A Profile of

National Institute of Malaria Research

National Institute of Malaria Research

(Indian Council of Medical Research)

Sector 8, Dwarka, New Delhi-110 077

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Preface

Soon after the resurgence of malaria in India, the erstwhile Malaria Research Centre, now renamed as National Institute of Malaria Research (NIMR) was set up in 1977 to undertake basic, applied and operational research on malaria, as well as to provide much-needed support to the National Vector Borne Disease Control Programme (NVBDCP) of the country in epidemiological research, situation analysis, capacity strengthening and investigation and containment of malaria epidemics. It was realized that malaria is a local and focal disease and its transmission is a dynamic process influenced by the changes in ecological conditions, agricultural practices, urbanization, socio-economical factors, cultural practices and meteorological/climatic conditions. This required evaluation and development of control strategies suited to local needs in various eco-epidemiological settings.

Whereas basic research was mainly undertaken at the NIMR Headquarters in Delhi, a major boost to field research was provided by setting up of an Integrated Disease Vector Control Project in 1986 under Science and Technology Mission mode by the initiative of the Prime Minister of India. This led to opening and operation of 13 field stations in different parts of the country as per the need of the programme. After reorganization, NIMR field units are now functionally operating in 10 malaria-endemic localities in India.

Over the past three decades, a number of technologies developed by NIMR were transferred to NVBDCP, which include insecticide-treated nets or long-lasting insecticidal nets, new insecticides, larvicides, larvivorous fishes, expanded polystyrene beads for larval control, environmental methods for mosquito control, rapid diagnostic kits, artemisinin-based combination therapy for the treatment of malaria, etc. NIMR has not only contributed immensely in providing research support in the fields of malaria, but also in other vector borne diseases, like filariasis, dengue and chikungunya, and in programme support by undertaking situation analysis of vector borne diseases, epidemic investigations, human resource development, advocacy, social mobilization, etc.

The present NIMR profile embodies outcomes of research and technical supports provided by the Institute since inception and highlighted on the benefit of the technologies and tools developed/evaluated at NIMR that have brought in alleviating the sufferings of masses from vector borne diseases in the country.

Meanwhile, the Institute has also strengthened its own capacity to fulfill its mandate and currently has a large number of dedicated scientists, technical staff and research students with facilities to conduct cutting-edge research on all aspects of modern biology. The new building of the Institute at Dwarka, New Delhi will provide physical infrastructure to ensure research of international standard facilities and ambience. NIMR is a 'WHO regional referral centre for the identification of *Anopheles culicifacies* sibling species' and ' WHO collaborating centre for laboratory testing and evaluation of public health pesticides', and has been identified as 'National referral centre for diagnosis of malaria' by National Vector Borne Disease Control Programme.

Thanks to the Director General and senior staff of ICMR for their immense and timely support to the Institute. I also take this opportunity to express my sincere thanks to Drs V.P. Sharma and S.K. Subbarao, former Directors of NIMR for their invaluable support and suggestions. I also wish to acknowledge the sincere efforts of all my scientist colleagues and staff, for their help at several stages in bringing out the current NIMR profile.

A P Dash
Director

Abbreviations & Acronyms

ABER	Annual blood examination rate
ACD	Active case detection
ACPR	Adequate clinical and parasitological response
AMA	Apical membrane antigen
An.	*Anopheles*
ANOVA	Analysis of variance
API	Annual parasite incidence
ACPR	Adequate clinical and parasitological response
ACT	Artemisinin-based combination therapy
AS	Artesunate
ASPCR	Polymerase chain reaction
BHC	Benzene hexachloride
BHEL	Bharat Heavy Electricals Limited
Bs	*Bacillus sphaericus*
BSC	Blood slides collected
BSE	Blood slides examined
Bti	*Bacillus thuringiensis israelensis*
CARE	Cooperative American Relief Everywhere
CDC	Centers for Disease Control & Prevention
CDRI	Central Drug Research Institute
CFR	Child falciparum rate
CHC	Community health centre
CMO	Chief Medical Officer
CPR	Child parasite rate
CQ	Chloroquine
CS	Capsule suspension
CSIR	Council of Scientific and Industrial Research
CSP	Circumsporozoite protein
CTDN	Conventionally treated deltamethrin net
CV	Coefficient of variation
CVC	Comprehensive vector control
Cx.	*Culex*
DALY	Disability adjusted life year
DBP	Duffy binding protein
DBT	Department of Biotechnology
DDC	Drug distribution centre
DDT	Dichloro diethyl trichloro ethane
DFID	Department for International Development, U.K.
DHA	Dihydroartesunate
DHF	Dengue haemorrhagic fever
DMO	District Malaria Officer
DND*i*	Drugs for Neglected Tropical Disease *initiative*
DRDO	Defence Research and Development Organization
DST	Department of Science & Technology
DT	Dispersible tablets
EC	Emulsified concentration
EDPT	Early case detection and prompt treatment
EI	Inhibition of emergence
EIR	Entomological inoculation rate
ELISA	Enzyme linked immunosorbent assay
EMCP	Enhanced malaria control project
EPS	Expanded polystyrene
ETF	Early treatment failure
EVBDCP	Enhanced vector borne disease control project
FTD	Fever treatment depot
GCP	Good clinical practice
GMP	Good maufacturing practice
G-6-PD	Glucose-6-Phosphate dehydrogenase
GIS	Geographical information system
GLC	Gas liquid chromatography
GPL	Glycophospholipids
GR	Geographical reconnaissance
GST	Glutathione S Transferase
HBI	Human blood index
HCH	Hexa-chloro-cyclo-hexane
HEC	Heavy Engineering Corporation
HIA	Health impact assessment
HPLC	High performance liquid chromatography
HPLC	High performance liquid chromatography
HRP	Histidine rich protein
HRP	Histidine Rich Protein
ICGEB	International Centre for Genetic Engineering & Biotechnology
ICMR	Indian Council of Medical Research
ICT	Immunochromatographic test
IDPL	Indian Drugs and Pharmaceuticals Pvt. Ltd

IDVC	Integrated disease vector control
IEC	Information, education and communication
IGH	Ispat General Hospital
IGR	Insect growth regulator
ILTP	Integrated long-term project
IOC	Indian Oil Corporation
IRCS	Indian Red Cross Society
IRMS	Institute for Research in Medical Statistics
IRS	Indoor residual spraying
ITN	Insecticide-treated nets
ITS-2	Inter transcribing Space 2
IVM	Integrated vector management
JE	Japanese encephalitis
LN	Long-lasting insecticidal net
LPF	Late parasitological failure
LTF	Late treatment failure
MCRP	Malaria control and research project
MHD	Man hour density
MLO	Malaria larvicidal oil
MMV	Medicines for Malaria Venture
MPI	Malaria parasite incidence
MPO	Modified plan of operation
MQ	Mefloquine
MRC	Malaria Research Centre
MSP	Merozoite surface protein
NAMP	National Anti Malaria Programme
NEDA	Non-conventional Energy Development Authority
NGO	Non-governmental organization
NICD	National Institute of Communicable Diseases
NIMR	National Institute of Malaria Research
NMEP	National Malaria Eradication Programme
NTPC	National Thermal Power Corporation
NVBDCP	National Vector Borne Disease Control Programme
OD	Optical density
OHT	Overhead tanks
PBO	Piperonyl butoxide
Pf	*Plasmodium falciparum*
PHC	Primary health centre
PPQ	Piperaquine
Pv	*Plasmodium vivax*
PWD	Public Works Department
RFLP	Restriction fragment length polymorphism
RMRC	Regional Medical Research Centre
RMRI	Rajendra Memorial Research Institute
RS	Remote sensing
RWH	Rainwater harvesting
SC	Suspension concentrate
SFR	Slide falciparum rate
SIDA	Swedish International Development Agency
SP	Sulphadoxine-pyrimethamine
SPR	Slide positivity rate
ssu RNA	Single stranded sub unit Ribonucleic acid
TLC	Thin layer chromatography
TPP	Triphenyl phosphate
TRAP	Thrombospondin-related anonymous protein
UGT	Underground tanks
UMS	Urban malaria scheme
UNDP	United Nations Development Programme
UV	Ultra violet
VCRC	Vector Control Research Centre
VSP	Visakhapatnam Steel Plant
WDG	Wettable dispersible granules
WHO	World Health Organization
WHO/SEARO	WHO/South East Asian Regional Organization
WHO/TDR	WHO/Tropical Disease Research
WHOPES	World Health Organization Pesticide Evaluation Scheme
WP	Wettable powder

Introduction

The return of malaria in the 1970s on a nationwide scale forced endemic countries to switch back from malaria eradication strategy to that of malaria control with a major aim to reduce morbidity and mortality due to malaria. In India, the National Malaria Eradication Programme (NMEP) (now renamed as National Vector Borne Disease Control Programme) launched a revised malaria control strategy known as the Modified Plan of Operation (MPO) in 1977. Resources under the MPO were inadequate and the infrastructure was insufficient to respond to the challenges in malaria control. The scientific community felt that a massive undertaking such as NMEP could not accomplish the goals of malaria control without a strong research support. In response to the challenge of re-emergence of malaria, the Indian Council of Medical Research (ICMR) reviewed the malaria situation and identified priority areas of research. Time bound research projects in specific fields of malaria were funded from the extra-mural grant of ICMR. Simultaneously in 1977, ICMR established the Malaria Research Centre (MRC) in Delhi to conduct basic and applied research, undertake field research in malariology and help to develop trained man power in the country. The Malaria Research Centre was renamed as National Institute of Malaria Research (NIMR) on 4 November 2005.

The research activities at NIMR were directed towards developing new and innovative practical methods of malaria control. The primary task was to find short-term as well as long-term solutions to the problem of malaria through basic, applied and operational field research. Therefore, the Instutute focused its research activities on vector biology and control, genetics, cellular and molecular biology,

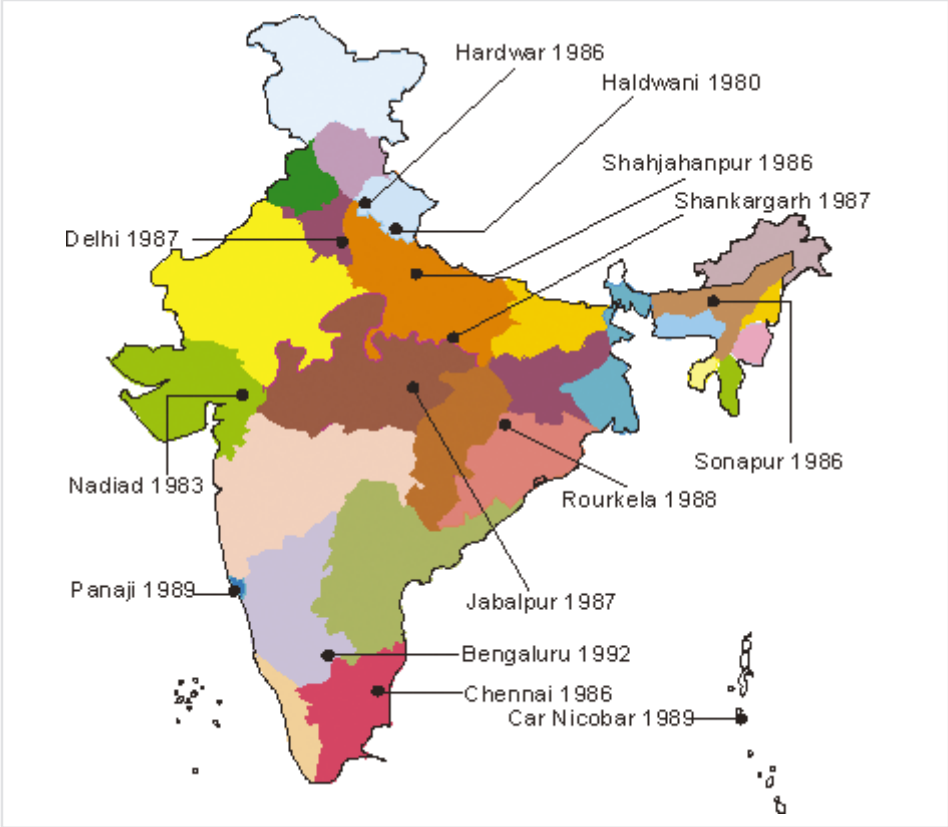


Fig. 1: Location and year of establishment of IDVC field units before re-organization

parasitology, biochemistry, pharmacology and epidemiology. A major programme on operational field research was also taken up by NIMR under the Science and Technology Project on the Integrated Control of Malaria, Filariasis and other Vector-borne Diseases. This project referred to as Integrated Disease Vector Control (IDVC) was launched in 1985 and its activities are spread over in many eco-epidemiological zones of the country. The IDVC project evaluated non-insecticidal methods for disease vector control, such as environmental modification and manipulation coupled with biological control of aquatic stages of vectors. In this approach, intersectoral collaboration, community involvement and cooperation were the key factors. The feasibility of this alternative strategy of malaria control was evaluated at 12 field sites (as shown in Fig.1), namely—Nadiad (Gujarat), Sonapur (Assam), Haldwani and Hardwar (Uttarakhand), Shahjahanpur and Shankargarh (Uttar Pradesh), Chennai (Tamil Nadu), Jabalpur (Madhya Pradesh), Rourkela (Orissa), Panjim (Goa), Car Nicobar (Andaman & Nicobar Islands) and Bengaluru (Karnataka).

A field unit in Delhi was also opened to control mosquito nuisance and malaria and to coordinate the activities of the field units. In March 2006, with the approval of the Ministry of Health and Family Welfare, the IDVC project was re-organised into ten field units (Fig. 2). The field units at Haldwani, Shahjahanpur, Shankargarh, Car Nicobar and Delhi were closed and two new field units at Ranchi (Jharkhand) and Raipur (Chhattisgarh) were opened.

Major areas of research undertaken by NIMR include mosquito fauna surveys, development of simple identification keys for adults and larvae, development of genetic maps using phenotypic and biochemical markers for important malaria vectors, cytotaxonomic studies for the identification of species complexes, laboratory and field studies to examine the biological variations among sibling species, development of molecular identification techniques for sibling species, monitoring of insecticide resistance through space and time, evaluation of new insecticides and biological control agents for vector control and reduction in malaria, evaluation of herbal products as mosquito repellents and larvicides, GIS and RS as tools to map the distribution and breeding site delineation of malaria vectors, etc. Malaria parasite bank has provided a rich resource of malaria parasites for various studies. These studies include molecular and biochemical characterization of parasites, drug resistance mechanisms, evaluation of new molecules/compounds for their antimalarial activity, parasite invasion and adherence mechanisms, etc. Epidemic investigations, clinical drug trials, monitoring of drug resistance, health impact

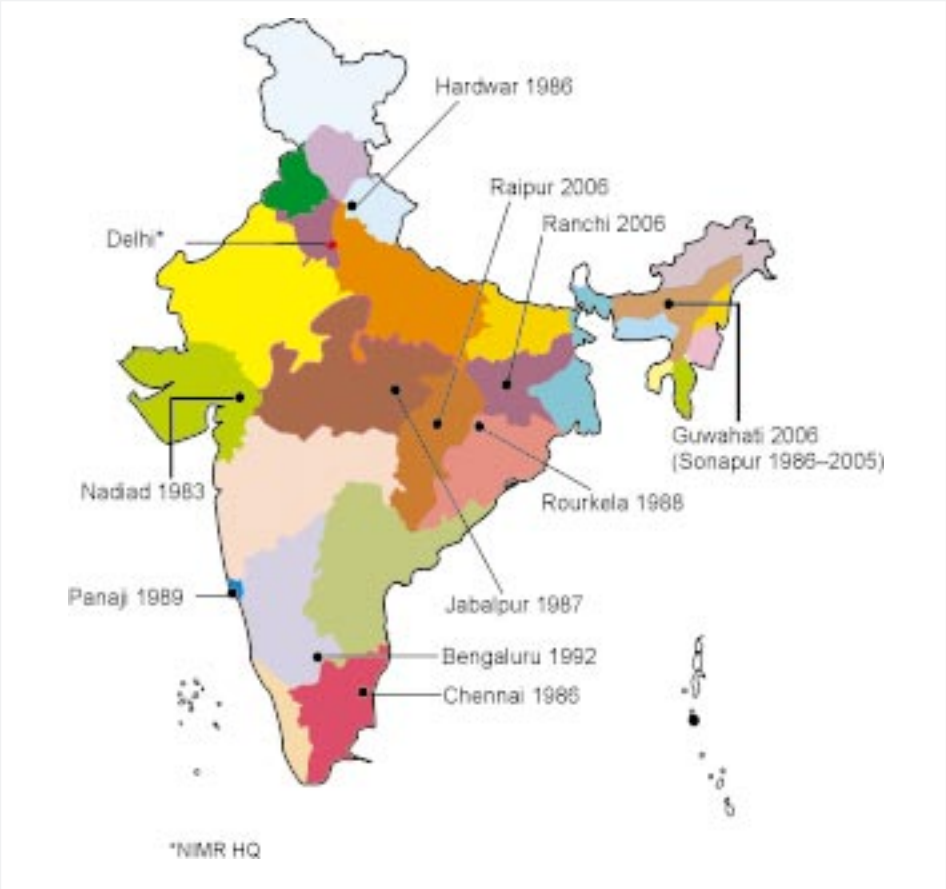


Fig. 2: Location and year of establishment of IDVC field units after re-organization in March 2006

assessment studies at developmental projects, preparation of action plans, etc. have yielded valuable information. Field evaluation of new insecticides, biolarvicides, insecticide impregnated bednets, long-lasting insecticidal nets and materials, drugs and parasite diagnostic kits have provided new armamentarium to malaria control and many of which have found place in the national malaria control programme. Malaria clinics at the headquarters and field units provided excellent diagnosis and treatment facilities to patients which made them very popular and many drug trials could be conducted very easily. Malaria clinics are also providing biological material for research.

At the field units feasibility and economic viability of bioenvironmental interventions was demonstrated in the control of industrial malaria at BHEL complex, Hardwar and IDPL, Rishikesh; rural malaria in Kheda, Shahjahanpur, Haldwani and Shankargarh; urban malaria in Chennai, Goa and Ahmedabad; and coastal malaria in Car Nicobar Islands. In areas not amenable to bioenvironmental interventions, such as in Sonapur (Assam), Rourkela (Orissa) and Mandla (Madhya Pradesh) insecticide treated bednets were highly successful. The network of field laboratories in endemic areas is serving as testing ground for new technologies and helping in the transfer of technology through field demonstrations.

Another important mandate for NIMR is man power development and transfer of technology to the end users. This was achieved very effectively by organizing training courses, workshops and meetings with health personnel and community. Audio-visual programmes/documentaries developed by the Institute are extensively used in training courses and to create community awareness. These are also being used by the State Health Officers in their training programmes. Several candidates have pursued post-graduate and doctoral research at NIMR that led to award of degrees by the national and international universities and institutions of higher learning.

Scientists of NIMR have participated in collaborative research and multinational training courses with several international organizations, undertaken consultancy assignments with WHO and other organizations, served as members of steering and expert committees and task forces, and have been invited to deliberate upon issues of topical interest in several scientific forums.

At the national level, scientists of NIMR have participated in planning of malaria control, in the in-depth reviews of the MPO/NVBDCP, reviewed the research projects in the background of current priorities, provided support for the expansion of malaria research in sister institutions by providing biological material and technical expertise. NIMR has undertaken research and training activities in consonance with the Global Malaria Control strategy and is an active partner in the Roll Back Malaria programme. The Institute had a number of fruitful collaborations with the R&D industry in evaluating the new drugs, insecticides and other tools and looks forward to more of these partnerships in future.

The studies carried out at the NIMR are inter-disciplinary, cutting across classical entomology, parasitology, epidemiology, genetics, immunology, biochemistry and molecular biology with the state-of-the-art tools and analytical procedures. Field operational researches are carried out following the national and international guidelines. Over the years, NIMR has carried out novel researches and the outcome of these researches found place in the planning and implementation of malaria control activities.

❑

Vector Biology

Anopheline Surveys and their Identification

The discovery by Sir Ronald Ross in 1897 implicating mosquitoes in malaria transmission created interest among entomologists for faunistic studies. Industrialization, water management projects, urbanization, deforestation, *etc*. have been the important developmental activities in post-independent era in India. Realizing the influence of environmental changes on species prevalence, the Institute carried out faunistic surveys to update the information on anopheline fauna and on other mosquitoes in different parts of the country (Fig. 1) (Nagpal and Sharma 1983, 1985, 1987; Nagpal *et al* 1983; Singh and Nagpal 1985; Singh *et al* 1985; Uprety *et al* 1983; Yadav *et al* 1989; Sharma *et al* 1985, 1999; Das *et al* 1998).

In addition, NIMR also took lead in publishing and authoring books describing anopheline species prevalent in India. The details of same are given in chapter–"Publications" of NIMR.

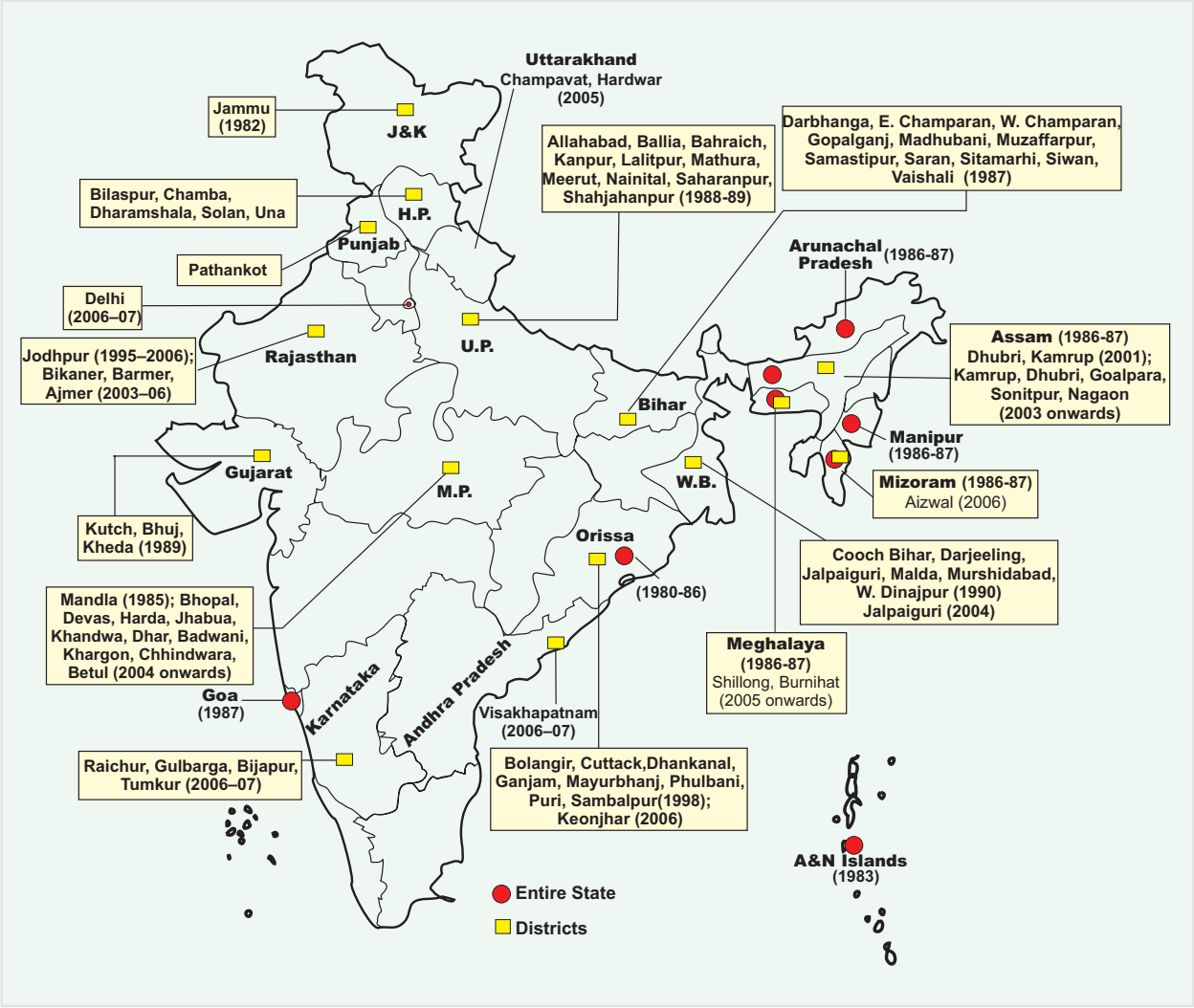


Fig. 1: Anopheline fauna surveys conducted by NIMR during 1980–2008

The references cited in the text are listed in the section "Research Articles Published by NIMR Scientists"

Highlights of Faunistic Surveys Carried out by NIMR

(i) Disappearance of *An. sundaicus* from coastal Orissa state and reappearance of *An. minimus* in northeastern states and Banbasa area of Champavat (Nainital district in erstwhile Uttar Pradesh state and now in Uttarakhand state)

(ii) Identification of *An. nivipes* (by morphological and cytotaxonomic methods) for the first time in India from northeastern states (Nagpal and Sharma 1987; Subbarao *et al* 2000)

(iii) A new focus of *An. sundaicus* in western coast (Kutchch, Bhuj) of India (Singh *et al* 1985); and

(iv) A number of morphological variations were recorded in more than 20 species in these surveys (Nagpal 1990; Nagpal and Sharma 1983).

Computer-aided Identification Tools for Indian Anopheline Species

Electronic Key for the Identification of Adult Anopheline Species

A computer-based identification key for all 58 female Indian anophelines belonging to subgenera *Anopheles* and *Cellia* has been developed. Besides being fast and easily upgradable, the added advantage of this electronic key over the earlier couplet keys is that it can identify the variant species. The software has been developed in Turbo Pascal ver. 6.0 and is menu driven (Nagpal *et al* 1995). The software is supported by computerized drawings (Fig. 2). The knowledge-base has been developed using the book by Nagpal and Sharma (1995). Mosquitoes are divided into two subgenera, namely *Anopheles* and *Cellia* depending on the number of pale areas on costa, subcosta and vein 1. The software of each subgenus consists of four modules. The module 1 is for beginners. Using a microscope or hand lens, the user enters his observations as prompted by computer menus, and species is identified. The module 2 is for experienced users who can recall the identification characters just by entering the species code assigned, these are displayed on the monitor. Characters for quick identification are also highlighted. The module 3 is for distant users in remote areas. The user completes a specially designed proforma and makes a string of coded numbers. As soon as the codes are entered, computer matches the string with the species code string stored. To identify the species, this task of computer can also be done manually using the species code given at the end of the proforma (Srivastava *et al* 1992). In module 4, based on successive characters, each subgenera, namely *Anopheles* and *Cellia* is divided in subgroups forming a nested sequence. At the end, the last subgroup contains only a few species with minor morphological variations leading to an approximate identification of the species.

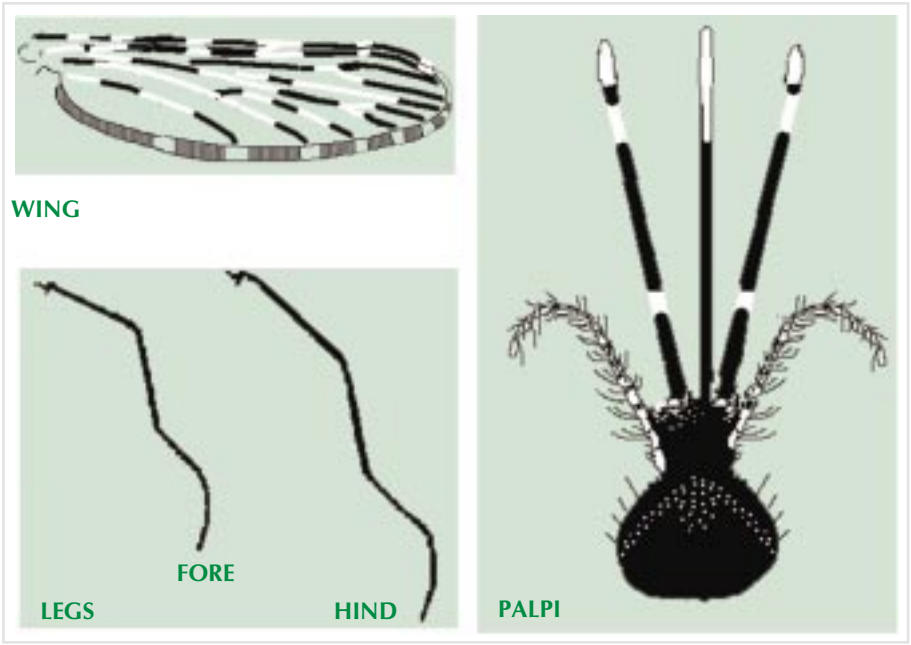


Fig. 2: Computerized drawings showing different characters for the identification of anopheline species through electronic key for identification

The software is very useful for beginners for quick and correct identification. It familiarizes the user with all required morphological characters. The software has proved to be an excellent training tool for entomologists, malariologists, researchers and public health workers.

Electronic Key for the Identification of Anopheline Larvae

A computer software for the identification of the IV instar larvae of 58 anopheline species has been developed. Knowledge-base consists of all the corresponding larval features of a species. The software has been divided into two modules. In the first module, user can enter the characters as seen under microscope through computerized menus and the larval species are identified. The second module is for an experienced user in which each species has got a unique code and by inputting the same, characters are displayed. One can cross-check the characters in the microscope and identify the species. Computerized drawings have also been linked to the modules. The software is user friendly and has been written in Foxpro 2.6. It has been field-tested and works accurately. The knowledge-base can be expanded and updated if new species are recorded. This software is a very useful tool for entomologists working in the field of malaria control in urban, foothill and forested areas of the country.

A Computerized Information System

An information system using updated knowledge-base on 58 species of Indian anophelines has been developed. The software is developed in dbase III plus. Species distribution, derivation of name, biology, biting habit, resting posture, preferred breeding sites, global occurrence, important references, *etc*. have been included in the database. The user can get any information about the Indian anophelines instantly. Also one can access state-wise information and species distribution in India. The software is easily upgradable to include new species. This information system is very useful for entomologists working in national and state health programmes.

Anopheline Identification Album

A CD has been prepared consisting of drawings of adult female and important identification characters, bio-ecology, vector status, Indian and global distribution, *etc*. for each Indian anopheline species (Fig. 3). To generate a tailor-made hard/soft copy album, a software in Visual Basic has been written, user can select the species codes of interest, the data would be processed and user can get album consisting of information on desired species. This album is extremely useful to the entomologists and field workers.

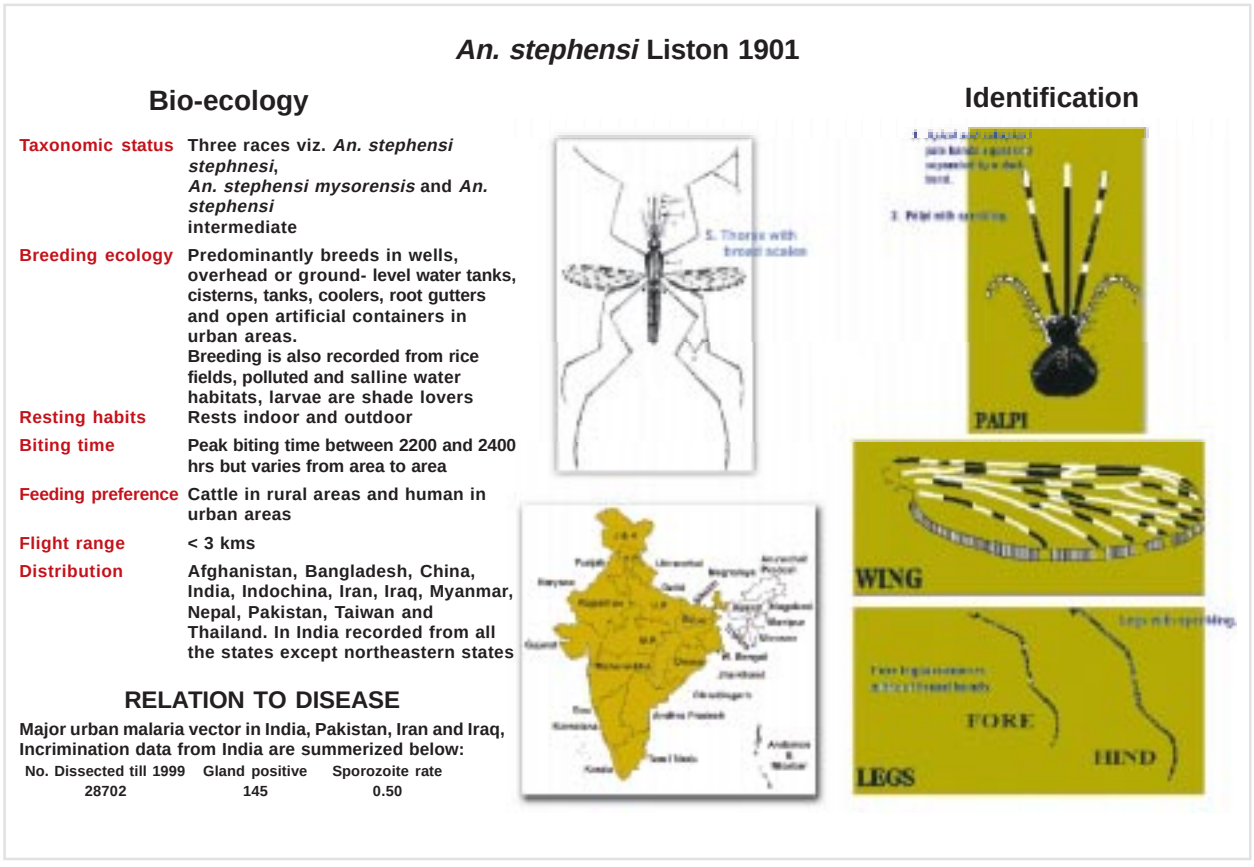


Fig. 3: A page of the album showing the identification characters, bio-ecology, distribution and relation to disease of *An. stephensi*

Pictorial Identification Key for Indian Anophelines

The pictorial identification key for 58 species of Indian anophelines has been published. The English version was released by the then Director General, ICMR (Fig. 4a) and Hindi version was released by the Director, NVBDCP, Delhi (Fig. 4b). The key was prepared on the request of Defence Research Laboratory, Tejpur, Assam and is meant for researchers, field workers and technicians. The pictorial key comprises of an introduction, checklist of Indian anophelines, morphological characters (pictures only) used for the identification and guidelines for using the key and pictorial identification. The breeding ecology of each species in brief is also given in the key.

Fig. 4a: Prof. NK Ganguly, the then Director General, ICMR releasing the pictorial identification key for Indian anophelines (English version)

Fig. 4b: Dr GPS Dhillon, Director, National Vector Borne Disease Control Programme releasing the pictorial identification key for Indian anophelines (Hindi version)

Morphometrics of *Anopheles stephensi*

Ecological Variants Based on Number of Ridges on Egg-float

Two variants which differ in egg-float ridge number and in egg length and width were reported earlier. These are referred as type form and var. *mysorensis*. To resolve the taxonomic status of these two forms, extensive surveys were carried in and around Delhi to collect *An. stephensi* and examine the egg-float ridge number. Several strains available in the insectary of NIMR were also examined (Subbarao *et al* 1987). The study grouped the *An. stephensi* into three categories—14–22, 12–17 and 9–15 ridges on the egg-floats respectively. The category with highest egg-float ridge number corresponded with the type form and the lowest with the var. *mysorensis* reported earlier, and the new egg-float category was designated as 'intermediate'. All the three forms were observed in semi-urban areas while only intermediate and var. *mysorensis* in rural areas. In this study typical urban localities were not surveyed. As genetic studies did not indicate any mating barrier between these forms, the three forms with different ridge number are best considered as ecological variants.

Spiracular Index to Identify Two Ecological Variants

To determine applicability of length of thoracic spiracle and its index as a taxonomic tool to identify two ecological variants of *An. stephensi* at adult stage, hand catches were made indoors and outdoors of *An. stephensi* populations during three seasons—summer, monsoon and post-monsoon. Gravid/semi-gravid females were allowed to lay eggs individually and batches of eggs-based on number of ridges on egg-float. *An. stephensi* type form with ≥15 and *mysorensis*≤14 ridges were separated. Corresponding females were subjected to measurement of thoracic spiracular indices to correlate with ridge counts of both variants. Studies clearly established statistically significant correlation between the ridge count with both thoracic spiracle length and spiracular index. In *An. stephensi* type form, the spiracle length ranged from 0.10 to 0.14 mm and spiracular index from 7–10 while in *mysorensis* these were 0.07 to 0.10 mm and 6–9 respectively. These parameters showed consistent variations in population of mosquitoes emerged during monsoon and summer seasons. The study established that the spiracle length and its index can be used to identify the two ecological variants of *An. stephensi* at the adult stage dispensing with the need of examining eggs.

Mapping and Distribution of Malaria Vectors and other Indian Anophelines using GIS and RS

There are 58 species of Indian anophelines out of which six—*Anopheles culicifacies, An. fluviatilis, An. stephensi, An. dirus, An. minimus* and *An. sundaicus* are the major vectors of malaria in different ecological settings in India. A GIS-based technique has been develolped to map Indian anophelines including malaria vectors. Thematic maps for ecological parameters which mainly govern the distribution of the species such as forest cover, rainfall, altitude, soil type and temperature published by National Thematic Mapping Organization (NATMO), Govt. of India on 1:6,000,000 scale were digitized (Fig. 5). Reported distribution was taken as baseline information. A software was developed to workout favourable conditions for existence of different species. Favourable conditions for continuous variables consisting of all values between minimum and maximum, whereas for discrete variables, individual values were pooled. A mathematical model was developed to extract the range of each parameter and integration. Digitization, overlaying and analysis was done using ESRI GIS software Arc/Info NT 8.1 and Arc View 3.2. Validation was done using reported distribution and field verification.

Anopheles sundaicus—A Species of Coastal Area

An. sundaicus is a coastal species and breeds in brackish water. Presently, it is confined only to Andaman & Nicobar Islands where it is the sole vector of malaria. In A & N Islands, the altitude ranges from sea level to 150 m, and the annual mean temperature is about 25°C. Since very high rainfall is not suitable for immature stages of the vector, areas having ≥1600 mm rainfall were considered as unfavourable. Sandy soil is the characteristic of coastal area, therefore, this soil was selected for the study. Integration of favourable themes resulted in areas favourable for *An. sundaicus* as shown in Fig. 6a (Srivastava *et al*

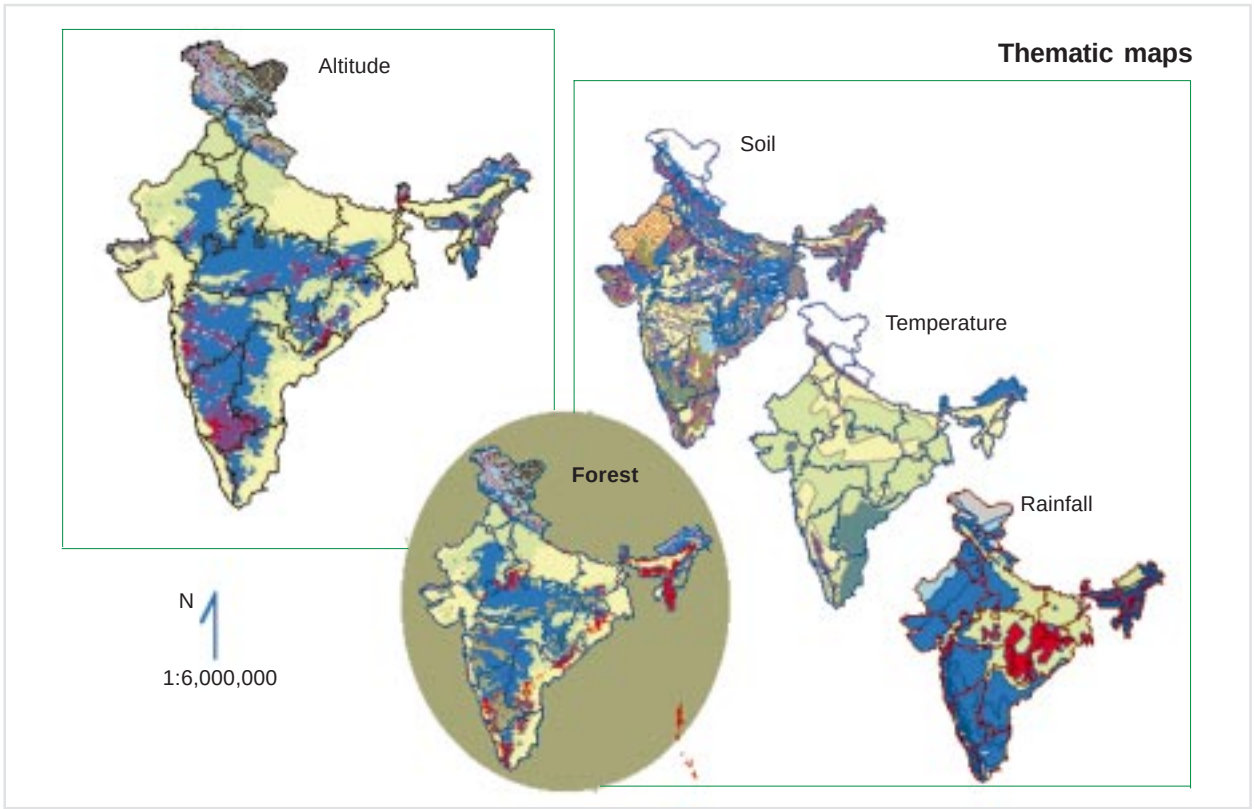


Fig. 5: Thematic maps of ecological parameters, namely altitude, rainfall, forest, soil and temperature plates of Land Resources Atlas of India, NATMO, Govt. of India (1996) in the scale of 1:6,000,000 were digitized by using Arc/Info NT 8.1 on Summagraphic A00 size digitizer

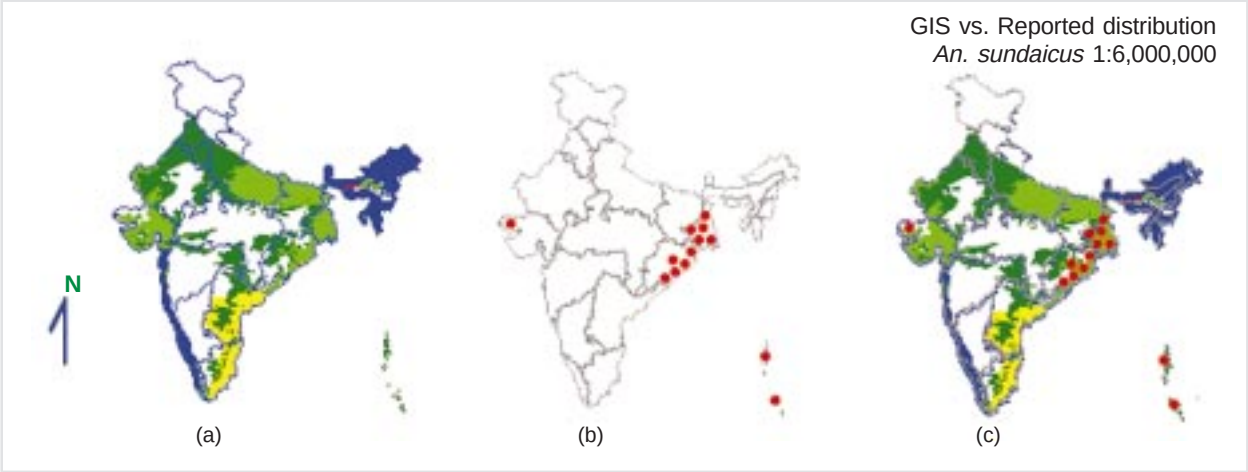


Fig. 6: (a) Map showing GIS-predicted favourable areas for *An. sundaicus* in India (light green colour); (b) Reported distribution of *An. sundaicus* in India; and (c) Validation of GIS-predicted areas favourable for *An. sundaicus*

1997, 1998). Comparison of GIS analyzed map with reported distribution (Fig. 6b) revealed a good geospatial correlation. In Fig. 6c, dots represent the actual sites where the species has been reported overlaid on GIS analyzed map which clearly reveals validity of results. Since it is a coastal species, the comparison was restricted to coastal areas only. For validity of the results a blowup of the Orissa state was taken (Fig. 7). It shows Chilka lake falling in GIS analyzed favourable zone and *An. sundaicus* was reported from this lake several times. In Visakhapatnam of Andhra Pradesh state which falls in favourable GIS zone the species was recorded earlier. On southwestern coast GIS studies revealed that a part of south Kerala is favourable for *An. sundaicus*, therefore, precision surveys are required to confirm the presence of this species.

Anopheles dirus—A Species of Deep Forested Areas

An. dirus is one of the most efficient vectors of malaria in forested areas of northeastern India. It

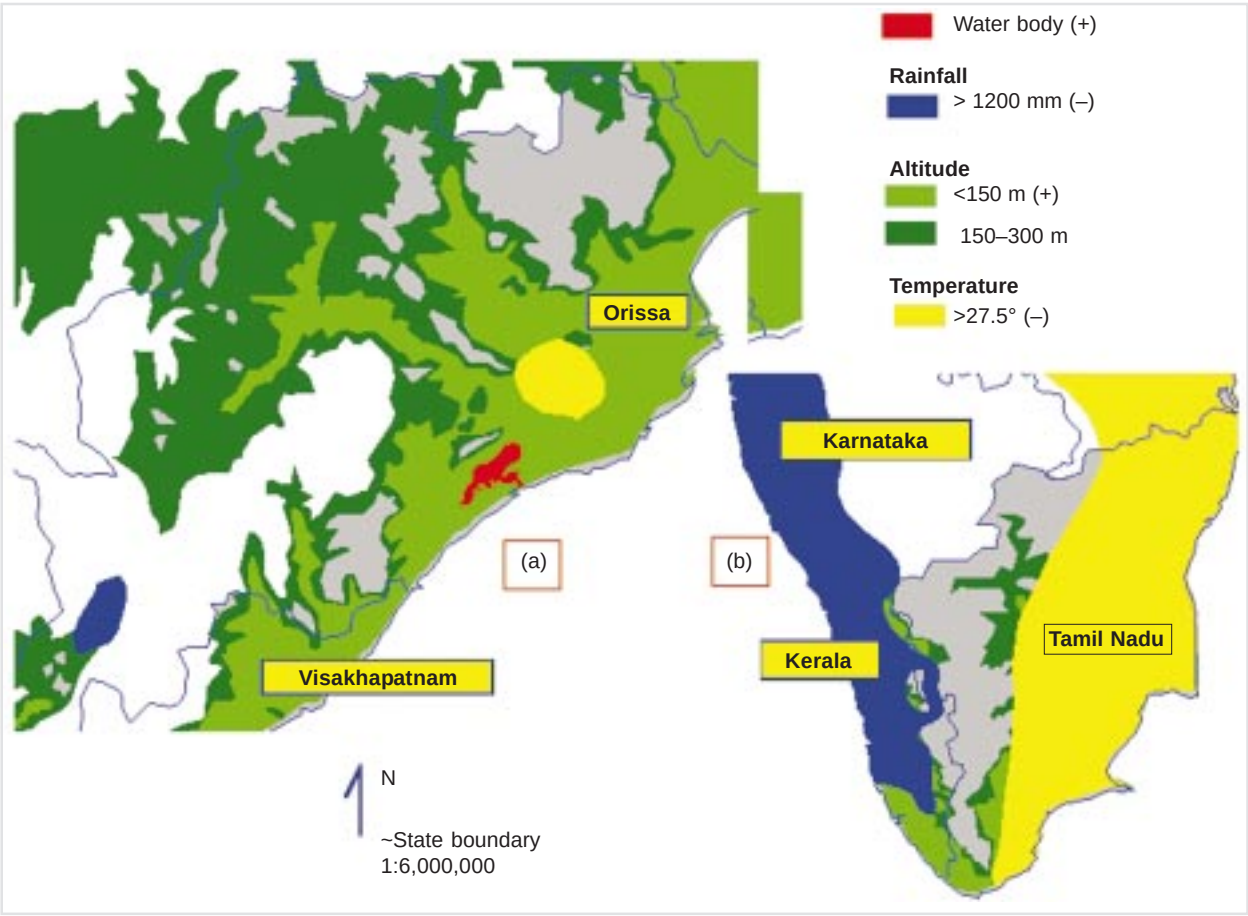


Fig. 7: GIS predicted areas of *An. sundaicus* in (a) Orissa, where the species has been reported several times; and (b) Kerala, no reports from this area

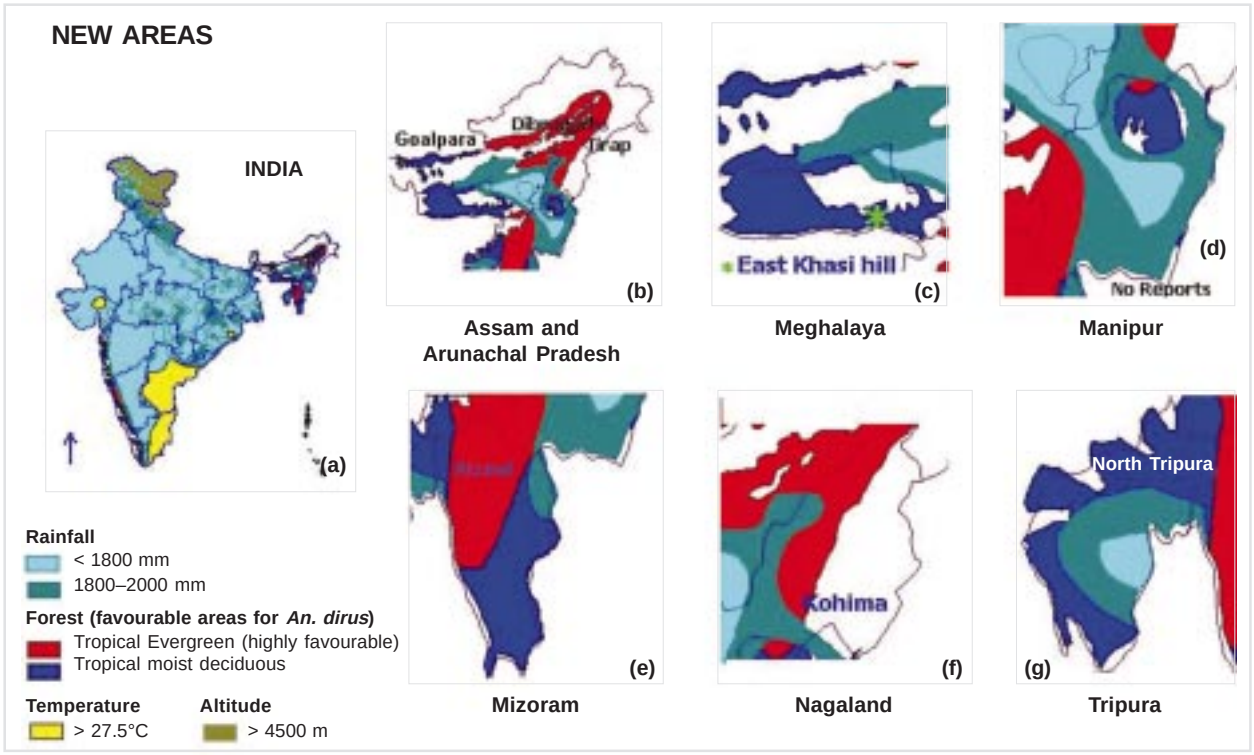


Fig. 8: GIS-predicted areas favourable for *An. dirus* in (a) India; and (b–g) northeastern states

breeds in pools, unused wells, borrow pits, hoof prints and drains covered with foliage in deep-forested areas. The resultant map obtained by integrating the four thematic maps of forest cover, rainfall, temperature and altitude is shown in Fig. 8a. The favourable areas for *An. dirus* are shown in red — evergreen forest and blue—deciduous moist forest. These are mainly located in northeast and western districts of India. In spot surveys, *An. dirus* has been reported from northeastern states and from Karnataka, Kerala, Tamil Nadu, Jammu & Kashmir and Andman & Nicobar Islands. It is observed that GIS-based distribution overlaps the areas where the species has been reported earlier (Srivastava and Nagpal 2000; Srivastava *et al* 2001). Besides these areas, there are some new areas where surveys have not been conducted and the species is likely to be found (Fig. 8a). For validation GIS-predicted areas were compared with reported distribution at micro level. In Assam, the large areas on northeast were found favourable for *An. dirus* through GIS (Fig. 8b). In western Assam, deciduous moist forest areas were found to be favourable for species occurrence and the species has been reported from Goalpara and Kamrup districts. Arunachal Pradesh envelopes Assam from the north, east and small portion on west. The species has been reported from Tirap district, Nampong, Changlang Tenga valley situated near Assam border. GIS also maps some areas favourable on Assam border (Fig. 8b). In Meghalaya, deciduous moist forest on eastern and western sides are favourable for *An. dirus* (Fig. 8c). There are reports from east Khasi hills, Burnihat. In Manipur, there are no reports of *An. dirus* prevalence (Fig. 8d) The entire state of Mizoram is favourable for *An. dirus* and it has been reported from Aizawl and south Mizoram (Fig. 8e). In Nagaland, favourable areas were found in Kohima, Mohokchung, Mon and Wokh, the species was reported from western side of Kohima (Fig. 8f). In Tripura favourable areas are due to deciduous moist forest, it forms a broken semi-circular ring on the western side (Fig. 8g). The species has been reported from north Tripura.

An. dirus has been reported from Jammu & Kashmir, A & N Islands and Kerala. Distribution through GIS also depicts areas favourable in these states. In Karnataka, it has been reported from Bijapur, Chitradurga, Hassan, Shimoga and north Kanara and Coorg, where GIS reconfirms the reports from these areas. From West Bengal, the reports of the species are from Jalpaiguri. Fig. 8 also shows that these areas are favourable for *An. dirus*. Besides new areas in Manipur falling in Indo-China zone, there are receptive areas for *An. dirus* in Madhya Pradesh, Uttar Pradesh and Maharashtra states falling in Indo-Iranian zone (Fig. 9 a–e).

Anopheles minimus—A Species of Forest-fringe Areas

An. minimus has been the most important vector of malaria along the foothills of Himalaya from Uttar Pradesh to northeast in India. The resultant map after integration of thematic maps of soil, forest cover, rainfall, temperature and altitude using GIS shows the areas favourable for *An. minimus* (Fig. 10).

GIS-predicts favourable areas not only in northeast but also in Uttarakhand, Bihar, Chhattisgarh, Madhya Pradesh, Orissa, Maharashtra, Kerala and

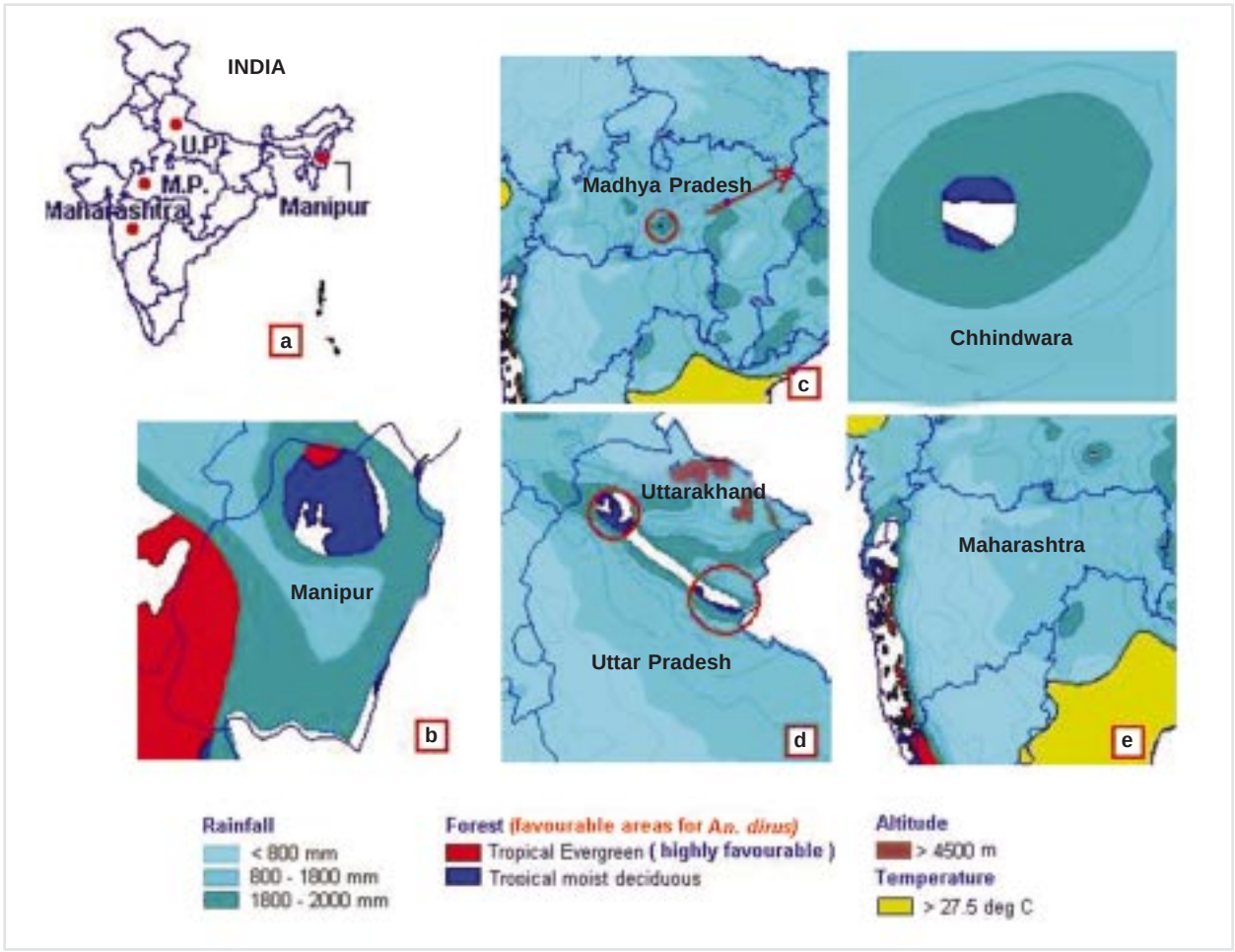


Fig. 9: GIS-predicted new areas for distribution of *An. dirus*: (a) states in India; (b) Manipur; (c) Madhya Pradesh—Chhindwara district is blownup to zoom in small favourable portion of the district; (d) Uttarakhand and Uttar Pradesh; and (e) Maharashtra

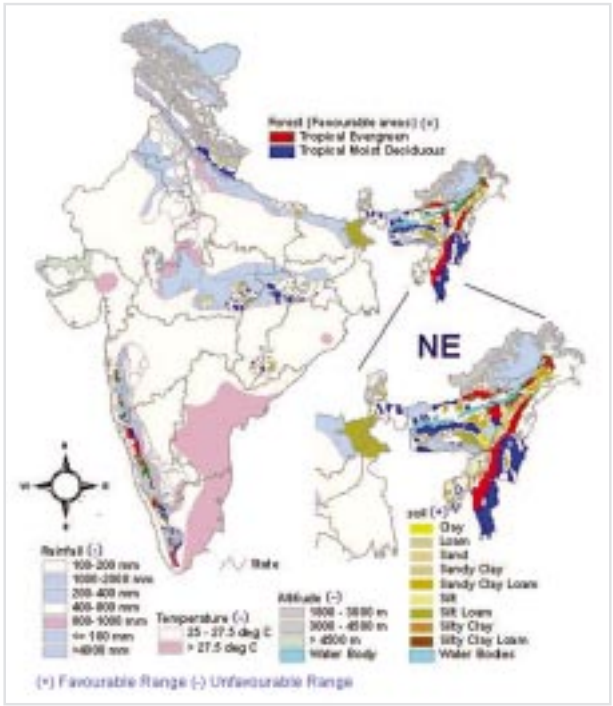


Fig. 10: GIS-predicted favourable areas for *An. minimus* distribution in India, shown in red and blue colours. Inset shows details of *An. minimus* distribution in malaria endemic states

Karnataka (Fig. 11 a–e). It reveals that except Andhra Pradesh all other states have the favourable areas for *An. minimus* distribution from where the species was recorded prior to 1960. In addition, some new areas are also exhibited in Kerala, Maharashtra, Himachal Pradesh and Sikkim.

The results were validated by reported distribution and carrying out precision field surveys at nine locations in four states, namely Uttarakhand, West Bengal, Assam and Meghalaya (Fig.12 a–b) and *An. minimus* was collected from all the locations. In two districts, namely Champavat (earlier Nainital) of Uttarakhand and Dhubri of Assam, the species was reported to have disappeared after 1950s in the former, and in the latter, it was not reported in earlier entomological surveys. In both the places *An. minimus* was encountered besides validation of GIS prediction, reappearance of *An. minimus* at Banbasa (Champavat) and the first report from Dhubri was established. Amazingly, GIS-predicted precisely the location in these districts to conduct entomological surveys and the species could be found there. Favourable areas for *An. minimus* in each state were also delineated using GIS and it was found that northeastern states of India are the most favourable and Mizoram has about 90% of its area favourable

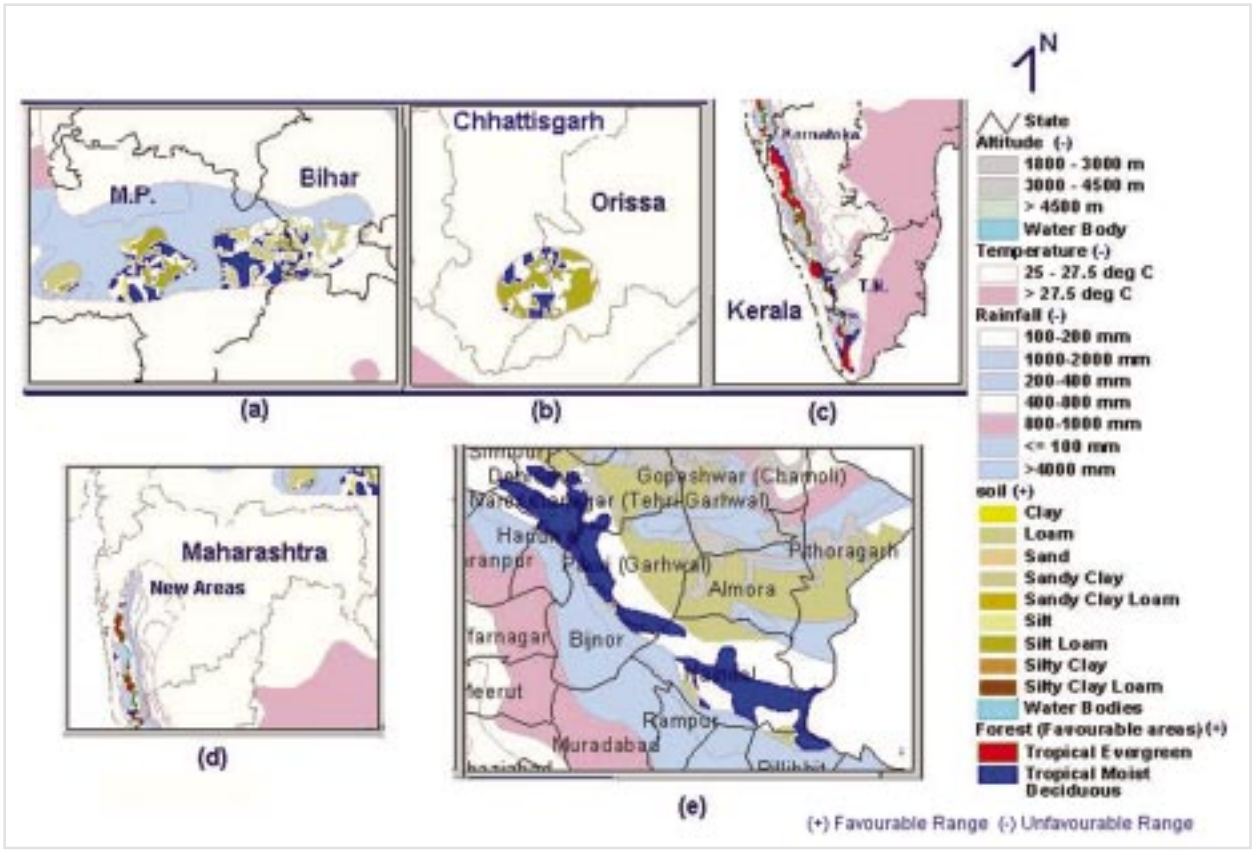


Fig. 11 a–e: GIS-predicted distribution of *An. minimus* in non-endemic areas and also in new areas

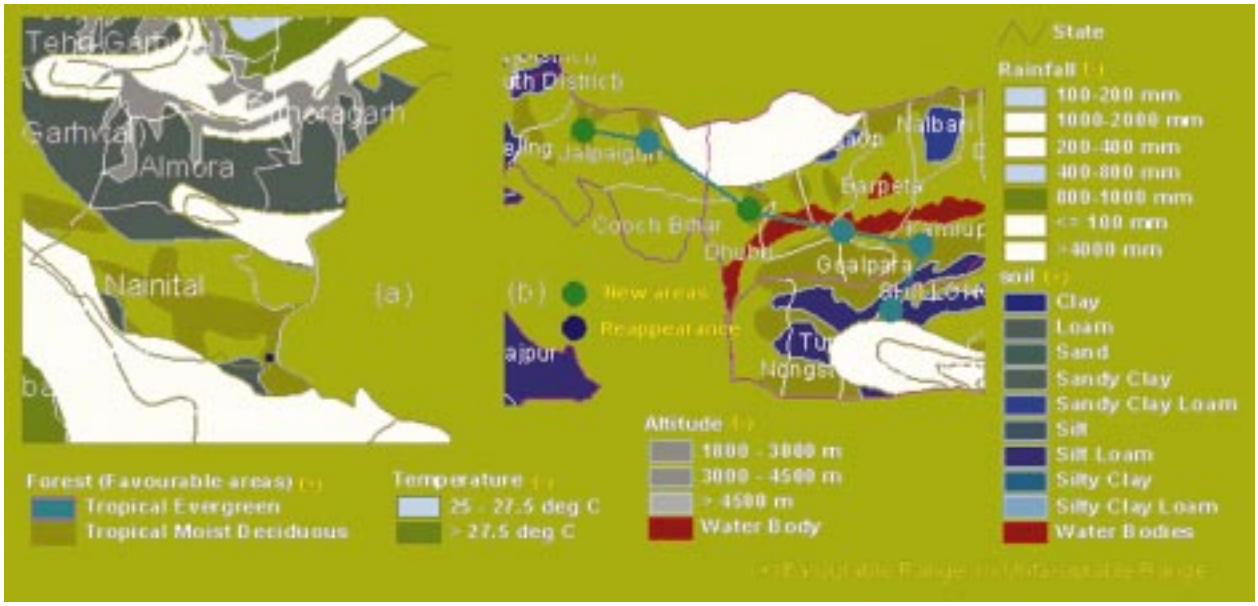


Fig. 12 a–b: Validation spots in GIS-predicted distribution areas of *An. minimus*. Red dots show areas where the species has been reported, pink dots show new areas

for *An. minimus*. There are a few favourable areas in Kerala and Maharashtra where the species could be found in these areas. However, till date no reports of *An. minimus* prevalence are available.

The technique can delineate the areas favourable for any species of flora and fauna, and is very useful for precision surveys. The technique is fast and can be easily duplicated in other parts of the country/ world. In any disease, once the vector distribution is known species-specific control measures can be formulated in a cost-effective manner.

Other Indian Anophelines

Besides mapping of vector distribution, database consisting of ecological parameters suitable for breeding, survival and longevity for non-vector

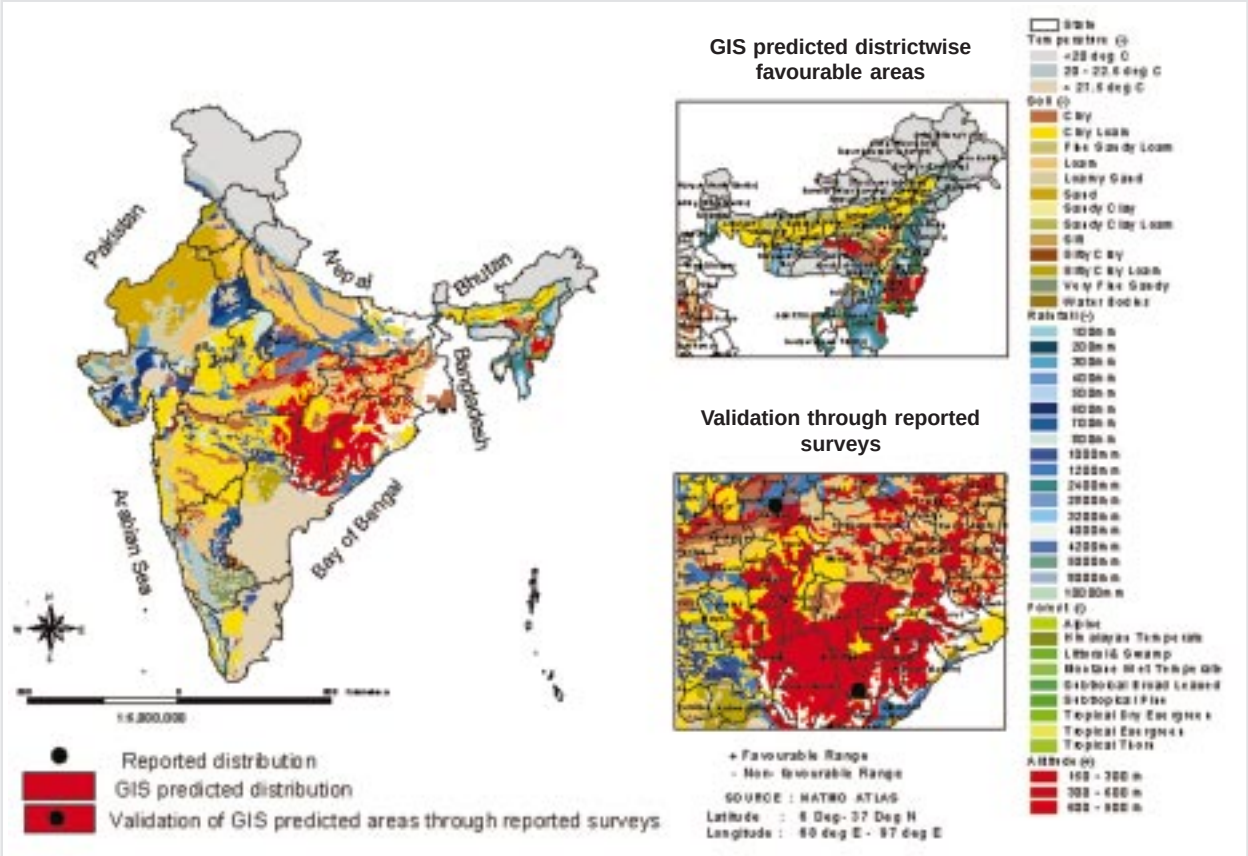


Fig. 13: GIS-predicted distribution of *An. sergentii* in India, a blowup of northeastern states and validation through reported distribution is also depicted in insets

species has been generated. Thematic maps prepared for vector distribution were used, using the software and the mathematical model developed, species-specific conditions were worked out, extracted, overlaid and integrated, the resultant maps showed favourable areas of respective species distribution. Reported areas have been overlaid to validate the GIS-predicted results. The results are reconciling well with the reported distribution. The work on 28 species in subgenus *Cellia* namely, *An. kochi, An. balabacensis, An. elegans, An. karwari, An. tessellatus, An. splendidus, An. pulcherrimus, An. jamesii, An. pseudojamesi, An. annularis, An. pallidus, An. philippinensis, An. nivipes, An. jeyporiensis, An. sergentii, An. moghulensis, An. subpictus, An. sundaicus, An. vagus, An. varuna, An. aconitus, An. majidi, An. maculatus, An. willmorei, An. theobaldi*, *An. dthali, An. multicolor* and *An. turkhudi* has been completed (Fig. 13). A compact disk (CD) has been prepared consisting of distribution of all Indian anopheline species, this is continuously being updated including more species, to be used as a training module.

Application of Remote Sensing (RS) at Village-level to Delineate the Breeding Habitats of *An. culicifacies*

With the advent of finer resolutions in Indian Remote Sensing Satellites, a pilot study was initiated in Tumkur district of Karnataka to delineate the breeding habitats of *An. culicifacies*, the major malaria vector, and to find out the suitable biotope for highly malarious/low malarious areas. Three Primary Health Centres (PHC) with highest, moderate and least malaria were selected for detailed study. Ten villages in each PHC were surveyed for entomological and ecological studies in peak and low malaria transmission seasons. False colour composite images from IRS1C LISS III and PAN data were generated and classification of land-use features was done village-wise (with buffer zone of 1.5 km radius, keeping in view the flight range of vector mosquito) into various land-use categories for peak and low malaria transmission seasons. It was found that delineation of water tanks, rivers, streams, ponds, marshy areas and some irrigation wells not covered by vegetation, was possible. The landscape features critical to malaria endemicity in May were found as water bodies, coconut/areca nut plantation, marshy areas, moist soil, rocks with vegetation and less barren area (Fig. 14). The study indicates that mapping of major breeding habitats of malaria vector and landscape features determining endemicity in similar ecotype is possible through satellite remote sensing technique. In India, the application of remote sensing technique in the field of malaria started in 1992. In this study the data from Indian remote sensing satellites IRS 1A and B with 36.5 meter

Fig. 14: IRS ID hybrid colour composite of Bukkapatna area of Tumkur district

resolution was used. It could be possible to detect water bodies, village boundaries, vegetation, barren areas *etc*. The smaller breeding habitats which are preferred by *Anopheles* mosquitoes were not detectable. With the advent of satellites with higher resolution (5.8 meter) estimation of mosquito larval production was also attempted.

The application of satellite remote sensing was attempted in inaccessible area of Car Nicobar with digital terrain modeling (Dhiman *et al* 2000). A study using a remote sensing and geographic information system was carried out in Car Nicobar, an island in the Bay of Bengal, to target mosquitogenic condition, particularly of *An. sundaicus*. Satellite data (IRS – 1B LISS II) provided a synoptic view of water bodies of inaccessible areas, marshy areas and coconut plantation as the possible breeding habitats preferred by *An. sundaicus*. A contour map and a digital terrain model helped in the prediction of areas prone to waterlogging. Integration of ground surveys, remote sensing and GIS provided comprehensive information in a short time, which would otherwise have been difficult to display and interpret.

Realizing that anopheline breeding is confined to smaller aquatic habitats and spatial distribution of habitats within the source of blood meal have important bearing on the transmission potential at village-level, the detection of landscape features at village-level was also attempted (Dhiman 2002) in three PHCs of Tumkur district (Karnataka) by selecting 10 villages in each category of high, moderate and no (least) malarious PHCs. Ground surveys for geographic reconnaissance of breeding habitats, larval density per dip, man hour density of adult *An. culicifacies* and other anophelines and ecological changes in landscape features were recorded in low (December/January) and peak transmission seasons of malaria. False colour composite was developed from IRS 1C/D LISS III and PAN data and classification was done for generating statistics for different land-use categories, like water bodies, coconut /areca nut plantation, moist land, barren areas, agricultural plantation rocks with and without vegetation, etc.

