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MALARIA RESEARCH CENTRE
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CONTENTS

AN OVERVIEW OF THE ACTIVITIES OF THE CENTRE

Malaria Research Centre has a network of well-equipped laboratories at Delhi and 12 field units in malarious areas. The Centre is engaged in finding short-term and long-term solutions to the problems of malaria through basic applied and operational field research and also aims at providing useful inputs for strengthening the national antimalaria programme. Research activities carried out during the reporting period have provided useful information in the fields of malaria entomology, parasite biology and epidemiology.

Entomological studies carried out at the Centre were directed towards developing molecular tools for the identification of sibling species of malaria vectors, studies on the bionomics of malaria vectors for better understanding of malaria transmission dynamics and to have alternate vector control options. A pictorial key for the identification of all 58 anopheline species of India was prepared for malaria field workers. Mapping the distribution of *An. culicifacies*, *An. fluviatilis* and *An. dirus* sibling species continued in different parts of the country. Population cytogenetic studies revealed the existence of a new species in *An. fluviatilis* complex. Molecular diagnostic assays developed for the differentiation of members of species complexes were validated with field-collected samples. The molecular basis of refractory mechanism in *An. culicifacies* to plasmodial infection is being worked out. Research activities for vector control included evaluation of new insecticides, biolarvicide formulations, IGR compounds, etc. against immature stages of vector mosquitoes. A large number of plant extracts were screened for their adulticidal, larvicidal and repellent properties.

Good knowledge of the biology of human malaria parasites is a prerequisite for development of new antimalarials and effective malaria vaccine and for proper understanding of malaria epidemiology. Field isolates of *P. falciparum* and *P. vivax* from different parts of India were analyzed for polymorphism of MSP-1 and MSP-2 microsatellite markers and MSP-3a to study genetic diversity and phylogenetic aspects. Studies were carried out on genetic polymorphism of T-cell epitopic region of circumsporozoite protein (CSP) of *P. falciparum* isolates from different regions which will have an important bearing on malaria vaccine development. Reactivity of monoclonal antibodies with *P. vivax* infected erythrocytes and parasite lysate was checked with an objective to develop *P. vivax* diagnostic kit. Likewise studies are in progress to use glycophospholipid (GPL) as a candidate antigen for diagnosing falciparum infection by laser immunoassay. Biochemical studies on parasite enzyme systems were undertaken. *P. vivax* aspartic protease has been purified and characterized for its use as potential drug target. The malaria parasite bank is supporting a large number of organizations working on various aspects of malaria. Screening of medicinal plant extracts/fractions for their antiplasmodial activity against chloroquine sensitive and resistant *P. falciparum* isolates was routinely carried out at parasite bank.

In view of antimalarial drug resistance problem in malaria control therapeutic efficacy studies with first and second line antimalarials (chloroquine and

sulfadoxine-pyrimethamine) were conducted in uncomplicated *P. falciparum* cases in different parts of the country including international borders, which indicate the need to review the drug policy in certain areas. Decreasing efficacy of existing anti-malarial drugs led to the search of new molecules / herbal products for antiplasmodial activity and some plant products screened at MRC have shown promising results. Rapid diagnostic test kits were evaluated for specificity and sensitivity for their use in national malaria control program. Testing of vaccines requires well-characterized field sites where epidemiology of disease is well understood. In this context Phase II activities were started for the preparation of field site for malaria vaccine trial in Sundargarh district, Orissa and a new site is being developed at Jabalpur, Madhya Pradesh. Remote sensing studies in Karnataka have shown good correlation between certain landscape features and malaria incidence, which can be used for malariogenic stratification in problematic paradigms of malaria.

Field units of the Centre actively participated in clinical drug trials, evaluation of diagnostic kits, new insecticides and insecticides treated nets. Besides some important projects have been initiated / completed during the year. A study on the health impact assessment of Sardar Sarovar Narmada water resources development project on mosquito-borne diseases was initiated by Nadiad field unit. Project to develop strategy for integrated control of vectors of malaria, JE and dengue in Gujarat, Madhya Pradesh and Karnataka states has been completed by Nadiad, Jabalpur and Bangalore field units. Evaluation of botanical pesticides was carried out at Hardwar field unit and use of larvivorous fish was scaled up in five talukas and four districts and Mangalore city, Karnataka under the supervision of Bangalore field unit. In addition technical advise, consultancy services were provided to local / state health authorities and other governmental / non-governmental organizations.

Centre celebrated National Science Day by organizing health camps and exhibitions, participated in "Science EXPO-2003" exhibition at Bangalore. "Hindi Week" was celebrated at MRC, Delhi by organizing workshop, symposium and competitions. The Journal of Vector Borne Diseases superseded Indian Journal of Malariology thus widening the scope for contributors. "Malaria Patrika" the popular Hindi magazine was brought out periodically to create awareness in the community about malaria and its control. Seven training courses for medical officers were organized by MRC in collaboration with NVBDCP. Students / researchers from different universities / institutes were provided training on various aspects of malaria by the scientists of the Centre. Nearly 55 research papers were published in national / international journals and MRC scientists participated and presented their research findings in various conferences / workshops held at national and international level.

DIRECTOR

1. Entomology

VECTOR BIOLOGY

Anopheles culicifacies Complex

Bionomics and Distribution Pattern

Anopheles culicifacies population from highly malarious villages in the District Angul (Orissa) was examined for sibling species composition. Results revealed that species B and C were sympatric in the study villages comprising 57.7 and 42.3% respectively of the total identified. Both the species were found primarily zoophagic. In Madhya Pradesh, cytological examination of *An. culicifacies* samples from hilly-forested areas of District Dindori revealed predominance of species C, an established vector of malaria. In study villages of Kutch and Surendranagar districts (Gujarat) species A was predominant and polymorphic for i^1 inversion. Predominance of species A correlated well with high malaria incidence in study areas of these districts.

Mapping the geographic distribution of *An. culicifacies* species revealed prevalence of sibling species B and C in hilly-forest areas of District Angul (Orissa); predominance of species C in District Dindori (M.P.) and species A in Kutch and Surendranagar districts (Gujarat)

Studies on detection and characterisation of organophosphate-resistance in *An. culicifacies* sibling species in Madhya Pradesh

Surveys carried out in villages of Mohkher and Pandhurna blocks in District Chhindwara (M.P.) in November and December 2001 and March 2002 on the M.P.– Maharashtra border revealed that the *An. culicifacies* population was resistant to DDT (36–84%) and malathion Mohkher population was 14–40% resistant, while in Pandhurna the resistance was in the range of 36–63%.

Further studies were carried out in October 2003 on this species to assess the susceptibility status to different insecticides, biochemical resistance mechanisms, differential susceptibility status of sibling species to malathion and synergist studies to determine resistance mechanisms. Complete mortality was observed in *An. culicifacies* against organophosphate-insecticides fenitrothion and fenthion, carbamates, propoxur, bendiocarb and to deltamethrin, a synthetic pyrethroid insecticide in insecticide susceptibility tests. To malathion the species was 50% susceptible and confirmed earlier results.

Synergist exposures with a carboxylesterase inhibitor, triphenylphosphate (TPP) followed by malathion have indicated continued synergism with different concentrations (5–25%) of TPP impregnated papers. The observed mortalities were in the range of 69–88% and with malathion alone it was 50%. While exposures with a mixed function oxidases (MFOs) inhibitor, piperonyl butoxide (PBO) followed by malathion indicated continued antagonism against different concentrations (5–25%) of synergist impregnated papers and non-involvement

of MFOs as a mechanism to confer malathion resistance. The observed mortalities were in the range of 27–38% and were less than the observed mortality (50%) with malathion alone.

Biochemical studies on malathion resistant *An. culicifacies* population from District Chhindwara indicated involvement of carboxylesterase as the major mechanism for conferring resistance. PCR assays indicated differential susceptibility status to malathion in *An. culicifacies* species B & C

Microplate assays for determining resistance mechanisms indicated non-involvement of general esterases and insensitive acetylcholinesterase in conferring malathion resistance. These assays have supported the observations made with synergist (TPP)-malathion bioassays indicating the involvement of carboxylesterase for conferring malathion resistance.

PCR assays were carried out to assess the prevalence of sibling species and species B and C were found sympatric in respective proportions of 73% (n=101) and 27% (n=37). The observed percent mortalities against malathion in species B and C were respectively 68 and 13.5% ($p > 0.001$) indicating increased resistance in species C. Similar observations were made in our earlier studies in Andhra Pradesh and Gujarat states where these two species are prevalent and sympatric.

Molecular diagnostic assays for the identification of members of *Anopheles culicifacies* complex

The two regions, inter-transcribed sequence 2 (ITS2) of rDNA and cytochrome oxidase II (COII) of mitochondrial DNA were analysed to find species-specific variations to differentiate the so far reported five sibling species of *An. culicifacies* complex. The sequence alignment of COII was utilised to design primers that could differentiate all the five species in two PCR assays on the pre-grouped A/D and B/C/E species by D3/D2-PCR assay. The approach followed for differentiating all the five members of *An. culicifacies* complex was—first, D3/D2 PCR assay to differentiate A/D from B/C/E; second, A-D-PCR to differentiate species A from species D; and third, B-C-E-PCR to differentiate the three species, B from C from E. These PCR assays were validated for field use on about 250 mosquitoes collected from five districts in five states having different sympatricites and the results of molecular assays well correlated with cytological identification.

Standardised PCR assays could differentiate so far reported all five sibling species of *An. culicifacies* complex. Results of molecular assays correlated with cytological identification

Population Genetic Analysis of *An. culicifacies* Species A

In addition to the existing 17 microsatellite markers, 14 new markers have been isolated during the year. Primers have been designed for these markers and were tested on positive controls (plasmids) for assessing the amplicon sizes. The size ranged from 104 to 184 bps.

Genotyping was done on two more *An. culicifacies* species A populations from Allahabad (n=24) and Udaipur (n=14) for eight markers. These populations have been found polymorphic for these markers.

In situ hybridization is being done with these markers to construct the physical map of the species A using biotin labelled probes of the clones on the polytene chromosomes. These studies are being done on laboratory reared and field collected species A females.

Anopheles fluviatilis Complex

Distribution, Bionomics and Biology of Sibling Species

Mapping the geographical distribution of *An. fluviatilis* sibling species continued. Samples examined from Kutch, Narmada, Vadodara (Gujarat) and Mysore (Karnataka) districts revealed prevalence of only species T whereas in district Bhopal (Madhya Pradesh) species T and U were found sympatric. *An. fluviatilis* collected from Iran Shahr, Baluchistan (Iran) were also examined and species T was found prevalent in that area. In contrast only species S was prevalent in Nuapada district (Orissa) which was found highly anthropophagic.

Population cytogenetic studies established the cytological variant observed in *An. fluviatilis* population in District Hardwar (Uttaranchal) as new species provisionally designated as species V

In order to resolve taxonomic status of the new cytological variant observed in *An. fluviatilis* complex, a longitudinal study was carried out in Laksar PHC of District Hardwar (Uttaranchal). Cytological examination of *An. fluviatilis* samples collected from villages Dargahpur, Auspur and Ismilepur during pre-monsoon, monsoon and post-monsoon months revealed that the new cytotype was prevalent in all the seasons and in sympatric association with species T and U. Detailed examination of ovarian polytene chromosomes revealed yet another inversion on chromosome arm 3 of the new cytotype. Thus, presence of two fixed paracentric inversions on polytene chromosomes with total absence of inversion heterozygotes unequivocally establish this cytological variant as new species provisionally designated as species V in the *An. fluviatilis* complex. Morphological identification of cytologically identified specimens confirmed them as *An. fluviatilis*. Species V was found sympatric with species T and U in all the study villages and majority of its specimens were found resting in human dwellings or mixed dwellings. Studies on the bionomics and vectorial potential of species V have been initiated. In addition, efforts are being made to colonise this new species for various laboratory studies.

Development of Comprehensive PCR-based Assay for the Identification of all the Members of *An. fluviatilis* Complex in Consequence of Discovery of New Species

Fourteen new microsatellite markers were isolated in addition to existing 17 markers for population genetic analysis of *An. culicifacies*

Previously an allele-specific PCR assay was developed for the identification of all three known members of the *An. fluviatilis* complex (species S, T and U), which is based on D3 domain of 28S rDNA (Singh *et al*, *Am J Trop Med Hyg* 2004; *70:* 27). The discovery of new species—species V, in *An. fluviatilis* species complex necessitated the development of a comprehensive molecular assay for the identification of all members of the complex. When new species was tested with the existing species specific diagnostic PCR assay, it was not possible to differentiate species V from species U.

To develop comprehensive PCR-based assay, D3 domain of 28S rDNA of new species was sequenced and aligned with the sequences of other three members

of the complex. The sequence of species V was found to differ from rest of the three species by at least four base pairs. However, these differences were not suitable to design species V-specific primer, therefore the sequences of all the four species were screened for presence of unique restriction sites. Species V was found to have three unique restriction sites which were absent in all other species. Thus, a PCR-RFLP assay was developed which can differentiate all the members of the complex (Fig. 1.1). The assay is to be validated using cytologically-identified specimens.

A new PCR assay was developed to differentiate *An. fluviatilis* species S, T, U & V. The newly identified species V has three unique restriction sites which the others do not possess

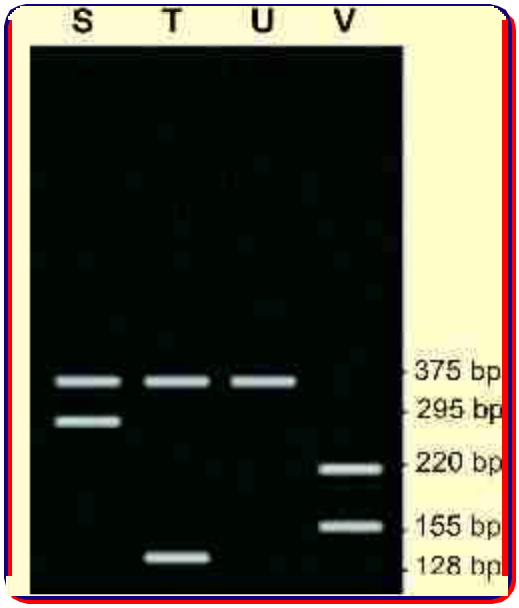


Fig. 1.1: Diagrammatic representation of PCR-RFLP assay for differentiation of all members of *An. fluviatilis* complex (S—Species S; T— Species T; U—Species U; V—Species V)

Anopheles minimus Complex

Morphologically identified specimens of *An. fluviatilis* collected from Assam were sequenced for D3 domain of 28S rDNA. Alignment of these sequences with database of GeneBank using BLAST and alignment with *An. minimus* sequence generated by MRC revealed complete homology with *An. minimus* species A. Keeping in view that the *An. fluviatilis* is sympatric with *An. minimus* species A in this area, investigation is on whether morphologically identified *An. fluviatilis* from Assam are *An. minimus* species A. Other variable regions of rDNA are also being sequenced for divergence and phylogenetic studies.

Anopheles dirus Complex

Identification of Members of *An. dirus*

Species distribution of *An. dirus* complex in Arunachal Pradesh and Assam was established by PCR assays using published primers designed from ITS2 region (Walton *et al*, *Med Vet Entomol* 1999; *13:* 24–32). The assays were carried out on few field-collected mosquitoes (n=11). Results indicated prevalence of species D (diagnostic fragment of 306 bp size) in the two collections made from Arunachal Pradesh and Assam states (Fig. 1.2).

Mosquito Fauna Survey and Identification Key

Mosquitoes of Deciduous Dry Area (Bhopal, Madhya Pradesh)

A mosquito fauna survey was carried out in deciduous dry area of Bhopal district during February 2003. A total of 23 villages were surveyed covering

the entire topography—hilly, plain, irrigated and urban areas of the district. A total of 6927 mosquitoes belonging to six genera—*Anopheles, Aedes, Culex, Armigeres, Mansonia* and *Toxorhynchites* were collected using the standard WHO techniques. The most dominant genus was *Anopheles* (5231 specimens) followed by *Culex* (933 specimens) and *Aedes* (247 specimens). In genus *Anopheles* 11 species were collected. The most dominant species was *An. culicifacies* followed by *An. subpictus* and *An. annularis*. Out of 11 species three vector species—*An. culicifacies* from rural areas, *An. fluviatilis* from foothill areas and *An. stephensi* from urban areas were collected. The larvae of eight anopheline species were collected from different habitats—ponds, streams, canals, wells, storage tanks, etc. Maximum number (37) out of 46 *An. fluviatilis* were collected during the night collection. All other species were found resting indoor and maximum number of specimens were collected during the day time. This is the first detailed report of mosquito fauna of Bhopal district.

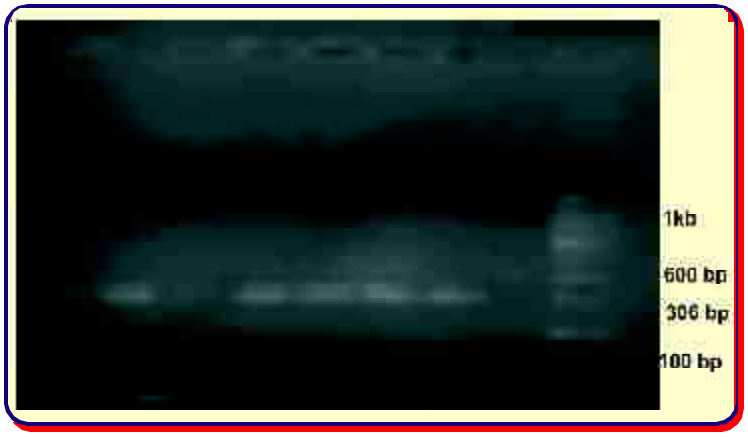


Fig. 1.2: Diagram showing diagnostic fragment for the identification of *An. dirus* species D

A pictoral key for the identification of all 58 anophelines was developed for use in the field

Anopheles Identification: Field Key

With the financial support of Defence Research Laboratory, Tezpur preparation of a pictorial key to identify the 58 species of Indian anophelines in the field by researchers, field workers and technicians is in progress. Drawings of 40 anopheline species have been completed and identification table for the species is also in progress.

