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MALARIA RESEARCH CENTRE
(Indian Council of Medical Research)
22 Sham Nath Marg
Delhi–110 054

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Contents

Anoverview of the Research Activities

It gives me an immense pleasure to present the activity report for the year 2004–05. The Malaria Research Centre has put intensive efforts to basic, applied and operational field research in different fields of malaria such as vector biology and control, vector parasite interactions, parasite biology, epidemiology, etc. Research activities carried out during the year provided useful inputs into the malaria control, understanding of biology of vectors and parasites and national programme.

Entomological activities included the study of bionomics and distribution, breeding habitat preference of *Anopheles culicifacies,* a principal rural malaria vector, development of PCR technique for the differentiation of all four members of the *An. fluviatilis* complex, evaluation of new formualtions for mosquito control, screening of plant products for their adulticidal, larvicidal and repellent properties, role of serine protease and prophenol oxidase in *Plasmodium vivax* refractory strain of *An. culicifacies*, etc.

In order to understand the biology of human malaria parasites, genetic diversity studies, assessment of molecular markers, monitoring of resistance in *P. vivax* to sulphadoxine-pyrimethamine combination, purification and characterisation of *P. vivax* monoclonal antibodies, characterisation of GPL antigen from *P. falciparum,* comparison of immuno cytochemical peroxidase test (ICPT) and dot immunobinding assay (DIBA) for the detection of antimalarial antibody, purification and charcterisation of haemoglobin degrading aspartic protease from *P. vivax*, etc. were undertaken during this year. Several plant extracts were screened for their antimalarial activity.

In order to ascertain the development of resistance in parasites to first and second line drugs, therapeutic efficacy studies on chloroquine, sulphadoxine-pyrimethamine were undertaken in Goa and Rajasthan. Project malaria has gained an importance now a days and in order to understand the malaria situation in project areas a helath impact assessment study was undertaken in SSP reservoir impundment areas in Narmada valley in Madhya Pradesh. Longitudinal entomological and epidemiological studies were undertaken in hyper- and meso-endemic areas of Orissa for the development of a suitable site for malaria vaccine trial. Malaria clinics located at 22 Sham Nath Marg and 2 Nanak Enclave premises provided diagnostic and treatment services to more than 2400 patients during the year.

Centre celebrated Science Day, Environment Day, Antimalaria month and Hindi Week by conducting exhibitions, workshops, meetings and competitions. During this year the *Journal of Vector Borne Diseases* and *Malaria Patrika* in Hindi were brought out timely. A monograph on Malaria Parasite Bank describing the objectives, activities, achievements and isolates characterised and stored in the bank was published. A pictoral key for the identification of 58 Indian anophelines has also been published. Strenous efforts were taken to to publish the document "Protocols for uniform evaluation of insecticides for use in vector control". Four training courses and one workshop were organised for malaria community during the reporting period. Apart from these several students, technicians and other personnel were imparted training on different aspects of malaria and its control by scientists of the Centre. Nearly 42 papers were published in peer reviewed journals during the year 2004 by scientists and MRC scientists participated in several national, international conferences and workshops to present their research findings.

(PROF. A.P. DASH)
Director

Entomology

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A. Taxonomy

Pictorial Identification Key for Indian Anophelines

The pictorial identification key for 58 species of Indian anophelines has been published. The key was prepared on the request of Defence Research Laboratory, Tejpur (Assam) and is meant for researchers, field workers and technicians. The pictorial key comprises an introduction, checklist of Indian anophelines, morphological characters (pictures only) used for identification and guidelines for using the key and pictorial identification. The breeding ecology of each species in brief is also given in the key.

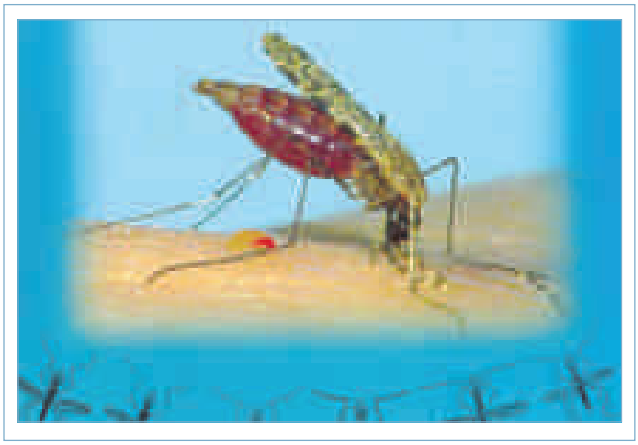


Fig. 1. A mosquito identification key for 58 Indian anophelines

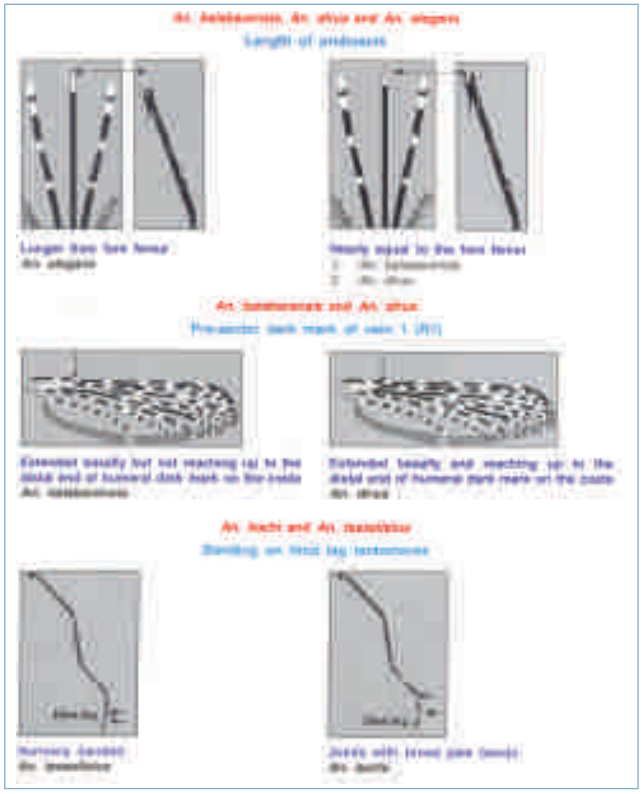


Fig. 2. Mosquito identification is done by nested sequencing through pictorial representation

B. VECTOR BIOLOGY

Anopheles culicifacies Complex

Bionomics and Distribution Pattern

An. culicifacies samples collected from Sangrur and Patiala districts (Punjab) were cytologically examined for sibling species composition. There was predominance of species A in Sangrur district and few specimens screened from District Paitala were species B. Examination of *An. culicifacies* population from Betul district (Madhya Pradesh), that witnessed malaria outbreak in 2000, revealed prevalence of species B and C in the district with predominance of latter. Blood meal source analysis of the cytologically identified specimens using counter current immunoelectrophoresis showed that the *An. culicifacies* sibling species were zoophagic in the above mentioned districts. A longitudinal study on the distribution and bionomics of *An. culicifacies* sibling species in malarious areas of Mandla and Dindori districts (Madhya Pradesh) is being carried

❖ The Pictorial Identification Key for 58 species of Indian anophelines has been published for use by researchers, field workers and technicians

out in collaboration with the Regional Medical Research Centre for Tribals, Jabalpur. Results obtained so far revealed predominance of species C (>80%) in both the districts whereas the relative proportion of species A, B and D was low. These sibling species were found to be primarily zoophagic. The study is in progress.

Studies on Breeding Sites Association of *An. culicifacies* Species A, B and E

A study was carried out in District Mandya of Karnataka state for possible association of *An. culicifacies* species A, B and E larvae to different breeding habitats. Major breeding habitats in the area include tanks, irrigation wells, draw wells, seepage water, riverbed pools and irrigation channels. Anopheline larvae were collected from breeding habitats and *An. culicifacies* adults emerged were identified to sibling species using AS-PCR assays.

The proportion of breeding of the three sibling species A, B and E varied in the breeding sites examined. The maximum prevalence of species A was in tanks (66%), while species B was found maximum in draw wells and streams (24%), irrigation wells (20%) and riverbed pools (27%), whereas species E was found in riverbed pools (28%), irrigation wells (19%) and tanks (14%). Species A was found in maximum numbers in tanks whereas species B and E are found in different breeding habitats. The data was subjected to statistical analysis to assess sibling species and breeding habitat association. The association of different breeding habitats and sibling species presence was found highly significant (χ^2 - 69.04, df=16, $p<0.005$) indicating each sibling species to have specific preference for breeding habitats. The individual sibling species associations with breeding habitats

❖ **Association of *An. culicifacies* sibling species A, B, and E to different breeding habitats showed that species B and E have common association and species A showed variation in preference**

suggests that among the breeding sites where the sibling species was found there was no specific preference and was found non-significant. Also it was significant for species A and E (χ^2 - 60.4, df=8, p<0.005), A versus B (χ^2-62.8, df=8, p<0.005) and A versus B and E (χ^2-60.4, df=8, p<0.005). Thus, this preliminary study indicated that B and E have common association for breeding habitats while other species have shown variations in breeding.

Anopheles fluviatilis Complex

Distribution, Bionomics and Biology of Sibling Species

Mapping the geographical distribution of *An. fluviatilis* sibling species continued. Samples examined from Dindori and Mandla districts (Madhya Pradesh) revealed prevalence of species S and T in study villages. There was predominance of species T which was found to be totally zoophagic. The proportion of species S was very low thereby indicating that *An. fluviatilis* has limited role in malaria transmission in these districts.

Consequent to the discovery of new species in *An. fluviatilis* complex, a longitudinal study has been initiated on the bionomics of species V and its role in malaria transmission in District Hardwar (Uttaranchal). Three villages namely Dargahpur, Purwala and Auspur have been selected in malarious Laksar PHC and monthly entomological and parasitological surveys are being carried out in these villages to study various parameters like seasonal prevalence, resting and feeding behaviour, vector potential, etc. with respect to new species. Observations made so far revealed that more than 70% of species V population was found resting in human and mixed dwellings thereby having greater chance to come in contact with humans. Blood meal source analysis using counter current immunoelectrophoresis showed species V with anthropophilic index (AI) around 4% whereas the other two sympatric species (T and U) were found to be totally zoophagic. It is noteworthy to mention that so far only species B, a non vector of *An. culicifacies* complex has been found in study villages along with *An. fluviatilis* sibling species. Therefore, ascertaining the role of species V in malaria transmission assumes greater importance in order to resolve the epidemiological paradox in this area. Efforts are also being made for laboratory colonisation of species V to study its phylogenetic relationship with other sibling species by cross-mating experiments and its susceptibility to plasmodial infection under laboratory conditions.

❖ **Predominance of species T in Dindori district (M.P.) suggests limited role of *An. fluviatilis* complex in malaria transmission in this area**

Molecular Assay for the Differentiation of Members of *the An. fluviatilis* Complex

Identification of sibling species is important in any vector control programme as they differ in biological characteristics such as vectorial competence, host preference or response to insecticides. *Anopheles fluviatilis*, which is second most important malaria vector in India, has now been recognised as a complex of four sibling species— species S, T, U and recently discovered species V. Earlier MRC has reported a molecular technique (PCR) for the differentiation of three known members of the complex, species S, T and U. Due to recognition of new species V, the existing species-diagnostic PCR

Fig. 3. PCR-RFLP assay for the identification of members of *An. fluviatilis* complex. (L= 100bp ladder; S= species S; T= species T; U= species U; V= species V; –ve= negative control)

❖ ***An. fluviatilis* species V which was found resting in human and mixed dwellings showed an Anthropophilic index of 4% indicating its importance in malaria transmission**

was modified into PCR-RFLP which can differentiate all four members of the complex. The assay was validated by comparing the results of with that of cytological method of species identification based on species-specific diagnostic inversions found in polytene chromosome. Over 500 specimens of *An. fluviatilis* were assayed with PCR-RFLP, out of which 98 samples were validated by comparing results with that of cytological technique. The PCR-RFLP unambiguously differentiates all the members of the complex and is ready for use in research laboratories/vector control programme.

C. Vector Control

Bio-efficacy of New Vector Control Agents

Efficacy of Vectron® 20 WP against Mosquitoes

Vectron® 20 W.P (etofenprox) was evaluated for its indoor residual efficacy in Shapur and Khandera villages of District Ghaziabad (Uttar Pradesh); Basantpur and Agwanpur villages in Districtt Faridabad (Haryana) and Rajeev Nagar and Deep Vihar in NCT area of Delhi. The experimental and control villages were selected on the basis of similar malaria incidence and vector productivity. Different wall surfaces such as brick, mud, cement and thatch walls were sprayed with Vectron® @ 0.05, 0.1 and 0.2 g/m^2. Results of bioassays tests revealed 100% corrected mortality against *An. culicifacies*, *An. stephensi* and *Cx. quinquefasciatus* @ 0.2 g/m^2 on different wall surfaces. Persistence of the insecticide was directly proportional to the dosages used. Field evaluation revealed drastic reduction in mosquito densities (MHD) after the spray and the impact was noticed up to a period of six months in the sprayed villages (Figs. 4–6). The results of the trial indicated that IRS of Vectron® 20 WP @ 0.1 g/m^2 can be used for malaria control and @ 0.2 g/m^2 can be used for comprehensive vector control.

❖ **A PCR-based assay was developed for the differentiation of all 4 members of the *An. fluviatilis* complex. The assay was field validated using classical cytotaxonomy and is ready for application in the field**

Bio-efficacy of Olyset® Nets Against Mosquitoes in Beel Akbar Village, District Ghaziabad (U.P.), India

Cone bioassays were performed by exposing 3-

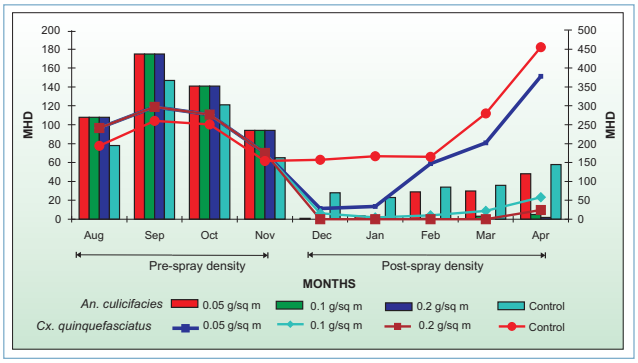


Fig. 4. Man hour densities of *An. culicifacies* and *Cx. quinquefasciatus* in experimental and control areas in District Ghaziabad

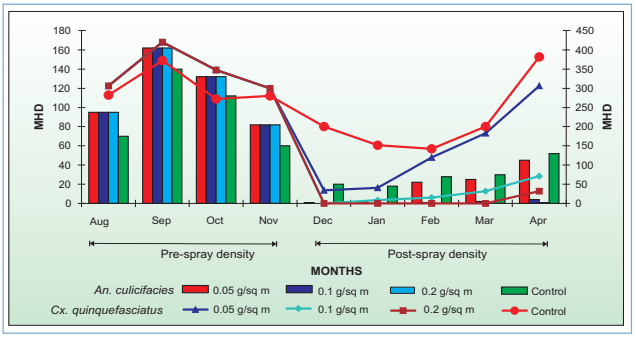


Fig. 5. Man hour densities of *An. culicifacies* and Cx. quinquefasciatus in experimental and central areas in District Faridabad

day old fulfed mosquito species such as *An. culicifacies, An. stephensi, An. subpictus, Ae. aegypti* and *Cx. quinquefasciatus* at fortnightly intervals to test the efficacy of Olyset® nets. Results revealed variable degree of susceptibility of different species of mosquitoes. Cent percent mortality was observed in field collected *An. culicifacies*, *An. stephensi* and *An. subpictus* in three minutes exposure time, while only 36% mortality was observed in case of *Cx. quinquefasciatus* in three minute exposure, however, 100% mortality was observed against this species when exposure period was extended up to 30

❖ **Vectron® 20 WP spray @ 0.2 g/m^2 resulted in drastic reduction in mosquito densities and the impact was observed up to six months**

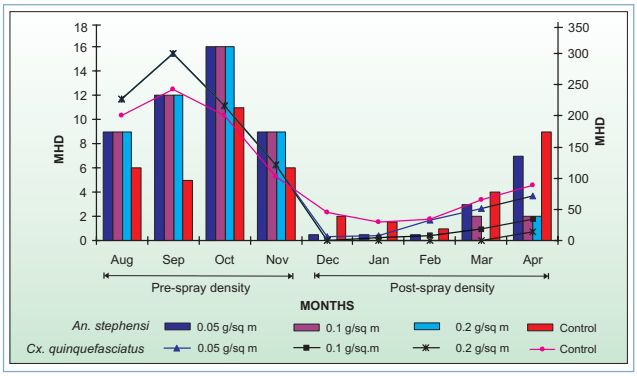


Fig. 6. Man hour densities of *An. stephensi* and *Cx. quinquefasciatus* in experimental and control areas in MCD area of Delhi

minutes, while 100% mortality in *Ae. aegypti* was obtained at five minutes exposure.

Field Evaluation

Results of field evaluation revealed that the average MHD of *An. culicifacies* in structures having Olyset® nets drastically reduced in comparison to that in structures where plain nets were used. The percent reduction in *An. culicifacies* density was 94% based on density in control and 47.2% in case of *Cx. quinquefasciatus*. It was further revealed that landing rate of female mosquitoes on Olyset® nets was drastically reduced and 100% corrected mortality was observed in those mosquitoes which landed on the Olyset® nets (Fig. 7).

Repellent action and excito repellent action of Olyset® nets are presented in Fig. 8. Results revealed

that Olyset® nets produced strong repellent action and it was more pronounced in *An. culicifacies* as compared to total anophelines and *Cx. quinquefasciatus*. The repellent action of the Olyset® nets was 55.2% in *An. culicifacies* as against 38.6%

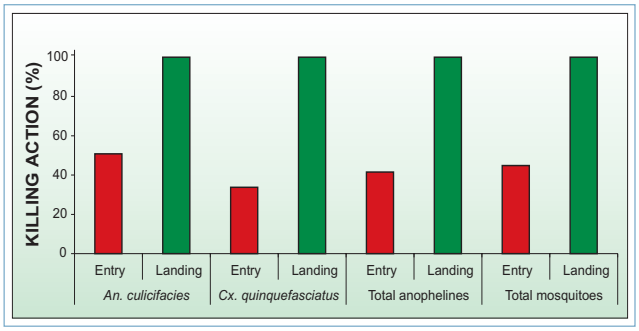


Fig. 7. Average killing action of Olyset® nets against different mosquitoes in field conditions

in *Cx. quinquefasciatus*. Results also revealed that excito repellency action (ERA) was almost 100% against all the mosquito species over a period of six months. Pilot studies are indicated to evaluate its impact on vector borne diseases particularly malaria and its cost-effectiveness in comparison to conventional indoor residual spraying.

Phase II Evaluation of Gokilaht®-S 5EC (Cyphenothrin) Space Spraying against Mosquitoes

Laboratory bioefficacy studies were carried out in a mosquito-free room against *An. culicifacies, An.*

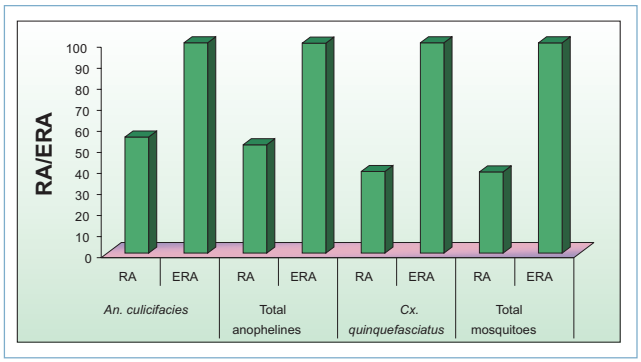


Fig. 8. Average repellent (RA) and excito repellent action (ERA) of Olyset® nets on different mosquito species in field conditions

stephensi, *Cx. quinquefasciatus* and *Ae. aegypti*. About 25 mosquitoes were released into cages of 12 cm^3. Four cages were kept in different corners of the room prior to fogging. Fogging was done with the help of Vanfog machine for one minute with the selected dose dissolved in kerosene. For control only kerosene was used. After 30 minutes, mosquitoes

❖ Olyset® nets showed high repellency and excito-repellency action against different mosquito species

knocked-down in the cages were scored and immediate mortality, if any, was recorded. Collected mosquitoes were kept for 24 h observation to record delayed mortality. Results showed that 100% mortality was observed @ 0.5 mg/m^3 in *An. stephensi, An. culicifacies* and *Cx. quinquefasciatus* and only 86.5% in *Aedes aegypti,* whereas 100% mortality was observed in all the four species @ 1.0 mg/m^3.

