंह रावत, उपनिदेशक राजभाषा विभाग को आमंत्रित किया गया था। प्रतियोगिता का विषय था— "घरेलू नौकर सुविधा या सिरदर्द"। इस विषय पर संस्थान के लगभग 15 कर्मचारियों ने जोशपूर्ण ढंग से अपने-अपने विचार प्रस्तुत किए। प्रतियोगिता के अंत में श्री नेत्र सिंह रावत ने कर्मचारियों के उत्साह एवं विचारों की प्रशंसा की एवं श्री सिंह ने परिणाम घोषित करते हुए वाद-विवाद में विषय के सही प्रस्तुतिकरण पर जोर देते हुए अपने विचार प्रकट किए।

इस पखवाड़े के दौरान उल्लेखित गतिविधियों के अलावा दिनांक 25 सितम्बर 2007 को एक और गतिविधि अर्थात् वाद-विवाद प्रतियोगिता (अधिकारी वर्ग) का आयोजन अपरान्ह् 3 बजे किया गया, जिसमें संस्थान के प्रशासनिक एवं विज्ञानीय अधिकारियों ने भाग लिया।

वैज्ञानिक संगोष्ठी

संबंधित प्रतियोगिता का सफलतापूर्वक संचालन डॉ. भूपेन्द्र नाथ नागपाल, उपनिदेशक ने किया। संबंधित प्रतियोगिता में निर्णायक एवं मुख्य अतिथि के रूप में जाने माने लेखक श्री हिमांशु जोशी एवं डॉ. कुसुमवीर सिंह, निदेशक, केन्द्रीय हिन्दी प्रशिक्षण संस्थान को आमंत्रित किया गया था। सर्वप्रथम कार्यक्रम का शुभारंभ करते हुए प्रतियोगिता के संचालक डॉ. नागपाल द्वारा प्रतियोगिता के नियमों पर प्रकाश डाला गया। प्रतियोगिता का विषय था— "वर्तमान पीढ़ी का भविष्य सरकारी या निजी"।

वाद-विवाद प्रतियोगिता की समाप्ति के पश्चात् अपरान्ह् 4 बजे से पुरस्कार वितरण समारोह का आयोजन किया गया था। इस समारोह का आरंभ करते हुए सर्वप्रथम संस्थान के निदेशक प्रो. ए.पी. दाश द्वारा मुख्य अतिथि श्री हिमांशु जोशी एवं निर्णायक महोदया डॉ. कुसुमवीर सिंह का पुष्पों से विधिवत् स्वागत किया गया। स्वागत समारोह के पश्चात् माननीय श्री जोशी को निदेशक महोदय द्वारा एवं डॉ. सिंह को डॉ. अरूणा श्रीवास्तव द्वारा शॉल भेंट कर सम्मानित किया गया। तत्पश्चात् श्री जोशी ने देश की एकता एवं अखण्डता को कायम रखने में राजभाषा हिन्दी के योगदान का गुणगान करते हुए सभी उपस्थित अधिकारियों एवं कर्मचारियों को इसका प्रयोग करने हेतु प्रेरित किया। उन्होंने कर्मचारियों के भीतर अपना स्वाभिमान जगा कर एवं पूरे आत्मबल से हिन्दी भाषा का वर्चस्व स्थापित करने हेतु प्रयास करने के लिए प्रेरित किया। उनके ओजपूर्ण एवं बहुमुल्य विचारों ने सभी को मंत्रमुग्ध कर दिया। डॉ. कुसुमवीर ने भी इसी दिशा में आगे बढ़ते हुए कहा कि पूरे विश्व में बोली जाने वाली भाषाओं में दूसरा स्थान रखने वाली हिन्दी भाषा विचारों एवं भावों को प्रकट करने वाली सर्वाधिक धनाढ्य भाषा होने के साथ ही वैज्ञानिक दृष्टि से भी बहुत विकसित भाषा है। उन्होंने बताया कि अपने छोटे-छोटे विवादों से ऊपर उठकर देश की अस्मिता की रक्षा हेतु हिन्दी भाषा का अधिकतम प्रयोग करना अत्यन्त आवश्यक है। इसके साथ ही संस्थान के निदेशक महोदय ने भी सभी को संबोधित करते हुए कहा कि संस्थान में दिन-प्रतिदिन राजभाषा हिन्दी में होने वाले कार्य में वृद्धि संस्थान में कार्यरत अधिकारियों की इच्छा शक्ति एवं कर्मचारियों की लगन का परिचायक है। उन्होंने मुख्य अतिथि महोदय को संस्थान से प्रकाशित

होने वाले प्रकाशनों की जानकारी प्रदान की और अंत में कहा कि जहाँ इच्छा प्रबल होती है वहाँ कठिनाइयां प्रबल नहीं हो सकतीं। अर्थात् गृह मंत्रालय द्वारा प्रेरणा एवं प्रोत्साहन की नीति द्वारा भी सफलता तभी मिलेगी जब आपके भीतर प्रबल इच्छा शक्ति हो। उन्होंने सभी अधिकारियों एवं कर्मचारियों की पूरे उत्साह के साथ राजभाषा हिन्दी संबंधी विभिन्न गतिविधियों में भाग लेने की भूरि-भूरि प्रशंसा की। मुख्य अतिथि महोदय एवं निदेशक महोदय के संबोधन के पश्चात् पूरे सप्ताह के दौरान आयोजित विभिन्न प्रतियोगिताओं के पुरस्कारों की घोषणा की गई।