Molecular stuidies on *P. vivax*-refractory *An. culicifacies* revealed quantitative difference in serine protease transcript in refractory and susceptible strains

VECTOR-PARASITE INTERACTIONS

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

Serine protease in recalcitrant and susceptible strains of *An. culicifacies*

Structure of gene: Earlier the cloning of serine protease gene from refractory and susceptible strains of mosquito was reported. A comparison of cDNA sequence from these strains did not reveal any difference. Nevertheless the northern blot analysis and quantitative RT-PCR clearly revealed differences in the abundance of the transcript, which co-related with the observed differences in the catalytic activity of serine proteases in susceptible and recalcitrant strains. To account for such an observation we isolated genomic clone from susceptible and recalcitrant

strains and screened for the presence of any intron. A 78 bp intron was detected at the 5' end of the serine protease. Junction and body sequence of intron from either strain did not reveal any difference, which could account for the reduced catalytic activity in the susceptible strain. We now propose to isolate promoter for serine protease from both the strains of mosquito and examine its efficiency using reporter genes. It is likely that a mutation in the promoter is responsible for reduced serine protease activity in the susceptible strain.

Regulation of serine protease and expression in *E. coli*

Serine protease is synthesised as a zymogen and is activated upon removal of propeptide located at its N-terminal end. It is generally believed that the propeptide inhibit their cognate enzyme specifically. We synthesised a peptide corresponding to the pro-region. Titration of serine protease with various concentrations of propeptide exhibited a strong inhibition by the propeptide. The Ki for the propeptide was calculated by plotting double reciprocal plots and compared with a routinely employed serine protease inhibitor, leupeptin. The Ki for the propeptide was 0.17µM and for leupeptin was 2µM. We are now exploring the possibility if the observed difference in the catalytic activity of serine protease is a consequence of differential release of propeptide from the zymogen.

In northern blot analysis it was revealed that upon blood meal the transcript abundance increased significantly in the recalcitrant females, while it was barely found in the susceptible females of *An. culicifacies*

Phenol oxidase activity in *An. culicifacies*

The 2.4 kb cDNA ORF of the proPO was sub cloned in a pET32a expression vector between NOT1 sites and was successfully expressed in BL21DE3 host strain of *E. coli*. The protein product of cloned proPO ORF corresponds to 97 kDa. Ac-proPO is expressed as a Trx fusion protein in pET32a vector. The expression

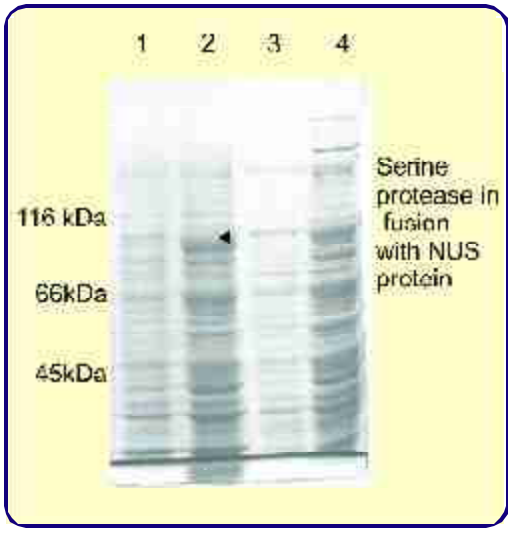


Fig. 1.3: Expression of 30 kDa serine protease in fusion with 65 kDa NUS protein using pET43a expression vector. Inclusion bodies prepared from uninduced culture (lane 1) and induced culture (lane 2). Supernatant prepared from uninduced culture (lane 3) and induced culture (lane 4)

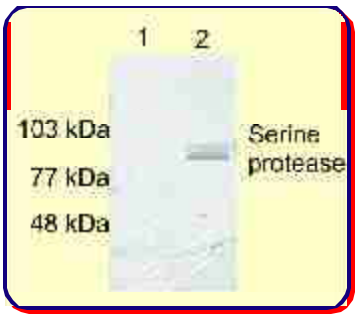


Fig. 1.4: Western analysis of recombinantly expressed serine protease using anti-His antibodies. Lane 1—Inclusion bodies prepared from uninduced culture and lane 2—induced culture

product was found to be present in inclusion bodies. At several growth regimen or IPTG induction conditions expression was detected in the soluble fraction only. To obtain the recombinant prophenol oxidase in catalytically active form, the 2.4 kb Ac-proPO was re-cloned in pET43a vector and expression was studied in BL21DE3 *E. coli* strain. In pET43a, a phenol oxidase is expressed as a NUS fusion protein at an expected size of 130 kDa. Expression was observed in the soluble fraction of the total *E. coli* proteins. On an incubation of soluble protein containing 30–80% phenol oxidase, with Dopamine or 4-Methyl catechol, we could not observe any activity of the phenol oxidase either before or after activation with trypsin (Figs. 1.3 & 1.4).

Northern blot analysis of Ac-proPO expressions

Using a 2.4 kb P^{32} labelled Ac-proPO as a probe, a transcript of expected size was detected in a Northern blot experiment of total body RNA from recalcitrant strain whereas a low expression was also observed in a susceptible strain. Adult males, which do not feed on blood, did not show any sign of proPo expression. To see the stage-specific expression of the prophenol oxidase, total RNA from different developmental stages of the mosquito was probed with the same probe as above. Result showed a high expression of Ac-proPO in the IV instar larvae, as it was low in the II and the III instar larvae. Upon blood meal the transcript abundance increased significantly in the recalcitrant females, while it was barely detectable in the susceptible female mosquitoes.

VECTOR CONTROL

Evaluation of VectoBac Tablets (Formulation of *Bti* H-14) against Larvae of Mosquito Vectors (Contract Research Project with M/s. Sumitomo Chemicals India Pvt. Ltd., Mumbai)

VectoBac tablets formulation of *Bti* H-14 provided complete control of immature & stages of vector mosquitoes up to two weeks.

A new anti-larval product VectoBac tablet was evaluated in small-scale field trials in specified breeding habitats of *An. stephensi* and *Ae. aegypti* in NCT of Delhi, Chennai and Nadiad.

In natural field conditions the testing was carried out at different doses in water storage cement tanks, iron drums, desert coolers and mud pots to ascertain the efficacy of the test larvicide which was assessed by measuring the larval density. VectoBac tablets were used at the dosage of $^1/_2$, 1 and 2 tablets per sq m and density was monitored up to 3 days and after an interval of 7 days.

Results of field testing in NCT of Delhi showed that dose of 2 tablets (0.76 g per sq m) in cemented tanks gave complete control of late instars and pupae of *An. stephensi* and *An. subpictus* up to 2 weeks period. Cent percent reduction in the densities of immatures was also achieved up to 2 weeks period against *Ae. aegypti* and *Cx. quinquefasciatus* in iron drums, desert coolers and mud pots when treated @ 2 tablets per habitat. VectoBac tablets were safe to non-target species *Gambusia affinis*, a larvivorous fish and notonectid bug *Anisops sordae.*

The results of this study would help in selection of appropriate dosage and frequency of application and the use of VectoBac tablets can be an additional tool for control of mosquito larvae and could be one of the choices in the larval control programmes in urban areas.

Laboratory and Field Evaluation of VectoBac WDG (*B. thuringiensis* var *israelensis*) Formulation against Immatures of Mosquitoes (Sponsored Research Project by M/s. Sumitomo Chemicals India Pvt. Ltd., Mumbai)

A study to evaluate the bioefficacy of a new formulation of *Bti*—VectoBac WDG provided by M/s. Sumitomo Chemicals Pvt. Ltd., was carried out in the laboratory against immatures of *An. culicifacies*, *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*. The biolarvicide formulation was most effective against larvae of *Cx. quinquefasciatus*, followed by *Ae. aegypti*, *An. stephensi* and *An. culicifacies*. The LC_{50} values against these four mosquito species were determined as 0.025, 0.046, 0.245 and 0.35 mg/l, respectively.

VectoBac WDG application @ >0.1 g/m² could produce 100% larval mortality in *An. stephensi* and *Ae. aegypti* up to five days and one week respectively

In field conditions the trial was carried out in breeding habitats ranging between 50 and 500 m^2 for the breeding of *An. culicifacies* and smaller breeding habitats such as cement tanks for the breeding of *An. stephensi* and desert coolers for the breeding of *Ae. aegypti*. Observations were made by counting immatures of target mosquito species in the experimental and control habitats, before and after the treatment up to 3 days and then after an interval of 7 days. Four doses were applied in the field @ 0.05, 0.1, 0.2 and 0.5 g/m^2 with the help of sprayer after dilution. Trials are in progress and initial results indicated that VectoBac application produced 100% control of L-3 and L-4 stages of *An. stephensi* up to Day 5 at all the dosages except 0.05 g/m^2 Around 80% reduction was however maintained up to two weeks at 0.05 g/m^2 The impact on *Ae. aegypti* breeding in coolers was 100% up to one week.

Bio-efficacy of Pirimiphos-Methyl 50% EC against Immatures of *Anopheles* and *Culex*

Results of laboratory evaluation of pirimiphos-methyl 50% EC against the larvae of *An. culicifacies*, *An. stephensi* and *Cx. quinquefasciatus* are shown in Table 1.1. The larvicide formulation was relatively more effective against immatures of

Table 1.1 Bio-efficacy of pirimiphos-methyl against late III instar mosquito larvae in the laboratory bioassay test

Mosquito species	Lethal concentration (ppm a.i.)		χ^2 (df)
	LC_{50} (95% Confidence limit)	LC_{90}	
An. culicifacies	0.032 (0.027–0.037)	0.057	1.79 (2)
An. stephensi	0.023 (0.019–0.027)	0.045	1.22 (2)
Cx. quinquefasciatus	0.040 (0.035–0.045)	0.114	10.54 (2)

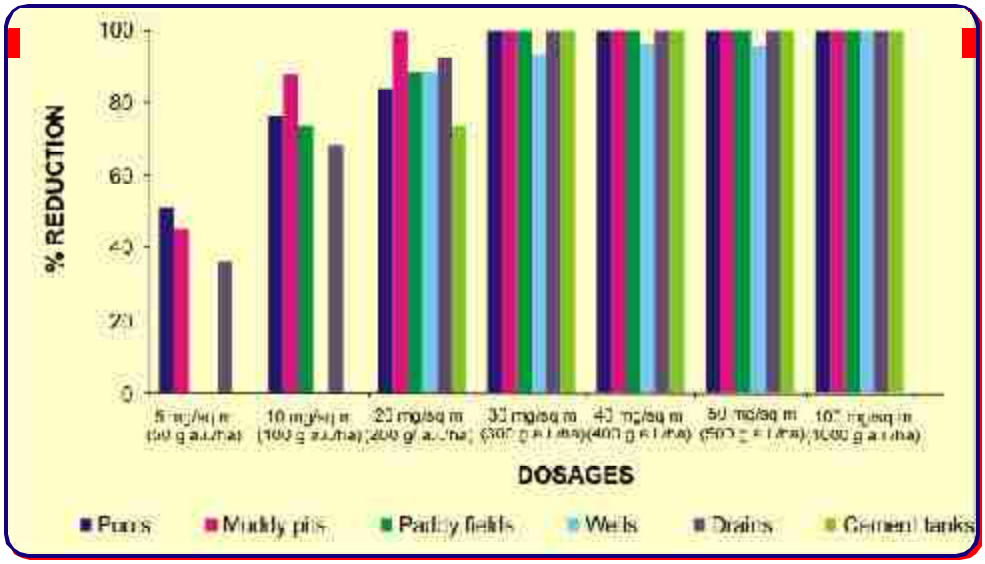


Fig. 1.5: Percent reduction in larval density of anophelines within 24 h after treatment

Anopheles species than *Culex* species. The LC_{50} and LC_{90} values against the larvae of *An. culicifacies* and *An. stephensi* were 0.032 and 0.05 ppm; and 0.023 and 0.045 ppm, respectively, as against 0.04 and 0.114 ppm for *Cx. quinquefasciatus.*

In laboratory and field trials, Pirimiphos-methyl 50% EC was found to be more effective against immature stages of anophelines in fresh water than culicines in polluted water

Fields trials were carried out in muddy pits and pools, irrigation channels, unused wells, cement tanks, waste commodes, polluted drains and paddy fields to determine the optimum dosage against immatures of *Anopheles* and *Culex* spp. Application of pirimiphos-methyl @ 100 g a.i./ha and above resulted in 100% reduction in the larval density of anophelines within 24 h after the treatment (Fig. 1.5). However, the bio-efficacy (percent reduction in the larval density on

Table 1.2 Bio-efficacy of pirimiphos-methyl 50% EC against larvae of anopheline immatures

Habitats	% Reduction after one week						
	5 mg/m^2 (50 g a.i./ha)	10 mg/m^2 (100 g a.i./ha)	20 mg/m^2 (200 g a.i./ha)	30 mg/m^2 (300 g a.i./ha)	40 mg/m^2 (400 g a.i./ha)	50 mg/m^2 (500 g a.i./ha)	100 mg/m^2 (1000 g a.i./ha)
Pools	51.2	76.3	83.4	100	–	–	–
Muddy pits	45.1	87.6	100	–	–	–	–
Paddy fields	–	74	88.2	100	–	–	–
Wells	–	–	88	93	96.5	96	100
Drains	36	68.4	92.3	100	–	–	–
Cement tanks	–	–	73.3	100	–	–	–

Surface area—Pools (8 to 15 m^2); Muddy pits (3.6 to 4.2 m^2), Paddy fields (542 to 916.6 m^2); Wells (2.4 to 3.6 m^2); Average area of waste commodes was 5.9 m^2 and depth was 6.45 inches. Average area of cement tanks in Goa was 5.6 m^2 and depth was 17 inches. Drains (14 to 106.2 m^2) and Cement tanks (5.5 to 8.6 m^2).

Day 7 after the treatment) of pirimiphos-methyl against immatures of anopheline species in different types of breeding habitats ranged between 64 and 100% @ 100 g a.i./ha and > 80% @ 200 g a.i./ha (Table 1.2). In pits and pools with *Anopheles* breeding consisting mainly of *An. culicifacies*, 100% reduction in immature density was obtained with a dosage of 200 and 300 g a.i./ha respectively. At lower doses @ 100 g a.i./ha, 76 to 87% reduction was observed on Day 7 in small shallow water bodies. At higher doses @ 400 and 500 g a.i./ha, 100% reduction was recorded up to three weeks. In paddy fields and irrigation channels, with anopheline breeding consisting mainly of *An. culicifacies* and *An. subpictus*, 100% reduction of immature density was observed @ 300 g a.i./ha. In wells used for irrigation purpose, supporting breeding of *An. culicifacies* and other species, 93 to 96% reduction of anopheline immatures density was observed @ 300 to 500 g a.i./ha, while cent percent reduction was observed @ 1000 g a.i./ha. In cement tanks where *An. stephensi* and *An. subpictus* breeding was observed, 100% reduction in density/dip was observed @ 100 to 200 g a.i./ha.

An. stephensi breeding was also commonly observed in waste commodes (WCs) in Goa. The efficacy of pirimiphos-methyl against breeding of *An. stephensi* in WCs, was also determined @ 100 and 200 g a.i./ha. Results revealed > 80% reduction @ 100 g a.i./ha as against 100% reduction @ 200 g a.i./ha up to one week (Table 1.2).

These trials clearly indicate that in fresh water habitats of *Anopheles* species pirimiphos-methyl @ 200 g a.i./ha will be required to obtain 80 to 100% reduction invariably in all the habitats at weekly intervals.