Field evaluation was carried out in selected urban localities of Ghaziabad, Faridabad and Delhi. Two doses of Gokilaht®-S 5EC—1.0 and 0.5 mg/m^3 were used for indoor evaluation and 1 and 3.5 g/ha were used for outdoor evaluation. Twenty-five female mosquitoes were exposed in separate cylindrical cages (12 X 18 cm) made of galvanised wire mesh of 20-mesh size. These were kept in different structures both in control and experimental areas prior to fogging. Cages were placed at different heights. All cages were collected after 30 minutes of fogging and brought to the laboratory. Total number of target and non-target species knocked-down during the indoor fogging was also collected from each structure. Mortality of the mosquitoes and non-target species were recorded after 24 h.

Results of indoor evaluation revealed that 100% mortality was recorded in *An. culicifacies* in Ghaziabad and Faridabad, and *An. stephensi* in Delhi @ 1.0 mg/m^3 and *Cx. quinquefasciatus* in all the three localities at this dose. However, in case of *Ae. aegypti* the average mortality was 99.5%. Almost similar results were obtained in outdoor evaluation against the test species in all the three study areas and the results demonstrated that 3.5 mg/m^2 is highly effective than 1.0 mg/m^2 in outdoor conditions. The results clearly indicate that Gokilaht-S 5EC is effective against *An. culicifacies* and *An. stephensi* @ 0.1 g/m^3 in indoors and 3.5 g/ha outdoors.

Efficacy and Persistence of Dimilin GR-2 (2% Granule formulation), 25% WP and Dimilin TB-2 (2% Tablet formulation) for the Control of Mosquito Larvae under Clear and Polluted Water Conditions

Dimilin GR-2, 25%WP and TB-2 formulations were evaluated in laboratory conditions against III instar larvae of *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus*. Results revealed high degree of larvicidal activity of three formulations of Dimilin and inhibition of the development of pupae and adult mosquitoes at lower dosages. Cumulative mean percent mortality of larvae at different intervals was by and large directly proportional to the dosage. Complete inhibition of emergence of *An. stephensi* and *Ae. aegypti* was observed at 0.008 ppm up to five and six weeks respectively. EC_{50} and EC_{90} values for Dimilin 25 WP, GR-2 and TB-2 were 0.0012 & 0.0026; 0.0014 & 0.0095 and 0.0014 & 0.0052 ppm against *Cx. quinquefasciatus*; 0.0015 & 0.0052, 0.0018 & 0.0072 and 0.0013 & 0.0045 ppm against *Ae. aegypti*; and 0.001 & 0.0034, 0.0012 & 0.0061 and 0.0012 & 0.0035 ppm against *An. stephensi* respectively. Field trials are still in progress.

Evaluation of VectoBac® WT, a Tablet formulation of *Bacillus thuringiensis* var. *israelensis* H-14 against Larvae of Mosquito Vectors at three different sites in Delhi, Chennai (Tamil Nadu) and Nadiad (Gujarat)

A multicentric trial of VectoBac, a tablet formulation of *Bacillus thuringiensis* var. *israelensis*, strain AM 65-52 having ITU 3000/mg was completed this year at three sites— Delhi, Nadiad and Chennai. The efficacy of VectoBac tablets used at different doses against two mosquito species, which were found to be breeding in a variety of habitats, showed that application of 2 tablets (0.76 g)/m^2 of water surface area resulted in very high to

❖ **Gokilaht® space spraying was found highly effective @1mg/m^3 in indoors and 3.5 g/ha in outdoors**

complete control of late instars and pupae of *An. stephensi* and *An. subpictus* up to two weeks period. Similarly, with a dose of 2 tablets/habitat caused cent percent reduction of late instars and pupae up to two weeks period against *Ae. aegypti* and *Cx. quinquefasciatus* in desert coolers, iron drums and mud pots. Application of 1–2 VectoBac tablets/50L water in domestic containers with mainly *Ae. aegypti* breeding caused high control of larvae up to nine days.

An important factor in using VectoBac tablet was the ease of its application in the containers, particularly those which generally remain inaccessible. The tablet formulation was found to be very useful particularly in desert coolers which are the main breeding sites for *Ae. aegypti* in urban areas. High rise buildings in urban areas, where a large number of desert room coolers are found fixed on the windows, VectoBac tablets will be very suitable for mosquito control during summer and monsoon. Moderate alkalinity of water in certain tanks did not have any significant adverse effect on the bio-efficacy of the tablet formulation. VectoBac tablets were found to be safe against non-target species, such as larvivorous fishes (*Gambusia affinis)* and notonectid bugs, *Anisops* spp. VectoBac tablet formulation would be very useful specially in water storage tanks and desert coolers for the control of *An. stephensi* in tanks and *Ae. aegypti* in coolers.

Evaluation of VectoBac® WDG, a Granular formulation of *B. thuringiensis* var. *israelensis* H-14 against Larvae of Mosquito Vectors

Trials in Delhi

Application of VectoBac WDG in paddy fields against breeding of *Anopheles* spp. mainly *An. culicifacies* gave high reduction in larval densities for one week at the doses of 0.5 and 1.0 g/m^2. Against *An. stephensi* breeding in cement tanks, there was 100% control up to one week with all doses applied. Efficacy was >77% at the doses of 0.2, 0.5 and 1.0 g/m^2 up to three week period. Against *Cx. quinquefasciatus* breeding in pools in vacant residential plots, the reduction of late instar larvae was 100% at the dose of 0.5 and 1.0 g/m^2 up to one week period.

Trials in Nadiad

VectoBac WDG was highly effective (80.2–100%) up to three weeks when applied at 0.5 and 1.0 g/m^2 doses in industrial tanks against late instars of *An. stephensi* and *Culex* breeding in industrial cement tanks and fabrication units. Treatment of domestic tanks with VectoBac WDG formulation gave 100% control of *Ae. aegypti* larvae up to one week. Application in rice-fields and waste water pools gave >80% control of anophelines and *Cx. quinquefasciatus* breeding at all four dosages up to one week and >75% control up to three weeks.

Trials in Bangalore

In stone quarry pits the efficacy of VectoBac WDG applied at 0.2 g and 1.0 g against mixed breeding of anophelines (mainly *An. culicifacies*) and culicines lasted up to three weeks. There was 96.2–100% control of breeding of *Culex* and *Aedes* species up to one week when applied at the dose of 0.2 g/m^2. The effectiveness of VectoBac WDG in ring well against *An. stephensi* larvae was 95.6–100% for one week period. VectoBac WDG was found safe to non-target species, *G. affinis* and notonectid bugs, *Anisops*.

Thus it is concluded that VectoBac WDG showed moderate to high control of mosquito larvae for 1–3 weeks in different situations when applied at the rate of 0.5 – 1.0 g/m^2. However, at lower doses varying degree of control was observed in different habitats and against different species. As the formulation had no adverse impact on non-target organisms it can be used as an additional tool in an integrated control approach.

Evaluation of Pyriproxyfen (Sumilarv 0.5% Granule) on Larvae of Mosquito Vectors at Nadiad (Gujarat), Haldwani (Uttaranchal) and Shahjahanpur (Uttar Pradesh)

Pyriproxyfen (Sumilarv 0.5G) (S-71639), a juvenile hormone mimic (JHM), was field tested at three

❖ **Dimilin GR-2, 25% WP and TB-2 formulations showed high degree of larvicidal activity against mosquitoes at low dosages**

❖ **VectoBac tablets were found to be useful in controlling mosquito breeding in storage tanks and desert coolers**

different sites. Pyriproxyfen was evaluated in different breeding habitats against various mosquito species—*An. stephensi, Cx. quinquefasciatus* and *Ae. aegypti* at Nadiad (Gujarat), Haldwani (Uttaranchal) and Shahjahanpur (Uttar Pradesh).

Based on the evaluation, following conclusions were made:

- Pyriproxyfen applied at 0.01 to 0.05 ppm a.i. caused produced 100% inhibition of adult emergence of *An. stephensi* and *Cx. quinquefasciatus* IV instar larvae for up to four weeks under laboratory conditions.
- Pyriproxyfen was found to be quite effective against malaria vector *An. culicifacies* even at the lowest dose of 0.01 ppm a.i. and inhibited 100% adult emergence up to four weeks equally at 0.01 and 0.02 ppm a.i. doses.
- In heavily polluted habitats against *Cx. quinquefasciatus* the same formulation at 0.01 to 0.05 ppm a.i. dose produced 100% inhibition of adult emergence for 1–6 weeks in a variety of habitats in different field trial sites.
- Under field conditions, application of pyriproxyfen at different doses, reduced the larval and pupal abundance in various habitats for a significantly longer duration. Its application also controlled *Ae. aegypti* breeding in domestic water storage tanks for an appreciable time although efficacy varied according to the use of water from such tanks.
- No adverse effects against non-target organisms were observed during the study period.

Table 1. Habitat-wise effective doses of pyriproxyfen

Breeding habitats	Target species	Effective dose range
Waste water pools	*Cx. quinquefasciatus*	0.25 kg/ha
Sewage drains	*Cx. quinquefasciatus*	0.4-1 ppm a.i.
Domestic ditches, pits	*Cx. quinquefasciatus*	0.05-0.2 ppm a.i.
Disused wells	*Culex* spp	0.25-0.5 kg/ha
River-bed pools, clean	*An. culicifacies*	0.2 ppm a.i.
Tyres with water	*Aedes* spp	0.02 ppm a.i.
Domestic cemented tanks	*An. stephensi, Ae. aegypti*	0.1 ppm a.i.
Industrial curing tanks	*An. stephensi*	0.1 ppm a.i.

Prospecting for Botanical Pesticides: Screening of Plant Extracts for Insecticidal and Repellent Activity against *An. stephensi*

Screening of plant extracts for their bioactivity against mosquitoes particularly against malaria vectors was continued in collaboration with other institutes. The study is focused to test larvicidal, insecticidal and mosquito repellent properties of the plant extracts/fractions/formulations against *An. stephensi*. Bioactivity of various herbal extracts/fractions/formulations received from five extracting laboratories was determined against mosquitoes particularly the malaria vector *An. stephensi* using standard protocol which included larvicidal, adulticidal and mosquito repellent activities. Preliminary screening of plant extracts was done at 250 ppm. The sample was considered to have larvicidal activity if it caused > 70% larval mortality within 24 hours of exposure. The samples causing 70–100% larval mortality in preliminary bioassays at a concentration of 250 ppm were tested further for determining LC_{50} and LC_{90} values.

Adulticidal activity was determined by exposing adult mosquitoes on impregnated paper with 10% solution (2.5 ml/paper of 180 cm^2 or 0.25 g/paper of 180 cm^2), equivalent to dose of 1.38 mg/cm^2. Samples giving 70–100% mortality were considered as positive for adulticidal activity. Repellent acticity was determined by applying 0.25 g sample mixed with equal amount of coconut oil on hands. The sample was considered to have repellent activity if no confirmed bite was received within one hour of exposure. The positive control, DEET solution 0.25 ml when used gave complete repellent activity for more than three hours.

Since beginning of this project a total of 625 coded samples of different plant extracts/fractions/formulations have been received at MRC for bioassays against mosquito species *An. stephensi* (Table 2).

Of these 99 samples showed larvicidal activity, 29 showed adulticidal activity and five showed repellent activity. However, during last one year 294 samples were tested. Of these 51 samples showed larvicidal activity and 15 samples showed adulticidal activity.

❖ Pyriproxyfen (Sumilarv) was found very useful in the control of mosquito larvae in different breeding habitats

Larvicidal Activity of *Zanthoxylum alatum* against *An. stephensi*

This study was undertaken in collaboration with IIT, Delhi. Essential oil from *Zanthoxylum armatum*, DC syn. *Z. alatum* Roxb (timur) an evergreen tree in the subtropic Himalayas, was extracted from its seeds using hydrodistillation and liquid CO_2 technique. Hydrodistillation gave 0.8% of oil (v/w) gas chromatographic analysis of the hydrodistilled oil resulted in the identification of 20 constituents, as compared to the 24 constituents by liquid CO_2 extraction technique. Linalool, limonene, terpene-4-ol, phellandrene and (Z)-methylcinnamate were the major components obtained from hydrodistillation method, whereas liquid CO_2 showed Linalool, limonene, methylcinnamate, palmitic acid and oleic acid as the major components. The essential oils obtained from both the techniques were tested for their larvicidal activity against *An. stephensi*. The essential oil obtained by using hydrodistillation technique was found to show better larvicidal activity than that obtained from liquid CO_2 extraction technique. These oils were fractioned to obtain linalool which is the major component of the essential oils and tested for the larvicidal activity. The activity of linalool against the larvae of *An. stephensi* was negligible which indicates that the activity shown by the essential oils is due to the presence of some other active component.

Table 2. Status of screening of of plant extracts/fractions/formulations of samples received at MRC

Laboratory/ Institute	No. of samples received	No. of samples screened	Positive results		
			L	A	R
RRL, Trivandrum	105	105	8	3	1
FRI, Dehradun	103	103	26	16	4
IIT, Delhi	101	101	12	2	-
RRL, Jammu	170	170	28	6	-
EID Parry, Bangalore	146	146	25	2	-
Total	625	625	99	29	5

L– Larvicidal; A–Adulticidal; R–Repellent activity.

Studies on Larvicidal Properties of Aqueous Leaf Extract of *Trianthema portulacastrum* (Family : Aizoaceae)

Larvicidal effect of crude aqueous extract of the leaf of a medicinally important plant *Trianthema portulacastrum* was assessed against *An. culicifacies* species A and C, *An. stephensi*, *Cx. quinquefasciatus* III/IV instars larvae in bioassays following standard WHO method for a range of concentrations (0.0025 to 0.3% in water). The calculated LC_{50} values (lethal concentration for killing 50% of treated larvae) for different species were: *An. culicifacies* species A–0.022%, *An. culicifacies* species C–0.028%, *An. stephensi* –0.023% and *Cx. quinquefasciatus* –0.017%. Studies will be carried out with different solvent extracts of different plant parts.

Biocontrol Agents

Effect of Formulation and Encapsulation on the Efficacy of Microbes against Mosquito Larvae

The bio-efficacy of three *Metarhizium anisopliae* strains MRCD-1, MRCD-2 and MRCD-3 was tested against II instar larvae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* (Table 3). The spores of *M. anisopliae* after 21 days growth in Emerson's YpSs medium were tested against the larvae. MRCD-3 was found relatively more effective than the other two strains and will be formulated to assess the effectiveness against different mosquito species.

❖ **The essential oil obtained from *Z. alatum* using hydrodistillation showed better larvicidal activity than that obtained from liquid CO_2 extraction**

Bio-efficacy of Culture Filtrates of *M. anisopliae* against *An. stephensi* and *Cx. quinquefasciatus*

Toxicity of cell-free culture filtrates of *M. anisopliae* (MRCD-1) was tested against larvae of *An. stephensi* and *Cx. quinquefasciatus*. Metabolites derived from the culture broth of Emerson's YpSs and chitin broth after 21 days of growth were tested. The metabolites derived from the chitin broth were relatively more effective than those from the Emerson's YpSs broth. The respective calculated LC_{50} values for different species are given in Table 4. The specific activity of chitinase from the dialysed culture filtrate of chitin broth was 6-fold more than the chitinase activity observed in dialysed Emerson's YpSs broth (Fig. 9).

Table 3. LC_{50} of *M. anisopliae* strains in spore/ml against II instar larvae of three mosquito genera after 96 hours of inoculation

Name of strains	*An. stephensi*	*Cx. quinquefasciatus*	*Ae. aegypti*
M.anisopliae MRCD-1	6.41×10^7 ($5.10 \times 10^7 - 9.09 \times 10^7$)	6.91×10^3 ($5.16 \times 10^3 - 3.62 \times 10^4$)	4.12×10^4 ($2.54 \times 10^4 - 1.06 \times 10^5$)
M. anisopliae MRCD-2	1.05×10^{13} ($1.03 \times 10^{13} - 1.42 \times 10^{13}$)	2.03×10^9 ($1.87 \times 10^9 - 2.90 \times 10^9$)	6.91×10^{12} ($6.69 \times 10^{12} - 9.22 \times 10^{12}$)
M. anisopliae MRCD-3	2.69×10^3 ($2.58 \times 10^3 - 2.80 \times 10^3$)	1.34×10^3 ($1.25 \times 10^3 - 1.51 \times 10^3$)	4.66×10^5 ($3.45 \times 10^5 - 7.39 \times 10^5$)

Figures in parentheses are 95% fiducial limits.

Further study is in progress to know the biochemical role of chitinase and other proteases in causing mortality.

Vector-Parasite Interactions

Studies on *P. vivax*-refractory *An. culicifacies*

Expression Profiles of Serine Protease and Prophenol Oxidase following injury and bacterial infection

Earlier, we reported cloning and characterisation of serine protease (*AcSp30* accession No. AY995188) and prophenol oxidase (*AcPPO6A* accession No. AF466196) gene from refractory strains of malaria vector *An. culicifacies*. Both the semi-qualitative and quantitative RT-PCR studies showed inherently higher levels of both these genes in refractory strains as compared to susceptible strains. To characterise the role of serine protease and prophenoloxidase in mounting immune response, the expression pattern of both the genes in adult naïve female refractory mosquitoes was temporally studied following injury and bacterial infection (Fig. 10). The mosquitoes were injured using a capillary glass needle and infected with gram-positive bacteria, *Micrococcus luteus*. The mosquitoes were collected at different time intervals and the levels were analysed by realtime PCR. Upto 2.5-fold increase in *AcSp30* transcript was observed upon injury in contrast to a 4-fold increase in *AcPPO6A* transcript under identical conditions (Fig. 10 a & b respectively). The observed increase in the transcript levels of the *AcSp30* was maintained throughout the 24-hour duration. A marginal increase in *AcSp30* transcript was observed upon challenge with *Micrococcus luteus*. The transcript levels of *AcSp30* were much lower than those compared to the levels obtained after sterile injury

Table 4. LC_{50} of culture filtrates of *M. anisopliae* against I–II & III–IV instars of *An. stephensi* and *Cx. quinquefasciatus* after 72 hours of inoculation

Name of the species	Instar	YpSs µl/ml (95% FL)	Chitin µl/ml (95% FL)	Relative potency (folds)
An. stephensi	I & II	7.23 (6.26–8.15)	3.94 (2.69–5.10)	1.83
	III & IV	5.23 (4.46–6.07)	5.62 (4.31–6.89)	0.93
Cx. quinquefasciatus	I & II	8.15 (7.20–9.06)	2.92 (1.49–4.32)	2.79
	III & IV	41.83 (31.91–59.22)	7.15 (5.49– 8.76)	5.85

Figures in parentheses are 95% fiducial limits.

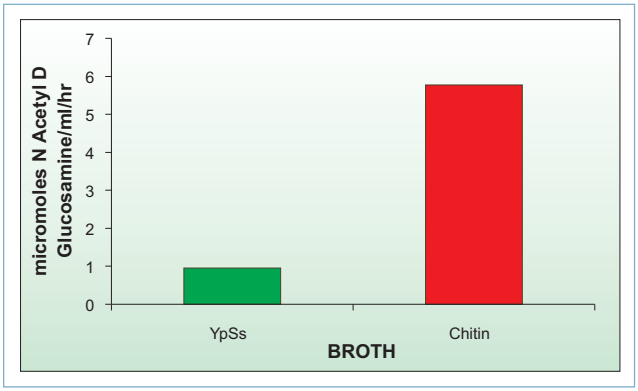


Fig. 9. Specific activity of chitinase present in culture filtrates of *M. anisopliae*

indicating that this serine protease is not involved in triggering the cascade for bacterial elimination. Upon injury, an increase in the *AcPPO6A* transcript levels was observed after two hours (2-fold) and continued to increase up to 12 hours (4-fold) and finally declined to the basal level in 24 hours. The modulation of PPO upon injury is suggestive of its role in wound healing. A temporally divergent regulation of PPO transcription was observed in mosquitoes challenged with gram-positive bacteria *M. luteus*. Interestingly, a 5-fold increase in *AcPPO6A* transcript was observed immediately after two hours of infection and maximum levels were attained six hours post-challenge (9-fold increase). Such a rapid induction of the *AcPPO6A* gene in response to bacterial infection is suggestive of some role in combating bacterial invasion.