It was found that tanks, ponds and streams are easily detectable by remote sensing while irrigation wells (which were found supporting mainly *An. barbirostris*) were rarely detectable. Presence of water in water bodies, rocks with vegetation, coconut/ areca nut plantations and less barren area were found as the landscape elements critical to malaria endemicity. It was found that remote sensing could be used for the detection in ecology of an area at village-level resulting into reduction/increase in malaria endemicity .

After tsunami attack in Car Nicobar Island, post-tsunami malariogenic conditions were mapped vis-a'-vis entomological, ecological and parasitological data using IRS P6 with LISS IV sensor having 5.8 m resolution in three bands. Presently, in order to make the use of satellite remote sensing operational, a study is underway in problematic districts of Karnataka.

❑

Bionomics of Malaria Vectors in India

Extensive work on the bionomics of the main malaria vectors, namely *Anopheles culicifacies*, *An. fluviatilis*, *An. minimus*, *An. sundaicus* and *An. stephensi* has been carried out at different locations in the country by the National Institute of Malaria Research and is briefly described below.

Anopheles culicifacies

Anopheles culicifacies is a major malaria vector in the plains of India and is a complex of sibling species A, B, C, D and E, details of which have been given in the chapter "Species Complexes in Malaria Vectors" in this publication. Its bionomics was studied in Delhi, Nadiad (Gujarat), Haldwani (Uttarakhand) and Rourkela (Orissa).

Abundance and Seasonal Prevalence

In Delhi, the study was done in a riverine zone of the River Yamuna and in a non-riverine belt in northwestern area during 1989–91 and the *An. culicifacies* (3.16%) was the third most prevalent species in this area. In the riverine zone, a higher peak of An. culicifacies was observed in April followed by another peak in October and in the non-riverine area, the peak density was observed in May and August (Fig. 15). Generally its density was more in the non-riverine area due to shifting of preferred larval habitat from the riverbed pools to large lakes/ channels and pumping reservoirs. This may be due to extensive pollution of the river zone by sewage discharged by 17 major stormwater drains. Breeding of *An. culicifacies* was maximum in the northern part of the riverine zone where water pollution was at minimal level. In the non-riverine area, *An. culicifacies* breeding was not observed in the western Yamuna canal which at present is cement lined and allows fast-flow of water. Earlier this canal used to be the main source of *An. culicifacies* breeding. The preferences of breeding water for *An. culicifacies* were ponds, pools, ditches, pits and lake (Batra *et al* 2001).

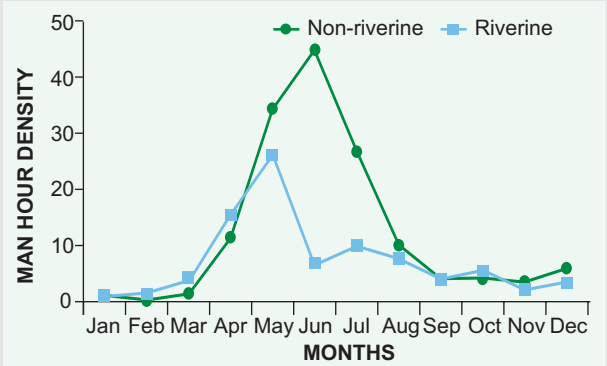


Fig. 15: Man hour density of *An. culicifacies* in Delhi

In a study carried out during 1989–91 in Gujarat, it was observed that *An. culicifacies* was most abundant in the villages situated on the bank of rivers (46%) followed by hilly (23%), canal irrigated (22%), coastal (7%) and non-canal-irrigated areas (2%) (Fig. 16). In general it was most abundant during the summer and monsoon months. In Orissa, stream and riverside villages had high densities.

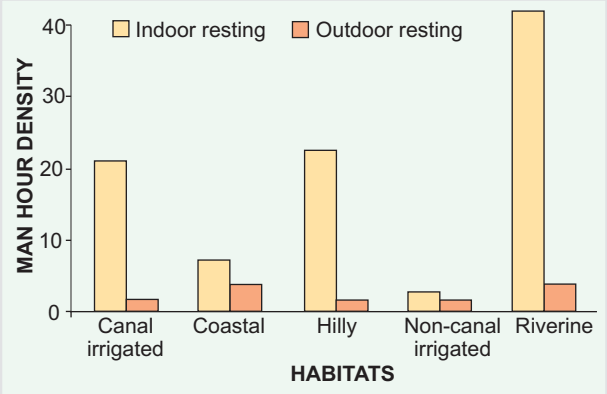


Fig. 16: Indoor resting density of *An. culicifacies* in various physiographical areas in Kheda, Gujarat

Resting Behaviour

Day-time resting preferences of *An. culicifacies* in human dwellings and cattlesheds in areas around Delhi did not differ significantly when subjected to ANOVA. Variations in the densities between the two zones (riverine and non-riverine) were observed which could be due to ecological factors. Though the two zones are in close proximity to each other, they are ecologically and physiogeographically distinct from one another. Thus, it is not the proximity and spatial continuity but the ecosystem which influences the prevalence and densities of *An. culicifacies*. *An. culicifacies* prefers to rest indoors and mainly in the cattlesheds than in the human dwellings in Gujarat and Orissa areas (Chand *et al* 1993). However, it has been observed to rest outdoors in natural shelters and artificial pit shelters as well in all the physiographic areas of Kheda district in Gujarat (Bhatt *et al* 1989).

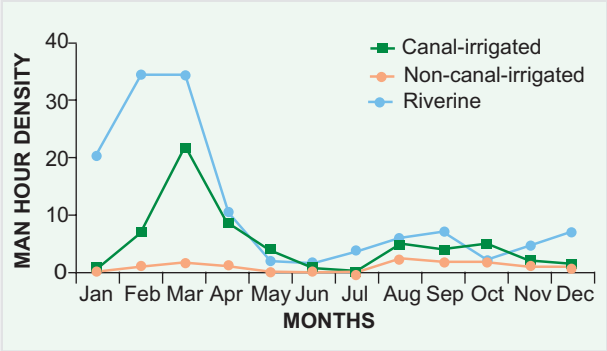


Fig. 17: Seasonal prevalence of *An. culicifacies* in Kheda district, Gujarat

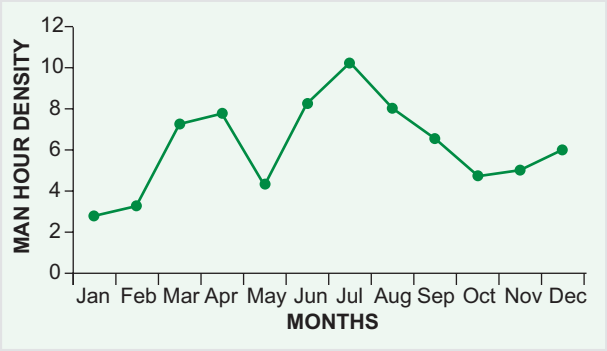


Fig. 19: Indoor resting density of *An. culicifacies* in Sundargarh, Orissa

Seasonal Prevalence

An. culicifacies is found throughout the year in the Kheda district in varying proportions. In the canal-irrigated areas, its density starts to build-up from February and reaches peak in March and thereafter it declines gradually till July. The rise during February is associated with the cultivation of the first crop of paddy. The second rise in the density though less pronounced, is associated with the onset of monsoon and it gets stabilized from August to October and thereafter it further declines in December. In the non-canal-irrigated areas, the density of *An. culicifacies* remains low throughout the year with less wider fluctuations. In the villages situated on the bank of the river, *An. culicifacies* is represented by nearly 67–75% of the all anophelines (Fig. 17). In *Bhabar* area of Uttarakhand in northern India, *An. culicifacies* density remains low during January to June and October to December (Fig. 18). It increases during monsoon reaching a peak in August. In *terai* its density picks up in March, remains high during April to August. In northern Orissa, *An. culicifacies* density shows a small peak in March–April and another in July (Fig. 19).

Host Preferences

Results of blood meal analysis showed that anthropophilic index (AI) of *An. culicifacies* in the riverine zone was 3.7% and in the non-riverine area it was 2.7%. Maximum AI was 5.7% in Mukundpur and 4.8% in Rithala locality of riverine and non-riverine zones in Delhi respectively. *An. culicifacies* is mainly a zoophilic species. Its anthropophilic index was found to be 0.62% which shows little variation between various physiographic areas in Kheda, Gujarat. In certain situations where there were few cattle to divert the vectors, its anthropophilic index has been observed to be considerably high (12.3%) (Bhatt *et al* 2002). In the forest area of Uttarakhand its human blood index was 0.01%.

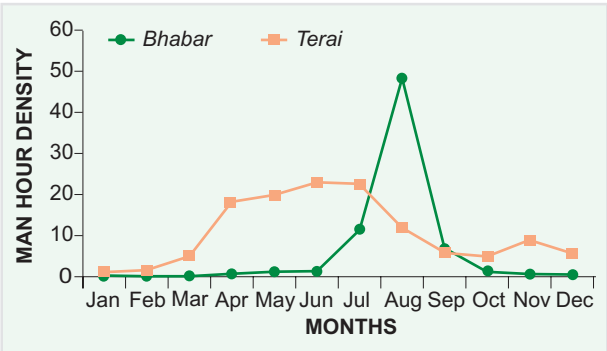


Fig. 18: Seasonal prevalence of *An. culicifacies* in *Bhabar* and *Terai* regions of Uttarakhand

Biting Behaviour

In Delhi, man biting rates of *An. culicifacies* was 0.07 only and a gross probability of biting a man was 0.0011 only. In Gujarat, biting activity of *An. culicifacies* starts in early part of the night from January to April, shifting by one hour from between 1800 and 1900 hrs in January to 2100 and 2200 hrs in April. About 70–90% of *An. culicifacies* population caught during the whole night was found to feed prior to midnight during these months. Bimodal activity was seen during June and July indicating a further shift towards the second and third quarters of the night. During August–September, most biting takes place in the later part of the night. Biting activity was positively correlated with temperature during January ($r = 0.762$; $p < 0.001$) and February ($r = 0.888$; $p < 0.001$) months. However, the biting activity was negatively correlated with the relative humidity during these months ($r = -0.734$ and -0.895; $p < 0.001$). For the rest of the months of the year, the biting activity was negatively correlated with both the parameters.

Human landing collections during the cold season in 1991–92 in a riverside settlement showed that *An. culicifacies* biting activity starts soon after dusk. More biting was recorded outdoors (26.7/man/night) compared to indoors (13.4/man/night), particularly during the early hours and in the last quarter of the night. During the hot season no definite biting rhythm was observed indoors or outdoors. The activity picks up only at the end of the first quarter (1800 to 2100 hrs) of the night and continues till dawn. More activity was observed in indoors (19.8/man/night) than in outdoors (11.7/man/night). In monsoon more activity was observed in indoors (23.5/man/night) than in outdoors (11.9/man/night). The mean indoor landing rates for the cold, hot and rainy seasons varies considerably ($F = 4.7$; $p = 0.0163$),

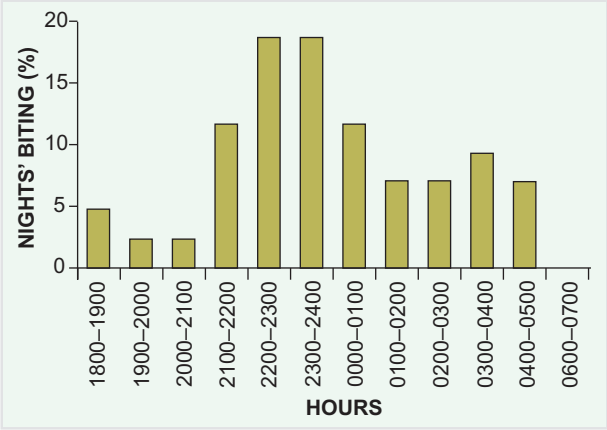


Fig. 20: Biting rhythm of *An. culicifacies* in northern Orissa

while the differences in outdoor landing rates were statistically non-significant (F = 0.4; p = 0.6).

In the forested area of Uttarakhand, it was found active throughout the night and indoor and outdoor landing rates were 0.5 and 1.4/man/night, respectively. In the plain area, *An. culicifacies* outdoor landing rate was 0.4/man/night. The average landing rate of *An. culicifacies* was recorded as 0.95 and 0.2/man/night in forest and dam areas, respectively. In Orissa, the mean landing rate was 0.86/man/night. Its biting rhythm is shown in Fig. 20.

Sporozoite Rate and Vectorial Capacity

In the riverine zone in Delhi, among 197 *An. culicifacies* dissected, the sporozoite rate was 0.5%. In the non-riverine zone, the sporozoite rate was 0.53% among 186 specimens dissected. A total of 59 *An. culicifacies* were assayed from the riverine area by IRMA technique and one specimen of *An. culicifacies* was found positive for CS antigen of *P. vivax*, giving a sporozoite rate of 1.69%.

Previous studies on incrimination of *An. culicifacies* in Delhi carried out in areas adjoining the River Yamuna and in south Delhi showed that *An. culicifacies* plays a role in malaria transmission in these areas but no infected specimen was detected in the non-riverine area of northwest Delhi. *An. culicifacies* plays a role in malaria transmission in the non-riverine area too. On the basis of these results it was found that in the riverine zone, *An. culicifacies* played a greater role in malaria transmission in only the north part of the zone where water pollution is at minimal level.

In a study carried out during 1991–92 in Galteshwar (Gujarat), the overall sporozoite rate was found to be 0.6% (10/1568), but in October the observed rate was 3.25 (4/125). In north Gujarat, the sporozoite rate of *An. culicifacies* during 2001–04 was found to be 1.74% (23/1319) by ELISA test. In this area maximum infective mosquitoes were found in October. The vectorial capacity (VC) estimates for *An. culicifacies* ranged between 0.0005 and 0.5649 for *Plasmodium vivax* and between 0.00001 and 0.3928 for *P. falciparum*. It was highest during November and lowest during January for both parasites. The combined VC for *Pv* and *Pf* showed positive correlation with the slide positivity rate (r = 0.0928; df = 10; p < 0.05). In a study carried out in Uttarakhand the sporozoite rates were recorded as 0.79, 2.4 and 6.0% during September, October and November 1982, respectively.

Ecology

In areas around Delhi, empty lands/marshy swamps, ponds and river water which were contaminated with open sullage/sewage pourings, did not support *An. culicifacies* breeding. Influence of *An. culicifacies* is restricted to northern areas of the riverine zone where water was not polluted with sewage pouring in the river compared to southern part of the riverine area. The changes were attributed mainly due to ecological changes, pollution of the Yamuna River and rapid developmental activities which contribute to the anopheline vector breeding habitats and malaria transmission.

Survey of breeding habitats in Gujarat have shown that *An. culicifacies* prefers to breed mostly in the canals, rivers, irrigation channels, riverbed pools and freshly inundated paddy-fields (Yadav *et al* 1989). Sample positivity rate of different habitats showed that *An. culicifacies* was present in 60.7% samples of immatures from river, 53.1% from irrigation canal, 34.8% from riverbed pools, 28.1% from paddy-fields, 19.8% from irrigation channels, 14.1% from wells, 12.4% from ponds and between 5 and 6% in domestic containers. Positive breeding association of *An. culicifacies* was observed with *An. annularis* in ponds and small pools, with *An. stephensi* and *An. barbirostris* in irrigation channels and with *An. stephensi* in paddy-fields in canal-irrigated areas of Kheda district. *An. culicifacies* was also observed breeding in water bodies infested with aquatic vegetation (Hydrilla).

In *Bhabar* area of Uttarakhand, it breeds predominantly in cemented tanks, pokhars (small pools) and paddy-fields throughout the year. In *terai* its breeding was observed in streams for the greater part of the year. In Orissa, main habitats are streams, riverbed pools, rainwater pools, and freshly inundated rice-fields. It does breed in treeholes filled with rainwater in the forest (Yadav *et al* 1997).

Anopheles stephensi

Anopheles stephensi is an important vector of malaria in urban areas. *An. stephensi* was first incriminated as a malaria vector from Bombay (now Mumbai), Maharashtra in 1911. Subsequently, it was also incriminated from Kutchch (Gujarat) and Madras, (now Chennai) Tamil Nadu in 1938, Ahmedabad City in 1943 and from Broach (now Bharuch) town in 1967. *An. stephensi* exists as two forms, the type form and the variety *mysorensis*, which are distinguished by differences in the egg length and width and by the number of ridges on the egg-float. The type form was

reported to be an efficient vector of urban malaria, whereas var. *mysorensis* was considered to be a rural species and a poor vector. Recent works have indicated yet another variant; the intermediate form with reference to the ridge number on the egg-floats. National Institute of Malaria Research has undertaken several studies on some bioecological aspects of this vector in the rural area of Kheda and Kutchch districts, and Ahmedabad and Surat cities in Gujarat, Chennai City (Tamil Nadu), Panaji (Goa) and desert areas of Rajasthan.

Abundance and Resting Behaviour

Study on bionomics of *An. stephensi* was carried out in riverine and non-riverine areas of Delhi. Among anophelines, *An. stephensi* proportion was 15.7%. The densities of *An. stephensi* observed in riverine and non-riverine areas are shown in Fig. 21. *An. stephensi* was found throughout the year in both the areas. Mean man hour densities and peaks of *An. stephensi* showed wide variation over months in both the zones and thus it could be considered that *An. stephensi* density was regulated by seasonal factors. Dry summer conditions were found favourable for *An. stephensi* as also reported earlier. In a study carried out in rural areas of Kheda district between 1989 and 1991, it was observed that *An. stephensi* was most abundant species in the villages situated in hilly area followed by coastal, non-canal-irrigated, canal-irrigated and riverine areas (Fig. 22).

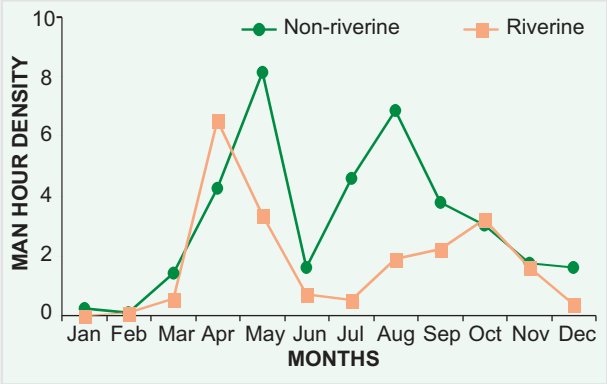


Fig. 21: Man hour density of *An. stephensi* in Delhi

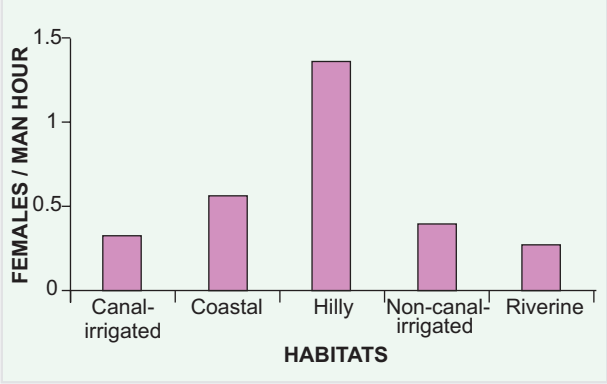


Fig. 22: Man hour density of *An. stephensi* in different physiographic areas of Kheda district, Gujarat

In a study carried out between 1985 and 1988 in the villages of Kheda district, it was observed that *An. stephensi* comprised 0.65% (270/41280) of the total anophelines collected indoors in the riverine villages followed by 0.32% (434/136495) in canal-irrigated and 0.19% (130/69678) in non-canal-irrigated villages. *An. stephensi* mainly rests indoors. However, a good proportion also rests outdoors in natural shelters as well as artificial pit shelters throughout the year. In a study during 1987–88 in five canal irrigated villages of Kheda district, *An. stephensi* proportion among all anophelines collected in outdoor shelters was observed to be 0.28% (14/4998) whereas, in the indoor collections it was 0.24% (98/40681).

In urban areas in Ahmedabad City, *An. stephensi* comprised 9.4% of all anophelines collected indoors by pyrethrum spray collections in five municipal zones between April 1997 and December 2000. In Surat City, collections made from July 1999 to December 2000 revealed that *An. stephensi* population comprised 4.8% of all anophelines collected by pyrethrum spray collection method in human dwelling rooms. Its proportion in the light-trap collections during the same period was 3.3%. A study in Panaji showed that *An. stephensi* rested between 30 cm and 2.4 m height indoors but one-third of these rested above 1.2 m height from the ground level (Sumodan *et al* 2002).

Seasonal Prevalence

In rural areas of Gujarat, *An. stephensi* is prevalent round the year in canal-irrigated, non-canal-irrigated and riverine villages. Its density in riverine villages remains <2 per man hour whereas, in the canal-irrigated and non-canal-irrigated villages it remains <1 per man hour throughout the year (Fig. 23). In urban areas it is collected throughout the year in varying proportions and is most abundant between June and August, which coincides with the onset of transmission season. In Surat City (Fig. 24), it was also collected in light-trap collections throughout the study period and followed a similar trend observed in pyrethrum spray collections. In Kutchch, pyrethrum space spray and

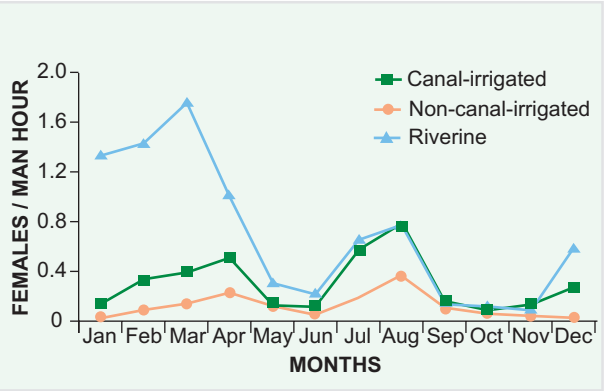


Fig. 23: Density of indoor resting *An. stephensi* in villages of Kheda district, Gujarat

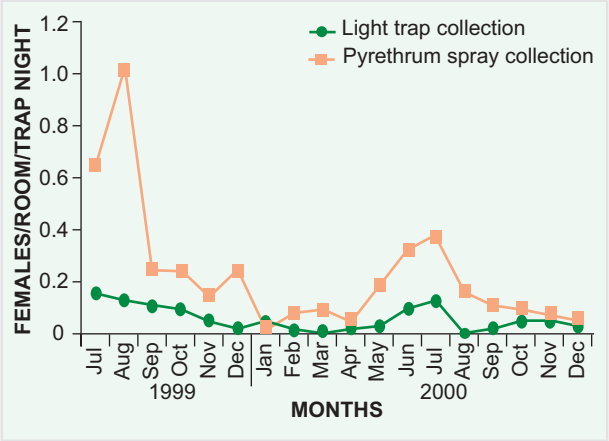


Fig. 24: Seasonal abundance of *An. stephensi* in Surat City, Gujarat

light-trap collections during 2006–2007 of the species showed a high peak in March–April soon after the winters were over and relatively lower prevalence during the wet season.

Host Preferences

Results of blood meals of specimens collected in Delhi showed that anthropophilic index (AI) of *An. stephensi* in the riverine zone was 0.45% and in the non-riverine area it was 1.4%. *An. stephensi* in rural areas is essentially a zoophilic species. There is little variation in the anthropophilic indices between the various physiographic areas of Kheda district. In a study carried out between 1989 and 1991, its anthropophilic index was found to be 1.03% (1/97). In cities, it is the main malaria vector and has shown an increased tendency to feed on man than on cattle unlike in rural areas. In Ahmedabad City, the anthropophilic index of *An. stephensi* was recorded as 8.6% (75/875) and that in Surat City it was 4.9% (15/306). In north Gujarat, it showed an anthropophilic index of 0.8%.

Biting Behaviour

Experiments on mosquito landing collections of the animal baits in villages of Kheda district have shown that the species remains most abundant between February and April (75%) and was encountered throughout the year in low numbers except in July, October and December. Biting occurs mostly before midnight and maximum activity was observed in the first quarter of the night (1800–2100 hrs). Biting activity has been observed till third and fourth quarters of the night, though at a low rate. In all night mosquito landing collections on human baits from July 1999 to August 2000 involving 134 man nights in Surat City, average human landing rate of *An. stephensi* was found to be 0.4% and it represented 54% of all anophelines collected. *An. stephensi* was active throughout the night and 39% population fed before midnight. More activity was observed in outdoors (0.46/man night) as compared to indoors (0.34/man night). Studies on the biting rhythms of *An. stephensi* type form in Chennai (Tamil Nadu) indicated that there was no seasonal variation in the peak biting time during different months indoors, but there was a significant shift in the peak biting time outdoors during different months and the maximum biting occurred during the third quarter of the night and was more pronounced during all the months as compared to that in indoor collections.

Sporozoite Rate

In the non-riverine area in Delhi, the sporozoite rate was 0.78% and in the riverine area it was 0.53%. Samples of *An. stephensi* collected from July 1999 to August 2000 by all methods in Surat City were analysed by ELISA for the detection of sporozoites. In all, 1660 samples were tested and 29 were found positive giving a sporozoite rate of 1.75%. Experimental infection to study the infectivity rate of *An. stephensi* var. *mysorensis* was carried out using blood isolates from 13 patients in Chennai City. The gametocyte count of the patients ranged between 200/µl and 2520/µl. Gut and gland positivity was observed from 7th and 10th day onwards. Gut positivity rates observed on the 7th, 9th, 10th and 13th day were 33.3, 78.57, 50 and 80% respectively. Sporozoites were observed on the 10th and 13th day and the sporozoite positivity obtained was 33.3 and 25% indicating delayed infectivity. In a study in Kutchch, *An. stephensi* was also incriminated from a rural area by ELISA method where its sporozoite rate was 0.07%. The study clearly established the species as a potential vector of malaria in semi-arid zone where thousands of man-made water bodies are being created for water conservation and irrigation.

Ecology

An. stephensi was found mainly in clear water, except in some polluted, blocked cemented drains with grass growth in the non-riverine zone near Delhi (Batra *et al* 2001). In cities and towns, it breeds in all kinds of contained waters in houses, at construction sites, industries, cattle troughs, in sluice valve chambers with water-supply leakages, and ornamental waters. Observations carried out in Ahmedabad City revealed that it prefers to breed in curing chambers at the construction sites and sluice valve chambers. Nearly 36% of all anophelines emerged from the larval samples collected from these two sites were contributed by *An. stephensi*. A small proportion of its breeding was also detected from ponds and pools. In a survey of breeding habitats carried out in rural areas, between 1985 and 1988, it was found that *An. stephensi* prefers to breed mainly in wells and domestic water storage containers. Of the total adults emerged in the samples from wells, this species accounted for 48.3% and from domestic containers for 69.5%. Except wells, other peridomestic habitats were not preferred by it, though its breeding was recorded from river, riverbed pools, irrigation canal, irrigation channel, ponds, small pools and hoof prints.

In Chennai City, an increase in the abundance of type form and intermediate form during post-monsoon was observed in general suggesting that it might be governed by the availability of the types of breeding sites that result from monsoon rains. Oviposition in type form was confined to the period of 1800 to 0100 hrs while in var. *mysorensis* it was prolonged from 1800 to 0600 hrs. The egg-hatching rate was 76.5% being maximum in *mysorensis*. The time required for eggs of the three variants to hatch was found significantly different. Variety *mysorensis* showed higher mortality rate than type form. Studies on the immature stages of type form under ambient conditions in different seasons indicated that throughout the year, larval mortality was significantly higher in IV instar and instar-wise larval mortality did not differ through different seasons. A study in Kutchch, Gujarat showed profuse breeding of *An. stephensi* in irrigation farm ponds and concrete tanks throughout the year.

Bio-ecology of Ecological Variants of *An. stephensi*

Studies on the bio-ecology of two ecological variants—*An. stephensi* type form and var. *mysorensis* were carried out in different seasons—pre-monsoon, monsoon and post-monsoon in an arid zone (Jodhpur, Rajasthan). The study brought out that: (i) two varieties of *An. stephensi* are sympatric in rural areas; (ii) the type form breeds indoor in domestic and peridomestic containers and also rests indoor on unsprayed hanging objects, throughout the year while var. *mysorensis* co-breeds and rests indoors during the summer for lack of breeding waters but moves out with the onset of rain as feral species (Fig. 25); and (iii) it is only type form which is involved in the transmission of malaria. The *An. stephensi* var. *mysorensis* may not be involved in the transmission, basically because of largely zoophagic feeding behaviour and low parity rate during the transmission season. The findings have obvious implication for control of *An. stephensi* transmitted malaria by residual spraying as the species select hanging objects for resting. In Kutchch, >90% population of *An. stephensi* had egg float ridge counts of 12–14 while the remaining had 16 ridges.

Anopheles sundaicus

In India, this species has now disappeared from the main land except for a small focus in the Kutchch area of Gujarat (Singh *et al* 1985) and is found abundantly and widely only in Andaman and Nicobar Islands. Mosquito fauna survey in Car Nicobar Island revealed that *An. sundaicus* was the most predominant species comprising 58% of the total

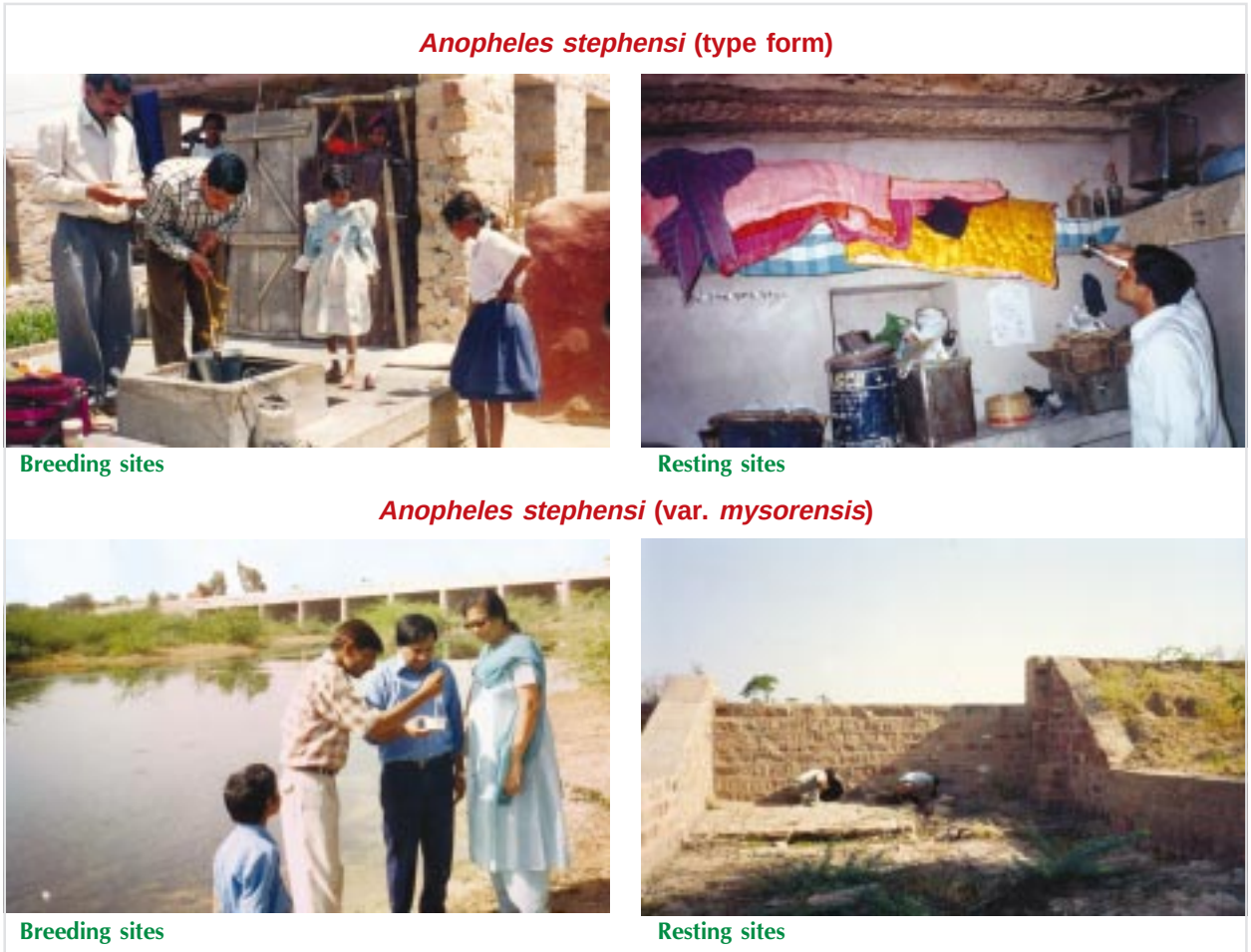


Fig. 25: Breeding and resting places of two variants of *An. stephensi* in Jodhpur, Rajasthan

mosquitoes collected (Das *et al* 1998). A wide variation in the resting and feeding behaviour of *An. sundaicus sensu lato* has been reported on this Island. Although substantial number of *An. sundaicus* rests outdoors, still the species prefers to rest indoors (Kumari and Sharma 1994). Studies on host-feeding behaviour showed that it was primarily a zoophagic species, however, a human blood index of 0.18 was observed in mosquitoes collected from the human dwellings (Kumari *et al* 1993). Indoor human landing rates were comparatively higher than the outdoor landing rates and the species showed bimodal biting rhythm with a peak activity observed between 2230 and 2330 hrs and second peak between 0130 and 0230 hrs (Kumari and Sharma 1994). The species prefers to breed in brackish waters such as creeks, mangrove swamps and marshy areas with a salinity range of 2–14 g/l but its breeding has also been reported from fresh waters such as ponds, wells and rainwater collections having salinity below 0.1 g/l (Sharma *et al* 1999). The wide variations in feeding and resting behaviour and breeding habits suggest that *An. sundaicus* is well-adapted to island ecosystem.

Anopheles minimus

With the resounding success in malaria control during 1960s using DDT as the residual insecticide, it was commonly believed that *Anopheles minimus* had disappeared from the northeastern states. Subsequently, the role in malaria transmission played by other vectors—*An. philippinensis* and *An. dirus* was highlighted. However, owing to persistent transmission of the disease in the region, studies were initiated by NIMR to identify the vectors and establish disease relationships in the changed ecological context. Under this initiative, detailed entomological investigations were conducted in malaria endemic pockets of the region. *An. minimus* was re-recorded in many districts of Assam and adjoining states. Data were collected *de novo* on its ecobiological characteristics including seasonal prevalence, sporozoite infection rates, feeding behaviour and breeding habitats. Its bionomics has been reported by Dev (1996).

Seasonal Prevalence and Resting Behaviour

In the day resting catches during 0900 to 1200 hrs from human dwellings indoor, *An. minimus*, was recorded in the non-intervention malaria endemic villages, and constituted fair proportion of the fauna (40%). They were found to rest on the darker corner of the house, hanging clothes and other articles, underside of beds/tables, *etc*. This species was recorded throughout the year, yet peak densities were observed during the months of March till August corresponding to wet season.

Sporozoite Infection Rates

From the day resting catches, *An. minimus* was reincriminated as vector by detection of sporozoites in the salivary glands. In the year 1990, sporozoite infections were recorded practically for all months of the year except August/September. Infection rate was lowest (0.7%) in March and highest in October (8.5%).

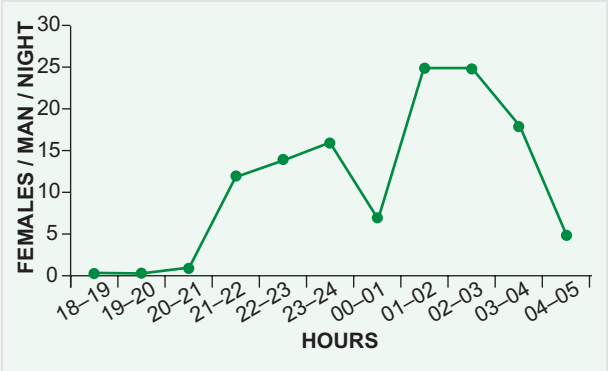


Fig. 26: Hourly landing pattern of *An. minimus* on human host in Assam

Feeding Behaviour

An. minimus was recorded to be highly anthropophagic (AI = 93%) based on hosts blood meal analysis. These mosquitoes landed all through the night on human host but landing was more pronounced between 1200 and 0400 hrs, and biting rate per person per night was 13.72. The hourly landing patterns based on nine all-night landing catches are shown in Fig. 26.

Ecology

Breeding of *An. minimus* was recorded throughout the year in slow-flowing seepage water streams with grassy banks in Assam.

Anopheles fluviatilis

Abundance and Seasonality

It is a main vector of malaria in the forested areas in central India, Himalayan foot hills and in eastern India (Nanda *et al* 2000). It is also found in other parts such as in Gujarat but its role in malaria transmission is doubtful. Studies in Orissa recorded that the species shows a small peak in its density in April just after the winter season when temperature rises, and a second higher peak in October during monsoon (Fig. 27). In *Bhabar* area of Uttarakhand, the adult *An. fluviatilis* density was observed to be low ranging from 0 to 1/man hour (PMH) but high during the month of October (1.7 PMH) and November (2.3 PMH). In the *terai* area, *An. fluviatilis* density was observed high in October (10.7 PMH) and March (9.7 PMH) whereas in rest of the months its density varied from 0.3 to 5.6. Average density of *An. fluviatilis* in the forest area ranged from 13.3 to 82.2. Adult resting catches revealed endophilic behaviour of this species.

In an entomological study, carried out in various

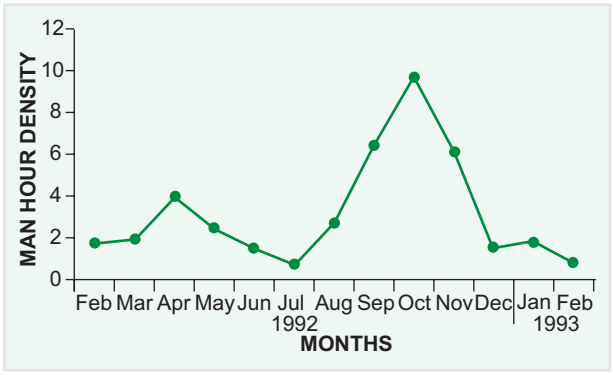


Fig. 27: Seasonality of *An. fluviatilis* in mining areas of Orissa

physiographic areas of District Kheda in Gujarat from 1989–91, the indoor resting density of *An. fluviatilis* was maximum in hilly area 0.57 PMH, followed by canal-irrigated area 0.03 PMH, coastal area 0.02 PMH and riverine area 0.01 PMH. Considerable resting was observed in outdoor natural shelters as well as artificial pit shelters. In the natural shelters, maximum density was recorded from hilly area 0.73 PMH and 2.48 PMH in artificial pit shelters. In a study carried out in the Sardar Sarovar Narmada Project area in Gujarat, indoor resting density of *An. fluviatilis* was high at the dam site than surrounding villages. Variable density was found in both areas throughout the year with two peaks, first during February–April and second in October. Density of *An. fluviatilis* ranged from 0.5 to 12 PMH at the dam site and 0.2 to1.8 PMH in the adjoining villages which reflects high breeding potential at the dam site. Further, it was observed that prevalence of *An. fluviatilis* was higher during winter season (Fig. 28).

Host Preference and Biting Behaviour

In Orissa, *An. fluviatilis* species S comprises nearly 98% and is mainly anthropophilic (Nanda *et al* 1996) whereas it has been found predominantly zoophagic in study areas of Nainital district, Uttarakhand (Shukla *et al* 1998). Its biting rhythm is shown in Fig. 29 indicating that most biting takes place during 2100 to 0400 hrs when most people are asleep. In the forest area in Uttarakhand, *An. fluviatilis* activity was recorded throughout the night with peak activity observed between 2100 and 2400 hrs. Indoor and outdoor landing rates on human host were found to be 1.1 and 3.0/man/night respectively. *An. fluviatilis* was not found in landing collections in the dam and plain areas. At the Sardar Sarovar Project site in Gujarat, the landing rate of *An. fluviatilis* was recorded as 0.28/man/night.

Sporozoite Rate

In the mining areas of District Sundargarh (Orissa), sporozoite rate based on dissections was 1.8%. In Uttarakhand, the sporozoite rates were 1.4, 0 and 62% during September, October and November in 1982, respectively (Choudhury *et al* 1983).

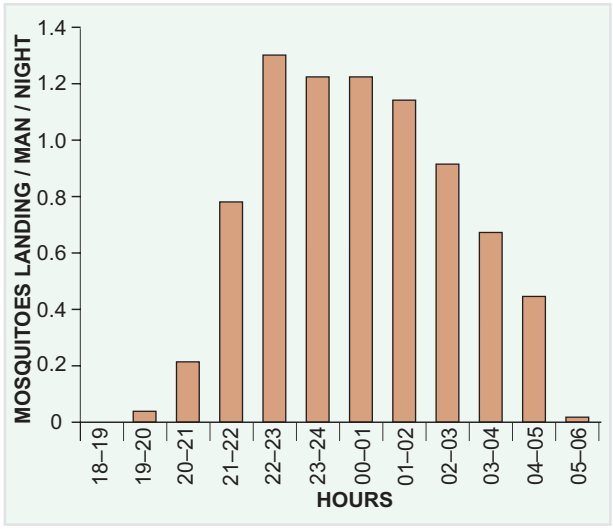


Fig. 29: Biting rhythm of *An. fluviatilis* in mining areas of Sundargarh, Orissa

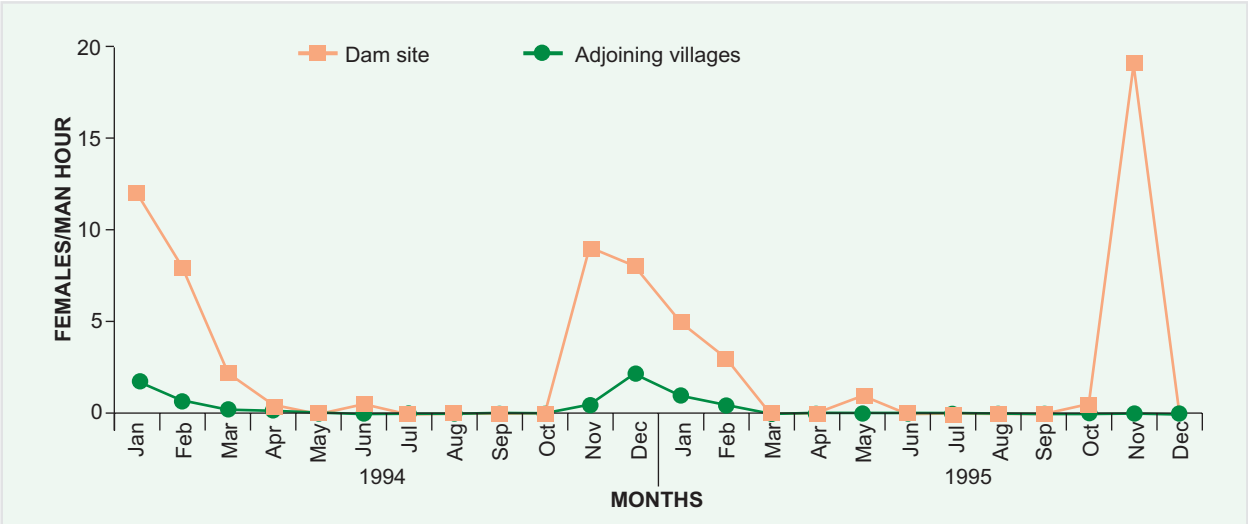


Fig. 28: Seasonal prevalence of *An. fluviatilis* at the Sardar Sarovar Project site and adjoining villages, Gujarat

Ecology

In the forested areas in Orissa, it breeds in sunlit slow running streams and freshly inundated paddy-fields. In Uttarakhand, *An. fluviatilis* was found breeding in artesian (irrigation) drains, streams and ponds (Shukla *et al* 1998). In districts of Orissa, *An. fluviatilis* was found resting predominantly in human dwellings. The preferred resting sites in foothill areas in Uttarakhand were cattlesheds.

❑

Species Complexes in Malaria Vectors in India

Species complexes are of common occurrence among *Anopheles* taxa. Since the discovery of *Anopheles maculipennis* as species complex in Europe, about 30 *Anopheles* taxa have been identified so far as species complexes and they are important vectors of malaria in different parts of the world. Members of a species complex, commonly known as sibling species/isomorphic or cryptic species, are reproductively isolated evolutionary units with distinct gene pools, and hence, differ in biological characteristics which determine their potential in the transmission of disease. Failure to recognize sibling species of anopheline taxa may result in failure to distinguish between a vector and a non-vector; hence the assessment of the impact of control measures may be seriously misleading if they are carried out on a morphologically defined taxon which could be a mixture of two or more sibling species. In addition, differences in the biological characteristics of the members of complexes have an important bearing on the malaria transmission dynamics.

NIMR is actively engaged in recognition of new species complexes in malaria vectors and in studying biology, distribution and transmission potential of the members of each species complex. Indian anopheline fauna comprises of 58 species, of which nine are vectors of malaria. *An. culicifacies, An. fluviatilis, An. stephensi, An. minimus, An. dirus* and *An. sundaicus* are the major malaria vectors. All these vectors except *An. stephensi*, are species complexes—each of these morphological species comprises a number of morphologically indistinguishable biological species commonly known as sibling species or cryptic species or isomorphic species. Among the vectors of secondary importance *An. annularis* and *An. (philippinensis) nivipes* specimens are species complexes. *An. subpictus* is not considered a vector but a few sporozite positive specimens have been found in this species in coastal areas of Puducherry where species B is found. The number of sibling species so far identified among the Indian anophelines is given in Table 1.

Recognition of Species Complexes

Pre-mating Barriers

The assortative mating observed between sibling species in nature is mainly due to pre-mating barriers which involve seasonal/ethological/mechanical isolating mechanisms. Cytotaxonomic studies of natural populations of malaria vectors carried out at NIMR led to the recognition of *An. culicifacies, An. fluviatilis* and *An. annularis* as species complexes. Fixed paracentric inversions in the ovarian polytene

Table 1. Species complexes among Indian anophelines

Species	No. of sibling species identified	Sibling species found in India
An. annularis	2	A, B
An. culicifacies	5	A, B, C, D, E
*An. dirus**	7	D, E (D in northeastern states and E in Karnataka)
An. fluviatilis	4	S, T, U, V
An. minimus	3	A
An. sundaicus	4	A new cytotype D
An. philippinensis-nivipes	3	*nivipes* A
*An. subpictus**	4	A, B, C, D

*These two complexes have not been studied by NIMR.

National Institute of Malaria Research is a WHO Regional Reference Centre for the identification of *Anopheles culicifacies* sibling species and variations

chromosomes were found in natural populations. Presence of alternate arrangements of an inversion—homozygous, standard and inverted, in a population with a total absence of the inversion heterozygotes indicated assortative mating (reproductive isolation). This was taken as evidence for designating the populations as distinct species (Atrie *et al* 1999; Subbarao *et al* 1983, 1994) and the fixed paracentric inversions are used to identify the species. This technique was used extensively to study the biological characteristics of sibling species of these complexes.

Post-mating Barriers

The pre-mating barriers generally break down in the laboratory and sibling species mate at random and produce hybrid progeny. Genetic differences between species are expressed in the form of non-viability of hybrid progeny or hybrid sterility which represent post-mating barriers.

In addition to pre-mating isolating mechanism observed in natural population, post-mating barriers were found to exist between the sibling species of *An. culicifacies* complex. Genetic crosses revealed bi-directional hybrid male sterility between species A and B demonstrating post-mating barriers between these species. In contrast, F_1 hybrid males of reciprocal crosses between species B and C were found fully fertile indicating the absence of post-mating barriers between species B and C. Similarly, fully fertile hybrid males were observed in reciprocal crosses between species T and U of *An. fluviatilis* complex.

Diagnostic Methods for the Identification of Sibling Species

Different methods for the identification of sibling species have been developed which are being used in various studies depending on the feasibility. In case of *An. culicifacies* complex, paracentric inversions readable on the polytene chromosomes have been identified which differentiate most of the members—species A, B, C and D at the population level (Fig. 30) (Subbarao *et al* 1983, 1988 & Vasantha *et al* 1991). Structural variations in metaphase chromosomes of mitotic and meiotic karyotypes (Fig. 31) along with biological variations have been used to differentiate species B and E.

Electrophoretic variations found at lactate dehydrogenase (LDH) locus (Fig. 32) could differentiate species A and D from species B and C. An allele-specific polymerase chain reaction (ASPCR) assay targeted to the D3 domain of 28S ribosomal DNA was developed which discriminates *An. culicifacies* species A and D from species B, C and E (Singh *et al* 2004). Similarly, a PCR-RFLP method targeting mitochondrial cytochromes oxidase subunit II and ITS2 of ribosomal DNA was developed which could differentiate species A and D from species B, C and E (Goswami *et al* 2005) (Fig. 33). Recently, two allele-

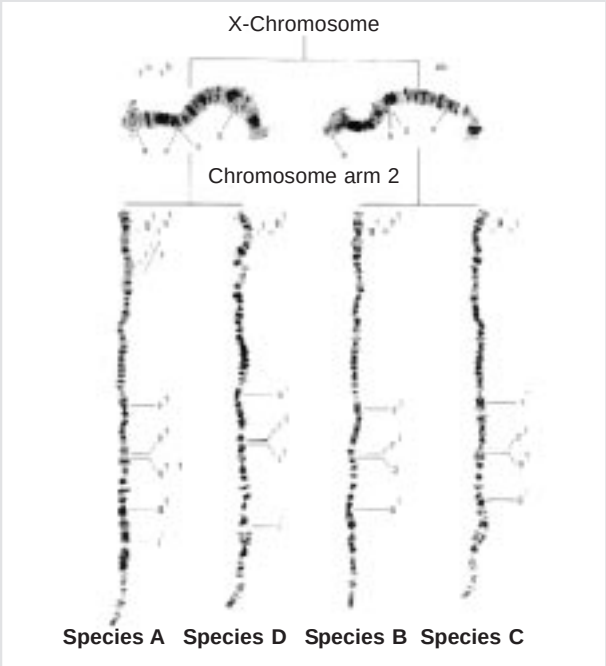


Fig. 30: Schematic representation of polytene chromosomes of *An. culicifacies* sibling species

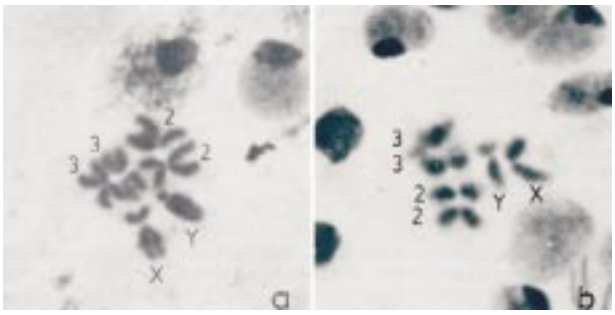


Fig. 31: Male mitotic karyotypes of *An. culicifacies* from Rameswaram Island: (a) Karyotype with acrocentric Y-chromosome (species B); (b) Karyotype with sub-metacentric Y-chromosome (species E)

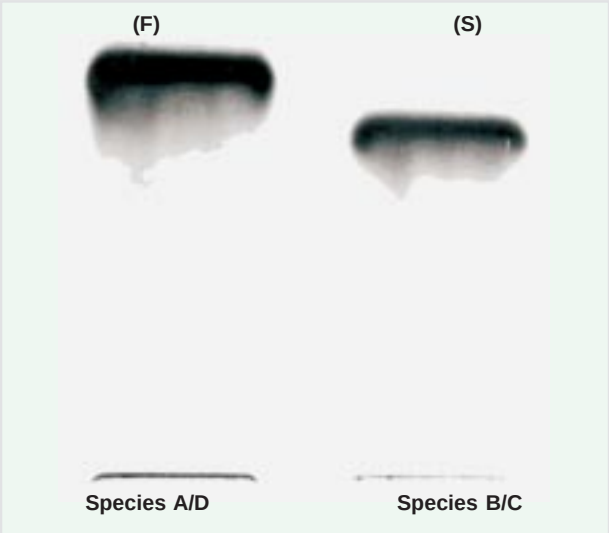


Fig. 32: Differentiation of the members of *An. culicifacies* complex by lactate dehydrogenase enzyme: the two forms of allozyme, i.e. Fast (F) and Slow (S) differentiate species A/D from species B/C of *An. culicifacies* respectively

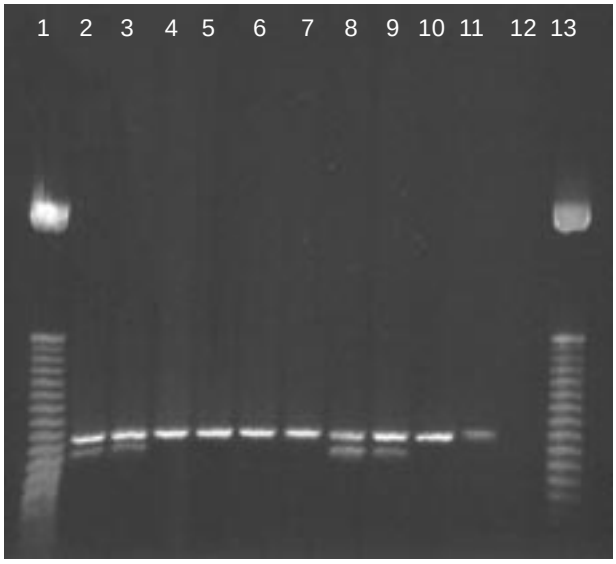


Fig. 33: PCR assay using primers designed from D2 region of 28S rDNA which differentiates species A and D from species B, C and E of *An. culicifacies*. Lanes 1 &13: DNA ladder; Lanes 2 & 3: species A; Lanes 4 & 5: species B; Lanes 6 & 7: species C; Lanes 8 & 9: species D; Lanes 10 & 11: species E, Lane 12: negative control

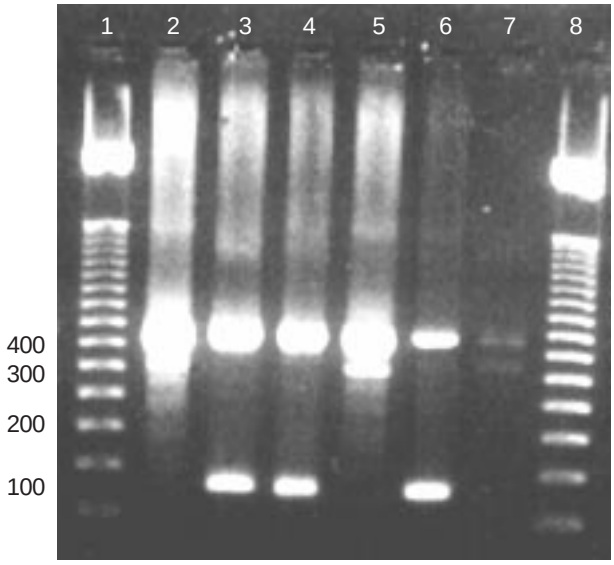


Fig. 34: PCR products obtained by the primers designed from D3 region of 28S rRNA electrophoresed on 2% agarose gel. Lanes 1 & 8: 50 bp marker; Lane 2: *An. culicifacies* sp. A; Lane 3: *An. culicifacies* sp. B; Lane 4: *An. culicifacies* sp. C; Lane 5: *An. culicifacies* sp. D; Lane 6: *An. culicifacies* sp. E from Rameswaram; Lane 7: Negative control

specific PCR assays (AD-PCR and BCE-PCR) using sequence differences in the mitochondrial cytochrome oxidase II (CO II) subunit have been developed. With a combination of two PCR assays, namely the D3-PCR/ITS2-RsaI assay, followed by either the AD-PCR or the BCE-PCR assay, it is possible to identify individual specimens of any of the species of *An. culicifacies* complex (Goswami *et al* 2006) (Fig. 34).

In *An. fluviatilis* complex, species S, T and U were identified by fixed inversions on polytene chromosome arm 2 which are species-specific (Subbarao *et al* 1994). The newly discovered species V in this complex can be identified by two fixed paracentric inversions on polytene chromosomes 2 and 3. Recently, an allele-specific PCR-based diagnostic assay has been developed which can differentiate all the three members of the complex (Fig. 35). The assay is based on the differences in nucleotide sequences of D3-domain of 28S ribosomal RNA in species S, T and U (Singh *et al* 2004).

In case of *An. annularis* complex, for species A and B the only method available is polytene chromosome examination for fixed paracentric inversions (Atrie *et al* 1999). *An. minimus* populations from northeastern states of India were identified as species A by using diagnostic Octanol dehydrogenase electromorphs. Polytene chromosome examination also identifies *An. sundaicus* sibling species. A new cytotype found in Andaman & Nicobar Islands can easily be distinguished from species A, B and C found in other southeast Asian countries by this method.

The discovery of species complexes adds new dimensions to vector control. Members of the complexes are generally isolated by pre-mating

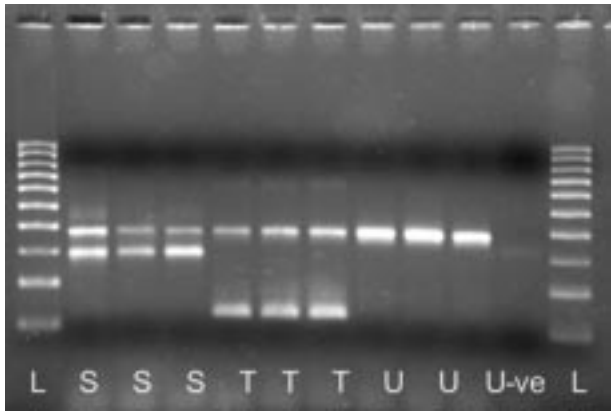


Fig. 35: Differentiation of the members of *An. fluviatilis* complex: PCR product as seen on 2% agarose gel containing ethidium bromide under UV illumination (L= 100 bp DNA ladder, S= Species S, T= Species T, U= Species U, –ve= Negative control without DNA)

barriers. Hence, the genetic structure of each species differs from the other and thus have to be taken into account for all types of control strategies.

Anopheles culicifacies Complex

In India, all five species of *An. culicifacies* complex have been found. Sites surveyed and the distribution of the species is given in Fig. 36. Species B was found almost throughout the country wherever *An. culicifacies* was encountered. In some areas, species B was found exclusively, whereas in other areas it was found sympatric with other species.

Field studies also demonstrated that the seasonal changes in the prevalence of different

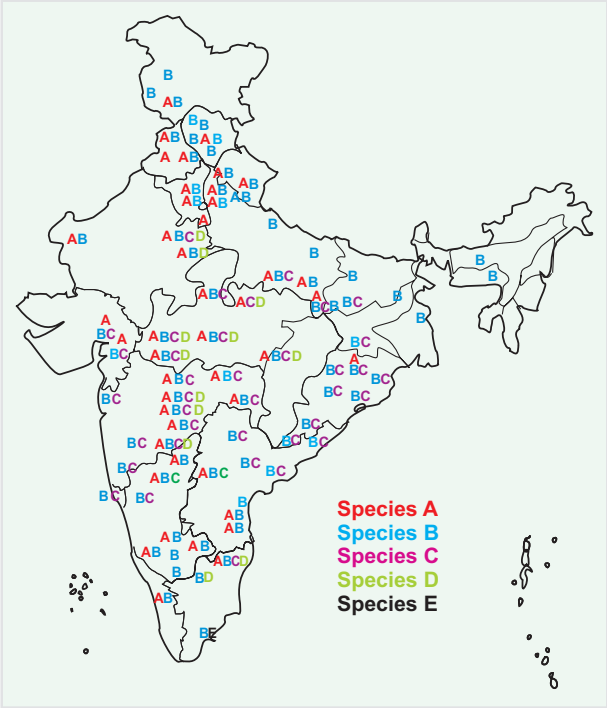


Fig. 36: Map showing the distribution of members of the *An. culicifacies* complex in India

sibling species in areas where more than one species occurred. In Alwar, Rajasthan, where four sibling species (A, B, C and D) were prevalent (Species E was not discovered at that time), all four species were found throughout the year with varying proportion. Species B increased in post-monsoon months while the proportion of species D remained the same throughout the year and densities of species C was very low.

Biological variations among species A, B, C, D and E are summarised in Table 2. Feeding preference, which is an important character that influences the vectorial potential is for cattle for species A, B, C and D while species E is highly anthropophagic. Incrimination studies using immunoradiometric analysis revealed species A, C and D to be vectors of *P. vivax* and *P. falciparum* malaria and species B to be a poor vector, if at all. Species E was found with sporozoites. These species also vary in the rate of development of resistance to different insecticides.

An. culicifacies s.l. was colonised in the laboratory for the first time in India in 1977 (Ansari *et al* 1977). After the discovery of sibling species in this taxon, laboratory colonies of species A, B and C were established from the cytologically identified field-collected isofemale progeny. Distinct differences were observed in laboratory studies with reference to insemination rates, fecundity, longevity, *etc.* among species A, B and C. Under laboratory conditions, the insemination rates were relatively low (< 60%) with species C showing the highest rates. Oviposition in all the three species was confined to the period of 2000 to 0800 hrs. The frequency of egg deposition during the seven-gonotrophic cycles showed normal distribution pattern in all the three sibling species. The egg hatching rate was >70% being maximum in species C. Species A had higher larval mortality rates, longer pupation time and longer emergence time than the other two species (Fig. 37). The effects of crowding which differed significantly among the three sibling species were reflected in higher larval mortalities, longer pupation and emergence time. Species B was the least adversely affected. In

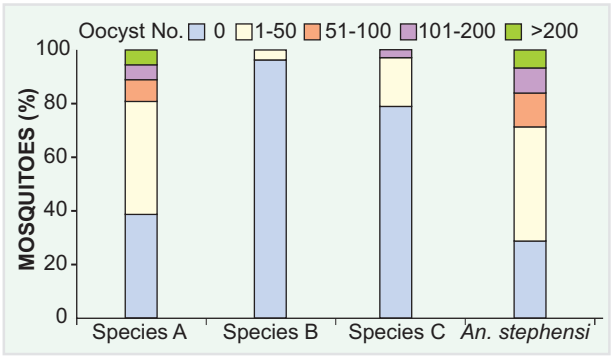


Fig. 37: Frequency distribution of *P. vivax* oocysts in different members of *An. culicifacies* complex and *An. stephensi*

Table 2. Biological variations among the *An. culicifacies* sibling species

	Sibling species				
	A	B	C	D	E
Anthropophilic Index (%)	0–4	0–1	0–3	0–1	High
Biting activity	All night	All night	All night	Up to midnight	–
Peak biting time	2200–2300 hrs	2200–2300 hrs	1800–2100 hrs	1800–2100 hrs	–
Vector potential	Vector	Non/poor vector	Vector	Vector	Vector
Sporozoite rate (%)	0.51	0.04	0.3	0.4	20
Resistance					
DDT	Slow	Fast	Fast	–	–
Malathion	Slow (9–10 yr)	Medium (6–7 yr)	Fast (4–5 yr)	–	–
Synthetic pyrethroids	–	Fast (4–5 yr)	Fast (4–5 yr)	–	–

general, food availability had a greater impact than larval density per se. Horizontal life-table experiments showed: (i) the adult survivorship patterns were similar among the three sibling species, but B had higher longevity than others; (ii) the longevity of males was shortest in species B; and (iii) the gross and the net reproduction rates as well as intrinsic rates of increase were highest in species B and lowest in species A. Species C had a significantly longer generation time than A and B. In addition to the biological variations examined in field population, variations under laboratory conditions were also studied.

In laboratory studies, susceptibility of three members of *An. culicifacies*, species A, B and C were determined against malaria parasites, *P. vivax, P. vinckei petteri* and *P. yoelii yoelii*, where it was found that species A had significantly higher oocyst load, oocyst rate and sporozoite rate as compared to species B and C. Species B was found least susceptible (Fig. 38).

A strain of *An. culicifacies* species B exhibiting complete refractoriness to *P. vivax* sporogony was isolated. In this line, late ookinetes are encapsulated with melanin like pigment within the midgut epithelia and further sporogony is completely aborted (Fig. 39). The strain is partially refractory to *P. falciparum* and rodent malaria parasite, *P. vinckei petteri* (Adak *et al* 2006). Genetic analysis revealed that the gene for refractoriness is dominant and autosomal. This strain is now being used to study the host parasite interaction at genetic, biochemical and molecular level. The gene(s) of this kind is(are) of great interest and research groups involved in the development of transgenic mosquitoes for malaria control are looking for such genes.

Molecular characterisation of a serine protease (*acsp*30) – encoding gene from *An. culicifacies* revealed that it was expressed in high abundance in the refractory (R) strain compared to the susceptible (S) strain. Gene organisation and primary sequence of *acsp*30 were found identical in R and S strains suggesting a divergent regulatory sequences of *acsp*30 in these strains. To examine this further, the

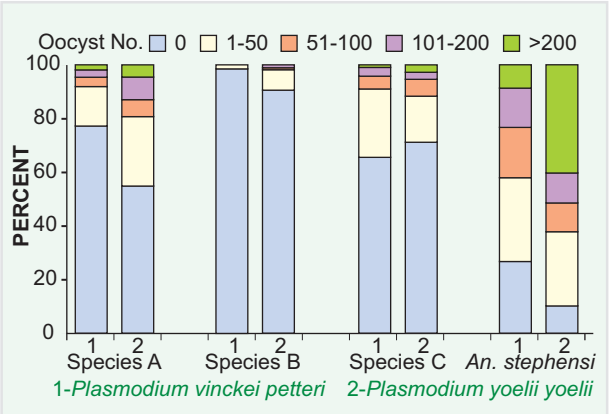


Fig. 38: Frequency distribution of rodent malaria oocysts (*P. v. petteri* and *P. y. yoelii*) in different members of *An. culicifacies* complex and *An. stephensi*

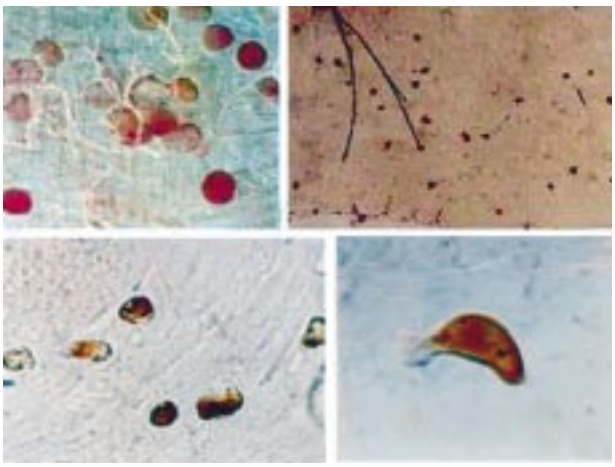


Fig. 39: Midguts of *An. culicifacies* species B refractory strain showing encapsulated *P. vivax* parasite

upstream regulatory sequences of *acsp*30 were isolated, cloned and evaluated for the presence of promoter activity. The 702 bp upstream region of *acsp*30 from the strains revealed sequences divergence. The promoter activity was found to be 1.5 fold higher in the R strain than in the S strain (Rodrigues *et al* 2007). Therefore, the specified upregulation of *acsp*30 in the R strain only in response to *Plasmodium* infection suggests its role in contributing the refractory phenotype to the *An. culicifacies* mosquito population.

Further, the role of prophenoloxidases, which are the key components in recognition and melanisation of invading organisms was investigated. The transcript levels of prophenoloxidase–encoding AcPPO6A gene were found to be higher in naive adult refractory female mosquitoes as compared to female susceptible mosquitoes. Upregulation of AcPPO6A transcription in response to parasite challenge exclusively in refractory strains suggests its role in melanotic encapsulation of the parasite (Rodrigues *et al* 2008).

The laboratory feeding experiments suggest that in addition to encapsulation and melanisation of oocysts in species B, there appears to be another more common genetic/physiological mechanism in species B, *i.e.* parasites in the ingested blood in the gut are destroyed before they enter into the midgut epithelium. Studies on life-table parameters such as longevity did not indicate that species B falls short of requirements to be an effective vector. Further, species A, B, C and D were all predominantly zoophilic. Thus, the genetic and physiological background is the main factor for species B to be a non-vector.

In epidemiological studies, a good correlation was observed between sibling species prevalence and malaria incidence. Broadly, it can be stated that as species B is a non-vector, there would be no malaria in areas where only species B is found. Also in areas where other sibling species are found in low proportions along with species B the incidence of malaria would be low. These findings coupled with geographical distribution of sibling species were used

to stratify the country and to recommend control strategies.

Anopheles fluviatilis Complex

Mapping geographic distribution of *An. fluviatilis* sibling species and studies on their bionomics and role in malaria transmission have been carried out by conducting spot surveys and longitudinal studies in different parts of India. Results revealed that species S, T and U have definite distribution pattern (Fig. 40) and exhibit distinct differences in their biological characteristics (Table 3). Species T is most widely distributed in India whereas species S is predominantly found in Orissa state. *An. fluviatilis* species T and U prefer to rest in cattlesheds and are primarily zoophagic (Nanda *et al* 1996). These species appear to be playing very minor role in malaria transmission (Sharma *et al* 1995; Shukla *et al* 1998). In contrast, species S prefers to rest in human dwellings and is highly anthropophagic. Vector incrimination studies have shown that species S is a very efficient vector of malaria in areas of its distribution (Subbarao 1998; Nanda *et al* 2000).

Susceptibility of *An. fluviatilis* species T, a non-vector, was compared with two established malaria vectors, *An. stephensi* and *An. sundaicus* in laboratory feeding experiments by feeding them artificially (through 'Parafilm' membrane) on the *P. vivax*-infected blood having mature gametocytes. Examination of gut of mosquitoes on Day 6 and salivary glands after 9th day of infective feeding (incubation temperature 27°C) revealed that all the three species had high oocyst and sporozoite rates and there were no significant differences in these rates among all the three species (Fig. 41) (Adak *et al* 2005). Similar results were obtained with *An. fluviatilis* species U in laboratory feeding experiments. These studies suggested that the *An. fluviatilis* species T and U which are not vectors in field, have inherent ability to support normal sporogony. These species are almost zoophagic in field and probably their preference to feed on cattle makes them poor vectors and may act as vector in the absence of cattle.

In recent years, cytogenetic studies carried out on *An. fluviatilis* population from villages under Laksar

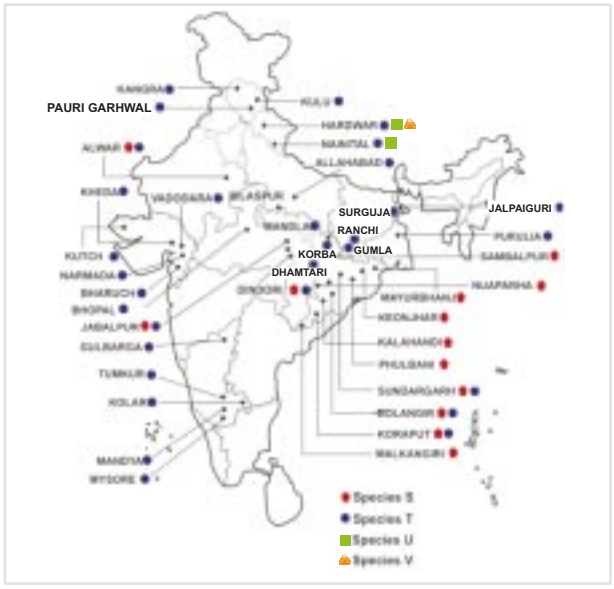


Fig. 40: Map showing the distribution of the members of the *An. fluviatilis* complex in India

PHC of District Hardwar (Uttarakhand) revealed existence of a new species in *An. fluviatilis* complex. The new cytotype observed differs from the reported species of the Fluviatilis Complex by two fixed paracentric inversions S^1 and S in polytene chromosomes 2 and 3, respectively. Longitudinal study carried out in study villages showed that the new

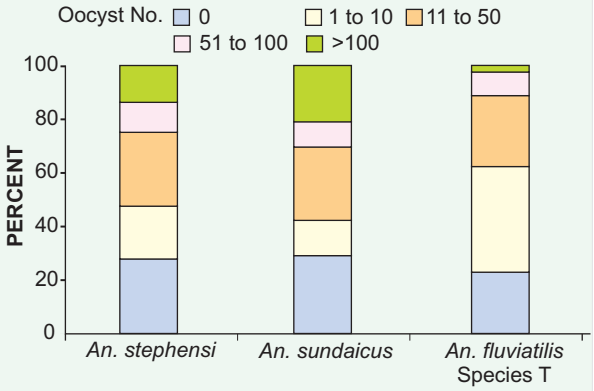


Fig. 41: Frequency distribution of oocysts in *An. fluviatilis* species T and other two vectors *An. stephensi* and *An. sundaicus*

Table 3. Biological differences and diagnostic characters of *An. fluviatilis* sibling species

Species	Inversion genotypes on Chromosome arm 2	Densities (MHD)	Feeding preference	Sporozoite positives	Preferred adult habitats	Observed in	
						Ecotypes	Epidemiological areas
S	$+q^1+r^1$	Low moderate (1–40)	Anthropophagic	Found	Human dwelling	Hilly forest & foothills	Hyper-endemic
T	q^1+r^1	High (up to 200)	Almost totally zoophagic	Not found	Cattlesheds	Foothills & plains	Hypo-meso-endemic
U	$+q^1r^1$	-do-	-do-	-do-	-do-	-do-	-do-

cytotype was sympatric with species T and U in all the seasons. The presense of two fixed paracentric inversions in polytene chromosomes with total absence of inversion heterozygotes unequivocally establishes this cytological variant as a new species (species V) in the *An. fluviatilis* complex. Analysing the DNA sequences of D3 domain of 28S rDNA has also shown that species V is distinctly different from species S, T and U. Preliminary observations made on the biological characteristics of species V revealed that it rests predominantly in human and mixed dwellings and has an anthropophagic index of about 4%. Studies are in progress to ascertain the role of species V in malaria transmission.

Anopheles minimus Complex

Out of the three recognized species of *An. minimus*—species A, C and E, only species A has been recorded from India based on isozyme analysis (Adak *et al* unpublished report). Mapping the distribution of *An. minimus* sibling species using molecular tools also revealed prevalence of species A in India. Recently, *An. fluviatilis* S was made synonym of *An. minimus* C by certain investigators, as a result the distribution of *An. minimus* C was shown in India. However, further molecular studies by NIMR revoked the synonymy of *An. fluviatilis* S with *An. minimus* C. Pair-wise distance and phylogenetic analysis using ITS2 sequences of the members of the Minimus and Fluviatilis Complexes revealed that *An. fluviatilis* S and *An. minimus* C are genetically distant and independent species (Singh *et al* 2006) (Fig. 42).