Results of field trials against *Culex* species, carried out in polluted pools and muddy pits, unused wells and drains are given in Fig. 1.6 and Table 1.3. The efficacy of pirimiphos-methyl against immatures of *Culex* species was determined at different doses ranging from 50 to 1000 g a.i./ha. Results revealed almost 100% reduction in the density of immatures @ 200 g a.i./h with in 24 h after the

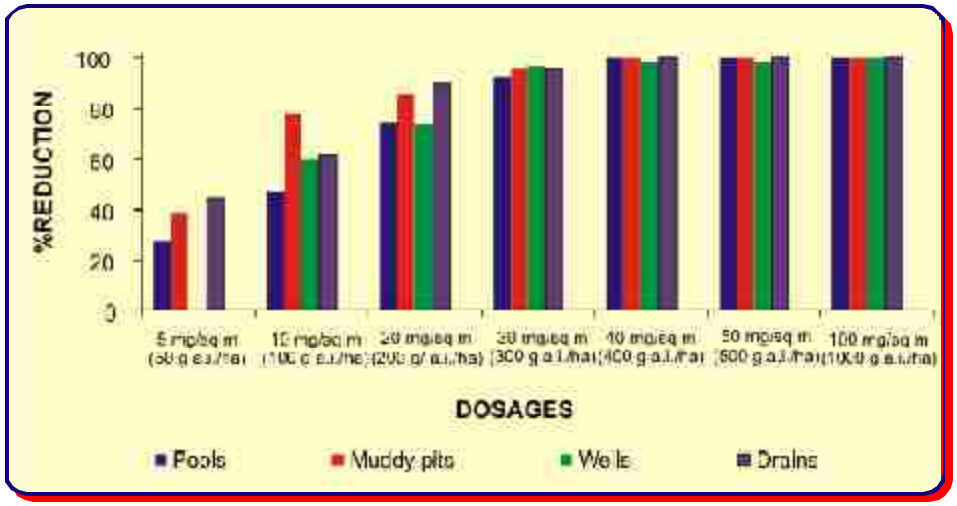


Fig. 1.6: Reduction in larval density of *Cx. quinquefasciatus* within 24 h after treatment

Table 1.3 Bio-efficacy of pirimiphos-methyl 50% EC against larvae of culicine immatures

Habitats	% Reduction after one week						
	5 mg / m^2 (50 g a.i. / ha)	10 mg / m^2 (100 g a.i. / ha)	20 mg / m^2 (200 g a.i. / ha)	30 mg / m^2 (300 g a.i. / ha)	40 mg / m^2 (400 g a.i. / ha)	50 mg / m^2 (500 g a.i. / ha)	100 mg / m^2 (1000 g a.i. / ha)
Pools	27.6	47.3	74.6	93.1	100	–	–
Muddy pits	39.2	78.4	86	95.4	100	–	–
Wells	–	59.7	74	96.9	97.9	98.4	100
Drains	44.1	61.4	90.4	95.8	100	–	–

Note: Surface area—Pools (8 to 21.1 m^2); Muddy pits (3.4 to 4 m^2); Unused wells (1.69 to 3.3 m^2); and Polluted drains (79 to 110 m^2).

treatment in different habitats (Fig. 1.6). However, the reduction @ 200 g a.i. / ha was even < 80% after one week but > 80% reduction was obtained at the dose of 300 g a.i. / ha and above in all the habitats (Table 1.3). In unused wells supporting *Culex* breeding, 100% reduction in the density of immatures was observed at a dose of 300 g a.i. / ha. At higher doses similar impact, however, persisted for three days to four weeks. In polluted drains supporting *Culex* breeding, 90% reduction was observed on Day 4 @ 200 g a.i. / ha and higher doses. These trials clearly indicate that in case of culicine species in different types of polluted water habitats, 90–100% reduction in the density of immatures can be obtained @ 200–400 g a.i. / ha by weekly application of the larvicide. These results also suggest that pirimiphos-methyl is more effective against anopheline immatures in clear water than culicine immatures in polluted water habitats.

Laboratory bioassays carried out to determine toxicity of pirimiphos-methyl against *Gambusia affinis* revealed that pirimiphos-methyl is not toxic to fish at a

Table 1.4 Toxicity of pirimiphos-methyl 50% EC against the larvivorous fish *Gambusia affinis* under laboratory condition

Dosage (ppm)	No. of fish in each replicate	% Mortality after (h)		
		24	48	72
1.0	25 x 4	24(24)	24(24)	24(24)
0.5	25 x 4	12(12)	12(12)	12(12)
0.25	25 x 4	4(4)	4(4)	4(4)
0.125	25 x 4	0(0)	0(0)	0(0)
0.0625	25 x 4	0(0)	0(0)	0(0)
0.3125	25 x 4	0(0)	0(0)	0(0)
Control	25 x 4	0(0)	0(0)	0(0)

Figures in parentheses indicate number exposed.

concentration of < 0.25 ppm, as no mortality was observed in any of these concentrations tested up to 72 h. However, at higher concentrations there was some mortality in larvivorous fish in the laboratory bioassays and therefore, doses higher than 0.25 ppm are not safe to this non-target species (Table 1.4). Toxicity test of pirimiphos-methyl to the main non-target organism—*Gambusia affinis* which is being used extensively under EMCP, suggest that this larvicide should not be used at concentration above 0.25 ppm in habitats harbouring the larvivorous fish.

Evaluation of IGR-Triflumuron against Larvae of Mosquito Vectors (Contract Research Project with M/s. Bayer India Ltd., Mumbai)

Field trial carried out in Delhi and Sonepat district (Haryana) to evaluate IGR- Triflumuron was completed this year. In small-scale field trials Triflumuron was applied at doses of 0.25, 0.5 and 1 ppm in breeding habitats of *An. culicifacies* and *Cx. quinquefasciatus*. Results showed that a dose of 1 ppm was most effective up to two weeks against *An. culicifacies* in paddy field.

Prospecting for Botanical Pesticides: Screening of Bio-activity of Plant Extracts against Mosquitoes particularly Anopheles spp. (DBT Funded Collaborative Project)

This multi-institutional collaborative project funded by DBT was carried out in collaboration with various laboratories as shown in Table 1.5. Bio-activity of various herbal extracts / fractions / formulations received from five laboratories was determined against mosquitoes particularly the malaria vector *An. stephensi* using standard protocol which included larvicidal, adulticidal properties and mosquito repellency.

Since beginning of this project a total of 331 samples have been received at MRC for bioassays against malaria vector (*An. stephensi).* Of these 48 samples showed larvicidal activity (70–100% mortality) at 250 ppm, while 14 samples

Table 1.5 Activities of various laboratories involved in the project

Activity	Responsible laboratories
Collection, preservation and taxonomic identification of plants	RRL, Jammu; RRL, Trivandrum; NBRI, Bangalore
Extraction and fractionation of herbal products	IIT, Delhi; FRI, Dehradun; EID Parry (India) Ltd, Bangalore; RRL, Trivandrum ; RRL, Jammu
Bioassay testing of the efficacy against agricultural pests	IHBT, Palampur; EID Parry, Bangalore
Bioassay testing of the efficacy against mosquitoes *(An. stephensi)*	MRC, Delhi & Hardwar
Formulation of the selected samples	IIT, Delhi; IPFT, Gurgaon

showed insecticidal activity against adult mosquitoes and five samples have shown repellent activity for more than one hour (Table 1.6). Tables 1.7 to 1.9 show the results of preliminary screening of larvicidal, adulticidal and repellent activity of different samples received from different laboratories.

Table 1.6 Samples received at Malaria Research Centre and their screening status

Laboratory / Institute	Received	Screened	Under screening	+ve results		
				L	A	R
RRL, Trivandrum	76	76	0	8	1	1
FRI, Dehradun	77	77	0	16	10	4
IIT, Delhi	56	56	0	5	2	–
RRL, Jammu	66	66	0	11	1	–
EID Parry, Bangalore	56	41	15	8	0	0
Total	331	316	15	48	14	5

L—Larvicidal; A—Adulticidal; R—Repellent property.

Of the 331 herbal extracts screened for pesticidal activity, 48 showed larvicidal, 14 insecticidal and five repellent activity when tested against An. stephensi

Table 1.7 Plant extract samples showing larvicidal activity within 24 hours

S. No.	Sample code number	Larvicidal activity		
		Show	LC_{50}	LC_{90}
1.	NBDB (4)-002B-07-P-13a	(+)	*	*
2.	NBDB (4) 022B-08-P-10a	(+)	*	*
3.	NBDB (4) 023B-07-P-10a	(+)	*	*
4.	NBDB (4) 042B-07-P-10a	(+)	*	*
5.	NBDB (2) 008A-06-P-01a	(+)	166	234
6.	NBDB (2) 010A-06-P-10a	(+)	*	*
7.	NBDB (2) 010A-06-P-10b	(+)	153	261
8.	NBDB (4)-001B-08-P-13a	(+)	117	276
9.	NBDB (4)-033B-07-P-10a	(+)	250	450
10.	NBDB (3)-022i-08-P-11e1	(+)	119	182
11.	NBDB (2)-005A-07-P-10a	(+)	138	230
12.	NBDB (2)-005A-07-P-10b	(+)	*	*
13.	NBDB (2)-005A-07-P-10c	(+)	53	266
14.	NBDB (2)-005A-07-P-04a	(+)	58	113
15.	NBDB (2)-005A-07-P-04b	(+)	77	133
16.	NBDB (2)-005A-07-P-04c	(+)	124	250
17.	NBDB (2)-055D-11-P02oil	(+)	87	132
18.	NBDB (4)-022B-08-P-11b	(+)	100	175
19.	NBDB (4)-023B-08-P-11b2	(+)	150	225
20.	NBDB (3)-022I-08-P-11E1	(+)	*	*
21.	NBDB (5) 021D-04-P-11b	(+)	100	150
22.	NBDB (1) 058T-12-P-01b	(+)	150	250

contd...

Table 1.7 *(contd...)*

S. No.	Sample code number	Larvicidal activity		
		Show	LC_{50}	LC_{90}
23.	NBDB (1) 058T-12-P-01c	(+)	120	190
24.	NBDB (1) 022-T-12-P-01b	(+)	75	175
25.	NBDB (1) 022-T-12-P-01c	(+)	160	200
26.	NBDB (1) N13 positive control	(+)	23	43
27.	NBDB (2) 048-A-10-P-10a	(+)	125	200
28.	NBDB (2) 048-A-10-P-10b	(+)	70	175
29.	NBDB (2) 055-D-12-P-02a	(+)	100	200
30.	NBDB (2) 055-D-12-P-02b	(+)	175	250
31.	NBDB (3) 005-D-10-P-10e1	(+)	72	121
32.	NBDB (3) 005-D-10-P-10a1	(+)	97	278
33.	NBDB (3) 005-D-10-P-10b2	(+)	156	248
34.	NBDB (1) 001-K-03-P-02C	(+)	202	287
35.	NBDB (1) N14-K-03-P-09a	(+)	26	57
36.	NBDB (1) N14-K-03-P-09b	(+)	*	
37.	NBDB (5) 056-A-07-P-04a	(+)	142	232
38.	NBDB (5) 056-A-07-P-04b	(+)	113	250
39.	NBDB (5) 056-A-07-P-04cd	(+)	55	125
40.	NBDB (5) 017-K-08-P-01a	(+)	175	317
41.	NBDB (5) 017-K-08-P-01b	(+)	*	
42.	NBDB (5) 017-K-08-P-01cd	(+)	*	
43.	NBDB (5) 052-Q-09-P-04a	(+)	*	
44.	NBDB (5) 052-Q-09-P-04b	(+)	*	
45.	NBDB (5) 042-E-06-P-08a	(+)	38	68
46.	NBDB (5) 042-E-06-P-08b	(+)	137	155
47.	NBDB (2) 005-A-07-P-10b	(+)	139	264
48.	NBDB (2) 008-A-06-P-01a	(+)	166	234

*To be determined after confirmation; (+) Positive activity.

Table 1.8 Plant extract samples showing insecticidal activity against adultmosquitoes

S.No.	Sample code number	Adulticidal activity
1.	NBDB(2)008A-06-P-01a	(+)
2.	NBDB(2)-005A-07-P-10a	(+)
3.	NBDB(2)-005A-07-P-10b	(+)
4.	NBDB(2)-005A-07-P-10c	(+)
5.	NBDB(2)-005A-07-P-04a	(+)
6.	NBDB(2)-005A-07-P-04b	(+)
7.	NBDB(2)-005A-07-P-04c	(+)
8.	NBDB(2)-055D-11-P02oil	(+)
9.	NBDB(1)- N-13	(+)
10.	NBDB(3)-005-D-10-p-10e1	(+)
11	NBDB(5)- 042 E06 P08a	(+)
12.	NBDB(3)- 005-D-10P-10a	(+)
13.	NBDB(2)-005-A-07-P-10b	(+)
14.	NBDB(2)-055-D-11-P-02 oil	(+)

Table 1.9 Samples showing mosquito repellent activity

S.No.	Sample code number	Repellency activity
1.	NBDB(2)-008A-06-P-01a	(+)
2.	NBDB(2)-005A-07-P-10b	(+)
3.	NBDB(2)-005A-07-P-04a	(+)
4.	NBDB(2)-055D-11-P02oil	(+)
5	NBDB (1)-N13 positive control	(+)

Larvicidal Activity of Crude Aqueous Extract of *Tribulus terristris*

Larvicidal effect of crude aqueous extract of the leaf of a medicinally important plant *Tribulus terristris* (Family: Zygophyllaceae) was tested against *An. culicifacies* species A and C, *Cx. quinquefasciatus* and *Ae. aegypti*. Third and fourth instar larvae were used for bioassays following standard WHO method for a range of concentrations (0.0025 to 0.3% in water). The calculated LC_{50} (lethal concentration for killing 50% of treated larvae) for different species were respectively: *An. culicifacies* species A–2100 ppm, *An. culicifacies* species C–4200 ppm, *Cx. quinquefasciatus*–3800 ppm and *Ae. aegypti*–3200 ppm. The calculated lethal values were at least 10 x more than the earlier recorded values for larvicidal effect of *Solanum nigrum* (Singh *et al*, *Curr Sci* 2001; *81:* 1529–30). It prompted to test extracts from other parts in different solvents.

Spinosins mixture DKVR-0001 mats provided >90% protection against *An. culicifacies*, *An. stephensi* and *Cx. quinquefasciatus*

Effectiveness of Spinosins Mixture DKVR-0001 Mats in Repelling Mosquitoes

Variety of mats and coils are marketed in India to prevent mosquito bites. Mats prepared with spinosins mixture marketed by De Nocil, Mumbai were tested for their efficacy (commercial name–Tracer Cardboard mats 35 x 25 mm). Mats were placed in the electrical device in the room. After putting electrical device a volunteer was asked to place left arm inside the cage covered with nylon net for mosquito feeding. Concurrently control cages were used with simple cardboard soaked in water.

Results revealed that mixture of spinosins mats had strong repellent action against mosquitoes. Against *An. stephensi* a principal vector of malaria, the mats showed 94.7% protection. Similar degree of protection was obtained against *An. culicifacies* (90.6%). However, 91.8% protection was obtained against *Cx. quinquefasciatus* a pest mosquito and vector of filariasis in the country. It was interesting to note that there was low protection against *Ae. aegypti* (21.6%). The results were compared with the results of Good Night mats and there was no significant difference in the efficacy of both mats.

Larvicidal and Mosquito Repellent Activity of Chir (*Pinus longifolia*, Family: *Pinaceae*) Oil

Results of the larvicidal activity of Chir oil against different species of mosquitoes are presented in Table 1.10. In terms of lethal concentration for 50% mortality (LC_{50})

Table 1.10 Larvicidal activity of Chir oil against different mosquito species

Concentration (ppm)	No. tested	No. larvae dead		
		An. stephensi	*Cx. quinquefasciatus*	*Ae. aegypti*
200	100	84	88	96
100	100	38	50	50
50	100	6	24	24
25	100	8	10	10
12.5	100	2	4	4
6.25	100	0	0	0
Control	100	0	0	0

Chir oil was effective at doses ranging between 80 and 112 ppm against larvae of the three mosquito species. Of the three species tested Chir oil was most effective against *Aedes aegypti* (LC_{50}= 82 ppm) followed by *Culex quinquefasciatus* (LC_{50}= 85.7 ppm) and *An. stephensi* (LC_{50} = 112.6 ppm).

Chir oil mats provided ~ 94% protection against *An. culicifacies*. Chir oil provided 100% protection up to 11 h against *An. culicifacies* and > 97% protection up to 9 h against *Cx. quinquefasciatus*

Table 1.11 shows the number of female mosquitoes, which landed on treated and untreated baits in eight night periods from dusk-to-dawn. The Chir oil

Table 1.11 Efficacy of Chir oil and lemon grass oil as mosquito repellent on human volunteers in village Dehra (PHC Dhaulana)

Repellent oil	*Cx. quinquefasciatus*		*An. culicifacies*	
	% Protection	Av. Protection time (h)	% Protection	Av. Protection time (h)
Chir oil	97.4±6.8	9	100	11
Lemon grass	98.5±5.5	9.6	100	11

provided 100% protection for 11 hours against *An. culicifacies*, a principal vector of the northern rural plain areas of India. Against *Culex quinquefasciatus* it provided 97% protection for nine hours. The results were compared with results of lemon grass oil and there was no significant difference in the efficacy of these two types of oils.

Mats prepared from Chir oil were also tested in the field. These mats provided 94.1% protection against bites of *An. culicifacies* with a protection time of 10.3 hours and 88% protection against *Cx. quinquefasciatus* with an average protection time of 7.6 hours.

INSECTICIDE RESISTANCE

WHO Collaborative Study for the Establishment of Diagnostic Concentrations for Bifenthrin and Alphacypermethrin for Resistance Monitoring in Malaria and Dengue Vectors

MRC was identified by WHO for determining the diagnostic concentration of synthetic pyrethroids for field testing of susceptibility status of various mosquitoes

Malaria Research Centre was identified for carrying out the studies to establish the diagnostic doses against two pyrethroid insecticides with six graded doses, alphacypermethrin (0.001 to 0.05%) and bifenthrin (0.01 to 0.5%). *An. culicifacies* species C, *An. stephensi* and *Ae. aegypti* were tested using standard WHO protocols. *An. culicifacies* registered 98% mortality against 0.0025% alphacypermethrin and 0.1% bifenthrin. Similarly for *An. stephensi* 94% mortality was registered against 0.05% alphacypermethrin and 99% against 0.25% bifenthrin. Likewise *Ae. aegypti* registered only 91% mortality against 0.05% alphacypermethrin and 99% against 0.25% bifenthrin. Results have been communicated to WHO for the determination of diagnostic doses against these insecticides.

OTHER STUDIES

Entomological and Epidemiological Field Investigations in Jaisalmer district, Rajasthan

Entomological studies in Jaisalmer district showed that *An. culicifacies* and *An. stephensi* the major malaria vector species in this area were susceptible to DDT, malathion and deltamethrin

District Jaisalmer of Rajasthan state was visited in the month of March 2004 for carrying out malaria situation analysis and to identify malraia risk factors. Epidemiological data of the district for the last 10 years (1994–2003) indicated that 142 sub centres had API >2 and were recognised as high risk areas.