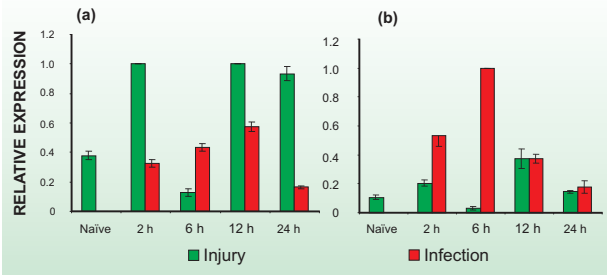


Fig. 10. *AcSp30* and *AcPPO6A* expression profiles following injury and bacterial infection

Refractory adult female mosquitoes were subjected to sterile injury using finely drawn glass capillary needle and to infection with gram-positive bacterium, *Micrococcus luteus* using a needle dipped in bacterial cell pellet. *AcSp30* (a) and *AcPPO6A* (b) transcript levels were measured at different time intervals (2, 6, 12 and 24 hours) post-injury (open bars) or post-infection (shaded bars) by real-time PCR using the Comparative CT Method and were normalised to the internal control transcript for β-actin gene. The data (mean ± SD) are from three independent experiments.

Immune Responses to *Plasmodium* Infection in Refractory and Susceptible Mosquito Strains

Further the role of the genes in encapsulation phenotype of the refractory strain was investigated by feeding model rodent malaria parasite *P. vinkei petteri,* using susceptible strain as control (Fig. 11). Both, refractory and susceptible 4–6 day old adult female mosquitoes were separately fed on blood of Balb/c mice, which had been infected with *P. vinckei petteri*. The refractory *An. culicifacies* mosquito strain is partially resistant to the rodent malarial parasite. Mosquitoes fed on un-infected mice served as blood-fed controls. Temporal expression of both the genes was monitored after blood meal, at regular intervals over a period of 24 hours by real-time PCR (Fig.11). In the refractory strain, although a 68-fold increase in *AcSp30* transcript was observed 24 hours post-blood meal, the invasion of *Plasmodium* resulted in a 300-fold increase in the transcript levels within the same time duration, when compared to transcript levels in naïve unfed female mosquitoes. Similarly, a significant up-regulation of *AcPPO6A* transcript levels was observed in response to parasite invasion. A 22-fold increase was observed in the expression levels of *AcPPO6A*, 6 hours after infective feeding as compared to blood feeding alone (16-fold). These levels almost doubled after 18 hours of infected blood meal thereby demonstrating the induction of *AcPPO6A* in response to parasite. This result is in conjunction with the microscopic observation where-in melanotic encapsulation of *Plasmodium* ookinetes was observed 16 to 24 hours post, infective blood feeding. On the other hand, in the susceptible strain, the expression levels of the *AcSp30* gene remained at the basal level upon blood feeding and parasite-

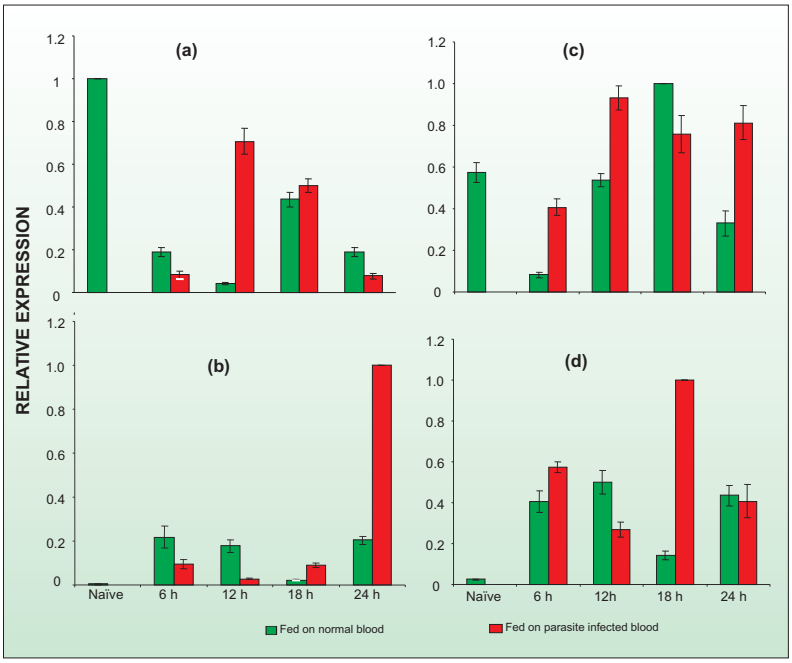


Fig. 11. Temporal induction of *AcSp30* and *AcPPO6A* upon *Plasmodium* infection
Susceptible (S) and refractory (R) mosquitoes were fed on uninfected blood (open bars) and on *Plasmodium vinckei* (shaded bars) infected blood and transcript levels of *AcSp30* in S strain (a) and R strain (b) were measured by real-time PCR at different time intervals post-blood meal (PBM). Transcript abundance of *AcPPO6A* was similarly determined in S strain (c) and R strain (d). Transcript levels were normalised to the internal control transcript for β-actin gene. The data (mean ± SD) are from three independent experiments

infected blood feeding. Unlike the *AcSp30* gene, the *AcPPO6A* transcript levels in the susceptible strain were insignificantly up regulated (1.4-fold increase), in response to blood and parasite. But these induction levels were substantially lower when compared to the refractory strain.

These results demonstrate the inherent high levels of these genes was not sufficient to combat the invading parasite and a tremendous induction was required to block the development of parasite. Noticeably, the up-regulation of *AcPPO6A* and *AcSp30* transcript levels at 18 and 24 hours post-parasite feeding respectively coincided with the appearance of melanotic capsules in the gut of *An. culicifacies* refractory strain. Such a coordinated response suggests that *AcSp30* and *AcPPO6A* enzymes are part of melanisation cascade that is triggered in response to *Plasmodium*-infected blood. A further validation of this fact is that the non-melanising susceptible strain showed no induction of transcript levels when fed on *Plasmodium*-infected blood.

Differential Expression of Serine Protease Gene: Role of Promotor

Earlier, we have reported differential expression of serine protease gene but no structural difference was observed in the gene. The cDNA corresponding to the *AcSp30* was cloned from R and S strains and sequenced. A comparison of the two sequences did not reveal any mutational differences in the two strains. Since the cDNA sequence of *AcSp30* was identical in both the strains, we isolated its genomic clone using PCR. An 887 bp amplicon was obtained in both the strains. The gDNA fragment was slightly bigger than cDNA indicating the presence of an intron. Sequence analysis revealed that the gene has a single intron of 71 bp at its 5′ end and is a phase 0 intron. Since the location and the sequence of the intron were identical in both the strains, the upstream sequences of *AcSp30* were isolated and explored for promoter activity.

The upstream region of serine protease gene is trapped using a set of nested gene specific reverse primers and a set of four forward universal walker primers WP1, WP2, WP3 and WP4, which differ from each other at their 3′ end. Their 5′ end is identical and comprises of the T7 primer sequence. In refractory strain, a 1.4 kb amplicon was obtained using WP4 and in susceptible strain, a 702 bp amplicon using WP2. A 702 bp fragment and a smaller 333 bp fragment of upstream sequences from both the strains were cloned into promoter-less vector pGL3-Basic (Promega) having a firefly luciferase reporter gene. The resulting reporter plasmids were named pGL3-Ref702 and pGL3-Ref333 for the refractory strain and pGL3-Sus702 and pGL3-Sus333 for the susceptible strain.

To understand the functional relevance of the upstream region a failed attempt was made to record the activity of region by cloning into eukaryotic promoter-less vector which was further sub-cloned into mammalian cell line. The inactivity of promoter region in the cell line was thought to be because of distantly related cell line. Relative strength of the

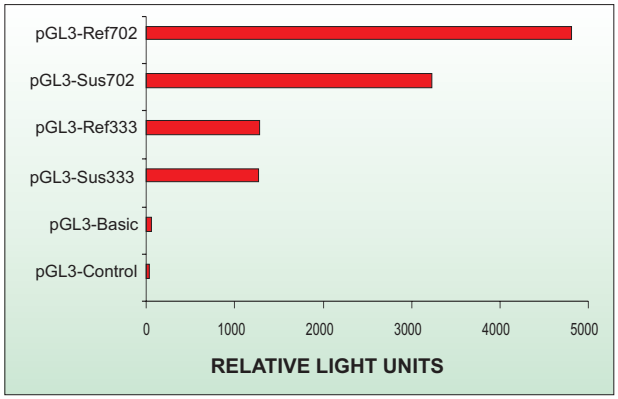


Fig. 12. Assessment of the activity of serine protease promoter from R and S strains of *An. culicifacies* using luciferase-based reporter assay in S2 *Drosophila* cell line. Mean luciferase (±S.D.) activities from pGL3-Ref (702 bp and 333 bp) and pGL3-Sus (702 bp and 333 bp) following transfection in the *Drosophila* Schneider (S2) cell line using Lipofectin. These activities were compared to those obtained using promoter-less vector pGL3-Basic and to the pGL3-Control plasmid having the SV40 promoter as controls. Reported activities were based on three independent transfections and the data were recorded in relative light units (RLU)

promoter was evaluated by transfecting these recombinant reporter constructs into *Drosophila* S2 cells. The luciferase activity from all the four constructs was higher than that of the vector (pGL3-Basic) without a promoter and pGL3-Control with an SV-40 based promoter, thereby confirming that these upstream gene sequences, of both the R and the S strains, contain the necessary regulatory elements for their respective promoters (Fig. 12). The two constructs pGL3-Ref333 and pGL3-Sus333 yielded similar levels of luciferase activity. However, pGL3-Ref702 and pGL3-Sus702 displayed difference in luciferase activity. The pGL3-Ref702 from the refractory strain showed a 1.5-fold increase in luciferase activity compared to the susceptible strain. We can attribute such a differential luciferase activity to differences in the upstream gene sequences between the 333 and the 702 bp regions in both these strains.

The 702 bp upstream sequences obtained from the refractory and susceptible strains were aligned (Fig. 13). A high degree of sequence similarity (94.2%) was observed in the refractory and susceptible sequences up to the 333 bp regions, upstream of the translational start site (ATG). Beyond this region there was a considerable divergence in the gene sequence. This difference could directly account for the observed difference in promoter activity in both these strains (Fig. 13). Analysis of the upstream sequences of serine protease gene revealed characteristics of RNA polymerase II-transcribed promoters.

Using the computer-based promoter prediction tool at http://www.fruitfly.org/, two putative transcription start sites were predicted to be located at –31 bp and –40 bp upstream of transcription start

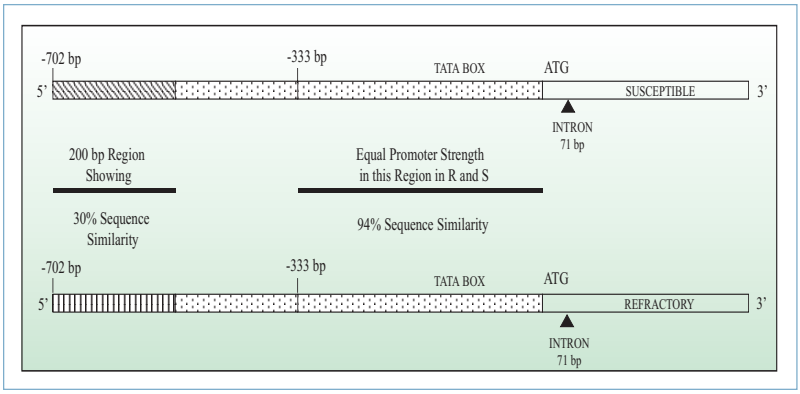


Fig. 13. Schematic diagram depicting the comparative analysis of the *AcSp30* coding and noncoding regions in the refractory and susceptible strain

site in refractory and susceptible strains. The region surrounding this transcription start point corresponded to the arthropod initiator sequence. This reinforced the prediction of this site being the true transcription start site. In both the strains, two

❖ **Serine protease and prophenol oxidase are involved in conferring refractory phenotype to *P. vivax* refractory strain of *An. culicifacies***

TATA motifs were found at position 53 and –189 from the ATG (translation start site), and two arthropod transcription initiator motifs TCAGT were present at position –12 and –152. Using the consensus DCAKTY, two putative capsites were found at position –31 and –152 respectively in both the strains. The sites constituted the putative core promoter elements.

Molecular Characterisation of Nitric Oxide Synthase (NOS) in *An. culicifacies*: Relevance for Refractory Mechanism

Global efforts to control malaria, caused by parasitic protozoa of the genus *Plasmodium*, have been hindered by insecticide resistant mosquitoes, drug-resistant parasites and socioeconomic obstacles. The drive to identify novel control strategies has, in part, focused on identifying mosquito gene products that impart refractory phenotypes. Our aim is to characterise the gene and gene elements that may be associated with NOS biology in *An. culicifacies* sibling species to understand the biochemistry of mosquito-parasite interaction and to evaluate the potential of manipulating AcNOS gene expression as a means of generating the refractory phenotypes. Our goal is to develop tools for altering the vector competence of *An. culicifaces* which requires understanding the mechanism of vector resistance to the malaria parasite including biochemical and molecular studies of vector parasite interactions. In a way that the vertebrate antiparasite immune responses have been used to identify vaccine and transmission blocking targets, we plan to use AcNOS response in mosquito vectors to *Plasmodium* as a tool to explore critical components of parasite development in mosquitoes and correlate it to mechanism of refractoriness.

An. culicifacies sibling species namely A, B were maintained at 27^{0}C and 75% humidity under a 12 h light/dark cycle. *An. stephensi* mosquitoes were also treated similarly and were used as controls. Samples (midguts and carcases without midguts) and haemolymph have been collected from *An. culicifacies* species A and B and *An. stephensi*.

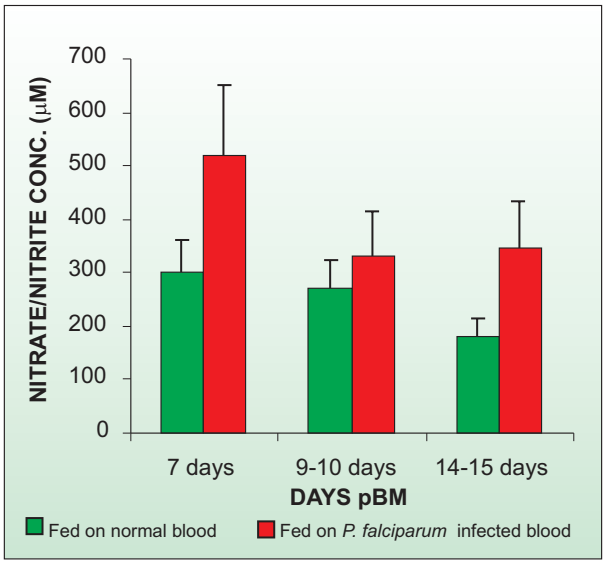


Fig. 14. Hemolymph nitrite/nitrate of blood-fed uninfected and *P. falciparum* infected *An. culicifacies* species B was determined at 7, 9-10 and 14-15 days pBM by using Cadmium reduction/ Griess reagent microassay. Means were analysed by using a paired *t*-test. p-values are depicted above the bars

Haemolymph nitrate/nitrite (NO_2^-/NO_3^-) concentrations were measured using a modified cadmium reduction/griess reagent microassay after collection from the group of 100–200 infected and uninfected mosquitoes at 7, 9–10 and 14–15 days pBM. At day 7 pBM NO levels were higher in *Plasmodium* infected mosquitoes than in uninfected mosquitoes at all time points (Fig. 14). Elevated levels of NO_2^-/NO_3^- at this time may be the result of sustained production of AsNOS induction at nine days.

❖ **Differences in the promoter sequences of serine protease are responsible for its differential expression in refractory (R) and susceptible (S) strains of *An. culicifacies***

Parasite Biology

2

Molecular Characterisation

Assessment of Molecular Markers for the Identification of Recrudescent Infection in *P. falciparum* from Fresh Infection

Blood spots collected from *P. falciparum* positive patients after microscopic examination during therapeutic efficacy studies of various antimalarials (chloroquine and sulphadoxine-pyrimethamine) were analysed for molecular markers namely MSP-1, MSP-2 and GLURP. During the study, paired samples of Day 0 and the day of recrudescence were analysed from Orissa, Goa, Tamil Nadu and West Bengal. Analysis revealed same genotype of all the three marker systems in about 56.4% of isolates in Orissa (Sundargarh district), 86.7% in Goa, 87.0% in Rameswaram, Tamil Nadu and 63.6% in Darjeeling district of West Bengal (Fig. 15). The study revealed highest efficiency of MSP-2 marker for identification of different genotypes in paired samples of Day 0 and the day of recrudescence (Fig. 16).

Genetic Diversity Studies of Human Malaria Parasites

P. falciparum isolates collected from Nadiad taluka (Kheda), Gujarat; Sundergarh district, Orissa; and Kamrup district (Sonapur), Assam were analysed for polymorphism in MSP-1 and MSP-2 to get information about extent of diversity existing among the field isolates.

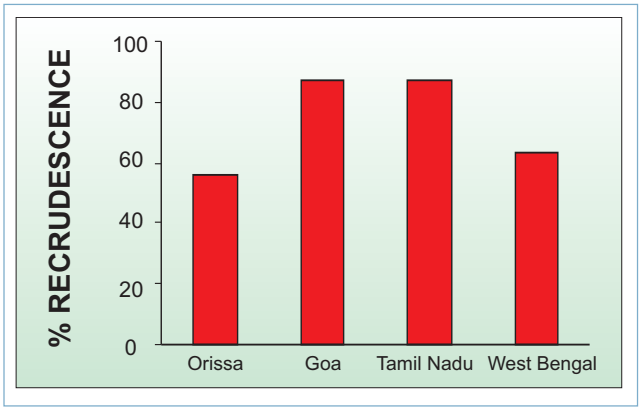


Fig. 15. Proportion of isolates with identical genotypes of MSP-1, MSP-2 and GLURP

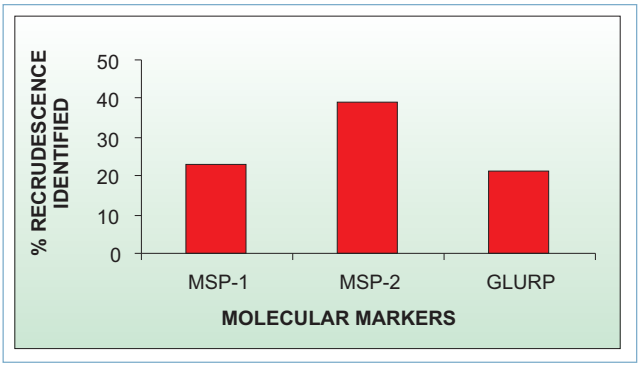


Fig. 16. Efficiency of markers for the differentiation of recrudescence

In all the three areas, both the markers MSP-1 and MSP-2 were polymorphic with three families of MSP-1 namely (K1, MAD20 and RO 33) and two of MSP-2 (FC27 and 3D7). Proportion of multiclonal isolates was 26.3% in Kheda, 35% in Sonapur and 52% in Orissa (Fig. 17).