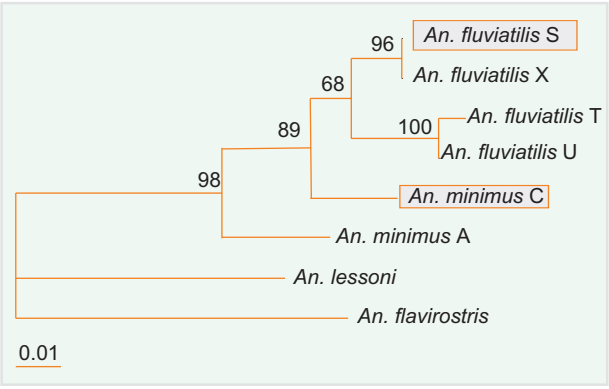


Fig. 42: Maximum Likelihood (ML) tree inferred from ITS2 sequences from the members of Fluviatilis and Minimus Complexes. Numbers above the branches are bootstrap values. Scale bar represents 0.01 nucleotide substitutions per site. *Anopheles lessoni* and *An. flavirostris* are taken as outgroup

Anopheles sundaicus Complex

In India, *An. sundaicus* is abundantly found only in Andaman and Nicobar Islands, where this species is the sole malaria vector. Studies carried out in Thailand and Indonesia have established *An. sundaicus* as a complex of three isomorphic species (species A, B and C) identifiable on the basis of

Table 4. Comparison of polytene and mitotic chromosomes of cytotype D with other forms in *An. sundaicus* complex

Forms	Polytene banding patterns				Mitotic chromosomes	
	Xa	Xb	2a	2b	Y	Ch
A	+	–	+	–	Y_1	Normal
B	–	+	+	–	Y_2	Normal
C	–	+	–	+	Y_1	Large
D	+	–	–	+	Y_1	

Note : +presence; – absence; Y_1—telocentric with 2 heterochromatin blocks; Y_2— telocentric but longer than Y_1 and with three heterochromatin blocks; Ch—pericentromeric heterochromatic blocks in autosome 2.

cytological variations together with enzyme polymorphism analysis. Another species in *An. sundaicus* complex has been identified from Malaysia on the basis of sequence variations in the regions of cytochrome b and cytochrome oxidase 1 of the mitochondrial DNA and this has been designated as *An. sundaicus sensu stricto*.

Cytogenetic characterization of *An. sundaicus* population from Car Nicobar Island, India was carried out. All the samples screened for ovarian polytene chromosomes had X-chromosome of Xa type as reported in case of species A and chromosome 2 (2b type) similar to that in species C. This combination revealed the existence of a new cytogenetic variant, *i.e.* cytotype D, reported for the first time in the Indian subcontinent. Examination of male and female mitotic karyotypes further substantiated these results (Table 4).

The above observations were strongly supported by molecular studies carried out on *An. sundaicus* samples from four geographically isolated areas — Teressa, Nancowry, Car Nicobar and Katchal islands. PCR-amplification and nucleotide sequence analyses were performed for ITS 2 and domain-3 (D3) of 28S rRNA. The ITS2 region of *An. sundaicus* from all four islands was identical but different from *An. sundaicus* A of Vietnam and *An. sundaicus sensu stricto* of Malaysia. Similarly, the D3 sequences, reported for the first time for a species of the Sundaicus Complex, were identical among all samples analysed from the four islands. These observations suggested the existence of *An. sundaicus* D in Andaman and Nicobar Islands.

Examination of *An. sundaicus* populations from other Andaman and Nicobar Islands revealed the prevalence of only cytotype D both in fresh water and brackish water areas, indicating wide adaptability of this form to different habitats.

Anopheles annularis Complex

An. annularis has wide distribution in India and is considered an important vector in certain parts of Orissa state. It is a secondary vector in certain localities and is sometimes found abundantly.

Cytogenetic studies carried out in different parts of India established *An. annularis* as a species complex comprising species A and B. These species were identified on the basis of differences in banding pattern on arm 2 of the polytene chromosomes complement. Species A is characterized by $+j^1$ arrangement and species B by j^1 arrangement on chromosome arm 2. The X-chromosome and the autosomal arms 3, 4 and 5 are homosequential in both the species. The male and female mitotic karyotypes have also been found to be the same in species A and B. Recently, ribosomal DNA PCR-RFLP methods have been developed, based on sequence analysis of ITS2 and domain 3 (D3) of *An. annularis* that can differentiate species A from B (Alam *et al* 2007).

Mapping the distribution of *An. annularis* sibling species in different geographical areas of India revealed the prevalence of species A in Districts Alwar (Rajasthan); Ghaziabad & Shahjahanpur (Uttar Pradesh); Sonepat (Haryana); Sundargarh and Koraput (Orissa); and Kamrup (Assam). Whereas, species B has been reported only from Districts Shahjahanpur and Ghaziabad in Uttar Pradesh state where it was found sympatric with species A. Blood samples from the gut of mosquitoes that were identified to sibling species in the above mentioned districts were analysed for determining the blood meal source. Almost all the samples belonging to species A and B had bovine blood strongly indicating that these species are primarily zoophagic.

❑

Genetics of Malaria Vectors

The fact that resistance developed to insecticides in mosquitoes after their extensive use in the 1950s in disease control/eradication programme and that resistance is genetically controlled, have stimulated studies in mosquito genetics and created awareness among entomologists on the importance of genetics in control strategies. The need for mutant markers and their genetic analyses, and well-developed genetic maps was realised, particularly in the 1960s when genetic control of mosquito population was considered as an alternative strategy in disease control programmes. Genetic manipulation of mosquito population using molecular approaches now being considered for reducing or eliminating vector-borne diseases, once again are underscoring the importance and need for well-developed genetic map and markers for studying the population structure and gene-flow.

Genetic Markers

Markers are useful as tools for genetic studies to map genes for insecticide resistance, resistance to malaria parasites, feeding preference, *etc*. in malaria vectors. Markers are also used in genetic studies to estimate the genetic distance and gene-flow between populations. Various types of markers are used for genetic study of mosquitoes such as morphological mutants, biochemical—isozyme

Table 5. List of genetic markers in malaria vectors

Linkage group	Markers	No. of alleles	Inheritance pattern	Reference
An. culicifacies Species A				
II	*Vermilion eye (v)*		Recessive	Adak *et al* 1983
An. culicifacies Species B				
I	*White eye (w)*		Recessive	Subbarao *et al* 1982
I	Malic enzyme (Me)	2	Co-dominant	Adak *et al* 1988
II	*Creamish larva (cr)*		Recessive	Subbarao *et al* 1982
III	*Red thorax (rt)*		Dominant	Subbarao *et al* 1982
An. stephensi				
I	*Red eye (r)*		Recessive	Sharma *et al* 1979
I	Malic enzyme (Me)	2	Co-dominant	Adak *et al* 1993
I	*Creamish white eye (cr)*		Recessive	Adak *et al* 1999
II	*Colourless-eye (c)*		Recessive	Sharma *et al* 1977
II	EST-4	3	Co-dominant	Adak *et al* 1984
II	IDH-2	2	Co-dominant	Adak *et al* 1991
III	*Green larva (g)*		Recessive	Subbarao *et al* 1978
III	*Golden yellow larva (gy)*		Recessive	Adak *et al* 1990
III	*Black larva (Bl)*		Semi-dominant	Adak *et al* 1990
III	6-PGD	2	Co-dominant	Adak *et al* 1992
III	MDH	2	Co-dominant	Adak *et al* 1992
III	AAT	2	Co-dominant	Adak *et al* 1996
An. sundaicus				
	Yellow larva (yl)		Recessive	Das *et al* 1997
An. minimus				
	ODH	1	Co-dominant	
	MDH-1	2	Co-dominant	
	MDH-2	2	Co-dominant	
	LDH	2	Co-dominant	
	MPI	1	Co-dominant	
	HAD	2	Co-dominant	

electrophoretic variations, microsatellites, RFLPs, *etc*. Some of the molecular markers have additional advantage that they can be physically mapped on chromosome, which would establish linkage relationship and indicate exact location in the genome.

Genetics of Phenotypic and Isozyme Electrophoretic Markers

We have isolated various phenotypic and isozyme electophoretic markers in some important malaria vector species—*An. culicifacies* (species A and B), *An. stephensi, An. sundaicus* and *An. minimus* and their inheritance pattern was also studied. A synoptic list of these markers and nature of inheritance is appended in Table 5.

Genetics of Egg-float Ridge Number in *Anopheles stephensi*

In *An. stephensi*, two races—type form and variety *mysorensis* were described on the basis of differences of the egg length, width and the number of ridges on the egg-float. The type form was reported to inhabit urban areas and an efficient vector of malaria, whereas var. *mysorensis* inhabits rural areas and considered as a poor vector. In order to resolve the taxonomic status of rural and urban *An. stephensi* populations and the genetic basis of egg ridge number, crosses were made between laboratory colonies of type form (egg ridge number >16) and var. *mysorensis* (egg ridge number < 13) established from rural and urban localities of India. Reciprocal genetic crosses between these two forms indicated no post-copulatory barriers between populations. Likelihood analysis of the results of crosses and backcrosses indicated that variation in egg ridge number is controlled by more than one genetic factor (Subbarao *et al* 1987).

Genetics of *Bacillus sphaericus* Resistance in Mosquitoes

Culex quinquefasciatus

The *Bacillus sphaericus* was used in the breeding sites of *Cx. quinquefasciatus* in Ghaziabad at fortnightly intervals. Within a year of spraying of *B.sphaericus,* the *Cx. quinquefasciatus* developed 7-fold resistance. This strain was further selected in laboratory for resistance against *B. sphaericus.* As a result, 52000-fold (LT_{50}) resistance was developed in this strain. To study the inheritance pattern of resistance the reciprocal and backcrosses were carried out between homozygous resistant and susceptible strain of *Cx. quinquefasciatus*, which revealed that the resistance was recessive, autosomal and controlled by more than one gene (Adak *et al* 1995). No maternal effect was observed in the expression of resistance.

Anopheles stephensi

A resistant strain of *An. stephensi* was selected in laboratory against *B. sphaericus.* The resistance strain (LC_{50} > 1600 mg/l) was crossed reciprocally to a susceptible strain *golden yellow larva* (LC_{50} > 0.08 mg/l). The F_1 progenies of both the reciprocal crosses were inbred and backcrossed. Results of these crosses revealed that the resistance is recessive and autosomally inherited. No linkage between *B. sphaericus* and *golden yellow larva* was established. As the resistance gene is autosomal and *golden yellow larva* is on chromosome 3, this gene has been assigned to linkage group II.

Development of Microsatellite Markers in *Anopheles culicifacies*

Microsatellite markers are simple tandem repetitive sequences, mostly of 2 to 6 nucleotides that are randomly distributed throughout the genome. The fact that are highly polymorphic in a population, good number of markers can be isolated in comparatively short duration, can be physically mapped on chromosomes and can be used for positional cloning of specific genes, makes them attractive markers for genetic studies.

Not much work has been done on microsatellite markers except in *An. gambiae, An. funestus, An. maculatus* and *An. dirus*. In India, NIMR for the first time isolated microsatellite markers from *An. culicifacies* species A. Partial genomic library was prepared and clones were screened for the presence of microsatellite loci using poly GA/GT probes. The clones positive for poly GT/GA probes were sequenced and primers were designed for amplification of 17 such microsatellite loci (Table 6). These primers have been tested in laboratory populations, out of which 12 microsatellite loci were found to be polymorphic which have been selected for further genetic analysis of *An. culicifacies* field populations. A few of these markers tested on species B population were found working and polymorphic too.

Population Genetic Analysis of Malaria Vectors

Using Microsatellite Markers in *Anopheles culicifacies*

Three populations of *An. culicifacies* species A collected from Gujarat, Haryana and Karnataka states were genotyped using 12 microsatellite markers isolated by NIMR. All the markers were found to be highly polymorphic in all the populations. The number of alleles found with each of the markers in the three populations are shown in Fig. 43. The study is in progress to test two more populations and genetic analysis of the results.

These microsatellite markers were tested against *An. culicifacies* species B from Gujarat and Haryana. Nine sets of primers developed for amplification of microsatellite loci in species A were successfully used for species B also, and all the nine markers were found to be multi-allelic having 3 to11 alleles.

Table 6. Microsatellite markers and amplifying primer pairs for *An. culicifacies* species A

S. No.	Locus	Gene bank accession No.	Repeat motif	Primer sequence (5'-3')	Sequenced allele size units
1.	*AC43C30*	–	$(CA)_{38}$	CCTTGGAGAGGGCTGTAGAA ATCACAACACGCGGTACAGA	200
2.	AN36	–	$(CA)_{8}$	GGGCAAACTGAAAAAGGTTG CACTGATGACGTTTCGTTGC	206
3.	*AN59*	–	$(CA)_{6}$	TCCCACATACCGATACACCA GCGTAGGTCAACCGTAATGC	210
4.	*AN61*	–	$(CA)_{3+4+3+4}$	TTCCTACTCACCAGCCGAAC CGAATGCATTTCGCTTGATA	204
5.	*AN75*	–	$(GT)_{10}$	TCTGGAGATTGAGCACGAGT AACGCAGTCACAAGGCAGTA	91
6.	*ACIIB5*	AJ417869	$(CA)_{3+1+2}$	CGGAAAACGTGCAACAAAATC ATCCAACCGTAGCCATAACAAGC	110
7.	*ACIIB71*	AJ417870	$(GT)_{5}$	GCAGGCAGACCACTCACAATCTG GACTCTGCTGCTGCCACACTTG	149
8.	*ACIVB129*	AJ417871	$(TG)_{7}$	TCTCCTTTTTGCATATCTTTCGTG TAGATTCGGTTGTAGTTTTCCTGC	107
9.	*ACVB93*	AJ420078	$(GTG)_{3+4+1}$	GTCCTTTGCAAATCACATCGG TTAATGACTTCAATCCACAAACCC	140
10.	*ACVB93A*	AJ420079	$(CA)_{2+3}$	GTGGCCGTTGTTCGTCCTTTTG CAGTGCTCGTGGCGTTCGCG	117
11.	*ACVB194*	AJ420081	$(GT)_{6+1}$	TGTCGTGAAGGCATGTTTGAG ATTATTGCATTCTAGCGGGTGA	184
12.	*ACVB221*	AJ420073	$(CT)_{3+7}$	ACTCACGGGAAGCCAAAATACC AAGGAGAAGGATACATCGATGGAG	115
13.	*ACVIB134*	AJ420074	$(CA/AC)_{3+1+2+6}$	CTGGCGATGATGATGATGGCG CAGCAGTTTGCCGGAAGGAGAG	168
14.	*ACVIB213*	AJ420076	$(GA)_{7+1+5}$	ATAAAACGCCCCGCATCATACATG CACGGCACATTCCCTCCCATA	116
15.	*ACVIIB46*	AJ420075	$(CAA)_{1+4}$	AACCGGAAGCAGTATCGCACAC GAGGCTCCTTCGTTATCCG	140
16.	*ACVIIIB40*	AJ420077	$(CA)_{5}$	TCAAGCTGGACAATGTAACTCAAC GTTCAATCAAACCCAGCCAAAC	118
17.	*ACVIIIB182A*	AJ420080	$(GA)_{1+6}$	GTTTAGCTTCGGGCCTTTCCATAC GAGATACAACCGGTGCGTCAGC	170

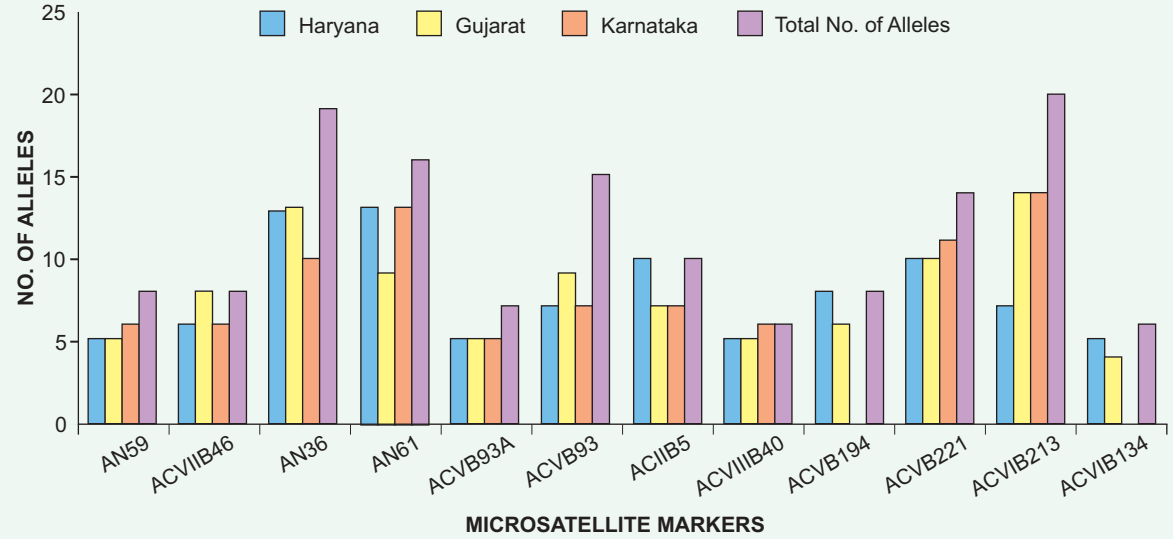


Fig. 43: Allele distribution of 12 microsatellite loci in three populations of *An. culicifacies* species A

Using Paracentric Inversion Markers in *Anopheles annularis*

Populations of *An. annularis* from six localities of India (Districts Shahjahanpur, Ghaziabad, Alwar, Sundargarh, Sonepat and Kamrup) were genotyped for inversion polymorphism by examining ovarian polytene chromosomes (Atrie *et al* 1999). A total of nine autosomal paracentric inversions located on different arms of chromosomes were found to be polymorphic. In Districts Shahjahanpur and Ghaziabad there were no heterozygotes for inversion j^1 on chromosome arm 2, which was taken as the evidence for reproductive isolation between these two forms. The two forms were provisionally designated as species A and B with diagnostic arrangements $2+j^1$ and $2\,j^1$. The BIOSYS-1 computer programme of Swofford and Selander revealed two clusters of the phenogram (Fig. 44), one of six populations with $2+j^1$ (species A) and another of $2j^1$ population (species B). The analysis further showed a good correlation between genetic and geographical distances among populations of species A (loc. cit.).

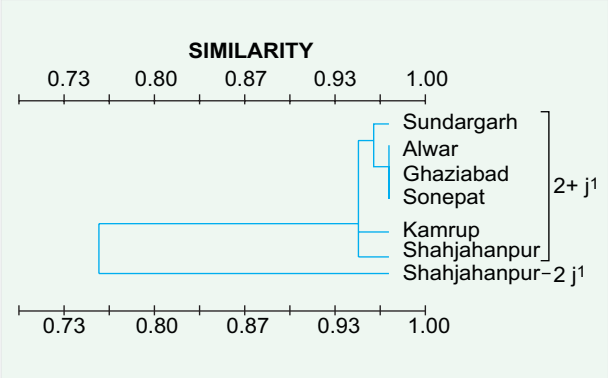


Fig. 44: A phenogram of *An. annularis* population of India produced by the unweighted pair group method of Nei with arithmetic average (UPGMA) cluster analysis of the average similarity matrix (Cophenetic correlation = 0.989)

Using Paracentric Inversions in Urban and Rural Populations of *Anopheles stephensi*

An. stephensi is one of the anopheline species in which inversion polymorphism is extensive. So far 26 floating paracentric inversions have been identified in this species. In a population studied from Delhi (urban areas), we have found 10 floating inversions. A photomap of polytene chromosome of this species with inversion break points of all the inversions marked is given elsewhere (Subbarao 1996). In addition to urban population, population from a rural area near Delhi was examined. While this species exhibits extensive inversion polymorphism in urban area, in rural population one very common inversion, 2b (found in urban area) and another inversion $2h^1$ in one specimen in heterozygous state were found. This study has shown that urban and rural *An. stephensi* populations are distinctly different. This confirmed earlier finding that rural and urban populations are distinct with reference to prevalence of two ecological races—in rural areas variety *mysorensis* (characterized by low egg-float ridge number) is prevalent while in urban areas type form (characterized by high ridge number) is prevalent (Subbarao *et al* 1987). ❑

Insecticide Resistance: Monitoring and Biochemical Mechanisms

Indoor residual spraying (IRS) with insecticides such as DDT, HCH and malathion has been the main strategy to control malaria vectors in India under the National Anti Malaria Programme (Now National Vector Borne Disease Control Programme). Continuous usage of insecticides under National Anti Malaria Programme has resulted in the development of resistance to different insecticides in major malaria vector species.

Resistance Monitoring

Monitoring of insecticide resistance in malaria vectors is an important activity performed along with other entomological studies. Resistance monitoring will be of use in formulating suitable situation-specific insecticide-based vector control strategies and most importantly for the management of insecticide resistance in malaria vectors.

Results of insecticide susceptibility tests carried out by NIMR using WHO diagnostic dose bioassays or by dose-response bioassays, during the past decades have shown that *An. culicifacies*, the major vector of malaria in most of the rural plain areas of India has developed varying degree of resistance to DDT and HCH in different parts of the country (Ansari *et al* 1986, 1988, 1990; Dhiman *et al* 2001; Sharma 1996; Sharma *et al* 1982, 1986; Shukla *et al* 1995; Singh and Sharma 1989; Singh *et al* 1989; Srivastava *et al* 1995; Subbarao *et al* 1984, 1988). It has also become resistant to malathion in most parts of Maharashtra and Gujarat and also in various districts of Andhra Pradesh, Madhya Pradesh, Haryana, Punjab, Karnataka and Tamil Nadu (Ansari *et al* 1988, 1990; Batra *et al* 1999; Raghavendra *et al* 1992, 1997, 1998). In Andhra Pradesh, our studies have shown that *An. culicifacies* developed resistance to malathion in the absence of IRS for malaria control owing to selection by pesticides used to protect cash crops like chilli, cotton and tobacco (Raghavendra *et al* 1991). Recently in District Chhindwara, Madhya Pradesh, a focus of malathion resistance was found in *An. culicifacies*. As in Andhra Pradesh the species has developed resistance in the absence of indoor spraying of malathion in public health and probably due to the use of pesticides in agriculture and forestry. During the surveys in Gujarat a focus of synthetic pyrethroid-resistant *An. culicifacies* has been found in some PHC areas of District Surat (Singh *et al* 2002). Monitoring studies in Rameswaram Island in Tamil Nadu state have also shown reduced susceptibility to deltamethrin in *An. culicifacies* (Mittal *et al* 2002).

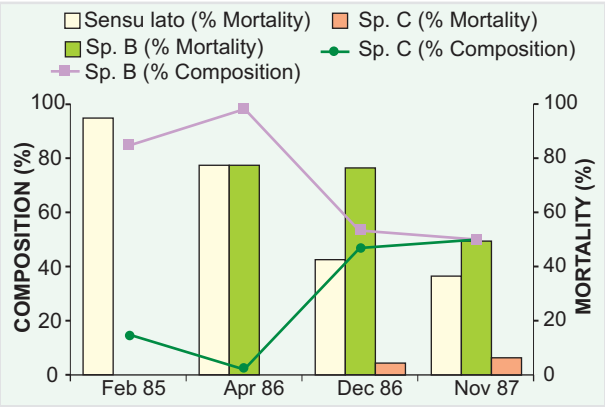


Fig. 45: Differential development of malathion resistance in sympatric *Anopheles culicifacies* sibling species B and C under the selection pressure of agriculture pesticide sprays on cash crop cultivation in District Mahabubnagar, Andhra Pradesh

We have also studied resistance status in sibling species of *An. culicifacies.* These studies have shown differential response to DDT and malathion in sympatric species A and B prevalent in Uttar Pradesh and Haryana respectively. Species A was found more susceptible to DDT and malathion than species B (Raghavendra *et al* 1992; Subbarao *et al* 1988). On the contrary, in Gujarat, Maharashtra and Andhra Pradesh, where species B and C are sympatric, species C became resistant to malathion within 2–3 years, while species B was still half as resistant as species C (Fig. 45) (Raghavendra *et al* 1991, 1998).

Susceptibility tests carried out at NIMR and elsewhere have also shown that *An. stephensi*, a predominant vector of malaria in urban areas in India has developed wide-spread resistance to DDT in different parts of the country (Sharma 1996). Resistance to malathion has also been reported from Maharashtra, Gujarat, Karnataka and Tamil Nadu. Further, studies carried out at NIMR have shown development of resistance to malathion in Haryana and Goa (Subbarao *et al* 1984 ; Thavaselvam *et al* 1993).

An. fluviatilis, another major vector was resistant to DDT in *terai* region of Uttar Pradesh (Sharma *et al* 1999), while it was found susceptible to DDT in Orissa (Chand and Yadav 1991). Susceptibility tests of *An. minimus* in District Kamrup in Assam and *An.*

sundaicus in Car Nicobar Islands with DDT and other insecticides have shown complete susceptibility. Field studies on insecticide resistance were carried out in November 2005 and November 2006 in villages of Ukalda PHC area (District Surat, Gujarat), which were under regular IRS till 2000. Malathion replaced with DDT in IRS in 1970s, and later pyrethroids were introduced in mid 1980s. Our earlier studies in 1987, 1992 and 2002 have indicated development of resistance to DDT, malathion and also to deltamethrin in 2002. Insecticide resistance status to different insecticides was determined using WHO bioassays, resistance mechanisms by microplate biochemical assays and species composition by ASPCR assays.

In 2005, this species was reported resistant to DDT (60%) and malathion (34%) but was susceptible to other organophosphates (fenitrothion 97%), carbamates (propoxur 95%; bendiocarb 100%) and pyrethroids (deltamethrin 98%; lambda-cyhalothrin 97%; cyfluthrin 96.8% and permethrin 99.2%). While in 2006, it was relatively more susceptible to DDT (80%) and malathion (43%) but has registered similar susceptibility to organophosphates, carbamates and pyrethroids. On the contrary, it was 31–40% resistant to deltamethrin in 2002 and to malathion it was 84% in 1987. Though DDT was withdrawn from regular sprays in 1970s, resistance in populations is maintained and may be due to the presence of DDT on walls which imparted continued selection or might have genetically weighed down the negative selection of DDT by removing the negative fitness costs of the resistant genes.

Synergistic bioassay with triphenyl phosphate (TPP), a specific inhibitor of carboxyl esterase indicated complete synergism with 10% TPP impregnated paper indicating involvement of carboxylesterase as major mechanism for conferring malathion resistance in this species as observed earlier. Field-collected female *An. culicifacies s.l.* mosquitoes and the dead and alive mosquitoes in the insecticide exposures in WHO susceptibility tests were stored in isopropanol and later identified to sibling species using ASPCR assays (Goswami *et al* 2006). Major sympatricity of species B (59.4%) and E (39.2%) was found while of species C it was only 1.2 %. Prevalence of species E from this area is being reported for the first time. Identification of dead and alive mosquitoes in the insecticide bioassays to sibling species indicated differential susceptibilities in sibling species to DDT and malathion. To DDT, species B was 31.5% susceptible while to malathion it was 64% and species E was 56.2 and 75% susceptible to these insecticides respectively. Thus, species B registered relatively more resistance to DDT and malathion.

In November 2006, species composition registered an increase in species C and was, species B (55%), E (32%) and species C (13%). Susceptibilities to DDT and malathion respectively in species B were 24 and 54%; species E 14 and 52% and species C, 50 and 48%. Withdrawal of IRS for 5–6 years has shown reversal of resistance especially to pyrethroids. This could be a strategy for the management of resistance in malaria vectors and if needed another insecticide with different mode of action can be used and reduce the continued selection pressure of insecticides in use.

Diagnostic concentrations for alpha-cypermethrin and bifenthrin for malaria and dengue vectors

Six graded doses of alpha-cypermethrin (0.001 to 0.05%) and bifenthrin (0.01 to 0.5%) were tested using standard WHO protocols. Three-day old sugar-fed mosquites were used for the assays. *An. culicifacies* registered 98% mortality against alpha-cypermethrin at 0.0025% concentration and against bifenthrin at 0.1% while *An. stephensi* registered 94% mortality against 0.05% alpha-cypermethrin and 99% against 0.25% bifenthrin. Likewise, *Ae. aegypti* registered only 91% mortality against 0.05% alpha-cypermethrin and 99% against 0.25% bifenthrin. Results were communicated to WHO for determination of diagnostic doses against these insecticides taking into view the results from other participating Institutes.

Biochemical Mechanisms of Resistance

Detection of biochemical mechanisms responsible for the development of resistance indicate the possible early onset of resistance, and from the type of mechanisms detected, one could predict the cross- and multiple-resistance patterns, the resistant insect would exhibit.

Organophosphate Resistance

Microplate biochemical assays carried out on field-collected malathion resistant *An. culicifacies* species A, B and C indicated the non-involvement of elevated levels of non-specific esterases (Fig. 46) and insensitive acetyl cholinesterase (Fig. 47) which are responsible for organophosphate resistance. Bioassays with synergist triphenyl phosphate (a specific carboxylesterase inhibitor) have indicated the involvement of carboxylesterase as the major

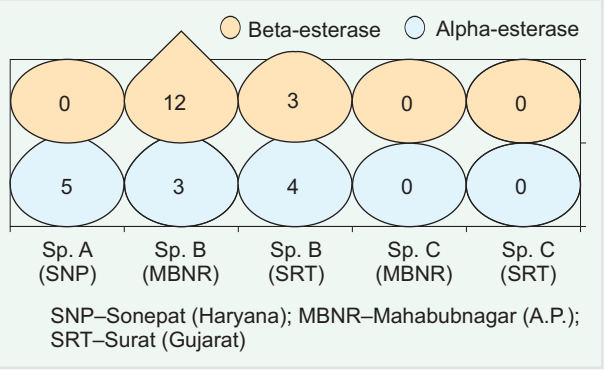
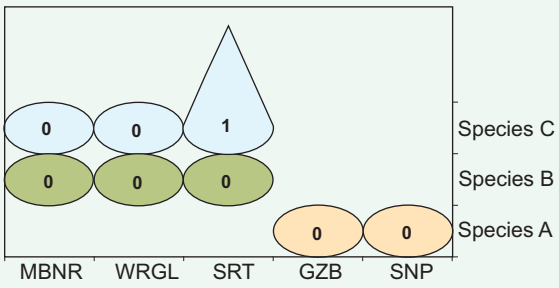


Fig. 46: Proportions of the *An. culicifacies* sibling species populations showing activities below the observed threshold values (as observed for the susceptible strains) of esterases



MBNR–Mahabubnagar (A.P.); SRT–Surat (Gurajat); WRGL–Warangal (A.P.); GZB–Ghaziabad (U.P.); SNP–Sonepat (Haryana)

Fig. 47: Proportions of sympatric sibling species populations showing uninhibited activity above 100% activity of acetyl cholinesterases

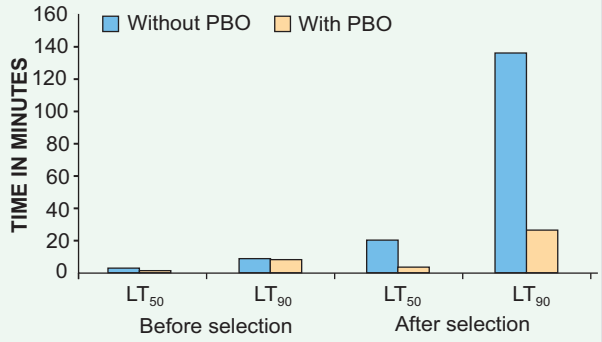


Fig. 49: Susceptibility of *An. culicifacies* Rameswaram (Rmr) strain to deltamethrin (0.05%) before and after laboratory selection with deltamethrin and in the presence of PBO

mechanism of malathion resistance in these species (Fig. 48) (Raghavendra *et al* 1998).

Similar profile of resistance mechanism for organophosphate insecticides was observed in *An. stephensi*. Further, study on malathion metabolism in malathion resistant and susceptible strains of *An. culicifacies* revealed the metabolism of malathion to mono- and di-carboxylic acids in malathion resistant strain which confirmed the involvement of malathion carboxylesterase in malathion resistance in *An. culicifacies*. The selection of this mechanism results in narrow spectrum resistance and in such a situation malathion can be replaced with other organophosphorous and carbamate insecticides to control malathion resistant vector species.

Pyrethroid Resistance

Synergistic studies on deltamethrin resistant strain of *An. culicifacies s.l.* with piperonyl butoxide (PBO—a monoxygenase inhibitor) indicated the involvement of monoxygenases in conferring deltamethrin resistance (Fig. 49).

DDT Resistance

Microplate assays on DDT resistant *An. culicifacies* species B and *An. culicifacies s.l.* strain from Rameswaram and DDT susceptible species A, for Glutathione-s-transferase (GST) activity, revealed a significantly higher titres of GST activity in DDT resistant strains than in DDT susceptible strain, indicating the involvement of GSTs in conferring DDT resistance in *An. culicifacies*. Further studies on DDT metabolism using HPLC showed enhanced metabolism of p-p' DDT to p-p' DDE in the DDT resistant strains in the presence of reduced Glutathione, which confirmed the involvement of GSTs in DDT resistance in *An. culicifacies* (Fig. 50).

This information on biochemical resistance mechanism(s) in conjunction with results of insecticide bioassays will be of use to develop suitable insecticide spray strategies for insecticide resistance management in disease vectors.

Surveys were carried out in November 2001, March 2002, March 2003 and October 2003 in two groups of villages (3 and 4 villages) in District Chhindwara for detection and characterisation of organophosphate-resistance in *An. culicifacies* sibling species in Madhya Pradesh. First group of villages was under regular spray of DDT in public health while the second group was on the Madhya Pradesh-Maharashtra state border about 100 km from the first group and not under IRS since last ten years. Malathion was never sprayed in the public health sprays in this district. Standard WHO methods were

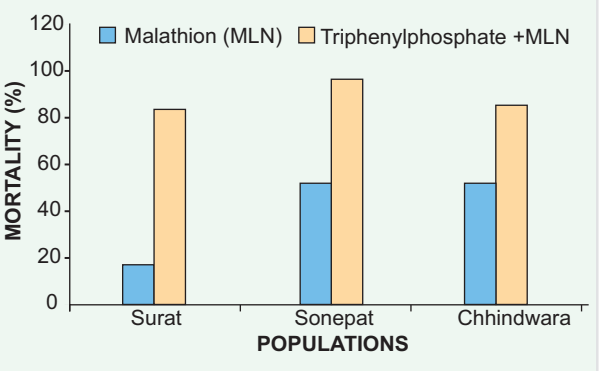


Fig. 48: Results of synergistic bioassays to determine the malathion resistance in mosquitoes

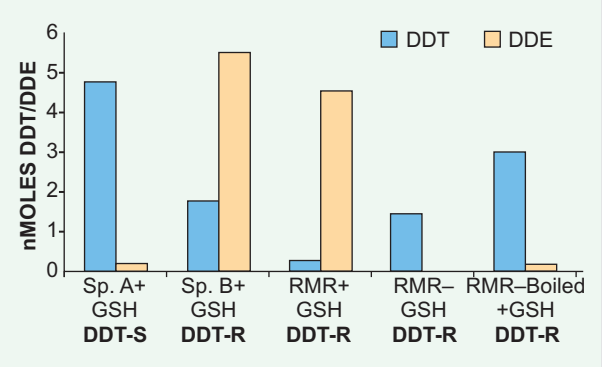


Fig. 50: Relative ratio of DDT metabolised to DDE *in vitro* in *An. culicifacies* with different susceptibility status to DDT and in the presence or absence of reduced glutathione (GSH)

used for assessing the resistance, microplate assays for the detection of resistance mechanisms and PCR assays for identifying the sibling species.

An. culicifacies, the major vector of malaria was resistant to DDT (~20–30%) to malathion it was ~ 52.6% resistant in susceptibility tests in both the areas (LT_{50} 62.44 min and LT_{90} 250.35 min). Pesticides of different groups including organophosphates were in regular use in this district in agriculture and forestry. The species was found completely susceptible to carbamates—propoxur (LT_{90} 22 min) and bendiocarb (LT_{90} 36.54 min) and to synthetic pyrethroid—deltamethrin (LT_{90} 19.44 min) indicating narrow spectrum resistance, *i.e*. to malathion alone. Synergistic bioassays with carboxylesterase inhibitor TPP (10, 15 and 20%) and mixed function oxidase inhibitor, PBO (10, 15 and 20%) revealed synergism with TPP indicating involvement of carboxylesterase for conferring malathion resistance which was also confirmed in biochemical assays. Species B and C were sympatric comprising, 73 and 27% (n = 138) respectively. In bioassays these were respectively 68 and 13.5% susceptible to malathion ($p > 0.001$). Thus, Species C was relatively more resistant to malathion than species B as was observed in our earlier studies in Andhra Pradesh where similar prevalence of sibling species and selection was found, *i.e.* by agriculture pesticides.

❑

Vector Evolutionary Genomics

Fine-scale Evolutionary Genetic Insights into *Anopheles gambiae* X-chromosome

Understanding the genetic architecture of individual taxa of medical importance is the first step for designing disease preventive strategies. To understand the genetic details and evolutionary perspective of the model malaria vector, *Anopheles gambiae* and to use the information in other species of local importance, we scanned the published X-chromosome sequence for detailed characterization and obtain evolutionary status of different genes. The telocentric X-chromosome contains 106 genes of known functions and 982 novel genes. Majorities of both the known and novel genes are with introns. The known genes are strictly biased towards less number of introns; about half of the total known genes have only one or two introns (Fig. 51). The extreme sized (either long or short) genes were found to be most prevalent (58% short and 23% large). Statistically significant positive correlations between gene length and intron length as well as with intron number and intron length were obtained signifying the role of introns in contributing to the overall size of the known genes of X-chromosome in *An. gambiae*. We compared each individual gene of *An. gambiae* with 33 other taxa having whole genome sequence information. In general, the mosquito *Aedes aegypti* was found to be genetically closest and the yeast *Saccharomyces cerevisiae* as most distant taxa to *An. gambiae* (Fig. 52). Further, only about a quarter of the known genes of X-chromosome were unique to *An. gambiae* and majorities have orthologs in different taxa (Fig. 53). A phylogenetic tree was constructed based on a single gene found to be highly orthologous across all the 34

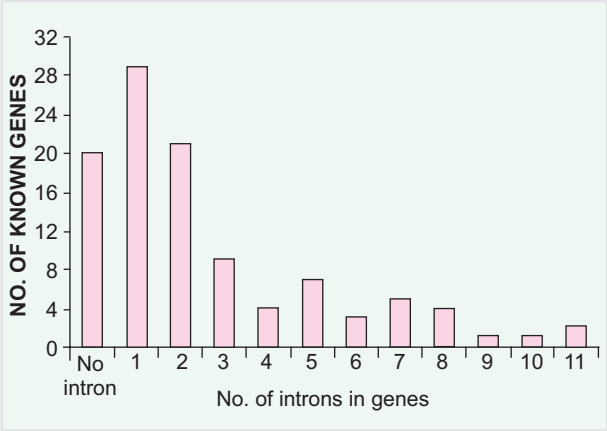


Fig. 51: Distribution of *An. gambiae* X-chromosome known genes according to the number of introns

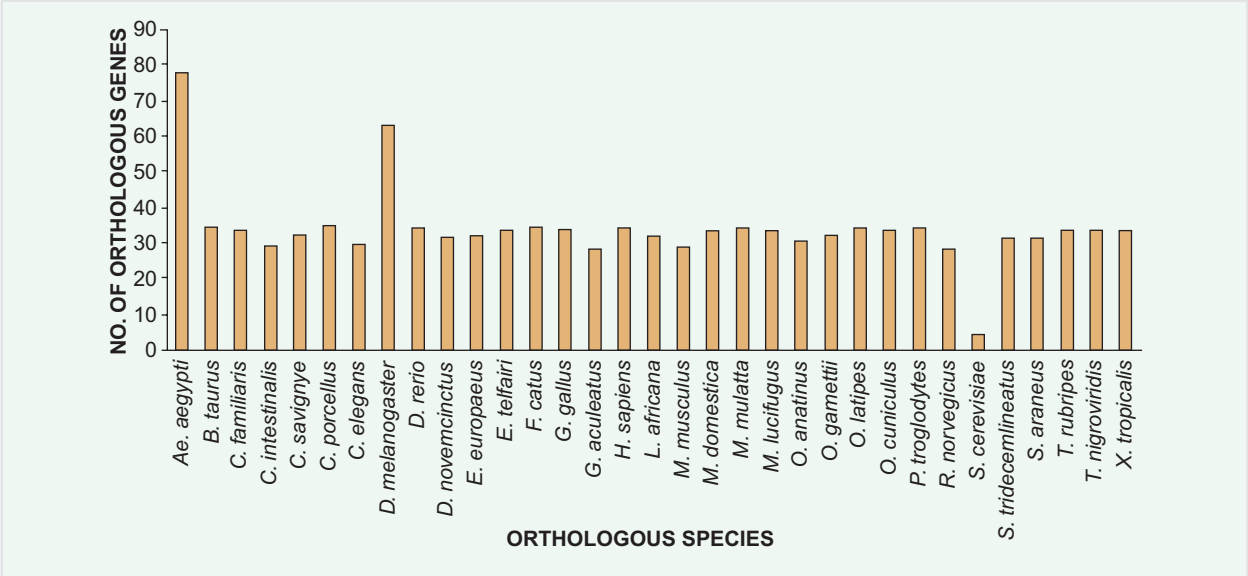


Fig. 52: Distribution of different taxa showing number of shared genes with *An. gambiae*

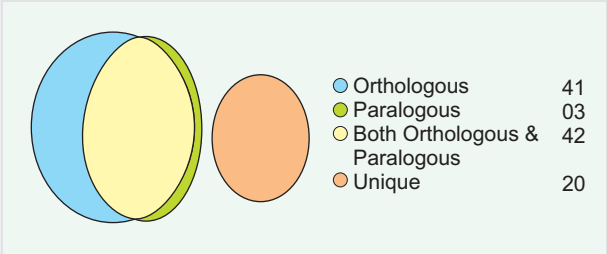


Fig. 53: Distribution of different gene types (based on homology prediction) in X-chromosome of *An. gambiae*

taxa (Fig. 54). Evolutionary relationships among 13 different taxa were inferred which corroborate the previous and present findings on genetic relationships across various taxa.

An Evolutionary Genetic Insight of Insecticide Resistance Gene Families in *Anopheles gambiae*

Insecticide resistance mechanism developed by malaria vector species is one of the major obstacles in vector control strategies and disease control and is known to be genetically controlled. Three major gene families (*Cyp*, *Gst* and *Coe*) are determined for the insecticide resistance mechanisms that encode various proteins to metabolize endogenous as well as exogenous compounds in insects. Since, insecticides are in excessive use (and misuse) in the

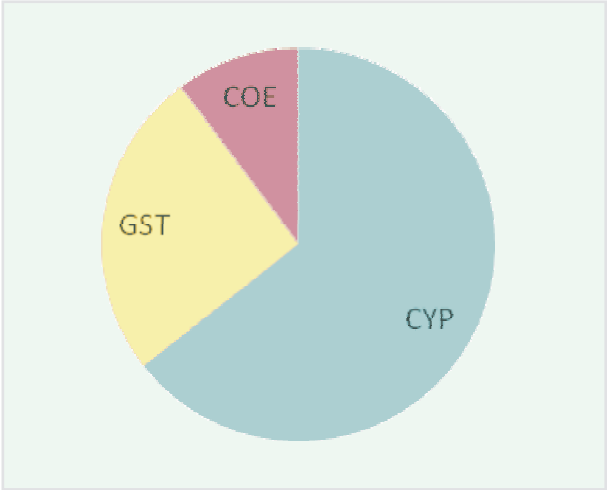


Fig. 55: Contribution of each insecticide resistance gene family in genome of *An. gambiae*

field putting enormous pressure for the evolution of more suitable and efficient insecticide resistance mechanisms in insects, it is important to have fair knowledge on how genetic basis of insecticide resistance genes evolve in these three different gene families. This is enormous importance to malaria research, as vector control and thus to control malaria has been grossly hampered by emergence and evolution of insecticide resistance in malaria vectors. We herewith studied the contribution of all three insecticide resistance gene families by utilizing

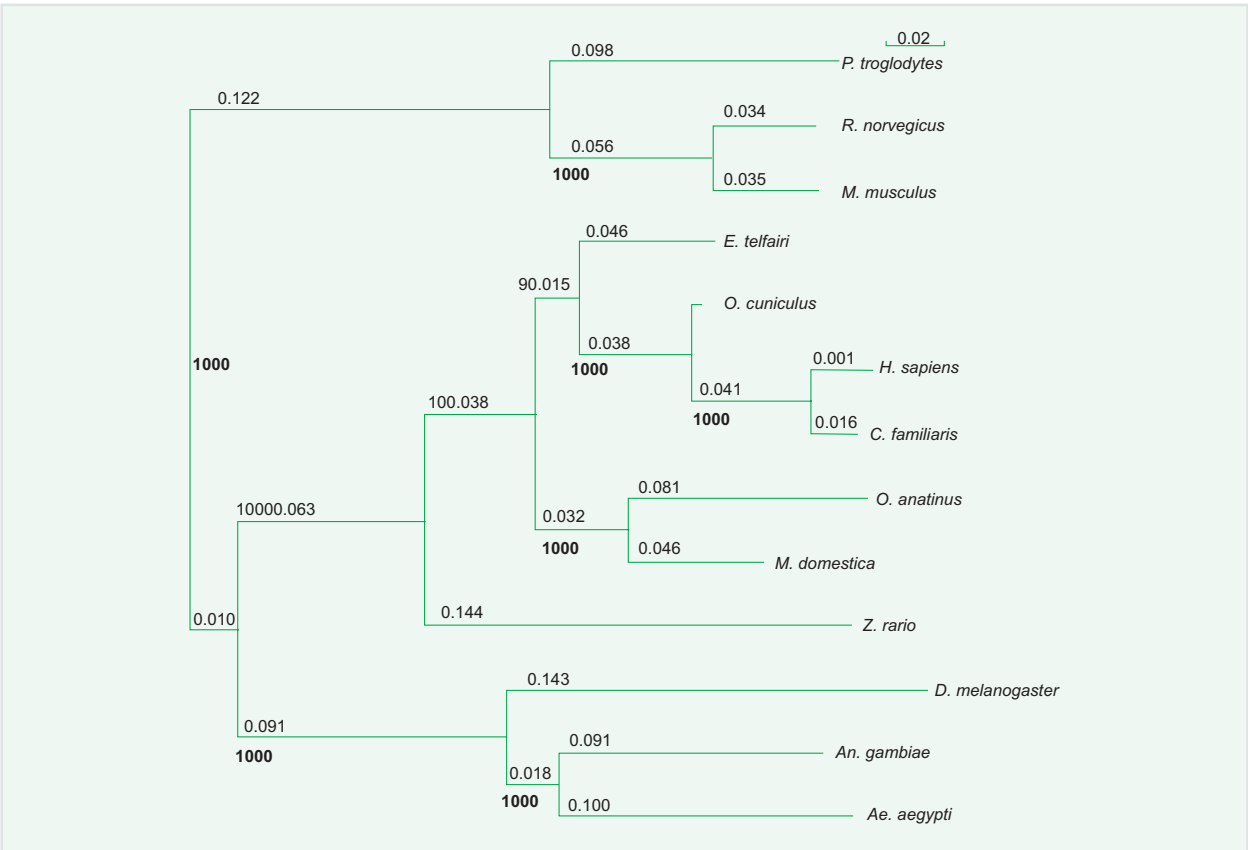


Fig. 54: Phylogenetic tree with bootstrap values (in bold font) and branch length (in normal font) in 13 different taxa

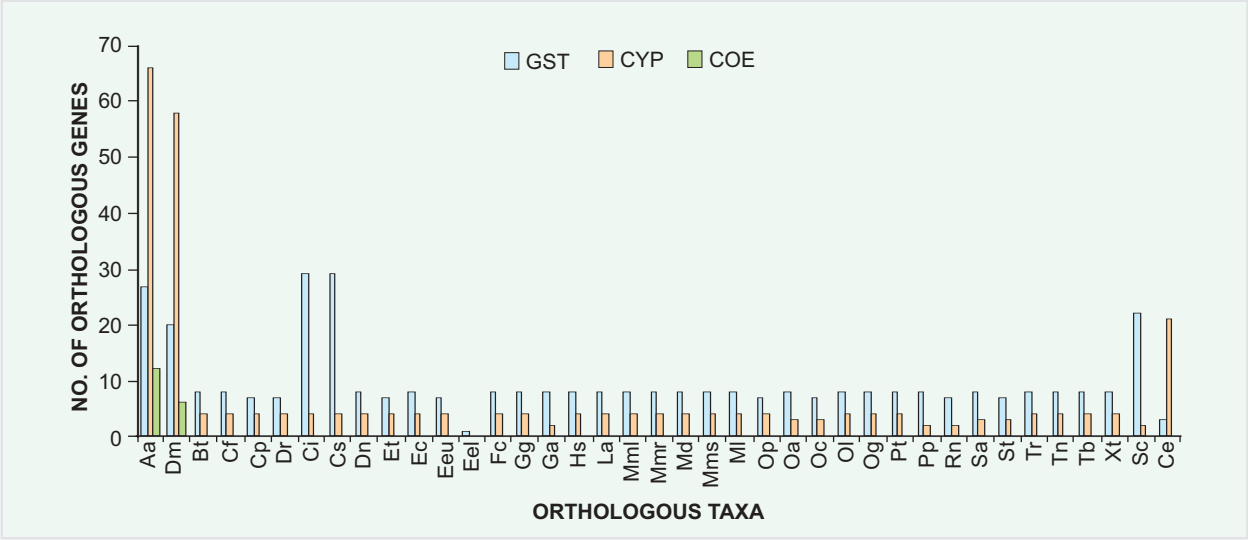


Fig. 56. Orthology of genes of insecticide resistance gene family *Cyp, Gst* and *Coe* to 39 different taxa

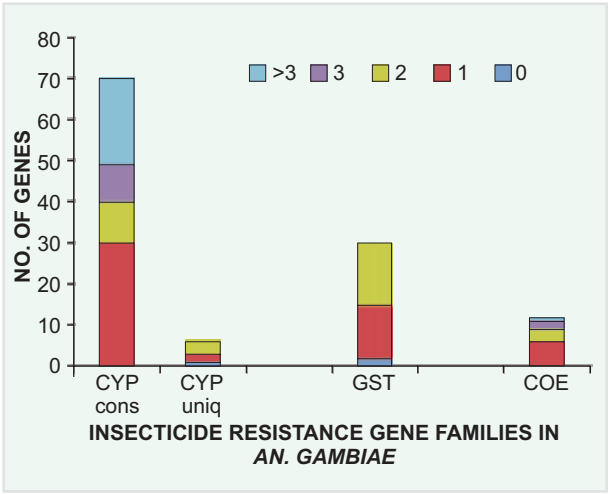


Fig. 57. Number of genes of each insecticide resistance gene family of *Anopheles gambiae* classified on the basis of intron number

genome sequence information of African malaria vector *An. gambiae* (Fig. 55). The pattern of conservation of insecticide resistance genes across various taxa (Fig. 56) and organization of introns in the genes (Fig. 57) have been determined to infer the present evolutionary status of the three gene families. We mapped each individual gene of all three insecticide resistance gene families in all chromosomes (Fig. 58) and measured distribution of genes across chromosomes (Fig. 59). Further, phylogenetic relationships were reconstructed within each gene families (Figs 60–62) and correlated the location of the genes with their position on chromosomes that provide the evidence for mode of expansion of gene families in genome. The results, as a whole in different gene families provide clues to evolutionary mechanisms evolve differently in each gene families of vectors. The knowledge on the

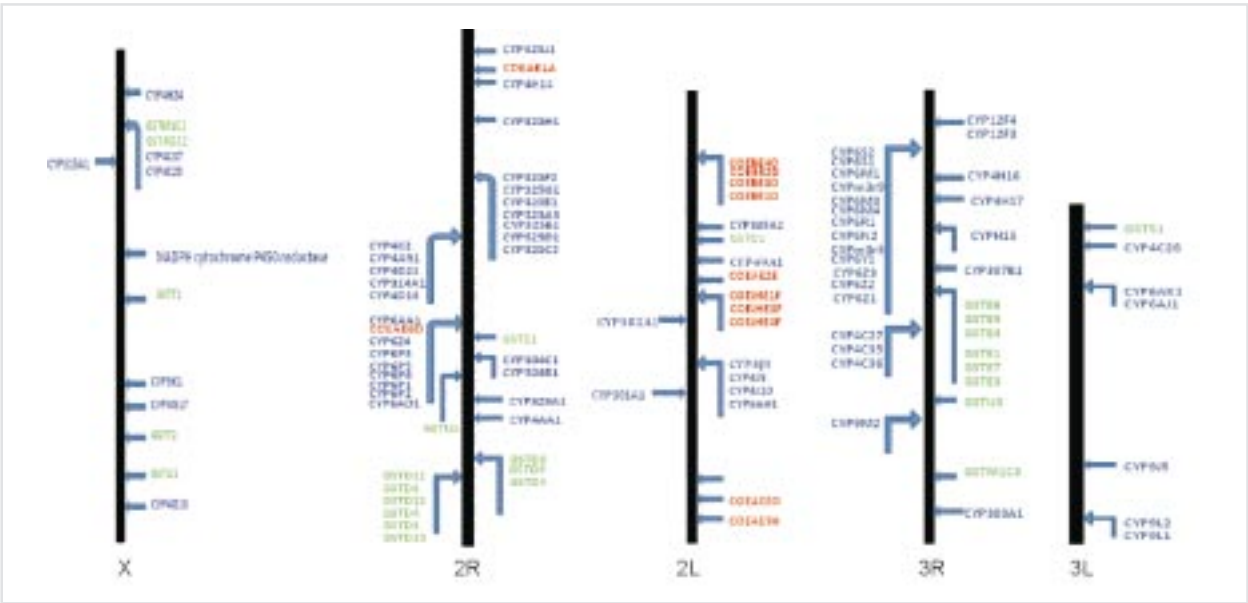


Fig 58: Location of different genes of insecticide resistance gene families *Cyp, Gst* and *Coe* on the chromosomes of *An. gambiae*

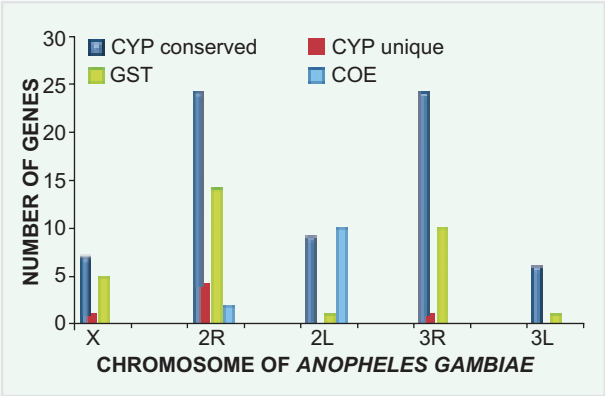


Fig. 59: Chromosomal distribution of genes of three gene families *Cyp*, *Gst* and *Coe* in *An. gambiae*

evolutionary architecture of these three insecticide resistance gene families in *An. gambiae* will be helpful to understand the genetic basis of resistance in malaria vectors of local and focal importance.

Phylogenetic Reconstruction of Indian Malaria Vectors using Multilocus DNA Sequences

Understanding the evolutionary status of closely related species of health importance is the first step in disease management. Malaria is one of the deadliest vector borne diseases, and the taxonomic status of several malaria vectors of *Anopheles* genus and Celia subgenus has been well-documented based on morphological features and gene sequences of ribosomal and mitochondrial regions. However, phylogenetic studies based on multilocus nuclear DNA sequences in *Anopheles* are still in dearth. Moreover, no concrete information on molecular phylogenetic status of major Indian malaria vector species is available till date; though India majorly contributes to the global malaria vector species diversity. We screened the available whole genome sequence information of *An. gambiae* to find six orthlogous nuclear genetic regions and sequenced these regions in six species of Indian malaria vectors. The sequence information of seven species of *Anopheles* (six Indian, and *An. gambiae*) was utilized to reconstruct phylogenetic trees for each individual genetic regions and the time of divergence among these species was calculated based on COII gene sequences. Although tree topologies with COII, ITS2 and one of the nuclear gene responsible for Carboxyl Esterase (*Coe*) genes mirror-imaged each other, for no other genetic region, similar tree topologies in all the seven species were observed (Fig. 63 a–h). Although Indian malaria vectors show gene-specific tree topologies which might be due to differential function-dependant evolutionary cons-traints, in principle, the reconstructed phylogenetic status follow the pattern based on morphology and that of the COII and ITS2 genetic regions. These results on one hand provide evidence for robustness of COII and ITS2 for phylogenetic inference in closely related species, on

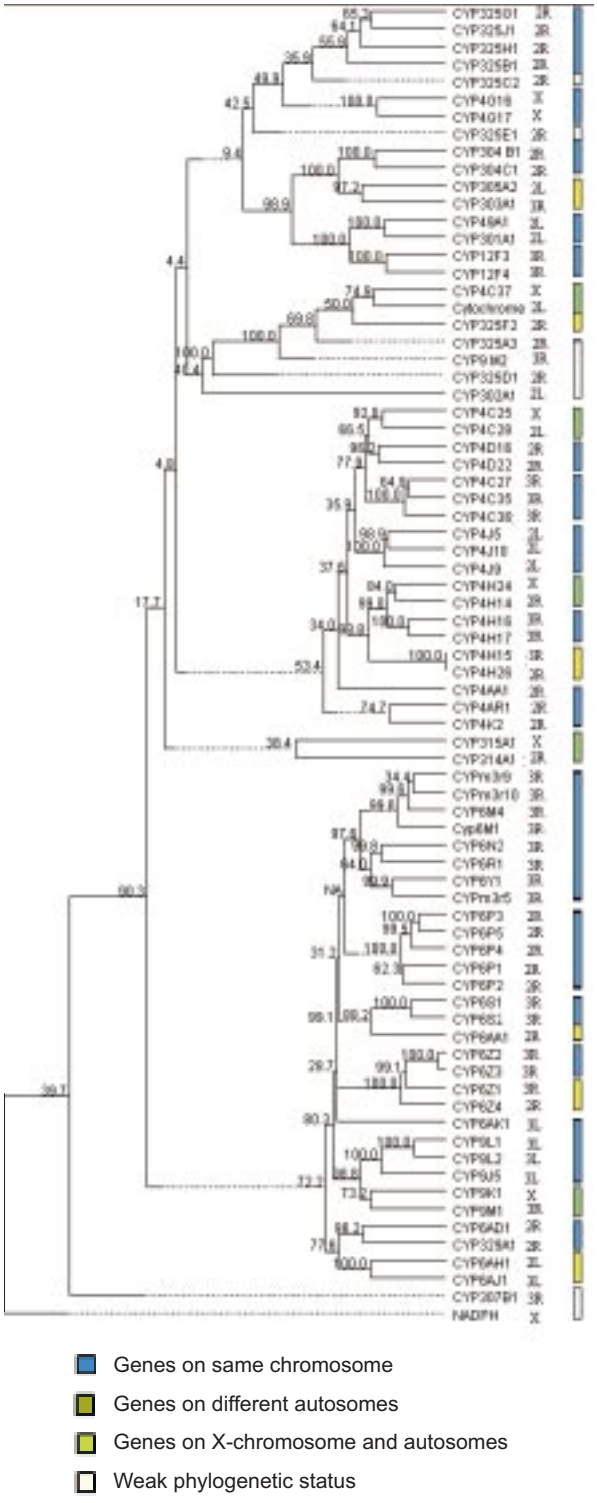


Fig. 60: Neighbor-Joining phylogenetic tree among the genes of *Cyp* gene family

the other hand signifies the utility of multilocus DNA sequence information in dissemi-nating gene-specific from global tree topologies. The divergence times calculated between species further corroborates the earlier theories about the major radiation of species belonging to *Cellia* subgenus in cretaceous period (Fig. 64). The information could be utilized in malaria management not only in India but also in places where some or all of these species are endemic.

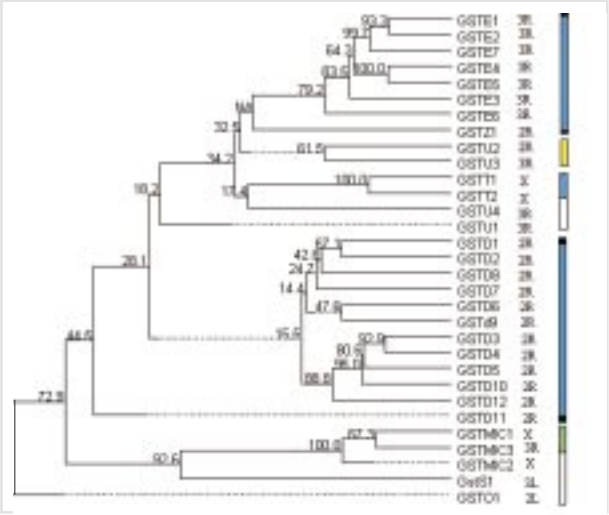


Fig. 61: Phylogenetic relationship based on neighbor-Joining method between genes of *Gst* gene family

Population Genomics of Indian *Anopheles minimus*

Malaria spreads through mosquitoes, belonging to genus *Anopheles* and ability to transmit malaria is uniquely present within this genus, with only ~30 out of 500 species, are major vectors. In addition to this,

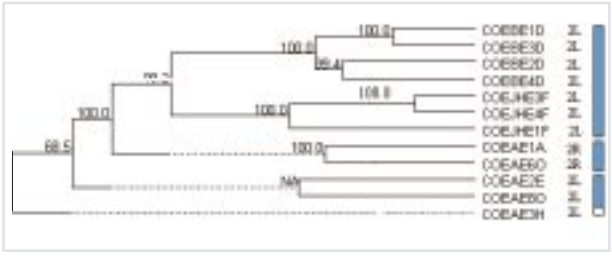


Fig. 62: Neighbor-Joining phylogenetic tree showing phylogenetic relationship among all genes of *Coe* gene family

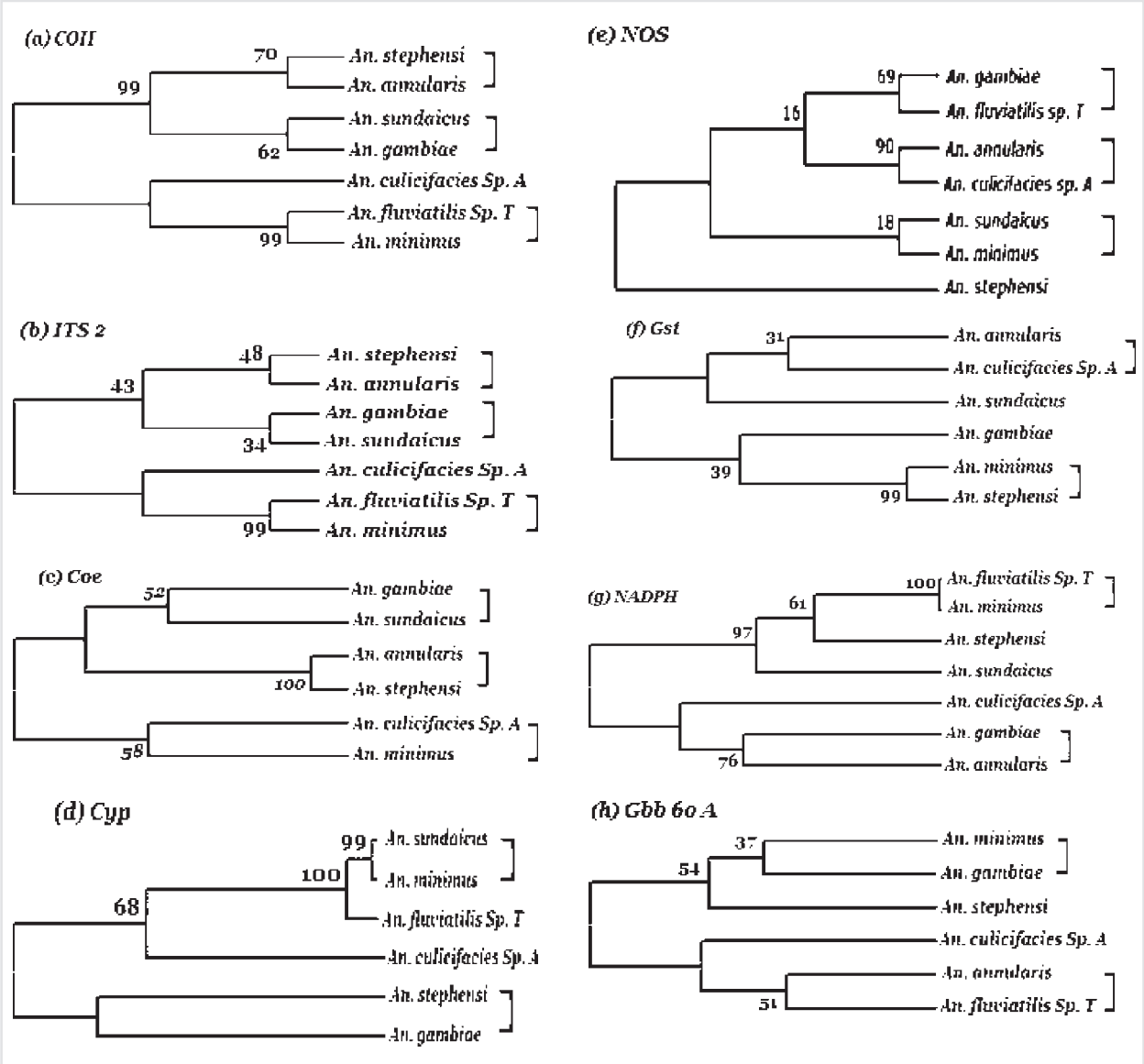


Fig. 63: The consensus tree resulting from maximum parsimony analysis based on the (a) mitochondrial Cytochrome oxidase II (COII) gene sequences (b) nuclear non-functional *ITS2* sequences (c) *Coe* gene sequences (d) *Cyp 450 4 G 16* gene sequence (e) *NOS* gene sequences (f) *Gst* gene sequence data (g) NADPH gene sequence data (h) *Gbb 60 A* gene sequences data. Tree was obtained by Max-mini branch and bound search option of MEGA 4.1.

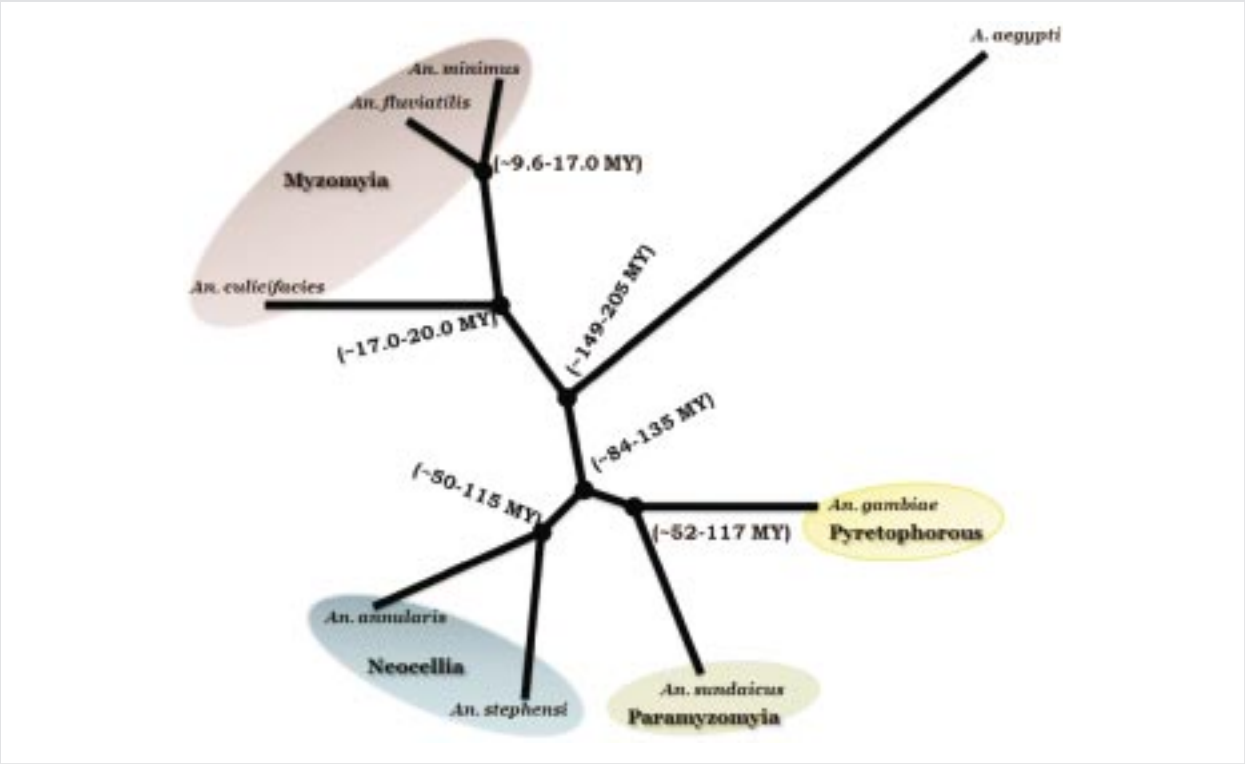


Fig. 64: Phylogenetic status and divergence time estimations of six Indian malaria vectors. Values in parentheses indicate the approximate divergence time between two vector species. Divergence time between *Anopheles* and *Ae. aegypti* (~145–200 MY) has been taken as the calibration point for estimation of divergence times

differential vectorial capacity within members of the same *Anopheline* species complex also exits. The possible reason behind this anomaly is the difference in the genetic makeup and population history of different members of a species complex. Without the knowledge of these aspects of a vector population, one cannot devise new measures to control malaria spread. However, till now very few studies have been done to explore the genetic structure and population history of vector species populations. To this respect we very recently have initiated the population genetic structure and demographic history of one of the main malaria vectors of our country, i.e. *An. minimus* species A. We have applied a comparative genomic approach in designing the putatively neutral nuclear DNA markers in the genome of this Indian vector species by taking the available genome sequence of *An.*

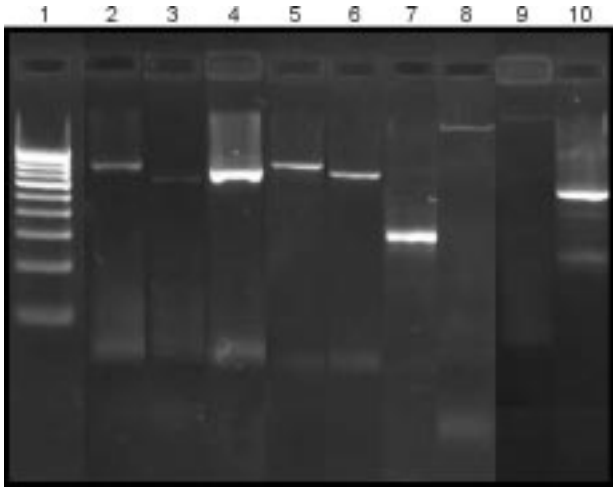


Fig 65: PCR amplified DNA fragments in nuclear genome of *An. minimus*

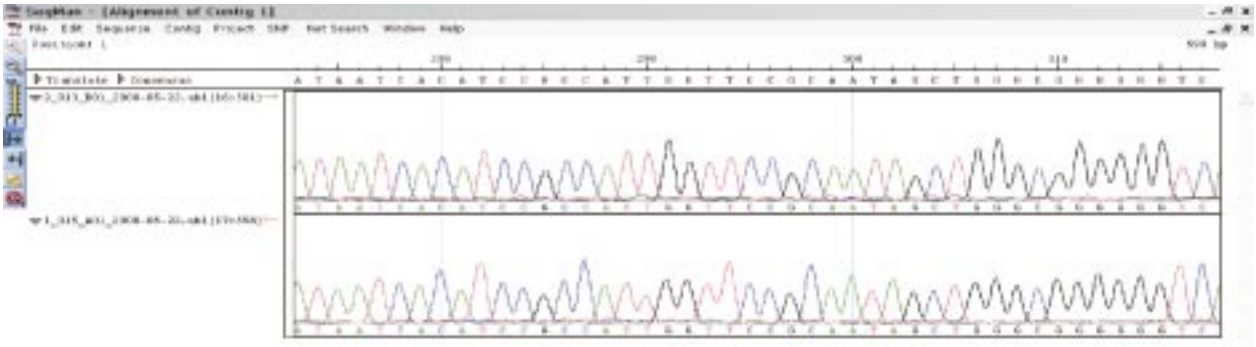


Fig. 66: Sequence chromatogram of a DNA fragment in Indian *An. minimus*

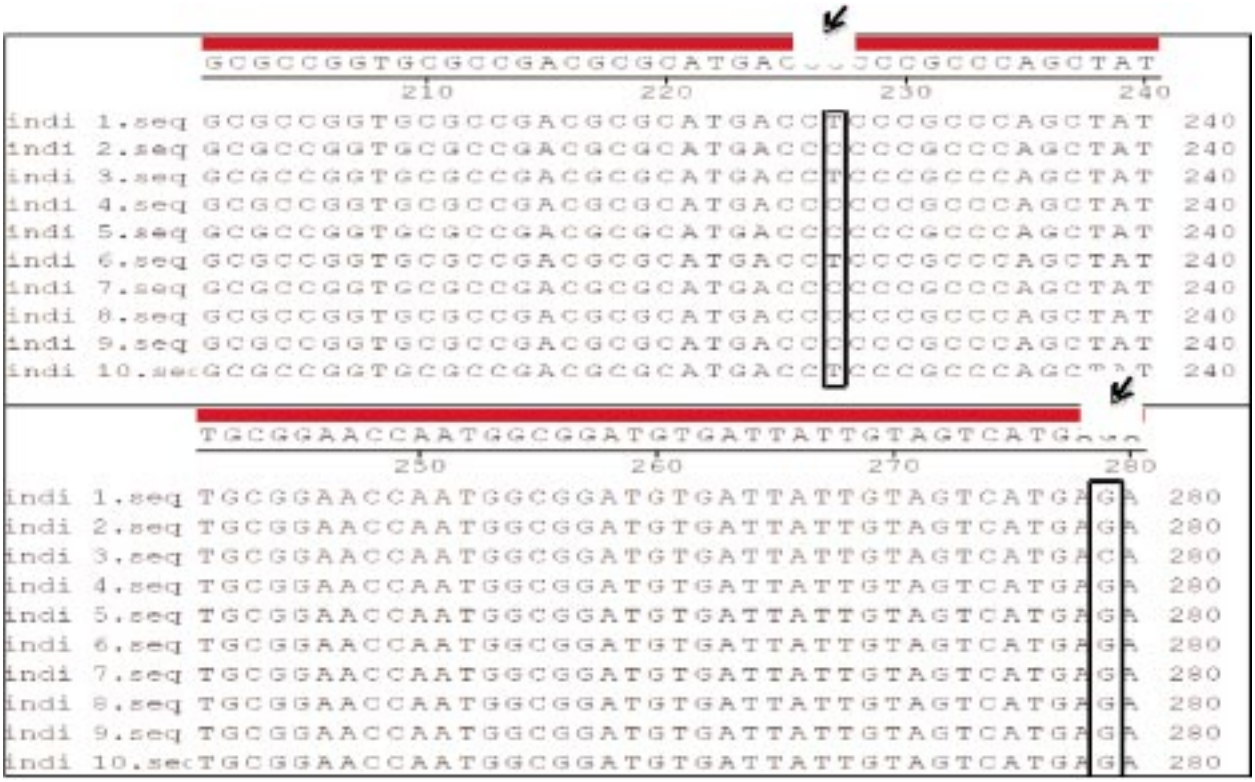


Fig. 67: DNA sequence alignment and SNP detection in Indian *An. minimus*

gambiae as reference (Fig. 65). The sequences for these markers have been generated in the lab (Fig. 66). Till now we have developed and studied three fragments as putatively neutral fragments in the genome of *An. minimus* taken from five different population samples of this species from the northeastern states of India. With the help of computer programmes we have detected 23 single nucleotide polymorphisms in the genome of *An. minimus* (Fig. 67). Neucleotide diversity, Tajima's D and Fu and Li's D were calculated for all the fragments. Except P22 fragment (for Moregaon population), statistical neutrality was observed in majority of the cases implying that all the three fragments could be considered as putatively neutral markers for population genetic studies in *An. minimus*.