इसमें सर्वप्रथम निबन्ध प्रतियोगिता के पुरस्कारों की घोषणा डॉ. सी.पी. बत्रा, सहायक निदेशक द्वारा की गई एवं संबंधित पुरस्कार मुख्य अतिथि श्री हिमांशु जोशी के कर-कमलों द्वारा प्रदान किए गए, जिसमें प्रथम पुरस्कार श्रीमती रेखा सक्सेना, सहायक निदेशक, द्वितीय पुरस्कार श्री जी.एल. पुरी, प्रवर श्रेणी लिपिक, तृतीय पुरस्कार श्री यू. श्रीहरि, सहायक संपादक और सांत्वना पुरस्कार श्रीमती कमला नेगी, तकनीशियन एवं श्री हरिओम त्यागी, फील्ड वर्कर को प्रदान किए गए। इसके पश्चात् टिप्पण-प्रारूपण प्रतियोगिता की घोषणा श्री आर.एन. यादव, सहायक अनुसंधान अधिकारी द्वारा की गई, जिसमें प्रथम पुरस्कार श्री जी.एल. पुरी, प्रवर श्रेणी लिपिक, द्वितीय पुरस्कार श्रीमती वीना, सहायक, तृतीय पुरस्कार श्री सुनील कुमार गुप्ता, सहायक एवं सांत्वना पुरस्कार श्री ए.के. द्विवेदी, सांख्यिकी सहायक व श्री रमेश कुमार जंडवानी, अवर श्रेणी लिपिक को मुख्य अतिथि डॉ. कुसुमवीर के कर-कमलों द्वारा प्रदान किए गए। इसके साथ ही श्रुतलेख प्रतियोगिता के पुरस्कारों की घोषणा डॉ. अरूणा श्रीवास्तव, उपनिदेशक (प्रवरण कोटि) द्वारा की गई, जिसमें प्रथम पुरस्कार श्री ए.के. द्विवेदी, सांख्यिकी सहायक, द्वितीय पुरस्कार श्री विजय पाण्डेय, तकनीशियन, तृतीय पुरस्कार श्री एस.पी. पाण्डेय, तकनीकी सहायक एवं सांत्वना पुरस्कार श्री जितेन्द्र कुमार, एवं श्री आर.एस. भारद्वाज, सहायक को प्रदान किए गए। इसी क्रम में आगे बढ़ते हुए वाद-विवाद प्रतियोगिता (कर्मचारी वर्ग) के पुरस्कारों की घोषणा डॉ. नूतन नन्दा, सहायक निदेशक द्वारा की गई, जिसमें प्रथम पुरस्कार श्री के.सी. सेहरा, सहायक, द्वितीय पुरस्कार श्री हरिओम त्यागी, फील्ड वर्कर, तृतीय पुरस्कार श्री शैलेन्द्र पाण्डेय, एवं सांत्वना पुरस्कार डॉ. पदमावती त्यागी, अनुसंधान सहायक एवं श्री दिनेश चन्द्र लोहनी, अनुसंधान सहायक को विशेष अतिथि डॉ. विजय लक्ष्मी दाश के कर-कमलों द्वारा वितरित किए गए।

तत्पश्चात् संस्थान में हिन्दी में अधिकाधिक कार्य करने हेतु लागू वर्ष 2006-07 की प्रोत्साहन योजना के पुरस्कारों की घोषणा निदेशक महोदय द्वारा की गई। संबंधित पुरस्कार मुख्य अतिथि श्री हिमांशु जोशी के कर-कमलों द्वारा प्रदान किए गए, जिसमें प्रथम पुरस्कार श्री के.सी. सेहरा, श्री मोहन लाल, द्वितीय पुरस्कार श्री रामदेव, श्री एच.सी. पाण्डेय, श्रीमती सुदर्शना छावड़ा, तृतीय पुरस्कार श्रीमती आशा सहगल, श्री रमेश कुमार जंडवानी, श्री जितेन्द्र कुमार, श्री दिलवर सिंह नेगी, श्री रामफूल मीणा को प्रदान किए गए। इसके अलावा हिन्दी में अधिकाधिक डिक्टेशन देने वाले अधिकारी का पुरस्कार श्री जय प्रकाश वर्मा, वरिष्ठ प्रशासन अधिकारी को प्रदान किया गया। इसके साथ ही पखवाड़े के दौरान आयोजित अंतिम प्रतियोगिता वाद-विवाद प्रतियोगिता (अधिकारी वर्ग) के पुरस्कारों की घोषणा डॉ. भूपेन्द्र नाथ नागपाल, उपनिदेशक द्वारा की गई, जिसमें प्रथम पुरस्कार डॉ. नीना वलेचा, द्वितीय पुरस्कार डॉ. आलोक सुमन, तृतीय पुरस्कार श्रीमती रेखा सक्सेना, तथा सांत्वना पुरस्कार डॉ. के. राघवेन्द्रा व श्री जी.पी. माथुर को निदेशक महोदय के कर-कमलों द्वारा वितरित किए गए।

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

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11

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Names are listed in alphabetical order by surname; Staff position as on 31 March 2008.