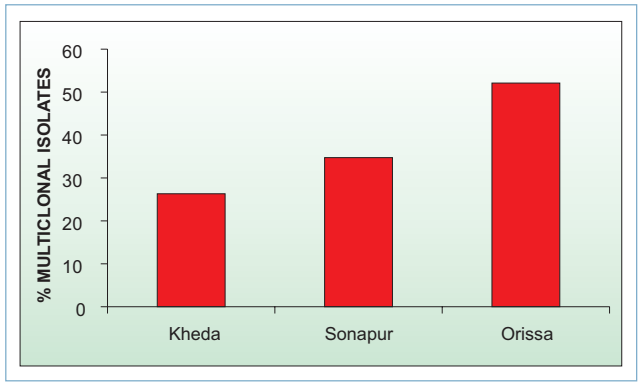


Fig. 17. Proportion of isolates with multiclones among the study samples

Molecular Monitoring of Sulphadoxine-Pyrimethamine Resistance among *P. vivax* Field Isolates

A total of 69 *P. vivax* infected blood spots were analysed for mutations of the *Pvdhfr* gene, associated with sulphadoxine/pyrimethamine resistance. Mutations are good markers for the molecular epidemiological studies of drug resistance. Maximum number of isolates (69.6%) showed mutation at 117

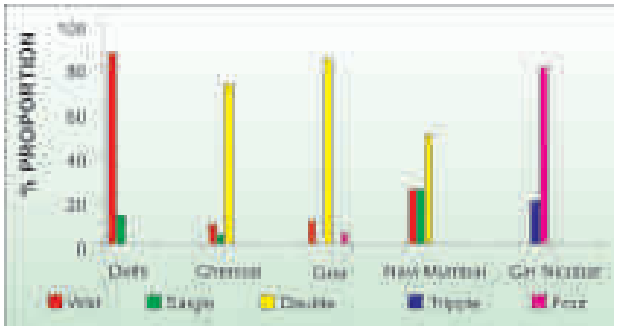


Fig. 18. Distribution of Pvdhfr genotypes among Indian field isolates

residue followed by at 58 residue (62.3%). Limited number of isolates showed mutations at residue 57 (10.1%) and 61 (7.2%). Among the study samples, proportion of double mutants was highest (56.5%) followed by wild type (27.5%). Wild type genotype was maximum in Delhi region (86.6%), while Chennai and Goa isolates had double mutants in 86.4 and 84.2% of isolates respectively. Navi-Mumbai isolates had shown in between picture with 50% isolates being double mutants.

Car Nicobar isolates had totally different picture with tripple and quadruple mutants. Fig. 18 shows the areawise distribution of *Pvdhfr* mutations in *P. vivax* field isolates.

❖ **Genotyping of treatment failure cases has revealed that MSP-1, MSP-2 and GLURP could be used to identify fresh infection from recrudescence infection in case of *P. falciparum***

Immunological Characterisation

P. vivax Monoclonal Antibodies: Purification and Characterisation

Ten hybridomas rose against *P. vivax* erythrocytic stages showed reactivities in immunofluorescence and enzyme immunoassays. These lines were expanded *in vitro* for producing large volume of culture. Culture supernatant from 10 hybridoma lines was tested for immunoglobulin isotyping. These 10 antibodies were found to be IgG_1 type. After testing by IFA, individual batch of supernatant was subjected to ammonium sulphate precipitation and affinity adsorption with Protein-A sepharose for isolation of IgG fraction. Purified IgG fractions were labelled with Fluorescine isothiocyanate and Rhodamine. Labelled conjugates were tested in *P. vivax* smears by IFA to check their differential reactivities.

One earlier batch of monoclonal antibodies reactive to *P. vivax* erythrocytic stages has been tested in clinical isolates to establish its potential as a diagnostic reagent. Antibody secreted by the clone, demonstrated reactivity with *P. vivax* isolates by Inhibition ELISA. This antibody detected patients' blood samples, positive with *P. vivax* at or above 1000 parasites per microlitre. Work is continued on isolation of *P. vivax* proteins reacting with monoclonal antibodies and isolation of high affinity parasite antibodies from existing panel of hybridomas to develop diagnostic reagent.

Antimalarial Antibody Profile against Defined *P. falciparum* Antigens in Chloroquine Responder and Non-responder Group of Patients

The study subjects included the inhabitants of Kathiatali, Nowgaon and Sonapur, Kamrup districts of Assam. Finger-prick blood samples were collected from 90 patients found positive with *P. falciparum*

❖ **Monitoring of S/P resistance revealed point mutation in *Pvdhfr* gene, a predominance of wild type gene among Delhi isolates, while isolates of South (Chennai), Coastal (Goa) and Central (Navi-Mumbai) regions had shown predominance of double mutants**

infection during July to October 2002. Of the 90 patients, 61 responded to CQ as no asexual parasite was observed during 28 days follow-up. This group was denoted as responder who showed ACR. The other group of 29 patients showed treatment failure (TF) since their blood smears detected positive with falciparum ring during 28 days follow-up. Sero-reactivity of these patients were compared with known falciparum patients from Delhi and Ghaziabad (n=24), who showed adequate clinical responses (ACR) to CQ; also with known malaria negative healthy individuals (n=16). Sera were tested for antimalarial IgG antibody against five *Pf* stage-specific synthetic peptides (CSP, MSP-1/19, EBA175, AMA1 and PfG27; procured from Molecular vaccine Section, CDC, Atlanta) and *P. falciparum* infected erythrocyte lysate (*Pf* crude).

The sero-reactivity of TF and ACR groups was compared. Sera of TF patients showed lower antibody profile against all six antigens. The differential reactivity profiles of these two groups found to be significant ($p<0.01$). Antibodies detected against CSP, MSP-1/19, AMA1 and PfG27 were lower in patients from study group than known *Pf* positive group. However, average level of anti-EBA175 antibody was almost alike in two groups. The healthy normal subjects showed very low sero-reactivity.

Immunocytochemical Peroxidase Test (ICPT) and Dot Immunobinding Assay (DIBA) for the Detection of Antimalarial Antibody in Patients' Sera

Finger-prick blood samples were collected from 46 subjects of age group 5–25 yr belonging to Nanoo village of PHC Loni, Distt. Ghaziabad during October–November 2003, after obtaining informed consent. They reported with fever and came to mobile clinic for malaria diagnosis. Among them, three were positive for *P. falciparum*. Almost all of them had previous history of malaria.

Indirect ELISA was done to estimate antimalarial IgG antibody. Of the 46 samples, 19 had high, 23 had moderate and 4 had low level of *Pf*-antigen specific IgG. The ELISA results of 46 individuals were compared with that of healthy individuals (negative sera) and malaria immune subjects (positive sera). In this group of 46, none showed very high titre of antibodies as compared to immune sera.

In ICPT, sera were allowed to react with the whole parasite on a microslide, whereas in DIBA sera were allowed to react on antigen blotted nitrocellulose membrane after inactivation of endogenous peroxidase. Parasite antigen-antibody complexes were trapped with antihuman IgG-HRPO conjugate. The assay was read after addition of enzyme-specific substrate, amino ethyl carbazol/hydrogen peroxide. The results of both the assays were comparable. However, DIBA found to be more sensitive than ICPT.

Partial Characterisation and Growth Inhibition Reaction of a Glycophospholipid Antigen from *P. falciparum* Culture Supernatant

Glycophospholipids (GPL), a distinct class of antigens are particularly abundant in parasites where they are found as free lipids and attached to proteins. It has been found that *P. falciparum* synthesise GPL and its biosynthesis is crucial for the development and survival of the parasites. Determination of a detailed structure requires isolation of pure GPL which is released in the spent media during schizogony is difficult to obtain in adequate amounts from host cell free components. We are able to purify *P. falciparum* GPL to homogenecity and partially characterised the structural components. Parasite culture supernatant was collected, concentrated, dialysed, gradient centrifuged and lyophilised. Parasite antigen was purified by differential chloroform extraction to remove most nonglycosylated lipids, and the extract containing free GPL was subjected to Folsch's wash. Finally the GPL was purified by successive fractionation using HPLC and silica gel column chromatography. One

❖ **One IgG_1 type monoclonal antibody demonstrated reactivity in *P. vivax* infected patients' blood samples by Inhibition ELISA**

❖ **The sero-reactivity of chloroquine responder (ACR) and non-responder (TF) groups were compared. Sera of TF group showed lower antibody profile against all six antigens than ACR group**

gram of lyophilised culture supernatant yielded 60 mg of GPL by differential extraction method.

The mass spectrometry of the purified three GPL fractions isolated from silica gel column chromatography showed the presence of glycerophosphorylcholine, nonadecanoyl, tetradecasanoyl and docasanoyl moieties. Each fraction contains glycophospocholine, nonadecanoyl and docasanoyl as core components. Identification of GPL fractions by HPLC revealed the presence of rhamnose, galactosamine fucose, mannose, galactose and glucose. Partial structural analysis indicates that GPL is a new kind of antigen so far has been published.

Among three GPL purified fractions, 50 and 70% fractions were used for parasite growth inhibition assay. This test suggests GPL fractions have toxic effect on parasite growth. A small amount of GPL can inhibit 50% growth in comparison to control GPL fractions.

The variation in parasite count between replicate wells was estimated. In treatment groups, count was lower when the concentration of GPL antigen was increased. We also determined that the schizonts started surviving when antigen concentrations were lowered from 25 μg/ml to 0.62 μg/ml. A 50% growth inhibition with the parasitised 1 μg/ml GPL was noticed while in control GPL 50% growth inhibition was observed when the concentration was increased to 26 μg/ml. Our experiment suggests GPL from parasitised culture supernantant is more lethal than the control GPL.

Growth Inhibition of *P. falciparum* in the Presence of 50 and 70% GPL Fractions

Silica gel column chromatography of 50 and 70% methanol eluted fractions isolated from glycophospholipid were tested for schizont growth inhibition. In *in vitro* parasite culture, dose dependent GPL antigen fractions were added in different concentration (μg/ml). GPL isolated from parasitaemic and nonparasitaemic (control GPL) culture supernatant were used. About 26 μg/ml control GPL was needed for inhibiting 50% schizont growth while 1.0 μg/ml GPL was good enough to reach to the above condition. The test indicates GPL has toxic effect on parasite growth.

Biochemical Characterisation

Purification and Characterisation of a Haemoglobin Degrading Aspartic Protease from *P. vivax*

Elucidation of structure/activity and biochemical strategies of the enzyme is necessary to facilitate the development of potent specific inhibitor for potential application as antimalarial drugs. In order to validate whether recombinant forms of plasmepsins are appropriate for use in systematic investigation into inhibitor drug design and development it was considered vital to isolate, characterise and establish the properties of the naturally occurring enzymes in terms of activity and specificity to reflect those in their recombinant forms. Thus isolation, characterisation and inhibition of haemoglobin catabolism catalysed by aspartic proteases in *P. vivax* offers attractive target for chemotherapeutic intervention studies if these classes of enzymes are to be exploited as drug targets. We have now defined the purification and characterisation of *P. vivax* aspartic proteases and have shown that they also share properties similar to that of *P. falciparum* and may play a pivotal role in the development of/as new drugs/drug targets.

❖ **Dot immunobinding assay found to be more sensitive than immunocytochemical test for detection of antimalarial antibody in patients' sera**

❖ **Glycophospholipid from parasitised culture supernatant is more lethal than the nonparasitised one and the GPL has toxic effect on parasite growth**

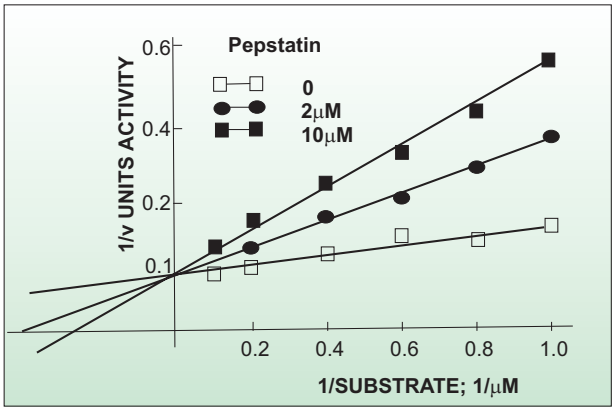


Fig. 19. Kinetic analysis of inhibition of the aspartic protease activity Lineweaver Burk double reciprocal plot of various concentrations of pepstatin (0,2 and 10 μM)

Kinetic Analysis of the Aspartic Protease Activity

To understand the kinetics of inhibition of the aspartic protease activity by pepstatin we have conducted experiments with increasing concentrations of pepstatin (2, 5, 10 μM) and plotted the inhibition kinetics by Lineweaver Burk double reciprocal plot (Fig. 19). Our results demonstrate that the inhibition is competitive with respect to substrate.

Haemoglobin Degradation

To compare the fragments generated by the haemoglobin degradation by purified aspartic protease with those of the fragments produced by the parasite *in vivo* we have carried out experiments with purified haemoglobin. Haemoglobin was incubated for 30 min with our isolated aspartic protease and also in the presence and absence of pepstatin and then analysed on SDS-PAGE (Fig. 20). Arrow 1,2 in the figure indicates the two peptide fragments with the increasing time the two primary cleavage products accumulated and can be seen in absence of pepstatin. The hydrolysis was found to be abolished by the inclusion of pepstatin.

Fig. 20. SDS-PAGE of the proteolysis products: Haemoglobin (4 μM) was incubated with *P. vivax* malaria aspartic protease (0.14 p moles/min) for 30 min at pH 4 in the presence and absence of aspartic protease inhibitor, pepstatin. An 8–25 % gradient gel was run under denaturing reducing conditions. Haemoglobin control (Lane A); Haemoglobin + aspartic protease + 10 μM pepstatin (Lane B); and Haemoglobin + aspartic protease (Lane C). Arrows 1 and 2 mark the two peptide fragments

Effects of the Antimalarials

The effect of antimalarial drugs on the enzyme activity was also investigated. The enzyme was incubated with each drug at different concentrations for 15 min at 30^0 C followed by the measurement of the residual activity under standard assay conditions (Table 5). The protease activity was found to be insensitive to known classess of antimalarials. Mefloquine ((1.0 mM) inhibited the enzyme activity to some extent.

Table 5. Inhibition of aspartic proteinase from *P. vivax* by antimalarial drugs

Drug	Concentration (mM)	% Activity
Quinine	1.0	80
Chloroquine	1.0	100
Primaquine	1.0	85
Mefloquine	1.0	65

Parasite Killing in *P. vivax* Malaria by Nitric Oxide: Implication of Aspartic Protease Inhibition

The generation of NO as a direct result of circulating cytokines may mediate the host pathology seen in malaria infections and in the production of reaction nitrogen intermediates (RNI). The antiparasitic effects of the cytokines observed in different murine malaria infections may be mediated via the production of RNI and may be due to the inhibition of protease activity. Thus, RNI production may be important for understanding the pathophysiology of *P. vivax* infections.

The catalytic activity of proteolytic enzymes is modulated by NO through binding to metal centres and also by chemical modification of the reactive residues. In our efforts to establish the role of NO compounds in malaria we have conducted

Table 6. Killing of *P. falciparum in vitro* by reactive nitrogen intermediates

Reactive nitrogen intermediates*	IC_{50} µM (Mean ± SEM)	n#
$NaNO_3$	36270 + 7,240	5
$NaNO_2$	10,450 + 3,490	5
Sodium nitroprusside	>25000	3
ON-S-Glutathione	41.8 + 18.45	4
ON-S-Cysteine	38.20 + 10.75	4

*Nitric oxide-releasing Compounds: ON-S-, nitrosothiol group; #No. of experiments.

experiments *in vitro* with *P. falciparum* in culture (Table 6). We have observed that the most potent compounds tested were S-nitrosoglutathione and S-nitroso compounds, the reaction mixture which requires 1000 times less material on a molar basis than for either nitrate or nitrite. The parasiticidal effect of these compounds is primarily because of their nitrosothiol contents, and they were found to be a thousand times more active (50% growth inhibitory concentration, approx. 40 µM) than nitrite. Once the nitrogen oxides have diffused into erythrocytes, nitrosothiol groups are formated on proteins, or more toxic chemical species such as peroxynitrites or hydroxyl radicals, which could lead to inactivation of enzymes and thus changes in protein functions are produced. The constant generation of nitric oxide may be required for it to be parasiticidal because it reacts to cross-linked sulfhydryl groups, therefore, we are now conducting experiments to purify and express the nitric oxide synthase in order to test this hypothesis.

The present data thus indicate that the plasmepsins of *P. vivax* are inactivated by NO donors and also NO, probably through S-nitrosylation, thus representing a novel approach for the inhibition of *P. vivax* infections. GNSO and nitroso-L-cysteine are known to kill *P. falciparum in vitro*, probably through the inhibition of a cysteine protease (falcipain). The concentrations of GNSO, NOR-3, SIN-1 and SNP used in the present study are consistent with the concentrations of GNSO and nitroso-L-cysteine used to kill malaria parasites (4.0×10^{-5} µM).

NO has been shown to exhibit direct microbiocidal activity by interacting with enzymes, sulphydryl groups or superoxides. Moreover, it has also been shown that NO regulates IL2 and IFN-γ production in Th-1 cells, and might be important in the regulation of switching between Th-1 and Th-2 type immune responses. This can facilitate the design of strategies for upregulating NO-mediated S-nitrosylation of proteases in *P. vivax* infections, and for *P. falciparum* antimalarial chemotherapy through blocking of essential metabolic pathways for haemoglobin degradation by the parasite. The demonstration of the involvement of proteases and NO inhibitors of this activity could constitute a new approach for the treatment of malaria.

Screening of Medicinal Plants for their Anti-malarial Property

Due to the development of resistance in parasites to almost all antimalarials available, efforts are being made to explore the possibility of having new antimalarials from indigenously available medicinal plants. About 25 medicinal plant extracts were tested and some of them showed good anti plasmodial activity *in vitro* against *P. falciparum*. Three extracts

were tested *in vivo* also. These extracts were giving up to 75% inhibition with 50 mg/kg body weight. Further work is in progress for the purification of compounds from these extracts.

The collaborative project entitled, "Primary screening of the medicinal plants from Northeastern states of India for their anti plasmodial activity" is an ongoing project. Under this project 25 crude extracts and eight fractions were tested *in vitro* till now. Out of this one crude extract tested *in vivo* gave about 50%

❖ **Plant extracts received from different parts of India are being tested *in vitro* and *in vivo* for their antimalarial properties**

inhibition with 50 mg/kg body weight. Further studies are in progress.

Three compounds (endoperoxidase) were received from the Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar, Punjab for testing their antiplasmodial activity *in vitro*. These compounds showed very high anti plasmodial activity. The work is in progress.

Epidemiology & Chemotherapy 3

Health Impact Assessment

Health Impact Assessment of Indira Sagar Dam and Resettlement and Rehabilitation Colonies in SSP Reservoir Impoundment Areas in Narmada Valley in Madhya Pradesh

During this period three surveys, i.e. in May 2004, July 2004 and October–November 2004 were carried out in the command area (villages of District Dhar and Jhabua) and in villages likely to go in submergence (totally, partially and rehabilitated villages of District Khandwa). Both entomological and epidemiological surveys were carried out in these villages.

In the month of May 2004, 231 slides from Dhar district were collected, of which 13 were found positive (all were *Pv*). Age-wise analysis revealed that none of the infants was found positive. In District Jhabua 328 slides were collected, of which 17 were found positive (7 *Pv* and 10 *Pf*). Two positive cases were detected in infants indicating active transmission. The vector species (both as adult and immatures) collected from both the districts were *An. culicifacies* and *An. stephensi* (malaria), *Ae. aegypti* (dengue) and *Cx. quinquefasciatus* (filariasis). The major breeding sites were cement tanks, river-bed pools, streams, etc. No *Culex vishnui* group and sand-flies were collected during the survey.

Second survey was carried out in the month of July

2004 in nine partially submerged and new rehabilitated villages of District Khandwa. Out of 205 slides examined none was found positive for malaria. The vector species collected from the district were *An. culicifacies*, *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. During the survey the baseline information about the dam site, command area and rehabilitation centres was also collected.