In parasitological studies carried out during the field study a total of 64 blood smears were collected during active surveillance and 19 were positive giving SPR of 29.68. Out of 267 blood samples collected during mass blood survey, 25 were positive for malaria.

In seven PHCs surveyed two malaria vectors *An. stephensi* and *An. culicifacies* were prevalent. Density of *An. culicifacies* was maximum in canal area. *An. stephensi* was prevalent in high density in almost all the PHCs surveyed. Larval surveys were carried out in selected PHCs. *An. culicifacies* breeding was maximum in Indira Gandhi Canal distributaries, while *An. stephensi* was found breeding in all the PHCs surveyed. The most common breeding sites of *An. stephensi* were underground tanks locally called “tankas”. About 64% breeding of anophelines was exclusively in tankas and rest in other sites such as concrete cisterns and cemented tanks.

Insecticide susceptibility tests were carried out against both the malaria vectors and these were found susceptible to DDT, malathion and deltamethrin insecticides in high risk PHC, Ramgarh. Based on survey, risk factors were identified and recommendations were made to contain malaria in the district.

Entomology

Aedes aegypti Survey in Certain Dengue Affected Localities of Municipal Corporation of Delhi

A survey of five dengue affected localities in urban and sub-urban areas under MCD revealed maximum breeding of *Ae. aegypti* in overhead cemented tanks

As per the request of the Municipal Health Officer (MHO)-cum-Director Health Services (DHS), Municipal Corporation of Delhi (MCD), an entomological survey was carried out from 6–12 November 2003 by a team of Malaria Research Centre, Delhi in five localities reporting dengue fever cases as identified by MCD. These localities, namely Dayanand Colony (Lajpat Nagar Phase IV), Lajpat Nagar (Phase I and II), Kotla Mubarakpur are urban whereas Dayalpur Extension and Harsh Vihar are sub-urban. The survey was carried out during day time for both larvae and adults of *Aedes* mosquitoes in all the five identified localities by using WHO standard techniques. To assess the levels of *Ae. aegypti* infestation in four localities surveyed—Lajpat Nagar, Kotla Mubarakpur, Dayalpur Extension and Harsh Vihar; two indices—container index (CI) and breteau index (BI) were calculated.

During the survey seven types of breeding habitats—overhead tanks (cement and syntax), underground tanks (cement and syntax), ground cement tanks, ornamental fountains and mud-pots containing drinking water for birds were found supporting the breeding of *Ae. aegypti*. The maximum breeding was recorded in overhead cemented tanks (58.49%) out of 53 tanks checked in five localities. On the other hand, 17.36% of syntax overhead tanks were found positive out of 190 checked. Two ornamental fountains inside the drawing room in Lajpat Nagar Phase I and three mud-pots (two in Kotla Mubarakpur and one in Dayanand Colony) containing the water for birds were supporting heavy breeding of *Ae. aegypti*. It is also noteworthy to mention that in all the four colonies surveyed majority of overhead tanks were found inaccessible because they do not have fixed ladders, therefore, breeding in these tanks could not be checked. The maximum level of infestation of *Ae. aegypti* was found in Lajpat Nagar Phase I, II (CI = 8.93, BI = 17.33) followed by Kotla Mubarakpur (CI = 7.29, BI = 11.29) and minimum was recorded in Harsh Vihar (CI = 0.89, BI = 1.47). A total of 23 adult *Ae. aegypti* were collected from three localities, namely Kotla Mubarakpur (six specimens), Harsh Vihar (seven specimens) and Dayalpur Extension (10 specimens). The species was found resting inside the houses and cattlesheds on floor, under the furniture, cupboards and water storage tanks. The detailed report of the survey was submitted to ICMR (HQ) and MCD.

2. Parasite Biology

MALARIA PARASITE BANK

Parasite Bank is supporting a large number of organisations working on various aspects of malaria. Biological materials including nonhuman and human plasmodia preserved / maintained at the Malaria Parasite Bank were supplied to various research organisations. The nonhuman parasites, especially *Plasmodium berghei* (both chloroquine resistant and sensitive) are being used for the *in vivo* screening of extracts / fractions of medicinal plants. During 2003 *P. falciparum* isolates adapted to *in vitro* culture conditions and characterised for drug susceptibility to different antimalarials have been supplied to scientists / researchers from Institutes / Universities for collaborative studies. A total of 48 *P. falciparum* isolates were supplied for studies on genetic variation of T-cell epitopes and another 20 isolates were supplied to IISc, Bangalore for molecular characterisation (Pfcrt). Few of the isolates characterised for their CQ sensitivity status, erythrocyte invasion properties, etc. were again cultivated *in vitro* to expand the number of vials cryopreserved at the Parasite Bank for future use / supply.

P. falciparum isolates from Goa, Maharashtra, Car Nicobar, Gautam Budh Nagar, Assam and Orissa showed high multiplicity of infection and prevalence of K1 and MAD20 of MSP-1, FC27 and 3D7 of MSP-2; R033 (< 25%) proportions.

One CQ resistant isolate has been cloned this year. Eleven clones were separated from this isolate and these clones were given for checking their monoclonality by molecular analysis. After expanding these clones were tested for CQ sensitivity. All the clones were found to be resistant to CQ in varying concentrations (8-32 p moles).

A study has been initiated to see the morphological changes taking place in cerebellar Purkinje cells and in the surrounding parenchymal cells during cerebral malaria. The study aims to see the effect of plant extracts with antimalarial properties in cerebral malaria in experimental animal model using Swiss albino mice and *P. berghei ANKA* strain. The initial studies showed that there is a reduction in the number Purkinje cells in *P. berghei* ANKA infected mice compared to uninfected normal animals.

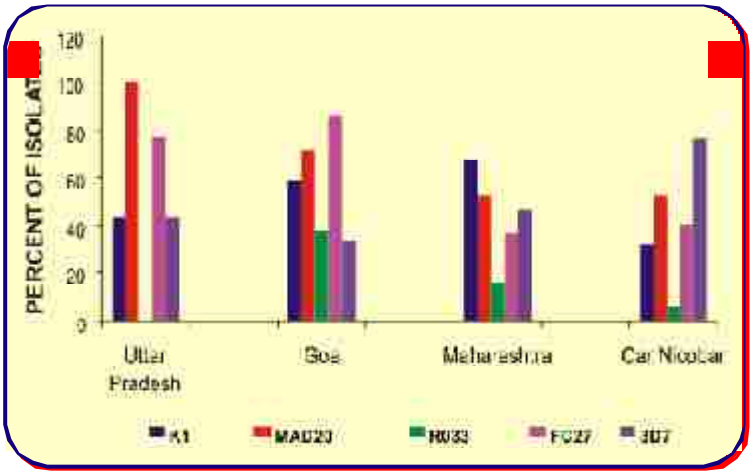
Characterisation of Human Malaria Parasites—*P. falciparum* and *P. vivax*

In continuation of earlier work, field isolates of *P. falciparum* collected from Goa, Maharashtra, Car Nicobar and Gautam Budh Nagar (U.P.) were analysed for polymorphism of MSP-1 and MSP-2, microsatellite markers and *P. vivax* from Rourkela and Goa were analysed for MSP-3α housekeeping gene (ribosomal protein L_{35}e) and SSU rRNA pattern. In addition, *P. falciparum* samples from the Parasite Bank were also analysed for MSP-1 and 2 polymorphism to check their clonal nature.

P. falciparum

MSP-1 and MSP-2: MSP-1 and 2 exhibited high degree of polymorphism among the isolates studied from different areas. In MSP-1, families K1 and MAD20 were prevalent in all the study areas, while R033 was present in proportions less than 25% except in Goa where it was observed in about 40% of the isolates. In MSP-2, both the families FC27 and 3D7 were prevalent in the study areas. High multiplicity of infection was observed among the isolates. Fig. 2.1 shows the areawise distribution of various families among the isolates.

Six out of 10 pairs of *P. falciparum* samples showed different genotypes of MSP-1 and MSP-2 on the day of recrudescence



Microsatellite markers: For the first time, microsatellite markers were used for analysis of the Indian *P. falciparum* isolates. Eleven microsatellite markers namely TA1, TA60, Polyα, Ara2, PfPg 377, 2490, TAA81, TAA109, TAA87, TAA42 and PfPK 2 were used. Analysis revealed highly polymorphic nature of Indian isolates and number of alleles observed ranged from 4 to 10.

Microsatellite markers have been used for the first time for the analysis of Indian *P. falciparum* isolates. Field isolates analysed are found to be highly polymorphic and number of alleles observed were minimum of four and maximum of ten

Parasite Bank isolates: Samples received from the parasite bank were genotyped for MSP-1 and MSP-2 using family specific nested PCR assays. All the samples were observed to be monoclonal in nature (Fig. 2.2).

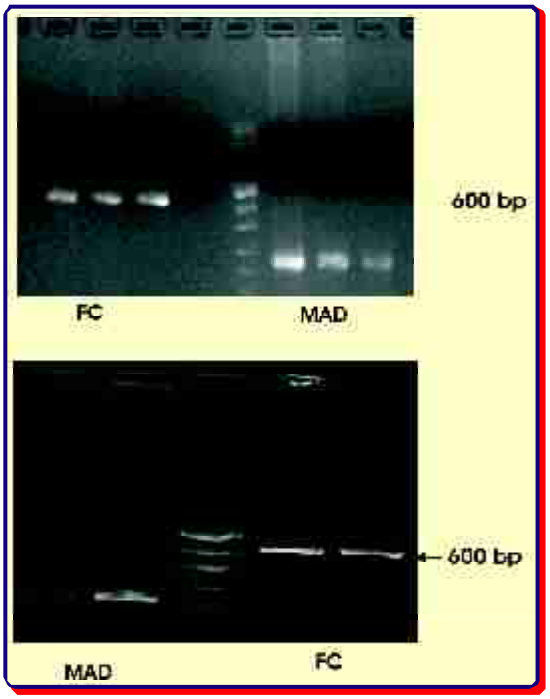


Fig. 2.2: Electrophoretogram showing MSP-1 and MSP-2 profiles of samples received from malaria parasite bank

Purpose of this was to select few pure clones which can be used in further research.

P. vivax

MSP-3α: Isolates from, Goa and Orissa were analysed using PCR-RFLP. Nested PCR revealed three common variants of sizes approximately 1.2, 1.4 & 1.8 kb with 1.8 kb variant being the most common. RFLP pattern with Alu I and Hha I revealed highly polymorphic nature of the isolates. During the study it was observed that H 1 pattern of Hha I digestion was observed frequently in 1.2 and 1.4 kb variants, while it was totally absent in the most common 1.8 kb variant. Therefore, in future efforts will be made to sequence this variant.

SSUrRNA: Geographically distributed population of *P. vivax* reveals that there are two separate sub populations of *P. vivax*. The populations of *P. vivax* from America and European countries form a group distinct from those of Asia and Africa. Parasites inhabiting in America and Europe are designated as new world isolates and those in Asian and African countries are designated as old world isolates. Comparing the 18S rRNA sequences of the new and old world isolates, a consistent polymorphism that separates the two according to geographic location was observed. Preliminary study carried out by MRC has shown the presence of both the forms— new and old world type in Indian isolates (Fig. 2.3). Sequence data has confirmed the two types in limited number of isolates.

SSU rRNA analysis of *P. vivax* field isolates revealed that in India both types of *P. vivax*, i.e. old world (Asian/ African) and new world (European/ American) isolates were found

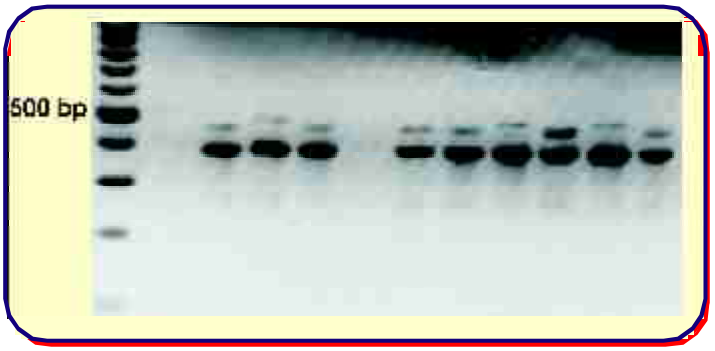


Fig. 2.3: Electrophoretogram showing SSU rRNA analysis of Indian *P. vivax* isolates

Genetic Diversity of *P. falciparum* and *P. vivax* in India using Molecular Markers (ICMR Funded Project)

Surveys were conducted for malaria prevalence in Sonapur district (Assam), Sundargarh district (Orissa) and Chennai (Tamil Nadu) to collect field isolates. In Delhi, patients attending the malaria clinic of Malaria Research Centre at Nanak Enclave campus were enrolled for the study. *P. falciparum* and *P. vivax* isolates have been collected from patients diagnosed positive for malaria by microscopy.

Field isolates of *P. falciparum* have been collected from Sundargarh district (Orissa) and Sonapur district (Assam) and field isolates of *P. vivax* from Delhi and Chennai (Tamil Nadu). Follow-up of the patients is being carried out to collect samples with primary and repeated episodes (recrudescence in *P. falciparum*

and relapse in *P. vivax*). A total of 18 paired samples of *P. falciparum* and 21 of *P. vivax* have been collected.

P. falciparum

Analysis of MSP-1 and MSP-2: A total of about 48 field isolates collected from Assam and Orissa have been analysed for polymorphism of MSP-1 (block 2) and MSP-2 (block 3). Results revealed polymorphism of both the systems among the isolates of both the areas.

Twenty-two isolates of *P. falciparum* from Assam have been analysed for family grouping and observations revealed the presence of all the three families of MSP-1 (K1, MAD20 and RO33) and both of MSP-2 (FC27 and 3D7). Proportional prevalence of family specific markers is 72.7% each for K1 and MAD20; 22.7% for RO33 of MSP-1 and 40.9% for FC27 and 81.8% for 3D7 of MSP-2. Out of twenty-two samples analysed 91% were multiclonal. Only two isolates were categorised as single clonal based on the genotyping of MSP-1 and 2.

Twenty-six isolates of *P. falciparum* from Sundargarh district (Orissa) have been analysed for MSP-1 and MSP-2 family grouping. All the three families of MSP-1 and two of MSP-2 were observed with a prevalence of 73% for K1, 38.5% for MAD20, 22.6% for RO33 of MSP-1 and 65.4% for FC27 and 96.1% for 3D7 of MSP-2. About 96% of the isolates were observed multiclonal based on genotypes of both MSP-1 and MSP-2.

Allelic polymorphism of MSP-1 and 2 observed is slightly more among Orissa isolates compared to that of Assam isolates, however, allele sizes observed were almost similar in both the areas. As observed in earlier studies, RO33 is monomorphic in both the areas.

Analysis of microsatellite markers: Thirty isolates from Sundargarh district, Orissa were analysed for eleven microsatellite markers using multiplex PCR and genotyping. Results revealed that more than 90% *P. falciparum* field isolates from Sundargarh district comprised of multiple infection of genetically different genotypes. All the eleven markers studied were observed to be highly polymorphic among Indian isolates.

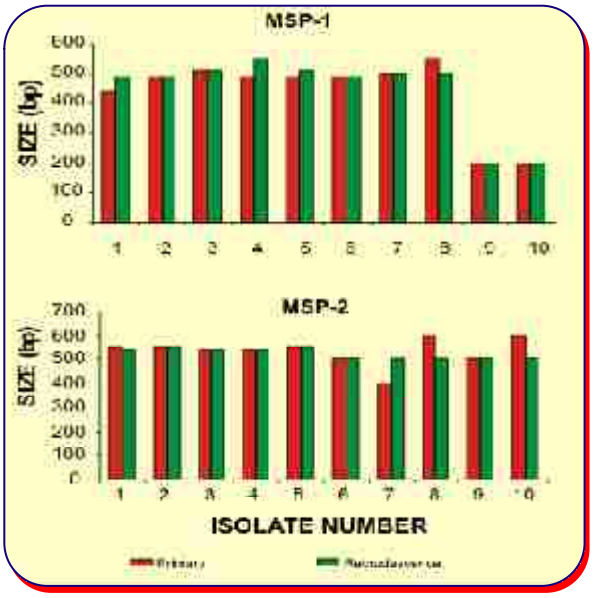


Fig. 2.4: Graphs showing the analysis of primary infection and recrudescence in *P. falciparum* isolates

Analaysis of recrudescence infection: A total of ten paired samples collected from *P. falciparum* positive patients were analysed for genotyping of MSP-1 and MSP-2. Results revealed that out of 10 pairs of samples analysed six showed different genotypes on the day of recrudescence. Fig. 2.4 shows the genotype analysis of *P. falciparum* samples collected on Day 0 and the day of recrudescence.

P. vivax

Analysis of MSP-3α: Isolates from Delhi and Chennai were analysed using PCR-RFLP. Nested PCR revealed three common variants of sizes approximately 1.2, 1.4 and 1.8 kb, of which 1.8 kb variant is common with frequency of occurrence being above 60%. A rare variant of 0.5 kb was also observed in one of the Chennai isolates. RFLP pattern with Alu I and Hha I revealed highly polymorphic nature of the isolates. During the study it is observed that H1 pattern of Hha I digestion was observed frequently in 1.2 and 1.4 kb variant, while it is totally absent in 1.8 kb variant and was observed in both the areas.

Therefore, in future efforts will be made to sequence samples showing this pattern to confirm its association with 1.2 and 1.4 kb variants. Fig. 2.5 shows the PCR amplified size variants of MSP–3α and their distribution among the isolates.