❑

Insectary

NIMR has a well-established insectary since 1977. Different strains of three important vectors of malaria— *An. stephensi, An. culicifacies* and *An. fluviatilis* collected from different geographical regions of India are being maintained. Various morphological mutants of *An. stephensi* maintained are *Red eye (re), Black larvae (Bl), Golden yellow (gy), Creamish white eye (cw), Reddish brown eye (rb)*. Resistant and susceptible lines of anophelines to different insecticides are also being maintained in the insectary.

In addition, there are laboratory colonies of *An. culicifacies* sibling species—A, B and C, *An. fluviatilis* species S, T and U initiated from cytologically identified mosquitoes collected from different parts of the country. This insectary is serving as a biological resource for several basic and applied studies being carried out in the Institute.

Mosquito Species

Anopheles stephensi

From urban and semi-urban areas

Nehru Place, Delhi
Okhla, Delhi
Chennai, Tamil Nadu

From rural areas

Ladpur, Haryana

Morphological mutants

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

Biochemical variants

Bahadurgarh (EST-2)

Insecticide resistant lines

Malathion resistant
Permethrin resistant
Lambda-cyhalothrin resistant
Deltamethrin resistant
Cyfluthrin resistant
Fenthion resistant

Anopheles culicifacies Complex

Species A

Dehra, Uttar Pradesh
Burari, Delhi
Rourkela, Orissa

Species B

Acrocentric Y-chromosome lines

Ladpur, Haryana
Haldwani, Uttarakhand

Submetacentric Y-chromosome lines

Rameswaram, Tamil Nadu
Rourkela, Orissa

Insecticide resistant lines

DDT resistant – Ladpur, Haryana
Malathion resistant – Ladpur, Haryana

Species C

Submetacentric Y-chromosome line

Jabalpur, Madhya Pradesh

Insecticide resistant line

DDT resistant – Jabalpur, Madhya Pradesh

Anopheles fluviatilis Complex

Species S and T – Rourkela, Orissa
Species T and U – Hardwar, Uttarakhand
Species T – Haldwani, Uttarakhand

Anopheles sundaicus

Cyclic colonies established from Car Nicobar, Katchal and Tressa (A & N Islands)

Anopheles annularis

Nathupura, Delhi

Aedes aegypti

Delhi

Culex quinquefasciatus

Delhi
Sonepat, Haryana
Mewat, Haryana

Insecticide resistant lines

Malathion resistant – Sonepat, Haryana
Permethrin resistant – Sonepat, Haryana
Lambda-cyhalothrin resistant – Sonepat, Haryana
Deltamethrin resistant – Sonepat, Haryana
Cyfluthrin resistant – Sonepat, Haryana
Fenthion resistant – Sonepat, Haryana

Morphological mutants

Red eye (re)
Scarlet eye (se) ❑

Parasite Biology

Malaria Parasite Bank

The Malaria Parasite Bank (MPB) was established in 1992. MPB is now functioning as a national resource facility and is involved in collection and characterization of field isolates of malaria parasites. Routine activities of the parasite bank include *in vitro* cultivation of *Plasmodium falciparum*, characterization of the isolates for susceptibility to antimalarials, cryopreservation, revival of adapted and non-adapted cultures, *etc*. Parasite isolates of *P. vivax* and *P. malariae,* malaria positive and negative sera, non-human malaria parasites in cryopreserved states and in their respective animal hosts, wherever possible, are also available in the bank. The details of malaria parasites and other biological material available in the parasite bank are given in Tables 1 and 2.

Table 1. Human malaria parasites preserved in the Parasite Bank

Parasite species	Collection sites		Years of collection			
	States	Districts	1992–2004	2005–06	2007–08	Total
P. falciparum	Andhra Pradesh	Visakhapatnam	12	—	—	
	Assam	Sonapur	20	—	—	
		Tezpur	6	—		
		Nalbari	—	1	—	
	Chhattisgarh	Jagdalpur	14	—	—	
		Bilaspur	—	26	—	
	Delhi		191	—	2	
	Gujarat	Anand	4	—	—	
		Kheda	7	—	—	
	Haryana	Gurgaon	25	—	—	
	Karnataka	Mangalore	0	14	—	
	Madhya Pradesh	Mandla/Jabalpur	14	—	—	
	Meghalaya	Tura	0	18	—	
	Mizoram	Kolasib	—	—	6	
	Orissa	Rayagada	29	—	—	
		Sundargarh	42	—	—	
	Rajasthan	Alwar	25	—	—	
		Bharatpur	35	—	—	
		Jaisalmer	38	—	—	
	Tamil Nadu	Chennai	0	4	—	
		Ramanathapuram	1	—	19	
	Uttar Pradesh	Baharaich	22	—	—	
		Gautam Budh Nagar	37	—	—	
		Ghaziabad	17	—	—	
		Allahabad	60	—	—	
	West Bengal	Kolkata	18	—	—	
		Midnapur	1	—	—	
		Total	618	63	27	708
P. vivax	Karnataka		0	6	—	6
	Delhi, Uttar Pradesh, Orissa		53	—	—	53
	Tamil Nadu		0	9	9	18
	Total		53	15	9	77
P. malariae	Orissa		4	—	—	4
	Delhi		1	—	—	1
Total			5	—	—	5
Total human malaria parasites						790

Malaria Parasite Bank is a National Resource for both Human and Non-human Malaria Parasites

Table 2. Total Non-human malaria parasites preserved in the Parasite Bank

Parasite species		Susceptibility to antimalarials
Simian malaria	*P. cynomolgi bastianelli* (CDRI)	Not done
	P. cynomolgi bastianelli (NICD)	–do–
	P. knowlesi (NICD)	–do–
	P. knowlesi (CDRI)	–do–
	P. fragile (CDRI)	–do–
Avian malaria	*P. gallinaceum*	Not done
	P. relictum	–do–
Rodent malaria	*P. berghei* (CDRI)	CQ-resistant
	*P. berghei**†	CQ-sensitive
	P. berghei	Quinine-resistant
	P. berghei ANKA	Not done
	P. berghei (NK65) (PGIMER, Chandigarh)	–do–
	P. chabaudi (Paris)	–do–
	P. vinckei petteri 279 BY	–do–
	P. yoelii nigeriensis (ICGEB)	–do–
	P. yoelii nigeriensis (CDRI)	Multi-resistant
	P. yoelii nigeriensis (LSHTM, London)**†	Not done
	P. yoelii yoelii (265 BY) (Paris)**	–do–

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

Screening of Drug Sensitivity Status

Since 1993, a total of 287 *P. falciparum* samples from different regions were tested for the sensitivity to chloroquine and 187 (67.03%) were found resistant to chloroquine (Table 3). Chloroquine (CQ) resistant and sensitive *P. falciparum* isolates were used for the studies on the role of *Pfmdr-1* gene in chloroquine resistance by studying the nucleotide changes at position 754, 1049, 3598, 3622 and 4234 in the coding region of *Pfmdr-1* gene and also to see whether any of the mutational changes can be used to study the detection of CQ resistance. The nucleotide changes in the said position were found ambiguous with strong association but incomplete correlation between

Table 3. Susceptibility/Resistance status of *P. falciparum* isolates to chloroquine (CQ) during 1992–2007

Place of collection	No. of samples tested	Response to chloroquine*	
		Susceptible	Resistant (%)
Delhi	74	17	57 (77.03)
Jaisalmer (Rajasthan)	22	1	21 (95.45)
Shankargarh (U.P.)	10	2	8 (80)
Gurgaon (Haryana)**	66	44	22 (33.33)
Sonapur (Assam)	18	5	13 (72.22)
Baharaich (U.P.)	11	6	5 (45.45)
Visakhapatnam (A.P.)	4	—	4 (100)
Gautam Budh Nagar (U.P.)	33	14	19 (57.57)
Bissam Cuttack (Orissa)	16	—	16 (100)
Rourkela (Orissa)	4	—	4 (100)
Jagdalpur (M.P.)	5	1	4 (66.66)
Tura (Meghalaya)	10	2	8 (80)
Mangalore (Karnataka)	1	—	1 (100)
Kheda (Gujarat)	1	—	1 (100)
Bilaspur (Chhattisgarh)	4	—	4 (100)
Kolasib (Mizoram)	6	6	—
Ramanathapuram (T.N.)	2	2	—
Total	287	100	187 (65.16%)

*WHO methods/kits were used; **Out of 66 samples tested from Gurgaon we could preserve only 25 *P. falciparum* samples, hence the difference in numbers.

chloroquine resistance and allelic variation in *Pfmdr-1* gene.

Erythrocyte Invasion Studies

Collaborative studies with ICGEB, New Delhi, aims to define the cytoadherence phenotypes and the invasion profile of Indian field isolates of *P. falciparum*, collected and cryopreserved in the parasite bank. Erythrocyte invasion by malaria parasites is mediated by specific molecular interactions. Sialic acid residues of glycophorin A are used as invasion receptors by *Plasmodium falciparum*. *In vitro* invasion studies have demonstrated that some cloned *P. falciparum* lines can use alternate receptors independent of sialic acid residues of glycophorin A. It is not known if invasion by alternate pathways occurs commonly in the field. In this study, we used in vitro growth assays and erythrocyte invasion assays to determine the invasion phenotypes of 15 *P. falciparum* field isolates. Of the 15 field isolates tested, 5 multiplied in both neuraminidase and trypsin-treated erythrocytes, 3 multiplied in neuraminidase-treated but not trypsin-treated erythrocyte, and 4 multiplied in trypsin-treated but not neuraminidase treated erythrocyte; 12 of the 15 field isolates tested use alternate invasion pathways are thus commonly used by *P. falciparum* field isolates (Table 4).

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

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

The experiments on erythrocyte invasion inhibition using anti EBA-175 showed encouraging results. Purified anti-EBA-175 R.II (F_2) antibody was used to test its ability to inhibit invasion of erythrocytes by parasites. One laboratory isolate 3D7 and two field isolates, RKL-9 and JDP-8, collected and preserved/maintained in parasite bank, with known invasion properties were selected for these studies. This antibody raised against EBA-175 showed about 80% inhibition compared to controls, indicating that this antibody is highly effective in blocking erythrocyte invasion by these parasites. Four more isolates were characterized for their cytoadherence properties, of which one from Assam and another from Delhi showed high binding to ICAM-1.

Cytoadherence

Cytoadherence refers to the ability of blood stage *P. falciparum* trophozoites and schizont to adhere to the vascular endothelium in the human host and bind to uninfected erythrocytes to form rosettes. Cytoadherence enables *P. falciparum* to avoid to passage through the spleen where infected erythrocytes are destroyed. The adhesion of *P. falciparum* infected erythrocytes in brain capillaries is implicated in the syndrome of cerebral malaria. The endothelial receptors used by *P. falciparum* for cytoadherence include CD36, ICAM-1, CD31, V-selectin, E-selectin and chondroitan sulfate –A (CSA). In our collaborative studies with ICGEB, we have screened few *P. falciparum* field isolates for their cytoadherence properties (Table 4). These studies will help in understanding the pathophysiological conditions during cerebral/complicated malaria.

Cultivation of Pre-erythrocytic Stage of *Plasmodium vivax in vitro*

For the first time in India, *P. vivax* pre-erythrocytic schizonts (liver stage) were developed in hepatoma cell line using the facilities of the parasite bank. National Institute of Malaria Research has well-established insectary facilities for the production of sporozoites in the laboratory. Mosquitoes were fed on infected blood through artificial membrane feeding apparatus and the fed mosquitoes were dissected on appropriate days for oocyst and sporozoites. These sporozoites from artificially fed mosquitoes were used for inoculating the hepatocytes/hepatoma cell line for the development of pre-erythrocytic stage parasites.

Cultivation of Erythrocytic Stage of *Plasmodium vivax in vitro*

Efforts have been made to cultivate and adapt erythrocytic stages of *P. vivax in vitro*, like *P. falciparum* in different combinations of media and culture conditions, with little success. A low-level parasitaemia could be maintained up to 52 days and growth of the parasites were observed for 2–3 cycles.

Table 5. Major Research Institutes/Universities which received biological material from the Malaria Parasite Bank

Andhra Pradesh
1. University of Hyderabad, Hyderabad

Assam
2. Defence Research Laboratory (DRL), Tezpur
3. Regional Medical Research Centre, Dibrugarh

Chandigarh
4. Post Graduate Institute of Medical Education and Research
5. Punjab University
6. Institute of Microbial Technology

Delhi
7. All India Institute of Medical Sciences
8. Department of Biochemistry, South Campus, University of Delhi
9. Department of Zoology, University of Delhi
10. Dr. B.R. Ambedkar Centre for Biomedical Research, University of Delhi
11. International Centre for Genetic Engineering and Biotechnology
12. Institute of Genomics and Integrative Biology
13. Jawaharlal Nehru University
14. Maulana Azad Medical College
15. National Institute of Communicable Diseases
16. National Institute of Immunology
17. Jamia Millia Islamia University
18. Jamia Hamdard University
19. Rapid Diagnostic Pvt. Ltd.

Gujarat
20. Medical College & SSG Hospital, Baroda
21. Sardar Patel University, V.V. Nagar, Anand
22. Veer Narmad South Gujarat University, Surat
23. Span Diagnostic, Surat

Haryana
24. Maharshi Dayanand University, Rohtak

Karnataka
25. Astra Research Centre, Bengaluru
26. Indian Institute of Science, Bengaluru
27. Regional Office for Health and Family Welfare, Bengaluru
28. University of Bangalore, Bengaluru
29. Banglore Genei, Bengaluru

Kerala
30. Cochin University of Science and Technology, Cochin
31. University of Calicut, Calicut

Madhya Pradesh
32. Defence Research & Development Establishment (DRDE), Gwalior
33. Department of Zoology, S.N. Jain Post Graduate College, Vidisha
34. University of Sagar (formerly University of Saugar)

Maharashtra
35. T.N. Medical College & B.Y.L. Nair Charitable Hospital, Mumbai
36. Wockhard Research Centre, Aurangabad
37. M.G. Institute of Medical Sciences, Sewagram

Orissa
38. Institute of Life Sciences, Bhubaneswar
39. Regional Medical Research Centre, Bhubaneswar
40. SCB Medical College, Cuttack

Punjab
41. Punjabi University, Patiala

Rajasthan
42. Birla Institute of Technology & Science, Pilani

Tamil Nadu
43. Manonmaniam Sundaranar University, Nagercoil, Kanyakumari
44. Bharathiar University, Coimbatore

Uttar Pradesh
45. Central Drug Research Institute, Lucknow
46. Central Institute of Medicinal and Aromatic Plants, Lucknow

West Bengal
47. Kalyani University, Kalyani
48. University of Calcutta, Kolkata
49. West Bengal University, Kolkata

This short-term culture system standardized in the parasite bank can be used for screening of anti-malarials *in vitro*.

Supply of Biological Materials

Providing malaria parasites to the scientific community has been one of the major activities of the parasite bank. Following biological materials were supplied to various institutes, universities and other research organizations (Table 5).

- Adapted and non adapted cryopreserved parasites isolates.
- Parasite isolates resistant/sensitive to chloroquine.

- Parasite isolates with rosetting properties.
- Parasite isolates with different cytoadherence properties.
- Isolates with different erythrocyte invasion properties.
- Sera/plasma from malaria positive patients.
- Different stages of parasites such as merozoites, ring forms, gametocytes from culture.
- Sporozoites harvested from artificially fed mosquitoes.
- Different species of avian, simian and rodent plasmodia.
- Rodent plasmodia infected rats/mice.
- Sera/plasma from respective vertebrate hosts.

Screening of Medicinal Plant Extracts for their Antiplasmodial Activity

As part of new drug (antimalarial) development, the parasite bank is involved in the primary screening of medicinal plants (extracts) collected from different parts of India. About 159 plant extracts/compounds were screened for their antiplasmodial activity. For this purpose the parasite bank is maintaining chloroquine resistant and sensitive *P. falciparum* and *P. berghei* isolates. Out of 159 samples tested, 39 showed antiplasmodial activity. The details of the samples tested are given in Table 6.

Table 6. Details of plant extracts/compounds screened for their antimalarial properties *in vitro*

Source	Total samples screened properties	No. of samples with antimalarial
NIMR, Delhi	20	9
RMRC, Dibrugarh	8	2
DRL, Tezpur*	40	12
DRDE, Gwalior	16	7
Kerala University**	72	6
Guru Nanak Dev University, Amritsar	3	3
Total	159	39

*Collaborative project (DRDO), **Collaborative project (DBT)

Human Resource Development

Imparting training is one of the mandates of NIMR. Expertise available for providing training in different techniques is listed in Table 7. Several scientists/research scholars were given training in parasite bank for one week to four months in different techniques.

A workshop on "Establishment of malaria parasite and screening of antimalarials" was conducted by late Dr. C. Usha Devi and Dr. C.R. Pillai at the Institute of Endemic Diseases, University of Khartoum, Sudan during 28 May to 12 June 2005 (17 scientists participated in the workshop). The details of training imparted from 1993–2008 are given in Table 8.

Table 7. Training facilities available in the parasite bank

- Collection, cryopreservation, revival and transportation of malaria parasite isolates/strains
- *In vitro* cultivation of erythrocytic stages of *P. falciparum*
- Short-term cultivation of *P. vivax* and other species of plasmodium
- *In vitro* cultivation of exo-erythrocytic stages of *P. vivax*
- *In vitro* testing for sensitivity of *P. falciparum* isolates to antimalarials
- *In vitro* and *in vivo* screening of medicinal plant extracts for antiplasmodial properties

Table 8. Training imparted to scientists/students (1993–2008)

Year	Indian	Foreign	Total
1993	1	—	1
1994	3	1	4
1995	2	4	6
1996	4	—	4
1997	4	—	4
1998	4	1	5
1999	2	1	3
2000	5	4	9
2001	—	—	—
2002	4	4	8
2003	8	1	9
2004	14	—	14
2005	4	17	21
2006	16	—	16
2007	17	2	19
Total	88	35	123

Cell Lines Available at the Parasite Bank

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

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Malaria Parasite Diagnostics

One of the most pronounced problems in controlling the morbidity and mortality caused by malaria is the limited access to effective diagnosis and treatment in areas where malaria is endemic. The most widely used routine method of microscopy needs laboratory infrastructure and expertise and is labour intensive. Therefore, development of rapid, sensitive and specific diagnostic tests to diagnose malaria is of paramount importance. NIMR has been making efforts to develop simple methods and recently, the Institute has been identified as the Referral Centre, and commercially developed diagnostic kits are referred from the Office of the Drugs Controller General of India for evaluation before marketing.

Detection of *Plasmodium vivax* in Human Blood using Synthetic DNA Probe

An oligonucleotide based on 19 tandem repeats of a nonapeptide published earlier was synthesized and an assay developed to detect *P. vivax* parasites in infected blood (Roy *et al* 1987). A 21 mer oligonucleotide representing repetitive sequences in genes coding for CS protein of *P. falciparum* was synthesized for comparison of specificity and cross-reactivity. The oligos were radio-labelled and tested as probe in DNA-DNA hybridization assay on nitrocellulose filters. These showed desirable specificity and the vivax probe was used in a simple dot blot assay for detecting parasite in patient's blood. Hybridization with 5 µl of *P. vivax* positive blood from patients spotted on nitrocellulose after lysis gave very strong signal with *Pv* probe but not with *Pf* probe. The results showed that synthetic DNA probe based on repetitive sequences can specifically discriminate *P. falciparum* from *P. vivax*. The importance of correct diagnosis for administration of radical treatment and interruption of transmission can not be over emphasized. Where rapid screening of a large number of samples is necessary, DNA probes will provide desirable speed, accuracy and sensitivity.

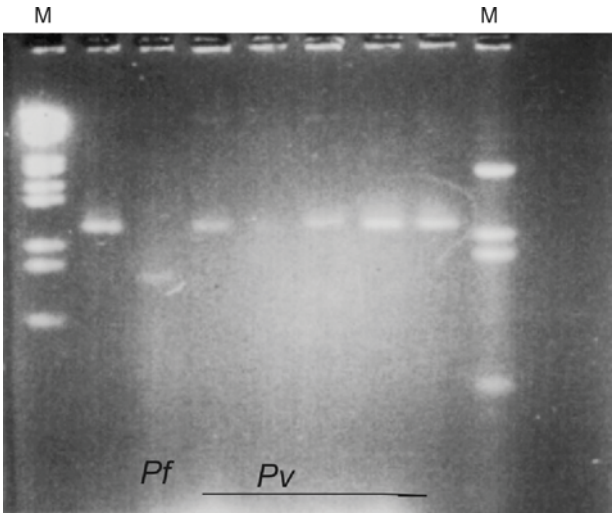

Fig. 1: PCR based diagnosis

PCR based Diagnostics

Simple PCR assay using primers derived from a highly repetitive DNA fragment was employed to amplify DNA fragment from dried blood spots on filter paper (Whatman 3 mm) (Fig. 1). Observed positivity of 97.1% for correct results (both positive and negative) using blood spots on filter paper suggests that the simple PCR assay can be used for the diagnosis of *P. falciparum* and *P. vivax*, the two human malaria parasite species commonly prevalent in our country. Sensitivity (97.6% for *P. falciparum* and 88% for *P. vivax*) and specificity (100% for both) is similar to the results reported by other diagnostic techniques. The study shows the feasibility of using field collected blood spots for the identification of malaria infections.

Production of Monoclonal Antibodies against Erythrocytic Stages of *P. vivax*

Twenty monoclonal antibodies, IgG1 type were raised against erythrocytic stages of *P. vivax*. Antibodies reacted with erythrocytic stages of *P. vivax* clinical isolates by indirect immunofluorescence assay. One clone Mab1B3 was studied for antigenic characterization. Antibody secreted by this clone showed its reactivity in inhibition ELISA when tested with blood samples collected from *P. vivax* patients (Fig. 2). This antibody showed the detecting ability in isolates having > 0.01% parasitaemia. By immunoblotting, this monoclonal antibody reacted

NIMR is a Referral Centre for Evaluating Commercially Developed Diagnostic Kits — Designated by the Drugs Controller General of India

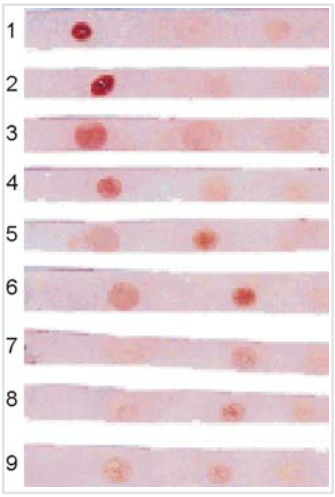

Fig. 2: Dipstick assay

with nearly 42 kDa protein of *P. vivax* parasite collected from different geographic locations of the country. An additional protein of nearly 65 kDa was also recognized by this antibody in some of the isolates collected from Rourkela, Chennai, Bhopal, Ghaziabad and Delhi. The epitope recognized by the antibody from crude extracts of erythrocytic stages of *P. vivax* might be common in these proteins. N-terminal sequencing of the protein identified by this clone produced significant alignments with one *P. falciparum* erythrocyte membrane associated antigen. Enzyme (HRPO)-antibody conjugate was prepared to develop a Dot blot/Dipstick ELISA for the detection of parasite in the patient's blood. The assay is to be evaluated in the field.

Immunodiagnostic Assay of *P. falciparum* Infection using a Glycophospholipid Antigen and Laser Immunoassay

Diagnostic potential of the GPL antigen was assessed by laser light scattering immunoassay (LIA) and ELISA methods. Immunoreactivity and specificity of the GPL antigen is compared with another previously reported RESA derived synthetic peptide antigen. We developed an assay using GPL coated latex beads which could be an excellent diagnostic method for *P. falciparum* infection. Agglutination assay is the simplest and cheapest immunoassay but has not been employed because of its low sensitivity. As the antigen-antibody complexes are often microscopic and not visible to the naked eye, laser immunoassay (LIA) based upon laser light scattering can detect microscopic agglutinates and can raise the sensitivity of agglutination assay enormously. LIA for malaria (Bhakat *et al* 1999) was developed which is as sensitive as ELISA but much simpler in principle and practice. Specific diagnosis of *P. falciparum* infection by LIA using a new glycophospholipid antigen isolated from *P. falciparum* culture supernatant was developed. The chemical composition of the antigen as analysed by HPLC, TLC and GLC showed it to be a glycophospholipid (GPL) with galactose, glucose, mannose and xylose as sugar residues (Mya *et al* 2001). GPL antigen was found to be very specific and could clearly discriminate between *P. falciparum* and *P. vivax* infections in clinically identified patients both from India and Myanmar (Mya *et al* 2002). Malaria diagnostic immunosensor method has been developed recently (Mya *et al* 2002). Specificity of the GPL antigen was also shown by LIA for serum from non-malarial patients. Out of forty finger prick blood samples collected on filter discs, sixteen samples each were from *Pf* and *Pv* positive cases, and eight sera from healthy individuals included as negative control. The results showed that anti GPL titers for *Pf* and *Pv* cases were about 10-fold and 4-fold higher than that of control sera. Studies are in progress to develop a cost-effective and sensitive LIA using glycolipid antigen.

Evaluation of Rapid Diagnostic Kits

In recent years, rapid antigen capture assays based on the detection of *Pf*HRP-2 antigen in peripheral blood for *P. falciparum* have been

Table 9. Evaluation of diagnostic kits

Name	Manufacturer	% Sensitivity/Specificity
ParaSight-F	Becton Dickinson, U.S.A.	93/92.4
ICT *Pf*/*Pv*	Becton Dickinson, U.S.A.	96/93.1 (*Pf*) 75.5/99 (*Pv*)
Rapid Test Malaria	Quoram Diagnostics, Canada	100/98.3
Pf Check-1	Veda Lab., France	87.7/98.9
Determine™ Malaria *Pf*	Dainabot Co., Japan	96.5/87.2
ACCU Stat Malaria	Millennium Bio-Technology Inc., USA	86.9/90.3
ParaCheck	Orchid Biomed. Systems (Goa)	95.8/85.7
OptiMAL	DiaMed, Switzerland	92.2/99.3 (*Pf*) 94.5/98.2 (*Pv*)
ParaHIT f	SPAN	90.9/91.4
First Response Malaria	PMC Ltd	96/95 (*Pf*)
Combo test		83/94 (Non *Pf*)

developed. The dipstick format kits for the detection of malaria antigens were compared with conventional microscopy for the detection of *P. falciparum* malaria. The overall sensitivity and specificity of these kits were more than 90% (Table 9). The advantages were: (i) less time consuming; and (ii) expertise and infrastructure not required. However, the most important limiting factor of these kits is the persistence of HRP-2 even after parasite clearance thereby making monitoring of therapeutic response difficult (Kumar *et al* 1996; Singh *et al* 1997; Valecha *et al* 1998; Singh and Valecha 2000; Ghosh *et al* 2000).

A kit—ICT (*Pf/Pv*) based on the same principle of detection of HRP-2 antigen for *Pf* and a panmalarial antigen for *P. vivax* was also evaluated. The sensitivity and specificity were 74 and 99.1% respectively for *P. vivax* while these were 96% for *P. falciparum*. OptiMAL, a test utilizing monoclonal antibodies against metabolic enzyme of parasite lactate dehydrogenase (LDH) has also been evaluated. This test does not have the limitation of persistence of antigen. The sensitivity of the test for *Pf* and *Pv* was 92.2 and 94.5% respectively. The test becomes negative in parallel with parasite clearance. Based on the data generated by NIMR most of these kits have been registered and marketed.

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Characterization of Human Malaria Parasites

Ultrastructural Studies on *Plasmodium vivax*

For the first time a detailed ultrastructural study was carried out on *P. vivax.* Fine structural analysis of growth and differentiation of successive stages involved in erythrocytic and sporogonic phases of development of this parasite was done and compared with those of other malaria parasites. In the erythrocytic phase, asynchrony during merozoite formation within the schizont and caveola-vesicle complexes and cytoplasmic clefts observed in all infected erythrocytes (Fig. 3) were important features of *P. vivax* (Nanda 1990). During sporogonic phase, oocysts on a single midgut exhibited differential rate of development. The invasion of sporozoites into the acinal cells of salivary gland resulted in depletion of rhoptries and changes in pellicular membranes (Nanda *et al* 1985). Morphologically two types of sporozoites were observed in salivary gland cells. Apart from providing better understanding of the parasite morphology, this study may provide basis for other investigations like mechanism of drug action, host-parasite interactions, *etc*.

Genetic Diversity Studies

Understanding the diversity, extent and distribution of variant forms of human malaria parasites (*Plasmodium* species) in different geographic locations as well as the complexity of infections they cause is crucial in developing effective control measures and in malaria surveillance. Therefore, with an objective to understand genetic structure and to estimate the type and the extent of genetic diversity existing among *P. falciparum* and *P. vivax* populations, studies on enzyme and DNA size polymorphism have been carried out in relation to space and time. The sites from where isolates have been characterized are shown in Fig. 4.

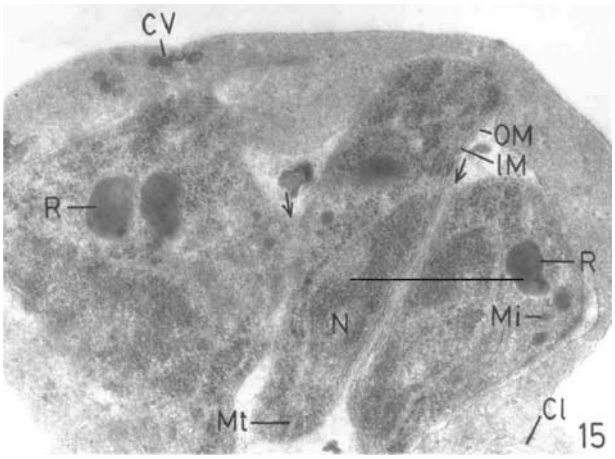


Fig. 3: Fully differentiated *P. vivax* merozoites, prior to host cell lysis, showing well-developed pellicular membranes (OM, IM), microtubules (Mt), rhoptries (R), micronemes (Mi), nucleus (N) and surface strands to bridge the adjacent merozoites (arrows). Note the caveola–vesicle complexes (CV) and cytoplasmic clefts (Cl) in the host cell

Plasmodium vivax

Population Genetic Structure

The polymorphic nature of Indian *P. vivax* isolates was initially established by isoenzyme typing (Glucose phophate isomerase, glutamate dehydrogenase and adenosine deaminase enzyme loci) studies in patient samples from Delhi (Joshi *et al* 1989). Later, longitudinal studies using *P. vivax* isolated from patients in Delhi between 1985 and 1993, together with samples collected from Sundargarh in the hyperendemic Orissa state in 1991, confirmed the extensive diversity of *P. vivax* in India (Joshi *et al* 1997), while simultaneously showing that allelic frequencies did not differ significantly in successive years. Equally important, these studies showed that a similar population structure existed in Delhi and Sundargarh and by extension in different geographical areas in India (because the Delhi samples were presumed to be representative of allelic variants from different regions of the country due to frequent migration). Subsequent studies have used PCR alone or in combination with sequencing and restriction fragment length polymorphisms (RFLPs) to describe the population structure of *P. vivax*. Markers used for the study are surface antigens (MSP-1, MSP-3α, CSP, AMA-1, DBP,), sexual stage antigens (GAM-1, 25S and 28S), 18s SSU RNA-S-type and neutral markers (mini and microsatellites and housekeeping genes).

The *P. vivax* population in India is highly diverse and is highly genetically variable according to studies of isoenzyme markers, antigen genes, DNA based markers (both asexual and sexual stages) as well as neutral markers. The high number of genetically mixed isolates indicate that in India, *P. vivax* is a

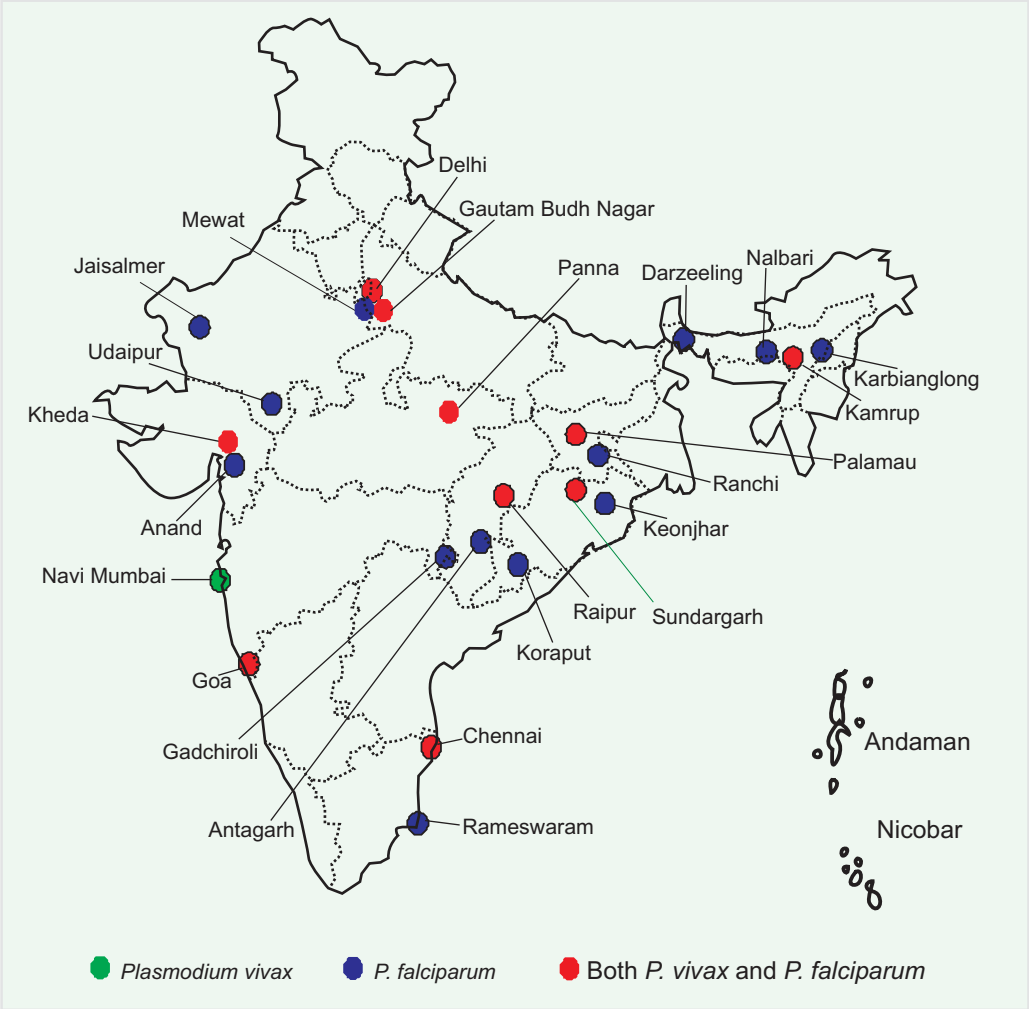


Fig. 4: Map showing study sites from where malaria parasites were collected for genetic diversity studies

randomly mating population, with the high level of genetic diversity likely due to prolonged periods of transmission along with population movement between different regions of the country. Indian isolates have 90 to 100% sequence similarity among themselves and average identity of Indian isolates with the isolates of other regions is in the range of 80 to 99%. Existence of common allelic composition in different parts of the globe and segregation of Indian isolates with the isolates of different regions suggests that Indian isolates have global allelic representation. Little evidence of population sub-structure was observed based on the mean synonymous and non-synonymous sequence diversity. Both synonymous and non-synonymous nucleotide diversity was higher between the populations than within the populations. High degree of allele sharing was observed among five geographical regions (Fig. 5) indicates good level

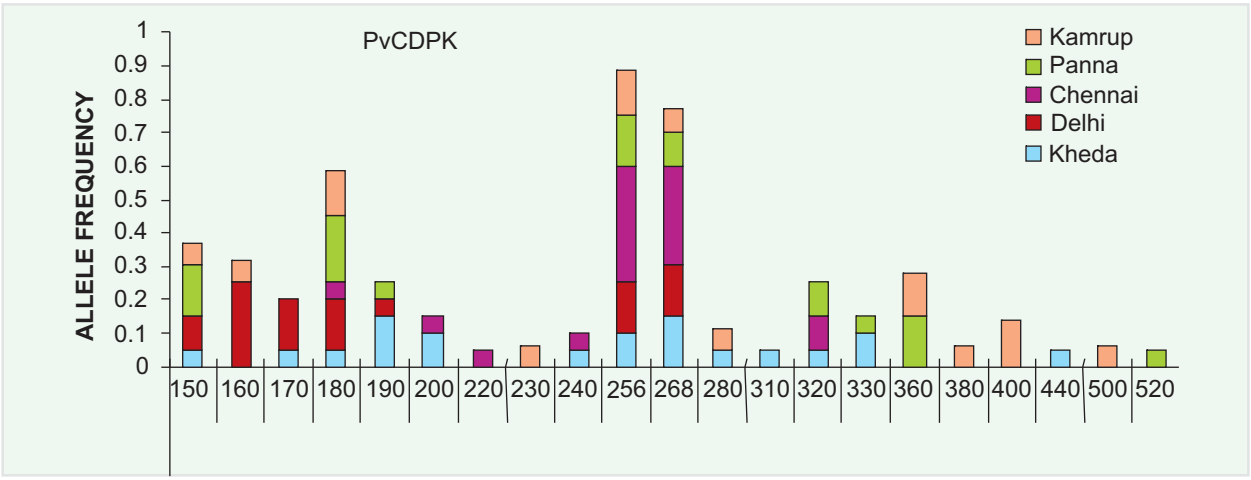


Fig. 5: Extent of genetic diversity and allele sharing at minisatellite locus

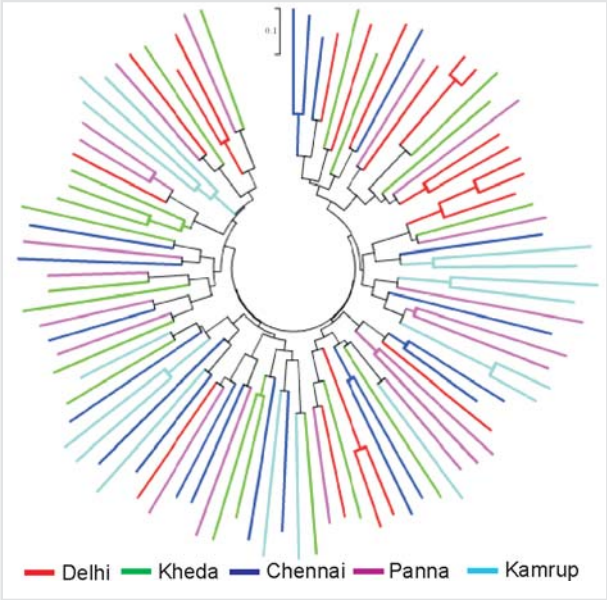


Fig. 6: Neighbor-Joining phylogenetic tree showing genetic relationship among Indian *P. vivax* isolates using ten minisatellites

of gene flow among the different geographical regions of India. Phylogenetic tree reflect that *P. vivax* isolates circulating in different geographical regions clustered together suggesting similar genetic structure among different geographical regions of India (Fig. 6). Observed genetic diversity reflects that Indian *P. vivax* isolates exhibit diversity very close to the Southeast Asian isolates but higher than the South American isolates (Prajapati *et al* 2006; Joshi *et al* 2008).

Evolutionary Parameters and Population History

Asia has been reported as the motherland for *P. vivax* origin (Escalante *et al* 2005, 2006). The expansion of Southeast Asian *P. vivax* population is more likely to led the introduction of this species in India. To understand the history between Southeast Asian and Indian *P. vivax* in real time scale, panels of genome wide neutral markers were explored, namely minisatellites, microsatellites and housekeeping genes. Analysis of microsatellites and minisatellites revealed that Indian isolates displayed extensive genetic diversity and this diversity is very close to the Southeast Asian isolates but is higher than the South American *P. vivax* isolates. Study indicated evidence of long-term population history of *P. vivax* in India suggesting ancient connection between Indian and Southeast Asian *P. vivax*. These findings were further supported by the extensive putatively neutral SNPs observed at ten housekeeping genes. Study indicated very high effective population size suggesting ancient population history of *P. vivax* in India. The coalescence analysis of TMRCA (Time to the Most Recent Common Ancestor) using housekeeping genes neutral SNPs suggest ancient signature of *P. vivax* in India. The TMRCA estimates in our study is overlapped with the earlier report of TMRCA using mitochondrial genome sequencing from world-wide *P. vivax* isolates suggesting Asia is the centre of *P. vivax* origin (Mu *et al* 2005, Escalante *et al* 2006). The study suggested that observed genetic diversity in respect of genome wide neutral markers reflects ancient population expansion of *P. vivax* in Southeast Asia which led to the introduction of this most prevalent human malaria parasite in India in ancient time scale.

Antigenic Repertoires of Vaccine Candidates

Antigenic diversity in the natural parasite populations is the major obstacle in the development and success of effective antimalarial measures. Antigenic repertoires of human malaria parasites have been widely studied, however, day-by-day new antigenic variants are being reported from different parts of the globe. Local antigenic variations which are hidden in smaller geographical regions are very crucial in the understanding of total antigenic repertoires in a country like India and in turn, planning effective control measures. Antigenic repertoires of *P. vivax* vaccine candidates were investigated in five widely separated geographical regions (Delhi, Panna, Kheda, Chennai and Kamrup) of India to understand the local antigenic repertoires. Sequence analysis of five vaccine candidates from asexual (*Circumsporozoite Protein (CSP), Apical Membrane Antigen-1 (AMA-1)* and *Duffy Binding Protein-II (DBP-II*) and sexual stages (*Pv25S* and *Pv28S*) revealed differential levels of antigenic repertoires. Asexual stage revealed high antigenic repertoires in comprison to sexual stage. Extensive non-synonymous and synonymous nucleotide substitutions were found and overall number of non-synonymous substitutions exceed over synonymous substitutions suggesting signature of diversifying selection at vaccine candidates. Extensive non-synonymous substitutions found in the vaccine candidates in comprison to the housekeeping genes explained that high antigenic variation among antigenic genes is the adaptive mechanism of parasite to evade the host immune response. Substantial amount of local antigenic repertoires for each candidate in all five regions have been uncovered. The antigenic repertoires of five vaccine candidates for entire regions were very high, however, each region showed a fraction of specific antigenic repertoires. The overall observed SNPs in five vaccine candidates revealed that a good amount of antigenic repertoires are shared among different regions, however, signature of region-specific antigenic-repertoire was also observed (Fig. 7).

Distribution of Two Subtypes

P. vivax has been categorized into two distinct lineages, the New and Old World, distinguishable by gene conversion in the SSU rRNA S-type and mutations in an open reading frame (ORF 470) in the apicoplast genome. These two populations of *P. vivax* also differed in their transmission potential. The

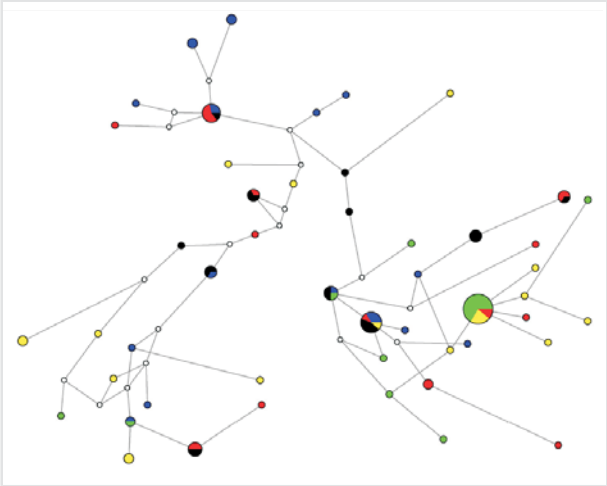


Fig. 7: Region-specific haplotypes and shared haplotypes of *P. vivax* as well as phylogenetic relationship between AMA-1 haplotypes. Each circle represents different haplotypes, colour of the circle represents geographical origin of isolates and different colours in a circle represent haplotypes shared between respective regions while colourless circle represents missing haplotypes. Haplotypes are connected with the mutational events, length of connecting line is proportional to the number of mutational events and size of circle is proportional to the frequency of haplotypes

distribution of two subtypes of *P. vivax* (Old and New world) based on S type 18s SSU rRNA was studied in field isolates from different locations in India. Comprehensive analysis revealed equal proportions of Old and New world *P. vivax* subpopulations across India, however, proportions of two subtypes were different according to geographical location (Fig. 8). Analysis of single clone isolates identified using MSP-3α marker has revealed similar pattern of mutations in *dhfr* and *dhps* genes, associated with resistance to pyrimethamine and sulphadoxine, respectively.

Drug Resistance Studies

P. vivax has been stated to be intrinsically resistant to pyrimethamine, but recent studies have suggested that acquired resistance is more likely, with mutations in three codons of the gene encoding for dihydrofolate reductase (DHFR): at residue 57 (F to L), 58 (S to R) and 117 (S to N), with double (residues 58 and 117) and triple (57, 58 and 117) mutation genotypes also implicated. The quadruple mutant including mutations affecting residue 61 (T to M) correlates with SP treatment failure *in vivo*. The use of SP to treat chloroquine (CQ) resistant *P. falciparum* might be creating selection pressure in *P. vivax* populations due to mixed species infections, therefore, a study has been undertaken to assess mutations in *dhfr* gene in field isolates. Interestingly, there was a gradual increase in the frequency of mutant genotypes from the north to the south (Fig. 9), with the double mutant genotype more prevalent in areas with sympatricity of *P. vivax* and *P. falciparum* infections and with perennial transmission, thus, suggesting the exposure of *P. vivax* to SP from treatment of CQ resistant *P. falciparum* (Prajapati *et al* 2007). However, Indian isolates show limited DHFR polymorphism as compared to isolates from other Southeast Asian countries where SP resistance among field isolates is very high. Based upon these studies, *P. vivax* appears to remain susceptible to SP treatment in India (Joshi *et al* 2007).

Plasmodium falciparum

P. falciparum, only species of malaria which causes death has shown an increasing trend in India. Therefore, a study has been taken up to understand

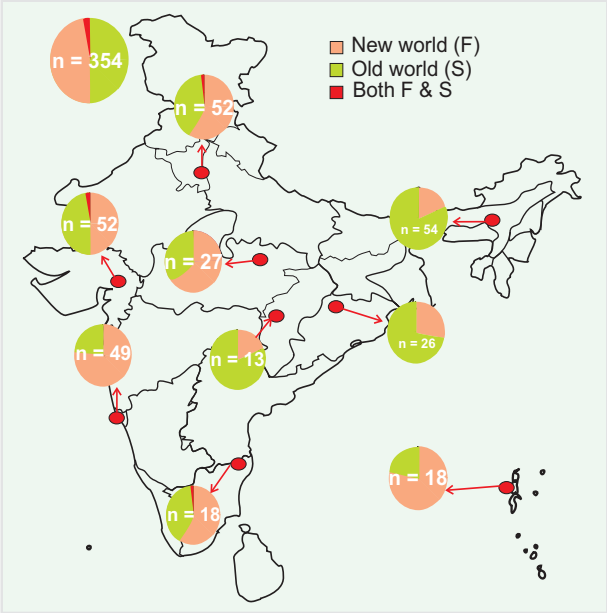


Fig. 8: Distribution of two *P. vivax* SSU rRNA subtypes in India

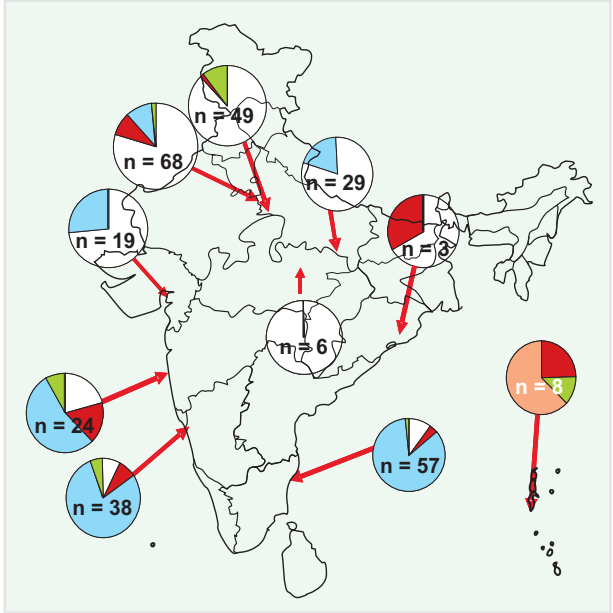


Fig. 9: Distribution of DHFR genotypes

the population structure of the field isolates. Isolates from different geographical regions were analyzed for surface protein markers, namely MSP-1 and MSP-2 for length as well as sequence variations and the analysis revealed highly polymorphic nature of Indian *P. falciparum* isolates on the basis length as well as sequence data. Three families of MSP-1 (K1, MAD20 & RO33) are prevalent in all the study sites with a few exceptions. K1 and MAD20 families have shown further allelic polymorphism while RO33 family was observed to be monomorphic with a single allele of 160 bp in all the study areas. In MSP-2, two families FC27 and 3D7 were observed and both the families have shown length variations. Both the families were observed in all the study sites and both were well represented. Fig. 10 shows the proportional prevalence of various families of MSP-1 and MSP-2.

Sequence analysis of K1, MAD20 and RO33 families of MSP-1 and FC27 and 3D7 families of MSP-2 supported the identical allelic composition of isolates in different areas. Further, identical length variants have shown novel deletions in addition to already reported ones. Sequence data had been submitted to genbank. The study suggested that Indian isolates represent a mixture of different alleles reported in different global regions. The study also revealed that Indian isolates present a level of diversity similar to Southeast Asia, Latin America and Papua New Guinea and a high degree of sequence homogeneity with isolates of other global regions. Clustering with isolates of different regions suggests that Indian isolates have global representation. Population sub-structuring was not observed on the basis of geographical location and allele frequency.

Characterization of *P. falciparum* Isolates from Jarawa, an Isolated Tribe of South Andaman

Analysis of *P. falciparum* samples from Jarawa tribe, an isolated tribe of South Andaman, Andaman and Nicobar Islands revealed 90–100% identity of these *P. falciparum* isolates with that of mainland as well as other regions in respect of MSP1 & 2 markers. Sequences were 100% identical to Kuwait isolate with identical deletion.

Molecular Genotyping of Clinical Resistance

Samples collected from *P. falciparum* patients on Day zero and the day of recrudescence during

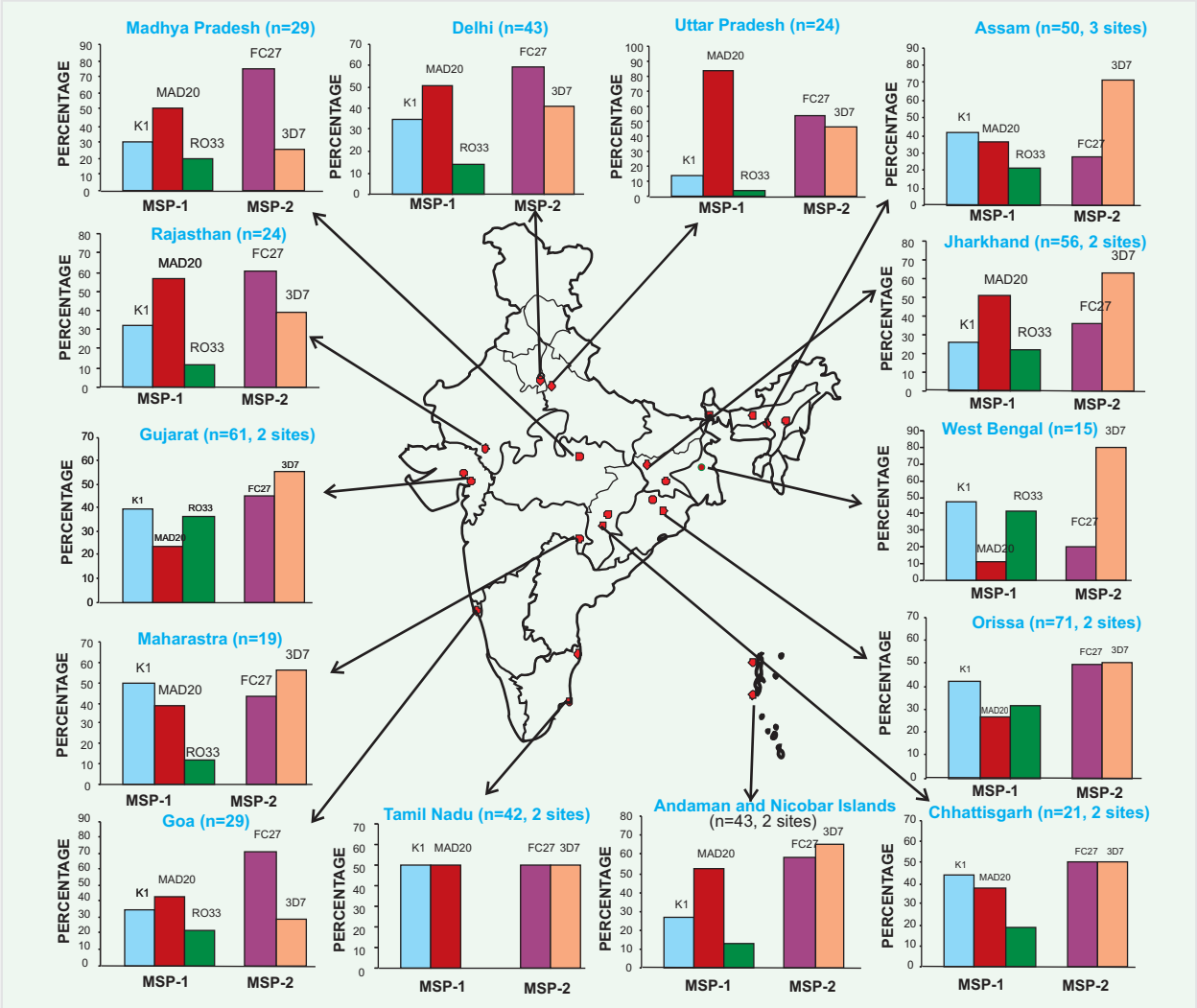


Fig. 10: *Plasmodium falciparum*-distribution of MSP-1 & 2 families among field isolates in India

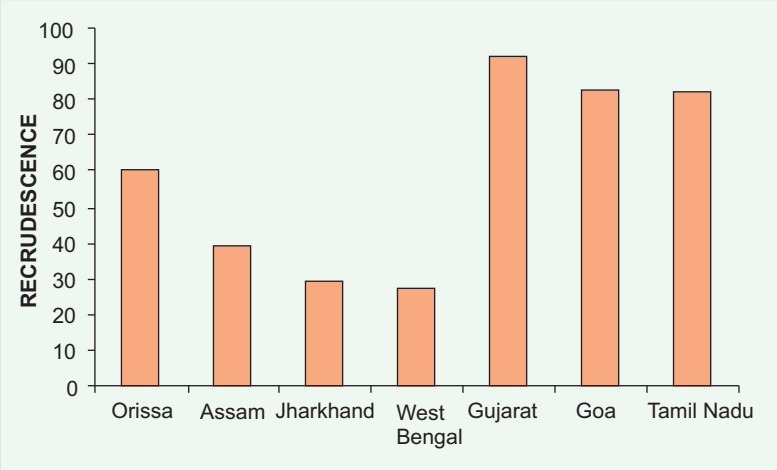


Fig. 11: Percentage of recrudescent infection in *P. falciparum* clinical samples

therapeutic efficacy studies of various antimalarials conducted in different regions of the country were analyzed for molecular genotyping using highly polymorphic surface protein markers. Results revealed high proportion of recrudescent infections with same genotype that of Day zero among field isolates in case of chloroquine (CQ) efficacy studies thus suggesting for resistance (Fig. 11).

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

Widespread use of chloroquine (CQ) for the past decades might have led to chloroquine resistant parasites and emergence of clinical failure of chloroquine treatment. Previous studies on the *Pfcrt* (*Plasmodium falciparum* chloroquine resistance transporter) gene, that is responsible for chloroquine

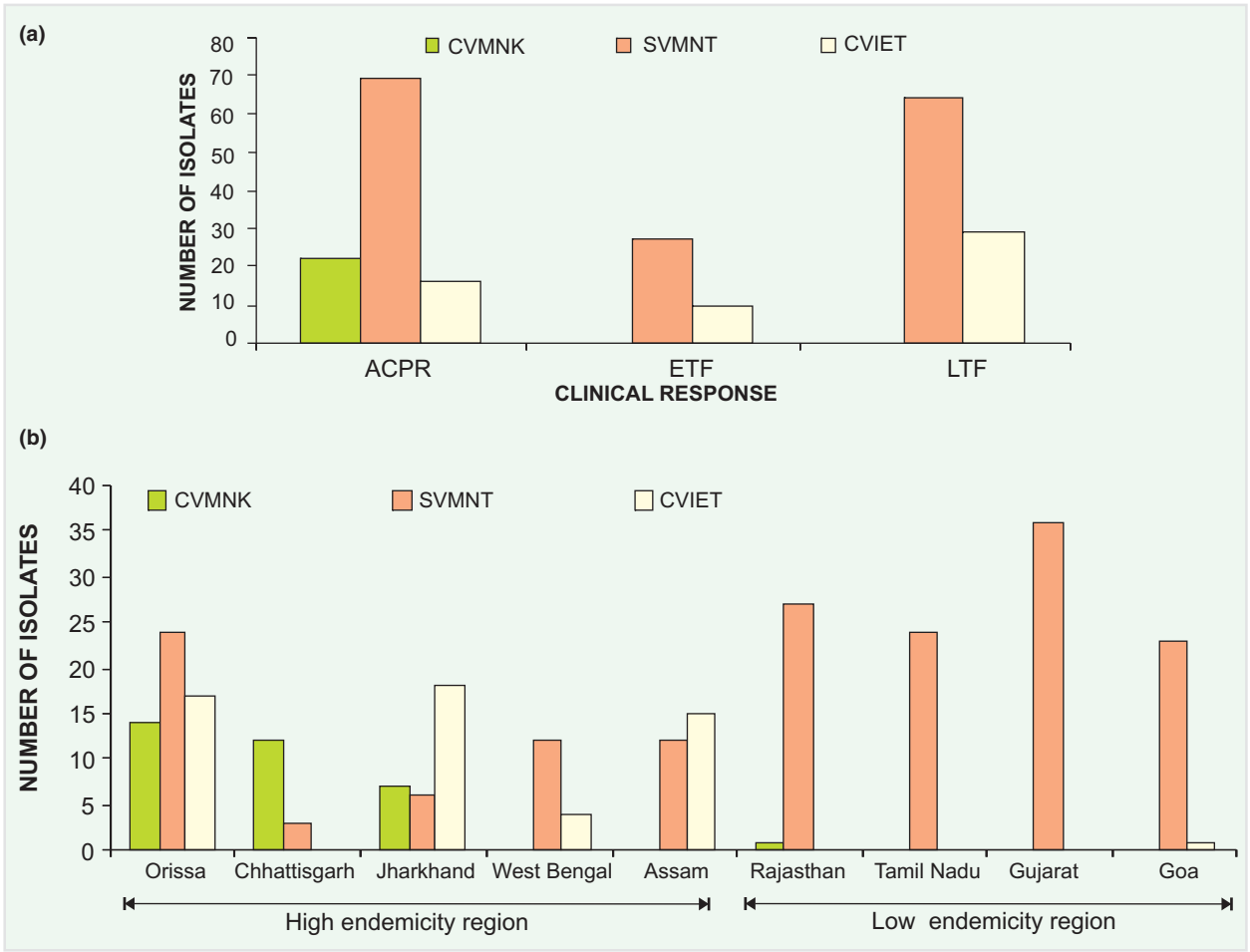


Fig.12: Distribution of Pfcrt haplotypes among field isolates. (a) Prevalence of haplotypes in chloroquine treated malaria cases. ACPR (adequate clinical and parasitological response), ETF (early treatment failure) and LTF (late treatment failure); (b) Prevalence of haplotypes in high and low malaria endemic area

resistance, revealed a heterogeneous situation of chloroquine resistance in Indian *P. falciparum* isolates. These studies analyzed the randomly collected samples and were not supported with clinical assessment of chloroquine efficacy, however, their results indicate *Pfcrt* gene as an attractive target for studying the epidemiological and molecular aspects of drug resistance. About 200 malaria patients enrolled for the chloroquine (CQ) therapeutic efficacy studies and 68 uncomplicated malaria patients from different geographical regions were assessed for genetic basis of chloroquine resistance using monitoring molecular markers. The status of point mutation responsible for CQ resistance was assessed by PCR amplification of *Pfcrt* gene. Three types of amino acid haplotype encoding 72aa-76aa (amino acid) of PFCRT protein, namely SVMNT, CVIET and CVMNK were observed. Results showed the prevalence of SVMNT haplotype in both clinically sensitive (70%) and in clinically resistant (74%) isolates. The wild type CVMNK was found only in clinically sensitive cases (20%) (Fig. 12a). Prevalence of SVMNT haplotype is observed in all geographical regions irrespective of malaria endemicity. Proportion of SVMNT were nearly cent percent in low endemicity areas while in high endemicity area all the three haplotypes were observed (Fig. 12b).

Studies on Genetic Variations in T-helper cell Epitopic Regions (Th2R and Th3R) of Circumsporozoite Protein (CSP) of *Plasmodium falciparum* Isolates from India

The role of T cells in malaria immunity has been appreciated for a long time. CSP has two T helper cell epitopes flanking the highly conserved region RII and spanning amino acid residue 326 to 343 (Th2R) and 361 to 380 (Th3R). However, these regions show polymorphism. Studies were performed to find out whether the genetic variations are regionally unbiased, polymorphism is restricted and can be categorized into groups because the T cell domains could be included in a polyvalent sporozoite vaccine and such a strategy might largely depend on the extent of polymorphism in these epitopes. Study revealed that majority of the Indian isolates are regionally unbiased and could be categorized into six groups and sequences of the two groups showed similarity with the sequences of *P. falciparum* isolates from other geographical regions of the world, although some of the isolates showed wide sequence variations and could not be categorized into any group. However, analysis of isolates from different transmission seasons revealed low diversity during pre- and post-transmission than peak transmission season (Table 10). Therefore, the prototype variants from each group could be included in a subunit polyvalent vaccine against sporozoites.

Study of immunogenicity of T-helper Cell Epitopic Region of *Plasmodium falciparum* CSP as a Recombinant Fusion Protein

The development of an effective antimalarial drug or vaccine has occupied a large part of antimalarial research. A prime and reported target for vaccine development is the sporozoite stage of the parasite life cycle. The T-helper cell sequence corresponding to circumsporozoite protein is termed as a universal epitope as it is examined by different vaccine trials to be highly immunogenic. T-cell epitopic region, residues 326–345 of the *P. falciparum* circumsporozoite (CS) was undertaken to study its immunogenicity. Since the peptide units are small and are not much stable in biological system, it was cloned and expressed as recombinant fusion protein fused to DHFR protein of *E. coli* vector pQE40. The recombinant cells were transformed and cloned into *E. coli* pREP-4 vector, under appropriate antibiotic selection. The recombinant fusion protein was expressed by IPTG induction. This expressed protein was purified and its immunogenic properties were studied by cell proliferation assay, using the peripheral blood mononuclear cells (PBMCs) isolated

Table 10. Categorization of Th2R and Th3R sequences into groups

Group	T-helper cell region of circumsporozoite protein	Pre-transmission period (%)	Peak transmission period (%)	Post-transmission period (%)
Group I	Th2R(QQKKKNTTQ)	26	16	24
	Th3R(DQQD)	23	15	24
Group II	Th2R(QQKKKEKNIIQL)	25	20	26
	Th3R(DQRDEAD)	26	21	25
Group III	Th2R(QHIEEYNTQNL)	24	17	25
	Th3R(QEENTQE)	27	23	27
Group IV	Th2R(QHKKRTL)	24	15	25
	Th3R(DQRQAD)	24	16	24
Group V	Th2R(QEENTQ)	–	16	–
	Th3R(A)	–	11	–
Group VI	Th2R(QKRQ)	–	17	–
	Th3R(DAD)	–	14	–

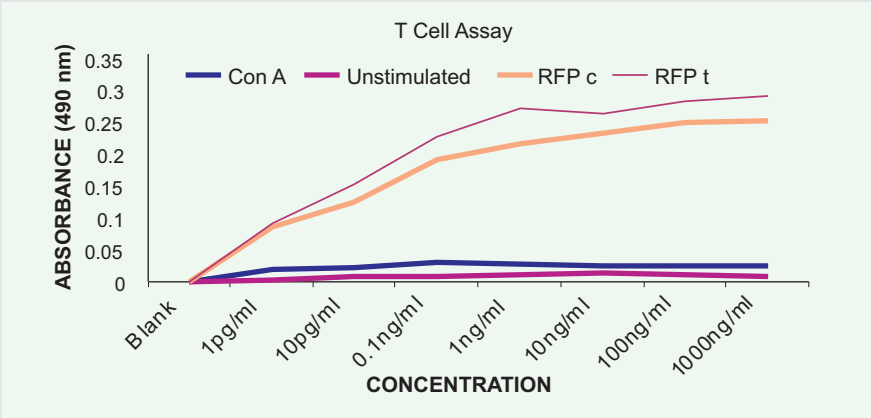


Fig. 13: Immunogenicity of recombinant fusion protein by cell proliferation assay

from the *P. falciparum* infected patients. The PBMCs from healthy individuals (who did not have any clinical history of malaria) and Con A served as negative and positive controls respectively in the cell proliferation studies. The immunogenicity study by T-cell proliferation study revealed that the recombinant fusion protein was more immunogenic than the corresponding peptide unit (Fig. 13). Therefore, to be effective in protection against malaria, T-cell epitopes could be included into a prototype subunit vaccine capable of eliciting protective immune response in genetically diverse population.

Molecular Analysis of Invasion of Indian *Plasmodium falciparum* Field Isolates and Cytoadherent Properties of Infected Erythrocytes

The invasion of erythrocytes by *Plasmodium* merozoites is mediated by specific molecular interactions between host receptors and parasite ligands. Most laboratory strains of *P. falciparum* use sialic acid residues on glycophorin A as receptors for erythrocyte invasion. A 175 kD *P. falciparum* protein known as EBA-175 (for erythrocyte binding antigen-175kD), mediates binding to sialic acid residues of glycophorin A during invasion. Some *P. falciparum* laboratory strains are known to possess alternative invasion pathways and can invade neuraminidase-treated RBCs. It is not known how commonly such alternative pathways are used by *P. falciparum* field isolates. We have studied the invasion phenotypes of *P. falciparum* field isolates collected from different regions of India (Okoyeh *et al* 1999). Out of 15 *P. falciparum* isolates tested, 5 showed invasion and multiplication in both neuraminidase and trypsin-treated erythrocytes, 3 in neuraminidase-treated but not in trypsin-treated erythrocytes and 4 in trypsin-treated but not in neuraminidase-treated erythrocytes. These studies indicate that *P. falciparum* field isolates commonly use alternative invasion pathways that do not depend on sialic acid residues of glycophorin A.

Cytoadherence refers to the ability of erythrocytes infected with blood stage parasites, trophozoites and schizonts, to adhere to the vascular endothelium in the human host and bind to uninfected erythrocytes to form rosettes. Cytoadherence of *P. falciparum*-infected erythrocytes in brain capillaries have been implicated in cerebral malaria and sequestration in the placenta results in complications in pregnancy. The endothelial receptors used for cytoadherence include ICAM-I, CD36, VCAM, E-selectin, CD31 and chondroitan sulfate A (CSA). Cytoadherent phenotypes of Indian *P. falciparum* field isolates collected from different regions of India have been studied. In a preliminary study, out of 13 isolates screened, 9 showed binding property and 4 did not. JDP-2 (*P. falciparum* isolates collected from tribal areas of Jagdalpur (Chhattisgarh) showed high binding with CD36 and JDP-8 showed high binding with ICAM-I and also forms rosettes. Another *P. falciparum* isolate RAJ-86 collected during Rajasthan epidemic in 1994, showed binding with CD36 and CSA (Chitnis *et al* 1998). Information is important for the development of novel strategies that block cytoadherence to receptors such as ICAM-I and prevent or reverse pathological outcomes such as cerebral malaria.

❑

Relapse Pattern in *Plasmodium vivax*

Plasmodium vivax malaria constitutes 60–65% of total malaria cases in India (Sharma 1996). Although the infection is benign except for a few case reports of severe malaria. But the morbidity is high especially due to relapses which is characteristic of vivax malaria. Blood schizontocidal drugs are not effective against persistent hypnozoites of the parasite in the liver. Primaquine (8-aminoquinoline) is the only available drug active against hypnozoites of relapsing malaria parasites. Indian national drug policy (2002) recommended 600 mg chloroquine on Day 0 and primaquine 15 mg/day for five days (adult dose) as radical treatment for *P. vivax* infection against WHO recommended schedule of 14 days treatment with primaquine. But due to logistics and operational reasons and potential side-effects of primaquine, five days of primaquine treatment was followed by NVBDCP in India. As per the current national drug policy on malaria (2008) microscopically positive *Pv* cases should be treated with chloroquine in full therapeutic dose of 25 mg/kg body weight divided over 3 days. Primaquine should be given in doses of 0.25 mg/kg body weight daily for 14 days to prevent relapse except in those with G-6-PD deficiency, infants and pregnant women.

To evaluate the efficacy of different dose schedules of chloroquine and or primaquine, number of studies have been carried out in different geo-epidemiological zones of the country where this species of malaria parasite is predominant. The list of these studies is given chronologically in Table 11. Most of these studies revealed that five days treatment of primaquine was inadequate to prevent relapses and relapse rates were highly variable, ranging between 2 and 30% depending on the duration of follow-up of patients. Most important information obtained was that 70% of the patients never had a relapse after the primary infection without any primaquine treatment.

Among the relapse patients, approximately 60% had only one relapse, while 25% patients had two and 7% had three and remaining 6% had four or more relapses during one year follow-up. Lag month of relapses within one year revealed approximately 80% had relapses within 12 months and 10% had in the following year. Although the intervals between primary attack and first relapse ranged widely, the most common intervals were 1–2 and 8–9 lag months.

Various studies on *P. vivax* relapses revealed existence of polymorphic *P. vivax* populations in different zones of the country, characterized by three main types of incubation periods following primary attack. Studies revealed existence of both tropical and temperate zone types of *P. vivax* populations with distinct incubation periods and existence of

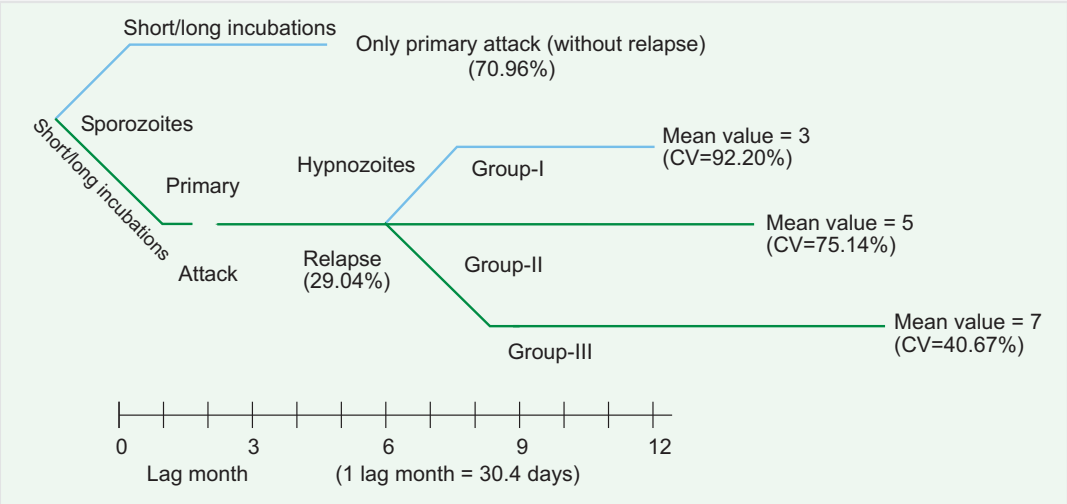


Fig. 14: Patterns of incubation interval in *P. vivax*. Group I = Primary attack between January and June (18%, n = 105); Group II = Primary attack between July and August (22.7%, n = 132); Group III = Primary attack between September and December (59.3%, n = 345); CV = Coefficient of variation (Source: Adak *et al* 1998)

Table 11. Relapse rates in *Plasmodium vivax* observed in different studies at NIMR

	No.*	Groups	Chloroquine	PQ**	Follow-up	Relapse rate %	Study site
Sinha *et al* 1989	725	1	900 mg over 2 days	Yes	395 days	6.9 (I relapse) 1.1 (II relapse) 0.27 (III relapse) 0.14 (IV relapse)	Hardwar (Uttarakhand)
Singh *et al* 1990	995	A (1987)	900 mg over 2 days	Yes	8 months	10.3 (I relapse) 0.01 (II relapse 0.002 (III relapse)	Mandla (M.P.)
	2500	B (1988)	900 mg over 2 days	No	8 months	8.9 (I relapse) 0.01 (II relapse) 0.002 (III relapse)	
Sharma *et al* 1990	1520	A (1984-88)	600 mg over 3 days	Yes	1 year (passive)	2.6	Kheda (Gujarat)
	264	B (1988)	1500 mg over 3 days	No		18.9	
Srivastava *et al* 1996	226	A	600 mg	No	1 year	28.3	Kheda
	173	B	600 mg	Yes		5.78	(Gujarat)
	136	C	600 mg+ 50 mg Pyrimethamine	No		27.7	
Adak *et al* 1998	316		900 mg over 2 days	No	5 years	44.3	Delhi
	487				4 years	30.2	
	497				3 years	26.2	
	524				2 years	28.4	
	669				1 year	23.3	
Valecha *et al* 2001	224	A	1500 mg over 3 days	No	1 year	40.1	Delhi
	220	B	1500 mg over 3 days	Yes	1 year	29.6	
	219	C	1500 mg over 3 days	Bulaquine†	1 year	26.8	
Adak *et al* 2001	224	A	1500 mg over 3 days	No	1 year	40.1	Delhi
	220	B	1500 mg over 3 days	Yes	1 year	29.6	
	219	C	1500 mg over 3 days	Bulaquine†	1 year	26.8	
Yadav *et al* 2002	723	A	1500 mg over 3 days	No	1 year	8.6	Sundargarh (Orissa)
	759	B	1500 mg over 3 days	Yes	1 year	6.5	

*Number of patients; **Primaquine 15 mg/day x 5 days; †New 8–aminoquinoline 25 mg/day × 5 days

subpopulations. The summary of relapse pattern derived from various studies is presented in Fig. 14.