During the survey of October–November 2004 it was found that the villages close to reservoir which had no mosquito/malaria problem are now facing a threat. Data on whole night/day biting collection, parity rate, gonotropic cycle, per man hour and room density of vectors of all the four diseases were collected. Besides this, susceptibility test for *An. culicifacies* was carried out against various insecticides as per WHO technique. The species was found resistant to DDT and mortality after 20 minutes was observed in deltamethrin and cyfluthrin. Larvae of all disease vectors were collected from various breeding habitats such as storage tanks, river-bed pools, canals, drains, etc. *Ae. aegypti* mosquitoes (127) would be sent to National Institute of Virology, Pune for dengue virus testing. In all the villages cross-sectional survey was carried out for malaria and

❖ **Entomological and epidemiological studies were carried out in command area of SSP reservoir and remedial measures were suggested**

filaria during day and night time. A total of 609 blood slides for malaria and 334 blood samples on filter paper for filariasis were collected.

Village-wise maps of the dam sites, command areas and rehabilitation centres has been procured and digitisation of canal network, land use, village location, forest area, etc. is in progress to develop a disease information system and map receptive areas

for various diseases. Data from various agencies was collected and it was found that no case of dengue, filaria, JE and Kala-azar is reported.

A meeting was held with the Vice Chairman, Narmada Valley Development Authority and state health authorities. The following points were highlighted — (i) the site selection for a few rehabilitation centres was not suitable; (ii) the site selection of labour colony was not suitable, these are close to drain under construction; (iii) there was a heavy vector breeding in the half finished canal which need immediate attention; and (iv) curing tanks at the rehabilitation centre, left unattended after construction, were supporting breeding of dengue and malaria vectors.

Situation Analysis of Malaria in Gadchiroli (Maharashtra) from the Viewpoint of Persistence of Malaria

In order to find out the reasons of persistence of malaria in Gadchiroli district, fieldwork comprising of entomological and parasitological survey was conducted in Dhanaura, Etapalli and Aheri Blocks of the district in November 2004, the peak transmission season of malaria. Data on socioeconomic conditions were generated through questionnaires. The parasitological and entomological findings revealed (gametocyte rate ranging from 14.2 to 31.2% and high density of *An. culicifacies*) that the intervention measures were still not effective in controlling transmission. Stratification of areas revealed that the breeding habitats and hilly areas within 1.5 km proximity of human settlements were highly malarious. Density of vector species was quite high in almost all the villages. Of 297 *An. culicifacies* exposed to ELISA test for detection of sporozoites, none was found positive.

❖ **Situation analysis in Gadchroli district revealed that the intervention measures are still not effective in controlling malaria**

Evaluation of the Pilot Programme for the Insecticide Treatment of Community Owned Bednets

A survey was undertaken in Karbi Anglong district of Assam and West Garo Hills (Meghalaya) in April 2004 to assess preparatory activities including surveys and measures for the involvement of PRIs, NGOs, SHGs; training and IEC activities; to document awareness in the community about insecticide treated mosquito nets and their keenness for getting the nets treated at the camps organised by the health department and NGOs; the willingness of the community to pay for the insecticide; operational details of the camps organised for the treatment of the nets; action taken for the procurement of the insecticide for treatment of the nets; to document coverage level achieved; the involvement of PRIs/NGOs and others; and budget available, expenditure statement and funds collected from the community. The information was elicited through questionnaires by contacting households in selected villages of two PHCs in each district. The observations indicate that the process indicators of the project are on. Efforts have been made to plan the programme, train the staff at various levels and create awareness in the community through newspapers, media, etc. Overall 3.2% of community owned bednets were treated by charging the community @ Rs. 12.50 for per 10 ml deltamethrin

❖ **Rainfall and canal irrigation were responsible for malaria transmission in southern and northern Karnataka**

flow in Karbi Anglong district while in West Garo Hills, 5.5% of community owned bednets were treated. A lot more is required to be done to achieve the target. Though there are many NGOs in the community but nobody came forward to help in the programme. Most of the persons could not afford to pay for the cost of insecticides.

Climate and Malaria

Impact of Climatic Factors on Malaria in Karnataka

To find out the relationship between malaria incidence and meteorological parameters for early warning of malaria, epidemiological data of malaria in respect of problematic districts of Karnataka and Rajasthan were procured from concerned state governments. Data on temperature, relative humidity and rainfall was collected from IITM, Pune. A field visit was made in February 2005 to Raichur district of Karnataka to find out the topographical conditions in canal-irrigated area. Monthly epidemiological data on *P. vivax* and *P. falciparum* in respect of Chitradurga, Raichur, Bijapur and Udupi districts of Karnataka were procured from the state government. Meteorological data on monthly minimum-maximum temperature, relative humidity and rainfall were procured for the period 1985–2000. Monthly data on total malaria cases in all the districts of Karnataka from 1994 to 2003 were also procured. Analysis of data revealed that in southern part of Karnataka (Chitradurga), transmission window of malaria is from April to November with peak of *P. vivax* in May/June, while for *P. falciparum* in October/November. On the other hand in northern Karnataka (Raichur and Bijapur), the peak of *P. vivax* is from August to October, while *Pf* reaches to peak in October/November. The analysis of results revealed that the local conditions of rainfall in southern Karnataka and canal irrigation in northern Karnataka were responsible for variation in malaria transmission windows.

Therapeutic Efficacy Studies

Operational Activity for the Assessment of Therapeutic Efficacy of Chloroquine and Sulphadoxine-Pyrimethamine in Uncomplicated *P. falciparum* Malaria

It is evident that although foci of resistance to chloroquine are present in the entire country, the problem is more pronounced in areas with intense *Pf* transmission like Northeastern states and Orissa; in areas where there is intermixing of population like project areas including construction sites, in big metros and along international borders. Overall, the early treatment failure to chloroquine or RII & RIII level resistance is still not widespread in the country but the low level resistance and recrudescence observed in many parts of the country favours continued morbidity and transmission of resistant strains. This is confirmed by increasing proportion of *Pf* cases in the country. Therefore, the studies were conducted in high transmission area of Orissa, tourist area in Goa and one low transmission area in Rajasthan. The studies in Orissa were completed in 2003 and the results of other two sites are reported here.

Malaria transmission in Goa is high due to increased construction and developmental activities which attracted labour population from surrounding states, being tourist place with natural beauty attracts tourists throughout the year and good rainfall coupled with favourable temperature (Max. 28–37°C; Min. 18–24°C) and relative humidity (75–95%) which are conducive for mosquito proliferation.

Udaipur district in Rajasthan shares its borders with seven other districts of Rajasthan. Aravali ranges from north to south enrich the district. There are two important passes in the Aravali range— Desuri Nal and Sadri which serve as link between Udaipur and Jodhpur districts. Udaipur has, on the whole, moderate and healthy climate without significant variation. The temperature ranges from 36.7°C in May and June to 16.08°C in January. PHC Rishabdev in this district was selected as study site (Fig. 21).

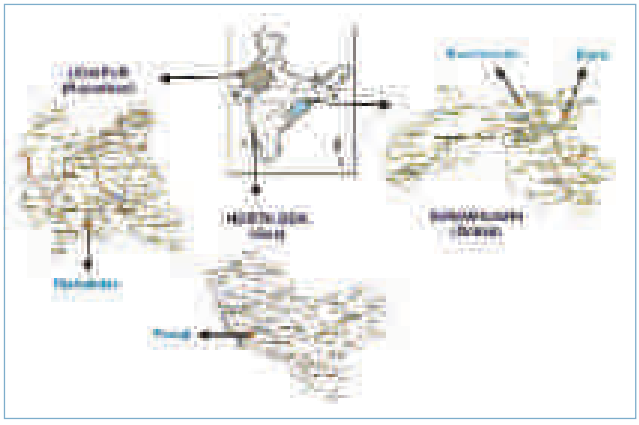


Fig. 21. Study sites: Study sites

All patients reporting to local clinic with complaint of fever were examined for prevalence of parasites in blood smear. Clinicians, taking special care to detect febrile disease other than malaria, evaluated those meeting basic enrolment criteria. The temperature, body weight and other demographic information was recorded. Peripheral smear was examined and those positive for *P. falciparum* were enrolled. Informed consent was obtained and case record form (CRF) was completed for each patient. WHO protocol was followed for inclusion/exclusion and results were analysed using WHO software.

In Udaipur (Rajasthan) 59 patients were enrolled for the present trial, 11 were lost to follow-up or withdrawn and 48 patients completed the study. The baseline characteristics are given in Table 7. The classification of therapeutic response is shown in Table 8 and Fig. 22.

Table 7. Baseline characteristics of patients

Drug: Chloroquine (Dose 25 mg/kg over 3 days)	Udaipur (Rajasthan)
No. of cases	59
M/F	33/26
Age in years (Range)	3–51 yr
Parasitaemia/µl on D0 (Range)	1000–10000

Table 8. Summary of classification of therapeutic response

Response	Number	Prevalence
Early treatment failure (ETF)	0	0
Late clinical failure (LCF)	1	0.021
Late parasitological failure (LPF)	1	0.021
Adequate clinical and parasitological response (ACPR)	45	0.957
Total analysis	47	
Withdrawal (With)	3	
Loss to follow-up (Loss)	9	0.203
Total	**59**	

❖ **Therapeutic efficacy studies of chloroquine revealed early treatment failure in Goa in contrast to late treatment failure in other parts of the country**

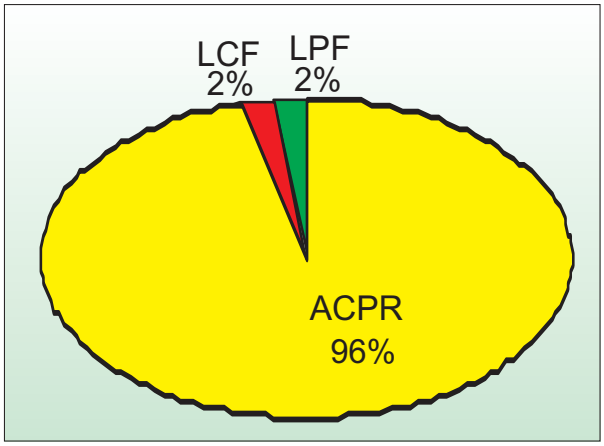


Fig. 22. Classification of therapeutic response in Udaipur

Table 9. Baseline characteristics of patients

Drug: Chloroquine (Dose 25 mg/kg over 3 days)	Panaji (Goa)
No. of cases	63
M/F	55/8
Age in years (Range)	9–55 yr
Parasitaemia/µl on D 0 (Range)	1040–98400

Table 10. Summary of classification of therapeutic response

Response	Number	Prevalence
Early treatment failure (ETF)	12	0.235
Late clinical failure (LCF)	7	0.137
Late parasitological failure (LPF)	24	0.471
Adequate clinical and parasitological response (ACPR)	8	0.157
Total analysis	51	
Withdrawal (With)	2	
Loss to follow-up (Loss)	10	0.2
Total	**63**	

Sixty-three patients were enrolled in Goa. High percentages of patients were visiting Goa from neighbouring states especially Karnataka, Maharashtra and Kerala. However, 95% of the enrolled patients were present at the study site for more than one month prior to diagnosis. This indicates that the infection was most probably acquired in the study area. The baseline data of the cases enrolled are shown in Table 9 and results are shown in Table 10 and Fig. 23.

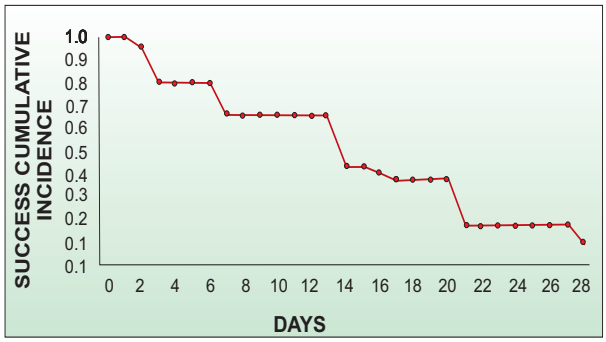


Fig. 23. Classification of therapeutic response in Panaji (Goa)

The data indicate high percentage of early treatment failures in this region in contrast to observations of late treatment failures in other parts of the country. This necessitates early intervention to limit the problem of chloroquine resistance in this state.

During 28-day follow-up reinfections can occur. To differentiate reinfections and recrudescence molecular markers (MSP-1, MSP-2 and GLURP) were used and correction factor was applied for classification of failure. The PCR corrected classification for Goa is given in Table 11.

Table 11. Summary of classification of therapeutic response

Response	Number	Prevalence
Early treatment failure (ETF)	12	0.245
Late clinical failure (LCF)	7	0.143
Late parasitological failure (LPF)	22	0.449
Adequate clinical and parasitological response (ACPR)	8	0.163
Total analysis	49	
Withdrawal (With)	4	
Loss to follow-up (Loss)	10	0.2
Total	**63**	

Vaccine Trial

Development of a Site for Malaria Vaccine Trial at Sundargarh District, Orissa

This is a collaborative project with International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi and is being funded by the Department of Biotechnology (DBT), Govt. of India under Jai Vigyan Mission. The studies are being carried out to understand the epidemiology of malaria in Sundargarh district, Orissa that will facilitate the field trials for *P. falciparum* malaria vaccines through collection of clinical, entomological and molecular epidemiological/immunological indicators from the study site. The longitudinal epidemiological studies were continued in two sets of villages in the forest and plain areas characterised by hyper and mesoendemic malaria situations respectively. Now there are 35 villages, 23 forest and 12 plain with a total population of 15,525. Longitudinal parasitological surveys were conducted in all the villages of phase-I study area. Weekly surveillance with the help of village volunteers was organised to measure malaria incidence. The annual parasite index (API) in the forest and plain areas was 241.8 and 14.1 respectively. Malaria is persistent throughout the year in both the areas but peak transmission was observed during post-monsoon months — September, October and November. The

❖ **Highest API (1106.5) is recorded in 1–5 years age group in forest area in contrast to plain area where all the age groups were equally affected**

proportion of different *Plasmodium* species in the forest area was 85.5, 13.7 and 0.8 for *P. falciparum, P. vivax* and *P. malariae* respectively, whereas it was 84.8, 15.2 and 0 respectively in the plain area.

In the forest area, the highest malaria incidence (API-1106.5) was recorded in the 1–5 years age group, whereas in the plain area, all the age groups were equally affected and API was ranging from 5.2 to 29.2. The infant parasite rate (IPR) and child parasite rate (CPR) in the forest area were high throughout the year with a yearly average of 62.3 and 49.3 respectively. The average IPR and CPR in the plain area were 0 and 2.8 respectively. The highest attack rate due to *P. falciparum* (number of episodes per person per year) in the forest area was recorded in 1–5 years age group (0.91 episodes per child per year). The average attack rate in the total population was found to be 0.21 and 0.01 in the forest and plain areas respectively.

Malaria prevalence in the study population during different transmission seasons was measured through cross-sectional point prevalence surveys in all the 35 study villages during March, June and November characterised by moderate, low and high transmission seasons respectively. About 40% of the houses were selected through computerised random numbers and all occupants of these houses were examined for malaria parasite irrespective of clinical symptoms. The parasite rate in the forest area during these surveys was found to be 10.1, 14.9 and 12.4 in March, June and November respectively, whereas it was 1.4, 1.7 and 0.5 respectively in the plain area. The highest parasite rate in the forest area during these surveys was found in 1–5 years age group with a gradual decline in the progressive age groups, whereas in plain area parasite rate was low and all the age groups were equally affected. The spleen rate in children and adults in the forest area was 79.7 and 16.2 respectively in March, June and November, whereas in the plain area it was 17.9 and 0.83 respectively. The average enlarged spleen (AES) in children in the forest and plain areas was 1.8 and 1.1 respectively. Studies on the parasite diversity and immune response during different transmission seasons were carried out by MRC (HQ) for which blood samples were collected during these surveys.

Longitudinal entomological surveys were conducted in two indicator villages each from forest and plain areas. A total of 11 anopheline species from forest area and 10 species from the plain area were recorded. *An. culicifacies* was the most predominant species and accounted for 41.2 and 36.5% of the total anophelines in forest and plain areas respectively. *An. fluviatilis* was restricted to only forest area and its prevalence rate was 1.1%. The density of *An. culicifacies* in forest and plain areas was ranging from 5.3–48.5 and 5–51 respectively. The density of *An. fluviatilis* in the forest area ranged between 0 and 2–3. The landing rate of *An. culicifacies* on human baits in the forest and plain areas was 1.04 and 1.5 bites per person per night, whereas the human landing rate of *An. fluviatilis* in the forest area was 1.2 bites per person per night. Higher sporozoite rate (annual) was recorded in the forest area (3.5%) as compared to that in the plain area (0.6%). The sporozoite rate (SR) in three different transmission seasons in forest and plain areas were found 0.016 and 0.004 (high transmission season); 0.029 and 0.007 (moderate transmission season); and 0.01 and 0.004 (low transmission season) respectively. Further, the entomological inoculation rate (EIR) estimated were 0.051, 0.13 and 0.02 in the forest area; and 0.005, 0.011 and 0.005 in the plain area respectively for high, moderate and low transmission season.

Analysis of 270 *P. falciparum* positive blood spots from two ecosystems, 230 from forest (10 villages) and 40 from plains (5 villages) for MSP-1 and MSP-2 polymorphs revealed highly polymorphic nature of both markers. A significantly high proportion of multiclonal isolates (53.91%) were observed among isolates from forested area compared to plain area (16.21%). Multiplicity of infection (MOI) was also higher for both MSP-1 (1.99) and MSP-2 (2.21) in isolates of forested area. At three transmission levels, MOI increased from low to high transmission level—

❖ **High sporozoite rate was recorded in the forest area (3.5%) in contrast to only 0.6% in the plain area**

❖ **The IgG profile against MSP-1_{19} EBA 175 and TRAP was higher in the population of forest area than that of in plain area during both low and high transmission seasons**

1.75 to 2.03 for MSP-1 and 1.89 to 2.66 for MSP-2 but was not significant. Percentage of single clone isolates for both MSP-1 and MSP-2 decreased from low to high transmission level while result was vice-versa with multiple clone isolates but was nonsignificant. MOI decreased with increasing age when analysed among four age groups. MOI in adults showed low MOI than infants and children which was found to be nonsignificant.

Finger-prick blood samples were collected from different age groups by repeated cross-sectional surveys at two sites each of forest and plain areas during low and high transmission seasons. Indirect ELISA was done to measure the antibody levels against *Pf* MSP-1_{19}, EBA 175 and TRAP antigens in 222 (110 from forest and 112 from plain areas) and 248 (138 from forest and 110 from plain areas) blood samples collected during low and high transmission seasons, respectively. Subsequently, 126 blood samples from 1–5 years age group of children were analysed for IgG profile against two vaccine candidate antigens MSP-1_{19}, EBA 175 during the low transmission period (June–July 2004). It was observed that overall IgG profile against MSP-1_{19}, EBA 175 and TRAP in both the studies was higher in the population of forest area than that of plain area in both low and high transmission seasons. The age-dependent increase of specific antibody levels was noticed in individuals of two areas in both seasons. The mean ELISA O.D. was significantly lower in children <5 years age as compared to adults ($p<0.001$). Proportion of high responders was more in adults than children ($p<0.01$). However, acquisition of antibodies during the time of high transmission phase was more as compared to low transmission. Thus the results suggest that there was a boosting in antibody production against these molecules by natural infections in these individuals. The level of antibodies in study groups appeared to be related to their exposures to the parasite during high transmission phase.

Malaria Clinics

At 22, Sham Nath Marg, Delhi

A total of 137 patients attended the Malaria Clinic at MRC, 22, Sham Nath Marg or were referred from hospitals for blood examination and treatment of malaria during January to December 2004. Out of 18 patients found positive for malaria, 12 were diagnosed as *P. vivax* and six as *P. falciparum* cases.

At 2, Nanak Enclave, Delhi

A total of 2,313 patients attended the Malaria Clinic at MRC, 2, Nanak Enclave during January to December 2004, of which 292 patients were found positive for malaria infection. Among all the positive malaria cases, 275 patients were positive for *P. vivax*, 16 for *P. falciparum* and one for mixed infection. Clinical examination was done and specific symptomatic treatment was given wherever necessary. Blood samples were collected for ongoing host-parasite interaction and genetic diversity studies. Monthly distribution of these cases and correspondingly slide positivity rate (SPR) and slide falciparum rate (SFR) calculated are given in Table 12.