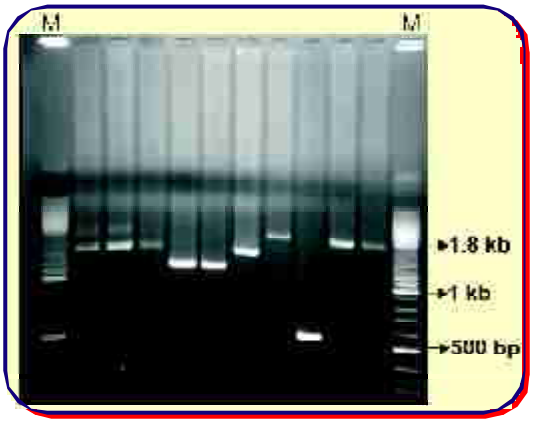


Fig. 2.5: Agarose gel electrophoretogram showing size variations in PCR amplified products of MSP-3α in *P. vivax* field isolates

A rare variant (approx. 0.5kb) of Pv MSP-3α was observed among Chennai field isolates of *P. vivax*. It is further observed that H1 genotype of Hha I digestion is associated with 1.2 & 1.4 kb variants

Rare variant (0.5 kb) observed in one of the Chennai isolates will also be sequenced to get structural details. Restriction digestion with Hha I revealed the absence of Hha I sites, while with Alu I revealed digested products.

Analysis of Ribosomal Protein L_{35}e: Isolates from Delhi and Chennai with single infection of *P. vivax*, ascertained by MSP-3α screening were amplified using specific primers and agarose gel electrophoresis revealed an amplified product of approximately 600 bp. Products were sequenced and analysed for single nucleotide polymorphism (SNP). SNPs observed were spread throughout the fragment length and not restricted to particular position. Fig. 2.6 shows the PCR amplified

Fig. 2.6: Electrophoretogram showing PCR amplified product of L_{35}e (ribosomal protein) in Indian *P. vivax* field isolates

product in the isolates. Alignment of sequences of $L_{35}e$ from field isolates of Delhi and Chennai showed a good alignment with SNPs.

DBP : Sequences of duffy binding protein (DBP) from Papua New Guinea, Colombia and Korea available from NCBI were aligned and based on consensus sequences of region II, primers were designed covering all 12 cystein residues of region II, functional domain. Isolates from Delhi and Chennai were amplified using hemi-nested PCR and an amplified product of approx. 1.0 kb was obtained. Fig. 2.7 shows the amplified DBP gene from Indian isolates. PCR products were purified and cloned in pGEMT vector. The recombinants when sequenced showed absolute homology with those of Papua New Guinea except for few single nucleotide polymor-phisms.

Studies have been initiated on the sequence diversity of house keeping gene, $L_{35}e$ and Duffy binding protein (DBP) of *P. vivax* field isolates

Fig. 2.7: Electrophoretogram showing PCR amplified product of DBP in Indian *P. vivax* field isolates

Studies on Genetic Polymorphism of T-cell Epitopic Region of Circumsporozoite Protein (CSP) of *P. falciparum* from India—Relevance for Vaccine Development (a CSIR Sponsored Project)

The importance of T-cells in malaria immunity has been appreciated for a long time. However, T-cell epitopes show variation. Two T-helper cell epitopes (Th 2R and Th 3R) have been identified in CSP. Genetic variation in Th-epitopes of CSP in *P. falciparum* was studied in 54 isolates collected from subjects from different regions of India—Delhi, Uttar Pradesh, Assam, Rajasthan and Orissa. The T-cell epitopic region was amplified by polymerase chain reaction (PCR) using primers corresponding to nucleotides 1008–1028 and 1323–1347 in the CSP gene sequence of 7G8 clone. The amplified products after purification were sequenced using the same primers which are used to amplify the T-helper cell epitopic regions.

Expression studies of different prototype variants of T-cell epitopic regions of Pf CSP have been initiated

An apparent trend of regionally unbiased restricted polymorphism was observed . The variations can be grouped. Therefore, different prototype variants from the groups could be included in a sub-unit polyvalent vaccine againt sporozoite. However, further studies are needed to establish this observation.

The amplified T-helper cell epitopic regions were cloned into pQE-40 vectors (QUIAGEN) at the Sma I site. Competent *E. coli* M15 strain (QUIAGEN) were transformed both with pQE-40 and pQE-40 I (pQE vector with insert). Positive clones were characterised by PCR using the same primers used to amplify the T-

helper cell epitopic regions. Expression of the T-helper cell epitopic regions is in progress.

Reactivity of Monoclonal Antibodies with *P. vivax* Infected Erythrocytes and Parasite Lysate

Ten hybridoma lines isolated earlier were grown in large volume. Culture supernatants were checked by the ELISA and IFA tests with *P. vivax* and *P. falciparum* crude lysates and erythrocyte smears. All ten antibodies showed reactivity with *P. vivax* erythrocytic stages in immunofluorescence assay (Figs. 2.8 a–d). Immunoglobulins were fractionated from an individual batch by ammonium sulphate precipitation. They were then passed through Protein-A sepharose for obtaining pure fractions. Supernatant of three clones were tested for their reactivity with *P. vivax* lysate. On western blot, these antibodies reacted with 40–42 kDa proteins of a pooled preparation of *P. vivax* parasites collected from different geographic areas of the country (Fig. 2.9). The affinity-purified fractions isolated from three different clones were coupled with 6MB-sepharose for the isolation of specific proteins from *P. vivax* parasitised erythrocyte lysate by affinity adsorption. Purified fractions were checked for reactivity with respective monoclonal antibody (Fig. 2.10). Proteins were then transferred onto

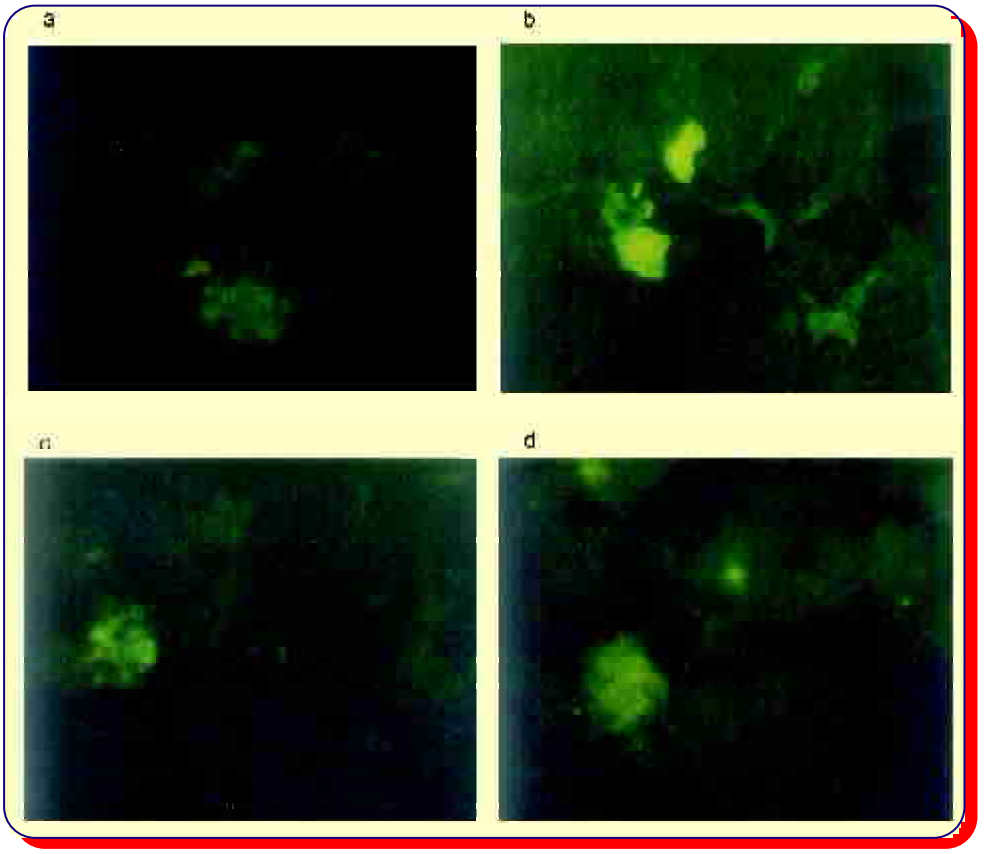


Fig. 2.8 (a-d): Reactivity of monoclonal antibodies with *P. vivax* in IFA

PVDF membrane and N-terminal sequencing was performed. The sequence homology search was done on available database. The protein sequences showed 45–75% homology with the family of *vir*-proteins of *P. vivax*.

Ten hybridoma lines showing reactivity with *P. vivax* erythrocytic stages in immunofluo-rescence assay and western blots are available

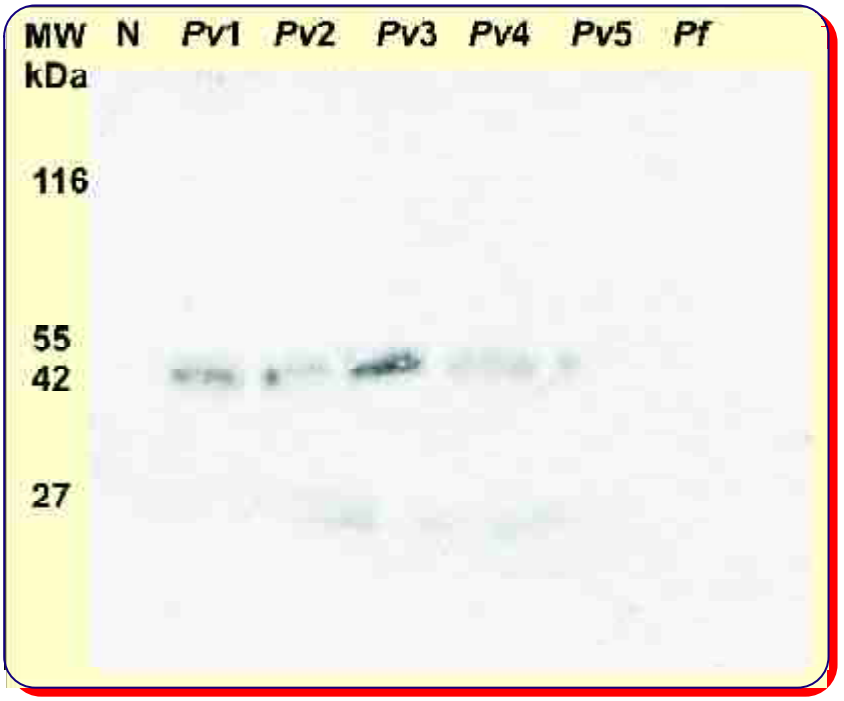


Fig. 2.9: Western blot of *P. vivax* lysates (*Pv*1–*Pv*5), *P. falciparum* lysate (*Pf*) and normal human erythrocyte lysate (N). A protein of ~40–42 kDa reacted with monoclonal antibody (MAb.1)

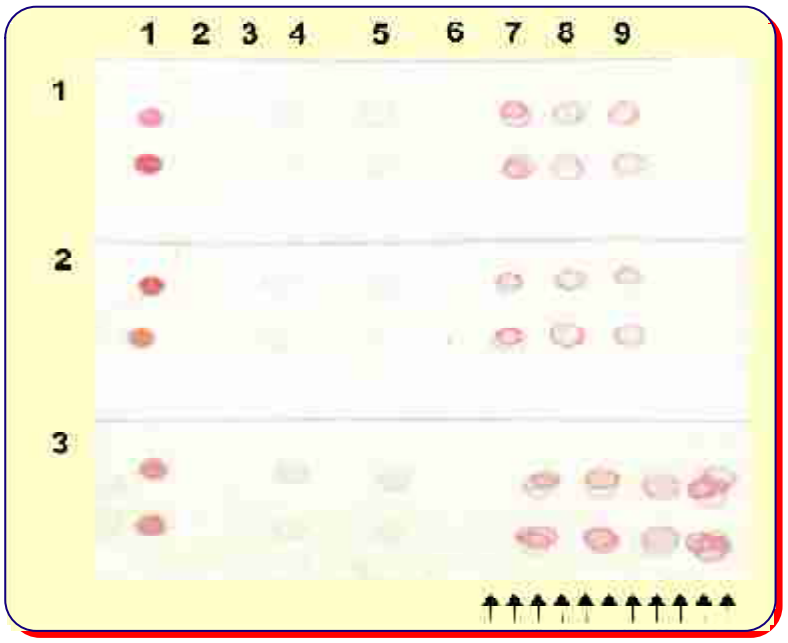


Fig. 2.10: Reactivity of purified proteins from *P. vivax* lysate with three different monoclonal antibodies. 1–MAb (1:10); 2–Eluent; 3–PvAg (1:100); 4–*Pv* (unbound; 5–Eluent; and 6-9–Fractions

Comparison between Serum and Filter Paper Absorbed Blood to Determine Immunoglobulin Isotype Profile by ELISA

Present study was aimed to assess the reliability of the use of filter paper absorbed blood for the estimation of immunoglobulin G and M isotypes and inter-test comparison with corresponding serum samples. Antimalarial IgG and IgM were detected by enzyme immunoassay using finger-prick blood samples collected in capillary tubes and also spotted on Whatman filter paper. Assay was done in 92 blood samples obtained from 53 falciparum malaria patients, 23 fever cases (malaria negative) and 16 healthy individuals. A simple indirect ELISA was done using *P. falciparum* lysate and MSP-1_{19} peptide as antigens. Total IgG and IgM contents were also estimated in individual serum and filter paper (FP) elute by single radial immunodiffusion (SRID). Assay results of both serum and filter paper were compared (Figs. 2.11 and 2.12). The sensitivity and specificity of the assays for IgG measurement were comparable between serum and filter paper ($p<0.001$), whereas in case of IgM, detection level was poor in filter paper as observed by ELISA and SRID.

Filter paper blood spots could be used for determining IgG profile without any significant loss and also in seroepidemiology

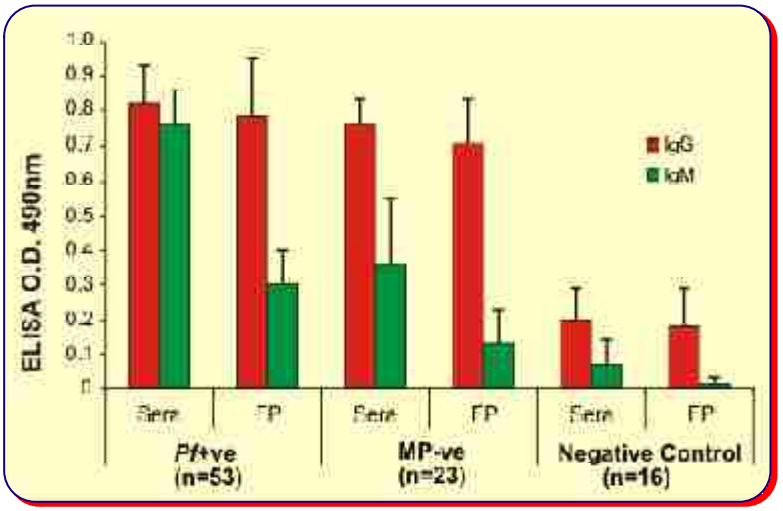


Fig. 2.11: Antimalarial IgG and IgM profiles in sera and *Pf* elutes against FP lysates

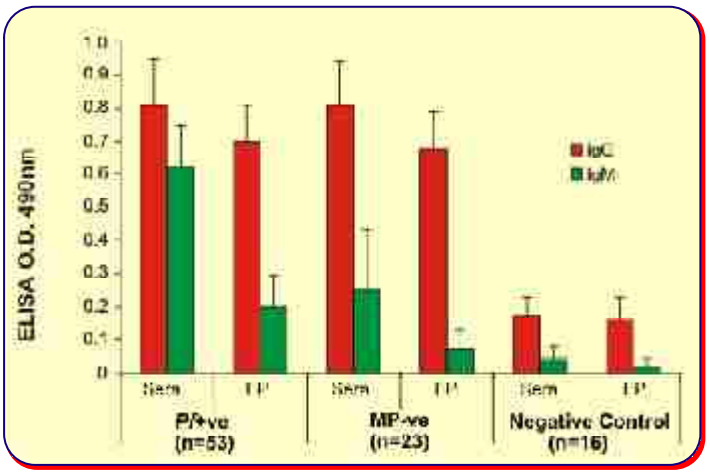


Fig. 2.12: Antimalarial IgG and IgM profiles in sera and FP elutes against MSP-1 peptide

For conducting serological surveys, field logistics are important for operational feasibility. Serological tests would be successful in producing relevant information in epidemiological surveys if the factors were well taken care of. Collection of blood samples and their transportation with proper storage from periphery to central facility carry a lot of importance in success of the test. Collection of finger-prick blood on filter paper from individuals of younger age group, especially children and infants is comparatively easier and acceptable than venipuncture. This advantage compensates for the slight loss in sensitivity when FP are titrated instead of sera. From the present study it may be concluded that filter papers to collect and handle blood samples are of great practical advantage in field studies. Blood samples absorbed on filter paper could be used for determining IgG profile without any significant loss in titre, and could also be applicable in seroepidemiological survey of other diseases. For determining primary or current or recent infection on the basis of IgM titre, serum samples are better than filter paper elutes.