Data from a double-blind randomized clinical drug trial were analysed to find the comparative responses of two antirelapse drugs, bulaquine and primaquine, against different forms of *P. vivax*. A one year follow-up study strongly suggested that the duration of pre-erythrocytic development of *P. vivax* is a polymorphic character, exhibited by two strains of hypnozoites responsible for early and late manifestations after the primary infection. Short-term relapses were significantly higher in the first half of the year than long-term relapses, and the reverse was true in the second half of the year. Clinical drug response data showed that the hypnozoites characterized for short-term relapse were not susceptible to either of the antirelapse drugs in the currently administered dose, whereas hypnozoites characterized for long incubation were significantly susceptible. However, there is no parasitological and clinical marker available at present which could be used to analyze the genetic diversity of the *P. vivax* population and correlate this with epidemiological finding. Therefore, there is a strong need for laboratory and field studies as well as the use of mathematical models to interpret the complex transmission dynamics of *P. vivax* so that appropriate control strategies, including chemotherapeutic measures can be devised. ❑

Drug Resistance

Monitoring Antimalarial Drug Resistance

In order to understand genetic composition of the isolates in respect to commonly used antimalarials, isolates from different geographical regions were tested for their drug response either by *in vitro* or *in vivo* assay. Status of drug sensitivity in malaria parasites is important to plan and identify appropriate chemotherapeutic regimens and drugs.

In vitro Studies

The drug sensitivity can be determined *in vitro* in *P. falciparum* culture by using standard 96 well microtitre plates, predosed or prepared with several dilutions of the test drug. The sensitivity of parasite to drug is assessed by schizont maturation inhibition of parasites over 24–48 h. At NIMR *P. falciparum* isolates from different parts of the country were tested to ascertain resistance to chloroquine (Fig. 15).

In vivo Studies

The standard WHO *in vivo* 28-day test system was originally developed for chloroquine and now is extended to other drugs with appropriate changes in the number of days for other drugs. In their performance, these tests follow set criteria for the administration of a standard treatment regimen of the appropriate drug, and daily parasitological blood examination for the stipulated period. As 28-day WHO *in vivo* method is time consuming and requires daily follow-up during the first week of treatment and also patients have to wait for at least seven days before starting the alternate treatment in resistant cases, a simplified *in vivo* 7-day test was validated by the Institute (Prasad *et al* 1990). Test requires blood examination on Day 0, 2 and 7 and infection can be declared resistant as early as on 2nd day of drug administration. Now this test is being used routinely in field studies. Both 28- and 7-day tests were conducted by Assam, Hardwar (Uttarakhand), Orissa, Car Nicobar, Madhya Pradesh field units of NIMR (Chaudhury *et al* 1987; Ghosh *et al* 1992; Dua *et al* 1993; Giri *et al* 1994; Singh *et al* 1989, 1995; Dua *et al* 2000). Variable response from RI to RIII was observed among isolates assayed from different areas. It may be mentioned that in Mathura (U.P.), during routine treatment, one case showed tolerance to chloroquine (Dua *et al* 1996).

Therapeutic Efficacy Studies

The standard *in vivo* tests consider mainly the parasitological responses for assessment. Thus, to take into consideration the clinical response as well, a simplified test system where the number of parasitological observations were reduced and complemented by standardized clinical observations has been introduced by the WHO. These studies

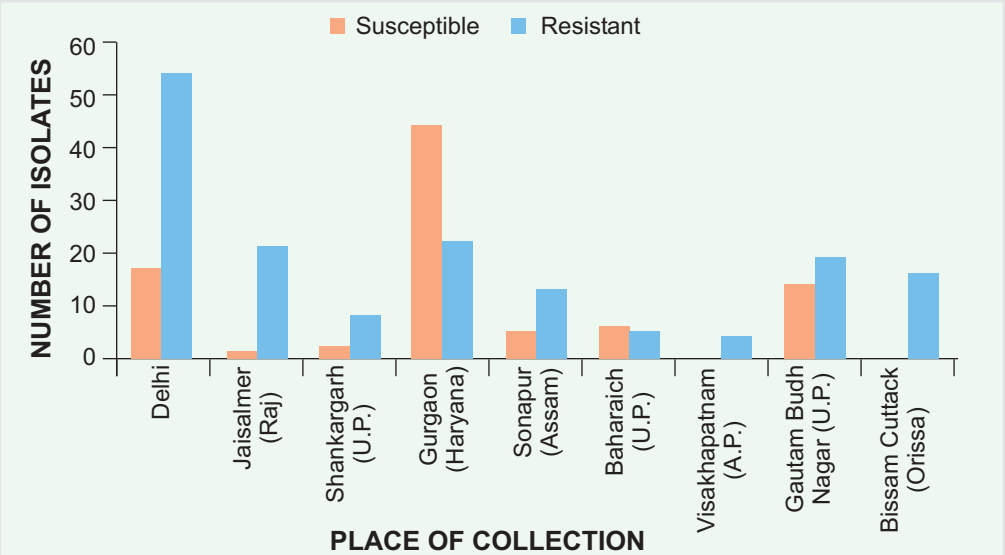


Fig. 15: Chloroquine sensitivity status of *P. falciparum* isolates *in vitro* (1992–2001)

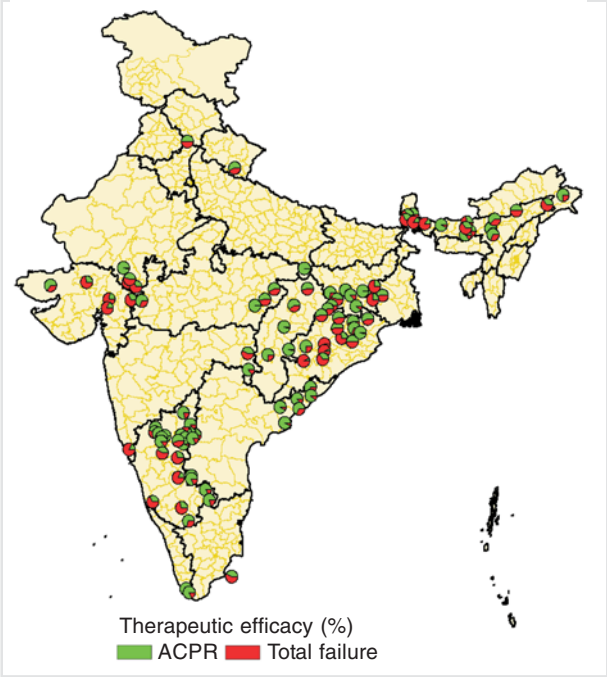


Fig. 16: Sites where antimalarial drug resistance monitoring conducted by NIMR

have been initiated by NIMR using the new WHO protocols (Fig. 16). Therapeutic efficacy of chloroquine in *P. vivax* and *P. falciparum* malaria was monitored at different sites in the country. Almost all the studies have shown high treatment failure to chloroquine and accordingly drug policy has been revised for *P. falciparum*. ACT has been recommended in about 200 districts. In addition, efficacy of ACT (AS + SP) will also be monitored.

Mechanism of Drug Resistance

Molecular Mechanism of Chloroquine (CQ) Resistance in *Plasmodium falciparum* Isolates

Polymerase chain reaction (PCR) polymorphism of 3' untranslated region of *Pfmdr 1* gene and mutational changes at nucleotide positions 754, 1049, 3598, 3622 and 4234 in the structural gene were attributed to CQ resistance. Our study revealed that PCR polymorphism of 3' untranslated region is not associated with CQ resistance. About 18 CQ sensitive and 22 CQ resistant isolates were studied to determine mutational pattern in Indian isolates. Analysis of mutations among CQ resistant isolates revealed mutations at 3 or more positions except for one isolate in which mutations at two positions were observed. In two of the isolates mutations were found at all five positions. In CQ sensitive isolates nucleotide changes were totally absent at four positions,1049, 3598, 3622 and 4234, while at 754 position mutation was present in five isolates only (Table 12). From this study, it can be said that among Indian *P. falciparum* isolates, CQ resistance is conferred by mutations at three or more nucleotide positions (mentioned above), indicating a strong association but incomplete correlation between mutational changes and chloroquine resistance. Analysis of more samples from different geographical regions may confirm the findings of the study (Bhattacharya *et al* 1999).

Biochemical Characterization of Chloroquine Resistance

Protein kinase C (PKC), a Ca++ and phospholipid dependent protein kinase which has a central role in the regulation of parasite growth, maturation and differentiation functions has been characterized from the trophozoite stage forms of the malarial parasite *P. falciparum*. PKC activity was found to be distributed in all the stages of the *P. falciparum* maturation. Activation of cytosolic PKC required Ca++, PSx and either diacylglycerol or phorbol esters (PMA). A nine fold increase in the activity was observed in schizonts as compared to the ring stage of the malaria parasite. Activation of the trophozoites with PMA resulted in the translocation of the PKC activity from cytosol to the membrane fractions. Our results showed that chloroquine (CQ) an antimalarial drug, directly inhibited the PKC activity in a dose dependent manner with an IC_{50} of 45 nM in trophozoites of chloroquine sensitive CQ(S) strains of the parasite whereas the activity was found to remain unaltered in the chloroquine resistant CQ(R) strain. Kinetic studies with Lineweaver-Burk double reciprocal plot showed that the inhibition of cytosolic PKC activity by CQ was noncompetitive with respect to ATP, histone and Phosphatidyl serine(PS). Above results indicated that PKC activity is developmentally

Table 12. Analysis of point mutations observed in *Pfmdr* gene of *P. falciparum* isolates

Nucleotide positions	CQ sensitive isolates		CQ resistant isolates											
	(13)	(5)	(1)	(3)	(1)	(1)	(3)	(1)	(3)	(2)	(2)	(2)	(1)	(2)
754	–	+	–	+	+	+	+	+	–	+	+	+	–	+
1049	–	–	+	–	+	+	–	+	+	+	–	+	+	+
3598	–	–	–	+	–	+	+	–	+	–	+	+	+	+
3622	–	–	+	–	+	–	+	–	–	+	+	+	+	+
4234	–	–	–	+	–	–	–	+	+	+	+	–	+	+

Figures in parentheses are number of isolates; with mutation (+) or absence (–).

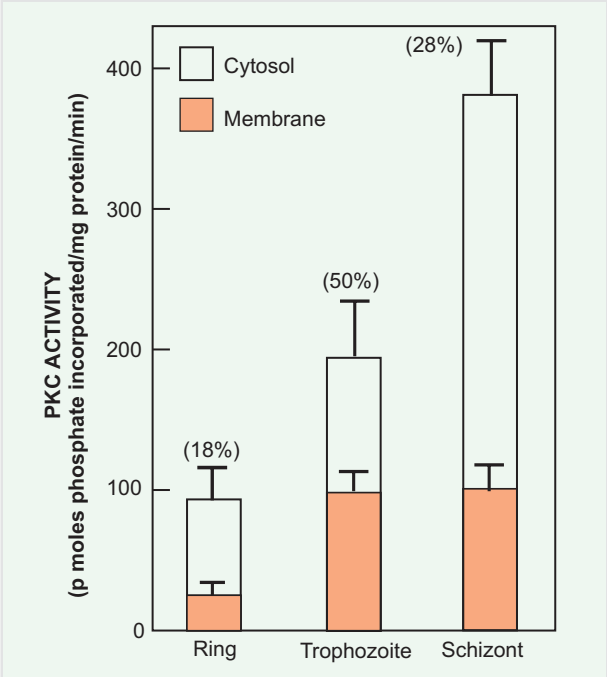


Fig. 17: PKC activity in the developmental stages of *P. falciparum* — (Solid bar–pellet; and Open bar–cytosol). Values in parentheses indicate percent of PKC activity in the membrane fraction of each parasite stage

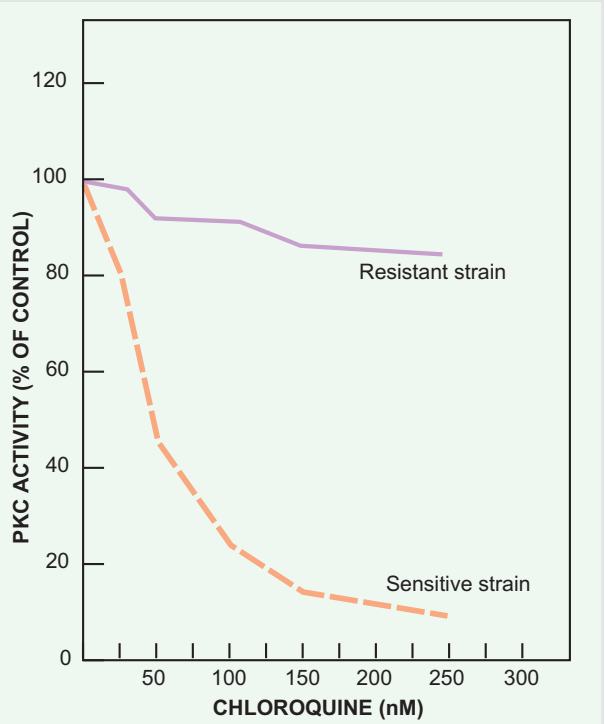


Fig. 18: Inhibition of PKC by CQ in cytosol of CQS and CQR strains of *P. falciparum* trophozoites. Each point is the average of triplicate determinations

expressed during parasite development and its inhibition by antimalarial drug chloroquine in the CQ(S) strain and no inhibition in CQ(R) strains (Figs. 17 and 18) may provide new insights into the possible explanation for the mechanism of action of CQ and development of resistance.

Protein tyrosine kinases (PTKs) are the principal signal enzymes enabling cell to cell communication, growth regulation and differentiation. PTKs are therefore potentially important drug targets due to their role as positive regulators of cell proliferation. Growing resistance of *P. falciparum* to chloroquine and other antimalarials has led to the search of other antimalarials including natural plant products. In this context, a search for naturally occurring plant products with inhibitory activity towards PTK could yield new antimalarials for the study of protein phosphorylation leading to new drug designs and more potent inhibitors. We have reported that one such compound piceatannol, an antileukemic principle in the seeds of *Euphoria lagascae*, has been found to be inhibitory to *P. falciparum* protein tyrosine kinase during the asexual maturation of the parasites in both CQ (S) and CQ (R) *in vitro* (Table 13). The results suggest that the PTK activity may be of use as chemotherapeutic drug target and new drug development in *P. falciparum* resistant malaria (Mishra *et al* 1999; Sharma *et al* 1999).

Detection of Mutations in Dihydrofolate Reductase (DHFR) and Dihydropteroate Synthetase (DHPS) Genes Associated with Resistance to Sulfadoxine-pyrimethamine in *Plasmodium falciparum*

The antimalarial compound sulfadoxine-pyrimethamine in combination is the drug of choice

Table 13. Distribution and inhibition of cytosolic protein tyrosine kinase activity in developmental stages of *P. falciparum*

Stages	Protein tyrosine kinase activity (p moles phosphate incorporated/mg protein/min)					
	Chloroquine sensitive strain (FJB-D9)			Chloroquine resistant strain (FJB-D4)		
	Status	Chloroquine (0.1 mM)	Piceatannol (0.25 mM)	Status	Chloroquine (0.1 mM)	Piceatannol (0.25 mM)
Ring	4.4 ± 0.4	3.6 ± 0.3	3.9 ± 0.3	4.3 ± 0.4	3.9 ± 0.4	3.4 ± 0.2
Trophozoite	6.1 ± 0.5*	3.7 ± 0.4	3.8 ± 0.2	5.6 ± 0.4*	5.6 ± 0.5	3.8 ± 0.2
Schizont	6.6 ± 0.5*	4.0 ± 0.3	4.1 ± 0.3	7.2 ± 0.5*	5.9 ± 0.4	4.0 ± 0.3
Merozoite	4.3 ± 0.3	2.7 ± 0.2	2.7 ± 0.2	5.0 ± 0.4	4.7 ± 0.5	2.5 ± 0.1

*$p < 0.05$; Values are for triplicate assays for five independent culture experiments and are expressed as mean ± SEM.

in patients suffering from *P. falciparum* malaria and fail to respond to chloroquine. They target the activities of folate biosynthetic pathways inhibiting DHFR and DHPS enzymes. A remarkable feature of *Pf*-DHFR and DHPS mutations is that the specific codon sequences involve changes at a number of sites. Pyrimethamine resistant parasites show mutations at codon sequences involving the changes—Ser 108–Asn 108/Thr 108; Ala 16 –Val 16; Ile 164 –Leu 164 and Cys 59 –Arg 59. In case of sulfadoxine resistant strains, mutations are observed in the DHPS domain altering Ser 436 –Phe 436; Ala 613 –Ser or Thr 613 and Ala 581 –Gly 581. Based on the mutations observed in the resistant strains PCR based studies have been designed to examine mutations in DHFR and DHPS genes in natural *P. falciparum* isolates collected from various parts of India. The results showed presence of 81% DHFR mutant type Asn 108 and only 6% DHPS mutant type Phe 436 and Ser 613. Though, the treatment failure against this combination drug has been reported from highly endemic areas of India, conditions are not yet so acute like in Thailand (Biswas *et al* 2000, 2001). ❑

Parasite Evolutionary Genomics

Malaria Parasite Genomics and Evolution of Drug Resistance

Development of Nuclear DNA Markers for Evolutionary Studies in *Plasmodium falciparum*

Recent researches in evolutionary genetics have revealed that estimation of genetic diversity is strongly dependent on the genetic markers used, thus making appropriate evolutionary inference at species and gene levels difficult. Considering these facts, we have used published whole-genome sequence information to develop nuclear DNA markers in the human malaria parasite, *P. falciparum* that would help in understanding the precise roles of demography and natural selection in the evolution of *P. falciparum*. For designing the putatively neutral DNA fragments (that bear no or very weak signals of past selection events) which would help in deciphering the demographic history of *P. falciparum*, we scanned the whole genome of *P. falciparum* available in the public domain and isolated introns in every kind of gene (known, putative and hypothetical). Specifically, we considered introns of 450–850 bp long and designed primers in the exons flanking these introns (exon priming intron crossing, or EPIC fragments). We could only predict 170 introns that could be considered as partially putatively neutral (Fig. 19). Further, we have divided the whole *pfcrt* gene into three different fragments for amplification by PCR and five internal fragments (within the three main fragments) for sequencing purposes. The development of nuclear DNA markers has far-reaching significance in malaria research.

Comparative Evolutionary Genetic Insights into *Plasmodium falciparum* Functional Genes with Reference to *P. vivax* genome

Complex and rapidly evolving behaviours of the two human malaria parasites, *P. falciparum* and *P. vivax* have always been mysterious to the evolutionary biologists as the former is the most virulent and the later is most prevalent malaria parasite species across the globe. With the availability of whole genome sequence data, it is now feasible to pinpoint genomic similarities and differences between the parasites with the comparative evolutionary genetic approaches, and thus, define new measures for malaria control. We herewith utilized available genome information of these two species and compared functional genes of *P.*

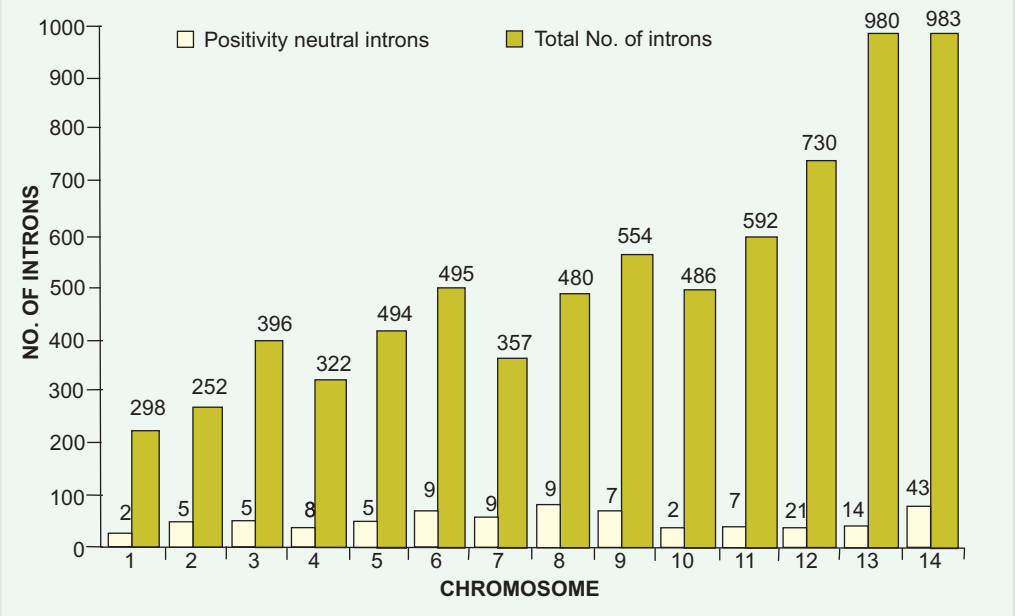


Fig. 19: Distribution of total number of introns and selected putatively neutral introns in *Plasmodium falciparum* chromosomes

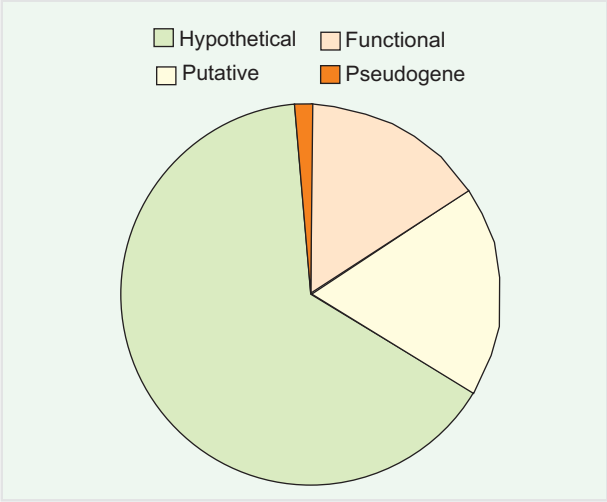


Fig. 20: Total genes in *Plasmodium falciparum* genome. Note that the putative and functional categories (together considered as functional) of genes have been utilized in the present study

falciparum with partially-assembled whole genome sequences of *P. vivax*. About 82% of total functional genes of *P. falciparum* were found to be conserved in *P. vivax* and rest 18% to be unique to *P. falciparum*. Although both types of genes were distributed across all 14 chromosomes of *P. falciparum*, the distribution was slightly biased towards two separate chromosomes for each category (Fig. 20). About a half of the conserved genes was intron-less, whereas almost all unique genes have introns. However, number of introns was comparatively higher (usually >2) in the intron-possessing conserved genes than in the unique genes (mostly <2). Statistically significant positive correlations between total intron length and gene lengths were detected in 11 chromosomes for unique genes, whereas only in three chromosomes for conserved genes. Three most conserved genes (Actin, Elongation factor alpha 1 and Ribosomal protein L 10 putative) between *P. falciparum* and *P. vivax* were found to be highly conserved in four other species of *Plasmodium* (except Actin gene in *P. chabaudi*) and were mostly intron-less. Phylogenetic trees were constructed separately for each of the three genes; in two genes (Actin and Elongation factor alpha 1) different *Plasmodium* species were placed in almost similar positions, whereas Ribosomal protein L 10 putative show different relationships between *Plasmodium* species. Three unique gene families in three *Plasmodium* species (*P. falciparum*, *P. vivax* and *P. knowlesi*) were studied in detail for total intron length and correlations between intron lengths and gene lengths, which corroborate findings on the overall patterns of whole unique genes of *P. falciparum*. The results are discussed in terms of chromosome and intron evolution in *Plasmodium* in general, relevance of introns in differential functions of *P. falciparum* genes and genetic similarities and differences between *P. falciparum* and *P. vivax* and its implications in malaria, in particular.

Fine-scale Genetic Characterization of *Plasmodium falciparum* Chromosome 7 Encompassing the antigenic *var* and the Drug-Resistant *pfcrt* Genes

The fact that malaria is still an uncontrolled disease is reflected by the genetic organization of the parasite genome. Efforts to curb malaria should begin with proper understanding of the mechanism by which the parasites evade human immune system and evolve resistance to different antimalarial drugs.

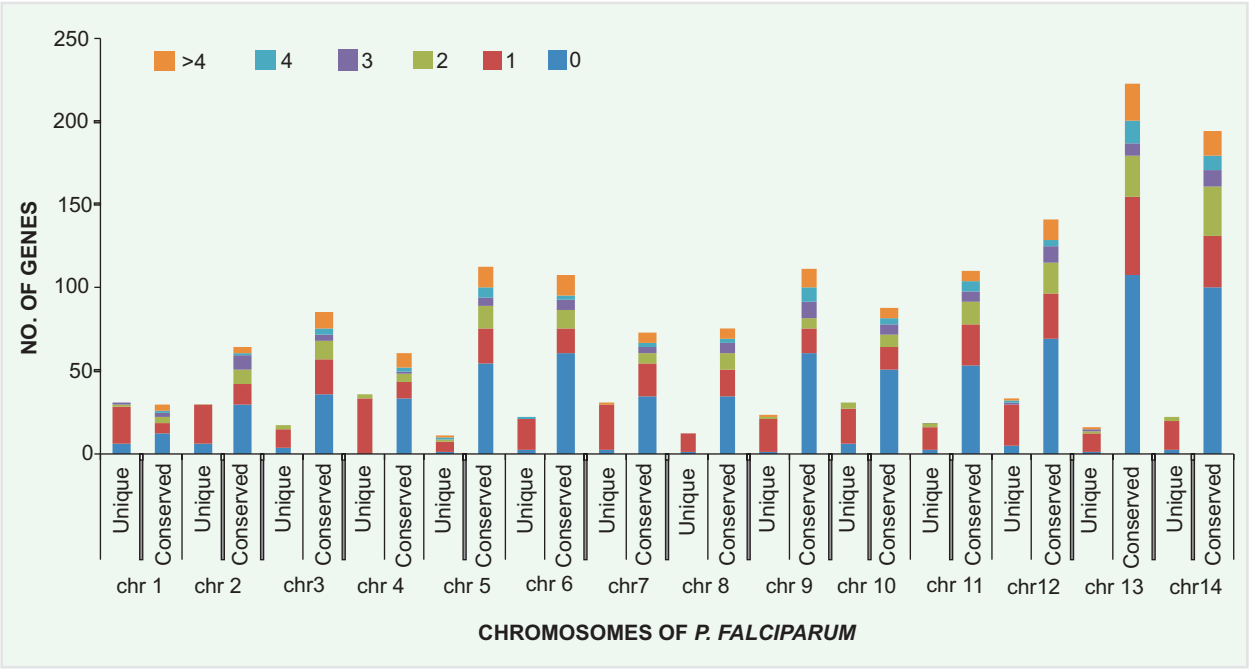


Fig. 21: Distribution of genes with different intron numbers in conserved and unique genes in chromosomes of *Plasmodium falciparum*

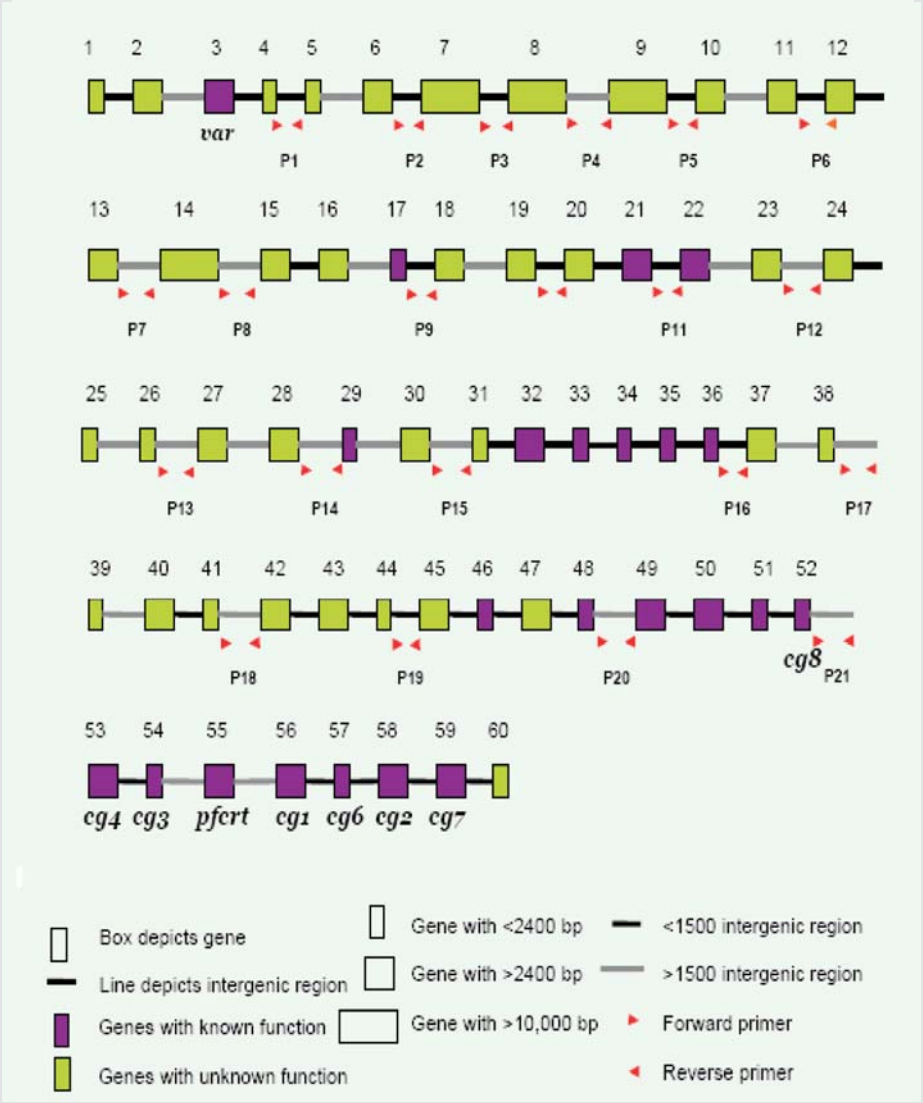


Fig. 22: Fine details of the specified region showing the genes, intergenic regions and locations of the primers designed for amplification of different regions

We have initiated such a study and presented here with the results from the *in silico* understanding of a seventh chromosomal region of the malaria parasite, *P. falciparum* encompassing the antigenic *var* genes (coding *pfemp1*) and the drug-resistant gene *pfcrt* located at a specified region of the chromosome 7. We found 60 genes of various functions and lengths, majority (61.67%) of them were performing known functions. Almost all the genes have orthologs in other four species of *Plasmodium*, of which *P. chabaudi* seems to be the closest to *P. falciparum*. However, only two genes were found to be paralogous. Interestingly, the drug-resistant gene, *pfcrt* was found to be surrounded by seven genes coding for several CG proteins out of which six were reported to be responsible for providing drug resistance to *P. vivax*. The intergenic regions, in this specified region were generally large in size, majority (73%) of them were of more than 500 nucleotide bp length. We also designed primers for amplification of 21 non-coding DNA fragments in the whole region for estimating genetic diversity and inferring the evolutionary history of this region of *P. falciparum* genome. (Figs. 21 and 22).

Evolutionary Paradigm of Chloroquine-Resistant Malaria in India

Drug pressure in the field is believed to be responsible for the emergence of drug-resistant *P. falciparum*. Variants of the *P. falciparum* chloroquine resistance transporter (*pfcrt*) gene have been shown to be responsible for conferring resistance to the commonly used drug chloroquine. In particular, an amino acid mutation, K76T, was shown to have a strong positive correlation with the chloroquine resistant varieties of malaria parasites. Global studies have reported highly reduced genetic diversity surrounding K76T in the pfcrt gene, which indicates that the mutation has been a target of positive Darwinian natural selection. However, two recent studies of *P. falciparum* in India found high genetic diversity in the *pfcrt* gene, which, at first sight, do not support the role of natural selection in the evolution of chloroquine resistance in India (Fig. 23).

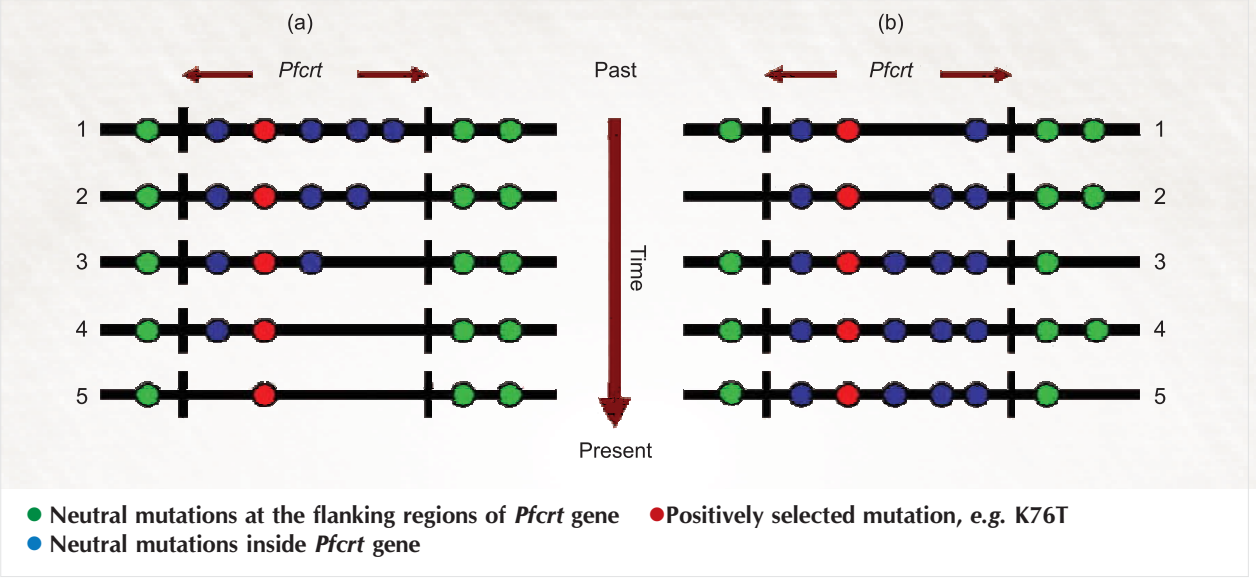


Fig. 23: Evolutionary pattern of the *Pfcrt* gene in chloroquine-resistant *Plasmodium falciparum* populations. (a) Over generations and drug selection pressure, the positively selected mutation (e.g. K76T: red circles) is swept away by natural selection along with the linked neutral genetic variations (blue circles) (1–5). However, variations that are distant from the selected mutation are unaffected by the selective sweep. Thus, neutral variations in the flanking regions of *Pfcrt* (green circles) are maintained (5). (b) An opposite evolutionary trend in which neutral mutations progressively accumulate over time (1–5) in *Pfcrt*. This is especially true in and around the positively selected mutation (*e.g.* K76T) (5).

Human Immunogenic Response to Malaria

Evolutionary Insights into Duffy Gene in Mammalian Taxa with Comparative Genetic Analysis

Evolutionary analyses of genes conserved across taxa are keys to understand the complexity of gene and genome variation. Considering malaria as a devastating infectious disease to humans, and host-parasite interaction mechanism is quite complex, detail evolutionary understanding on human gene responsible for parasite recognition and invasion of human system is the first step to understand the complexity of such interactions. The human duffy gene which is a erythrocyte chemokine receptor has been characterized in detail in this study and compared with eight other different mammalian taxa (*Pan troglodytes, Macaca mulatta, Pongo pygmaeus, Rattus norvegicus, Mus musculus, Monodelphis domestica, Bos taurus* and *Canis familiaris)*. While the genetic architechture of this gene was entirely different across all the nine taxa, a close similarity between human and chimpanzee was evident for several aspects of this gene. Comparisons on other aspects such as ratio of coding and non-coding regions, total gene length number and size of introns and difference of number of nucleotides in human and chimpanzees were also done. Phylogenetic trees were constructed on the basis of exon sizes and in total gene sizes. Most remarkably, human and chimpanzee were only 0.75% different in this gene. The results were discussed on the similarities between human and chimpanzee and gain of introns in human-chimpanzee clade with an inference on the role of evolutionary forces (mainly natural selection) in maintaining such variations across closely-related mammalian taxa. (Fig. 24).

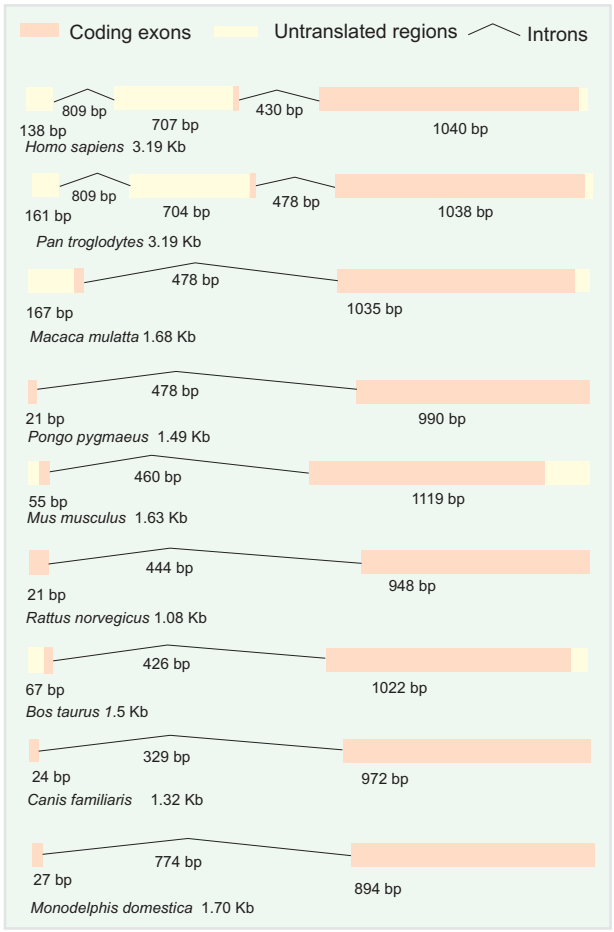


Fig. 24: Detailed characterization of the duffy gene across nine mammalian taxa

Genetic Characterization and Evolutionary Inference of TNF-α with Computational Analyses

TNF-α is an important human cytokine that imparts dualism in malaria pathogenecity. At high dosages TNF-α is believed to exhibit pathogenecity against cerebral malaria and at lower dosages TNF-α is protective against severe human malaria. In order to understand the human TNF-α gene closely and to ascertain evolutionary aspect of its dualistic nature on malaria pathogenecity, we first characterized this gene in detail in six different mammalian taxa. The avian taxa, *Gallus gallus* was included in the present study, as TNF-α is not present in birds, therefore, a tandemly placed duplicate of TNF-α (LT-α or TNF-α) was included in this study (Fig. 25). Comparative study was performed on nucleotide length variation, intron and exon size, number variation, differential compositions of coding to the non-coding bases, *etc.* to look for similarities/dissimilarities at the TNF-α gene across all seven taxa. The phylogenetic study revealed the pattern found in other genes, as human, chimpanzee and rhesus monkey were placed in a single clade and rat and mouse in another, with the *G. gallus* in a clearly separate branch. We further focused these three taxa and aligned the amino acid sequences and found less differences between human and chimpanzee but great differences in rhesus monkey from the other two

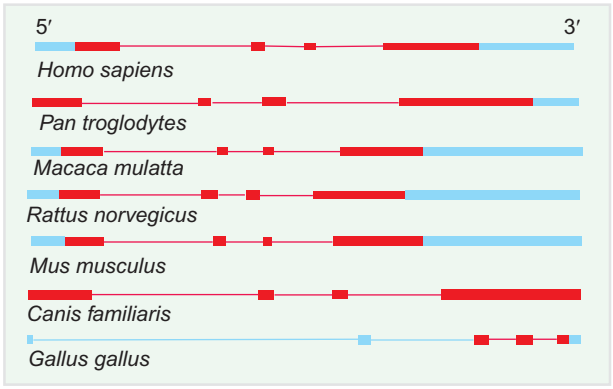


Fig. 25: Fine-scale characterization of TNF-α gene among six mammalian taxa with coding exons (red) and un-translated region (UTR) or non-coding exons (blue). For *G. gallus* information on the TNF-β has been provided. The length of non-coding exons, coding exons and introns are not in scale

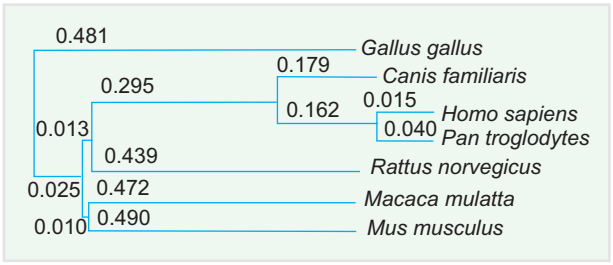


Fig. 26: Phylogenetic positions of seven different mammalian taxa at the human CD36 gene

taxa (Fig. 25). Further, comparison of coding and non-coding nucleotide length variations and coding to non-coding nucleotide ratio between TNF-α and TNF-β among these three mammalian taxa provided a first-hand indication on the role of TNF-α gene, not its duplicate TNF-β in dualistic nature of TNF-α in malaria pathogenecity.

Characterization and Comparative Analysis of Human CD36 Gene

Characterization and comparative analysis of genes of essential functions help understanding the detail composition and evolutionary pathways that the genes have passed through time. With the advent of computational biological tools and availability of whole genome sequences of different organisms, it is now possible to deeply understand the detail characteristics of such genes. We followed such approaches and characterized a human immune-system gene (CD36) responsible for malaria pathogenesis and compared with homologous genes of other six (five mammalian and one avian) taxa. We also studied distribution of CpG Islands across different types of introns (first, small and large) of the CD36 gene separately in each taxa and detected differential distribution patterns. Further, considering all the seven taxa, we constructed a phylogenetic tree on the basis of DNA sequence information of CD36 gene. Number of different copies of this gene was also determined in genome of each taxa and wide variations in copy number across taxa were detected. The detailed study on a human immune-system gene of high importance to malaria pathogenecity provides important information and paves new ways for further research on this gene (Fig. 26).

❑

Screening of Natural/Synthetic Compounds for Antimalarial Activity

Plants have been used as a traditional medicine for the treatment of malaria. Plants may provide drugs directly such as quinine from cinchona bark or they may provide template molecules on which to base further new structures by organic synthesis—artemisinin from *Artemisia annua*. At the NIMR, efforts are being made to do primary screening of crude extracts of plant products to screen different fractions isolated from various parts of plants and to isolate pure compounds having antimalarial properties.

Primary Screening of Plant Products

Aqueous extracts of *Azadirachta indica* (bark), *Phyllanthus niruri* (whole plant) and *Ocimum sanctum* (leaves) were tested *in vivo* against *P. berghei* following Peter's 4-day test. Antimalarial effect of three medicinal plants tested is shown in Fig. 27 (Usha Devi *et al* 2001).

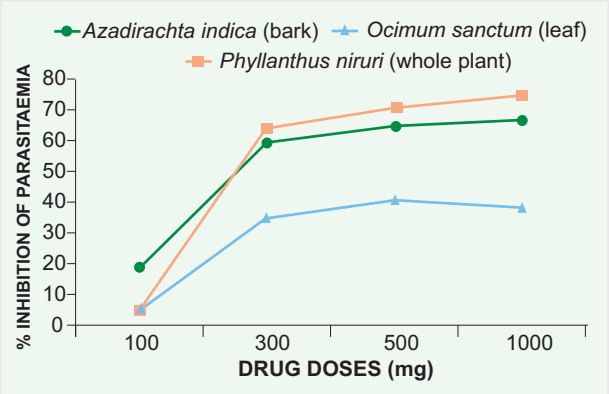


Fig. 27: *In vivo* antimalarial effect of three medicinal plants (aqueous extracts)

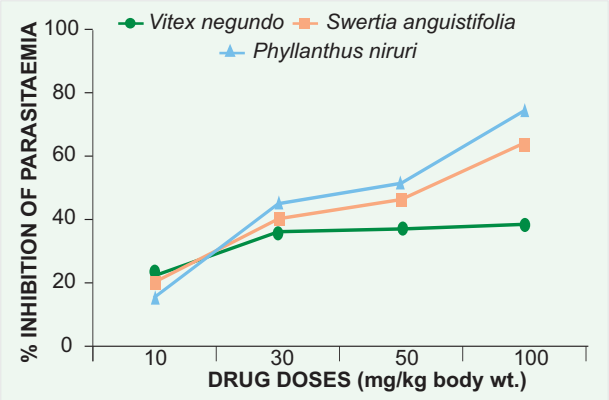


Fig. 28: *In vivo* antimalarial effect of three medicinal plants (50% ethanol extracts)

Ethanol extract (50%) of nine medicinal plants were tested *in vitro* for their antimalarial activities using CQ sensitive isolate. IC_{50} values ranged from 0.3–70.0 µg/ml. Some of these extracts, showing encouraging results with *in vitro* system, had also been tested *in vivo* against *P. berghei* following Peter's 4-day test. The study showed that, *Phyllanthus niruri* and *Swertia anguistifolia* plants have good antimalarial properties whereas *Vitex negundo* had less effect (Fig. 28).

Screening of Fractions

Andrographis paniculata

Andrographis paniculata is widely used as a folk medicine in China and southeast Asia. Leaves of *Andrographis paniculata* (local name *Bhuineem*) has been extensively used as a traditional medicine for the treatment of symptomatic malaria by the tribal population of Bastar district, Madhya Pradesh, India (Dua *et al* 1999). Therefore, a study was undertaken to investigate the antimalarial activity of this plant. The roots from the dried plants (Source: Gurukul University, Hardwar) were separated, washed with distilled water, dried under shade and solvent partitioned with four different polarity solvents—petroleum ether, methanol, chloroform and water using soxhlet apparatus. The four fractions so obtained, namely AG-1, AG-2 AG-3 and AG-4 were screened *in vitro* for schizontocidal activity (Fig. 29). Since AG-3 possessed promising antimalarial activity, it was selected for further studies.

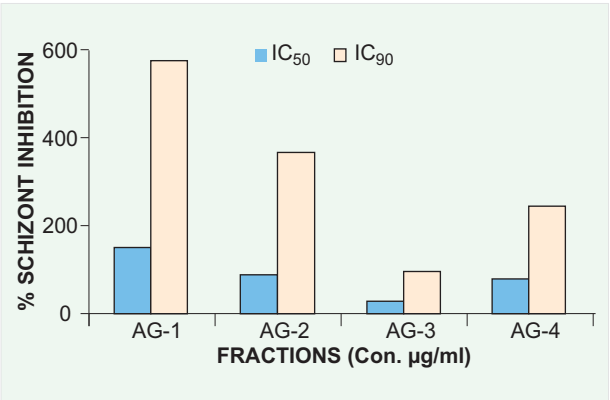
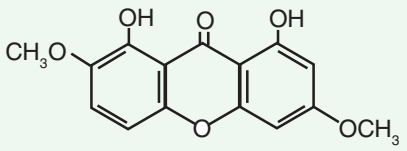
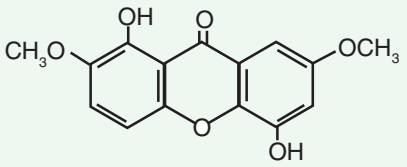


Fig. 29: *In vitro* schizontocidal activities of some fractions isolated from the roots of *Andrographis paniculata*

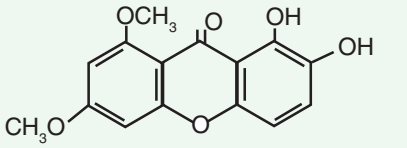
Silica gel column chromatography of fraction AG-3 eluted with chloroform resulted three distinct colour bands—yellow, greenish-yellow and pale-yellow. Two compounds with Rf values 0.7 (TDR 13008) and 0.45 (TDR 13009) were isolated from yellow coloured band by preparative TLC using benzene as mobile phase. TLC of greenish-yellow band gave four distinct spots with benzene-methanol (98:2, v/v). Compounds with Rf values 0.52 (TDR 13013) and Rf O (TDR 13011) were isolated by preparative TLC. Pale-yellow coloured band resulted four distinct spots and compound with Rf value 0.30 (TDR 130012) was isolated using benzene-methanol (95:5, v/v) by preparative TLC. Out of six compounds isolated by preparative TLC, the structure of four were determined by spectroscopic methods (Fig. 30).



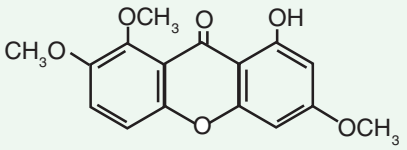
1,8-dihydroxy 3,7-dimethoxy-xanthene-9-one (TDR 13008)



4,8-dihydroxy-2,7-dimethoxy-xanthene-9-one (TDR 13009)



1,2-dihydroxy-6-8-dimethoxy- xanthene-9-one (TDR130011)



3,7,8-trimethoxy-1-hydroxy-xanthene-9-one (TDR 130013)

Fig. 30: Structures of compounds isolated from the roots of *Andrographis paniculata*

In vitro antimalarial studies showed compound TDR 13011 with maximum schizontocidal activity as compared to other compounds. However, it has exhibited moderate activity with IC50 value of 4 µg/ml-1 which has been much lower than chloroquine. Compound TDR 13011 was further assessed for its antimalarial properties *in vivo* against *P. berghei* infected mice. Results revealed that compound TDR 13011 gave substantial reduction (70%) in parasitaemia after treating animals with an intravenous dose of 30 mg/kg. Cytotoxic activity was done by WHO on MRC–5 (human lung fibroblast) showed the compound TDR 13008 to be cytotoxic with IC_{50} value 24 µg/ml-1 while all other compounds had IC_{50} values >32 µg/ml-1, indicating non-cytotoxic behaviour of TDR 13008. Our study clearly revealed that 1,2-dihydroxy-6,8 dimethoxy, xanthene-9-one isolated from the roots of *Andrographis paniculata* possessed antimalarial activity without cytotoxicity (Dua *et al* 1999).

Azadirachta indica A. Juss

Azadirachta indica A. Juss (neem) is known for its medicinal and insecticidal properties. Eight fractions from *Azadirachta indica* seeds were isolated using solvent partition and column chromatography and tested their antimalarial activity against *P. falciparum* in *in vitro* culture. Out of three fractions from seed cake, two fractions, code A-1 and A-2 showed significant activity with IC_{50} values of 4.8 and 5.0 µg/ml respectively. Similarly out of five fractions from *Azadirachta indica* oil, two fractions, code A-5 and A-6 had high antimalarial activities with their IC_{50} values of 2.25 and 2.30 µg/ml respectively while fraction code A-8 showed no antimalarial activity (Fig. 31).

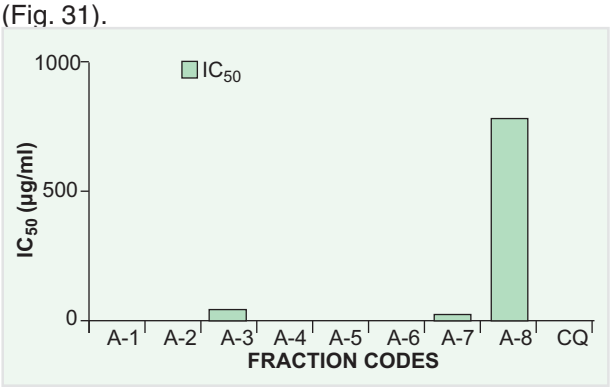


Fig. 31: *In vitro* antiplasmodial activity of *Azadirachta indica* fractions

Isolation and Testing Antimalarial Activity of Peroxydisulfate Oxidation Products of Primaquine

Primaquine, an 8-aminoquinoline, is the clinical drug of choice for the radical cure of relapsing malaria. However, its usefulness has been restricted by toxic side-effects, especially with patients deficient in glucose-6-phosphate dehydrogenase. We have isolated five compounds formed by the peroxydisulfate oxidation of primaquine using chromatographic methods and tested for antimalarial activity. *In vitro* gametocytocidal studies showed that two compounds have more gametocytocidal activity than primaquine, while *in vivo* results indicated only one compound with gametocytocidal activity against *P. yoelii* infected mice (Dua *et al* 2002).

Primaquine, on oxidation with peroxydisulfate ion in neutral medium gave pale-yellow to orange, violet and then yellow colour within one hour after initiation of reaction. Five compounds were isolated in >90% purity using Bio-Gel P-2 column chromatography and

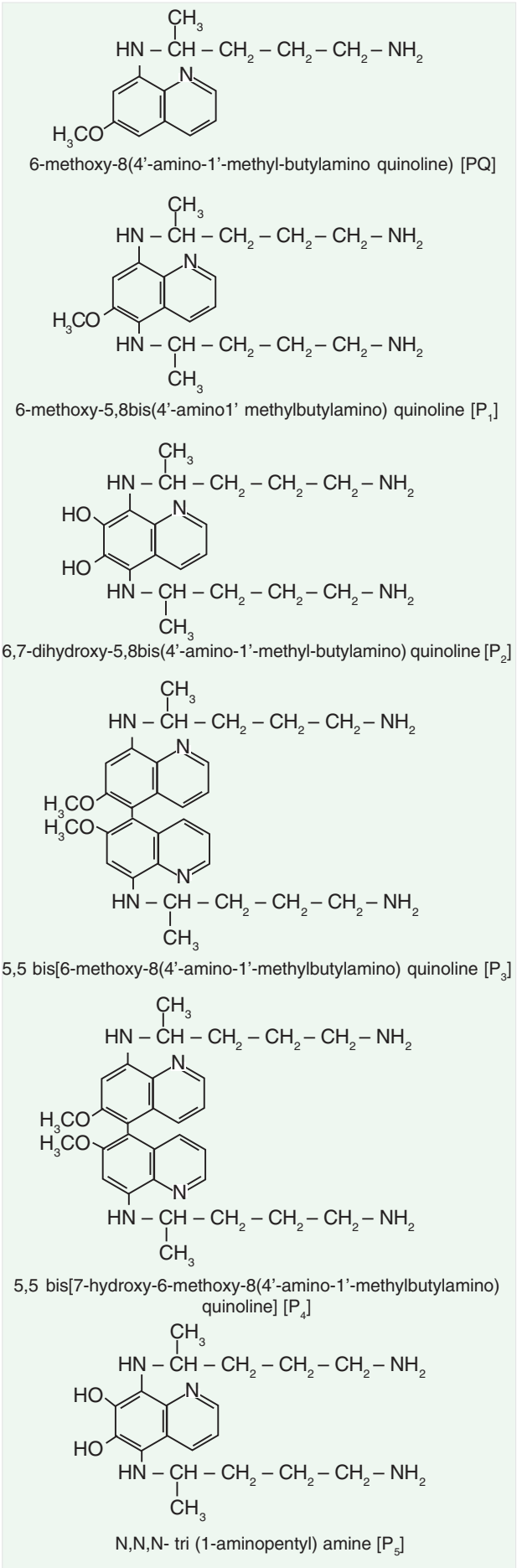


Fig. 32: Structure of oxidation products of primaquine

HPLC from the reaction mixture. The structures of all compounds were determined using IR, MS and 1H NMR studies which are given in Fig. 32.

In vitro

Five compounds isolated from the oxidation of primaquine were tested for their *in vitro* schizontocidal and gametocytocidal activities at different concentrations. Compounds P_1 and P_2 showed higher gametocytocidal activity than primaquine, while the compounds P_3, P_4 and P_5 had lower activity than primaquine or no gametocytocidal effects. The IC_{50} and IC_{90} of compound P_1 were 0.026 and 0.055 mg/well respectively, while of compound P_2 were 0.036 and 0.062 mg/well respectively. The schizontocidal activity of all five compounds were many fold lower than that of chloroquine. However, the schizontocytocidal activity of compounds P_1 and P_2 were more than primaquine.

In vivo

The compounds P_1 and P_2 were tested for *in vivo* gametocytocidal activity against *P. yoelii* infected mice. Compound P_1 showed good gametocytocidal activity in mice and there was no infectivity in mice after treatment with P_1 at the dose of 10 mg/kg. This was confirmed by feeding *An. stephensi* mosquitoes on *P. yoelii* infected mice before and after the treatment. Results showed that there was complete loss of infectivity in mosquitoes after treatment with compound P_1 while the infectivity was confirmed in mosquitoes fed on animals before treatment. Primaquine was taken as control compound. The compound P_2 did not possess any gametocytocidal effect against *P. yoelii* infected mice. In conclusion, compound P_1 [6-methoxy-5,8bis(4'-amino-1'-methylbutylamino) quinoline] is found to be a novel antimalarial compound with good gametocytocidal activity (Du*a et al* 2002).

Antimalarial Properties of Some Plants from Garhwal Region of North-west Himalaya

Studies were aimed to investigate the antimalarial properties of some plants from Garhwal region. Three plants were selected in consultation with Botanical Survey of India, Dehradun. Twelve fractions were isolated from these plants using solvent partition method. *In vitro* study was carried out on these fractions to investigate antiplasmodial activity and results are given in Table 14. Fractions isolated from plant code MRCHAR/04/3 possessed good anti-plasmodial activity while other two plants did not. Chromatographic methods are being developed for the isolation of pure compounds from plant code MRCHAR/04/3.

Antimalarial Properties of a Plant Code MRCHAR/03/04

Five compounds, coded as MRCHAR/03/04/1, MRCHAR/03/04/2, MRCHAR/03/04/3, MRCHAR/03/

Table 14. Antiplasmodium activity of some plant extracts from Garhwal region of North-west Himalaya

Fraction code	*P. falciparum* K1 IC_{50} µg/ml
MRC HAR/04/1/1	>5
MRC HAR/04/1/2	>5
MRC HAR/04/1/3	>5
MRC HAR/04/1/4	>5
MRC HAR/04/2/1	4.07
MRC HAR/04/2/2	3.7
MRC HAR/04/2/3	>5
MRC HAR/04/2/4	>5
MRC HAR/04/3/1	>5
MRC HAR/04/3/2	>5
MRC HAR/04/3/3	>5
MRC HAR/04/3/4	>5
Chloroquine	0.036

04/4 and MRCHAR/03/04/5 were tested for their antiplasmodial activity by *in vitro* method. Results revealed that fraction codes, MRCHAR/03/04/1 and MRCHAR/03/04/4 showed good activity with their IC_{50} values of 0.62 and 1.5 µg/ml respectively.

Apasmomycin Analogues

The antimalarial activity of 2-methylene-3-hydroxyalkyl (synthesized at IIT, Mumbai) propionic acid derivatives were evaluated and all of them displayed activity at 10–6 dose level in *in vitro P. falciparum* culture. Two compounds showed 100% schizont maturation inhibition at dose of 5 and 10 µmol/well respectively. *In vivo* studies of these derivatives in mice revealed antimalarial activity at 80 mg/kg dose level (Kundu *et al* 1999). Twelve t-butylperoxyamines were also synthesized at IIT, Mumbai and screened at NIMR. *In vivo* studies showed activity of one of derivatives at 80–160 mg/kg dose level (Sunder *et al* 2001). These synthetic compounds developed at IIT, Mumbai were screened *in vitro* and *in vivo* models and various levels of antimalarial activity were obtained (Kundu *et al* 1999; Sunder *et al* 2001).

Reversal of Chloroquine Resistance

Chloroquine has been the most effective and widely used drug in malaria therapy. Therefore, great hopes have been placed on development of agents, which can reverse resistance to chloroquine. Few such drugs, namely verapamil, cyproheptadine, ascorbic acid and few new compounds were evaluated *in vivo* in mice in combination with chloroquine using chloroquine resistant *P. berghei.* The results showed that these agents reversed resistance in animal models partially and that too when used in high doses, which may limit the clinical use of such systemically acting drugs (Valecha *et al* 1992, 1994). ❑

Epidemiology

Malaria Epidemic Investigations

A number of malaria outbreaks have occurred in the country. The Institute carried out investigations of epidemics either at the request of National Anti Malaria Programme (NAMP), State Government, Indian Council of Medical Research (ICMR) and/or on its own to find out the causes/factors responsible for the epidemic.

During 1981–82, investigations were carried out in Kharkhoda PHC of Sonepat district (Haryana) and Kichha PHC of Nainital district, Uttarakhand (erstwhile Uttar Pradesh) to assess the malaria incidence (Sharma *et al* 1983). In Gadarpur PHC of Nainital, resurgence of malaria was investigated in 1983 and slide positivity rate (SPR) was found to be 67.5 and slide falciparum rate (SFR) 71.5%. *Anopheles culicifacies* and *An. fluviatilis* mosquitoes were incriminated as vectors of malaria (Chaudhary *et al* 1983). In villages of Nigohi and Tilhar PHCs of

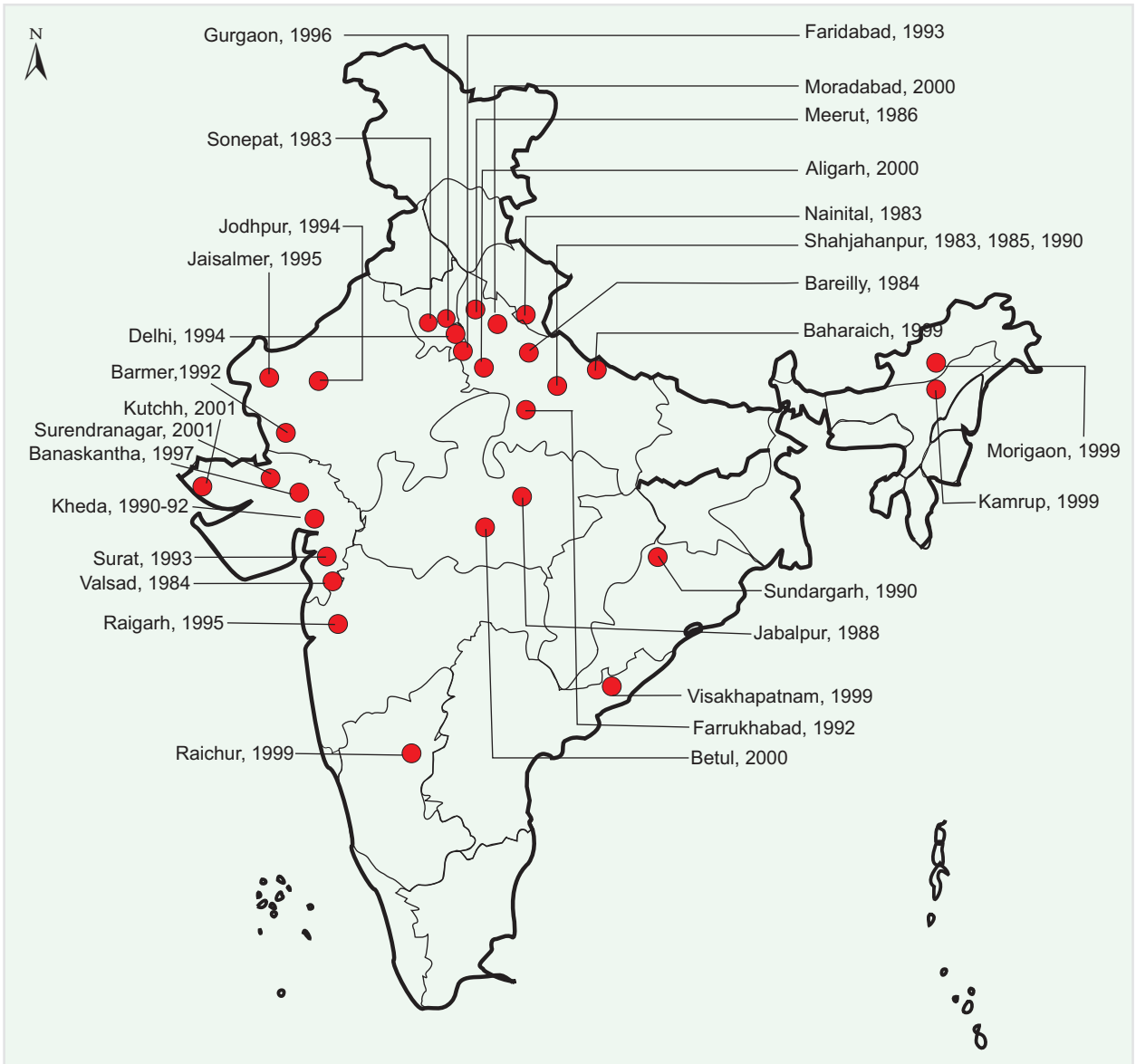


Fig. 1: Malaria epidemic investigations carried out by the National Institute of Malaria Research from 1981–2001

Table 1. Malaria epidemic investigations carried out during 1999–2001

Place and month of investigation	Findings *Recommendations
Morigaon, Golaghat (Kamrup, Assam) May 1999	Lack of surveillance. *Use of ITN and IEC activities suggested.
Paderu (Visakhapatnam, A.P.) July 1999	Lack of surveillance owing to difficult terrain, onset of early rains, detection of resistance in *An. culicifacies* to DDT, detection of resistance to chloroquine in *P. falciparum*. *Use of malathion/synthetic pyrethroids for residual spray, treatment schedule for drug resistant subjects as per NAMP policy and to explore the possibility of using ITN.
Jarwal (Baharaich, U.P.) Sep–Oct 1999	Shifting of surveillance workers resulted in low API (<2) hence, there was no spray and resistance in *P. falciparum* parasite to chloroquine. *Training of technicians, use of suitable drug for resistant cases as per NAMP policy and strengthening of surveillance also in other PHCs of the district.
Raichur (Karnataka) Nov 1999	High SPR 71.2% with *Pf* 95.1%, *An. culicifacies* resistant to DDT, *P. falciparum* parasite resistant to chloroquine. *Bio-environmental control through larvivorous fishes.
Moradabad (U.P.) (Sep–Oct 2000)	SPR 84.7; *Pf* 97%; *An. culicifacies* was resistant to DDT and susceptible to malathion and deltamethrin. *Deployment of health workers and strengthening of surveillance system were recommended.
Dadri CHC, Gautam Budhnagar (U.P.) Sep 2000	Mean SPR 44.6% (highest 52.1%); *Pf* 87.6%. *An. culicifacies* was found resistant to DDT (21.5% mortality). Insecticide spray was not done for last 10 years. *Strengthening of surveillance system, change of insecticide for IRS
Betul (Chhattisgarh) Oct–Dec 2000	35 affected villages of three PHCs were surveyed, SPR, SFR and *Pf* were 52.5, 50 and 93.3% respectively. *Synthetic pyrethroid/malathion to be sprayed in all the PHCs. Mobile health clinic/camp to be organised on a priority basis. All *P. falciparum* cases are to be treated with Fansidar (SP combination). People to be encouraged to use personal protective measures, i.e. bednets, skin repellents etc. In remote villages rapid diagnostic tests, such as OptiMAL/ICT are to be used. Pregnant women and infants are to be given chemoprophylaxis, and health education on top priority, and release of larvivorous fish in all breeding places. These recommendations were implemented by Govt. of Madhya Pradesh as a result there was over 60 and 70% reduction in malaria cases and in falciparum cases respectively in 2002. Besides, spleen rate in children declined from 72% in 2000 to 25% in 2002.
Chandausi and Iglas PHCs, Aligarh (U.P.) Nov 2000	SPR 41–73.6%; *Pf*% 94.7–100; and IRS was not done for last 10 years. *Strengthening of surveillance and IRS to be done regularly.
Surendranagar (Gujarat) 2001	Some villages were affected by the earthquake, poor surveillance, high rainfall leading to high densities of *An. culicifacies,* poor coverage of malathion spraying in some villages, high breeding of anophelines in domestic waters, susceptibility of *An. culicifacies* to malathion was 100%. Mopping up round of malathion sprayed in villages with poor spray coverage. *Since it is dry zone, use of fish in permanent waters and peridomestic breeding can be controlled in most months of the year, improvement in EDPT, training of PHC Medical Officers, detailed epidemiological study in Patadi taluka.
Kutchh (Gujarat) 2001	Increased vulnerability after earthquake due to breakdown of health services, excess rainfall, migrant labourers for reconstruction brought parasite load. *Strengthening of active and passive surveillance including role of mobile dispensaries, fogging in outbreak-affected villages, use of fish in permanent water bodies and check dams, use of rapid diagnostic kits, training of MO's, laboratory technicians, screening of labour population.

*The recommendations/suggestions were sent to the concerned state government and NAMP for necessary action.

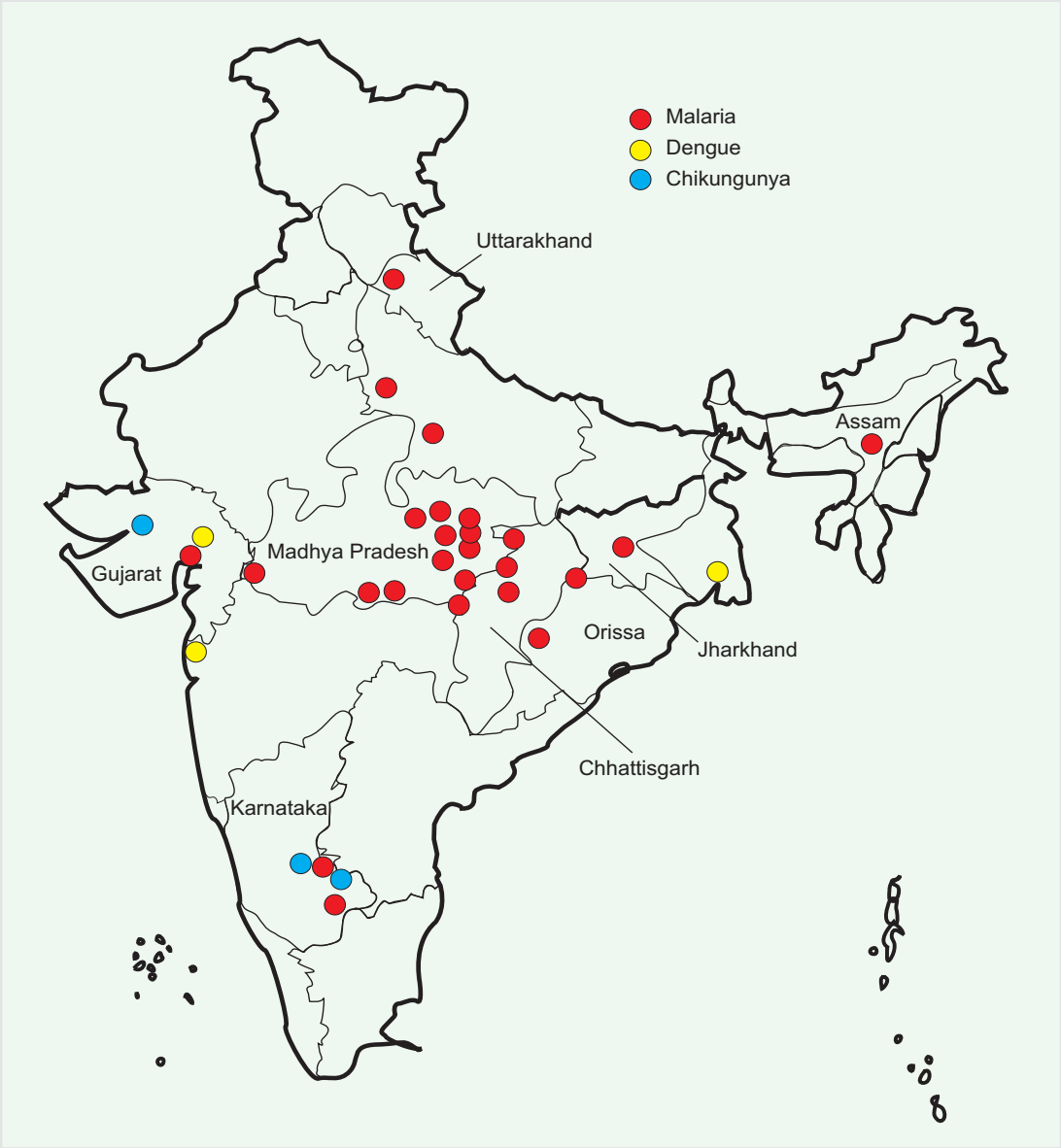


Fig. 2: Outbreaks of vector diseases investigated by NIMR from 2002–06

Shahjahanpur district, Uttar Pradesh (Chandrahas and Sharma 1983), in spite of three rounds of HCH spray and intensive efforts to control malaria, SPR was 75.3 and SFR was 95.1%. HCH was not effective against malaria vectors due to development of resistance. Remedial measures were suggested to the state government. Follow-up studies as well as subsequent epidemics of malaria in Banda PHC (Shahjahanpur) and Banyani PHC of Farrukhabad and Bareilly (Uttar Pradesh) were also investigated (Ansari *et al* 1984; Sharma *et al* 1985; Prasad *et al* 1992; Prasad and Sharma 1990). In 1986, in villages of Meerut district (Uttar Pradesh), it was found that about half of the patients were asymptomatic and HCH was found ineffective (Ansari *et al* 1986), and malathion for indoor residual spray (IRS) was recommended. Investigations were carried out in Madhya Pradesh (Singh *et al* 1988), Gujarat (Sharma and Gautam 1990; Srivastava *et al* 1995; Srivastava and Yadav 2000), Haryana (Sharma 1993; Raghavendra *et al* 1997), Rajasthan (Shukla *et al* 1995; Batra *et al* 1999) and Delhi (Adak *et al* 1994; Sharma *et al* 1985) . The locations of all study sites with years of investigations are shown in Fig. 1. Details of latest epidemic investigations carried out during 1999 to 2001 in Visakhapatnam (Andhra Pradesh) (Dhiman *et al* 2001), Baharaich (Uttar Pradesh) (Dhiman *et al* 2001), Morigaon and Golaghat (Assam), Raichur (Karnataka), Dadri, Aligarh and Moradabad (Uttar Pradesh), Betul (Chhattisgarh), Surendranagar and Kutchch (Gujarat) are given in Table 1.

On the request of NVBDCP or state governments, NIMR investigated the outbreaks of malaria, dengue and chikungunya in different parts of the country from 2002–06 (Fig. 2). Most of the outbreaks were confined to Madhya Pradesh, Karnataka, Gujarat and Assam. The findings/recommendations were sent to concerned state governments for remedial measures.

During 2000, an outbreak investigation under-

taken in Betul (Madhya Pradesh) and the follow-up of recommendations of NIMR became a success story for control of epidemic malaria.

Betul, a success story of malaria control using existing tools

Betul, a tribal forested district is highly malarious. An outbreak of malaria was recorded in Betul district, during October 2000, which caused very high morbidity and mortality. NIMR surveyed 40 villages in 3 PHCs on the request of Govt. of Madhya Pradesh. In view of the very high prevalence of falciparum malaria, intensive intervention measures were recommended by NIMR that DDT should be replaced by an effective insecticide (synthetic pyrethroid), prompt treatment of all fever cases with SP, release of larvivorous fishes in breeding sites, use of rapid test in remote areas for on the spot diagnosis and treatment and regular information, education and communication (IEC). These recommendations were implemented by the state government and post-intervention showed a sharp steady decline in number of malaria cases. There were 28, 32, 51, 52 and 52% reduction in SPR and 33, 40, 49, 49 and 48% reduction in SFR in 2001, 2002, 2003, 2004 and 2005 respectively. Spleen rate also showed decline, i.e. 5, 47, 60, 66 and 68% reduction in 2001, 2002, 2003, 2004 and 2005 respectively as compared to 2000. Monitoring of entomological results revealed a significant decline in both *Anopheles* and *An. culicifacies* ($p < 0.00001$). A combination of indoor residual spraying and early detection and prompt treatment complemented by rapid diagnostic tests and larvivorous fishes successfully brought malaria under control. These approaches could be applied in other regions of different endemicity to control malaria in India.