Table 12. Malaria cases reported at MRC clinic 2, Nanak Enclave, Delhi during the year 2004

Month	BSE	Total	*Pv*	*Pf*	Mix	SPR	SFR
Jan	53	0	0	0	0	0	0
Feb	83	0	0	0	0	0	0
Mar	155	1	1	0	0	0.65	0.65
Apr	176	3	3	0	0	1.70	0
May	118	4	4	0	0	3.39	0
Jun	153	8	8	0	0	5.23	0
Jul	195	10	10	0	0	5.13	0
Aug	359	89	88	1	0	24.79	0.28
Sep	497	139	135	3	1	27.97	0.60
Oct	281	25	20	5	0	8.9	1.78
Nov	128	8	5	3	0	6.25	2.34
Dec	115	5	1	4	0	4.35	3.48
Total	**2313**	**292**	**275**	**16**	**1**	**12.62**	**0.69**

❖ **Diagnostic and treatment services were provided to > 2400 patients**

IDVC Research Activities

4

Bangalore (Karnataka)

A project on "Development of strategy for integrated control of vectors of malaria, Japanese encephalitis (JE) and dengue" was accomplished in seven talukas comprising of 50 PHCs and 1846 villages in Mandya district of Karnataka. Based on geographical reconnaissance (GR), susceptibility of vector species, epidemiological analysis of data, control strategy for malaria, JE and dengue was suggested to the state government. Field evaluation of VectoBac against *Anopheles, Culex* and *Aedes* mosquitoes, in a variety of breeding habitats in Bangalore rural and urban areas was done. It was found 100% effective against anophelines for three days, for culicines up to 10 days; and against aedines up to seven days. Situation analysis of malaria was done in Bangalore (rural and urban), Kolar, Bellary, Chitradurga and Raichur districts. Trainings and workshops were organised for medical officers, entomologists and health workers, etc.

Car Nicobar (Andaman & Nicobar Islands)

The study on duffy blood group in the primitive tribe of Andaman and Nicobar Islands has brought forward that out of four primitive tribes found in Andaman & Nicobar Islands, Jarawas are duffy negative whereas Great Andamanese, Onges and Nicobarese are duffy positive. Study on diurnally sub-periodic filarial forms revealed that the infection was among the Nicobarese (tribal) and the nocturnally periodic form was observed among the settlers and migratory labourers. The field unit was devastated by Tsunami on 26 December 2004. The staff is being utilised by periodic visits to Andamans for situation analysis of malaria.

Chennai (Tamil Nadu)

During the year, study on bio-ecology of *An. stephensi* and its probable role in disease transmission in Chennai was completed. A report on the assessment of therapeutic efficacy of chloroquine for the treatment of vivax and falciparum malaria in Rameswaram, Ramanathapuram districts (Tamil Nadu) indicating adequate clinical and parasitological response (ACPR) in *P. vivax* and late treatment failure in *P. falciparum* has been handed over to the officials of the Directorate of Public Health and Preventive Medicine, Govt. of Tamil Nadu. Evaluation of VectoBac tablet formulation (*Bacillus thuringiensis* var *israelensis*) and Temeguard (Temephos 50% EC) as larvicides was carried out. Other activities included technical support to various centres/institutes and collaborative research/scientific work. Health education and training programmes were undertaken as routine activities. Malaria clinic continued to function, catering to the health needs of the general public by providing prompt diagnosis and treatment.

Haldwani (Uttaranchal)

Work on the project "Indepth study of entomological and parasitological factors responsible for malaria transmission in some areas of Bhabar region, District Nainital, Uttaranchal" was continued and completed. Reverse pattern of prevalence of *An. culicifacies* and *An. fluviatilis* (peak of *An. culicifacies* in July and *An. fluviatilis* in March). About 0.37% sporozoite rate (1/270) was found in *An. culicifacies*. In OPD, a total of 1489 blood slides were prepared and examined. Out of that 581 (417 *Pv*, 161 *Pf* & 3 mixed) were found positive for malaria showing 39 and 11% SPR and SFR, respectively. IEC activities were kept continued.

Hardwar (Uttaranchal)

Steam distillate fraction from plant code MRCHAR/03/05 showed excellent activity against *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes with their KDT_{50} values of 13, 12 and 18 min respectively on 2% impregnated test papers. Four plants coded as NBDB022, NBDB041, NBDB048 and NBDB056 have been short-listed to develop as novel bio-insecticides against mosquitoes. Fraction code MRCHAR/04/04/S possessed good adulticide activity against *Cx. quinquefasciatus* with LC_{50} and LC_{90} values of 0.5 and 0.97 mg cm^{-1}. Fractions MRCHAR/03/04/1 and

MRCHAR/03/04/4 of plant code MRCHAR/03/04 showed good antiplasmodial activity with their IC_{50} values of 0.62 and 1.5 μg/ml respectively. A total of 265 samples of soil, sediment, water, human blood and human milk collected from Garhwal region were processed for determination of organochlorine residues.

Jabalpur (Madhya Pradesh)

Laboratory bioassays were performed on field collected *An. culicifacies* to determine the efficacy of Olyset® nets after repeated washings with detergent. Support was provided to the programme by undertaking epidemic investigation in Jhabua district, situation analysis of malaria in Sidhi, Seoni and Betul districts and additionally, evaluated the pilot programme of NVBDCP for the insecticide treatment of community-owned mosquito nets in Districts Chandrapur (Maharashtra) and Mandla (Madhya Pradesh). Organised WHO sponsored international training workshop on "Rapid assessment tools for malaria in pregnancy for southeast Asia". Also organised an Indo-US workshop on "Cerebral malaria associated neurological disorders in central India", project funded by Fogarty International Centre. MRC clinic at Medicine Department in Medical College Hospital continued to provide diagnostic and treatment services to malaria patients

Nadiad (Gujarat)

Health impact assessment of Sardar Sarovar water resources development project during the pre-irrigation phase made a significant progress. Disease prevalence assessment and entomological surveillance were the main activities. A collaborative study titled, 'Randomised village-scale evaluation to compare the efficacy of lambdacyhalothrin CS with lambdacyhalothrin WP used in indoor residual spraying for malaria vector control' was initiated with support of WHO Pesticide Evaluation Scheme. New larvicide formulations (pyriproxyfen and VectoBac WDG) were evaluated in the field for mosquito larval control. Therapeutic efficacy study detected high level of chloroquine resistance in *P. falciparum* in malaria epidemic affected areas in Kheda and Anand districts. Technical support was given to the NVBDCP in epidemiological investigations of malaria, whereas work of malaria epidemic containment in various districts in Gujarat was also undertaken. Observation of antimalaria month and setting up new hatcheries for mass breeding of larvivorous fish were also undertaken. Scientists also participated in major meetings to plan or review the activities of the malaria programme on the request of the Gujarat government. Organised several training programmes for state health personnel.

Panaji (Goa)

Susceptibility status of Panaji and Candolim strains of malaria vector *An. stephensi* to DDT, malathion and deltamethrin revealed that both the strains are susceptible to deltamethrin but highly resistant to both DDT and malathion. GR of breeding habitats was done in the entire city of Panaji to assess the mosquitogenic potential of the breeding sites preferred by anophelines, culicines and aedines. Spot intervention measures were instituted by the NVBDCP, Goa team. The stratification of the city is being done to prioritise malaria control on the basis of situation analysis. Therapeutic efficacy of chloroquine in uncomplicated malaria revealed that ACPR was 15.7%, while failure rate was high (84.3%). The national programme has withdrawn chloroquine from Panaji and introduced SP in that area. Malaria clinic continued to provide EDPT to general public and private practitioners. Work is underway to estimate malaria burden in Jharkhand state. Training programmes were organised for students and professionals.

Rourkela (Orissa)

The work on development of a site for malaria vaccine trials was continued in Sundargarh district. Evaluation of Olyset® nets impregnated with permethrin revealed that even after 20 washes the efficacy of Olyset® nets was 80-95% against *An. culicifacies* and 100% against *An. fluviatilis*. Evaluation of bio-efficacy, persistence and impact of mosquito nets treated with tablet formulation of deltamethrin (K-O Tab) on malaria transmission was done. Independent assessment of the operational feasibility of the introduction of rapid diagnostic kits and blister combi packs for strengthening the early diagnosis and prompt treatment (EDPT) under EMCP in Sundargarh district, Orissa was done. Also concurrent evaluation of the pilot programme for the insecticide treatment of community-owned mosquito nets in Districts Nayagarh and Keonjhar (Orissa), Purulia (West Bengal) and Visakhapatnam (Andhra Pradesh) was carried out.

Shahjahanpur (Uttar Pradesh)

Field trials of pyriproxifen (0.5G) was carried out in various mosquito breeding habitats of district Shahjahanpur. The compound was applied in three dosages 2 g/m^3, 4 g/m^3 and 10 g/m^3 of water capacity of breeding sites. The compound was tested against mosquito immatures of *Cx. quinquefasciatus* and *An. culicifacies*. Successful inhibition of adult emergence was obtained with all three doses in various mosquito breeding sites. The effect was more pronounced on the larva to pupal metamorphosis. Analysis of malaria in Shahjahanpur district to identify transmission risk factors and GR for planning vector control was also studied. Situation analysis of malaria was undertaken in Jharkhand. IEC activities were also undertaken.

Shankargarh (Uttar Pradesh)

Malaria clinic continued to serve as a source of sentinel site for monitoring of trend of malaria in Shankargarh PHC and surrounding areas. In the year 2003, the SPR showed a rising trend (22.1%) and in 2004, the SPR has gone up to 45.4%. Malariogenic stratification of Allahabad district is being attempted based on physiography, vector distribution and parasite load.

Sonapur (Assam)

The major thrust areas of research included: (i) the situation analysis of malaria endemic districts of Assam to recommend situation-specific intervention strategies to contain the spread of drug resistant malaria; (ii) to ascertain the treatment seeking behaviour and health care access in ethnic communities of Assam; (iii) to ascertain the therapeutic efficacy of sulphadoxine-pyrimethamine (SP) as primary treatment in districts under alternate therapy replacing chloroquine; (iv) to characterise the malaria parasite strains prevalent in the region for genetic diversity; and (v) to ascertain the therapeutic efficacy of alpha-beta arteether in pediatric malaria for treatment of *P. falciparum* malaria. Other activities included health education and capacity building measures, observation of antimalaria month, and mass propagation and distribution of larvivorous fishes (Guppy) in town areas of Assam. Technical support to the malaria control programme is being provided through World Bank assistance for transfer of technology (TOT) on ITNs to the northeastern states of India.

Repository of Biological Material 5

Mosquito species

Anopheles stephensi

From urban and semi-urban areas

Nehru Place, Delhi; Gurgaon, Haryana; Nanak Enclave, Delhi; Hardwar, Uttaranchal; Nathupura, Delhi

From rural areas

Ladpur, Haryana; Badhdhana, Haryana; Single Line, Punjab; Faridkot, Punjab

Morphological mutants

Red eye (re)	–	sex-linked recessive
Black larvae (bl)	–	autosomal semi-dominant
Golden yellow (gy)	–	autosomal semi-dominant
Creamish white eye (cw)	–	new mutant
Black larva white eye (blew)	–	new mutant

Biochemical variant

Bahadurgarh, Haryana (EST-2)

Anopheles culicifacies Complex

Species A

Dehra, Uttar Pradesh; Burari, Delhi; Rourkela, Orissa; R6-Rourkela, Orissa; RM-4-Chennai, Tamil Nadu

Species B

Acrocentric Y-chromosome lines

Ladpur, Haryana; Haldwani, Uttaranchal; R39-Rourkela, Orissa

Species C

Submetacentric Y-chromosome lines

Jabalpur, Madhya Pradesh; Rourkela, Orissa

Anopheles fluviatilis Complex

Species T

Rourkela, Orissa; Hardwar, Uttaranchal; Haldwani, Uttaranchal

Species U

Hardwar, Uttaranchal

Anopheles sundaicus Complex

Brackish water, A&N Islands; Nancourie, A&N Islands; Katchal, A&N Islands; Tressa, A&N Islands

Morphological mutant

Dark green (Larvae) mutant

Aedes aegypti

Delhi

Culex quinquefasciatus

Delhi

Morphological mutants

Red eye (re); Scarlet eye (se)

Parasite Species

Human Plasmodia

- Nonadapted cryopreserved isolates of *P. falciparum, P. vivax* and *P. malariae*
- Sera/plasma from infected patients

P. falciparum

- Adapted/Characterised isolates
- Different stages of the parasite from culture
- Merozoites (from culture supernatant)
- Ring (by synchronisation)
- Gametocytes (by Hypoxanthine treatment)
- Free parasites for antigen preparation (by Saponin lysis and ultrasonication)

P. vivax

- Sporozoites harvested from artificially fed mosquitoes

Cell Lines

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

Nonhuman Plasmodia

- Different species of avian, simian and rodent plasmodia

Details of characterised *P. falciparum* Isolates

Species/Strains of parasite	No. of isolates
Adapted isolates susceptible to chloroquine	54
Adapted isolates resistant to chloroquine	52
NF-54 : an infective gametocyte producing strain of *P. falciparum*	1
3D 7A : a clone of NF-54	1
A-4 : a clone with binding property to CD36	1
Dd2 : a clone which can invade trypsin treated erythrocytes	1
Field isolates which can invade trypsin treated erythrocytes	3
Field isolates which can invade neuraminidase treated but not trypsin treated erythrocytes	3
Field isolates which can invade normal erythrocytes but not neuraminidase or trypsin treated erythrocytes	3
Field isolates which can invade both neuraminidase treated and trypsin treated erythrocytes	5
Field isolates which can form rosettes	3
Field isolates which can bind to CSA	1
Field isolates which can bind to CD36	9
Field isolates which can bind to ICAM-1	2
Isolates with isoenzyme profile of GPI, GDH, ADA and LDH markers	22
Isolates with MSP-1, MSP-2 and GLURP markers	40

Nonhuman malaria parasites available at the Parasite Bank

Parasite species	Source	Susceptibility to antimalarials
Simian malaria parasites		
P. cynomolgi bastianelli	NICD, Delhi	Not done
P. knowlesi	–do–	–do–
P. fragile	CDRI, Lucknow	–do–
Avian malaria parasites		
P. gallinaceum	NICD, Delhi	Not done
P. relictum	Wild, Delhi	–do–
Rodent malaria parasites		
P. berghei NK-65	PGI, Chandigarh	Not done
P. berghei NK-65*†	CDRI, Lucknow	CQ sensitive
*P. berghei**	–do–	CQ resistant
P. berghei	–do–	Quinine resistant
P. chabaudi	INSERM, Paris	Not done
P. vinckei petteri 279 BY	–do–	–do–
P. yoelii yoelii 265 BY**	–do–	–do–
*P. yoelii nigeriensis***†	LSHTM, London	–do–
P. yoelii nigeriensis	CDRI, Lucknow	Multi-resistant
P. yoelii	ICGEB, New Delhi	Not done

*Oocyst positive in *An. stephensi*; **Oocyst and sporozoite positive in *An. stephensi*; †Infective gametocyte producing strain.

- Rodent plasmodia infected rats/mice
- Sera/plasma from respective vertebrate hosts

Animal House Facility

Rabbits, pigeons, domestic fowls, laboratory mice, etc. were procured, maintained and utilised for research purpose throughout the year as per the guidelines issued by the concerned authorities. These animals were housed at 22, Sham Nath Marg and 2, Nanak Enclave buildings and were used as blood meal source to mosquitoes of different species and strains maintained at the Centre. Laboratory mice were used in screening the antimalarials, host-parasite interaction studies and maintenance of rodent plasmodia at the parasite bank. Experiments on animals were performed with the approval of the Scientific Advisory Committee (SAC) and Institutional Animal Ethics Committee (IAEC) of the Centre.

Information, Education and Communication (IEC)

6

National Science Day Celebration

A speech-cum-discussion was organised at the Centre on 28 February 2005 on Environment factors affecting mosquito borne diseases. The speaker was Dr. R.K. Suri, Deputy Director, Ministry of Environment and Forests, New Delhi. All scientists, technical officers and research fellows attended and participated actively in the lecture/discussion.

Preparation of Exhibition

An exhibition consisting of 10 panels highlighting the major research activities of Malaria Research Centre was designed and prepared using computer softwares. These exhibits were later displayed at ICMR (HQs) during the visit of Hon'ble Health Minister to ICMR. The scientific informations received from some other institutes of ICMR were also made into exhibition display panels. Basic details of ICMR (HQs), like numbers of institutes/centres functions, budget, achievements, logo, etc. were also designed and converted into exhibition panels for display.

Exhibition in Vigyan Rail

Associated and collaborated with ICMR in preparation and display of exhibition on ICMR's major achievements through its esteemed institutes/centres. The train also displayed the exhibition of other scientific departments. It later moved to all the major towns of India and stopped at these towns for 4–5 days to enable school children and general public to see the exhibition.

Exhibition, Live Demonstration and Malaria Clinic in Indian Science Congress

MRC associated with ICMR in organising the biggest ever exhibition of ICMR and its institutes on the occasion of Annual Indian Science Congress presided by Prof. N.K. Ganguly, Director General, ICMR, and held at Ahmedabad, Gujarat from 1 to 5 January 2005. In addition to the exhibition panels depicting the major activities and achievements of the Centre, MRC, along with the collaboration of its Nadiad field unit organised its services like, on the spot malaria detection and treatment. MRC also displayed live demonstration of two larvivorous fishes which turned out to be centre of attraction to local people and students. Other live exhibits included all stages of mosquito life-cycle (eggs, larvae, pupae

and adults of vector species of *Anopheles, Culex* and *Aedes*). Instruction to prevent mosquito breeding in domestic and peridomestic habitats was imparted and pamphlets/handouts were distributed. Video films on prevention and control, and remedial measures of mosquito borne diseases were displayed continuously. Several thousand people including students attended the exhibition-cum-live demonstration and viewed the video films on health and expressed their curiousness and satisfaction. There was an earnest demand from the Hon'ble Chief Minister of Gujarat to extend the show for few more days.

Health Education Camp

A health camp on mosquito borne diseases, their prevention and control, self protection and creation of general awareness was organised at Kendriya Vidhyalaya, Dwarka, New Delhi in March 2005. School children were briefed about the life-cycle of mosquito and parasite, prevention of mosquito breeding in and around houses, self protection from mosquitoes, first-aid during onset of high fever, blood examination and treatment etc. Exhibition, video-films and live exhibits were displayed and explained. Brochures and handouts were distributed and questions raised by the visitors were answered. Few books and charts were donated by MRC to the school library.

Hindi Exhibits

On the occasion of celebration of "Hindi Diwas Saptah" panels on MRC's activities were displayed in Hindi language. These were designed and prepared at the Centre.

Still Photography

To cover the scientific research activities of the Centre, still photography of the ongoing research work of all the laboratories including parasite bank, insectories, infected blood meal to vectors, bio-chemistry, genetics, immunology etc. was highlighted in the photographs.

Documentary Videos

(i) Researching, scripting, story-board and some field shooting of a documentary on the role of Olyset nets in controlling mosquito borne diseases has been undertaken in collaboration with Dr. M.A. Ansari. The evaluation of this product is being undertaken in some villages of Ghaziabad. Evaluation aspects would be shot in night and effectiveness of this product would be assessed and highlighted in this documentary film.

(ii) Visited Betul, Madhya Pradesh in March along with a team from National Vector Borne Disease Control Programme to facilitate filming of two videos on larvivorous fish and bednets.

Publications

Periodicals

Journal of Vector Borne Diseases

The Centre has been successfully publishing an English quarterly the *Journal of Vector Borne Diseases*. The journal is completely updated and it is being published in time. Further, it is worth mentioning here that contribution of research articles by scientists in abroad has been increased and the journal is being uploaded in the web for easy access to the scientists working in the field of vector borne diseases.