Age-related Prevalence of Antibody Response against Five Defined *P. falciparum* Antigens

Age related prevalance of antibody response against Pf CSP, MSP-1, AMA-1 EBA-175 and Pfg 27 revealed an age-wise increasing pattern in antibody response

The occurrence of the humoral response to defined *P. falciparum* antigens was studied in 86 falciparum malaria patients from villages of Loni PHC, Ghaziabad. The antigens used for indirect ELISA were synthetic peptides derived from *Pf* circumsporozoite protein (CSP), merozoite surface protein (MSP-1), apical membrane antigen (AMA1), erythrocyte binding antigen (EBA175) and gametocyte antigen (PfG27).

Finger-prick blood samples were collected for sera from patients to conduct this test. Sera were tested for determining antimalarial IgG antibody against five stage-specific peptides by ELISA. An age-wise increasing pattern in antibody responses has been observed against all five antigens (Fig. 2.13). However, there was difference in the amount of antibody reaction as observed in ELISA O.D. values. The humoral response to these specific antigens occurred concurrently. Antibodies were detected in most of the adult sera compared to children. The

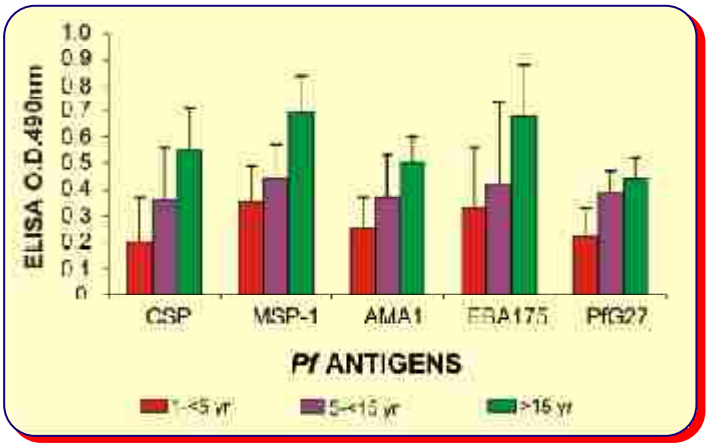


Fig. 2.13: Mean ELISA values for *P. falciparum* patients of different age groups

frequency of seropositivity increased with age approaching, but not reached adult levels by the age of 15 years.

Purification and Characterisation of a Haemoglobin Degrading Aspartic Protease from the Malarial Parasite *Plasmodium vivax*

Aspartic proteases of the human malaria parasite, *P. falciparum* are believed to play a key role in the essential pathways of merozoite release, invasion and haemoglobin degradation during the intraerythrocytic stages of its life cycle. Therefore, we have purified and characterised *P. vivax* aspartic protease to determine if this enzyme can be used as potential drug target and its inhibitors as potential antimalarial drugs. A *P. vivax* aspartic protease has been purified by a combination of ion exchange size exclusion chromato-graphy and HPLC. Its properties were examined to define their role in the haemoglobin degradation process. The purified enzyme migrated as a single band on the native PAGE and SDS / PAGE with a molecular mass of 40 kDa (Fig. 2.14). Gelatin zymogram analyses revealed a clear zone of proteolytic activity, which corresponded to the band obtained with native PAGE and also SDS / PAGE (Fig. 2.15 a & b). The enzyme had an optimal *pH* of 4.0 and exhibited its highest activity at 37°C. The enzyme was inhibited by pepstatin and IC_{50} value was found to be 3.5 mM, but not inhibited by other inhibitors namely *0*-phenanthroline, EDTA, PMSF or E-64

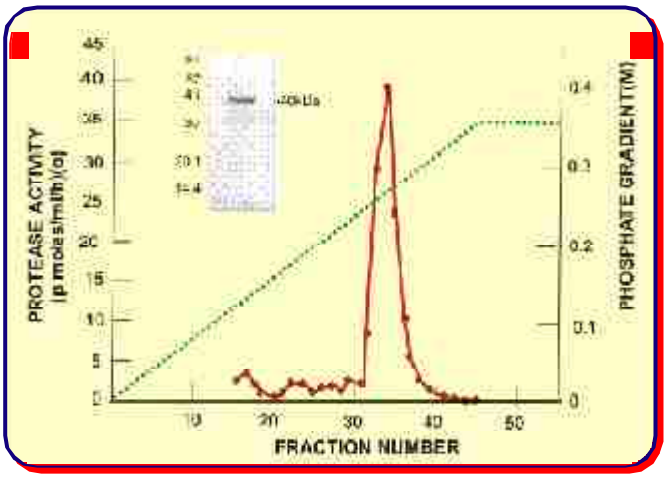


Fig. 2.14: HPLC purification of aspartic protease activity on a Biogel HPHT column using a Shimadzu 10A apparatus. The column was eluted with a sodium phosphate gradient, 0.01–0.35 M, at *pH* 6.8. The flow rate was 0.8 ml / min, and 0.8 ml fractions were collected and assayed for aspartic protease activity. *Inset* : SDS-PAGE analysis. A 50 µl aliquot of the HPLC peak was subjected to electrophoresis on a 12% SDS PAGE gel under reducing conditions. The gel was developed with Coomassie blue.

***P. vivax* aspartic protease has been purified and charecterised for field isolates**

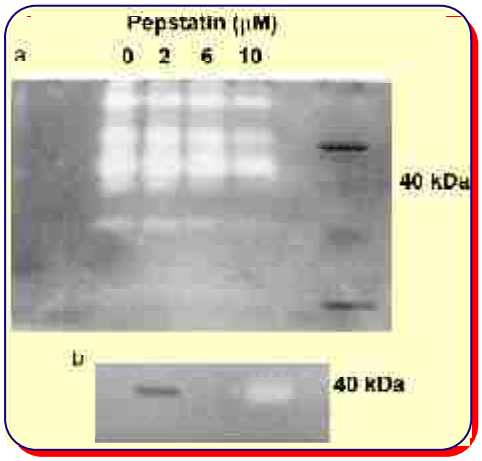


Fig. 2.15 (a): Zymogram (Gelatin gel PAGE) showing the aspartic protease activity (40 kDa) and its inhibition by different concentrations of pepstatin (2, 6, 10 µM); **(b):** SDS-PAGE and Zymogram (Gelatin gel PAGE) showing the purified aspartic protease protein and activity (40 kDa)

supporting its designation as aspartic protease. A lineweaver burk double reciprocal plot with pepstatin showed that the inhibition was competitive with respect to the substrate. Ca^{+2} and Mg^{+2} ions enhanced the protease activity whereas Cu^{+2} and Hg^{+2} ions were found to inhibit the enzyme activity. The pivotal role of aspartic protease inhibitors in development of / as new drugs in *P. vivax* may allow rational drug design for a new class of antimalarials. The results of this study have been compiled and ready for publication.

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

In the present study, we examined the abilities of NO donors and NO producers to inhibit the plasmepsin activity in purified *P. vivax* extracts in a dose-dependent manner . The results provide new insights into the regulation of NO production in *P. vivax* malaria and also the mechanism of killing of malaria parasites via inhibition of protease activities, and may help us design novel strategies for selectively upregulating NO production for the inhibition of *P. vivax* malaria (Figs. 2.16 and 2.17).

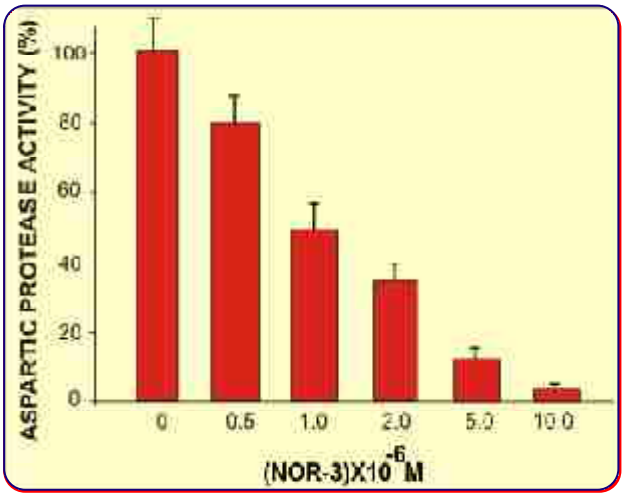


Fig. 2.16: Effect of NO donor NOR-3 on the activity of *P. vivax* aspartic protease and dose dependent inhibitory effect of NOR-3 on the enzyme activity.

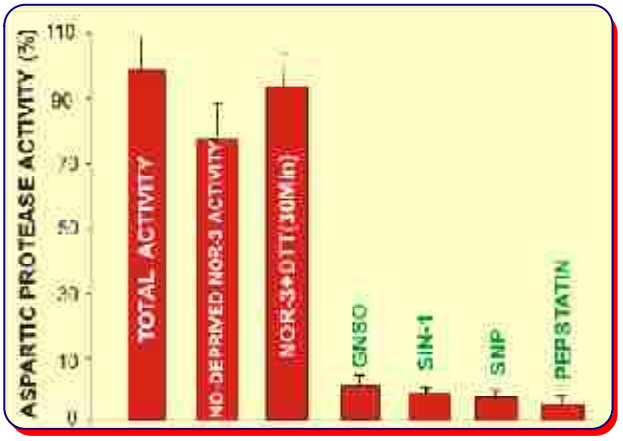


Fig. 2.17: Effect of NO on the catalytic activity of *P. vivax* aspartic protease. NO-deprived NOR-3 (NOR-3*; 1.0 x 10^{-5} M) does not affect the aspartic protease activity. The addition of DTT (1.0 x 10^{-3}M) to the inactive enzyme restores its activity. GNSO, SNP and SIN-1 block the aspartic protease activity. As a control, pepstatin, a typical aspartic protease inhibitor, suppresses the aspartic protease activity.

Glycophospholipid (GPL) as a Candidate Antigen for Diagnosing *Pf* Infection by Laser Immunoassay

GPL is very specific and sensitive to determine *Pf* positive case detection. Crude GPL was fractionated by thin layer chromatography, silica gel glass column chromatography. With increasing methanol concentrations it was possible to fractionate crude GPL into three fractions by column chromatography. Preparative thin layer chromatography also gave three bands. Each fraction showed the presence of docohexanoyl (lipid), sugars and phosphate. By mass spectrometry, three fractions (10, 50 and 70%) from methanol elute showed four core lipid components. Nuclear magnetic resonance spectroscopy was done in CDCL3 indicated 50 and 70% fractions have carbohydrate components. High performance liquid chromatography indicated the presence of six different sugar moieties. Glycophospholipid crude as well as pure fractions were tested against *P. falciparum*, *P. vivax* and control samples. Each component was highly specific and sensitive towards *Pf* infection. Antigenicity determined by ELISA technique showed poor specificity and sensitivity compared to that of LIA. Attempts are being made to develop matrix for enhancing binding with GPL antigen for large-scale immunodiagnosis purposes.

Screening of Medicinal Plants for their Antimalarial Property

Four out of 50 plant extracts tested from different parts of the country showed good antiplasmodial activity.

There is an urgent need for new antimalarials particularly to those parasites which are showing resistance to the existing antimalarials. The medicinal plants or parts of plants used for the treatment of fever in rural / tribal areas were collected from different geographical regions of India and tested for their antimalarial properties. A total of twenty-two medicinal plants were tested *in vitro*, for their antiplasmodial activity after preparing the crude ethanol extract. The *in vitro* test was done using both chloroquine sensitive and resistant isolates of *P. falciparum*. Schizont maturation inhibition (SMI) assay was used for the study. *In vivo* schizontocidal activity was done for one of the plant extracts, which was found effective in *in vitro* screening. Out of these, two extracts were further fractionated with chloroform, hexane, ethyl acetate and butanol. Chloroform fractions of these extracts were showing good activity. This material has been given for further processing by HPLC. Result is awaited.

Based on the results of the schizontocidal activity, one compound having four ingredients was prepared and schizontocidal assay was conducted *in vivo* using *P. berghei* and Swiss albino mice. Six groups, of five animals each, were taken for the study. All the animals were inoculated with *P. berghei* on Day 0. The drug in different doses (10, 30, 50 and 100 mg) were given to four experimental groups. The drug was administered orally for four consecutive days starting from Day 0. One group received no drug which served as control. The parasitaemia was monitored on Day 4. The inhibition percentage in the four experimental groups was 2.58, 52.9, 75.48 and 64.57% respectively. It is interesting to note that even though the group received 50 mg / kg body weight had an inhibition of

75.48% in parasitaemia on the Day 4, all the five animals survived for a very long time whereas the animals from the other groups died. Further studies are in progress.

Twenty extracts received from DRDO have been tested and out of these two were having very good antiplasmodial activity. Besides these, as a collaborative work, eight fractions of medicinal plants were tested *in vitro* for RMRC, Dibrugarh, Assam. One of these fractions was found to be having very good antiplasmodial activity.

3. Epidemiology

MALARIA CLINICS

At 22-Sham Nath Marg, Delhi

A total of 67 patients attended the Malaria Clinic at 22-Sham Nath Marg or were referred from hospitals for blood examination and treatment of malaria during the period of January to December 2003. Of these, five patients were positive for malaria, one was diagnosed as *Plasmodium vivax* and four as *P. falciparum* infection.

At 2-Nanak Enclave, Delhi

A total of 2575 patients attended the Malaria Clinic at 2, Nanak Enclave during January to December 2003, of which 56 patients were found positive for malaria. Among these 43 were positive for *P. vivax* and 13 were positive for *P. falciparum*. Slide positivity rate (SPR) and slide falciparum rate (SfR) are given in Table 3.1. Clinical examination was done and specific and symptomatic treatment was given wherever necessary. Blood samples were collected for host parasite interaction studies from 37 patients (34 *Pv* and 3 *Pf*) and for genetic diversity studies from 46 patients (34 *Pv* and 12 *Pf*). The month-wise distribution of malaria cases are given in Table 3.1.

Diagnosis and treatment services were provided to more than 2600 patients at Malaria Clinics

Table 3.1 Data from Malaria Clinic, Nanak Enclave (2003)

Month	BSE	Total	*Pv*	*Pf*	Mix	SPR	SFR
Jan	45	0	0	0	0	0	0
Feb	123	0	0	0	0	0	0
Mar	177	1	0	1	0	0.56	0.56
Apr	225	2	1	1	0	0.89	0.44
May	191	1	0	1	0	0.52	0.52
Jun	88	5	5	0	0	5.68	0
Jul	170	3	3	0	0	1.76	0
Aug	266	9	8	1	0	3.38	0.37
Sep	410	22	17	5	0	5.36	1.21
Oct	603	11	8	3	0	1.82	0.50
Nov	196	2	1	1	0	1.02	0.51
Dec	81	0	0	0	0	0	0
Total	2575	56	43	13	0	2.17	0.50

CLINICAL TRIALS

Operational Activity for the Assessment of Therapeutic Efficacy of Chloroquine and Sulfadoxine-pyrimethamine in Uncomplicated falciparum Malaria

Antimalarial drug resistance is a major public health problem, which hinders control of malaria. In India, after the detection of first case of *P. falciparum* resistant to chloroquine in 1973, resistance has been reported to this drug from several parts of the country. Twelve *P. falciparum* monitoring teams from National Anti Malaria Programme (NAMP) now NVBDCP monitor resistance to chloroquine and in case RII/RIII resistance exceeds 25%, second line drug sulfadoxine-pyrimethamine is introduced in that particular PHC/district. However, the data is still limited and needs updating. Recently new protocols have been developed by WHO to have uniform data globally on the problem of drug resistance. Thus the present studies envisage generating data systematically in different parts of the country.

Therapeutic efficacy studies revealed only 50% cure rates with chloroquine in CHCs of Sundargarh district Orissa and still lower rates in Goa. However, chloroquine is still effective in Udaipur district (Rajasthan)

The present study was conducted at urban and rural sites– Districts Sundargarh (CHC Bisra and Kuarmunda) in Orissa; District North Goa (Panaji) in Goa and District Udaipur (PHC Rishabdev) in Rajasthan, which are located in different ecoepidemiological zones of the country.

Since study was also aimed at developing skills at local level in addition to generating data, therefore, Malaria Research Centre conducted orientation training for technical staff and medical officers of PHC, at the respective sites at the time of initiation of the study. The objectives were : to familiarise the team with the protocol; to orient the medical staff about the study procedures and their role in case of emergencies and setting up the study site and field laboratory.

In Orissa, preinitiation meeting was conducted on 20 August 2003 at MRC, Rourkela, to discuss study protocol and logistics. Co-investigators and staff of Malaria Research Centre, Doctors of Primary Health Centres, Consultants of IGH Hospital, District Malaria Officers of Sundargarh, Kuarmunda and Bisra

Table 3.2 Baseline characteristics of patients in Sundargarh district (Orissa)

Classification	Dose 25 mg/kg over 3 days	
	Bisra CHC	Kuarmunda CHC
No. of cases	63	70
Male/Female	37/26	28/42
Age in years (Range)	3.5–65	9 months–58 yrs
Parasitaemia/?l(Range)	1040–88800	1120–88800

Table 3.3 Classification of therapeutic response

Classification	Bisra CHC		Kuarmunda CHC	
	No. of patients	Prevalence	No. of patients	Prevalence
ETF	1	0.018	2	0.034
LCF	10	0.179	17	0.288
LPF	14	0.250	8	0.136
ACPR	31	0.554	32	0.542
Loss	6	0.1	3	0.2
Withdrawl	1	–	8	–
Total	63		70	

CHCs attended the meeting. In Goa, meeting was held on 21 August 2003 at the Office of the Director, Health Services, Goa. The meeting was attended by the Director of Health Services Goa, Deputy Director (Malaria & Filaria), Deputy Director (Public Health), Health Officer (Malaria) and State Entomologist. In Rajasthan, an orientation workshop was conducted at District Udaipur on 1 September, 2003. The Additional Chief Medical Officer, Dy. CMHO, Senior Medical Officers, Medical Officers of PHCs and technical staff attended the meeting.