❑

Estimation of True Malaria Burden in India

Malaria imposes great socio-economic burden on humanity and with six other diseases like diarrhoea, HIV/AIDS, tuberculosis, measles, hepatitis B and pneumonia account for 85% of Global infectious disease burden. About 36% of the world population, i.e. 2020 million is exposed to the risk of contracting malaria in ~ 90 countries. World Health Organization estimates 300–500 million malaria cases annually and 90% of this burden is in Africa alone. In addition, the estimated annual mortality attributed to malaria ranges from 700,000 and 2.7 million globally and over 75% of them are African children and expectant mothers. Doubts have been expressed about reliability of these estimates as most of the hyper- and holoendemic countries, especially in Africa lack credible diagnostic facilities and reporting system.

In the south-east Asian Region of WHO, out of about 1.4 billion people living in 11 countries (land area 8,466,600 km^2, i.e. 6% of global area), 1.2 billion are exposed to the risk of malaria and most of whom live in India (Kondrachine 1992). However, the south-east Asia contributed only 2.5 million cases to the global burden of malaria. Of this, India alone contributed 76% of the total cases. Taking into account clinical episodes, it has now been estimated with the help of epidemiological models, geographical and demographic data that *P. falciparum* estimates outside Africa, especially in south-east Asia are 200% higher than that reported by the World Health Organization, i.e. 118.94 million out of global estimates of 515 million cases (Snow *et al* 2005). In addition to this, burden of *P. vivax* malaria in the world has been calculated at 71–80 million cases of which south-east Asia and western pacific countries contributed 42 million cases (Mendis *et al* 2001).

Malaria Scenario in India

Even a century after the discovery of malaria transmission through mosquitoes in India by Sir, Ronald Ross in 1897, malaria continues to be one of India's leading pubic health problems. In the 1930s, a treatise written by Sinton (1935) on 'what malaria costs India' recorded that the problem of the very existence in many parts of India was in fact the problem of malaria. In those days, it constituted one of the most important causes of economic misfortune, engendering poverty which lowered the physical and intellectual standards of the nation and hampered prosperity and economic progress in every way. In 1935, it was estimated that 100 million malaria cases and 1 million deaths occurred in India. Another estimate in 1947 suggests that 75 million cases (21.8% population) occurred in the post-independence population of 334 million with some

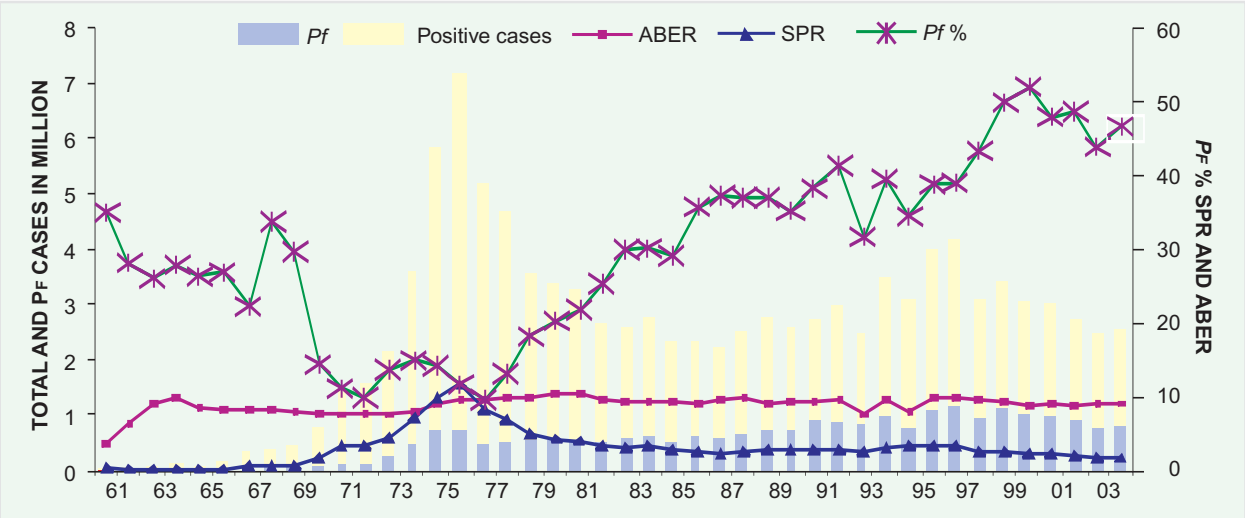


Fig. 3: Trends of malaria incidence in India from 1960 to 2005. Nearing eradication in 1960s (< 100,000 cases) to resurgence in the mid 1970s (~6.4 million cases) and stabilizing trend to around 2 million cases in the 1990s. *Plasmodium falciparum* proportion has steadily risen to around 50% in the recent years and the remaining incidence is of *P. vivax* and a small proportion of *P. malariae*. (Source: NVBDCP data)

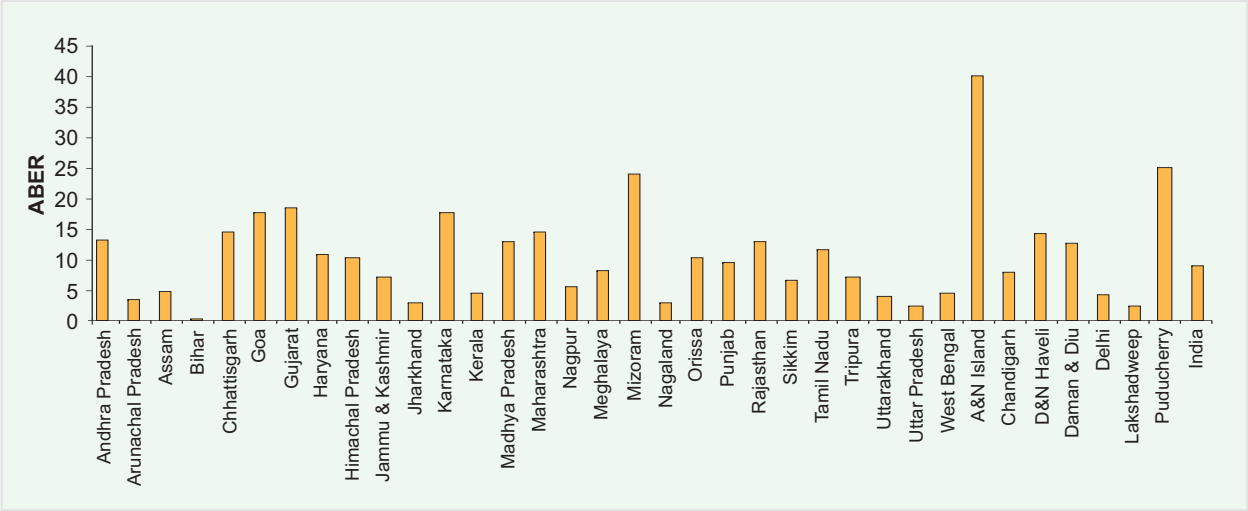


Fig. 4: Showing annual blood examination rates (ABER) for the detection of malaria in different states of India in 2004. About 10% is considered adequate to reflect true picture of malaria but there are some highly endemic states where ABER is much less than the prescribed norms. Even national average was 9% (Source: NVBDCP, India)

800,000 deaths. From this point, India achieved spectacular gains in malaria control during the 'Eradication Era' in the 1950s till the mid 1960s when reported cases were reduced to 64,000. In the post-resurgence phase, for many decades reported cases of malaria fluctuated between 1.5 and 3.0 million against the backdrop of rising population of India (Fig. 3).

There were a number of attempts to arrive at the true burden of malaria morbidity and mortality in India. Whereas, NMEP reported 5.2 and 2.9 million cases in 1975 and 1980 respectively, the Indian Drug Manufacturers Association estimated 12 million cases in 1975 and 20 million in 1980. From 1990s to date the reported malaria incidence in India has been around 1.5 to 2.6 million cases and 666–1000 deaths/annum, whereas estimated incidence by WHO was 15 million malaria cases with 19500 to 20000 deaths/annum (WHO SEARO website).

In 1990, it was estimated that out of a population of 843.7 million in India, 75 million, 240 million and 500 million people were respectively at high, moderate and low risk of contracting malaria. Situation has not changed much since then except for the population growth in each risk category (Sharma 1996). It is now well-accepted that the reported incidence of malaria at the national level on the basis of surveillance carried out in the primary health care system at best reflects a trend and not the true burden of malaria. Some studies carried out by the Malaria Research Centre (now NIMR) have also revealed a huge gap between reported and the true incidence of malaria. Sharma *et al* (1983) found that malaria incidence in PHC Kichha in District Nainital (erstwhile in U.P.) and Kharkhoda in District Sonepat (Haryana) was much high 95% (1784 cases) and 97% (7117 cases) higher than reported [76 and 183 cases respectively] (Table 2). Similarly, Malhotra *et al* (1985) detected 2623 cases as against 49 reported in Gadarpur PHC (Uttarakhand) showing once again a gap of 98%. Another study in PHC Bisra in District Sundargarh, Orissa state reported a slide positivity rate (SPR) of 33% by adopting weekly surveillance during 1988–89, whereas the SPR recorded by fortnightly surveillance during 1981–97 in Bisra PHC ranged between 9 and 18.5% (Yadav

Table 2. Some examples of incidence gap between routine surveillance system and longitudinal/point prevalence studies

Area	Pop	Surv	Cases	SPR	SFR	% Dif	Ref.
Kichha PHC,	97183	RS	76	4.7	NA	95	Sharma *et al*
Nainital	97183	LS	1784	22.1	NA		1983
Kharkhoda PHC,	91806	RS	183	12.6	5.5	97.4	Sharma *et al*
Sonepat	91806	LS	7117	43.2	30.46		1983
Gadarpur	6475	RS	492	5.27	1.61	98.1	Malhotra *et al*
UHC (U.P.)	6475	LS	623	58.66	34.58		1985
Bisra PHC,	6918	RS	825	7.6	3.8	68.0	Ghosh *et al*
Rourkela	NA	PP		26.3	15.8		1989

NA—Not available LS—Longitudinal studies; RS—Routine surveillance; PP—Point prevalence; Pop—Total population surveyed; Surv—Type of surveillance; Dif—Difference; Ref.—Publication reference

et al 1990). Yet another study in the mining areas of Orissa reported that at any given point of time about 13% population harboured malaria parasites and about 200 persons suffered from new malaria episodes per 1000 population per year (Haque 1998).

One of the reasons for under reporting is the low Annual blood examination rate which is reflection of inadequate disease surveillance by the states. The NVBDCP prescribes that annual blood examination rate for malaria should at least be 10% on a presumption that 10% of the population in a year will have fever at one point of time or the other. It is assumed that if all or most of the fever cases are examined for malaria, most of the incidence of malaria could be captured during fortnightly active surveillance. A look at the 2004 data (Fig. 4) show that the average ABER was 9% in India. In 14 out of 29 states, some of which were highly endemic to malaria, ABER ranged from 1 to 8% and in the remaining 15 states and union territories, ABER ranged from 10 to 40%.

Other reasons attributed to the gap besides inadequacies in surveillance are the quality of smear examination and underreporting of malaria cases. Underreporting of malaria due to misdiagnosis has been observed in Gujarat, where re-examination of blood smears in 9 primary health centers revealed that 6.7% of them had been misdiagnosed. As a result, 1262 malaria cases went undetected and unreported. Consequently, the annual parasite incidence (API) of malaria should have been 9.0 instead of 5.9 reported (Gautam *et al* 1992). How reliable was the clinical diagnosis alone for the treatment of malaria was shown in a hospital-based study. While there were 24% malaria cases on the basis of clinical judgement alone, the cases were actually 52% when microscopic diagnosis was done showing a gap of 28% (Gautam *et al* 1991). In a more recent study conducted in Ahmedabad metropolitan city in Gujarat state, it was estimated that there were on an average 25,465 malaria cases/annum as against 4119 cases reported and at least 22 malaria deaths/million population as against 0.3/million reported (Yadav *et al* 2003). This situation would have been further different if data of patients treated by all the private practitioners was available and computed to find out the true incidence in the city.

In three hospitals under the Steel Authority of India Limited in the mining areas in the interior forest of Sundargarh district in Orissa, a large number (52–68%) of outpatients with fever were treated for clinical malaria and a subsequent study showed that a third of all fever cases indeed had malaria (Yadav *et al* 1990). None of these cases were, however, captured in the PHC statistics due to lack of reporting system and even *P. malariae* parasites were not recorded due to misdiagnosis. It is a recognized fact that a large number of patients avail medical care at private institutions which do not keep or report disease statistics to health services.

Burden of malaria in different States of India

The annual parasite incidence (API) is a malariometric index to express malaria cases per thousand population. As per the NVBDCP incidence records, in most parts of of India the API was <2, whereas 2–5 API was in scattered regions, while regions with >5 API were scattered in the states like Rajasthan, Gujarat, Karnataka, Goa, southern Madhya Pradesh, Chhattisgarh, Jharkhand and Orissa, and in the northeastern states (Fig. 5).

The proportion of *P. vivax* and *P. falciparum* varies in different parts of India. Although most of the indo-gangetic plains and northern hilly states, northwestern India and southern Tamil Nadu state have <10% *P. falciparum* and the rest *are P. vivax* infections; in the forested areas inhabited by ethnic tribes, the situation is reverse and *P. falciparum* proportion is 30–90% and in the remaining areas it is between 10 and 30% (Fig. 6).

In India, maximum malaria is contributed by the Orissa state (Fig. 7). Although Orissa has a population of 36.7 million (3.5%), it contributed 25% of total 1.5 to 2 million reported annual malaria incidence, 39.5% of *P. falciparum* malaria and 30% of deaths due to malaria in India (Source: NVBDCP, India). Similarly, in the other states inhabited by ethnic tribes mainly in the forest ecosystems, meso- to hyper-endemic conditions of malaria exist with the preponderance of *P. falciparum* to the extent of 90% or even more.

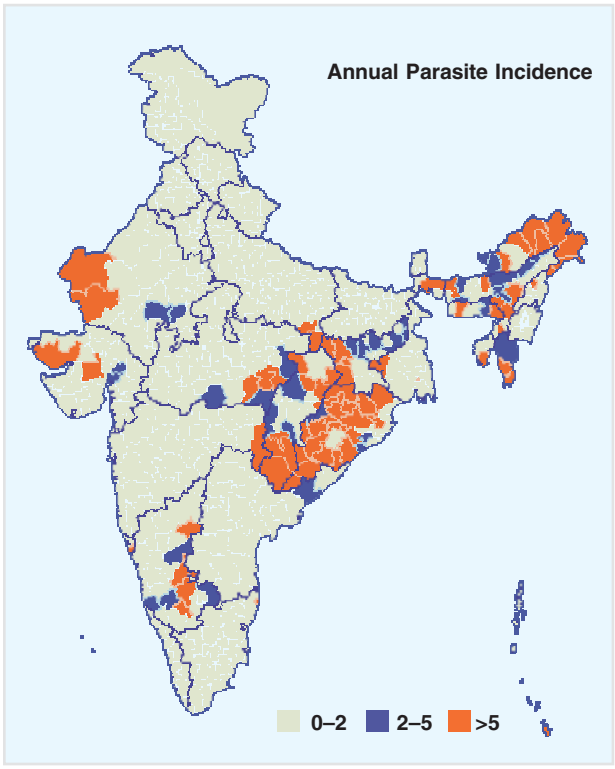


Fig. 5: Distribution of Malaria Incidence in India according to API in 2004 (Data Source: NVBDCP). Majority of India had less than 2 cases per 1000 population, 2–5 cases in some scattered regions and >5 cases where ethnic tribes live and stable malaria conditions prevail

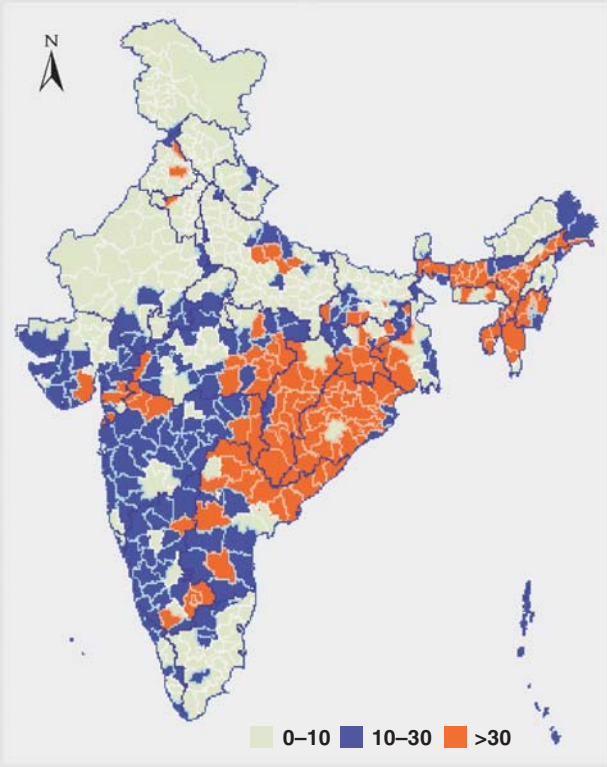


Fig. 6: *Plasmodium falciparum* proportion distribution in India. High proportion of *P. falciparum* up to 90% is seen in zones inhabited by ethnic tribes in forest ecosystems where stable malaria conditions occur

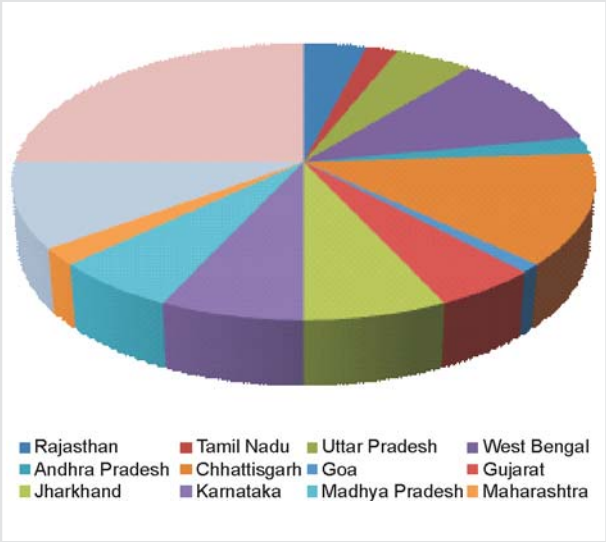


Fig. 7: Contribution of different states to malaria in India. Orissa, Chhattisgarh, West Bengal, Jharkhand and Karnataka contributed the most

Malaria Prevalence according to Age and Gender in India

Most of the point prevalence studies in India have been carried out for outbreak/epidemic investigations. There is very limited information on age and gender specific seasonal prevalence of malaria in different paradigms in the country. In the available studies, age and gender classification used is arbiterary (Das *et al* 1997; Dev and Sharma 1995; Prakash *et al* 1997; Dutta *et al* 1999; Shukla *et al* 1995; Dhiman *et al* 2001; Shrivastva *et al* 1995). The burden is generally higher in males than females in all age groups. These studies showed that children in the states like Assam, Arunachal Pradesh and Rajasthan had higher incidence of malaria than adults, whereas in the indo-gangetic plains the situation was reverse.

The Burden of Drug Resistant Malaria

In India, chloroquine resistance in *P. falciparum* was first reported from Manjha in Karbi Anglong district in 1973 (Sehgal *et al* 1973) and then from Nowgaon in 1974 in the northeastern state of Assam. More cases were then detected in next 3–4 years in Assam, Arunachal Pradesh, Mizoram and Nagaland. Although foci of resistance to chloroquine are present in the entire country, the problem is more pronounced in areas with intense *P. falciparum* transmission like northeastern states and Orissa; in areas where there is intermixing of population like project areas including construction sites, in big metros and along international borders (Fig. 8). In most of the studies, only late treatment failure to chloroquine has been observed probably because of semi immune nature of the population.

The problem of drug resistance has also been investigated using molecular markers. Molecular studies in 274 Indian *Pf* isolates have detected K76T mutations in all cases who did not respond to chloroquine and 96% of cases who were cured with chloroquine showing lack of co-relation between K76T mutation and clinical cure (Vinayak *et al* 2003). However, in this study, significant association of K76T mutation was observed with *in vitro* response to chloroquine in *P. falciparum*. Alleles of *Pfmdr1* gene showed strong association but incomplete correlation with CQ resistance (Bhattacharya *et al* 1997).

Although the available data on sulfadoxine pyrimethamine (SP) resistance is limited, it appears that the efficacy of this drug is within acceptable limits except in limited areas like Indo-Myanmar border in Arunachal Pradesh and some parts of Assam and West Bengal (NVBDCP 2002; Mohapatra *et al* 1997). In a study, out of 40 clinical isolates, 87.5% had Dihydrofolate reductase (DHFR) and 15% had Dihydropteroate synthase (DHPS) mutations (Biswas 2004). Parasites carrying double or single mutants also showed increased minimum inhibitory concentration (MIC) value for both pyrimethamine and sulfadoxine.

Only limited reports of chloroquine resistance in *P. vivax* malaria are available from India. Two cases from Mumbai did not respond to full dose of chloroquine (1500 mg) and peripheral smear continued to be positive despite adequate blood concentration of drug (Garg *et al* 1995). Similarly, there is another case report from Mathura (U.P.) of non-response to standard dose of chloroquine as confirmed by repeated blood examination (Dua *et al*.1996). Recently, 16% RI and 6.7%, RII resistance

Fig. 8: Areas shown in Grey (triangles and patches) where chloroquine resistance in *P. falciparum* has been confirmed qualifying for use of second line drug SP (Source: Data from NVBDCP, India)

in *P. vivax* was reported in a study conducted in 75 patients in Bihar (Singh 2000). In addition, multi-drug resistance has also been reported (Kshirsagar *et al* 2000). Contrary to these reports, a study in West Bengal and Orissa during 1998–2001, observed 100% cure rates by Day 7 in 480 vivax malaria patients (Nandy *et al* 2003). Incidentally, these areas, where *P. vivax* is still sensitive to chloroquine, have high drug pressure and chloroquine resistance in *P. falciparum.* Similar findings were confirmed in therapeutic efficacy studies with chloroquine in vivax malaria, in Gautam Budh Nagar (Uttar Pradesh) in the north, Navi Mumbai (Maharashtra) in the west and Chennai (Tamil Nadu) in south India in 287 patients in 2002. Curative efficacy of chloroquine was 100% in these patients of vivax malaria. Rapid parasite and fever clearance was observed in all cases and the drug was well tolerated (Valecha *et al* 2006). From the data available so far, it is evident that the problem of drug resistance in *P. vivax* is not of major concern, however, one needs to be vigilant as *P. vivax* produces relapsing type of infection and is a predominant species in India.

Based on the results of 28-day *in vivo* studies till 2001 and therapeutic studies from 2002 onward conducted by NVBDCP and research institutes including National Institute of Malaria Research, drug policy has been revised in 241 PHC'S of 71 districts in 20 states of India.

The Burden of Complicated Malaria

In India, reports suggest that mortality in complicated *P. falciparum* malaria in Vellore in southern state of Tamil Nadu was 7.9%, while in Jabalpur (Madhya Pradesh) and Rourkela (Orissa) it was 25.6 and 30% respectively (Herris *et al* 2001; Shukla *et al* 1995). In Jabalpur Medical College, 1783 patients were admitted with complicated *P. falciparum* infection of which 152 (8.5%) had cerebral malaria. Of these, 39 (25.6%) died and majority of them were in 16–40 years age group. Mortality was significantly higher in patients with hyper-parasitaemia and hypoglycemia. Delayed diagnosis and comatose condition were the main determinants of death. In a

tertiary care industrial hospital at Rourkela, a comparative analysis revealed that total number of patients admitted with complicated malaria significantly increased from 14.15% (62/431) in 1995–97 to 23.69% (236/996) in 2000–02. Similarly, cases of acute renal failures doubled from 22.5% (47/369) to 44.15% (117/265) and deaths in patients without renal involvement also increased from 12.7% (47/369) to 16.8% (119/731) [Unpublished data, courtesy Ispat General Hospital, Rourkela]. A general shift in the clinical profile in patients with complicated malaria has been observed and multiple organ dysfunction/failure is becoming common feature. For example in a tertiary care hospital in Cuttack only 10.9% (96/879) cases admitted were without complications, while 382 (43.5%) had either cerebral or renal or hepatic involvement, 298 (33.9%) had cerebral malaria with either renal or hepatic involvement and 103 (11.7%) had multi-organ failure and 138/783 (17.6%) died due to malaria.

Complications due to hitherto considered benign species *P. vivax* have been reported from Bikaner, India as from elsewhere in the recent years (Kochar *et al* 2005; Beg *et al* 2002; Valecha *et al* 1992; Patial *et al* 1998). It was observed that 72 of the 440 patients with microscopically and PCR confirmed mono infection of *P. vivax* had severe manifestations which included jaundice [33 (47%)], severe anemia [11 (15.7%)], respiratory distress with acidosis [8 (11.42%)], acute renal failure [7 (10%)], cerebral dysfunction with multiple convulsions [6 (8.6%)], abnormal bleeding [6 (8.6%)], shock (hypotension) [5 (7.1%)], pulmonary edema [3 (4.2%)] and hemoglobinuria [3 (4.2%)]. Many combinations of severe manifestations were observed in 35 of the 72 *P. vivax* cases followed. In 12 pregnant women with *P. vivax* infection, there were 2 abortions, 2 stillbirths and 4 pre-term deliveries.

The Burden of Malaria in Pregnancy in India

It is well-known that pregnant women constitute an important risk group for malaria infection particularly in hyper and holoendemic situations. The well known effects include effectiveness of placental barrier, parasite sequestration in placenta, suboptimal nutrition of the fetus, congenital malaria, intrauterine growth retardation, low birth weight, premature interruption of pregnancy, infant mortality and maternal death (Egwunyenga *et al* 1997; Melba 2002; Singh *et al* 2005; Singh *et al* 1999). Besides it may be the cause of cerebral malaria and severe anemia. In low transmission areas maternal mortality is about 1% while in Africa it could be between 84 and 2000 per 100,000 live births (0.00084–2%).

In the southeast Asia, malaria is a serious burden in pregnancy with spectrum of ill effects as shown by slide postivity rate (1.1–58%, n = 45–365), parasitaemia (1–70%, n = 55–365), cerebral malaria (7–76%, n = 45–365), anaemia (8.6–90%, n = 45–365), maternal mortality (7–66.6%, n = 45–365), placental malaria (18–29%, n = 256–365), abortions (2–11%, n = 45–365) and intrauterine fetal development impairment (2–31%, n = 45–322), stillbirth (2–13%, n = 45–365), pre-term (4.2–60%, n = 45–322) and low birth weight (5.4–89%, n = 55–365) (Singh *et al* 2005).

In the northwestern India in a hospital-based study in Bikaner, it was found that mortality rate in 45 pregnant women with *P. falciparum* infection was highly significant (37.8%) in comparison to non-pregnant women with *Pf* infection (14.81%) at p <0.001. Similarly, cerebral malaria (75.55%), severe anaemia (<5 g%) 20%, hepatic (13.3%) and renal failure (20%) were significantly more in pregnant women than non-pregnant females at 32.92, 4.11, 9.05 and 6.17%, respectively, (Kochar *et al* 2005).

From Central India, it has been reported that pregnant women (n = 365) suffer significantly more from both *P. vivax* (n = 121) and *P. falciparum* (n = 244) malaria than non-pregnant women (n = 150) (Singh *et al* 1999). The weight of neonates born to infected mothers was 300–350 g less on an average than neonates born to non-infected mothers(n = 1762). The weights continued to be significantly lower till the first six months affecting the growth of babies in infancy. It was found that rates of malaria infection reduced from first to third pregnancies. The mean parasitaemia in pregnant women suffering from *P. vivax* ($p<0.05$) or *P. falciparum* ($p<0.0001$) malaria was much higher than non-pregnant malaria infected women. Similarly, women with *P. falciparum* infection were significantly more anemic than the non-infected pregnant women ($p<0.0001$) or infected non-pregnant women ($p<0.001$). The pregnant women with *P. falciparum* malaria were significantly more anaemic than those suffering from *P. vivax* infection. Of the 244 pregnant women who had *P. falciparum* infection, 3 (1.22%) died, while in another 3 abortions were recorded and in two others still births were recorded. Only one still birth and abortion each were recorded in *P. vivax* infected women who were primigravidae. Among non-infected women, however, one abortion (in a primigravida) and one stillbirth (in a multigravida) were recorded.

Mortality Attributable to Malaria and Gaps

In India, malaria is one of the most important causes of direct or indirect infant, child and adult mortality. In pre-independent India, death toll due to malaria was estimated at one million during normal years and two million during epidemic years (Sinton 1935). Malaria mortality steeply declined after National Malaria Eradication Programme was launched in 1958. The National Programme reported 879, 666, 1057, 946 and 938 deaths due to complicated *P. falciparum* malaria from 1997 to 2001 showing a Specific Malaria Mortality Ratio (SMMR) of 0.30 to 0.48 in these years which was one of the lowest in the world. However, as per the WHO SEARO, 19500 to 20000 deaths occurred annually

Table 3. Estimates of deaths due to malaria in 15 states and Union Territories (UT) in India based on report of medically certified deaths in 1998[18]

State/UT	Proportion of deaths medically certified to total reported deaths (a)	No. of certified deaths attributable to malaria (b)	Total no. of estimated deaths due to malaria* (c) =b ×100/a
Puducherry	53.5	8	15
Nagaland	4	7	175
Manipur	32.7	10	31
Meghalaya	15.6	37	237
Haryana	10.5	80	762
Goa	89.9	87	97
Gujarat	4	95	2375
Arunachal Pradesh	69.5	119	171
Andhra Pradesh	6.6	165	2500
Delhi	58.5	212	362
Rajasthan	12.8	245	1914
Maharashtra	33.6	326	970
Karnataka	13.8	407	2949
Madhya Pradesh	4.9	890	18163
Orissa	9.4	1793	19074
Total	14.9	4481	49796

*Assuming malarial deaths were uniformly distributed in the entire sample of deaths due to all causes.

in India. Other than these sources, there are scanty reports on deaths due to malaria which are primarily based on outbreak or epidemic investigations. Age, gender and cause specific deaths are most extensively covered in the Govt. of India report on the basis of Medical Certification of Cause of Death (MCCD) (Anonymous 2001, 2002). The most recent available report is for 1998 during which there were 4481 certified malarial deaths reported from various categories of hospitals in rural and urban areas of India (Kochar *et al* 1998). Significantly, in this report only 14.9% of the total registered deaths were medically certified and to which specific cause of death was attributed. A simple conversion to 100% certification would mean that the deaths due to malaria could be 49,796 assuming that the malarial deaths were uniformly distributed in the remaining 85.1% sample (Table 3). During the same year, i.e. 1998 only 666 deaths were reported by the NVBDCP hence, these estimates were incomparable. It may further be noted that MCCD-1998 report contained death statistics from only 15 states and union territories out of total of 29 states and seven union territories. Certified death data from many malaria endemic states, such as Uttar Pradesh, Bihar, Assam, West Bengal and Tamil Nadu were not available. Had there been reporting of deaths from these states, the malarial deaths would have been much more than estimated 49,796. Hence, available data on deaths are incomplete and there appears to be a huge gap between reported and actual deaths due to malaria in India.

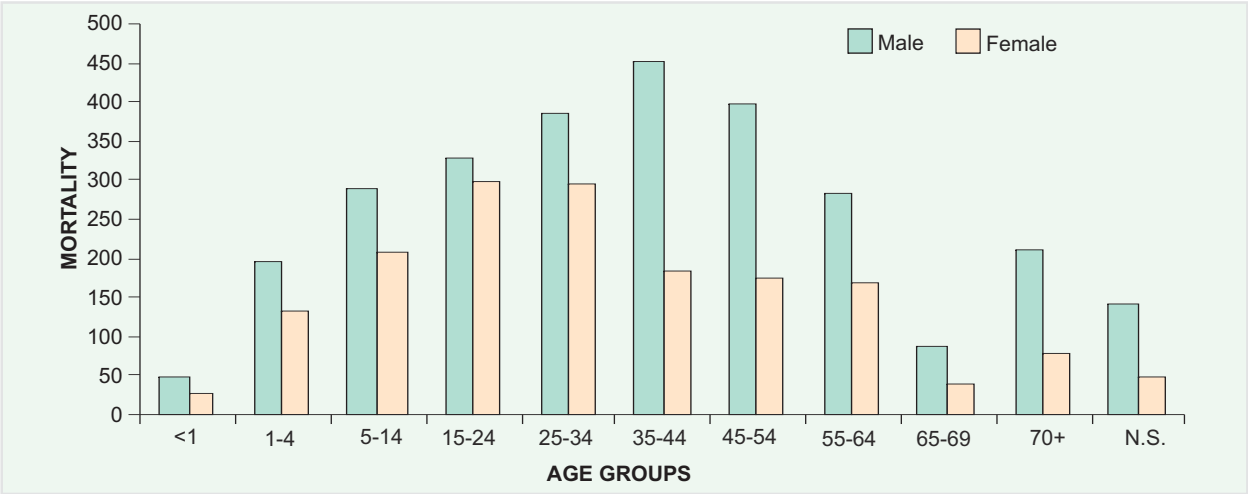


Fig. 9: Age and sex distribution of malaria mortality in India in 1998. The deaths are more in males than in females across all the ages while middle productive ages in general have much higher mortality than in children. N.S.= age not specified

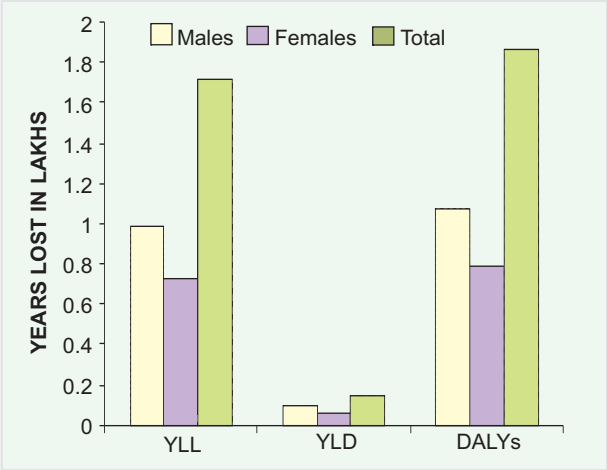


Fig. 10: Years of life lost (YLL), years lost due to disability (YLD) and disability adjusted life years lost (DALYs) due to malaria in both sexes in 1997 in India

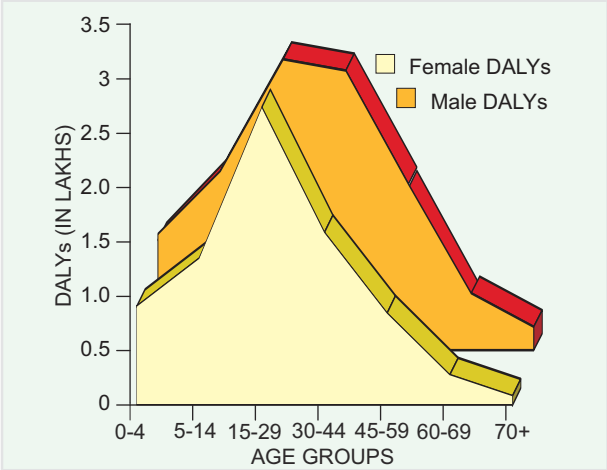


Fig. 11: DALYs lost according to age and sexes in India in 1997

Age and Sex-wise Distribution of Malaria Mortality

Age-sex distribution of malaria deaths shows that in general malaria mortality across all ages was comparatively higher in males than in females (Fig. 9). This mortality gap in genders widens after the age of 25 years (Anonymous 2002). Overall number of deaths in males were 2827 (63.1%) as compared to 1654 (36.9%) in females with a male: female ratio of 1:0.56. Unlike in Africa, where most of the deaths are reported in infants and children, it is seen that in India malarial deaths increased up to the age of 44 years in both the genders and then declined thereafter. Although the deaths in infants and children <14years of age accounted for 20.6%, in the higher ages (15–54 years), it accounted for 56.1% and the rest 23.3% were in >55 years of age. Hence, most of the burden of malarial mortality was borne by the economically productive ages.

The Burden of Malaria in terms of DALYs Lost in India—A Preliminary Estimate

In 1993, the Harvard School of Public Health in collaboration with World Bank and WHO assessed the Global Burden of Diseases (GBD) (Murray and Lopez 1997). The GBD study introduced a new metric–the disability adjusted life year (DALY)-to quantify the burden of the disease. One DALY means one lost year of healthy life on account of disease and is a common currency for disease morbidity and mortality expressed in time. This concept has gained importance in the past decade and WHO had undertaken GBD study of 135 major causes for the year 2002 and estimated DALYs for each cause in different regions and the countries (WHO 2004).

DALYs lost due to malaria in India for the year 1997 have been computed (Kumar *et al* 2007). The deaths due to malaria were estimated at 71,396 based on MCCD 1997 report (Anonymous 2001). Deaths were proportionately distributed according to age and gender based on MCCD data. From population Census of India 1991 report (Anonymous 1991), mid year population was calculated and assigned to different ages of both genders. Incidence of malaria was taken as per the WHO estimates of 15 million. Disability weights estimated in Global Burden of Disease Study 2000 for episodes (0.172 to 0.211), anaemia (0.012 to 0.013) and neurologic sequelae (0.581) were used. Duration of episode of malaria was taken as 7 days. DALYs were estimated using GBD template with age weighting and discounting. The total DALYs lost due to malaria were worked out to 1.86 million years. Among the females, DALYs lost were 0.786 million as against 1.074 million in the males (Fig. 10). The maximum DALYs lost (53.25%) were in the middle productive ages from 15 to 44 years followed by children <14 years of age (27.68%) and rest 19% in >45 years of age (Fig.11).

Health planners and administrators need estimates of true burden of malaria for allocation of much needed resources for interventions. The current reported incidence of around two million/annum in India at best reflects trend and given the gaps identified in various studies, the actual incidence is definitely far more than presently known. The reasons attributed to such a gap are deficiencies in coverage, collection and examination of blood smears and reporting system. Moreover, in India, the government health sector which provides free or highly subsidized health care caters to the needs of 20% population mainly in rural areas while the rest of the population seeks health care in private sector as their first point of contact where bulk of malaria is generally treated empirically Zwi *et al* 2001. The clinically treated cases never or rarely find place in the official statistics. This gap needs to be bridged to build burden estimates. Coupled with this, there is likelihood of sizable population acting as asymptomatic carriers of plasmodial infection, particularly in hard core malarious areas inhabited by ethnic tribes in India where meso- to hyper-endemic conditions exist. In such areas, inaccessibility and insurgency appear to be major causes of deficient routine surveillance services. In many such remote places, DDC (Drug

distribution centres) have been opened in India where malaria is symptomatically treated by trained community volunteers without accounting for the treated cases. Similar doubts have been expressed about the validity of estimates available for Africa because of inadequate detection and reporting and general inadequacies in the surveillance in malarious countries. The known and missing incidence of malaria in affected countries has been compared with the ears of hippopotamus which are visible above water while the bulk lies unseen underneath. This statement may also apply to many parts of India.

The true incidence of morbidity and mortality are of paramount importance in estimation of DALYs lost. In the absence of true burden estimates, we computed DALYs lost for India using WHO projections and mortality estimation on the basis of MCCD data. Although our DALYs estimates are conservative, they are much higher at 1.86 million years lost as compared to WHO estimates of 0.844 million years for the year 2002 (WHO 2004). India, therefore, must initiate burden estimation studies based on primary incidence and prevalence data to highlight the actual malaria burden in the country.

Malaria is well-known for its debilitating, demoralizing and impoverishing consequences and, therefore, estimation of its true burden and control is central to addressing these issues with the final aim of lifting the human resource above poverty line. The poor may find it hard to deal with persistent malaria problem, as coping with it is economically disastrous for the communities living on the edge. A good investment in malaria control not only makes public health sense but also economic sense in the present era of economic liberalization and surge in India. A firm malaria control is imperative for human resource development which in turn is imperative for equitable and sustained economic growth.

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Malaria during Pregnancy

Pregnant women form a high risk group for malaria infection which may cause abortions, still births and premature labour. Malaria in pregnancy is a significant health problem in India and requires systematic studies. Almost all the published literature on the topic refers to Africa, which presents data referring principally to *Plasmodium falciparum*—the commonest cause of infection. We examined the relationship between malaria infection and pregnancy in central India. This region is of special interest because the population is exposed to both *P. vivax* and *P. falciparum* infections.

Epidemiology of Malaria in Pregnancy

A study was carried out in collaboration with the Government Medical College, Jabalpur, which caters to mixed rural, tribal and urban population (Singh *et al* 1999). A malaria clinic was established at the hospital to examine pregnant women for malaria. Analysis of three years data showed significantly higher ($p<0.001$) malaria prevalence among pregnant women, especially primigravid than non-pregnant women. Mean parasite densities were significantly higher in pregnant women compared to non-pregnant women for both *P. falciparum* ($p<0.001$; df = 137) and *P. vivax* ($p<0.05$; df = 72) infections. Pregnant women with falciparum or vivax malaria were significantly more anaemic than non-infected pregnant women or infected non-pregnant women (Table 4). Cerebral malaria was a common complication of severe *P. falciparum* infection, with a high mortality rate during pregnancy.

The average weight of 155 neonates from infected mothers was 350 g less than that of 175 neonates from non-infected mothers. This difference in birth weight was statistically significant for both *P. falciparum* ($p<0.0001$; df = 278) and *P. vivax* ($p<0.0001$; df = 223) infection. Congenital malaria was not recorded.

In an another study during malaria epidemic (Singh *et al* 2001) among 151 malaria infected pregnant women, *P. falciparum* was the predominant species (88%) with highest prevalence in II trimester (59.4%) irrespective of parity status. About 3% abortions, 3.7% stillbirths and 2.2% neonatal deaths were documented in *P. falciparum* infected women. Out of six cases examined, three of these samples (1 *Pv*; 2 *Pf*) showed evidence of placental infection. The proportion of low birth weight babies was also significantly higher among those born to infected women than those born to the non-infected (95.2% vs 68%; $\chi^2 = 13.09$; df = 1; $p<0.01$). Four neonates died before Day 20 post-partum—three born to multigravidae infected with *P. falciparum* and one to an apparently uninfected second gravida.

A study was also done to evaluate the efficacy

Table 4. Malaria parasitaemia, anaemia during pregnancy and low birth weight babies/neonates among the study subjects in central India

	All cases[a]	Cases with *P. vivax* infection	Cases with *P. falciparum* infection	Control 1[a]	Control 2[a]
No. tested	2127	365	365	1984	
No. selected	365 (17%)	121 (33%)	244 (67%)	150 (8%)	1762 (i.e. 2127-365)
Pregnant	Yes	Yes	Yes	Yes	Yes
Fever	Yes	Yes	Yes	Yes	Yes
Malaria	Yes	Yes	Yes	Yes	No
No. with Hb data available	271 (74%)	83 (69%)	188 (77%)	85 (57%)	88 (5%)
Mean Hb ± SD (g/dl)	–	9.05 ± 1.39	6.42 ± 1.98	9.68 ± 1.43	10.03 ± 1.11
No. with birth weight available	155 (42%)	50 (41%)	105 (43%)	–	175 (10%)
Mean wt ± SD (kg)	2.18 ± 0.25	2.22 ± 0.30	2.15 ± 0.21	–	2.53 ± 0.43

[a]Women cases with fever; Control 1— Infected non-pregnant women; Control 2—Non-infected pregnant women.

of CQ (25 mg/kg body weight) in the treatment of *P. falciparum* in pregnant women in a malaria meso-endemic area of District Mandla (Singh *et al* 2001). Out of 21 positive patients enrolled, six (28.6%) women (2 primi + 4 multi) had a RIII type response (95% C.I. 9–48%), one (4.7%) multigravida showed partial response (RI early/RII). Remaining (66.7%) women (3 primi + 11 multi) had a late RI/S type of response. Thus, the cumulative failure rate in this study was 95% (95%C.I. 86.13–100%). The 13 women (2 primi + 11 multi) who did not respond and were treated again with CQ, 10 (1 primi + 9 multi) failed (77%) again (95% C.I. 48–95%) on Day 28 and 35.

To evaluate the feasibility of delivering malaria chemoprophylaxis to pregnant women in urban settings of District Jabalpur, 155 pregnant women were enrolled of which 100 were with malaria and 55 without malaria. Out of 100, 27 were *P. vivax* (parasitaemia ranged from 1025–19,700 parasites/µl) and 73 were *P. falciparum* (1175–35,000 parasites/µl) (Singh *et al* 2002). The results revealed that the chemoprophylaxis to pregnant women was possible only in 30 patients (19.3%)—20 with malaria and 10 without malaria. None of these women developed malaria during the study period. Average birth weight of 11 babies born to women with malaria was 2.41 ± 0.21 kg and of the five babies born to women without malaria was 2.48 ± 0.2 kg. This difference was not statistically significant. Studies showed that there is limited understanding of the drug policy at district level and even if the drugs are prescribed for chemoprophylaxis, the compliance among pregnant women for the same is poor.

Evaluation of a Rapid Diagnostic Test for Assessing the Burden of Malaria at Delivery

Plasmodium falciparum sequester in placenta, it is very difficult to assess the true burden of diseases without the examination of placenta after delivery. Therefore, we used rapid diagnostic tests (RDTs) for on the spot diagnosis and treatment.

All pregnant women who came for delivery at a district hospital in Mandla and a civil hospital in Maihar were screened for *P. falciparum* (placental parasitaemia using a rapid test and microscopy and peripheral and umbilical cord parasitaemia using microscopy alone). Two rapid diagnostic tests (RDTs), ParaCheck *Pf* and ParaHITf, were used. At Mandla, the sensitivity and specificity of the ParaCheck *Pf* for *P. falciparum* were 93 and 84%, respectively. The positive predictive values (PPVs) and negative predictive values (NPVs) were 50 and 99%, respectively. At Maihar, the sensitivity and specificity of the ParaHITf for *P. falciparum* were 87.5 and 97%, respectively. The PPVs and NPVs were 75.4 and 98.7%, respectively. Placental infection was significantly associated with low birth weight. The RDTs for the identification of *P. falciparum* were more sensitive in placental blood than the placental blood smear by microscopy. Thus, the RDTs should be useful for rapid assessment of malaria at delivery.

We also organized training workshops on malaria in pregnancy for national and international programme managers. The first workshop was conducted at Jabalpur in 2004 for four southeast Asian countries, i.e. Bangladesh, Myanmar, Indonesia and India with the financial assistance of WHO and technical assistance of CDC. Subsequently, WR India provided funds for conducting four workshops in the state. These were carried out in Jabalpur, Katni, Satna and Bhopal for bringing awareness to policy makers and programme managers. Additionally, "Burden of malaria in pregnancy" is being assessed in Jharkhand and Chhattisgarh states with the financial assistance of USAID, Washington and ICMR, New Delhi in collaboration with US investigators. The study is in progress.

❑

Clinical Drug Trials

Drug resistance to chloroquine in *P. falciparum* was reported in India for the first time from Assam in 1973. Since then the foci of resistance have spread to many more states all over India. The situation has further deteriorated in the recent past due to parasite becoming resistant to other available drugs in addition to chloroquine. Sulphadoxine-pyrimethamine, a second line drug for *P. falciparum* is not effective for *P. vivax* malaria. Quinine is still effective but as oral monotherapy it has limited role in mild malaria because of 7-day regimen. Mefloquine and artemisinin have specific indications. Therefore, new drugs and treatment strategies need to be developed as a priority.

Development of new drugs involves extensive pre-clinical and toxicological studies followed by well-planned clinical trials. At NIMR, a number of new drugs have been screened in clinical trials for evaluation of safety and efficacy. Based on these data, the drugs have been registered with Drugs Controller General of India for commercial marketing and also for use in national programme under NVBDCP.

α,β-Arteether

Artemisinin is an active constituent of plant *Artemisia annua*, a Chinese herb (Qinghaosu). Artemisinin and its derivatives are most rapidly acting schizonticides valuable for emergency treatment of complicated malaria as well as multi-drug resistant falciparum malaria. Sodium artesunate, artemether and arteether are three formulations registered in India. Arteether is oil soluble ethyl-ether derivative of artemisinin. α, β- Arteether (30:70 mixture racemic) was developed jointly by Central Institute of Medicine and Aromatic Plants (CIMAP) and Central Drug Research Institute (CDRI) and clinical efficacy trials were conducted and co-ordinated by NIMR. Phase III multicentric trial was conducted at seven centres on 267 uncomplicated falciparum malaria patients and 211 complicated falciparum malaria cases (Valecha *et al* 1997; Asthana *et al* 1997). It was prospective, open and non-crossover study. Adult dose of drug was 150 mg once a day intramuscularly for three consecutive days. The cumulative cure rates ranged from 93–100%, parasite clearance in 24–40 h, while fever clearance was in 36–75.6 h (Figs. 12 and 13). Based on the results of these Phase III trials the drug α, β-Arteether marketed by M/s. Themis, India as EMAL was registered for use in India in 1996. Now the drug has been included in the National Vector Borne Disease Control Programme.

Bulaquine (80/53)

Plasmodium vivax malaria constitutes 60–65% cases of malaria in India. Although mortality is low due to this infection, relapses occur due to persistence of hepatic forms. The only drug available for preventing these relapses is primaquine, which can cause haemolysis in G-6-PD deficient cases. In the quest for safer substitute, CDRI developed activated enamine of primaquine which is chemically N-(3-acetyl 4-5-dihydro-2-furanyl)-N-(6-methoxy-8-quinlinyl) 1,4-pentadiamine. This new drug is safer than primaquine as evidenced by toxicological and haematological studies in beagle dogs and *in vitro*.

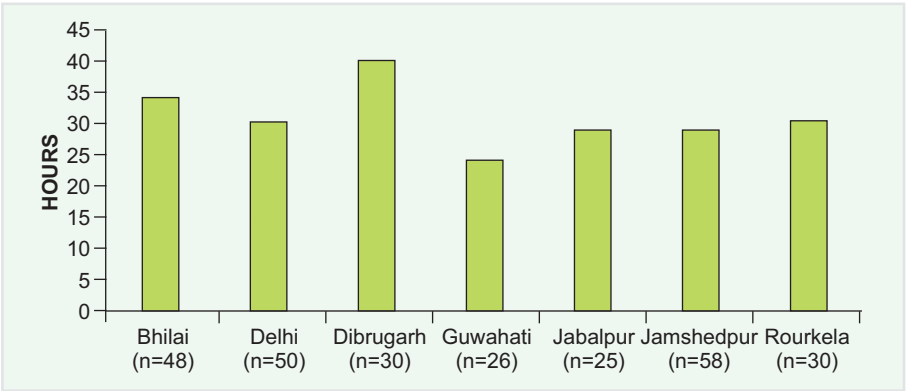


Fig. 12: Mean parasite clearance time in uncomplicated *P. falciparum* malaria treated with α–β arteether

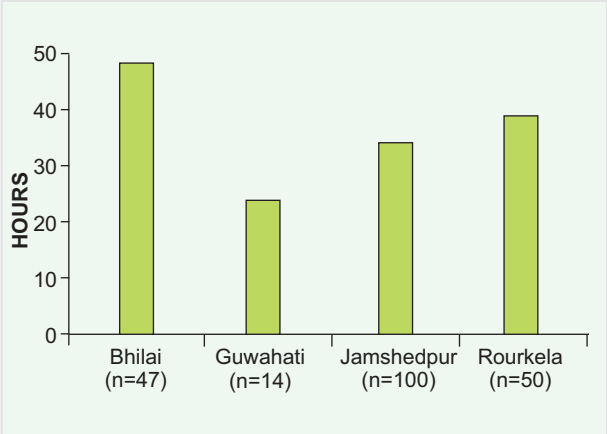


Fig. 13: Mean parasite clearance in complicated *P. falciparum* malaria treated with α–β arteether

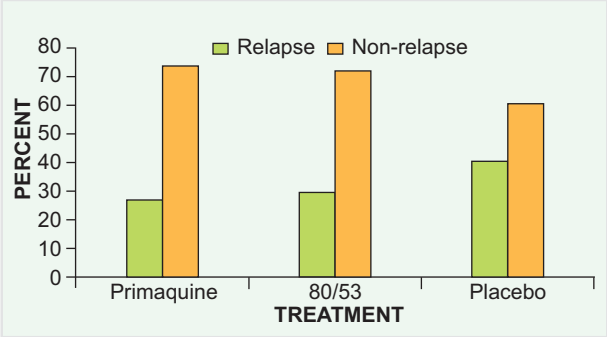


Fig. 14: Per cent relapse rates in primaquine, 80/53 and placebo groups

Phase III, double blind, prospective and non-crossover clinical trial was conducted at NIMR, Delhi comparing placebo, primaquine and 80/53 for anti-relapse activity during one year follow-up (Valecha *et al* 2001; Adak *et al* 2001).

A total of 697 patients of *P. vivax* malaria were enrolled and drugs were given for 5 days after treating acute episode with chloroquine. The doses used were primaquine 15 mg and bulaquine15 mg orally daily. During one year follow-up, relapse rates in placebo group were 40.18%, in primaquine group 26.8%, while in 80/53 group 29.6%. This shows that bulaquine was better than placebo and as effective as primaquine in preventing relapses (Fig. 14).

The drug has been registered and marketed for use as anti-relapse drug for *P. vivax* malaria in India. Further studies are being planned by MMV in collaboration with CDRI, NIMR and Nicholas Piramel.

Ayush-64

Ayush-64 is a combination of four plants namely *Alstonia scholaris* (aqueous extract of bark–1 part) *Picrorhiza kurroa* Royle (aqueous extract of rhizome–1 part), *Swertia chirata* (aqueous extract of whole plant–1 part) and *Caesalpinia crista* Linn (fine powder of seed pulp–3 parts).

The drug was patented by the Central Council of Ayurveda and Siddha and to confirm the efficacy in well-designed scientific trial, open prospective, non-crossover, randomised clinical trial was conducted

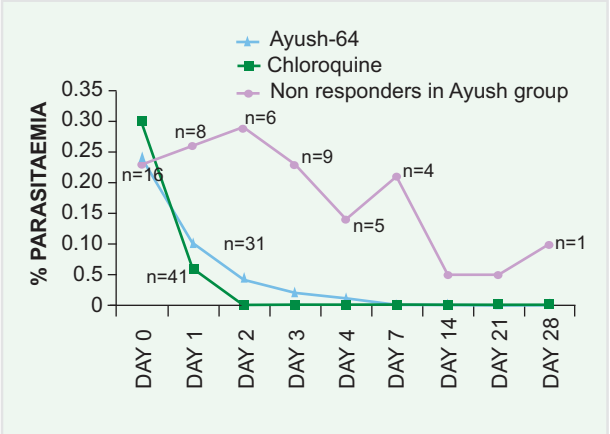


Fig. 15: Comparative parasite clearance time with chloroquine and Ayush-64

in *P. vivax* malaria patients at the Centre in collaboration with NAMP (Valecha *et al* 2000). Results showed that with Ayush-64 cure rate on Day 28 was 48.9% at a dose of 1 g three times a day for 5–7 days as against 100% with chloroquine 1500 mg over three days (Fig. 15).

Azithromycin

Azithromycin, an azalide antibiotic similar to erythromycin has been shown to possess good antimalarial activity in human malaria challenge studies. In addition, when used for treating other diseases the numbers of episodes of febrile parasitaemia due to *P. falciparum* were reduced. The drug has, therefore, been used in a number of studies and shown protective efficacy of 71–98% in falciparum and vivax malaria. The drug has an additional advantage that it can be given to pregnant women and infants. Considering the potential of the drug and the limitations of existing drugs to treat malaria, a randomised, double-blind, comparative Good Clinical Practice (GCP) study was undertaken to explore antimalarial activity of azithromycin in the dose of 1g/day for 3 days in vivax malaria in comparison to standard chloroquine treatment in the dose of 1500 mg over 3 days.

The results indicate that by the Day 7 more patients given chloroquine had resolved their fever compared with those who received azithromycin [99% (101 of 102) versus 88% (87 of 97), respectively; 95.1% CI on the difference: –18, –4] (Table 5) (Dunne *et al* 2005).

The clinical outcome measures are supported by parasitologic response. Patients receiving azithromycin were slower to resolve their parasitaemia compared with those getting chloroquine with 44 and 93% having negative peripheral blood smears at Day 3, respectively (Fig. 16).

In conclusion, azithromycin given as 1 g per day for three days resulted in an 88% clinical response rate by Day 7 but was not as active as chloroquine. Although it appeared to be better tolerated overall, the slower onset of action of azithromycin delayed the time

Table 5. Clinical response in patients treated with azithromycin and chloroquine

	Variable			
	Azithromycin n = 97 (%)	Chloroquine n =102 (%)	Difference (95.1% CI)	P-value
Clinical response				
Day 3	72 (74)	96 (94)	20 (–30, –10)	< 0.001
Day 7	85 (88)	101 (99)	11 (–18, –4)	0.003
Day 28	86 (89)	101 (99)	10 (–17, –3)	0.006
PC_{50} (h)	55	20		
PC_{90} (h)	96	40		

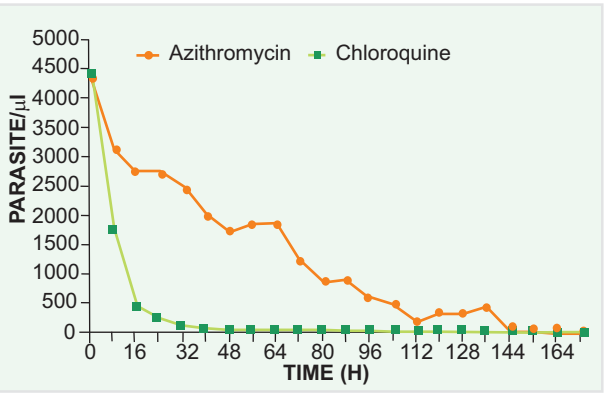


Fig. 16: Parasitaemia (mean) for patients treated with azithromycin and chloroquine (Ref.: *Am J Trop Med Hyg 2005; 73(6): 1108–1111).*

to clinical improvement. These data provide support for further study of azithromycin to better define its role in the treatment of *P. vivax* malaria, either alone as second line treatment or in combination with other active therapies.

Azithromycin has also demonstrated *in vitro* and *in vivo* activity against *P. falciparum*, but small treatment studies have given mixed results. A multicentric study of azithromycin, alone and in combination with chloroquine, for the treatment of acute uncomplicated *P. falciparum* malaria was undertaken in India. Participants with fever and with both a blood smear and a rapid diagnostic test positive for falciparum malaria were randomly assigned to groups that were treated with either azithromycin or chloroquine or to matched groups receiving a placebo. After an interim analysis, open-label combination therapy with both drugs was initiated.

On Day 28, 5 (33%) of 15 participants in the azithromycin-treated group had remained free of fever, compared with 4 (27%) of 15 in the chloroquine-treated group. All subsequently enrolled participants then received combination therapy with azithromycin and chloroquine. In 61 (91%) of 67 participants, resolution of fever and parasitaemia had occurred by Day 7, and through Day 28, no clinical or parasitologic relapse had occurred in them (Table 6) (Dunne *et al* 2005). In conclusion, resolution of parasitaemia was inadequate with monotherapy with either azithromycin or chloroquine, but combination therapy provided substantially improved clinical and parasitologic outcomes.

Arterolane (RBx 11160)

A phase II, double-blind, parallel-group, randomised, dose-ranging study assessing the antimalarial activity and safety of RBx 11160 administered for 7 days in patients with acute uncomplicated *P. falciparum* malaria was undertaken.

RBx 11160 (Arterolane) a new peroxide, is a synthetic trioxolane that is easy to synthesise, inexpensive, achiral and orally rapidly acting with high anti-malarial activity. It is a potential new anti-malarial

Table 6. Clinical-response data of monotherapy and combination therapy with chloroquine and azithromycin

Outcome	Monotherapy		Combination therapy		
	Azithromycin proportion (%)	Chloroquine proportion (%)	Difference 95% CI	Azithromycin and chloroquine	95% CI
Day 3					
Cure	9/16 (56)	14/16(88)	–63 to 7	61/64 (95)	87 to 99
Failure	7	2		3	
Day 7					
Cure	10/16 (63)	14/16 (88)	–58 to 13	61/63 (97)	90 to 99
Failure	6	2		2	
Day 28					
Cure	5/15 (33)	4/15 (27)	–29 to 46	61/63 (97)	90 to 99
Failure {relapse}	10 {4}	11 {9}		2	

agent with demonstrable activity in pre-clinical models and a substantial safety margin between an effective dose for malaria and the toxic dose. Mechanism of action is by reductive activation by haem, released as a result of haemoglobin digestion and irreversible redox reaction produces carbon-centered free radicals, leading to alkylation of haem and proteins (enzymes). One enzyme is the sarcoplasmic endoplasmic reticulum ATPase, PfATP6.

Pharmacological studies carried out indicate that RBx 11160 is safe and does not produce any clinically significant effect on behavioural parameters and cardiovascular systems. The present study was designed to assess the clinical safety and efficacy of three dose levels of RBx11160 (50 mg, 100 mg or 200 mg), administered for 7 days in patients with acute uncomplicated *P. falciparum* malaria. Primary objective of the study was to compare 3 (50, 100 and 200 mg) RBx 11160 dose levels administered orally for 7 consecutive days on time to 90% parasite clearance (PC_{90}) in patients with acute uncomplicated *P. falciparum* malaria and to identify the most appropriate dose of RBx 11160 for further investigation.

A double-blind, multicentric, randomised, parallel-group, dose ranging study of the antimalarial activity and safety of 3 (50, 100 and 200 mg) RBx 11160 dose levels administered as a single dose orally for 7 consecutive days was conducted as part of multicentric study in Thailand, Africa and India. Patients were randomised to 1 of 3 dose groups. The trial was conducted according to GCP guidelines.

Male or female patients aged 13 to 65 years, with no clinical evidence of severe malnutrition and presence of acute uncomplicated falciparum malaria with asexual parasitaemia between 1000 and 100,000 asexual parasites/µl blood were included.

The multicentric study was completed in 80 patients at Rourkela field unit (Table 7) in collaboration with Ispat General Hospital, Orissa. All the three doses resulted in parasite clearance in all the patients by Day 7 and drug was well-tolerated. Further studies are planned in combination with long-acting antimalarial. In conclusion, RBx 11160 (Arterolane) is an effective synthetic alternative to Artemisinin and further studies in combination with long-acting partner drugs are important.

Table 7. Final global enrolment status of the arterolone trial

Study centre	Patients enrolled
Bangkok, Thailand	90
Rourkela, India	80
Bagamoyo, Tanzania	43
Kivunge, Zanzibar	17
Total	230

Combination Therapy

Artesunate + Sulpha-Pyrimethamine

Artesunate with SP in the dose of (AS 4 mg/kg/day × 3 days; SP 500+25 mg/kg single dose) has been recommended as second line regimen in areas with >10% treatment failure with chloroquine in the national drug policy. The efficacy of regimen was evaluated in different epidemiological settings in India using standard WHO efficacy protocols. Although there is evidence of SP resistance in some areas and DHFR mutations are present (Sharma 2005), the regimen at present shows high cure rates of >95% in study sites in Madhya Pradesh, Orissa and Assam (Fig. 17). However, since this regimen is available as blister pack and not as fixed dose formulation, there may be issues of compliance.

Fig. 17: Study sites for efficacy of artesunate and sulpha-doxine-pyrimethamine

Artemether-lumefantrine

Artemether-lumefantrine is the only widely available co-formulated artemisinin combination therapy that is produced to international good manufacturing practice standards and has been adopted by many countries. Although one study was conducted in India in the year 2000 with four dose regimen of co-artemether, the drug was registered for restricted use only and has not been included in national programme till date. The present study is the first report on efficacy of co-artemether in two endemic regions of India following the WHO therapeutic efficacy protocols. The study was undertaken in Kamrup district, Assam and Keonjhar district, Orissa (Fig. 18). It was open single arm prospective trial based on 2003 WHO therapeutic efficacy protocols. The primary end point was 28 day cure rates. Secondary end points included proportion

Fig. 18: Study sites for efficacy of artemether-lumefantrine

of parasitaemic patients on Days 1, 2 and 3 and post-treatment gametocyte carriage.

Patients with microscopically confirmed *P. falciparum* malaria (asexual parasites 100–1000/µl) with fever or history of fever in proceeding 24 h and who gave voluntary consent were enrolled in local clinics. Pregnant or lactating women and children under 5 kg body weight were excluded. After complete physical examination, patients were given six dose regimen of artemether-lumefantrine with biscuits and glass of water. The treatment was directly observed and follow-up was done on Days 1, 2, 3, 7, 14, 21 and 28.The cumulative risk of failure was assessed by survival analysis with the Kaplan Meir method. The results indicated excellent efficacy and safety of the combination. On the 28 day, cure rate was 100% in Assam and 98.6% in Orissa.

DHA + Piperaquine

Phase III, randomised, non-inferiority trial, to assess the efficacy and safety of Dihydroartemisinin + Piperaquine (DHA+PPQ, Artekin) in comparison with Artesunate+Mefloquine (AS+MQ) in patients affected by acute, uncomplicated *Plasmodium falciparum* malaria was conducted.

Artekin™ was developed in China and is registered in China and Cambodia. It has been evaluated extensively in clinical trials in Thailand, Vietnam, Cambodia and China. Efficacy has been high and tolerability uniformly excellent in all trials in these multidrug-resistant areas. Artekin™ is a second generation artemisinin-based combination therapy (ACT) with similar efficacy to that of Coartem (artemether+lumefantrine) or Artesunate+Mefloquine but with a simpler dosing scheme that will aid better compliance. Moreover, its good safety profile and affordable cost make it ideal for resource constrained countries. DHA + PPQ is highly effective and tolerated by all age groups. DHA is active metabolite of Artemisinin Piperaquine related to CQ and has similar mechanism of action and is long-acting. The dose regimen includes 3-day therapy (2.1 mg/kg DHA and 16.8 mg/kg piperaquine). Reported side-effects are vomiting, dizziness and nausea.

The primary objective of the study was to measure the Day 63, PCR corrected cure rates of Artekin and AS+MQ and demonstrate that the cure rate of Artekin is non-inferior to that of AS+MQ (non-inferiority margin = 5%). The study was conducted at three sites in India, namely Goa, in collaboration with Goa Medical College & Hospital; Mangalore in collaboration with Wenlock District Hospital, Mangalore, and Guwahati in collaboration with Down Town Hospital (Fig. 19). Investigators meetings were held before starting the study.

This was a phase III, randomised, open-label, two arms study to include 1050 patients (700 DHA+PPQ and 350 AS+MQ) at all the sites in India and other countries. In order to ensure concealment of treatment allocation and avoid other biases the randomisation was under blind conditions and treatment allocation was concealed until the final recruitment of the patients. The primary endpoint was the PCR-corrected adequate clinical and parasitological response (PCR corrected ACPR) on Day 63. Patients classified as failures by clinical and parasitological criteria were considered ACPR if the

Fig. 19: Study site in Goa (above) and laboratory investigations in progress in Guwahati (below)

Table 8. Enrolment status for DHA + Piperaquine at study sites in India

	Guwahati	Mangalore	Goa
Number enrolled	69	57	28
Screen failures	1	3	0
Number finally enrolled	68	54	28
Number of patients completed the study	65	30	27

PCR analysis showed a new infection rather than a recrudescence.

Males and females aged ≥18 years having microscopically confirmed, monoinfection of *P. falciparum* (asexual forms parasitaemia ≥ 1000/µl ≤100,000/µl or mixed infection), history of fever or presence of fever (temperature at ≥ 37.5°C) were included. Tablets containing 40 mg of dihydro-artemisinin and 320 mg of piperaquine for adult patients or containing 20 mg of dihydroartemisinin and 160 mg of piperaquine for infants were administrated for three days. Altogether 154 cases of *P. falciparum* were enrolled at all the sites (Table 8). Artekin appears to be an effective and well-tolerated combination regimen.

Artesunate + Amodiaquine

Multicentre, open-label, randomised clinical trial of efficacy and tolerability of the fixed-dose artesunate/amodiaquine (AS/AQ) combination therapy and amodiaquine (AQ) monotherapy for treatment of uncomplicated falciparum malaria in India

DND*i* (Drugs for neglected diseases *initiative*) is working to test, register, and introduce fixed dose combinations of artesunate/amodiaquine (AS/AQ) for use in Africa and artesunate/mefloquine (AS/MQ) for use in Asia and Latin America. These combinations have already demonstrated excellent efficacy and rapid cure rates with acceptable tolerability when used as free associations. At present only one ACT (artemether- lumefantrine – Coartem®) is available as a fixed dose drug in which both compounds are co-administered. Co-packaging of the combination partner drugs in a blister is highly recommended in order to make them user-friendly, and to increase adherence to the complete therapy. DND*i* in association with UNICEF-UNDP-World Bank-WHO (TDR) is developing a new fixed-dose combination

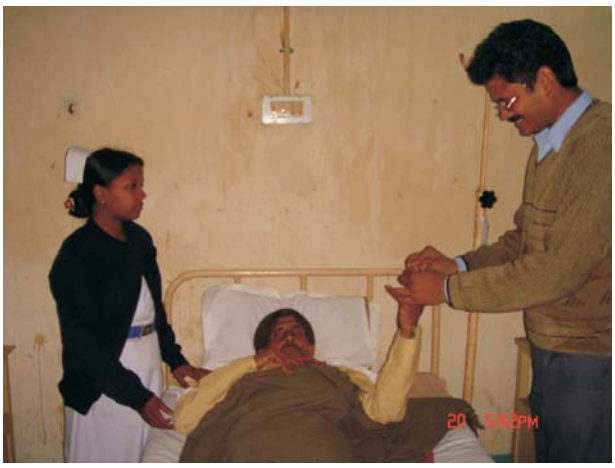


Fig. 20: Monitoring of patient at Mahadevi Birla Hospital, Ranchi

of artesunate and amodiaquine that will allow a simple treatment of just three days, with a single daily administration of two tablets. Since WHO has decreed that artemisinin-based combinations be used in malaria endemic countries, the testing of AS/ AQ is relevant for informing drug policy makers. Comparing AS/AQ with chloroquine, the current first line drug, would not be an optimal choice because chloroquine will have to be replaced in future. The present study with AS/AQ and AQ will generate data on amodiaquine for the first time in India.

Primary objective of the study was to measure the clinical and parasitological efficacy of the fixed-dose artesunate/amodiaquine combination therapy among children and adult patients suffering from uncomplicated falciparum malaria, by determining the proportion of patients having negative peripheral smear for malaria without relapse before 28 days (cure rate).

The study design was multicentre, open-label randomised clinical trial in two parallel groups: fixed-dose AS/AQ combination therapy (group A) and AQ monotherapy (group B). The study was conducted at Ranchi (Fig. 20) and Rourkela in collaboration with Community Welfare Society Hospital, Rourkela. Investigators meeting was held at Ranchi. The inclusion criteria were children and adults from 6 months to 60 years of age and *P. falciparum* parasitaemia in the range of (1000–100,000 asexual parasites/µl). Patients with any other concomitant condition, infection by *P. vivax* pregnancy, anaemia and deranged liver function tests were excluded. Although both the regimens were effective of enrolled 300 patients but the combination was superior to monotherapy.

❑

Development of Field Sites for Malaria Vaccine Trial

A study has been initiated to understand the epidemiology of malaria in Sundargarh, Orissa and Jabalpur, Madhya Pradesh with an objective to develop field sites for vaccine trial.

Site I: Sundargarh, Orissa

Sundargarh district is located in the Garhjat hills of eastern Deccan plateau between 21°35'N and 22°35'N latitudes, and between 83°32'E and 85°22'E

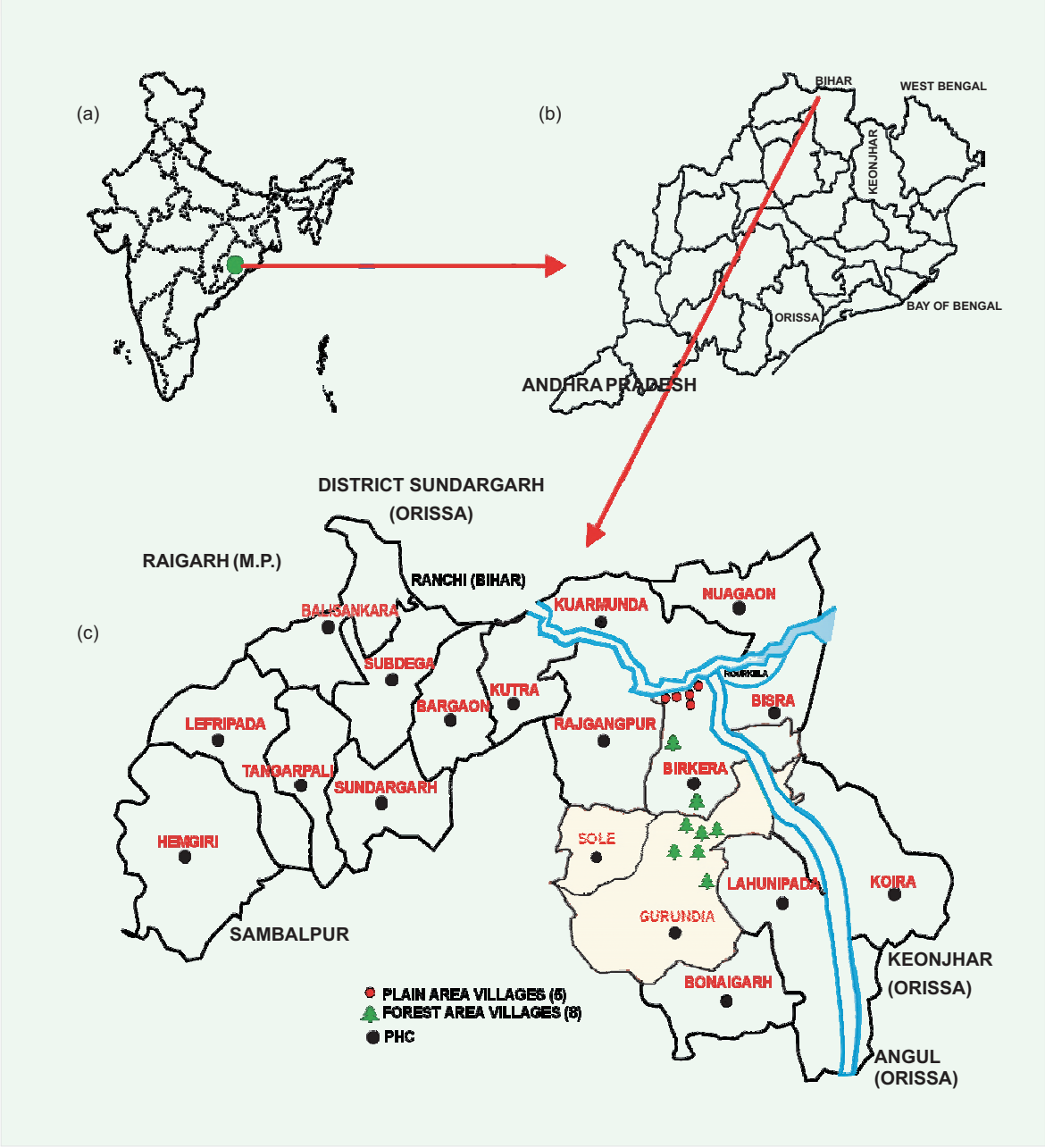


Fig. 21: Study area (a) India map showing location of Orissa state; (b) Orissa state showing location of District Sundargarh; and (c) District Sundargarh showing villages in two PHCs—Gurundia and Birkera

longitudes, at an altitude in the range of 200 to 900 m above the sea level. Topographically, the area presents ideal ecological conditions for malaria transmission with undulating uplands intersected by forested hills, rocky streams, and paddy fields. The area is characterized by a tropical humid climate and receives rainfall between June and September from the 'south-west monsoon' and in December and January from the 'northeast monsoon'. Average annual rainfall ranges between 160 and 200 cm and mean annual temperature ranges between 22 and 27°C. The weather seasons are: hot dry summer from April to mid-June, monsoon from mid-June to September, autumn from October to November, winter from December to January, and spring from February to March. The maximum temperature during summer rises to 40–45°C and the minimum temperature during winter falls to 5–10°C. A total of 51% of the area is covered with forests and is inhabited predominantly by tribals, that constitute 62% of the total population. The area is rich in mineral resources and industrialization based on these resources has lead to the development of new settlements, deforestation and ecological changes resulting in changes in malaria transmission patterns.