Malaria Patrika

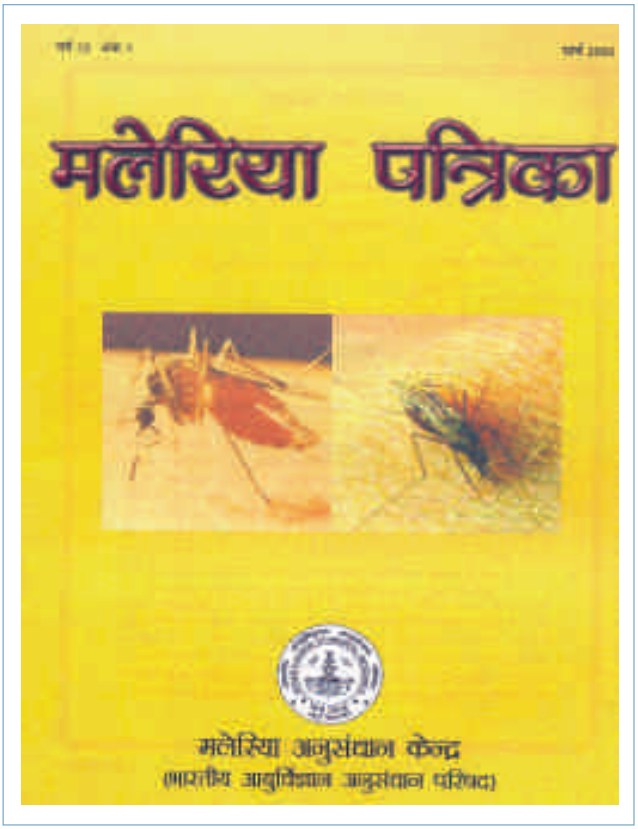
Malaria Patrika a quarterly periodical in Hindi language is also published regularly. Various activities relating to the Centre, news and latest developments in the field of malaria research are being covered in this periodical apart from scientific articles. The *Patrika* is also being applauded by the community.

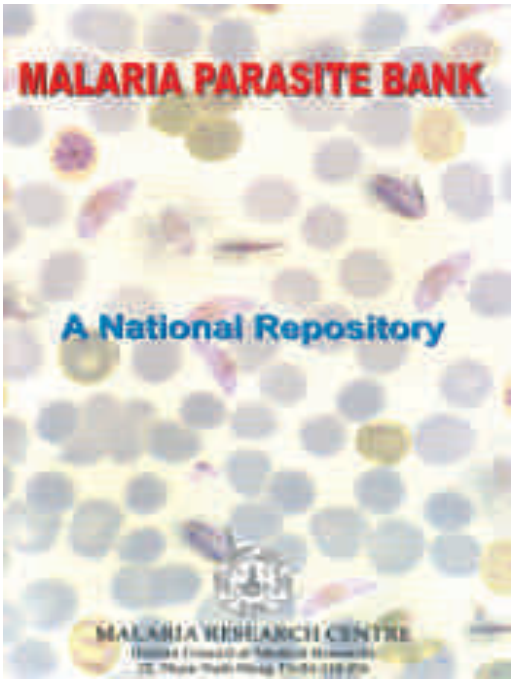
Malaria Parasite Bank: A National Repository

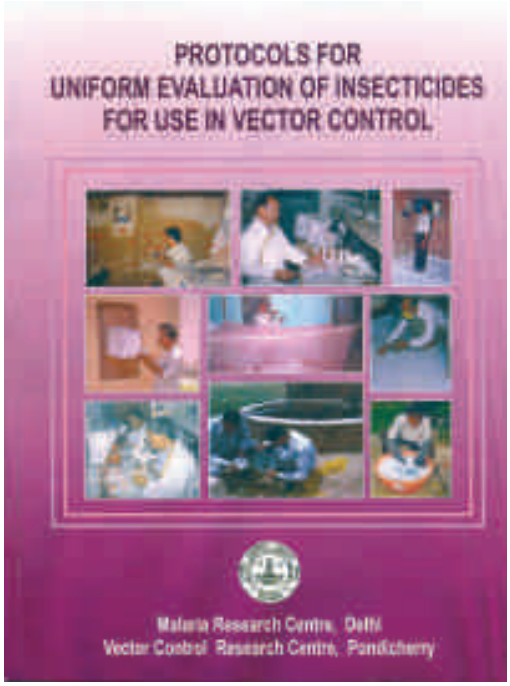
A monograph on the activities of Malaria Parasite Bank—operational at the Centre, has been published. Various aspects related to the collection, adaptation to *in vitro* culture, preservation, characterisation, supply of parasite material etc. have been included in this book. Details of parasite isolates collected from different parts of India are also included in this document.

Protocols for Uniform Evaluation of Insecticides for Use in Vector Control

A book containing protocols for evaluation of insecticides which are used in vector control has been published in collaboration with Vector Control







Research Centre, Pondicherry. Detailed procedures for evaluation of insecticides in indoor residual spray, impregnation of bednets, fabrics and plastic sheetings, space sprays, larvicides, repellents, biolarvicides, mono molecular films and insect growth regulators, have been given in this document. The book is being circulated to all the Institutes involved in the evaluation of insecticides.

Websites

Centre's Website http//www.mrcindia.org

The website contains information on research activities being carried out by MRC, publications of the Centre, research activities of the field units of MRC, audio-visual unit activities and directory of scientists, etc.

Website of *Journal of Vector Borne Diseases* http//www.mrcindia.org/journal of vector borne diseases

A separate webpage is being maintained for Centre's English periodical the *Journal of Vector Borne Diseases*. The website contains information on guidelines for contributors, subscribers and contact information of editorial office. Further complete articles published in the journal are also updated regularly on the website.

Library

The Centre has one of the best libraries in the country in the field of malaria having 6607 books, 3005 bound journals, 3559 reprints, 18 video cassettes, 27 audio cassettes, five microfilms, 13 theses and 100 national and international reports. A total of 60 journals (50 foreign and 10 national) are being subscribed besides 10 journals which are being received on exchange or complimentary basis. Three magazines and 12 newspapers (seven in English and five in Hindi) are also being subscribed. In the financial year 2004–05, the library has added 18 new books and four new journals.

The Library renders its services not only to the scientists/research scholars of the Centre, but also to various national and international universities and organisations. During the year library has provided its services to the scientists of 16 national/international institutes/universities. In the process of modernisation during the year 2004–05 data of 3000 books and 50 Journals have been entered in the library software—Libsys, while other cataloguing work is in progress. Library is affiliated with DELNET (Developing Library Network) to access the various database like union catalogue of books/periodicals to provide the required material to the scientists and its users.

Library also provides abstracts, references, CAS and SDI services. Medline CD search and internet facility to access online journals is also available to the users. Library also provides science citation analysis services to its scientists through INSDOC. Library has provided this facility to the scientists of the Centre. The library also provides inter-library loan facilities and reprographic services on demand. ICMR (HQ) has procured web-based product, viz. J-Gate and JCCC (J-Gate Custom Content for Consortia) for its 24 centres and institutes. MRC library will provide this facility in near future to its users as well as to all its field units located in different parts of the country. Proquest medical database has been procured by ICMR (HQ) for Malaria Research Centre. The library is gaining access to the product.

"Hindi Week" Celebrations

For compliance of official language policy of the central government Malaria Research Centre has celebrated Hindi week from 14–21 September 2004. Many activities took place during the week, which included—Hindi workshop for administrative staff, scientific seminar for scientists, noting-drafting competition, debate competition for staff and officers. All these competitions were organised by the members of the Official Language Implementation Committee.

The first activity of the week started on 14 September with Hindi workshop for the administrative staff. The guest on the occasion was Shri Satyendra Singh, Assistant Director, Central Translation Bureau. The programme was conducted by Shri S.C. Sharma, Administrative Officer of the Centre. Shri Satyendra Singh, in his lecture talked in detail about the use of administrative terms in official work and gave examples of complete change of meaning of the sentences by using inappropriate or wrong words. The workshop continued till 1 p.m. and after the lunch break another workshop started at 2.30 p.m. The guest on the occasion was Dr. Raj Kumari Dev, Joint Director, Ministry of Water Resources. Mrs. Dev explained about Rajbhasha Rules & Regulations and various incentive schemes of the central government.

On the second day, 15 September 2004, two competitions were held. In the morning session, noting-drafting competition for the Administrative staff was conducted by the Administrative Officer of the Centre and many staff members participated in it. In the afternoon session Hindi essay competition was held in which 18 staff members participated. The topics for the essay competition were *Shiksha Ka*

Vyavasaikaran (Commercialisation of Education) or *Suchna Kranti* or The Impact of Explosion of Population in Metros. The essay competition was conducted by Dr. Mantosh Malhotra, Deputy Director of the Centre.

On 16 September scientific seminar for scientists was organised and the topic of which was *Swasthya aur Vatavaran* (Health and Environment). The seminar was organised by Dr. R.C. Dhiman, Deputy Director and chaired by Prof. Puran Singh Dabas, Reader (Retd.) of Delhi University. In this seminar 10 scientists of the Centre presented their views on this topic and chief guest appreciated the scientific views and intellectual discussion of the scientists.

In addition to above mentioned activities, on 20 September 2004 Dr. Nutan Nanda, Assistant Director organised a very interesting debate competition for staff on the topic *Aikal Parivar Banam Sanyukt Parivar* (Nuclear Family vs Joint Family). On this occasion Dr. Jagdeep Saxena from ICAR and Dr. (Mrs.). Aruna Srivastava, Deputy Director of the Centre were invited as judges. The chief guest and the judge Dr. Jagdeep Saxena appreciated the debate competition and organiser of the competition. He suggested to organise this type of function on Science Day and Environment Day also.

On 21 September 2004 the last activity of the week debate competition for officers was held which was organised by Dr. B.N. Nagpal, Assitant Director of the Centre. The topic of debate was *Mobile Phone: Suvidha ya Sirdard*. On this occasion Chief guest and the judge respectively were Director of Central Translation Bureau and Professor from Kendriya Hindi Sansthan. This competition was held in successful and fruitful manner. Just after the debate competition valedictory session was organised by Dr. Vandana Sharma, Hindi Officer, and the Director of the Centre Prof. A.P. Dash chaired the session. In this concluding session Prof. A.P. Dash, addressed the officers and staff of the Centre and requested them to

use Hindi in official work. He congratulated all the organisers for conducting these activities successfully and thanked the staff for their active participation.

After this, the prize distribution function started and the prizes of the essay competition were given by Dr. M.A. Ansari, Deputy Director (S.G.) to Smt. Kamla Negi, 1st winner, Dr. Padmawati Tyagi, 2nd winner, Shri Vanshidhar, 3rd winner and consolation prizes to Shri Dan Singh Sontiyal and Shri Shailendra Pandey. The prizes of noting and drafting competition were given by Dr. Aruna Srivastava to Shri Pradeep Dutta, 1st winner, Smt. Monika Malhotra, 2nd winner, Shri K.C. Sehra, 3rd winner, and consolation prizes to Shri G.L. Puri and Shri A.K. Dwivedi. After this the prizes of debate competition were declared and distributed to Shri Hari Om Tyagi (1st), Shri K.C. Sehra (2nd), Shri Vanshidhar (3rd) and Shri Satpal and Shri M.D. Tiwari got conslation prizes. Then the prizes of debate competition for officers were given by Prof. A.P. Dash, Director to Dr. K. Raghavendra (1st), Shri S.C. Sharma (2nd), Dr. Neena Valecha (3rd) and Dr. Nutan Nanda and Mrs. Rekha Saxena got the consolation prizes.

In addition, the prizes under incentive scheme which has been implemented during the year 2003–04, was given to Shri S.C. Sharma for maximum dictations in Hindi. For carrying out maximum work in Hindi, prizes were given to Shri Ramdev and Monika Malhotra (1st prize), Shri Mohan Lal (2nd), Shri Raghvendera and Shri Mohan Singh Bisht (3rd). At the end of this valedictory function Dr. R.C. Dhiman, Deputy Director proposed vote of thanks to the Director, Senior Officers and to all the organisers and participants.

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Workshops/Symposia/Seminars/Conferences Attended

Name of Scientist/Designation	Name and Place of Event	Period
Prof. A.P. Dash Director	Workshop on Methodologies in Medical Research and Epidemiology at RMRC, Bhubaneswar	16–18 August 2004
-do-	Workshop on Epidemiology of Viral Hepatitis in Tribal Population of India	24 August 2004
-do-	Indo-US Workshop on Cerebral Malaria, RMRCT, Jabalpur	3–5 October 2004
-do-	International Symposium on Emerging Viral Infections at NIV, Pune	11–13 October 2004
-do-	V Joint Annual Conference of Indian Society for Malaria and other Communicable Diseases and Indian Association of Epidemiologists, New Delhi (Chaired oration lecture)	19–21 November 2004
-do-	Indian Science Congress, Ahmedabad	3–7 January 2005
-do-	Workshop on Clinical Management of *P. falciparum*, RMRCT, Jabalpur	1 March 2005
Dr. M.A. Ansari Dy. Director (SG)	Workshop on Malaria Diagnosis and Control, Sagar (M.P.)	15–16 April 2004
-do-	Integrated Strategy to Control Malaria and other Vector Borne Diseases, Nadiad, Gujarat	28–29 April 2004
-do-	IEC Workshop, Car Nicobar	3–9 May 2004
-do-	National Dissemination Workshop on the Development of Integrated Vector Control Strategies, Bangalore	22–24 July 2004
-do-	V Joint Annual Conference of Indian Society for Malaria and other Communicable Diseases and Indian Association of Epidemiologists, New Delhi	19–21 November 2004
-do-	Workshop on Systematic Reviews	24–26 February 2005
-do-	and Evidence based Medicine, VCRC, Pondicherry	
-do-	Seminar on Globalisation in the Asian Perspective at Conference Hall of Culture, House of Islamic Republic of Iran, New Delhi	4–5 March 2005
Dr. Arati Roy Dy. Director (SG)	XIX Carbohydrate Conference at Forest Research Institute, Dehradun	1–3 December 2004
Dr. T. Adak Dy. Director	Malaria and Arvivorous Disease Control in India: Current Approach in International Workshop on Malaria and Arvivorous Vector Control held at Bangkok, Thailand	28 March–1 April 2004
-do-	Organised Silver Jubilee Celeberation at Malaria Research Centre, Field Unit Car Nicobar, Andaman & Nicobar Islands	6–7 May 2004
-do-	WHO sponsored on National Dissemination workshop on Development of Integrated Vector Control Strategies, Bangalore	23–24 July 2004

Name of Scientist/Designation	Name and Place of Event	Period
Dr. Aruna Srivastava Dy. Director	Workshop on Malaria Diagnosis and Control, organised by MRC, Sagar (M.P.)	15–16 April 2004
-do-	V Joint Annual Conference of Indian Society for Malaria and other Communicable Diseases and Indian Association of Epidemiologists, New Delhi	19–21 November 2004
-do-	VII Annual ESRI India User Conference 2004, Noida	2–3 December 2004
-do-	Seminar on Changing Face of Libraries and Access to e-content, New Delhi	17 January 2005
Dr. R.C. Dhiman Dy. Director	Workshop on Malaria and Dengue, IMA, New Delhi	25 May 2004
-do-	WHO sponsored National Dissemination Workshop on Development of Integrated Vector Control Strategies, Bangalore	23–24 July 2004
-do-	XXII International Congress of Entomology, Brisbane (Australia)	15–21 August 2004
Mr. O.P. Singh Assistant Director	XXII International Congress of Entomology, Brisbane (Australia)	15–21 August 2004
Dr. B.N. Nagpal Assistant Director	Workshop on Malaria Diagnosis and Control organised by MRC field unit, Jabalpur at Sagar (M.P.)	15–16 April 2004
-do-	V Joint Annual Conference of Indian Society for Malaria and other Communicable Diseases and Indian Association of Epidemiologists, New Delhi	19–21 November 2004
-do-	VII Annual ESRI India User Conference 2004, Noida	2–3 December 2004
-do-	Seminar on Changing Face of Libraries and Access to e-content, New Delhi	17 January 2005
Dr. Hema Joshi Assistant Director	V Joint Annual Conference of Indian Society for Malaria and other Communicable Diseases and Indian Association of Epidemiologists, New Delhi	19–21 November 2004
Dr. K. Raghavendra Assistant Director	XXII International Congress of Entomology, Brisbane (Australia)	15–21 August 2004
-do-	V Joint Annual Conference of Indian Society for Malaria and other Communicable Diseases and Indian Association of Epidemiologists, New Delhi	19–21 November 2004
-do-	VII International Symposium on Vectors and Vector Borne Diseases, Punjabi University, Patiala, Punjab, India	18–20 February 2005
Dr. Nutan Nanda Assistant Director	V Joint Conference of Indian Society of Malaria and other Communicable Diseases and Indian Association of Epidemiologists, New Delhi	19–21 November 2004

Important Meetings Attended by the Scientists

Prof. A.P. Dash

- Attended expert group meeting on fellowship at ICMR (HQ) on 25 May 2004.
- Attended a meeting of expert group on Malaria disease burden estimation study in India at MRC, Delhi on 9 June 2004.
- Attended a larvicide meeting at National Vector Borne Disease Control Programme, Delhi on 24 June 2004.
- Attended a meeting on Epidemiology hepatistic in tribal population of India at ICMR, New Delhi on 13 July 2004.
- Attended Scientific Advisory Group meeting of the Division of ECD at ICMR from 20–21 July 2004.
- Attended a WHO meeting at Bangalore from 22–24 July 2004.
- Attended a meeting on Task force for hepatitis in tribal areas of India at ICMR, New Delhi on 24 August 2004.
- Attended the Scientific Advisory Committee meeting at RMRC, Bhubaneswar from 16–18 September 2004.
- Attended the Scientific Advisory Committee meeting of VCRC, Pondicherry on 4 and 5 November 2004.
- Attended the Scientific Advisory Committee meeting of CRME, Madurai on 26 and 27 December 2004.

Dr. M.A. Ansari

- Organised and coordinated a meeting on Concurrent evaluation of the pilot programme for the insecticide treatment of community-owned mosquito nets at study areas of Meghalaya and Assam from 24–26 April 2004.
- Actively participated in a meeting to finalise the National guidelines of fogging organised by the DGHS at Nirman Bhawan on 27 April 2004.
- Participated in a meeting of the Expert group on the issue of procurement of bednets for use under NVBDCP at Nirman Bhawan from 11–12 May 2004.
- Participated as an expert in the Assessment Board of DRDO for promotion of scientists on 19 May 2004.
- Visited VCRC, Pondicherry for discussions with the Director to develop a common protocol for testing of larvicide from 3–5 June 2004.
- Participated in Disease burden meeting 2004 at NICD, Delhi on 9 June 2004.
- Participated in the meeting at London School of Hygiene & Tropical Medicine and IV meeting of Global collaboration for development of pesticides for public health (GCDPP) from 21–23 & 24–26 June 2004.
- Participated as an expert in the Assessment Board of DRDO, Delhi at MetCalf House, Delhi on 10 July 2004.
- Participated in a meeting to discuss the Specifications of fogging machines at Seminar Hall, NVBDCP, Delhi on 14 July 2004.
- Attended the meeting of Scientific Advisory Group of ICMR from 21–22 July 2004.

Dr. Neena Valecha

- Meeting for finalisation of logo for National Vector Borne Disease Control Programme at NICD, Delhi on 22 and 28 January 2004.
- Meeting of drug for neglected diseases initiative (DNDI), India at ICMR (HQ), New Delhi on 31 January and 1 February 2004.
- Meeting on NICD Institutional Animal Ethical Committee at NICD, Delhi on 6 February 2004.
- Meeting on expert group on Chemotherapy of malaria at Directorate of NVBDCP, Delhi on 10 March 2004.
- Attended seminar on Olyset® net: long-lasting insecticide treated bednet at New Delhi on 9 April 2004.
- Meeting of MMV/Ranbaxy Synthetic Peroxide (RBx-11160/OZ 277) Project Development Advisory Board at Geneva from 17–19 May 2004.
- Meeting of expert group on Malaria disease burden estimation study in India at MRC, Delhi on 9 June 2004.