High percentage of treatment failure to first line drug sulfadoxine-pyrimethamine was found in areas along the West Bengal-Nepal border

The therapeutic efficacy of first line drug chloroquine was determined using WHO 28-day protocol. Subjects were enrolled according to the inclusion and exclusion criteria and weekly peripheral smears and clinical examination conducted. The results of the study indicate that cure rates with chloroquine were about 50% in study CHCs of Orissa (Tables 3.2 and 3.3) and still lower in Goa. However, chloroquine was found to be still effective in Udaipur district (Rajasthan).

Assessment of Therapeutic Efficacy of Antimalarial Drugs against Uncomplicated *P. falciparum* Malaria in West Bengal as Part of Indo-Nepal Cross Border Activity

Among the areas with high transmission of falciparum malaria, areas along international borders pose serious problem. India and Nepal share their border along Bihar and West Bengal and there is free population movement across the border. On both sides of the border the presence of drug resistance has been documented. In West Bengal the first line drug is still chloroquine except in PHCs Ajodhya hills and Uttarlatabari of Purulia and Jalpaiguri districts respectively. In Nepal, the total number of laboratory confirmed cases are 10,000 annually, out of which 6–20% infections are due to *P. falciparum*. Drug policy was modified in 1998 following reports of resistance to chloroquine. First line drug is now sulfadoxine-pyrimethamine (SP). Since it is well-known that




distribution and prevalence of drug resistance parasites can grow quickly, there is urgent need to generate data on drug resistance in these areas.

The sites selected for the study were Sukna and Naxalbari PHCs of District Darjeeling, based on the baseline epidemiological data and the logistics. The medical officers and technical staff of the block primary health centre (BPHC) were identified as Co-investigators for the study.

The standard procedures were followed for the study. A total of 91 patients were enrolled at two study sites (Table 3.4). The analysis of the data revealed high percentage of treatment failure in these PHCs of border district (Fig. 3.1). Thus from the results of the studies it can be concluded that efficacy of first line drug is compromised in some parts of the country and there is an urgent need to review drug policy in these areas.

Table 3.4 Baseline characteristics of patients

Drug: Chloroquine (Dose 25 mg / kg over 3 days)	Sukna	Naxalbari
No. of cases	50	41
Male / Female	29 / 21	21 / 20
Mean age in yrs [M±SD]	21.6±13.1	22.1±12.7
(Range)	(0.5–50)	(2.5–56)
Mean parasitaemia / ?l on D0	3894.4±2731.9	7453.7±7035
[M±SD] (Range)	(1000–9640)	(1000–32,280)

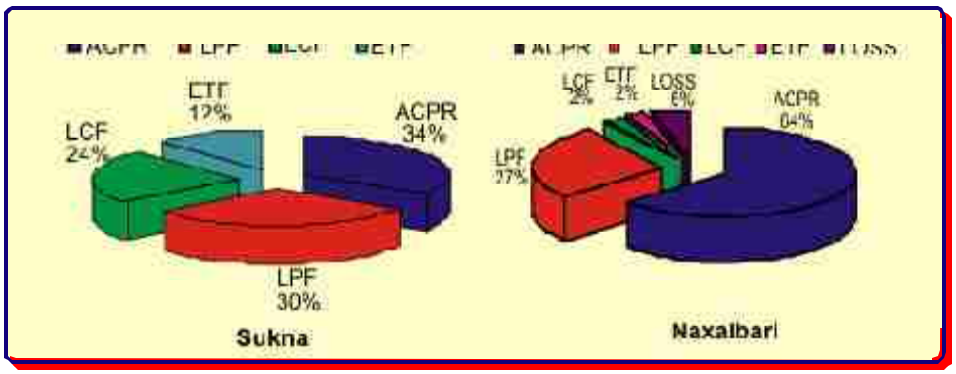


Fig. 3.1: Classification of response (% of total) in Sukna and Naxalbari PHCs, District Darjeeling

DIAGNOSTICS

Paracheck[â], a rapid diagnostic kit showed 100% sensitivity and > 90% specificity in the detection of P. falciparum

Quality check of rapid diagnostic test "Paracheck[?] " was conducted on request from NVBDCP/RITES. Approximately 56–58 test kits, each from seven batches of the dipstick were checked for sensitivity and specificity. The rapid test has 100% sensitivity for detection of *P. falciparum* for parasitaemia range of 100–19000/?l, which is within acceptable standards. The specificity is also more than 90% and is acceptable for HRP II based kits since the antigenaemia persists even after treatment. Thus the tests from above batches have been found to be of high quality in terms of sensitivity and specificity. The report has been submitted and kits were procured by NVBDCP.

Association of Leptospirosis in Patients of Severe falciparum Malaria

Severe and complicated malaria present with many complications like cerebral malaria, jaundice, renal failure, etc. Among these, acute renal failure occurs in less than 1% cases but the mortality from these cases is reported up to 45%. Data from Ispat General Hospital show nearly two fold increase in the number of patients with severe complications like acute renal failure and jaundice over a period of five years. Leptospirosis is an acute anthropozoonotic infection prevalent worldwide and is emerging as an important public health problem in India. The clinical picture of leptospirosis mimicks severe and complicated malaria especially that of acute renal failure and jaundice. The recent increase of acute renal failure and jaundice among malaria patients at Rourkela may be due to leptospirosis alone or in combination with malaria. Hence, a collaborative study was undertaken to rule out the presence and/or association of leptospirosis among these patients.

Detailed haematological and biochemical examinations were done in these patients. There were 23 severe malaria patients (microscopy/ICT +ve 12; microscopy/ICT –ve 11) and 16 uncomplicated malaria (microscopy/ICT +ve). Serum samples were tested for leptospirosis by leptospira IgM specific agglutination test and Lepto Tek Dri Dot test at NICD, Delhi. None of these were positive by serology for leptospirosis. The study will be continued with larger sample size.

REMOTE SENSING AND GIS

RS and GIS for Decision Support in Malaria Control

Blind field surveys in GIS predicted areas were conducted for *An. minimus* in Kamrup and Karbi Anglong districts of Assam. In GIS predicted favourable areas in Kamrup district *An. minimus* was found whereas in non-favourable areas in Karbi Anglong, the species could not be found. A team of experts visited Banbasa, Champawat district of Uttaranchal during May 2003. The team validated the site for collection of adults and larvae of *An. minimus*. Adults and larvae were collected and identified in Haldwani Field Unit laboratory by the expert team. Out of 117 larvae collected, 103 (88%) belonged to *An. minimus* group and in 45 adult specimens of *An. minimus* group collected, 2 specimens were identified as *An. minimus*. As per the recommendation of the group the specimens of *An. minimus* have been given for confirmation by molecular technique– PCR.

Electronic album on GIS predicted distribution of 58 anophelines was prepared

Electronic album on GIS based distribution of Indian anophelines was tested and final product on CD was prepared. This album consists of 58 maps each showing the GIS predicted distribution of individual species in India along with the blow-up map of GIS predicted districtwise favourable areas and the validation through reported surveys.

Delineation of Breeding Habitats and Landscape Features Suitable for *An. culicifacies* Abundance using Satellite Data

In continuation of ongoing work, satellite data of January 2002 and May 2001 was analysed in respect of 27 villages of high, moderate and non-malarious areas (or lowest) of three PHCs for generating statistics of landscape features. The difference in extent of land use features in villages of high and least malaria categories indicated that in villages of high malarious area, presence of water in water bodies (0.36–35.78%), permanent vegetation cover ranging from 24.9–85.72%, less barren area and scrub (0–12.9%), less barren rocks (0.13–9.68%) as compared to 1.44–3.2% water bodies, 15.07–34.19% vegetation cover; 8.57–30.21% barren area and scrub, and 0.35–20.33% barren rocks in least malarious area (Byalya). It was found that the presence of water in water bodies in the month of May is the most important landscape feature followed by less barren area and acacia plantation associated with high malarious area. Based on landscape features regrouping of villages from high risk malarious area to low risk malarious and vice versa is desirable.

Comparision of NDVI images of years 2000 and 2001 was also done to find out the significance of vegetation index in predicting malaria. It was observed that apparently in low malarious areas, vegetation index is low as compared to high malarious area, however, mapping of vegetation index at village level is desired for application of NDVI parameter in predicting malaria.

Remote sensing may be used for stratification of malaria at village level

necessitating the need for operationalisation of this technique in different problematic paradigms of malaria.

Impact of Climate Change on Malaria in India

In continuation of work, field visit was undertaken in Banaskantha district to find out the terrain features and distribution of breeding habitats and vector species supporting malaria endemicity. Meteorological data for 1985 to 2000 was procured from IITM, Pune. Correlation coefficient between malaria incidence and meteorological parameters was calculated. It was found that in monthly correlation between rainfall and *P. vivax* cases, positive correlation was found in general, while with *P. falciparum* it was negative.

Outbreaks of malaria occurred due to fluctuations in rainfall and not due to temperature fluctuations as evidenced in meteorological data of Tumkur and Bikaner districts

Retrospective analysis of temperature increase in 15–20 years was studied vis-a-vis malaria incidence in respect of Banaskantha, Tumkur and Bikaner districts. It was found that there was only 0.9°C increase in annual temperature over the years. Preliminary analysis indicated that outbreaks of malaria occurred due to fluctuations in rainfall (indirectly increasing RH) and not because of increase/decrease in temperature. Study is in progress for identifying transmission windows of malaria and correlation of meteorological parameters and malaria in all problematic districts of Rajasthan and Karnataka.

Independent Assessment of the Status of the Use of Larvivorous Fish as an Integrated Vector Control Measure under the Enhanced Malaria Control Project in Kota district

The project was undertaken to: (i) assess and describe the process of the establishment and distribution of larvivorous fishes in malaria endemic areas; (ii) to assess the impact of fishes in the control of mosquito breeding, adult densities and malaria-incidence; and (iii) to describe the sustainability and reproducibility of the fish introduction programme within the integrated vector control framework of NVBDCP.

IEC activities, community participation and GR at subcentre level are desired for use of larvivorous fish for malaria control in Kota district

Two Block PHCs namely Kaithun which reported highest API (7.03) and Pipalda where least GR work was done and fish hatcheries were selected for detailed survey under rural area. In Kota city also a few sites were selected for evaluation. The entomological surveys were conducted as per WHO procedures. Pre-designed questionnaires to elicit information on the establishment of fish hatcheries, method of distribution of fish, fish density, infrastructure available, involvement of other sectors, monitoring and evaluation of epidemiological and entomological parameters at district and PHC level were filled up.

The establishment of fish hatcheries in Kota city and endemic rural areas of Kota district is in process since August 2003. Hatcheries comprising mainly Guppy fish (*Poecilia reticulata*) and *Gambusia affinis* have been established in available step wells/irrigation wells/bavries mainly up to PHC level. Geographical reconnaissance (GR) of potential breeding habitats of mosquitoes has been done

to some extent in all the Block PHCs. Ponds, road-side ditches, seepage from canals, riverine stagnant pools (Chambal river which passes through Kota city) are the prevalent breeding habitats of anophelines. Therefore, release of fish up to village level and in all the habitats– Roadside ditches, ponds, pools, etc. still remains to be done.

It is premature to study the impact of fish on adult anopheline density / malaria incidence keeping in view that the fish are not released in all the prevalent breeding habitats of mosquitoes. As regards sustainability and reproducibility of fish introduction programme within the integrated vector control framework of NVBDCP, the programme of fish hatcheries established / release done so far in the available aquatic habitats was found successful. However, detailed GR of breeding habitats is required at sub-centre level and fish hatcheries should be established in ponds, specially constructed tanks at all sub-centres. From sub-centres the fish may be released in potential breeding habitats. IEC activities and involvement of other sectors like fishery department, irrigation and village Panchayats are needed for sustainability of fish introduction programme.

Serological assessment was found well-correlated with slide positivity rate and API in determining the malaria endemicity in Goa

Sero-epidemiological Assessment of Malaria in Migratory Population in Goa

Sero-epidemiological survey was conducted in a large number of migratory labour populations engaged in construction work in Goa. Results of the antimalarial seroreactivity against *P. falciparum* crude antigen and synthetic peptide AR1 along with the slide positivity rate (SPR) and annual parasite index (API) have been compared. A total of 841 samples from nine areas of Goa like Panaji, Porvarim, Caranzalem, Mapusa, Parnem, Vasco-da-Gamma, Margaon, Canacona and Ponda were collected during January 1998. API from 1999–2003 was also collected. API value for 1997–98 was found to be 42.7, ELISA O.D. against AR1 and *Pf* were 0.67 $\pm$ 0.23 and 0.65 $\pm$ 0.22 respectively. SPR value before and after sample collection was compared with ELISA O.D. Comparison of SPR (parasite load) and malarial antibody response (Table 3.5) in migratory population have been analysed as follows:

Table 3.5 Classification of malaria transmission in different study sites based on serological assessment

Area	SPR (1994-97)	AR1 ELISA O.D.	SPR (1999-2003)	Malaria transmission
Panaji	+++	+++	+++	High
Caranzalem	+++	+++	+++	High
Mapusa	+++	+++	+	Low
Parnem	+	+++	+	Low
Vasco-da-Gamma	++	+++	+	Moderate
Margaon	+++	++	+++	High
Canacona	++	++	+	Moderate
Ponda	+	+	+	Low
Porvorim	+++	+++	–	High

+++	High ELISA O.D.	>0.7	SPR >2
++	Moderate ELISA O.D.	0.4–0.7	SPR=2
+	Low ELISA O.D.	<0.4	SPR<2

The results confirm Panaji, Caranzalem and Mapusa had high malaria transmission, while in Parnem and Vasco-da-Gama antibody levels were higher compared to parasite load indicating labourers had high antibody titre though they had low infection rate. In Margaon and Canacona ELISA O.D. were moderate but parasite load was high. The possible explanation is that these groups of labourers are susceptible to malaria infection. In Ponda, transmission was very low both serologically and parasitologically, confirm the same. The results indicate that serologically it is possible to study malaria transmission dynamics.

Development of a Field Site for Malaria Vaccine Trial (A Collaborative Project with International Centre for Genetic Engineering and Biotechnology, New Delhi –Funded by Department of Biotechnology, Govt. of India)

Phase II studies were initiated for preparation of site for malaria vaccine trial in Sundargarh district, Orissa. Longitudinal studies indicated API—280.9 and 21.8, IPR—69.2 and 12.5, CPR—49.7 and 89 in forest and plain area villages respectively.

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. Longitudinal epidemiological studies were continued in two sets of villages in forest and plain areas characterised by hyper- and meso-endemic malaria situations respectively. During the year, new study villages for phase II studies were identified on the basis of baseline surveys and 22 new villages (forest–15 and plain–7) with a total population of 11,304 were included in the study area. Now there are 35 villages (forest–23 and plain–12) with a total study population of 15,525. The demographic information of the new villages was collected through census surveys and the demographic information of phase-I study villages was updated.

Entomology and Parasitology

Longitudinal parasitological surveys were conducted in all the 13 villages of phase-I study area. The malaria incidence was measured through weekly surveillance with the help of village volunteers. The month-wise malaria incidence in forest and plain areas was ranging from 7.7 to 43.1 and 0 to 3.8 respectively. The annual parasite index (API) in forest and plain areas was 280.9 and 21.8 respectively. The age-wise distribution of malaria incidence in forest and plain areas showed highest malaria incidence in 1–5 years age group and the annual parasite index (API) was 1053.3 and 36.5 respectively. In forest area the malaria incidence was inversely proportional to the increase in age but in plain area, malaria incidence was more or less the same in all other age groups and the difference was not very significant. Infant parasite rate (IPR) and child parasite rate (CPR) in the forest area were high throughout the year with a yearly average of 69.2 and 49.7 respectively. The average annual IPR and CPR in plain area

were 12.5 and 8.9 respectively. The highest attack rate due to *P. falciparum* – number of episodes per person per year in forest area was recorded in 1–5 years age group (0.82 episodes per child per annum). The average attack rate in the total population was found to be 0.24 and 0.02 in forest and plain area respectively. During the year peak transmission was observed during post monsoon months– October–November with another peak during March–April which was due to spring transmission. The proportion of different *Plasmodium* species in forest area was 85, 14 and 1 for *P. falciparum, P. vivax and P. malariae* respectively whereas it was 75, 25 and 0 respectively in plain area.