A study has been initiated to understand the epidemiology of malaria in Sundargarh, Orissa with an objective to develop a field site for vaccine trial. Initially, a study was conducted in 13 villages with a total population of 4221 under Gurundia and Birkera PHCs of Sundargarh district, out of which eight villages with a population of 2058 are located in deep forests and five villages with a total population of 2,163 are located in plain area (Fig. 21). The study villages are predominantly inhabited by ethnic tribals— Oram, Munda, Khadia, etc. Later, the study was extended to 35 villages (23 forested and 12 plain) with a total population 15,847.

Epidemiology of Malaria

Malaria is persistent throughout the year in both the areas but peak transmission was observed during post monsoon months–September, October and November. The proportion of *P. falciparum, P. vivax* and *P. malariae* species in the forest area was 85, 14 and 1 respectively, whereas it was 75, 25 and nil respectively in the plain area. A malaria episode was defined as a case where an individual had an axillary temperature more than 37.5°C and asexual forms of *P. falciparum*, *P. vivax* or *P. malariae* were detectable in thick blood smears. A second episode of fever occurring within 28 days of first episode was considered as recrudescence and treated as a single episode. A total of 3993 and 379 malaria cases were reported from forest and plain area villages respectively during the study period from January 2001 to December 2007. Over the 7-year study period an average of 65% of malaria cases were detected through active surveillance each year from the study area. The remaining malaria cases were detected through passive surveillance. The month-wise incidence of malaria in forest and plain areas during the study period is shown in Fig. 22. In the forest area, malaria transmission is perennial and fresh *P. falciparum* malaria cases are reported throughout the year. A similar pattern is seen in the plain area but the incidence is markedly low as compared to the forest area. The average number of *P. falciparum* cases/1000 population/year in the forest area was 225.4, whereas it was 17 in the plain area.

In the forest area, the average malaria incidence rate of *P. falciparum* (only first episode per individual per year) was ranging between 9.1 and 18% over different years and the highest incidence rate of 63.9% was recorded in the 1–5 years age group and it was inversely proportional to increasing age, whereas in the plain area, the incidence rate was

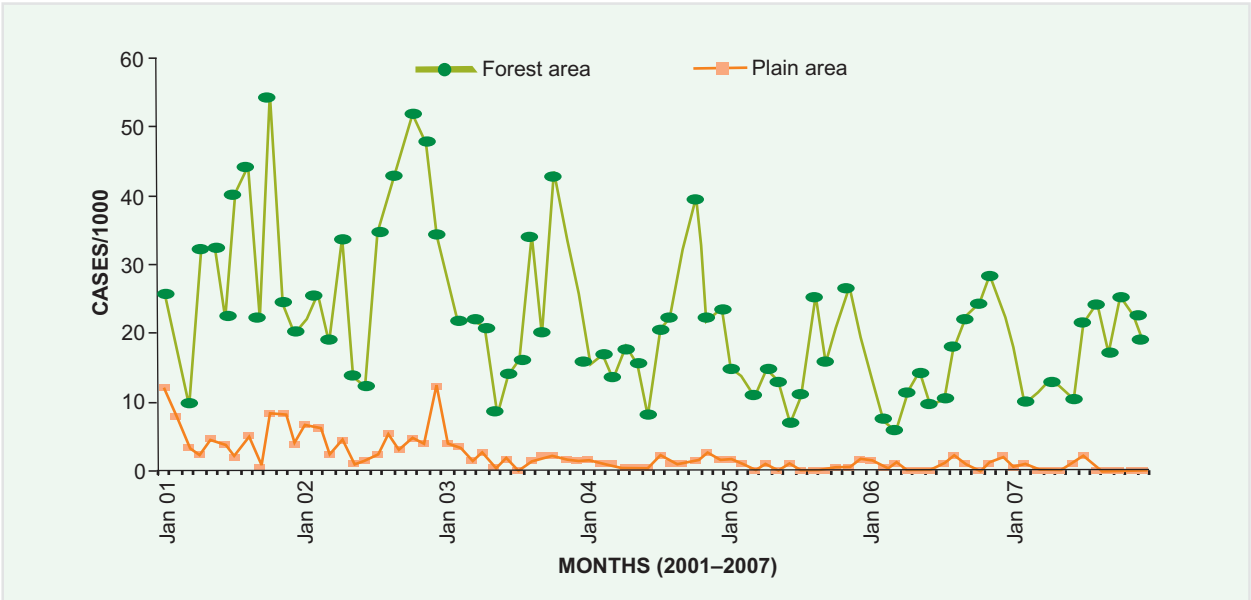


Fig. 22: Month-wise malaria incidence in the forest and plain area in Sundargarh district (2001–07)

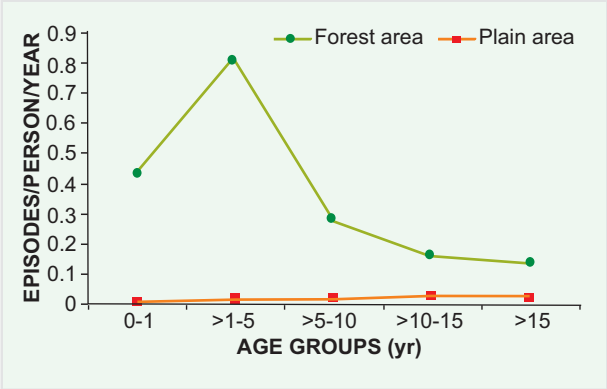


Fig. 23: Malaria attack rate due to *P. falciparum* in different age groups in the forest and plain area study villages during 2001–07

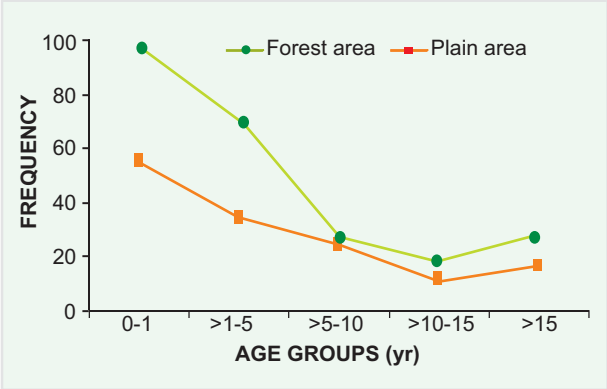


Fig. 25: Frequency of severe and moderate anaemia (< 70–100g Hb/l) in different age groups in the forest and plain area study villages recorded through cross-sectional malaria prevalence surveys

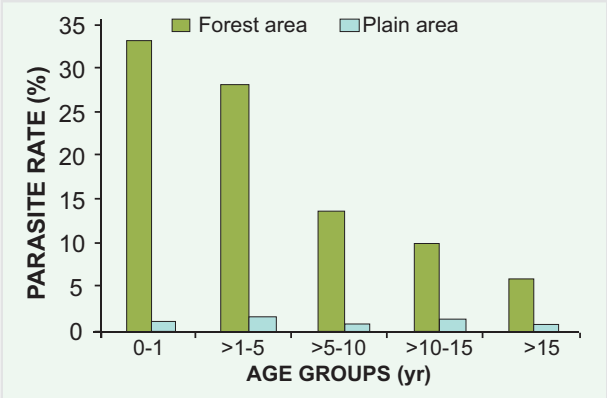


Fig. 24: Parasite rate in different age groups in the forest and plain areas in Sundargarh district as recorded through cross-sectional prevalence surveys during 2001 to 2006

low and ranged between 0.16 and 1%; and all the age groups were equally affected. The malaria attack rate due to *P. falciparum* infection (number of episodes per person per year) was determined in different age groups in both the study areas based on the weekly surveillance data collected over seven years from January 2001 to December 2007. The average attack rate in the forest area for all age groups over the 7-year period was 0.23 episodes/person/year respectively, whereas the average attack rate in the plain area for all age groups was 0.016. The difference in *P. falciparum* malaria attack rate between forest and plain areas was significant ($p<0.01$). In the forest area, the *P. falciparum* malaria attack rate varies with age (Fig. 23). The attack rate was found to be highest in 1 to 5 year old children during all the seven years of study. There was a gradual decline in the attack rate with increase in age. In the plain area, there was no correlation of attack rate with increase in age (Fig. 23).

Cross-sectional surveys were carried out each year in March, June and November representing intermediate, low and high malaria transmission seasons respectively. The average annual parasite rate in the forest and plain area was 12 and 1.3 respectively. The highest parasite rate in the forest area during these surveys was found in 1–5 years age group with a gradual decline as the age progresses, whereas in the plain area parasite rate was low and all the age groups were equally affected (Fig. 24). Out of total parasite positive cases found in the forest area during cross-sectional surveys, about 40% of the cases were asymptomatic and 9% were found gametocyte carriers. The average spleen rate in children and adults in the forest area was 82 and 20.5% respectively, whereas in the plain area it was 15.0 and 0.70% respectively. Estimation of haemoglobin in the study population showed that about 33% of the population in the forest area had moderate to severe anaemia, whereas in the plain area 20% population have anaemia of varying degree. About 18% of the population in both the areas was found to be G-6-PD deficient and 6% population in the forest area was found with sickle-cell trait (Table 9).

In the forest area, anaemia was absent in 26.7% of the persons screened, while 40.3% had mild anaemia and 33% had moderate to severe anaemia. The distribution of moderate to severe anaemia in

Table 9. Prevalence of G-6-PD deficiency in the forest and plain areas

Area	Population	No. tested	Normal	Deficient (%)	Malaria (*Pf*)	
					Normal	deficient (%)
Forest	2204	1133	926	207 (18.3)	301 (18.3)	92 (44.4)
Plain	2341	857	702	155 (18.1)	18 (2.6)	6 (3.9)

different age groups in the forest area is shown in Fig. 25. The highest prevalence of moderate to severe anaemia was found in 0–1 year old children (94.7%) followed by 1–5 year old children (68.2%). The frequency of incidence of moderate to severe anaemia decreased with age with incidence dropping to 16.9% in 10 to 15 year old children. In the plain area, 29.6% of the population screened had normal haemoglobin values, while 49.1% had mild anaemia, 20.8% had moderate anaemia and 0.5% had severe anaemia. The distribution of moderate to severe anaemia in different age groups in the plain area is shown in Fig. 25. The prevalence of moderate and severe anaemia in different age groups was similar to the forest area but the frequency of anaemia was significantly lower in comparison to the forest area ($p<0.05$).

Entomological Studies

During the study period, 15 anopheline species from the forest area and 13 species from the plain area were recorded and the percent composition of different *Anopheles* species in both the areas is shown in Table 10. Two primary vector species, *An. culicifacies* and *An. fluviatilis* were found in the forest area whereas the latter species was altogether absent in the plain area. *An. culicifacies* was most prevalent species and constituted 39.1 and 36.1% of all the total anopheline species in the forest and plain area respectively. *Anopheles fluviatilis* constituted 7.1% of all the anopheline species recorded from the forest area. *Anopheles annularis*, which is a secondary malaria vector in some parts of India but not incriminated as a vector in Sundargarh district was also found in good numbers and comprised 11.6 and 16.4% of the total species in the forest and plain areas respectively.

The month wise person-hour density (PHD) of *An. culicifacies* and *An. fluviatilis* in both the areas is shown in Fig. 26. The relative abundance of *An. culicifacies* was high throughout the year, although there were wide seasonal fluctuations in the density of *An. culicifacies* in both the areas. In the forest area, highest density of this vector species was observed between February and August and lowest during September to January. In the plain area, although the density fluctuations of this species were similar but peak was observed during January. The distribution pattern of this species in the forest and plain areas was almost similar and small density variations were insignificant. *Anopheles fluviatilis,* which was found only in the forest area, maintained low density throughout the year (range: 0.01 to 16.3). The highest prevalence of this species was recorded during post-monsoon months of September to December and lowest during hot dry months of May and June.

Mosquito blood meal analysis of the vector species revealed that the human blood index (HBI) of *An. culicifacies* and *An. fluviatilis* was 0.007 and 0.98 respectively, showing that *An. culicifacies* was

Table 10. Percent composition of anopheline species in forest and plain area of Sundargarh district as recorded through indoor resting collections from January 2001 to December 2007

S.No.	Species	Forest area (%)	Plain area (%)
1.	*An. culicifacies*	6737 (39.1)	7781 (36.1)
2.	*An. fluviatilis*	1222 (7.1)	0
3.	*An. annularis*	2001 (11.6)	3540 (16.4)
4.	*An. subpictus*	3414 (19.8)	4218 (19.6)
5.	*An. vagus*	2148 (12.4)	2649 (12.3)
6.	*An. pallidus*	940 (5.4)	1409 (6.5)
7.	*An. aconitus*	147 (0.8)	1323 (6.1)
8.	*An. nigerrimus*	277 (1.6)	348 (1.6)
9.	*An. barbirostris*	111 (0.6)	109 (0.5)
10.	*An. varuna*	32 (0.2)	5 (0.02)
11.	*An. splendidus*	178 (1.0)	112 (0.5)
12.	*An. tessellatus*	23 (0.1)	46 (0.2)
13.	*An. ramsayi*	5 (0.03)	2 (0.009)
14.	*An. jamesi*	2 (0.01)	1 (0.005)
15.	*An. jeyporiensis*	4 (0.02)	0
	Total	17241	21543

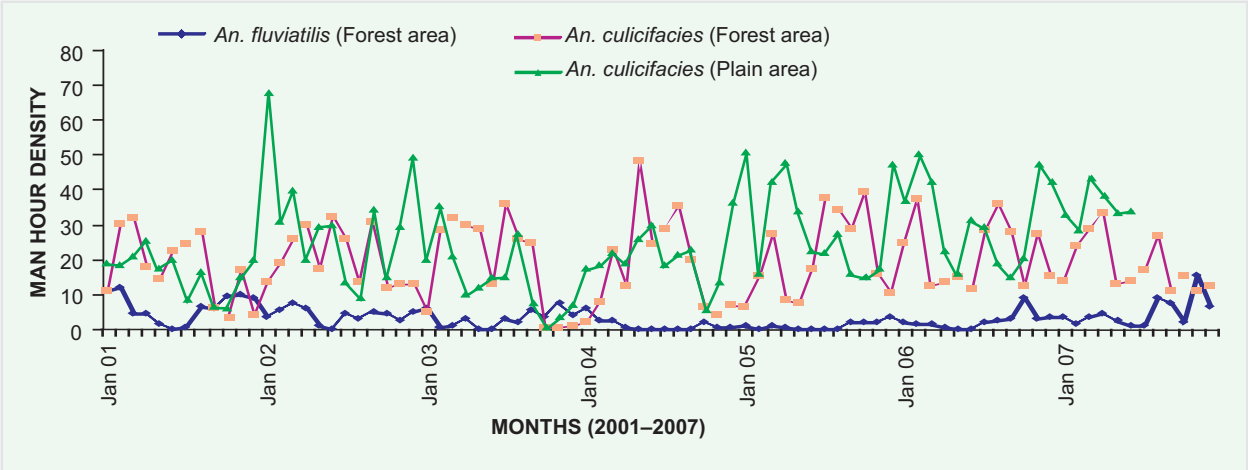


Fig. 26: Density of *An. culicifacies* and *An. fluviatilis* in the forest and plain areas of Sundargah district (2001–07)

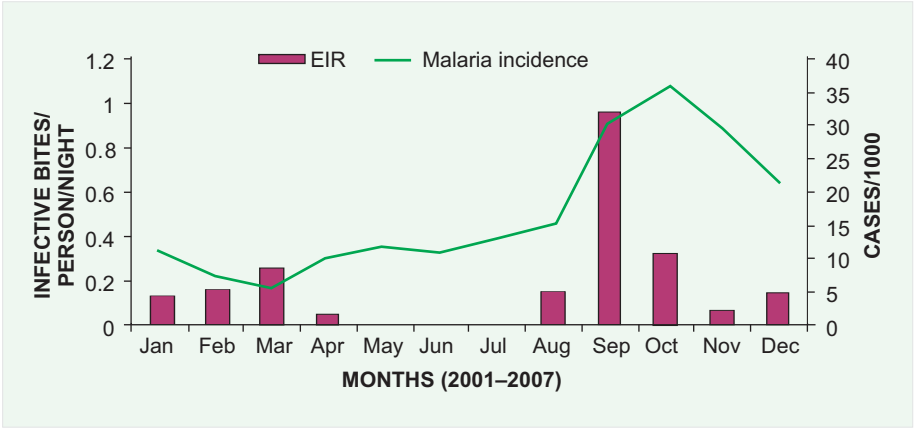


Fig. 27: Relationship of entomological inoculation rate (EIR) with malaria incidence (2002–06)

primarily a zoophagic species whereas *An. fluviatilis* is strongly anthropophagic. The results of blood meal analysis were also supported by direct observations on the biting activities of these species during whole night human bait collections. Transmission load in two areas during different transmission seasons was estimated by calculating the entomological inoculation rate (EIR). The EIR in the forest area varied significantly during different seasons of the year. The EIR during low, intermediate and high transmission seasons was 0.02, 0.085 and 0.35 infective bites per person per night respectively thereby showing high transmission load during most part of the year. In the plain area, EIR was 0.005, 0.005 and 0.014 infective bites per person per night respectively in low, intermediate and high transmission seasons. There was direct correlation of EIR with the malaria incidence in the study area (Fig. 27).

Genetic Diversity Studies

Plasmodium falciparum field isolates were collected in two ecosystems i.e. forest and plain with differential malaria transmission of District Sundargarh, Orissa. A total of 270 field isolates of *P. falciparum* were collected on filter paper strips and of which 230 were from villages of forest and 40 from villages of plain ecotypes. These isolates were collected at different transmission seasons and the genotypes of the parasite population were determined by amplification of polymorphic regions of two *P. falciparum* antigen genes: *MSP-1* (block 2) and *MSP-2* (block 3) by family specific nested polymerase chain reaction (PCR).

Study revealed highly polymorphic nature of both markers namely *MSP-1* and *MSP-2* in study isolates of two ecotypes, however, allelic diversity observed was slightly higher in the forest ecotype compared to the plain ecotype. *MSP-1* was represented by three reported families, namely K1, MAD20 and RO33 and two families, namely FC27 and 3D7 of *MSP-2*. It is revealed that composition of *MSP-1* and *MSP-2* families was same among the isolates of two ecotypes though variations in the proportional prevalence of families were observed.

A good number of isolates showed multiple infections of *MSP-1* and *MSP-2* judged on the basis of presence of more than one family or presence of more than one PCR fragment (allele) of the family in the same individual. Proportion of isolates with multiclone was significantly higher in the forest ecotype with high malaria transmission ($p<0.002$ for *MSP-1* and $p<0.026$ for *MSP-2*) and vice versa for single clone isolates in plain ecotype ($p<0.003$ for *MSP-2*) with low malaria transmission (Fig. 28).

Among 270 samples at *MSP-1* locus, 8 alleles of the K1 family ranging from 125 to 300 bp, 8 alleles of MAD20 ranged from 110 to 270 bp and monomorphic RO33 family having single allele of 160 bp were observed. For *MSP-2* locus, 12 alleles of FC27 ranged from 200 to 600 bp and 13 alleles of 3D7 ranging from 370–660 bp were observed. Fig. 29 shows the agarose gel electrophoretogram showing allelic variations in families of *MSP-1* and *MSP-2*.

Presence of all the allelic families of *MSP-1* and *MSP-2* among the isolates of both the ecotypes suggests for the same population in both the ecotypes. Absence of any significant difference in the prevalence of *MSP-1* and *MSP-2* families among isolates of forest and plain ecotypes could be due to the close proximity of both the ecotypes (located within a distance of 20–30 km) and population

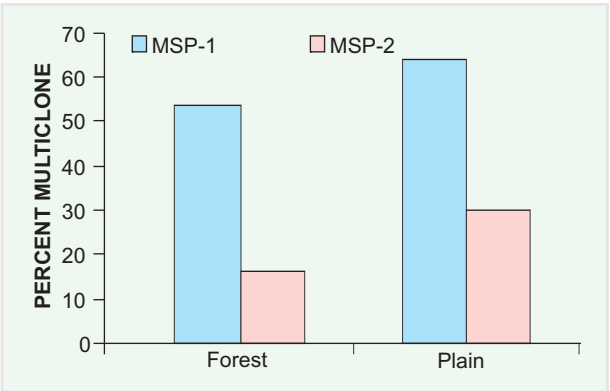


Fig. 28: Proportion distribution of multiclone isolates in forest and plain ecotypes of Sundargarh, Orissa

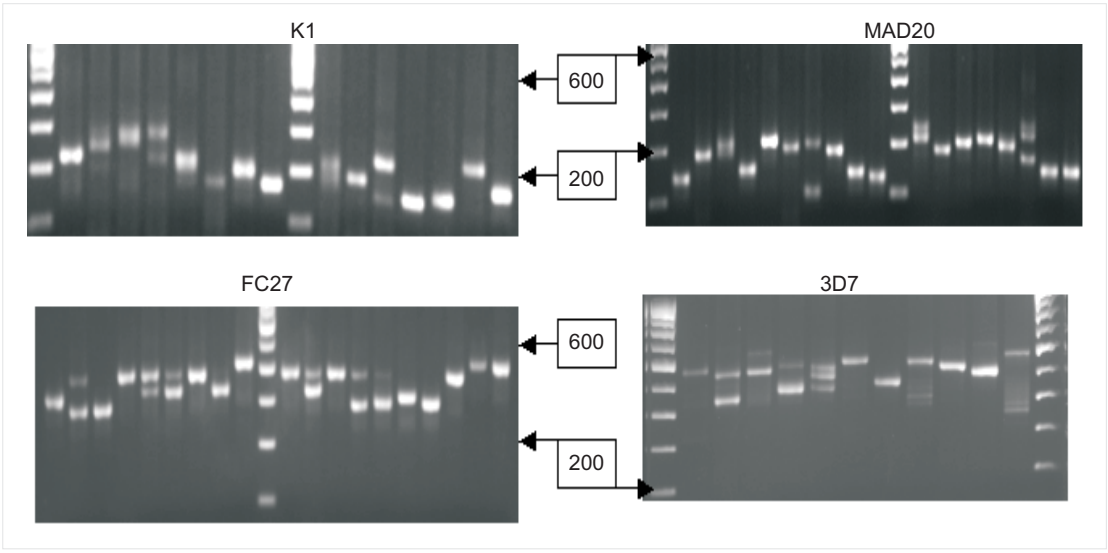


Fig. 29: Allelic polymorphism observed among *Plasmodium falciparum* field isolates of District Sundargarh, Orissa

movement between the two ecotypes thus supporting a common pool of parasites in both the ecotypes.

A high level of genetic diversity was present in isolates of both forest and plain ecotypes of District Sundargarh, Orissa. A significantly higher proportion of multiclone infections of different genotypes in isolates of forest ecotype, compared to plain ecotype may be attributed to the differential vector potential of the species (*An. fluvitilis*) prevalent in the area which is reflected in the malaria transmission pattern also, thus, supporting positive association of multiclone infection with high malaria transmission. Of both the marker systems, *MSP-2* has shown generally a higher level of polymorphism, revealed higher proportion of multiple genotype infections as well as higher multiplicity of infection (MOI) in both the transmission seasons as well as both ecotypes. MOI has shown an increase from lower to higher transmission season.

Sequence Diversity

The sequence diversity in three malaria vaccine candidates, namely MSP-1_{19} (C- terminal 19 kDa fragment of MSP-1), EBA 175-RII and TRAP was determined in *P. falciparum* isolates collected from forested villages. Sequencing of 16 field isolates for MSP-1 has shown polymorphism only at 5-amino acid positions. Out of which four were reported earlier by other workers. Sequencing of TRAP N-terminal region in field isolates showed polymorphism at 25 sites, and three were reported for the first time. Sequencing of EBA-F2 region in 16 field isolates has shown polymorphism at 19-amino acid positions. Only five of these polymorphisms were reported between different strains. The study further revealed that a few selected amino acids are targeted for change. This selection may be to maintain non-synonymous polymorphism in EBA region II, thus, not affecting the functional aspects. Significant levels of cross-reactive antibodies are generated against different PfMSP-1_{19} allelic forms in a *P. falciparum* infected natural human population. Studies further indicated that antibodies elicited by the vaccine candidate, region F2 of EBA-175 (Camp strain), can block the binding of variant F2 regions observed among the field isolates with similar efficiency suggesting that the binding site within F2 may be conserved. Observation that anti-F2 (Camp) sera blocks binding of diverse variants with comparable efficiency provides support for the development of recombinant F2 as a blood stage vaccine for *P. falciparum* malaria.

Immunological Profile

Finger-prick blood samples were collected from different age groups by repeated cross-sectional surveys at two sites each of forest and plain areas during low and high transmission seasons. Indirect ELISA was done to measure the antibody levels against *P. falciparum* MSP-1_{19}, EBA-175 and TRAP antigens in 222 (110 from forest and 112 from plain areas) and 248 (138 from forest and 110 from plain areas) blood samples collected during low and high transmission seasons, respectively.

In the forest area, *P. falciparum* infection was detected in 12.7% (14/110) persons during low transmission phase, whereas in plain area among 112, only 3 (2.7%) were found positive. During high transmission, *P. falciparum* positivity was detected in 28.2% (39/138) in the forest area and 8.2% (9/110) in the plain area. It was observed that overall IgG profiles against MSP-1_{19}, EBA-175 and TRAP were higher in study subjects of forest area than plain in both low and high transmission seasons (Figs. 30–33). The age-dependent increase of specific antibody levels was noticed in individuals of two areas in both the seasons. The mean ELISA O.D. was significantly lower in children <5 years compared to adults ($p<0.001$). Proportion of high responders was more in adults than in children ($p<0.01$). However,

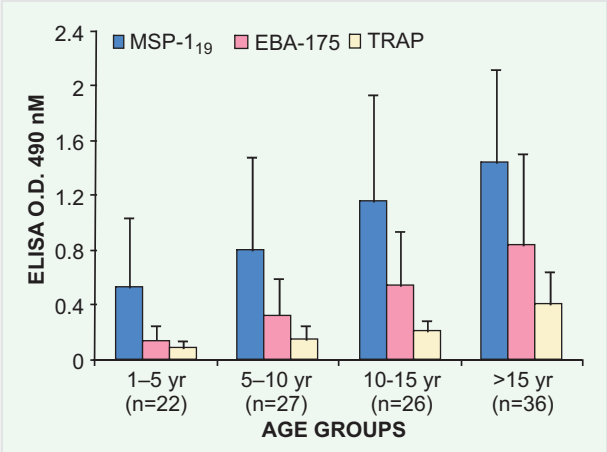


Fig. 30: Prevalence of MSP-1, EBA-175 and TRAP in different age groups in low transmission forest area in Sundargarh district, Orissa

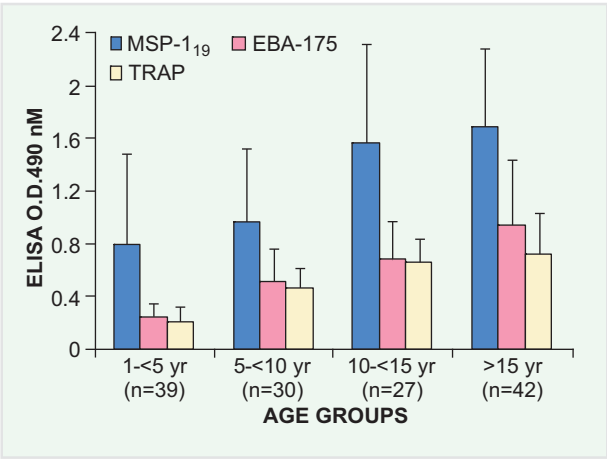


Fig. 31: Prevalence of MSP-1, EBA-175 and TRAP in different age groups in high transmission forest area in Sundargarh district, Orissa

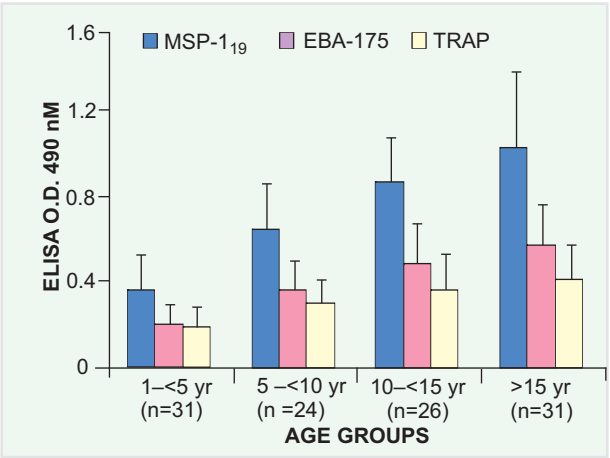


Fig. 32: Prevalence of MSP-1, EBA-175 and TRAP in different age groups in low transmission plain area in Sundargarh district, Orissa

acquisition of antibodies during the time of high transmission phase was more compared to low transmission (Fig. 34). A total of 127 and 68 blood samples, collected from the forest and plain areas during mass survey were tested for their IgG subtypes. Age-wise increase in IgG level has been observed in individuals of both forest and plain areas.

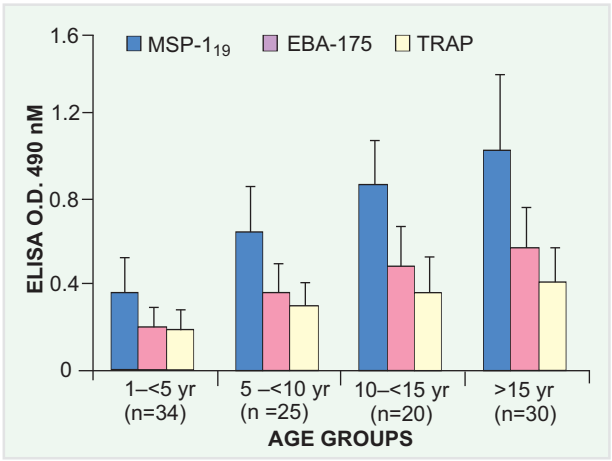


Fig. 33: Prevalence of MSP-1, EBA-175 and TRAP in different age groups in high transmission plain area in Sundargarh district, Orissa

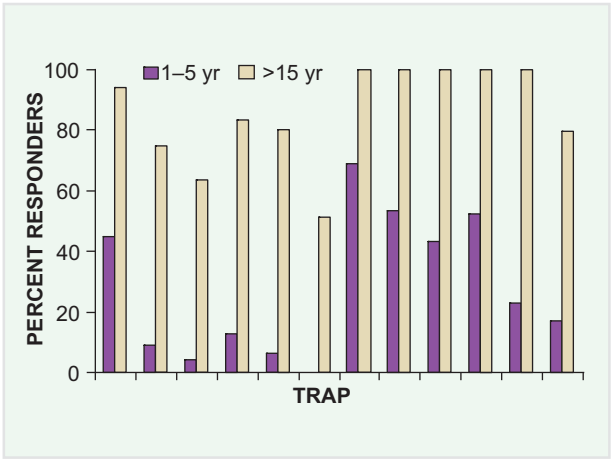


Fig. 34: The responder frequency of children and adults for TRAP in District Sundargarh, Orissa

The IgG1 and IgG2 were the predominant subclass responses to all three antigens (Figs. 35–37).

In another set of repeated cross-sectional surveys at four sites each of forest and plain areas, individuals were categorized on the basis of fever and/or presence or absence of *P. falciparum* infection. In afebrile (healthy) and febrile/non-malarial subjects, IgG levels against all three antigens were higher than febrile/*Pf*+ patients. However, antigen specific IgM was higher in this group compared to afebrile/healthy and non-malarial fever cases.

Overall IgG profiles against MSP-1$_{19}$, EBA-175 and TRAP were higher in study subjects of the forest area than plain in both low and high transmission seasons. The age-dependent increase of specific antibody levels was noticed in individuals of two areas in both seasons. Boosting in antibody production has been observed against these molecules by natural infections.

On the basis of existing epidemiological data as well as immune status of the study population, the children in the age group of 1–5 years are eligible for vaccine trial in the forest area. The required number of target children for vaccine trial will be sufficiently met out of the existing study population.

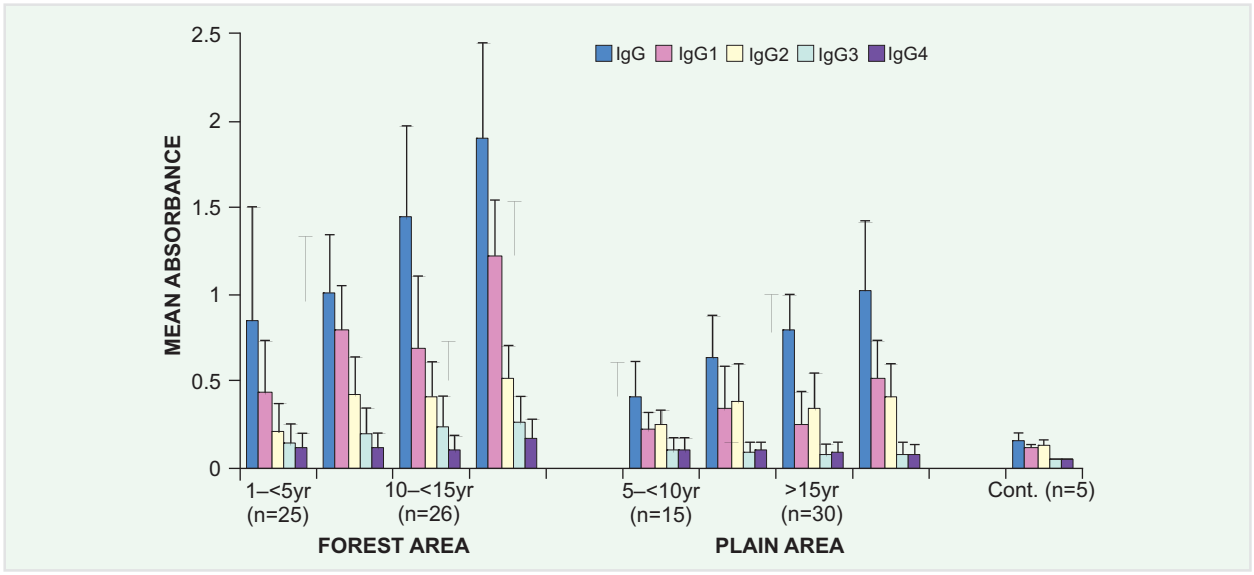


Fig. 35: IgG subtypes profile in different age groups against MSP-1_{19} in Sundargarh district, Orissa

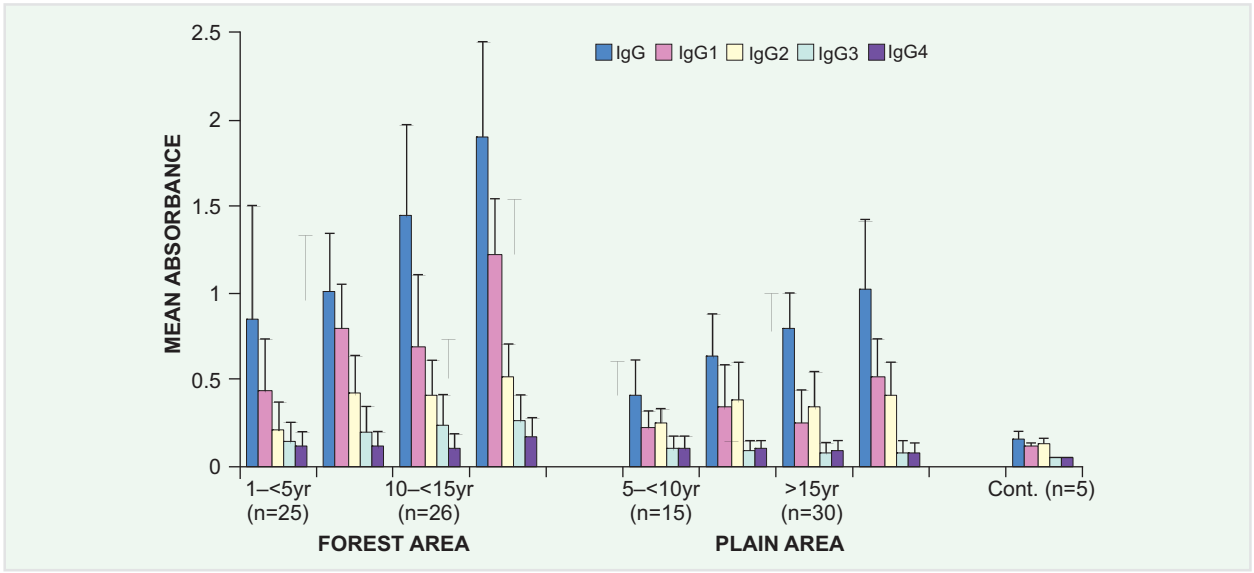


Fig. 36: IgG subtypes profile in different age groups against EBA-175 in Sundargarh district, Orissa

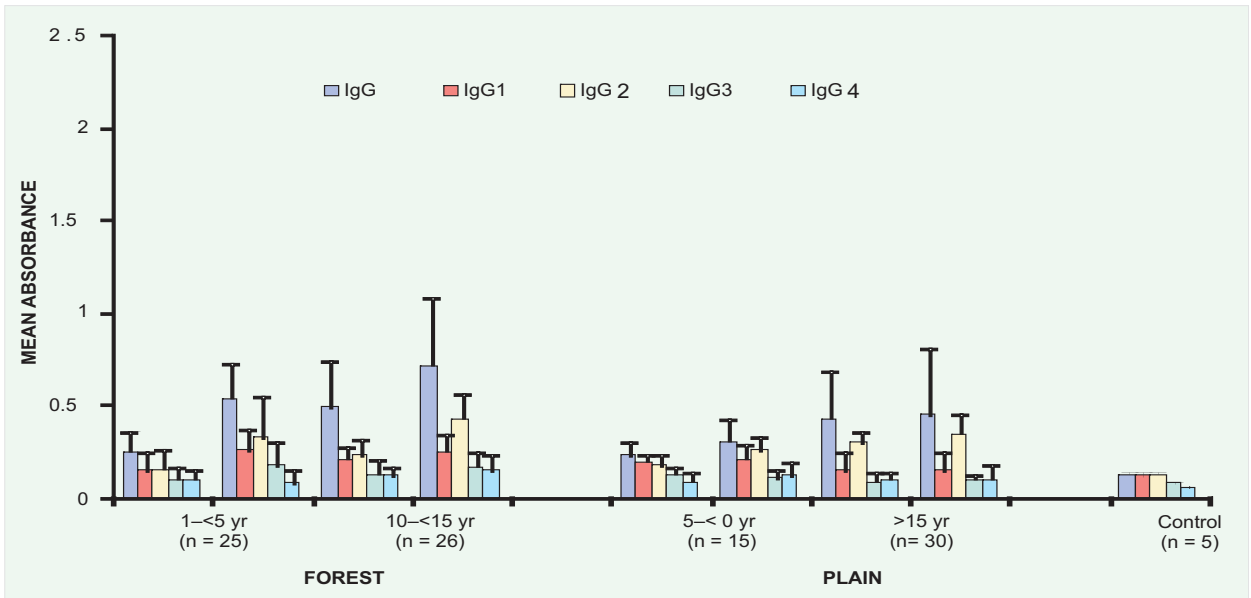


Fig. 37: IgG subtypes profile in different age groups against TRAP in Sundargarh district, Orissa

GIS Database

Village boundaries of plain area villages, namely Balupatra, Chikatmati, Sarala, Mahaliapalli and Mallikpalli were digitized showing landscape features such as highways, village roads, walk ways, rivers, canals, branch canals, water bodies, houses, schools, shops, clubs, churches, industries, open space, rice-fields, *etc*.

Data Architecture

A three-tier GIS database has been generated. First level— village-wise data, which include census information and malaria data; second—house-wise data, where data of individual house pertaining to house number, number of rooms in the house, type of house—kuchcha or pucca/human dwelling or mixed dwelling, name of the headman, number of persons in the house, their names, age, sex, religion, tribe, income, *etc*, number of animals in the house and malaria history (houses have been depicted by square blocks on the village boundary); and third level—personal level data, namely name, age, sex, marital status, education level, occupation, malaria history up to four malaria episodes of each person in the village have been included. Persons in the houses have been shown by dots. Number of dots in a house (shown by boxes) show number of persons (Fig. 38).

Out of five plain area villages house-wise data of three villages have been obtained and put in the GIS database. Forest villages data are being processed for developing GIS data base.

Functionality of the System

1. Information of any village/house or person can be retrieved at the click of the mouse within village boundary/house/dots respectively on the map.
2. Using zoom-in facility one can blow up houses and can see number of persons, by assigning different colour to positive and negative cases both for houses or persons, one can see the house-wise malaria spread or in houses how many persons are sick to evaluate the disease scenario.
3. Malaria epidemiology can be studied both in space and time where change in malaria situation in any village can be correlated to any specific breeding site or the activity in that area to take situation-specific control measure.
4. Percent composition of any parameter can easily be mapped to review the situation. For example, if one needs to know the per cent parasite composition— *Pv* and *Pf*, instantly situation of the entire area/houses can be known.

Depending upon the requirement, database can be tailor-made and so is the analysis algorithm to achieve the desired result.

Site 2: Jabalpur, Madhya Pradesh

This study was initiated in June 2005 with the objective to develop a well characterized site, where the epidemiology of the disease, immune response to malarial antigens, diversity of parasite genes and vector characteristics are well understood. The study has four arms which are as under:

(a) Epidemiology
(b) Immunoepidemiology
(c) Molecular epidemiology and
(d) Entomology

Epidemiologic Study

The main objectives of the study were based on: (i) to measure the rate of morbidity and mortality in all age groups in a selected population particularly infants, children and pregnant women; (ii) to

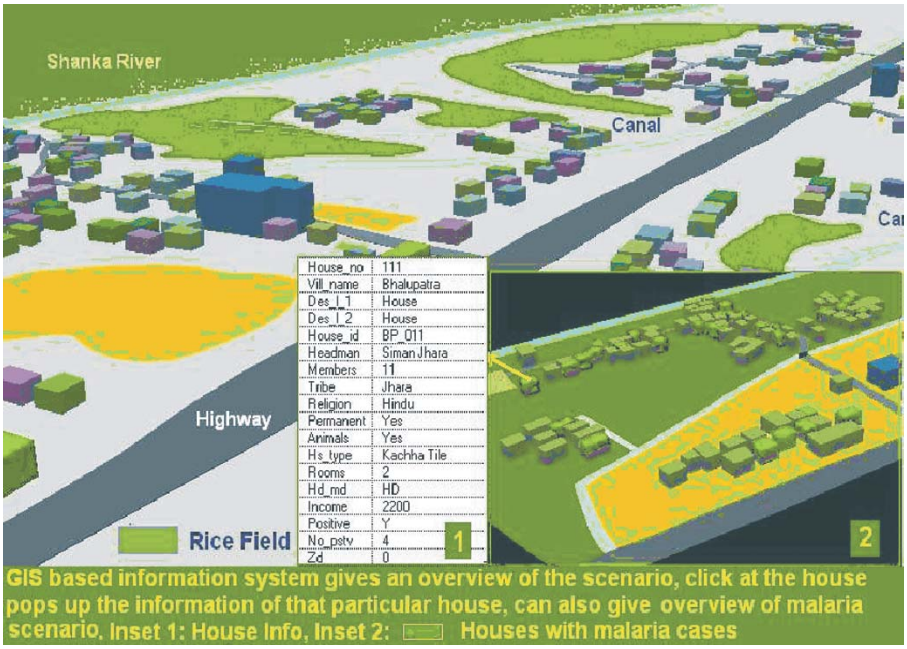


Fig. 38: GIS-based information system of a study village in Sundargarh district, Orissa

Fig. 39: Study villages in Jabalpur (M.P.)

determine the frequency and level of parasitaemia, onset of fever due to *P. vivax* and *P. falciparum* in different age groups; (iii) to examine the relationship between placental malaria and infant low birth weight and mortality.

This study was started in November 2005 in the Bargi PHC of District Jabalpur (Fig. 39). In the cohort, nine villages (2414 persons) were on the bank of Bargi dam reservoir, 17 in forested (8831 persons) and 36 (21,455 persons) in the plain area (Fig. 40). The cohort was established by conducting baseline census in the study area to generate a real picture of different settings.

Baseline census of study villages consisted of collection of demographic, socio-economic information of all the individuals in the study area. A household is defined as a group of people living under one roof and sharing the same cooking spot/pot. At registration an identity number is allocated to each household which is painted on top of the front entrance door frame of the household. Thereafter every cooking pot sharing unit is denoted by the unique identifier number as a single household (Fig. 41). Quality assurance was maintained by conducting re-interview of 5% randomly selected households. Locally designed software in ADO.NET is used for data entry. The system is developed based on the MS SQL 2000 along with data collection and entry guidelines of Household Registration System Software, Population Council (Flowchart 1).

Baseline population of the study area is 32,700 residing in 5813 households of 62 villages. Average family size is 5.6 per household. This population is

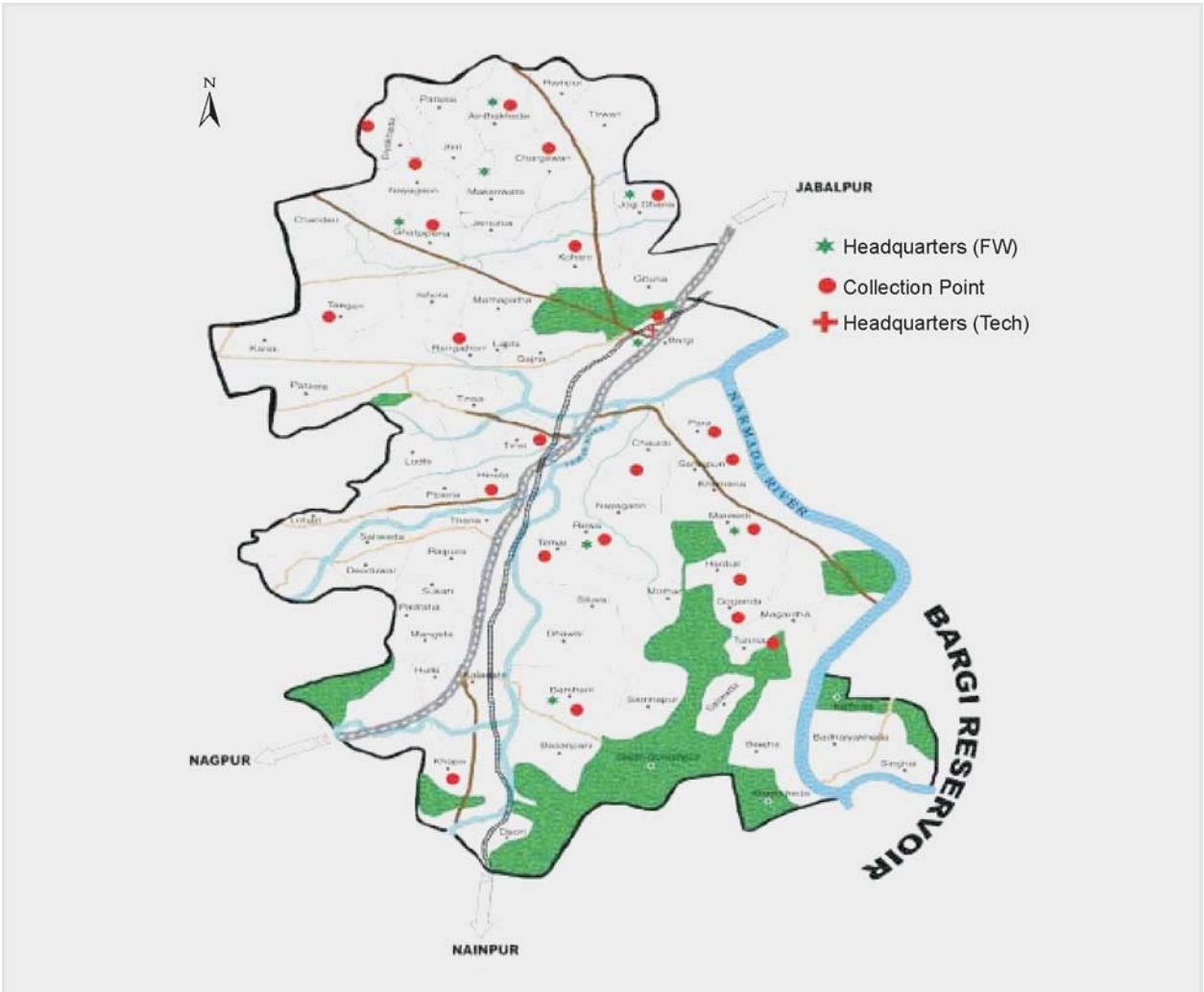


Fig. 40: Map of Bargi PHC showing study villages

Flowchart 1: Demographic database management

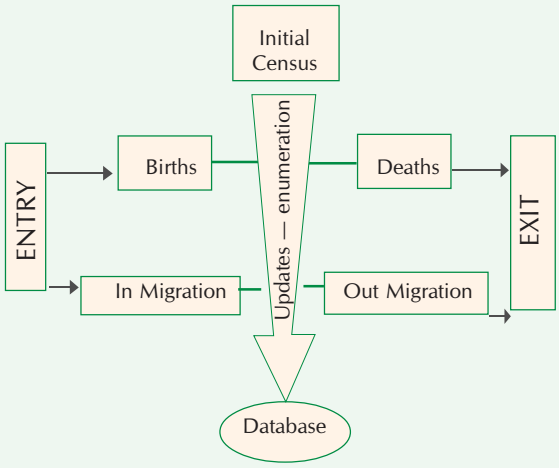


Table 11. Baseline census at a glance

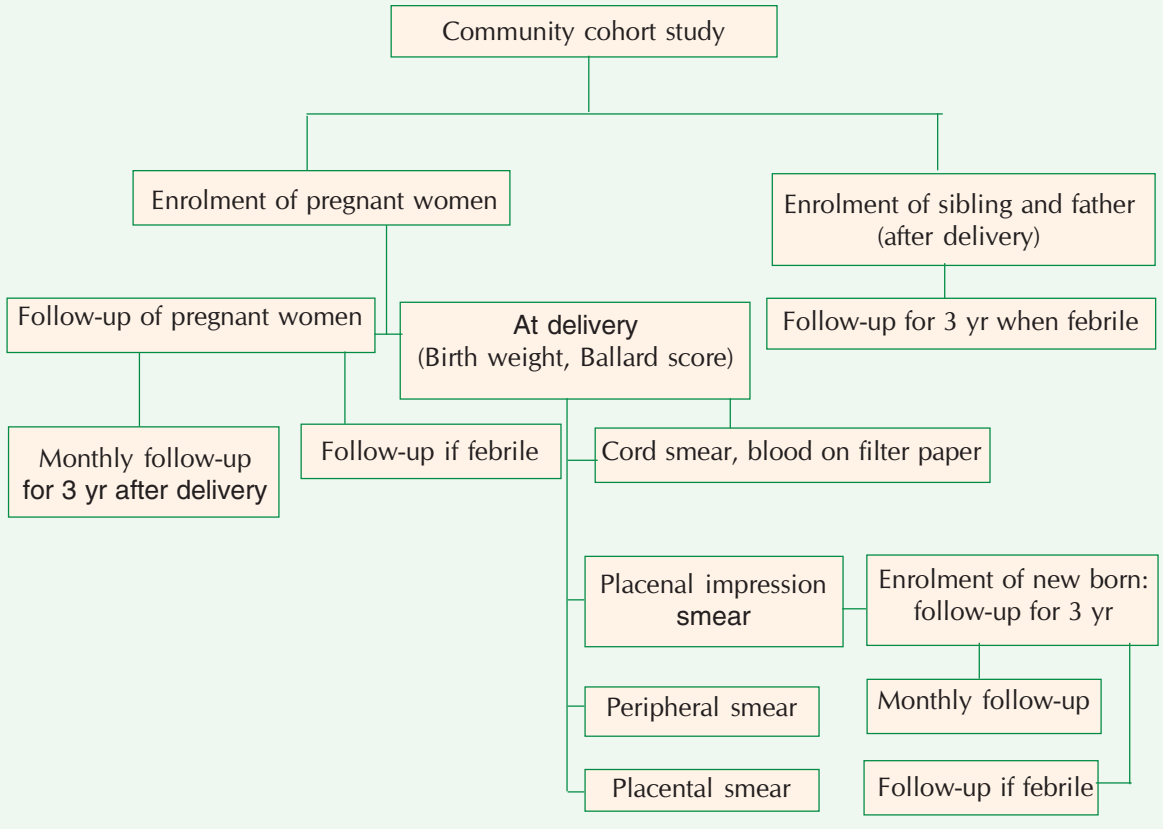
Age interval	Population	Male	Female
0–1	1357	701	656
>1–4	2636	1341	1295
>4–8	3600	1778	1822
>8–14	4691	2396	2295
>14–18	2332	1320	1012
>18	18084	9155	8929

mainly ethnic 'Gond' tribes (54.4%). Age-wise distribution of the total population under different age groups is given in Table 11. Sex ratio is 959 female/ 1000 male populations.

Pregnant women were identified and enrolled in the study with or without fever after obtaining written informed consent. At the time of enrolment, data concerning reproductive history indicators were collected. Each woman was followed every month during her pregnancy. Both current and recent clinical

Fig. 41: Numbering system for identification (Location ID) of houses

Flowchart 2. Community cohort study



signs and symptoms associated with malaria and antimalarial treatment was recorded. Thick and thin film prepared and finger-prick blood samples were collected for measurement of hemoglobin (Hb), immunological and molecular biological study during follow-up (Flowchart 2).

In all 23 sample collection points were established to collect placenta samples at the time of delivery with the help of traditional birth attendant and to keep them at our sample collection points. A cold chain was also established in these sample collection points. Delivery questionnaire was filled to collect information about the clinical sign of malaria and any other complications during delivery. Maternal peripheral blood smear, birth weight, gestational age and birth outcome was recorded.

Mother-infant pairs were followed-up every month after delivery. Questionnaire was filled during follow-up to obtain information on each child's health and axillary body temperature. Simultaneously, father and siblings were also enrolled after delivery and followed-up every month for clinical signs and as symptoms associated with malaria and antimalarial treatment. Blood samples collected from study subjects were used in immunologic and molecular biologic studies. G-6-PD and sickle-cell trait were examined in all enrolled subjects. In addition, fever surveys were also carried out weekly to measure the rate of morbidity and mortality in all age groups in the cohort area.

Hospital-based cross-sectional study was carried out at NSCB Medical College, Jabalpur and Civil Hospital, Maihar. Patients were screened for malaria parasite and malaria positive patients were enrolled in the study after obtaining written informed consent. All fever cases were given presumptive treatment and all subjects found positive for malaria were treated as per guidelines of NVBDCP within 24 h.

In all 1100 pregnant women (PW) were enrolled in the community cohort (Fig. 42). At the time of enrolment only 14.5% PW were having fever or history of fever and 37 PW were found positive for malaria (16 *Pv*, 21 *Pf*) of which only 14 were symptomatic and remaining 23 asymptomatic. During

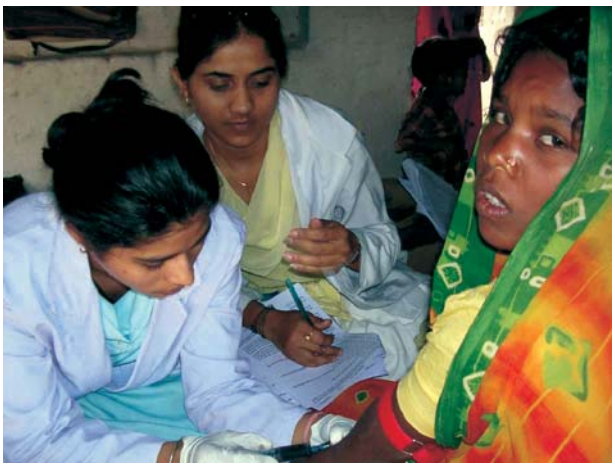


Fig. 42: Enrolment of pregnant women

the follow-up a total of 36 PW were found infected for malaria (17 *Pv*, 19 *Pf*) of which 37% were febrile (Table 12) while 38% infected PW having repeated episode of malaria during their pregnancy and post-pregnancy (Table 13). More than 90% PW were followed successfully till delivery while the overall follow-up rate among the PW was 68%. Mean haemoglobin of these PW were 10.04 ± 1.71 g% (4.7–16.6). Mild anaemia (9.86 ± 0.55 g%) was recorded in 52% of the subjects, moderate anaemia (8.30 ± 0.52 g%) in 23% and severe anemia in 3% (6.38 ± 0.66). Sickle-cell heterozygote and G-6-PD deficiency were found in 11.5 and 2% of women respectively in all the enrolled subjects.

A total of 678 infants were enrolled and followed subsequently every month. At the time of enrolment 8.7% were having fever or history of fever and 6.4% during subsequent follow-up. Two infants were found positive for malaria (1 *Pf*, 1 *Pv*) during enrolment and 5 (3 *Pf*, 2 *Pv*) during subsequent follow-up (Table 12) and all of them were symptomatic. Also 217 fathers and 113 siblings were enrolled and followed on monthly basis. Out of those 8.3% fathers and 6.1% siblings were found symptomatic and one from each group was positive for malaria during the enrolment (Table 12). Details of malaria episodes among enrolled pregnant women are shown in Table 13.

Table 12. Malaria infection in the study subject at enrolment and during follow-up

Study subjects	Enrolled (E)/Follow-up (F)	Malaria +ve	No. of parasite (Parasite density/ml)	
			Pv	*Pf*
Pregnant women (n = 1100)	E	37	16 (1800/ml)	21 (1080/ml)
	F	36	17 (2400/ml)	19 (3280/ml)
New born (n = 678)	E	2	1 (160/ml)	1 (1040/ml)
	F	5	2 (3360/ml)	3 (1080/ml)
Father (n = 217)	E	1	1 (3020/ml)	0
	F	0	0	0
Sibling (n = 113)	E	1	1 (160/ml)	0
	F	0	0	0

Table 13. Malarial episode among pregnant women

No. of pregnant women	Repeated episodes
8	2
4	3
1	4 (first 3 *Pf* and 4th one is *Pv*)
1	5 (first 3 *Pf* and 4th & 5th one is *Pv*)
23	1

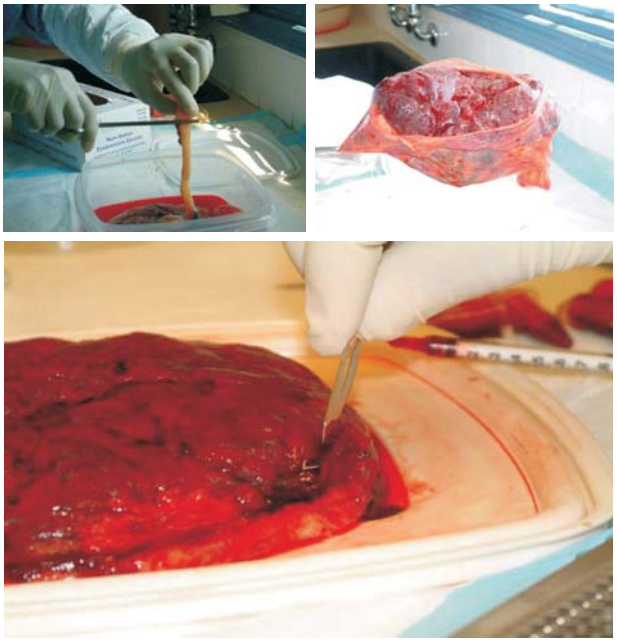


Fig. 43: Processing of placenta

Of the 1100 enrolled pregnant women, 735 deliveries took place. Out of 735 deliveries, 554 could be attended and 3.8% women had fever at the time of delivery. Peripheral smears of nine mothers were positive for malaria; 394 placentas were collected and processed successfully (Fig. 43). Still birth and neonatal death were recorded in 1.8 and 1.5% subjects respectively. Abortion was recorded in 26 (3.53%)

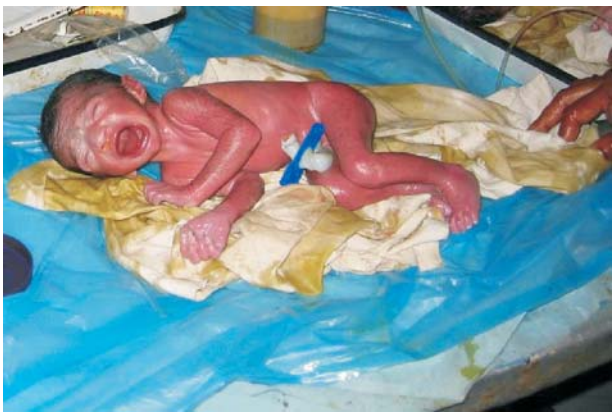


Fig. 44: New born baby (Low birth weight)

subjects. Only one maternal death was found. Low birth weight was found in 38% (2.14 ± 0.47 kg) cases (Fig. 44). The details of placental malaria are shown in Table 14.

Active fever survey was conducted in the cohort area. Both *P. vivax* and *P. falciparum* were prevalent in the study area and as the transmission season progressed, there was an increasing trend in *Pf* ratio from 28 to 83% (Fig. 45). Overall SPR was 14 and *Pf*% was 64%. The age group >4–14 years found to be highly susceptible for malaria (Fig. 46) as compared to other age groups combined (OR = 2.34;

Table 14. Placental investigation for malaria parasite

Smears (n = 394)	Positive (no. of cases)	Species (Parasite density)	
		Pf	*Pv*
Mother peripheral smears	9	5 (496/µl)	4 (4200/µl)
Cord blood smears	4	1 (200/µl)	3 (220/µl)
Placental smears	6	2 (12940/µl)	4 (547/µl)
Tissue impression smears	6	2 (12980/µl)	4 (1400/µl)

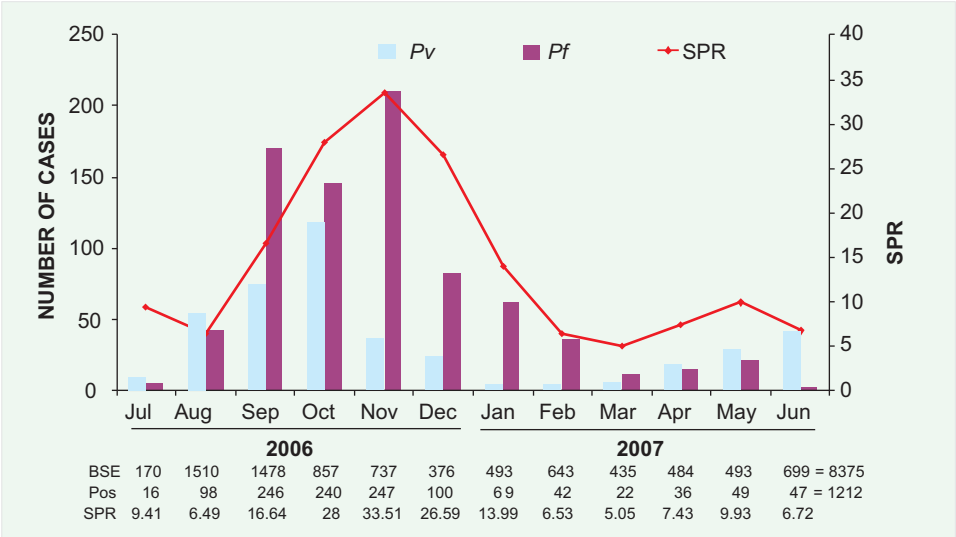


Fig. 45: Malaria prevalence in cohort area

Table 15. Hospital-based cross-sectional study

Case distribution (n = 75 in each category)	Samples collected
Severe malaria anaemia (Hb <5 g/dl)	31 (3.94 ± 0.67)
Moderate malaria anaemia (Hb ≥5 & ≤7.9 g/dl)	65 (6.65 ± 0.79)
Mild malaria anaemia (Hb ≥8 & ≤10.9 g/dl)	75 (9.37 ± 0.78)
Cerebral malaria	50
Uncomplicated malaria	74 (12.8 ± 1.56)
Acute renal failure	14

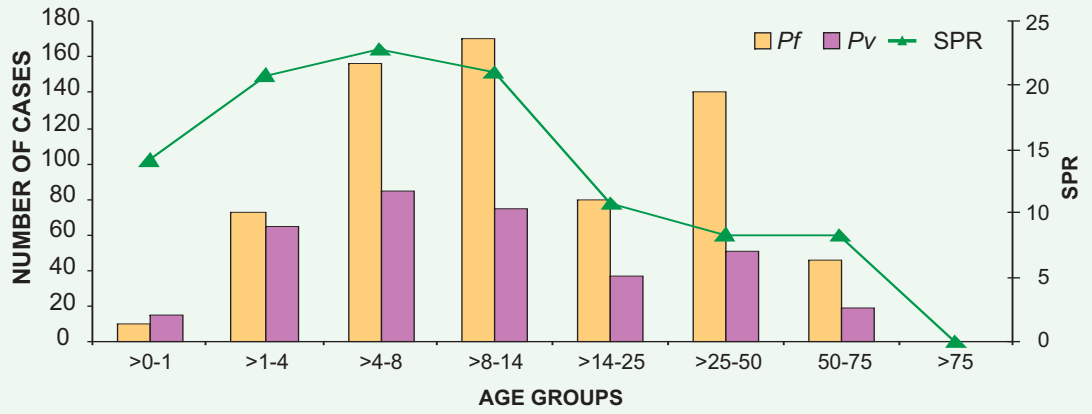
Fig. 47: Malaria prevalence in cohort area (Site-wise)

Table 16. Hospital-based cross-sectional study in pregnant women

Case distribution (n = 106 in each category)	Samples collected
Pregnant women with *Plasmodium falciparum* infection	53
Pregnant women with *Plasmodium vivax* infection	6
Healthy controls	36

95% CI 2.04–2.69). Further analysis revealed that the highest malaria prevalence was recorded in the villages surrounded by the dam reservoir (20.5%) as compared to the forested villages (15.4%) [OR-0.70; 95% CI-0.57–0.86] and plain villages (11.5%) [OR-0.50; 95% CI-0.43–0.60] as shown in Fig. 47. Similarly, the prevalence of *P. falciparum* was also found to be relatively higher in the villages surrounded by the dam reservoir (14.4%) than the forested villages (9.4%) [OR-0.62; 95% CI-0.48–0.78] and the plain villages (7.1%) [OR-0.45; 95% CI-0.37–0.55].

In the hospital-based cross-sectional surveys, the patients were also enrolled following the protocol and blood samples were collected for immunological and molecular biology study with particular reference to establish correlation with clinical severity of the disease and serum cytokine levels e.g. TNF-α, IFN-γ, IL-4, IL-10, IL-12 and IP-10. Enrolled patients were summarized in Tables 15 & 16. Samples from hospital-based cross-sectional study of the PW cases allowed us to generate data on immunological parameters, such as cellular and humoral immune responses profile of women with *P. falciparum* and *P. vivax* infections. In addition, all fever cases coming to Civil Hospital Maihar, were also screened for malaria (Fig. 48). Age group >4–14 years were highly susceptible for malaria as compared to other age groups combined (OR = 1.79; 95% CI-1.54–2.09) as shown in Fig 49.