- Meeting of national dissemination workshop on Development of integrated vector control strategies at Bangalore from 23–24 July 2004.
- Short-term consultant to assist programme manager to conduct Therapeutic efficacy of antimalarial drugs in Gelephu, Bhutan (APW) from 2–7 August 2004.
- Meeting of Development of a field site for malaria vaccine trial(s) in Sundargarh district, Orissa at MRC, Delhi on 12 August 2004.
- Meeting on Malaria brainstorming and lecture on Present drug policy and challenges for malaria treatment organised by M/s. Pfizer, India at Hotel (The Grand), Delhi on 10 November 2004.
- First meeting of Drug resistance, new drugs and combination therapy of the Regional Technical Advisory Group (RTAG) on malaria at Haryana from 15–17 December 2004.
- Ethics committee meeting of MRC at MRC, Delhi on 20 January 2005.
- Meeting of Expert group of drug policy at NVBDCP, Delhi

Dr. Aruna Srivastava

- Meeting of the technical experts to review and analyse the technical specifications of computer units organised by the Directorate of NVBDCP, Delhi on 21 September 2004.
- Attended meeting with Mr. Pradeep Bhargawa, IAS, Vice-chairman, NVDA on the progress of health impact assessment of ISP and SSP at NVDA and presented the tour report in November 2004.
- Attended malaria meeting in Science Congress at Ahmedabad organised by ICMR from 5–6 January 2005.

Dr. B.N. Nagpal

- Attended meeting on the Progress of health impact assessment of ISP and SSP at NVDA, Bhopal on 3 September 2004.
- Attended meeting with Mr. Pradeep Bhargawa, IAS, Vice-chairman, NVDA on the progress of health impact assessment of ISP and SSP at NVDA and presented the tour report in November 2004.

Training Courses Organised and Trainings Imparted

Trainings courses organised by MRC

1. Seven courses for Medical Officers of MCD, NDMC, Railways and Armed Forces under EMCP, jointly organised by MRC and NVBDCP at MRC, Delhi from January to March 2004.
2. Training on "Vector control" in advance level training programme for laboratory technicians on 1 October 2004.
3. Three trainers training for entomologists/ biologists jointly organised by MRC and NVBDCP from January to March 2005.
4. Workshop on "Clinical management of severe and complicated falciparum malaria" at RMRC, Bhubaneswar, jointly organised by MRC and NVBDCP from 21–23 March 2005.

Trainings imparted by scientists

Dr. Hema Joshi

1. Four students of B.Sc. (Biotechnology) of Amity University and Jamia Milia Islamia University were supervised for their project work on "Genetic characterisation studies of *P. falciparum*", and related thesis preparation.
2. Two students of B.Tech. (Biotechnology) from Sastra University and Bundelkhand University and one B.Sc. student of Jamia Milia Islamia University were imparted training on PCR based diagnosis of malaria parasites and genetic characterisation studies.

Dr. P.K. Mittal

1. Mr. Dilshad, a student of Jamia Milia Islamia University, Delhi was provided training on vector control.

Dr. B.N. Nagpal

1. Mr. Vanramliana, Research Scholar from Mizoram was provided training on "Anopheline species identification techniques/manual", during July–August 2004.
2. Mr. U. Pe Than Htun, Research Scientist from Myanmar was imparted training on the ongoing research activities at MRC, Delhi on 17 September 2004.

Dr. Nutan Nanda

1. Mr. M.L. Patel and Mr. S.R. Mishra from RMRCT, Jabalpur were provided training in cytotaxonomic techniques and mosquito blood meal analysis in May 2004.
2. Mr. Zaheenul Islam Siddiqui, student from Jamia Milia Islamia University was provided training in preparation of mitotic and polytene chromosomes and identification of sibling species. Supervised his dissertation entitled, "A study on the cytogenetics of malaria vector *An. culicifacies* Giles" for the partial fulfilment of B.Sc. degree in Biotechnology from June–July 2004.
3. Mr. U. Pe Than Htun, a WHO Fellow from Myanmar was provided training in cytotaxonomic techniques for identification of sibling species in malaria vectors in November 2004.
4. Dr. Sachin N. Tikar, Scientist B, DRDE, Gwalior was trained in cytotaxonomic methods of sibling species identification of malaria vectors in October and November 2004.
5. Dr. W.K. Wickremesinghe, Dr. A.M.G. Manel Yapabandara and Mr. D.A.R. Premasiri, WHO Fellows from Sri Lanka were trained in processing of samples for mitotic and polytene chromosome preparation and identification of *An. culicifacies* sibling species in December 2004.

Dr. C.R. Pillai and Dr. C. Usha Devi at Malaria Parasite Bank

1. Mr. Prabhakar, Deptt. of Biotechnology, Calicut University, Kerala, in *in vitro* cultivation of malaria parasite *P. falciparum* from 5 January 2004 for a period of four months.
2. Mr. Shiv Om Pratap, a student of M.Sc. Biotechnology, Sophia College, Jiwaji

University, Gwalior (M.P.) in *in vitro* cultivation of malaria parasite *P. falciparum* from 9 February 2004 for a period of four months.
3. Mr. Sunil Kumar (c/o Sh. Koel Chaudhury, Asstt. Professor) a Ph.D. student, School of Medical Science and Technology, Indian Institute of Technology, Kharagpur, in *in vitro* cultivation of malaria parasite *P. falciparum/P. vivax* and isolation and purification of parasites from 11–14 February 2004 for a period of three days.
4. Dr. C. Rajendran, Scientist 'B' from DRDO, Tezpur, Assam in *in vitro* cultivation of malaria parasite *P. falciparum* and basic training on malaria parasitology from 16–19 February 2004 for a period of three days.
5. Mr. Rama Krishna Kancha, Ph.D. Student, c/o Dr. Prakash Babu, Associate Prof., Deptt. of Animal Science, University of Hyderabad, Hyderabad (A.P.) in *in vitro* cultivation of malaria parasite *P. falciparum* and basic training on malaria parasitology from 23–25 February 2004 for a period of three days.
6. Ms. K.G. Purnima, Lecturer in the Deptt. of Microbiology, Coimbatore, in *in vitro* cultivation of malaria parasite *P. falciparum* and basic training on malaria parasitology for a period of 2 months from 26 April to 26 June 2004.
7. Mr. Gulam Mustafa Hasan c/o Prof. Prahalad C. Ghosh, University of Delhi, South Campus, Delhi, in *in vitro* cultivation of *P. falciparum*, basic training on malaria parasitology and handling of animals (mice) from 28 June to 7 July 2004.
8. Mr. Dipak Kumar Raj, SRF, Ms. Sarmita Mishra, JRF and Ms. Priyanka Kar, JRF, Institute of Life Science, Bhubaneswer, Orissa, c/o Prof. A.P. Dash, Director, MRC, Delhi, in *in vitro* cultivation of *P. falciparum*, basic training on malaria parasitology from 9–14 August 2004.
9. Mr. D. Bharathi c/o Lt. Gen. D. Raghunath, Sir

Dorabji Tata Centre for Research in Tropical Diseases, Innovation Centre, Indian Institute of Science Campus, Banglore, in *in vitro* cultivation of *P. falciparum*, basic training on malaria parasitology from 23–27 August 2004.
10. Mr. Farzin Parabic c/o Prof. I.L. Kothari, Deptt. of Bioscience, S.P. University, Anand, Gujarat, in *in vitro* cultivation of malaria parasite *P. falciparum* from 1 November 2004 for a period of two weeks.
11. Dr. N. Gopalan, Division of Entomology, DRDE, Gwalior, in *in vitro* cultivation of malaria parasite *P. falciparum* from 1 November 2004 for a period of two weeks.
12. Dr. Deepika Saraswat, Division of Entomology, DRDE, Gwalior, in *in vitro* cultivation of malaria parasite *P. falciparum* from 1 November 2004 for a period of two weeks.

Dr. K. Raghavendra

1. Dr. K. Chandrasekhar, Scientist "B", National Botanical Research Institute (CSIR), Lucknow was trained on Mosquito colonisation and

bioassay techniques from 17– 28 January 2005.

2. WHO fellows Dr. W.K. Wickremesinghe, Medical Officer, Dr. (Mrs). A.M.G.M. Yapabandara, Regional Malaria Officer and Dr. D.A.R. Premasiri, Regional Malaria Officer, Anti-malaria Campaign, Sri Lanka were imparted training on molecular and cytotaxonomical methods for identification of mosquito sibling species, vector control methods and epidemic forecasting using GIS from 29 November to 10 December 2004.
3. WHO fellow Mr. Pe Than Hunt, Research Scientist, Medical Entomology Research Division, Department of Medical Research, Myanmar was imparted training in Molecular and cytotaxonomical methods for identification of mosquito sibling species from 25 October–11 November 2004.
4. Dr. Sachin N. Tikar, Scientist "B", DRDE (DRDO), Gwalior was trained in Molecular and cytotaxanomical methods for identification of mosquito sibling species from 25 October–11 November 2004.
5. Mr. Noble Surendran, Lecturer, University of Jayawardenapura, Sri Lanka was imparted training in Molecular techniques for identification of *An. culicifacies* sibling species as part of an agreement for transfer of technology under the DBT funded Indo-Sri Lanka project from 20 December 2003 to 20 January 2004.

Dr. Arati Roy

1. Participants participating in "Course on malariology", had been imparted training on seroepidemiology conducted by NICD, Delhi from 14 February–11 March 2005.
2. Mohd. Shoeb Alam and Zulfequar Ahmed Arfi, Graduate students of Biotechnology, Jamia Milia Osmania University, Delhi, on sero-epidemiology and microdot ELISA from May to July 2004.

Dr. Aruna Srivastava

1. Imparted training to Mr. U. Pe Than Htun, Research Scientist from Myanmar on the ongoing research activities at MRC, Delhi on 17 September 2004.
2. Training imparted to three WHO fellows from Sri Lanka on latest development on application of GIS in forecasting malaria epidemics on 30 November 2004.

Dr. Neena Valecha

1. Imparted training to Mozambique delegation of four scientists under the "India-Mozambique Agreement" on 8 September 2004.
2. Imparted training on "Advance level training programme for Laboratory Technicians of Institute of Pathology (IOP)" on 5 October 2004.
3. Lecture on training course for "Assessment of therapeutic efficacy of antimalaria drugs for uncomplicated *P. falciparum* malaria in India" at Training of *Pf* monitoring team technicians at NVBDCP, Delhi on 27 December 2004.
4. Lecture on "Treatment of malaria" at Malariology training course at NICD, Delhi on 18 February 2005.

Ph.D. Programme

MRC runs a Ph. D. programme. The following students are working for their Ph. D. degree under various scientists.

Prof. A.P. Dash
Mr. S. Mishra
Mr. N. Marai
Ms. Priyanka Kar

Dr. M.A. Ansari
Mr. U. Sreehari

Dr. T. Adak
Mr. Anil Sharma
Mrs. A. Mehrunnisa

Dr. V.K. Dua
Mr. N.C. Gupta
Mr. A.C. Pandey
Mr. V.P. Ojha
Mr. Firoz Alam
Mr. Swapnil Roy

Dr. R.C. Dhiman
Mrs. Sharmila Pahwa

Dr. Ashwani Kumar
Dr. Keshav Prasad*
Mrs. Deeparani Prabhu
Mrs. Nandini Korgaonkar
Mr. M.B. Kaliwal

Dr. Arun Sharma
Mr. Suprio Ray
Miss Sonika Sharma

Dr. P.K. Mittal
Mr. Suresh Yadav

Dr. Hema Joshi
Mr. Surendra Kumar Prajapati
Ms. Gertrude Kiwanuka

Dr. Sarala K. Subbarao (Former Director)
Mr. O.P. Singh
Mr. Dinesh Chandra

* Awarded in 2004.

Advisory Committees of the Centre

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(1)	Prof. N.K. Ganguly, Indian Council of Medical Research, V. Ramalingaswami Bhawan, Ansari Nagar, New Delhi–110 029	*Director General*
(2)	Prof. R.C. Mahajan, Emeritus Professor, H.No. 276, Sector 6, Panchkula–134 109	*Chairman*
(3)	Dr. S. Pattanayak, B-91, Swasthya Vihar, Delhi–110 092	*Member*
(4)	Prof. M.K.K. Pillai, 37 Saakshara Apartments, A–3 Block, Paschim Vihar, New Delhi–110 063	*Member*
(5)	Prof. Ramesh Kumar, B-601 Rishi Apartments, Alaknanda, New Delhi–110 019	*Member*
(6)	Prof. Shobhona Sharma, Tata Institute of Fundamental Research, Deptt. of Biological Sciences, Homi Bhaba Road, Colaba, Mumbai–400 005	*Member*
(7)	Prof. K. Muralidhar, Head, Deptt. of Zoology, University of Delhi, Delhi–110 007	*Member*
(8)	Dr. B. Ravindran, Dy. Director (SG), Regional Medical Research Centre (ICMR), Chandrasekharpur, Bhubaneswar–751 023	*Member*
(9)	Dr. Shiv Lal, Director, National Institute of Communicable Diseases, 22 Sham Nath Marg, Delhi–110 054	*Member*
(10)	Dr. P.L. Joshi, Director, National Vector Borne Disease Control Programme, 22-Sham Nath Marg, Delhi–110 054	*Member*

(11)	Dr. J. Mahanta, Director, Regional Medical Research Centre, N.E. Region (ICMR), Post Box No. 105, Dibrugarh–786 001	Member
(12)	Prof. Anil Gore, Department of Statistics, University of Pune, Ganesh Khind, Pune–411 007	*Member*
(13)	Dr. Sarala K. Subbarao, Indian Council of Medical Research, V. Ramalingaswami Bhawan, Ansari Nagar, New Delhi–110 029	*Member*
(14)	Dr. Deepali Mukherjee, Dy. Director General (SG), Indian Council of Medical Research, V. Ramalingaswami Bhawan, Ansari Nagar, New Delhi–110 029	*Member*
(15)	Dr. Rashmi Arora, Dy. Director General (SG), Indian Council of Medical Research, V. Ramalingaswami Bhawan, Ansari Nagar, New Delhi–110 029	*Member*
(16)	Prof. A.P. Dash, Director, Malaria Research Centre, 22 Sham Nath Marg, Delhi–110 054	*Member Secretary*

Research Advisory Committees Vector Biology & Control

(1)	Prof. M.K.K. Pillai, 37 Saakshara Apartments, A–3 Block, Paschim Vihar, New Delhi–110 063	*Chairman*
(2)	Mr. N.L. Kalra, A-38 Swasthya Vihar, Delhi–110 092	*Member*
(3)	Dr. P.K. Das, Director, Vector Control Research Centre, Medical Complex, Indira Nagar, Pondicherry–605 006	*Member*
(4)	Dr. Sarala K. Subbarao, Indian Council of Medical Research, V. Ramalingaswami Bhawan, Ansari Nagar, New Delhi–110 029	*Member*
(5)	Prof. A.P. Dash, Director, Malaria Research Centre, 22 Sham Nath Marg, Delhi–110 054	*Member Secretary*

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(1)	Prof. Ramesh Kumar, B-601 Rishi Apartments, Alaknanda, New Delhi–110 019	*Chairman*
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(3)	Prof. Shobhona Sharma, Tata Institute of Fundamental Research, Deptt. of Biological Sciences, Homi Bhaba Road, Colaba, Mumbai–400 005	*Member*

(4)	Dr. B. Ravindran, Dy. Director (SG), Regional Medical Research Centre (ICMR), Chandrasekharpur, Bhubaneswar–751 023	*Member*
(5)	Prof. K. Muralidhar, Head, Deptt. of Zoology, University of Delhi, Delhi–110 007	*Member*
(6)	Prof. A.P. Dash, Director, Malaria Research Centre, 22 Sham Nath Marg, Delhi–110 054	*Member Secretary*

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(3)	Dr. Shiv Lal, Director, National Institute of Communicable Diseases, 22-Sham Nath Marg, Delhi–110 054	*Member*
(4)	Prof. Anil Gore, Department of Statistics, University of Pune, Pune	*Member*
(5)	Dr. B.K. Das, Associate Professor of Medicine, SCB Medical College, Cuttack–753 007	*Member*
(6)	Prof. A.P. Dash, Director, Malaria Research Centre, 22 Sham Nath Marg, Delhi–110 054	*Member Secretary*

Bench Review – IDVC Project

(1)	Dr. S. Pattanayak, B-91 Swasthya Vihar, Delhi–110 092	*Chairman*
(2)	Prof. R.C. Mahajan, Emeritus Professor, H.No. 276, Sector 6, Panchkula–134 109	

(3)	Prof. M.K.K. Pillai, 37 Saakshara Apartments, A–3 Block, Paschim Vihar, New Delhi–110 063	*Member*
(4)	Dr. Shiv Lal, Director, National Institute of Communicable Diseases, 22 Sham Nath Marg, Delhi–110 054	*Member*
(5)	Dr. P.L. Joshi, Director, National Vector Borne Disease Control Programme, 22 Sham Nath Marg, Delhi–110 054	*Member*
(6)	Sh. N.L. Kalra, B-38 Swasthya Vihar, Delhi–110 092	*Member*
(7)	Prof. Anil Gore, Department of Statistics, University of Pune, Ganesh Khind, Pune–411 007	*Member*
(8)	Dr. Sarala K. Subbarao, Indian Council of Medical Resarch, V. Ramalingaswami Bhawan, Ansari Nagar, New Delhi–110 029	*Member*
(9)	Dr. Deepali Mukherjee, Dy. Director General (SG), Indian Council of Medical Research, V. Ramalingaswami Bhawan, Ansari Nagar, New Delhi–110 029	*Member*
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(6)	Mr. Raju Dundani, Advocate, Chamber No. 144, Patiala House Court, New Delhi–110 001	*Member*
(7)	Prof. A.P. Dash, Director, Malaria Research Centre, 22 Sham Nath Marg, Delhi–110 054	*Member*

(8)	Dr. Neena Valecha, Dy. Director, Malaria Research Centre, 22 Sham Nath Marg, Delhi–110 054	*Member Secretary*

Animal Ethical Committee

(1)	Prof. S. Prabhu, Former Director, Pharmacology Division, Maulana Azad Medical College, New Delhi–110 002	*Chairman*
(2)	Mr. D.P. Jain, Ex-Manager, Charity Birds Hospital, Opp. Lal Quila, Delhi–110 006	*Member*
(3)	Dr. S. Jayaraman, Dy. Director, Institute of Pathology, New Delhi, *in lieu of* Mrs. Irani Mukherjee, Circle of Animal E-67, DDA Flats, Saket, New Delhi–110 017	*Member*
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(5)	Dr. T. Adak, Dy. Director, Malaria Research Centre, 2 Nanak Enclave, Delhi–110 009	*Member*
(6)	Dr. Neena Valecha, Dy. Director, Malaria Research Centre, 22 Sham Nath Marg, Delhi–110 054	*Member*
(7)	Dr. P.K. Atul, Senior Research Officer, Malaria Research Centre, 22 Sham Nath Marg, Delhi–110 054	*Member Secretary*

Building Committee

(1)	Dr. Sandeep Basu, Director, National Institute of Immunology, New Delhi–110 067	*Chairman*
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(4)	Dr. Deepali Mukherji, Chief of ECD, Indian Council of Medical Research, Ramalingaswami Bhawan, Ansari Nagar, New Delhi–110 029	*Member*
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(8)	Dr. B.N. Nagpal, Assistant Director, Malaria Research Centre, 20 Madhuban, Delhi–110 092	*Member*
(9)	Dr. Nutan Nanda, Assistant Director, Malaria Research Centre, 22 Sham Nath Marg, Delhi–110 054	*Member*
(10)	Mr. H.D. Mamgai, Accounts Officer, Malaria Research Centre, 22 Sham Nath Marg, Delhi–110 054	*Member*
(11)	Mr. S.C. Sharma, Administrative Officer, Malaria Research Centre, 22 Sham Nath Marg, Delhi–110 054	*Member*
(12)	Prof. A.P. Dash, Director, Malaria Research Centre, 22 Sham Nath Marg, Delhi–110 054	*Member Secretary*

Scientific Staff of the Centre (as on 31 March 2005)

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Senior Research Scientists

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