Malaria prevalence in the study population during different transmission seasons was measured through cross-sectional point prevalence surveys in all the study villages during March, June and November characterised by moderate, low and high malaria transmission seasons respectively. About 40% of the houses were selected randomly and all occupants of these houses were examined for malaria parasite irrespective of clinical symptoms. The clinical case history of each individual screened during point prevalence surveys was recorded on patient data sheet. The parasite rate in the forest area during these surveys was found to be 10.6, 11.5 and 14.5 respectively, whereas it was 1.4, 1.1 and 1.2 respectively in plain area. In forest area, about 40 percent of falciparum cases were asymptomatic out of which 5.2 percent were asymptomatic carriers. The proportion of children having >10,000 parasites / ?l was more as compared to adults. The spleen rate in children and adults in the forest area was 72.9 and 14.5 respectively, whereas in plain area it was 14.6 and 1.0 respectively. The average enlarged spleen (AES) in

The spleen rate in children was 72.9 and 14.6 in forest and plain areas respectively

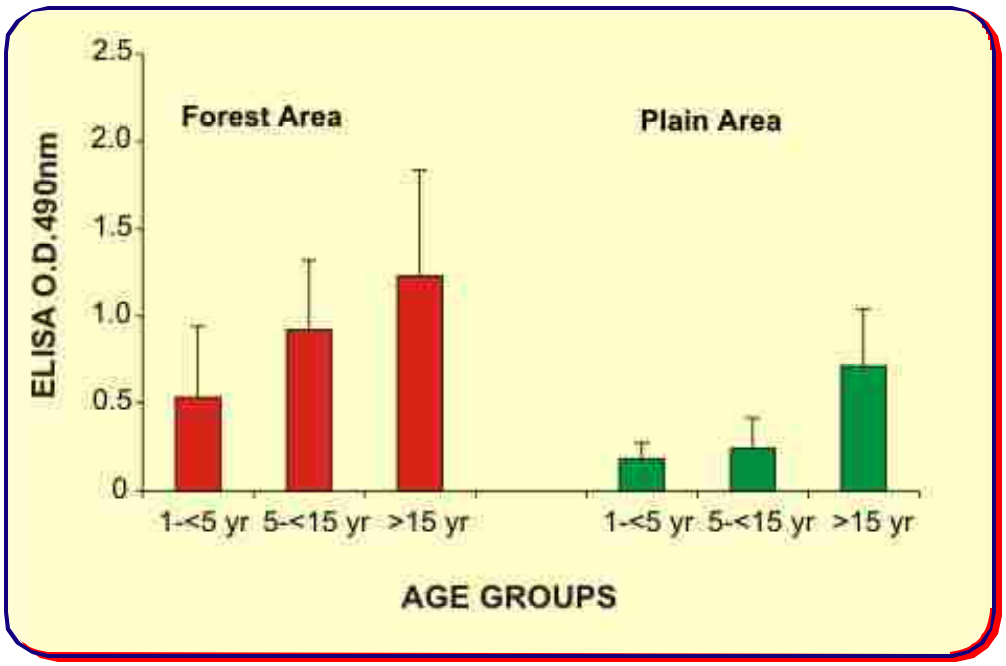


Fig. 3.2: Anti MSP-1_{19} antibody profile in different age groups of forest and plain areas during low transmission season

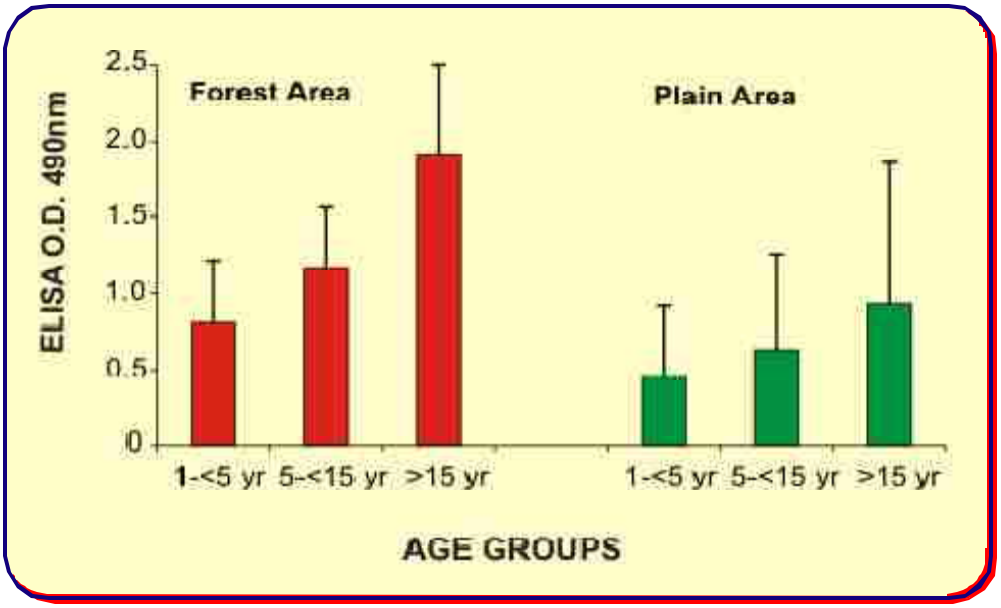


Fig. 3.3: Anti MSP-1_{19} antibody profile in different age groups of forest and plain areas during high transmission season

children in forest and plain areas was 1.8 and 1 respectively. During malaria prevalence surveys, blood samples were also collected for parasite diversity and immune response during different seasons and these studies were carried out at MRC, Delhi.

Age-dependent increase of specific antibody level was observed in individuals in both the areas during high as well as low malaria transmission seasons as revealed from host immune response studies.

Entomological surveys were carried out in two indicator villages each from forest and plain area villages of phase-I study area and one village each selected randomly from forest and plain areas of phase-II study villages. A total of 14 anopheline species from forest area and 10 species from the plain area were recorded. *An. culicifacies* was widely prevalent throughout the year in both forest and plain areas with prevalence rate of 38.8 and 24.9 per cent respectively. *An. fluviatilis* was restricted to the forest area and its prevalence rate was 6.3 percent. The density of *An. culicifacies* in the forest and plain areas was ranging from 0.5–36.2 and 0.7–35.7 respectively. The density of *An. fluviatilis* in the forest area ranged between 0–7.7. The mosquito-landing rate of *An. culicifacies* on human baits in both the areas was 0.50 bites per person per night, whereas that of *An. fluviatilis* in the forest area was 6.4 bites per person per night. The sporozoite rate and human landing rate of vector species were used to calculate entomological inoculation rate (EIR) in the study area during different transmission seasons. The EIR in plain area was nil whereas, in forest area it was 0, 0.085 and 0.35 infective bites per person per day during low, intermediate and high transmission seasons respectively.

Host Immune Responses

In continuation of earlier work, repeated cross-sectional surveys were conducted at two sites of forest area and two sites of plain area for the collection of finger-prick blood samples from different age groups during low and high

transmission seasons. In forest area, *P. falciparum* infection was detected in 11.25% (9/80) persons during low transmission phase, whereas in plain area among 57 none was found positive. During high transmission, 26.14% (23/88) were *P. falciparum* positive in forest area and 9.09% (5/55) in plain area. Indirect ELISA was conducted to measure the antibody level in 280 blood samples collected in both the surveys. It was observed that overall anti-MSP-1_{19} IgG profile was higher in study subjects of forest area than that of in plain area in both low and high transmission seasons. The age-dependent increase of specific antibody level was noticed in individuals of two areas in both seasons (Figs. 3.2 and 3.3). However, acquisition of anti-MSP-1_{19} antibodies during the time of high transmission phase was more compared to low transmission phase. The results demonstrated that during high transmission more than 95% of the sera contained antibodies to MSP-1_{19} in forest area, whereas about 89% of the sera contained anti-MSP-1_{19} antibody in plain area, though at low level. Thus the results suggest that there was enhanced antibody production against this molecule by natural infections among these individuals. The level of antibodies in study groups appeared to be related to their exposures to the parasite during high transmission phase.

MOI of 1.8 for MSP-1 and 2.0 for MSP-2 was reported among *P. falciparum* isolates.

Multiplicity of Infection

To understand the epidemiology of malaria it is important to understand the diversity existing among the malaria parasite species and its transmission intensity. Results obtained during 9th plan period has shown highly polymorphic nature of *P. falciparum* isolates in respect of MSP-1 and 2 gene with about more than 60% of the isolates harbouring more than one parasite genotype suggesting multiplicity of infection is mostly greater than 1. To confirm if phenomenon is universal in the area, analysis of multiplicity of infection among *P. falciparum* was continued and results confirmed our earlier observations. Analysis revealed MOI of 1.8 for MSP-1 and 2 for MSP-2 among the *P. falciparum* isolates.

Fifteen novel sequence variations of vaccine candidate antigens— MSP-$1_{19,}$ EBA-175 and TRAP were reported for the first time

Sequence Diversity in Vaccine Candidate Antigen Genes

For the success of development and testing of malaria vaccine it is important to know diversity existing among the parasite population of the area. Our earlier results revealed sequence variations in all the three vaccine candidate antigens MSP-$1_{19,}$ EBA175 and TRAP. Maximum variations were observed in EBA. Among 16 field isolates, 20 sequence variations were observed and of what 15 were novel, not reported earlier from any area. To answer the question if these variations effect the binding function of the parasite to human RBC. Isolates with mutations were expressed in expression vectors and results revealed binding pattern of sequence mutations is same as observed for control (camp-EBA-F2) sequence.

Also sera raised against control (camp-EBA-F2) sequence binding (rosette formation) was equal to the binding of the control sera. Studies provide support for the development of a sexual blood stage vaccine based on recombinant PfF2 expressed and purified from *E. coli* which may prevent free *P. falciparum* merozoites from entering into the red blood cells and thus prevent pathogenesis of malaria.

6. INFORMATION, EDUCATION AND COMMUNICATION (IEC)

Health Education

Information, education and communication (IEC) Division prepared the posters depicting activities and results of major scientific activities of the Malaria Research Centre and its various field units of the Integrated Disease Vector Control Programme under the administrative control of MRC.

National Science Day was celebrated by organising a health education camp in a school. The activities included display of exhibition on malaria, live demonstrations and video films.

Malaria Research Centre participated in the Science and Technology Exhibition "Science EXPO-2003—Pride of India". This exhibition was the part of the 90th Session of Indian Science Congress held at Bangalore from 3–7 January 2003. The theme of the Congress was "Frontier Science and Cutting Edge Technologies".

A lecture-cum-demonstration was given to trainees on health education and community participation.

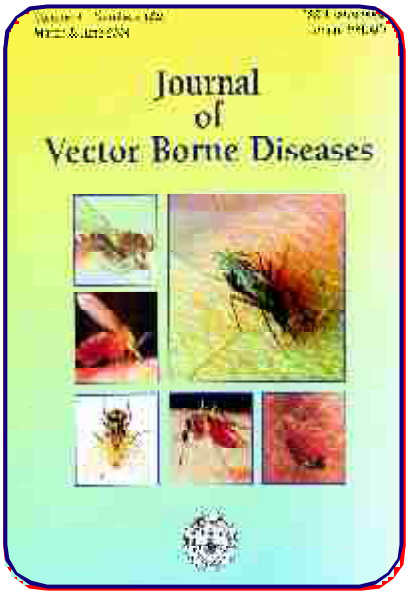
One health education-cum-blood examination camp was organised for the rural population of Tehsil Jewar, District Ghaziabad from 29–31 October 2003.

The exhibition in Hindi was also displayed in addition to display of other activities related to malaria prevention and control. Blood smears of fever cases were taken and examined on the spot and results communicated to the patients.

Publications

Publications

Journal of Vector Borne Diseases

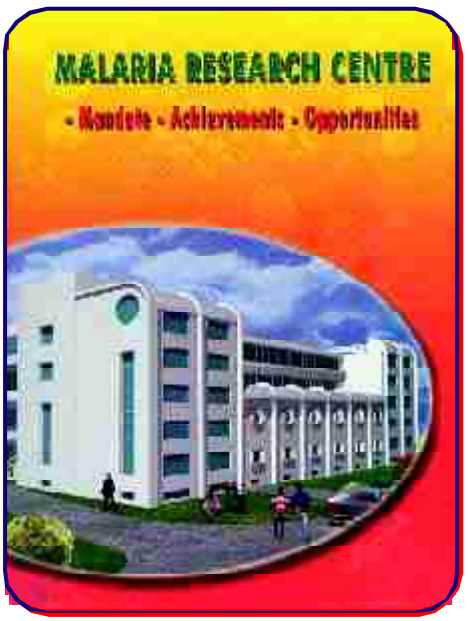


The *Journal of Vector Borne Diseases* superseded *Indian Journal of Malariology* in 2003. This periodical with a totally different elegant look, shape, International size and widened scientific scope is being brought out by the Centre regularly to serve the scientific community. Great emphasis had been made to bring out the periodical within its scheduled time span.

Malaria Patrika

The popular Hindi quarterly magazine of the Centre (consisting all the important activities and latest research information) has entered in its twelfth year of publication fulfilling its purpose of creating awareness in the community about malaria and its control.

Besides above periodicals, Annual Reports of the Centre; IDVC Project and multi-coloured brochures in English— *Malaria Research Centre : Mandate–Achievement—Opportunities;* and in Hindi—*Malaria Anusandhan Kendra : Uddeshaya –Uplabdhiyan–Avsar* had also been brought out.



MRC Website

The website of Malaria Research Centre launched on 28 October 2002 is being maintained successfully. The URL of website is **http//www.mrcindia.org.** The website contains information on:

1. Directory of all the IDVC project field units under the administrative control of MRC with separate page for each field unit describing their activities and contact information.
2. Directory of all scientists of MRC with their address, e-mail and research interest.
3. Information about publications of MRC including year-wise research publications for last 12 years (1993–2004).
4. Separate page for *Journal of Vector Borne Diseases (* Formerly *Indian Journal of Malariology).* In this page all information regarding the Journal such as subscription, guidelines to the authors, editorial board, latest status, and above all, all articles published in the journal as PDF files are made available to the readers separately.
5. Information about activities of MRC such as, activities of audio-visual unit, research activities and services rendered by MRC.
5. Pages with 'Search' form (within website) and 'Feed back' form.
6. Links to important malaria websites.

Additionally, following documents have been uploaded on the website:

1. Chapter-wise PDF files of document entitled, *A Profile of Malaria Research Centre* published on the occasion of the Silver Jubilee celebrations of MRC.
2. Annual Report 2002.

Database on research projects and their activities are under preparation.

The website has been included in DMOZ open directory project. The various pages of website can now be searched using Google® search engine.

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 thesis 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 2003-04, 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 2003-04 data of 1422 books and 70 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 on line 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 for its users as well as to all its field units located in different parts of the country.

"Hindi Week" Celebrations

For compliance of official language policy of Central Govt., MRC has celebrated "Hindi Week" from 15–19 September 2003. Many activities and competitions such as—release of MRC brochure (Hindi), scientific symposium & workshop, essay writing, noting & drafting, and debate competetion were held on this occasion.

On 15 September 2003 the first day of "Hindi Week", the inaugural function was organised and Dr. Vichar Das, Director of Central Hindi Teaching Scheme was invited as the Chief Guest. Programme was organised by Dr. Vandana Sharma, Hindi Officer of the Centre. After welcoming the Chief Guest, Dr. M.A. Ansari, Officer Incharge of the Centre requested Dr. Vichar Das to release the Hindi brochure which was published on the occasion of "Hindi Day". The Chief Guest appreciated and congratulated the Officer Incharge for the brochure and various Hindi activities. In the end of this formal inauguration, Hindi Workshop—first activity of the week was started.

This first activity was organised by Dr. Nutan Nanda, Assistant Director of the Centre. Shri Satyendra Singh, A.D. of the Central Translation Bureau had been invited as the Chief Guest for the workshop. Initially Dr. Nanda welcomed Mr. Singh and requested him to start the workshop. The leactures delivered in the workshop were very useful for the administrative staff.

On 16 September 2003, the second day of "Hindi Week" celebration, two activites- Noting & Drafting and Essay Writing Competetion were organised by Dr. M.C. Sharma, A.D. and Dr. M.S. Malhotra, D.D. The subject of essay writing competetion was *Pardushan aur Swasthya* or *Yuva Pidi aur Bujurg pidi ke Beech Badti Duri* and at the successful completion of competition the first, second, third prizes were given by Dr. Aruna Srivastava, Deputy Director of the Centre in the valedictory function to the following:

Dr. Padmavati Tyagi	First
Shri Pradeep Dutta	Second
Shri M.P. Singh	Third

The first, second and third prizes of noting & drafting competition were given by Dr. Arati Roy, Deputy Director of the Centre to the under mentioned in the valedictory function:

Shri Pradeep Dutta	First
Shri G.L. Puri	Second
Smt. Monika Malhotra	Third

On 17 September 2003, a scientfic symposium was organised by Dr. R.C. Dhiman, D.D. of the Centre. All the scientists of the Centre had participated in this symposium with full enthusiasm and presented their views.

Debate competitions for staff and officers were also organised separately on 18 and 19 September 2003 by Dr. B.N. Nagpal, A.D. of the Centre. The subject of debate competetion for staff was *Fast Food ka Chalan Kitna Saarthak*. Dr. Aruna Srivastava and Shri K.N. Pandey from ICMR (HQ) were the judges. The first, second and third prizes were given to the following in the valedictory function:

Shri Vansidhar	First
Shri Hari Om	Second
Shri K.C. Sehra	Third

The subject of debate competetion for officers was *Fitness Centre Banaam Prakritik Chikitsa*, which was held on 19 September 2004. The Chief Guest and judges were Prof. K.K. Gosoami of Kendriya Hindi Sansthan and Dr. Vijay Shrivastav, A.D.G. from ICMR. The Function was chaired by Dr. M.A. Ansari. First, Dr. B.N. Nagpal initiated the function by welcoming the Chief Guest and Judges. After that the competetion started and first, second and third prizes were given by Dr. M.A. Ansari, Officer Incharge, to the following participants in the valedictory function:

Dr. R.C. Dhiman	First
Dr. Nutan Nanda	Second
Dr. K. Raghavendra	Third

A valedictory function was also held on 19 September 2003 and prizes were distributed to all the winners of the above mentioned competetions. The prizes under incentive scheme for maximum work in Hindi were also distributed to the following:

Shri S.C. Sharma—for maximum Hindi dictation

Shri Ram Dev	First
Shri Mohan Lal	Second
Shri Rameshwar Gupta	Third
Shri Raghavendra Sharma	Third
Smt. Monika Malhotra	Third

At the end of the "Hindi Week" celeberation programme, vote of thanks was given by Dr. Vandana Sharma, Hindi Officer of the Centre.

7. Committees

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