BSE	176	658	1052	1166	1080	2289	783	34 = 7238
Pos	25	137	240	245	117	192	65	0 = 1021
SPR	14.20	20.82	22.81	21.01	10.83	8.39	8.30	0

Age group 4–14 is highly suceptible to malaria; OR = 2.34 (2.04 – 2.69); RR = 2.05 (1.83 – 2.29); χ^2 = 158.96; P<0.001

Fig. 46: Malaria prevalence by age groups in cohort area

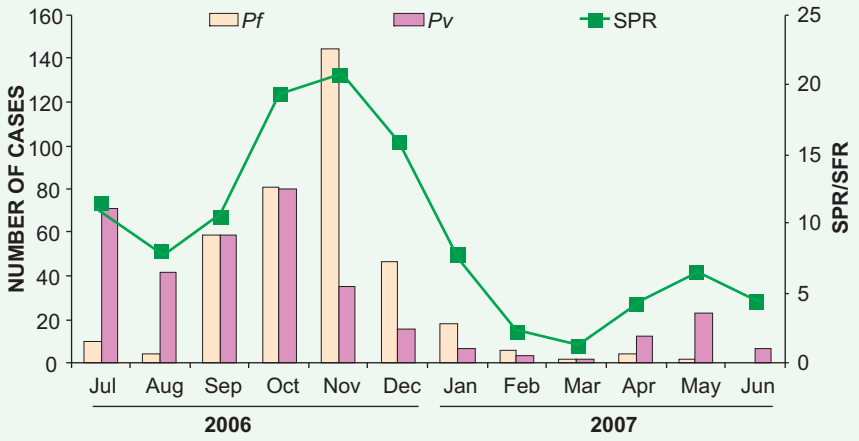


Fig. 48: Malaria prevalence in Maihar Civil Hospital, Satna

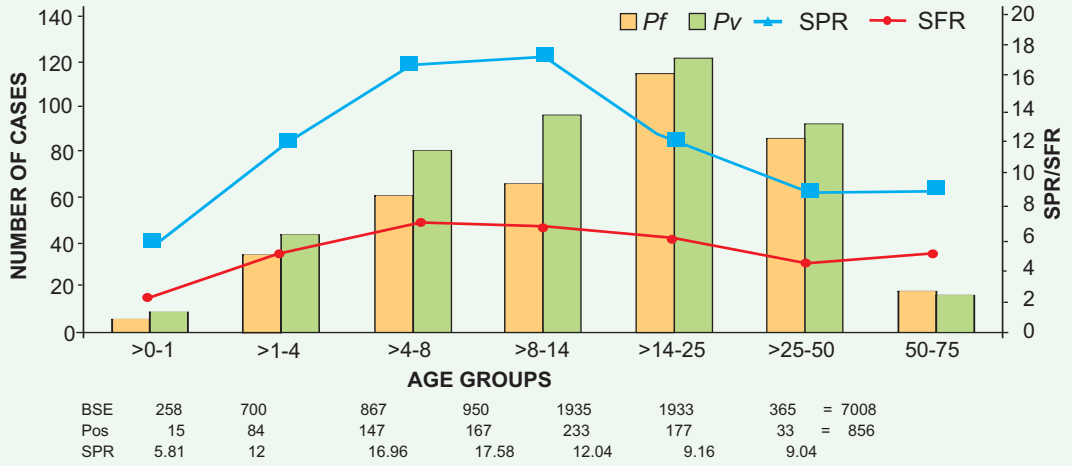


Fig. 49: Malaria prevalence by age groups

Immunoepidemiology

Naturally acquired immune responses to stage-specific *P. falciparum* and *P. vivax* antigens in a population of central India

The study has been initiated with an aim to characterize immune responses to stage-specific *P. falciparum* and *P. vivax* antigens in children and adults naturally exposed to malaria; to study the development and maintenance of immune responses in different age groups with emphasis of infants, their older siblings and mothers, including identification of epitopes that correlate with protection; to determine the role of stage-specific antigens in the development and maintenance of natural immunity to malaria; to evaluate the immune mechanisms, those are involved in pathogenesis of malaria, especially anaemia, cerebral malaria and placental malaria. Study was conducted in three populations. They are: (i) Infants, children and adults from the community; (ii) Pregnant women from the community; and (iii) Hospitalized patients with severe malaria. Peripheral blood, placental and cord blood at delivery were taken for determining antibodies against species and stage-specific antigens by enzyme immunoassay. The antibody levels were quantified using known antimalarial antibody positive controls and this allowed us to estimate antibody levels in O.D. values. Sera from non-endemic healthy subjects were taken as negative control (Figs. 50–53). Blood samples collected from a group of 149 malaria parasites positive (79 *P. falciparum* and 70 *P. vivax*) patients were tested for the determination of antibodies to species and stage-specific peptides. Proportionate sizes of malaria negative subjects (n = 50) were also taken for comparison. Seroprevalence was more in parasite positive cases. Differences in antibody responses between parasite positive and negative groups were categorized as high and low responders. Most of the parasite positive individuals showed high antibody responses to all the peptides, whereas the malaria negative individuals were mostly low responders (Figs. 54 and 55). The responders' frequency of *Pf* and *Pv* positive patients with malaria negative subjects was compared and results found significant between two groups. The level of

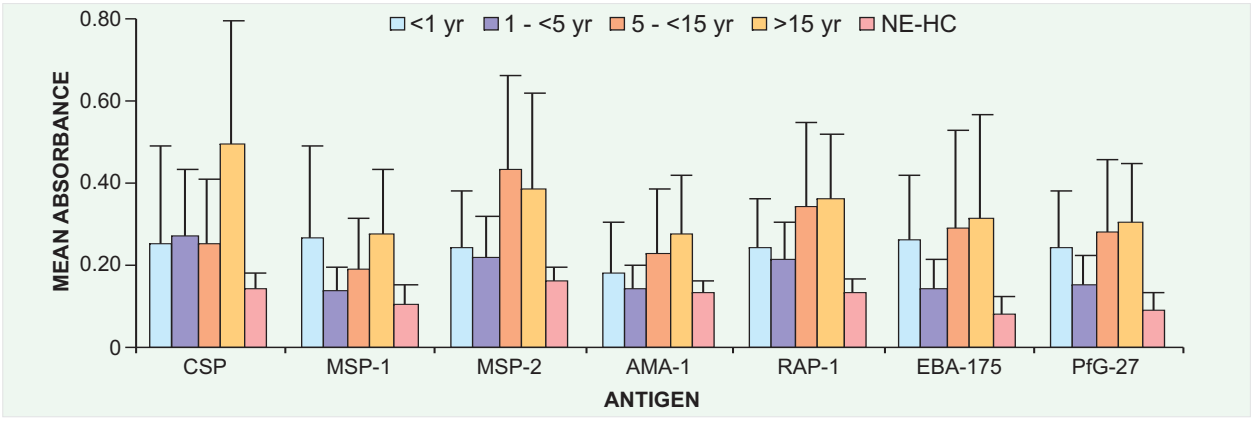


Fig. 50: Antibody profile with *P. falciparum* antigen

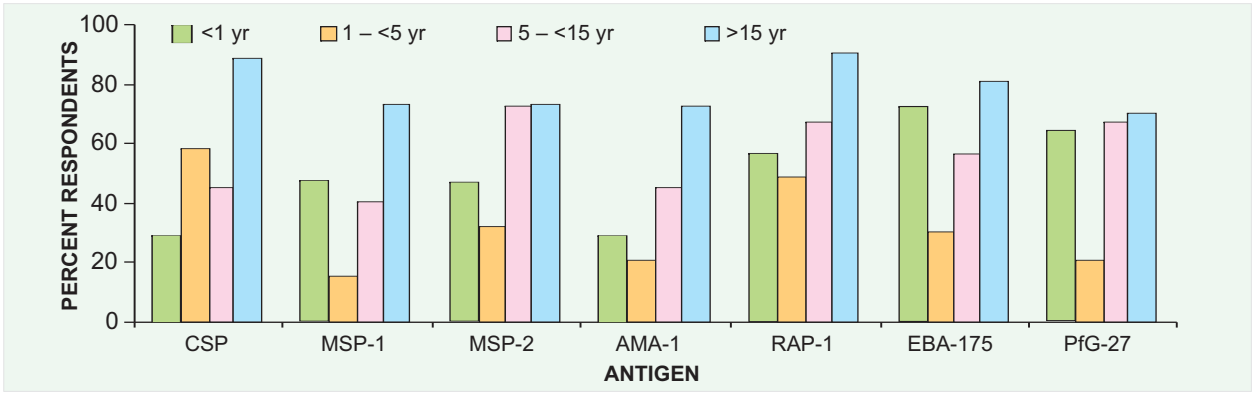


Fig. 51: Responder frequeny to *P. falciparum* antigen

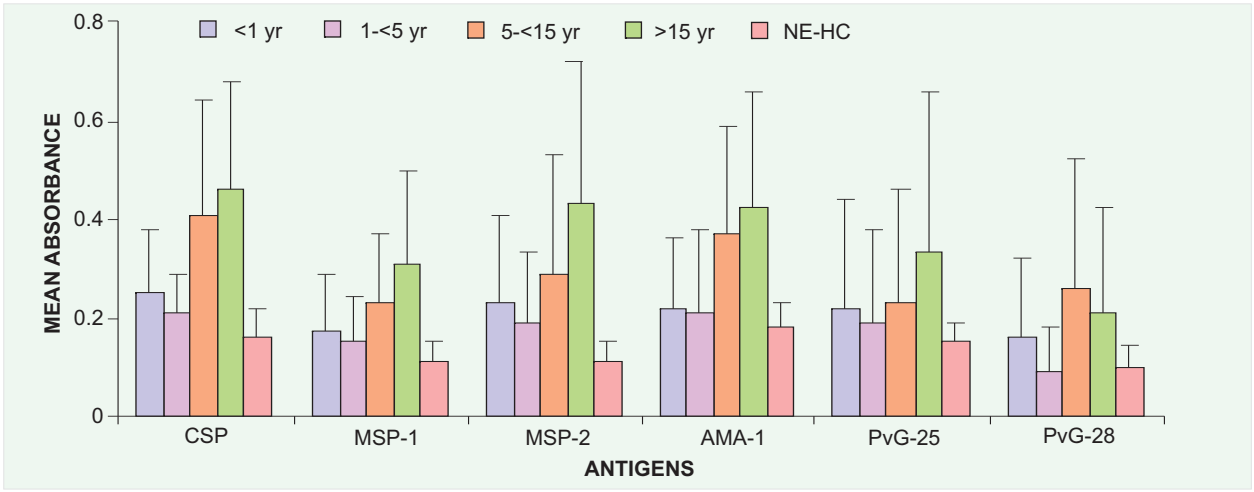


Fig. 52: Antibody profile with *P. vivax* antigens

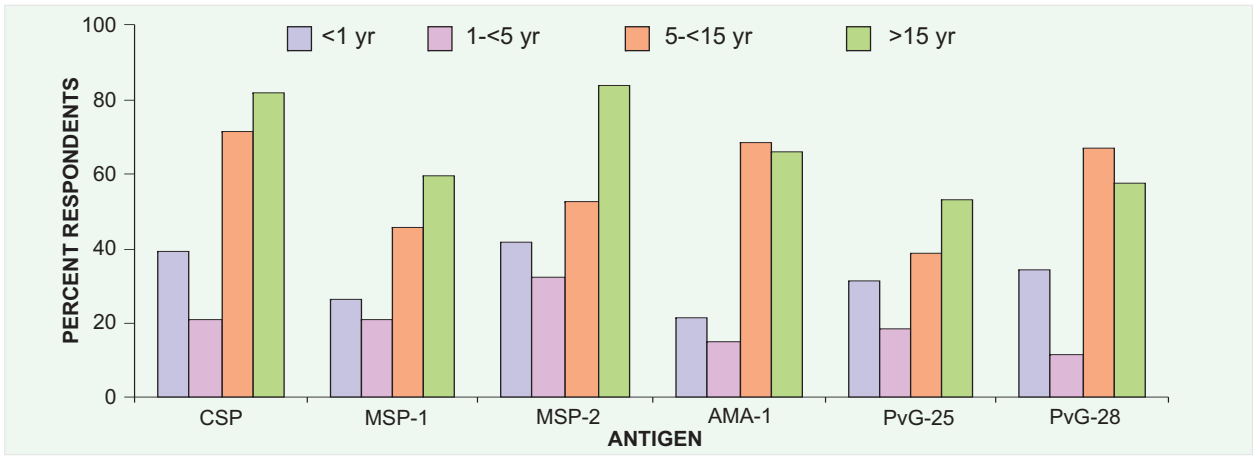


Fig. 53: Responder frequency to *P. vivax* antigens

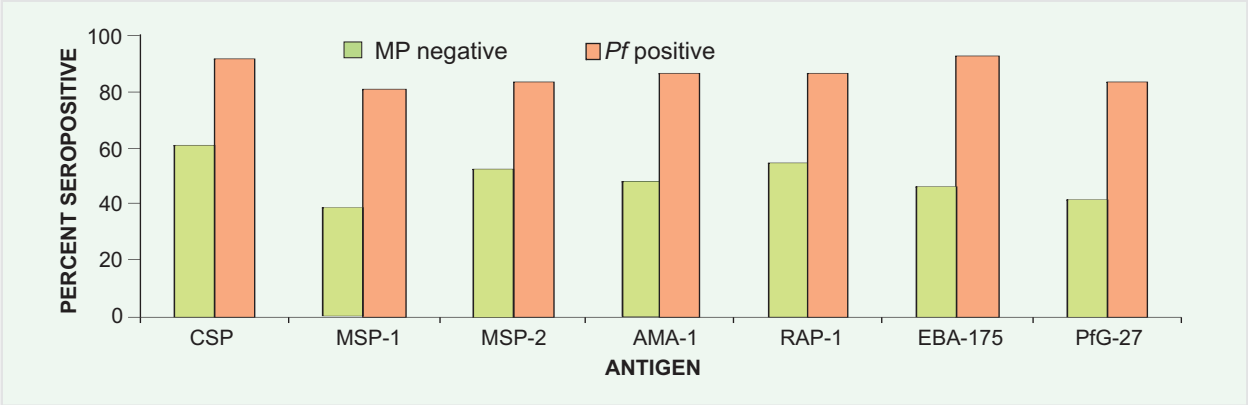


Fig. 54: Seroprevalence with *P. falciparum* antigen

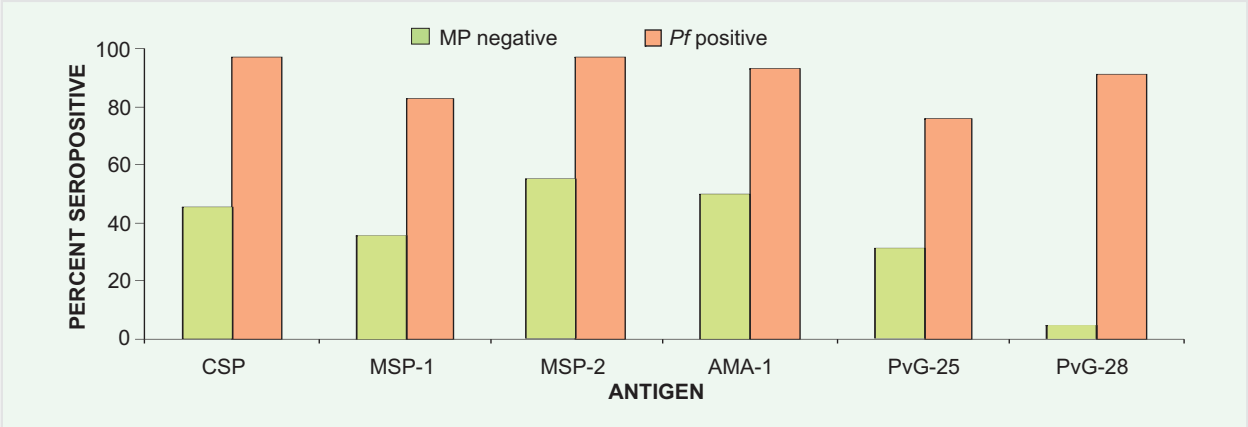


Fig. 55: Seroprevalence with *P. vivax* antigen

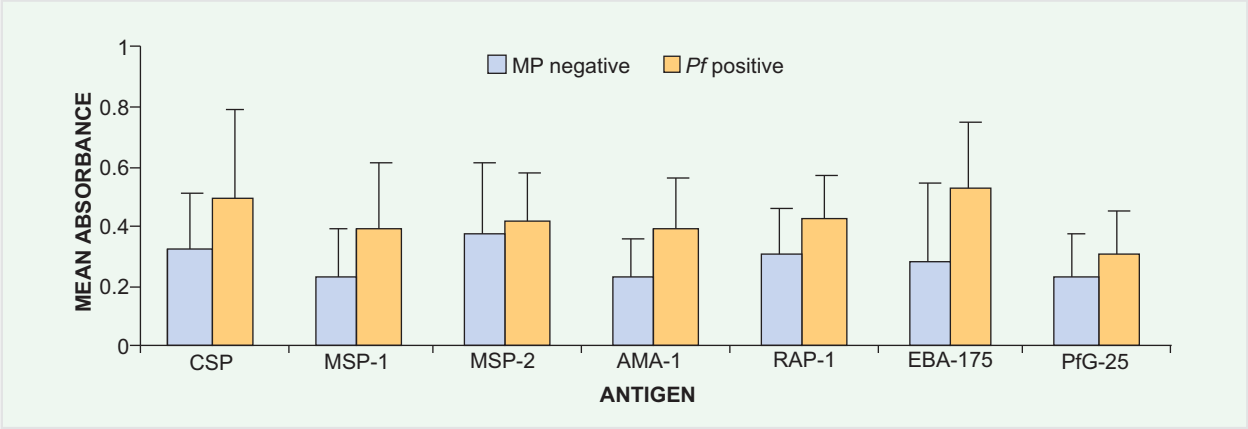


Fig. 56: Antibody profile of enrolled pregnant women with *P. falciparum*

antibodies appeared to be related to their exposures to the parasite during malaria transmission. Thus the results suggest that there might be natural boosting in antibody responses as a result of infection and that were measurable against these peptides.

Blood samples collected from enrolled pregnant women were checked (n = 600) for antibodies to species and stage-specific peptides. In this group also seroprevalence was more in parasite positive cases. Most of the parasite positive individuals (36 *P. falciparum* and 26 *P. vivax*) showed comparatively higher antibody response to all the peptides than the malaria negative individuals. The enrolled subjects were divided gravida-wise into three categories: primigravida (n = 180), secundigravida (n = 186) and multigravida (n = 234). Results of the antibody responses of three groups against individual peptides were compared. No significant differences have been observed between pregnant women of different gravidities (Figs. 56–59).

The infants' umbilical cord blood and mothers' placental blood were tested for antimalarial antibodies to both *Pf* and *Pv* antigens to determine the possible effects of maternal antimalarial IgG antibodies on protection against placental infection and infection in infants. The relationship between antimalarial antibodies in infants' cord blood (C) and mothers' placental blood (P) were drawn. Overall antimalarial IgG profile in mothers at the time of delivery was low and same response has been observed in respective

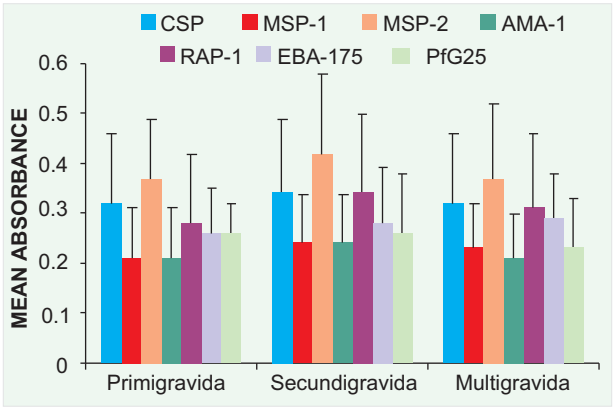


Fig. 57: Antibody profile with *P. falciparum* antigen

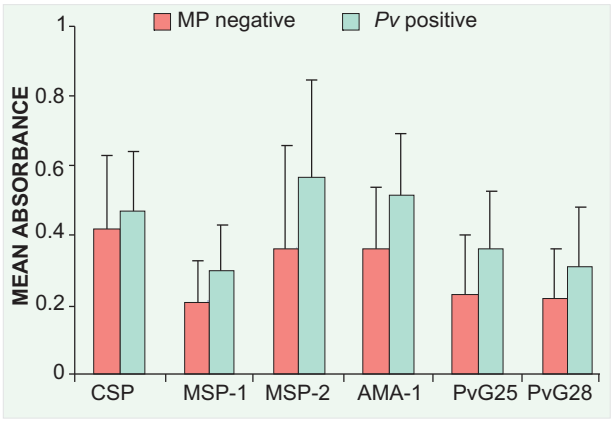


Fig. 58: Antibody profile of enrolled pregnant women with *P. vivax*

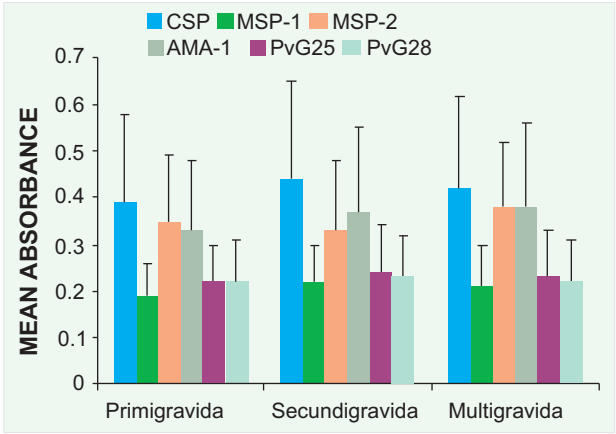


Fig. 59: Antibody profile with *P. vivax* antigen

infants. In these study cohorts, there was a trend for the higher antibody levels in maternal sample than cord against all *Pf* and *Pv* stage-specific peptides. We have also determined percent positive responders among different placental and cord blood samples. Among *Pf* peptides, *Pf*MSP-1 and *Pf*G-27 showed less seropositivity when compared to other peptides, whereas among *Pv* peptides *Pv*G-28 showed less seropositivity. Highest seropositivity was seen in *Pf* and *Pv*CSP.

Cellular response was determined in peripheral blood mononuclear cells by lymphocyte transformation test in the presence of *P. falciparum* and *P. vivax* antigens and cytokines (IL-4 and IFN-γ) level was estimated in activated T lymphocytes culture supernatant by sandwich ELISA. The peripheral blood mononuclear cells (PBMC) were isolated from venous blood of subjects who have past experience of malaria and the proliferative responses against *P. falciparum* and *P. vivax* antigens were determined in individual set of PBMCs. Only a subset of adults and older children above 14 year were participated in this study. In general, the study subjects responded to T-cell epitopes. The mean stimulation index (SI) was not significantly different among positive responders. The *in vitro* stimulation of T-cells from malaria-exposed donors results in the production of IL-4 and IFN-γ in concordance with the serum concentrations of antibodies specific for the antigens used for lymphocyte stimulation.

Peripheral blood from healthy cohort participants and hospital admitted *P. falciparum* malaria patients were collected in anticoagulant. Four categories of patients were identified: uncomplicated falciparum malaria (UFM), cerebral malaria (CM), severe malaria (SM) and severe malaria anaemia (SMA). Cytokines like IL-4, IL-10, IP-10, IFN-γ and TNF-α levels were estimated in plasma using commercially developed two-site ELISA assay kits. Our results revealed that IP-10, which is a proinflammatory chemokine, progressively increased with the disease severity. Its plasma levels were found to be significantly elevated during malaria illness compared to HC; with highest levels found among CM cases followed by SM, SMA and UFM and lowest levels among HC subjects. The levels of TNF-α and IFN-γ (both proinflammatory) were also increased with the severity of disease. In comparison to healthy controls, both were elevated among the severe cases of malaria. In our study, IL-10 was undetectable among the HC subjects but in patients it got up-regulated during the disease severity; in the SMA patients IL-10 level was low. Another anti-inflammatory cytokine IL-4 began rising in plasma during acute falciparum malaria and increased maximum among CM cases but in SM and SMA, IL-4 level was comparatively lower than CM. Cytokine levels were compared within groups and between groups and results were significant.

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

to differences in the malaria transmission and availability of both *P. falciparum* and *P. vivax*.

Molecular Epidemiology

Genomic DNA Extraction and Polymerase Chain reaction (PCR), Nucleotide Sequencing and Sequence Analysis

The genetic polymorphism in the vaccine candidate antigen genes (*MSP-1, MSP-2, MSP-3, TRAP, RAP-1, CSP, EBA-175* and *AMA-1*) and drug resistance genes (*pfcrt*, *pfdhfr* and *pfdhps*) were studied. Genomic DNA was extracted from *P. falciparum* infected blood. The aliquot of extracted DNA was used to amplify the vaccine candidate antigen genes and drug resistance genes using the respective gene-specific primers. Sequencing was performed using ABI Big Dye Terminator Ready Reaction Kit Version 3.1 on a 310 genetic analyzer (Applied Biosystems). Sequences were analyzed using BioEdit software and aligned by using GeneDoc (Table 17 and 18; Figs. 60 and 61).

P. falciparum MSP-1 gene was sequenced from 37 isolates. Majority (17 of 37) of the isolates had MAD20-type allele while 12 isolates showed K1-type MSP-1 alleles. Remaining eight isolates were showing RO33-type of alleles. It is noteworthy that MAD20 alleles further showed nine different variants (MI to MIX) among themselves whereas six variants (KI to KVI) were found of K1-type alleles. *MSP-2* gene was sequenced from 35 isolates. Majority (29 of 35) of the isolates showed FC27-type allele while, six isolates showed 3D7-type alleles. Eight variants of FC27-type (FI to FVIII) and three variants of 3D7-

Table 17. Details of the number of isolates sequenced for vaccine candidate antigens

Antigens	No. of isolates	Sequenced nucleotides (bp)	Results
MSP-1	85	555	K1–40%, MAD20–40% & RO33–20%
MSP-2	81	634	FC27–63% & 3D7–37%
MSP-3	32	550	K1–44%, FC27–34% & 3D7–22%
TRAP	67	757	G3–46% & (G8–G23)–54%
RAP-1	47	1133	GIII–36%, GII–23% & Rest (GI- GXI)–41%
EBA-175 (Region II)	31	1100	GIII–38%, GVIII–23% & Rest (GI–GX)–39%
CSP	30	1000 plus 450	Th2R GI–83% & Rest (GII–GV)–17% Th3R GI–87% & Rest (GII–GIV)–13%
AMA-1	90	540	GI–33%, GIII–20% & Rest (GII–GXI)–47%

Table 18. Details of the number of isolates sequenced for drug resistance loci

Drug loci	No. of Isolates	Sequenced nucleotides (bp)	Codons	Results
Pfdhps	96	653	436, 437, 540, 581 & 613	Wild–92% Single mutant–8%
Pfdhfr	93	542	16, 51, 59, 108 & 164	Wild–4% Single mutant–11% Double mutant–47% Triple mutant–38%
Pfcrt	78	582 plus 232	72–76 & 220	Wild–2% Single mutant–98%

Fig. 60: Electrophoretogram of merozoite surface protein-1

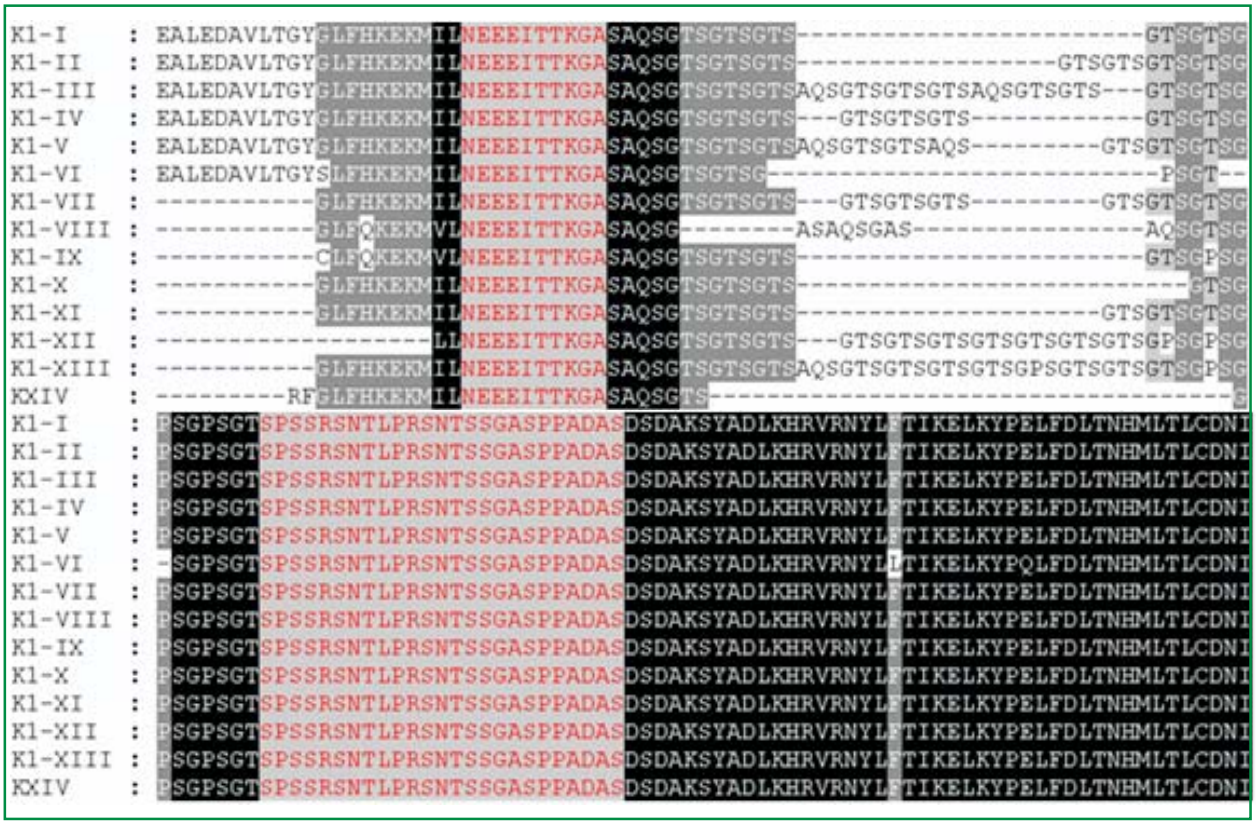


Fig. 61: Alignment of K1 like allele family (MSP)

type (3D7I to 3D7III) alleles were further observed. *MSP-3* gene was sequenced from 32 isolates. Majority (14 of 32) of the isolates had K1-type allele, while seven isolates showed 3D7-type MSP-3 alleles. Remaining 11 isolates were showing FC27-type alleles. Two variants each in K1 allele (K1-a and K1-b) and 3D7 alleles (3D7-a and 3D7-b) were found. TRAP gene was sequenced from 29 isolates. Seven allelic variants (GI to GVII) of the TRAP gene were observed. Majority (18 of 29) of the isolates belong to the GIII alleles. Five of the isolates showed GV alleles. Two isolates had GI alleles. The remaining four isolates had four different types of alleles GII, GIV, GVI and GVII. *AMA-1* gene was sequenced from 40 isolates. Those were classified into ten different alleles (GI to GX) according to their amino acid sequences. The predominant allele observed among the isolates was G1 allele.

Polymorphism in Drug Resistance Loci

Dhps gene of *P. falciparum* was sequenced from 35 isolates. Majority (32 of 35) of the isolates showed SAKAA (wild type) genotypes at the codons 436, 437, 540, 581 and 613 respectively. Two isolates had SGKAA (single mutant) at these codons while one isolate was having SA/EKAA genotype. *Dhfr* gene was sequenced in 36 isolates. Majority (25 of 36) of the isolates showed ANRNI (double mutant) genotypes at the codons 50, 51, 59, 108 and 164 respectively. Eight isolates had ANCNI (single

Setting up of experiment in molecular biology laboratory

Harvesting of labelled lymphocytes onto glass-fibre filters in Cell Harvester

Breeding site in forested villages

Breeding site in plain villages

mutant) at these codons while three isolates were having ANCSI (wild type) genotype. *Pfcrt* gene was sequenced in 35 isolates. All of the isolates showed SVMNTS genotype at the codons 72, 73, 74, 75, 76 and 220 respectively (Table 18).

Entomological study

Indoor Resting Mosquito Collections

Monthly collections of indoor resting anophelines were initiated from August 2006 in 10 study villages. Out of those, five are located in the plain area, three in the forest area and two near the dam site. Results of one year study revealed that the average per man hour density (MHD) of *Anopheles* mosquitoes was 42.59 (range 16 in June to 195.05 in August). Of which 66.8% were *An. culicifacies* (MHD 28.49, range 12.5 in May to 126.4 in August) (Fig. 62). The density of *An. fluviatilis* was recorded 0.48. Of which, the density in the forested villages was 0.97, which is significantly higher ($F = 9.23$; $p < 0.001$) as compared to that in villages located in the plain area and near the dam site (Fig. 63). *An. culicifacies* and total anophelines density was highest in the villages surrounded by dam reservoir and lowest in forested villages, although this difference is not significant statistically ($p > 0.05$).

Human Landing Collections

These collections were initiated from September 2006 by conducting all night collections in each month in indoor and outdoor sites of the study villages. Eleven months' results revealed that the average human landing of total *Anopheles* was 0.98 per man per night in indoors and 0.88 in outdoors. *An. culicifacies* was the most common species at both sites with 0.60 per man per night landing in indoors and 0.51 in outdoors (Fig. 64). *An. fluviatilis* landing was low (0.03 in indoors and 0.02 in outdoors). The

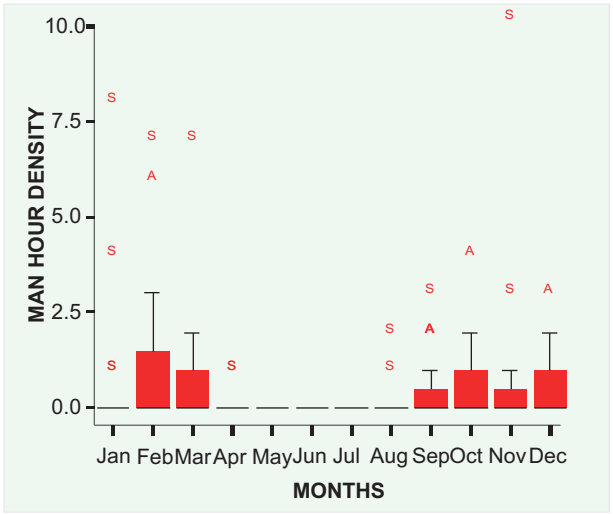


Fig. 63: Monthly man hour density of *An. fluviatilis*

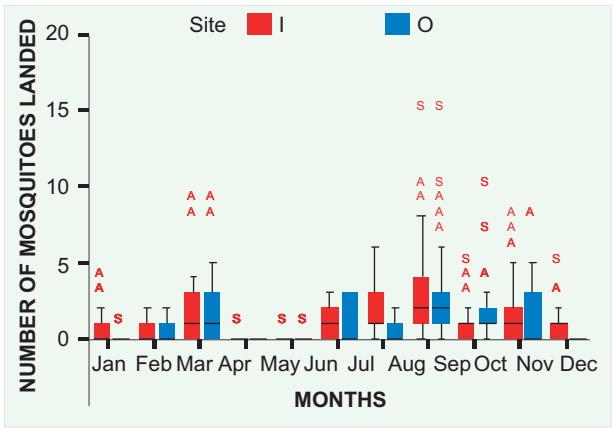


Fig. 62: Month-wise man hour density of *An. culicifacies*

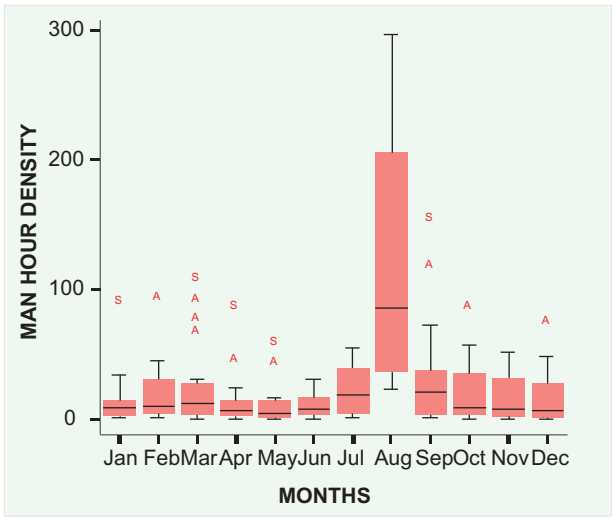


Fig. 64: Monthly human landing catches of *An. culicifacies*

Table 19. Results of vector incrimination by ELISA

Month of collection	No. of *An. culicifacies* tested	Results	No. of *An. fluviatilis* tested	Results
April 2007	135	1+ve (*Pf*) 2+ve (*Pv* mix of 247&210)	3	–ve
May	63	2 +ve (*Pv*247)	0	–ve
June	68	–ve	0	–ve
July	115	1+ve (*Pv*210)	1	–ve
August	210	–ve	8	–ve
September	215	1+ve (*Pv*247)	11	2+ve (*Pf*)
October	129	–ve	34	1+ve (*Pv*247)
November	117	–ve	34	–ve
December	83	–ve	22	–ve
January 2008	93	–ve	16	–ve
February	114	–ve	12	–ve
March	130	–ve	7	–ve
Total	1472	7 +ve (1*Pf*, 6*Pv*)	148	3+ve (2*Pf*, 1*Pv*)

landing rate of all anophelines at indoor sites was almost equal in villages located at plain, forest and dam sites, however, at outdoor sites it was higher in plain and dam sites as compared to forest villages. Further analysis revealed that significantly more *An. culicifacies* land between 1800 and 2200 hrs than after 2200 and 0600 hrs (t=4.73; p<0.0001).

Light Trap Collections

Light trap catches revealed that the average per trap per night catch of *Anopheles* mosquitoes was higher at outdoors (20.84) as compared to that at indoors (11.78). *Anopheles culicifacies* and *An. fluviatilis* were 4.44 and 0.84 at indoors and 6.18 and 1.28 at outdoors respectively. Area-wise results revealed that most of the anophelines were trapped in the plain villages. Further analysis revealed that significantly more *An. culicifacies* were trapped between 1800 to 2200 hrs than after 2200 hrs (t = 4.73; p<0.0001). The trend was similar for *An. fluviatilis* (t = 2.19; p<0.05). This would have direct implication for bednet as preventive measure.

Determination of Sporozoite Rate

The sporozoite determination by ELISA could be initiated in June 2007 after obtaining Monoclonals from CDC and standardization of the techniques at the field unit. The proportion of *An. culicifacies* and *An. fluviatilis* with positive salivary glands by ELISA is shown in Table 19. Monthly entomological surveillance carried out in 10 study villages revealed that average indoor resting density (per man hour) of anophelines during the year was 37.3 of which *An. culicifacies* was the dominant species (18.4) followed by *An. subpictus* (10.9), *An. annularis* (5.8) and *An. fluviatilis* (0.5). A total of 1472 *An. culicifacies* and 148 *An. fluviatilis*, collected from different localities were assayed during the year for sporozoite detection by ELISA technique of which seven *An. culicifacies* (1 *Pf* and 6 *Pv* strain) and three *An. fluviatilis* (2 *Pf* and 1 *Pv* strain) were found positive for the presence of sporozoites (Table 19).

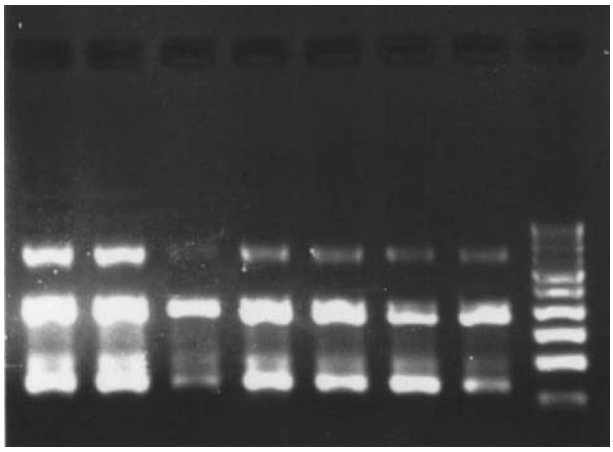


Fig. 65:Polymerase chain reaction assay for differentiation of members of *An. fluviatilis* complex

Sibling species determination

The *An. culicifacies* was further identified into the sibling species using cytotaxonomy and *An. fluviatilis* by using polymerase chain reaction (PCR) techniques at NIMR, Delhi. Results revealed that the majority of the *An. culicifacies* tested were of sibling species 'C' (66%). Species 'D' was detected in 24.6% and species 'B' in 9.1% of *An. culicifacies.* Area-wise results also showed the highest prevalence of sibling species 'C' in all the plain, forest and dam areas. All the 101 *An. fluviatilis* tested were of sibling species 'T' of which three were detected as sporozoite positive (Fig. 65).

As proposed in the project, data on all above parameters would be generated to fulfil the objectives in large number of samples. Once fully characterized, this site would be useful for testing of any tools available for the control and prevention of malaria, such as antimalarial vaccines and diagnostic reagents. The study is in progress. ❑

Impact of Climate Change on Vector Borne Diseases with Emphasis on Malaria

The role of environment is an important component of epidemiology of vector borne diseases. In recent years, more precisely since 1990 there is greater awareness about the threat of climate change on human health in addition to various other sectors. Climate change refers to a statistically significant variation in either the mean state of the climate or in its variability, persisting for an extended period (typically decades or longer). It may be due to natural internal processes or external forces, or to persistent anthropogenic changes in the composition of the atmosphere. The United Nations Framework Convention on Climate Change (UNFCCC) defines climate change as 'a change of climate which is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to natural climate variability observed over comparable time periods'.

As per Intergovernmental Panel on Climate Change's (IPCC) Third Assessment Report, about 3.8°C rise in temperature and 7% change in precipitation (increase as well as decrease) are projected by the year 2080 (IPCC 2001). The fourth Assessment Report of IPCC (2007) also projects rise in temperature up to 4°C and sea level rise up to 0.59 m by the year 2100. IPCC concludes that climate change is likely to expand the geographical distribution of several vector-borne diseases, including malaria, dengue and leishmaniasis to higher altitudes (high confidence) and higher latitudes with limited public health defences and to extend the transmission seasons in some locations. In some locations, reduced rainfall or incremental temperature may cause decrease in transmission of some vector borne diseases.

International bodies like IPCC, UNFCCC, and World Health Organization (WHO) have accelerated efforts to generate, collate and compile the data on assessments of impacts of climate change on different sectors like geology, forestry, agriculture, water resources, biodiversity, health and so on. UNFCCC through Global Environmental Facility (GEF) has also funded projects for national assessments in different parts of the world.

Ministry of Environment and Forests, Govt. of India has taken initiatives to assess the vulnerability due to climate change in different sectors, like agriculture, geology, hydrology, forestry, energy, health, etc and possible adaptation measures. National Institute of Malaria Research in 2001 has also participated in the first National Communication Project to assess the impact of climate change on malaria in India.

Load of Vector Borne Diseases

India is afflicted with six major vector borne diseases (VBDs), namely malaria, dengue, chikungunya, filariasis, Japanese encephalitis and leishmaniasis of which malaria ranks at number one with about 1.48 million cases and 1173 deaths. As per NVBDCP, in 2007, cases and deaths due to Japanese encephalitis, dengue and visceral leishmaniasis were 4022/963, 5534/69 and 44001/189 respectively. Around 60,000 suspected cases of chikungunya were also reported in 2007. In addition to mortality, vector borne diseases cause morbidity of millions of persons resulting in loss of man-days causing economic loss.

The Logic of Impact of Climate Change on VBDs

The role of climatic factors has been studied extensively in the epidemiology of malaria due to its global public health importance. The minimum temperature required for development of *P. vivax* parasite in anopheline mosquitoes is 14.5–16.5°C while for *P. falciparum* it is 16.5–18°C. At 16°C it will take 55 days for completion of sporogony of *P. vivax* while at 28°C, the process can be completed in seven days and at 18°C it will take 29 days. The duration of sporogony in *Anopheles* mosquitoes decreases with increase in temperature from 20 to 25°C.

From 32 to 39°C temperature, there is high mortality in mosquitoes and at 40°C, their daily survival becomes zero. At increased temperatures, the rate of digestion of blood meal increases which in turn accelerates the ovarian development, egg-laying, reduction in duration of the gonotrophic cycle and more frequency of feeding on hosts, thus, increasing the probability of transmission.

Thus, climatic conditions play important role in the distribution, degree of endemicity and epidemicity of diseases in an area. Some areas, which have most favourable conditions of temperature and rainfall, experience transmission of disease throughout the year, while in areas experiencing colder months, transmission is seasonal and does not take place throughout the year.

Studies Undertaken by NIMR

The Ministry of Environment and Forests launched a mega project—India's initial National Communication to UNFCCC in 2002. Impacts of climate change on malaria was one of the projects under which vulnerability assessment and adaptation measures in view of climate change on malaria were studied by the NIMR.

Vulnerability Assessment and Future Scenario

Based on monthly incidence of malaria in different states of India, it was found that northern states, such as Jammu & Kashmir, Himachal Pradesh, Punjab, Haryana, Uttarakhand and northeastern states, *etc* are more vulnerable to climate change whereas southern states, such as Karnataka, Kerala, Orissa, Tamil Nadu and Andhra Pradesh are less vulnerable, as the climatic conditions are already suitable for malaria transmission almost throughout the year .

Preliminary studies undertaken by NIMR (taking one district from each state of India), a baseline map showing transmission windows (TW) of malaria open for different months in different states of India was generated based on minimum required temperature (19°C) and RH (55%). It was found that in Rajasthan, Punjab, Haryana, Himachal Pradesh, and Meghalaya, the TWs are open for 4–6 months, while in Gujarat, Orissa, West Bengal and southern states for 10–12 months. In view of the projected rise in temperature, the future scenario by 2080 is likely to extend the TWs in Punjab, Haryana, Jammu & Kashmir, Uttarakhand, Himachal Pradesh and northeastern states, while in Orissa, Andhra Pradesh and Tamil Nadu, it is expected that there may be reduction in number of months of TWs due to projected high temperatures. However, in such situations mosquitoes may adapt to micro niche as evidenced in Rajasthan (where adult mosquitoes prefer to rest in "tankas" to avoid high temperatures). As malaria transmission dynamics depend on a number of factors like agricultural practices, ecological changes, developmental activities, social development, types of intervention measures undertaken and existing health infrastructure, *etc* further studies are being undertaken to assess the negative impact of climate change on different vector borne diseases.

Evidence of Climate Change

Studies have been undertaken in Nanital district of Uttrakhand to find out the earlier evidence of climate change. Results have revealed very high densities of *An. fluviatilis* and presence of *P. falciparum* malaria cases in the month of March–April from the hilly parts which did not report malaria earlier. In-depth studies are being undertaken.

Early Warning of Malaria

As an adaptation measure to reduce the negative impacts of climate change, development of tools for early warning of malaria/diseases is warranted. Studies between climate variability and malaria with emphasis on different sites in India show that rainfall is an important indicator for early warning of malaria in Rajasthan and Gujarat. Work on relationship between El Nino Southern Oscillation (ENSO), vegetation index and malaria is being undertaken to find out the indicators for early warning of malaria. The case studies undertaken in selected districts of Gujarat, Rajasthan and Karnataka reveal that rainfall, ENSO and satellite derived Normalized Difference in Vegetation Index (NDVI) may be used for early warning of malaria in some epidemic prone states. Efforts are being made to develop such a system in India by using meteorological and satellite derived parameters.

Ongoing Studies at NIMR

Following studies are being undertaken to see the impact of climate change on malaria and dengue and developing early warning for malaria:

1. Assessment of the impacts of climate change on malaria and dengue at national scale and adaptation strategies for short, medium to long-term scales.
2. Impact of climate change on dengue in Delhi and environs.
3. Developing a framework for predicting malaria outbreaks in rural and urban Gujarat and Rajasthan, India (2007 to 2009) in collaboration with Michigan University, U.S.A.

❑

Malariogenic Stratification

Malaria is endemic in most parts of the country. There is a lot of diversity in terrain features, ecological conditions, biology of vectors and immunological aspects. In order to use the limited resources available effectively, areas with high potential for malaria transmission with some similarities need to be identified. Stratification of areas for suitable and effective malaria control is one of the best strategies. Different entomological, parasitological and environmental parameters can be and have been used for malariogenic stratification. At NIMR several tools for stratification of mosquitogenic conditions and malaria, like sibling species prevalence, remote sensing (RS), geographical information system (GIS) and seroepidemiology have been used.

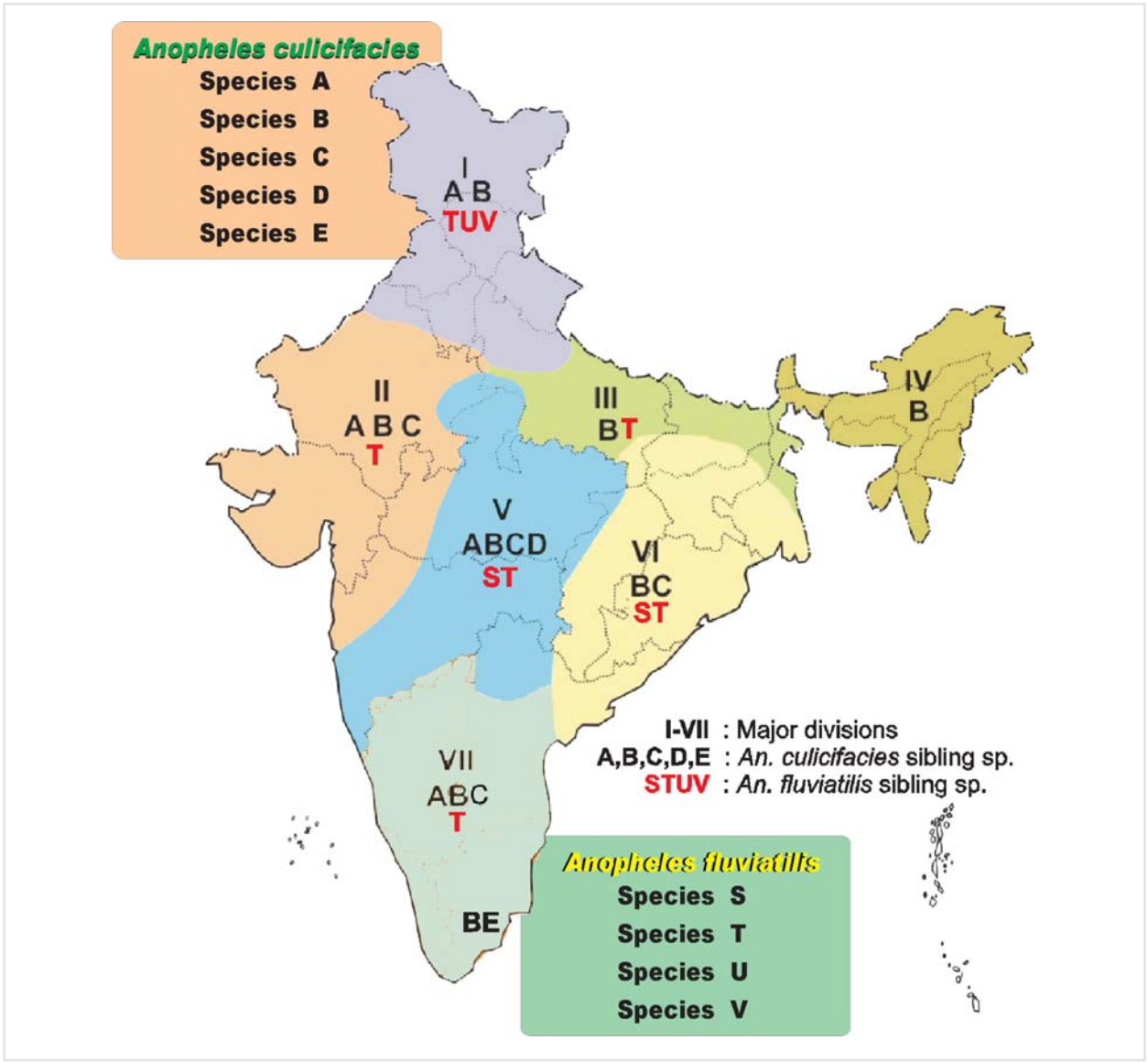


Fig. 66: Stratification of India based on *An. culicifacies* and *An. fluviatilis* sibling species distribution

Sibling Species Prevalence and Control Options

Anopheles culicifacies sibling species distinctly vary in biological characters. Species A, C, D, and E are vectors of both *Plasmodium vivax* and *P. falciparum* malaria as determined by immunoradiometric assay in areas wherever they are prevalent (Subbarao *et al* 1988, 1992). Both in laboratory and field, species B has been found to be a non-vector (Subbarao *et al* 1980). Among *An. fluviatilis* sibling species, only species S is anthropophagic and a vector, and the other two species T and U are non-vectors. Based on these distinct biological characters, their specific distribution pattern and sympatric association of *An. culicifacies* and *An. fluviatilis* sibling species prevalence, the whole country has been divided into seven major divisions (Subbarao *et al* 1999) (Fig. 66). *An. culicifacies* and *An. fluviatilis* transmit 60–70 and 15% of malaria in the country respectively (Sharma *et al* 1998). Recommended control measures in seven divisions of India, based on vector species prevalence are summarised in Table 20. Urban areas where *An. stephensi* is responsible for malaria are not being considered under these divisions.

To illustrate further possible stratification of a division to develop situation-specific strategies, stratification of Uttar Pradesh (in Divisions I and III) and Bihar (in Division III) is presented here (Fig. 67). In Uttar Pradesh and Bihar, where longitudinal studies were carried out, a good correlation has been observed between *An. culicifacies* sibling species prevalence and malaria incidence. Based on these observations Uttar Pradesh has been stratified into four zones, northern zone 1, (now falls in Uttarakhand state, western (zone 2), eastern (zone 3) and southern (zone 4) (Fig. 68). Bihar has been stratified into two, northern and southern zones (now in Jharkhand state). In zone 1, *An. culicifacies* A and B are found with high proportion of species B and malaria endemicity is low; in zone 2 species A and B with high proportion of species A and malaria endemicity is high; in zone 3, complete prevalence of species B and no indigenous malaria; and in zone 4 species A, B, C and D and in most of the areas proportion of B is high and malaria is low. In districts of northern Bihar only species B is found and there is no malaria while in southern Bihar (now in Jharkhand state) species B and C are prevalent and area is endemic for malaria. Following this stratification, location-specific control measures have ben proposed.

Geographical Information System (GIS) and Remote Sensing (RS) as Tools for Malariogenic Stratification

Case study 1: Nadiad, Kheda district (Gujarat)

A study using RS and GIS for mapping the receptivity of malaria was undertaken in Nadiad taluka comprising of 100 villages with unstable malaria and periodic epidemics. Using topo-sheets and satellite imageries, thematic maps on water table, water quality, hydrogeomorphology, soil type, relief, irrigation channels, etc. were prepared and stratified in 2–3 categories. These maps were sequentially overlaid and integrated using ARC/INFO software. The composite map resulted in 13 contours. Contours 1–12 falling in non-irrigated tract exhibited 95% matching with the ground realities, *i.e.* annual parasite incidence (API) of malaria. Contour 13, an irrigated area did not show an obvious matching but the ground verification resulted in complete reconciliation of cause and effect relationship in explaining malaria epidemiology in the region (Fig. 69). The study revealed that the parameters for high malaria in the villages of Nadiad were—high water table, soil type, irrigation and water quality (Srivastava *et al* 1999; Sharma and Srivastava 1997; Malhotra and Srivastava 1994). The technique can be used for mapping of malaria receptivity in larger areas.

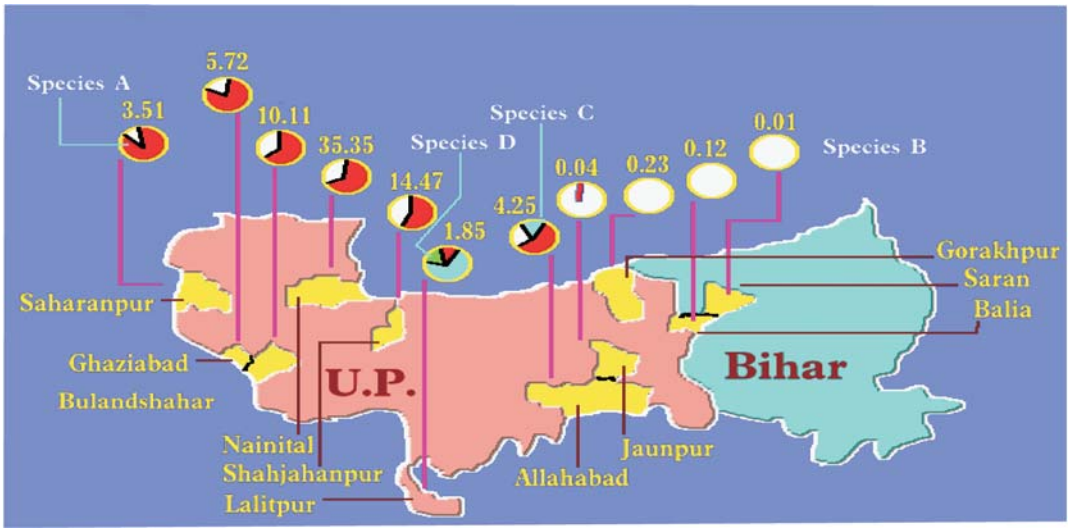


Fig. 67: Relationship between *An. culicifacies* sibling species prevalence and malaria incidence (Nos. indicate API) (undivided Uttar Pradesh and Bihar states)

Fig. 68: Stratification of Uttarakhand and Uttar Pradesh states and suggested control measures

Table 20. Recommended control measures in seven divisions of India based on vector species prevalence

Divisions	Vector species	Malaria endemicity	Recommended control strategy	Remarks
Division I				
J&K, H.P., Punjab, Haryana, Delhi, Uttarakhand, north-west U.P. & Part of north-west Rajasthan	*An. culicifacies* A, B *An. fluviatilis* T, U	Low	• No routine vector control activities. • Regular monitoring of densities of vector and passive parasitological data.	Major influence of *An. culicifacies* species A. If insecticides are to be used, susceptibility status of this species to be checked. Today this species is fully susceptible to synthetic pyrethroids and susceptibility to malathion is variable and fully resistant to DDT.
Division II				
Gujarat, parts of north-west and southern Rajasthan, western M.P. and north-west Maharashtra	*An. culicifacies* A, B, C *An. fluviatilis* T	Moderate to high	• Insecticide spray. • Selection of insecticide based on susceptibility status of *An. culicifacies* species A or C.	Major influence of *An. culicifacies* species A or C or A & C. *An. culicifacies* is mostly resistant to DDT and in some areas to malathion and even to synthetic pyrethroids.
Division III				
Parts of eastern U.P., southern U.P., Bihar and northern region of Jharkhand	*An. culicifacies* A, B *An. fluviatilis* T	Very low	• No vector control measures. • Effective chemotherapy to treat imported cases.	Most prevalent *An. culicifacies* species B, is a non-vector.
Division IV				
All seven northeastern states	*An. dirus* *An. minimus* *An. fluviatilis* *An. nivipes* / *An. philippinensis*	High	• DDT spray to continue.	Major influence of *An. minimus* and *An. dirus*, which are susceptible to DDT. Other vectors play a secondary role.

(contd...)

Table 20. ***Contd...***

Divisions	Vector species	Malaria endemicity	Recommended control strategy	Remarks
Division V				
Most districts of M.P., Chhattisgarh, northern A.P., southern U.P. and western Maharashtra	*An. culicifacies* A, B, C, D *An. fluviatilis* S, T	High	• Insecticide spray (DDT not recommended). • Selection of insecticide-based on susceptibility status of *An. culicifacies* species C.	Major influence of *An. culicifacies*. Species A, C, D and S are vectors. Selection of insecticide can be based on species C as this species has developed resistance to most insecticides to variable levels.
Division VI				
Orissa, most of the districts of Jharkhand, northeastern districts of A.P. Madhya Pradesh	*An. culicifacies* B, C *An. fluviatilis* S, T, U	Moderate to high	• Insecticide spray. • Selection of insecticide-based on prevalence of the major species. • In *An. culicifacies* prevalent areas, selection of insecticide depending on the susceptibility of species C. • In *An. fluviatilis* areas DDT spray.	Major influence of *An. culicifacies* in plains and of *An. fluviatilis* in hilly forested areas. Species C and S are vectors.
Division VII				
Southern A.P., Karnataka, Kerala, Tamil Nadu	*An. culicifacies* A, B, C, E *An. fluviatilis* T	Low to moderate	• Insecticide spray, larvivorous fishes. • In Kerala chemotherapy and in Karnataka larvivorous fishes are very effective.	Major influence of *An. culicifacies*. Species A, C and E are vectors and B and T are non-vectors. Species E has been identified in Rameswaram Island and a few areas on mainland.

- Insecticide-treated mosquito nets are recommended in the areas where vector species are prevalent, depending on the feasibility, acceptability and sustainability of this intervention.
- It is assumed that treatment of malaria cases are done routinely in all the areas.
- Recommended strategy is based on the present knowledge on the prevalence of vector species and their susceptibility status.

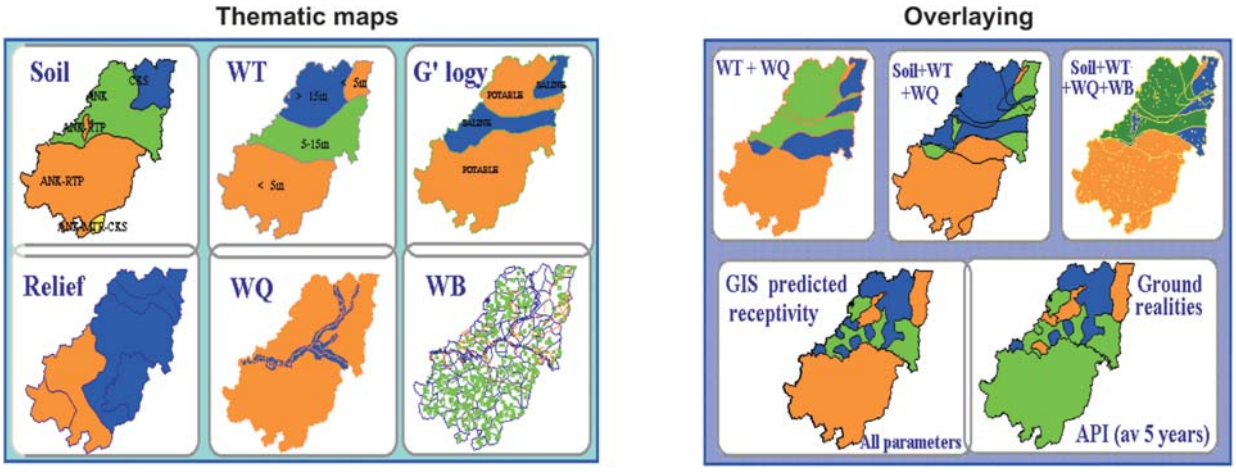


Fig. 69: Thematic maps of Nadiad taluka, Kheda district, Gujarat; and overlaying and integration of maps

Case study 2: Mewat Region, District Gurgaon (Haryana)

Mewat region, Haryana situated at Lat. 26° and 30°N, Long. 76° and 78°E, comprised of six blocks Nuh, Nagina, Taroru, Ferozepur Jhirka and Punhana of Gurgaon district and a small portion of Hatin block of Faridabad district of Haryana (Fig.70a and b). The total population of the region is 0.8 million spread over 491 villages under 84 sections.

A GIS-based study was initiated to aim at the objectives: (i) delimitation of malaria paradigms at macro-level and their epidemiological characteristics; (ii) situation analysis of each paradigm to identify transmission risk factors and to suggest mitigating measures at micro-level; and (iii) identify epidemic risk factors and development of epidemiological information system to assist forecasting of epidemics. The study was conducted in collaboration with Haryana Space Application Centre, Hissar, Haryana.

As per NVBDCP records, malaria in Mewat follows a cyclic epidemic pattern. Soon after resurgence during 1975–76 malaria decreased gradually from 62 API to about 3 API in 1980. In 1981 and 1982 there was a spurt of malaria cases and API went up to 6 and in later years it decreased steadily and remained below 2 API between 1984 and 1994. In 1996, this region experienced an epidemic. In post-epidemic period (1997–99) using GIS, sections having high malaria were identified. It was found that the section Sunehra consistently had high API in all the five years (1995–99), while Gulata (150) for last four years from 1996–99, Nuh and Kherla for three years out of 5 years. Rest of the sections had high malaria in two years out of five year period. As the malaria had decreasing trend, sections with persistent malaria during 1998–99 were identified as sections with residual malaria. These are Sunhera (157), Gulata (150), Sihari (145), Bisro (152), Dudoli (154), Naheda (158), Tirwara (163), Indana (165), Neemka (166) of Punhana block and Kherla (87) of Nuh Block (Figures given in parentheses indicate section number) (Fig. 70b).

Based on geographic reconnaissance, ecological and socioeconomic profile, five malaria paradigms were identified, namely, Irrigation command area, Catchment area, Mining area, Urban area and Flood prone areas (Fig. 71).

Sections falling in each paradigm were extracted, maps were prepared and overlaid successively on high API/residual malaria sections to study their ecoepidemiological characteristics (Fig. 72). Analysis revealed that section Akera (83) has three ecoepidemiological characteristics—irrigation command area, low-lying topography within catchment area and flood prone area. Section Malab (84) has irrigation, whereas sections Nuh (86) and F. Namak (88) have urban/mining and mining malaria ecoepidemiological profiles respectively. Sections Gulata (150), Bisro (152) and Sunhera (157) fall within catchment area. It is worthy to mention that section Sunhera (157) which consistently had high malaria since last five years has four ecoepidemiological characteristics, namely low-lying area within catchment area, mining, urban centre and flood prone areas. Other problematic sections are Kherla (87), Sihari (145), Dudoli (154), Naheda (158) and Tirwara (163) fall in flood prone areas except sections Indana (165) and Neemka (166) which have problems due to Bichchore minor of Gurgaon canal. The surveys are being conducted in Mewat area in the villages of residual malaria sections to identify risk factors at the micro level.

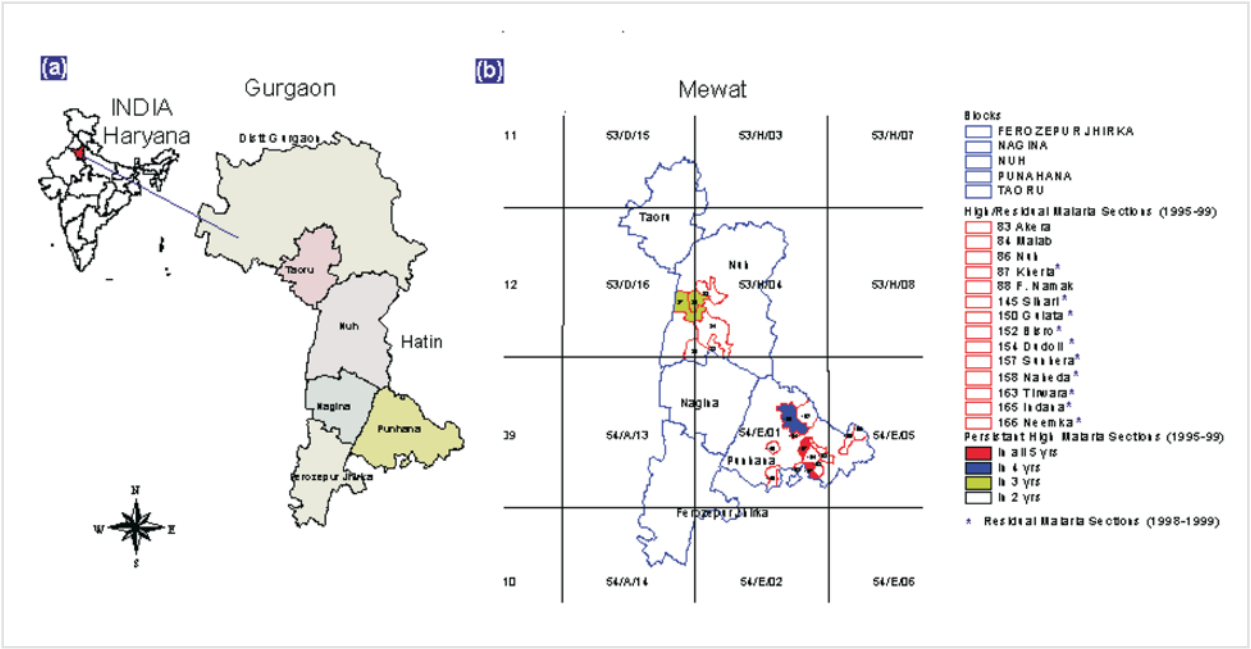


Fig. 70 a & b: Map showing (a) location of study area Mewat in Gurgaon, Haryana; and (b) sections with high/residual malaria (1995–99)

Peptide ELISA: A Simple Indicator of Malaria Endemicity in Communities

Microscopic examination of blood slides, though excellent for clinical diagnosis, is not a practical tool for mass malaria survey in a community. Annual parasite index (API) measurement in a huge population during transmission season is time consuming and greatly dependent on diligence and expertise of primary health workers and microscopists. As a result, API values may not be exact due to human error or shortage of manpower and it is recognised that malaria incidence is under

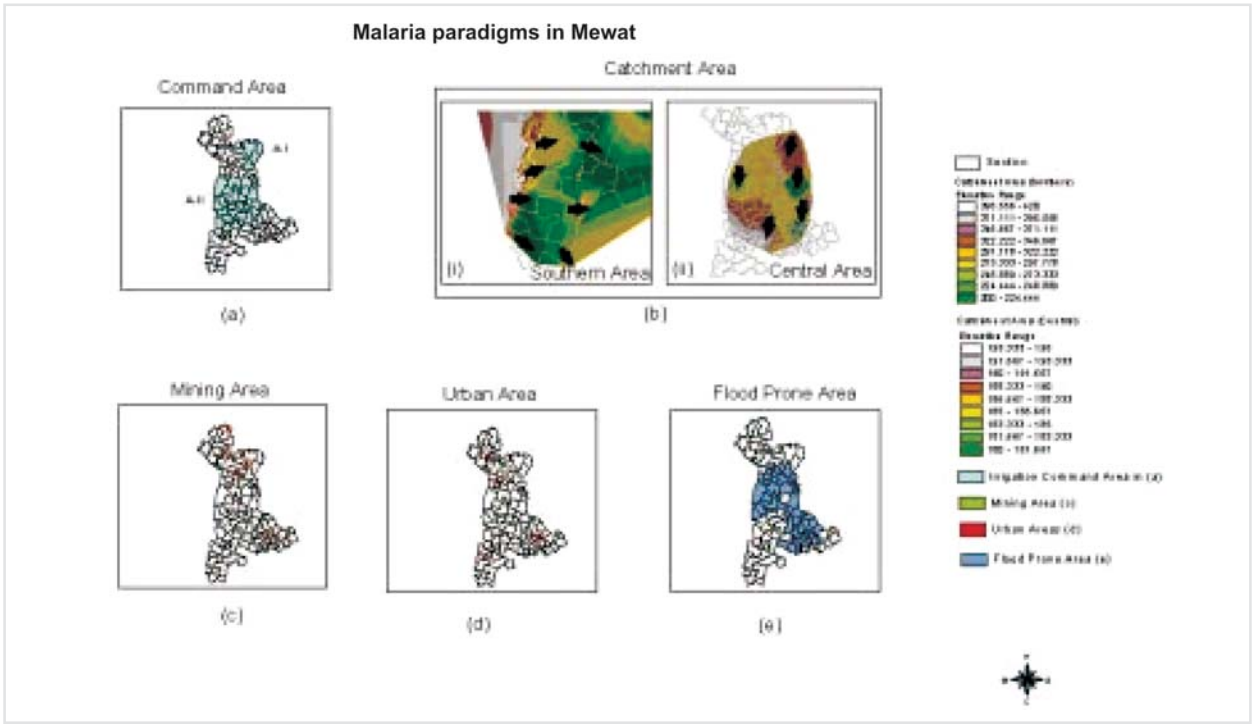


Fig. 71: (a) Showing the two command areas A-I and A-II in Mewat; (b)– (i) 3-D Tin model shows depression areas in part of southern and central Mewat, and (ii) Tin model was regenerated for central Mewat by taking dense contour lines; (c) & (d) Mining and urban areas respectively; and (e) Flood prone areas

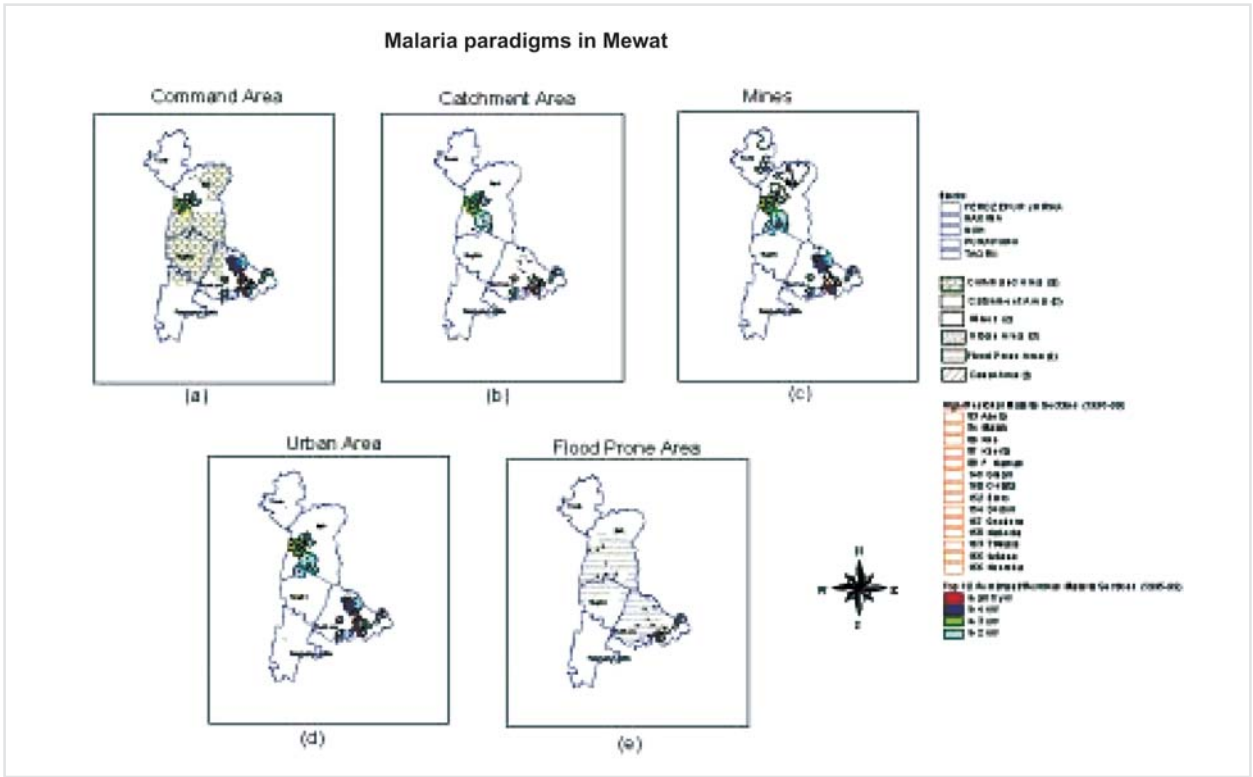


Fig. 72: Persistent high malaria and residual malaria (1995–99) and their eco-epidemiological characteristics as revealed by overlaying maps of each paradigm on high risk sections

Table 21. Comparison of seroreactivity with different malarial peptides and blood samples under different malarial situations

Area	History of malaria exposure	No. examined	Current status of malaria endemicity	ELISA OD of malarial peptide antigens						
				AR1	*Pf*	LSAR	HRP	CSP-60	$EENV_4$	SC-5 non-malarial peptide
Hindu Rao Hospital, Delhi	(0-2) yrs infants, no history of malaria	42	Non-endemic locality	0.141 ± 0.03	0.205 ± 0.024	0.191 ± 0.050	0.192 ± 0.047	0.165 ± 0.048	0.100 ± 0.035	0.164 ± 0.049
Jaisalmer, Rajasthan	Non-endemic	62	Epidemic	0.153 ± 0.058	0.162 ± 0.126	0.273 ± 0.089	0.160 ± 0.102	0.195 ± 0.061	0.147 ± 0.104	0.242 ± 0.134
Raigarh Hospital O.P.D.	Non-endemic	42	Epidemic	0.151 ± 0.059	0.159 ± 0.059	0.091 ± 0.03	0.082 ± 0.03	0.16 ± 0.05	0.069 ± 0.046	0.366 ± 0.076
Ghaziabad (village-Piyawali)	Endemic perennial transmission	42	Highly endemic	0.677 ± 0.075	0.452 ± 0.059	0.498 ± 0.119	0.47 ± 0.170	0.494 ± 0.069	0.355 ± 0.047	0.277 ± 0.054

AR1 —Non-apeptide (EENVEHDACYS of RESA—*Pf*155 of *P. falciparum; Pf*—Crude parasite antigen; LSAR—Liver specific antigen repeat; HRP—Histidine rich protein; CSP-60—Circumsporozoite protein-60; $EENV_4$—Synthetic peptide of *Pf* 155/RESA tandom repeats of 4–amino acids.

Table 22. Comparison of entomological, parasitological and serological data in three different riverine areas of Allahabad district

Area (No. of villages)	*An. culicifacies* sibling species A (%)	Density per structure (HD only)	Human blood index species A	Malaria incidence SPR (Parasite Index)	Infant parasite rate	Child parasite rate	Serological indices AR1 ELISA OD (No. examined)	Status of endemicity
Gangapar (5)	4 (12.5)	2.3	0 1.8	(1.9)	0	1.5	0.194 ± 0.11 (534)	Low
Doaba (9)	26 (9.7)	11.2	0 2.8	(3.1)	0	1.7	0.119 ± 0.12 (817)	Low
Yamunapar (13)	192 (36.9)	17.1	0.035 8.8	(14.4)	7.7	5.0	0.718 ± 0.18 (1105)	High

HD—Human dwelling.

Table 23. Derivation of ETI values from (AR1) ELISA OD for comparison with API value (ETI = 270.55 × AR1 ELISA OD ± 7.4079)

Area (Endemicity)	Village	Sample size	AR1 ELISA OD	API observed	ETI calculated
Haldwani (Low)	Baira Pokhra	13	0.125 ± 0.06	19	41.23
	Badi Mukhani	14	0.242 ± 0.15	5.8	72.88
	Ratanpur	9	0.099 ± 0.04	35	34.19
	Manpur	16	0.138 ± 0.08	3	44.74
	Jeetpur Negi	14	0.136 ± 0.07	9	44.20
	Anandpur	17	0.134 ± 0.07	22	43.66
	Himmatpur	13	0.160 ± 0.04	42	50.70
	Gusaipur	17	0.134 ± 0.06	31	43.66
Mandla (Moderate)	Chargaon	69	0.42 ± 0.07	100	121.04
	Ghota	81	0.44 ± 0.22	189	126.45
	Somnopur	90	0.29 ± 0.10	291	85.87
	Vijaypur	118	0.32 ± 0.16	81	93.98
Jabalpur (High)	Tarwani	58	0.85 ± 0.11	235	237.38
	Dandwa	58	0.85 ± 0.19	210	240.08
	Magardha	116	1.00 ± 0.13	195	277.96
	Chargaonkala	103	1.19 ± 0.05	293	329.36
	Majhgaon	92	0.91 ± 0.10	381	254.42

reported in our country. We developed a malaria surveillance system (Roy *et al* 1994, 1995) by employing ELISA technique to estimate malaria-specific antibodies in the blood using RESA derived nonapeptide (AR1) as antigen. This nonapeptide has been found to be superior to parasite lysate and several other synthetic peptide epitopes (Table 21). A comparison of parasitological and serological data under different malaria situations led us to develop a hypothesis. Stratification of malaria endemicity (Ansari *et al* 2001) by ELISA method indicates low, moderate and high status depending on AR1 ELISA OD values, <0.3, 0.4–0.7 and >0.7 respectively (Table 22). It is shown that anti-AR1 antibody level is a simple indicator of malaria transmission dynamics in the recent past.

Reliability of the test was evaluated by comparing entomological, parasitological and serological data in Gangapar, Doaba, Yamunapar, three different ecosystems in District Allahabad, Uttar Pradesh (Tiwari *et al* 1994), and a longitudinal study has been done in Piyawali village of District Ghaziabad (Roy *et al* 1998) and Haldwani from 1989–93 in order to check the effect of a control programme (Roy *et al* 1996). AR1 ELISA OD has been used to derive a new parameter—equivalent transmission index (ETI) for determining malaria situation. All the parameters (ETI, API, AR1 ELISA OD) have good correlations (Table 23). Association between infection and seropositivity in individual level from high, moderate and low endemic population has been done. Serology is more meaningful for malaria surveillance in moderate and high endemic areas as well as malaria outbreak situation. Seroreactivity is directly related with the number of malaria episodes for detecting intensity of malaria transmission which can stratify endemicity. Further, the method does not need year long mass survey, sample collection is required once in a year only during non-transmission season. Introduction of new technology is a need. The emphasis of technology improvement is warranted for the communities affected by the incidence of communicable diseases. ❑

Surveys of Human Genetic Markers in Malaria Endemic Areas

Human blood polymorphic systems are important biochemical markers in anthropological surveys especially in relation to disease distribution. G-6-PD deficiency and certain haemoglobinopathies are known to confer a selective advantage to the subjects against falciparum malaria. However, certain antimalarials such as primaquine and other 8-aminoquinolines increase the oxidant stress in G-6-PD deficient individuals resulting in haemolytic crisis which can be fatal if not checked in time. Therefore, information on the frequency and distribution of these variants would help in the administration of proper drugs.

Haemoglobinopathies and G-6-PD Deficiency

Studies carried out by us on mapping of these disorders in various tribal/non-tribal groups living in malarious areas of the country have shown variable frequencies of G-6-PD deficiency (0–17.6%) among tribals of Andhra Pradesh, Assam, Gujarat, Madhya Pradesh, Orissa, Uttar Pradesh and Uttarakhand. Similarly frequencies for sickle-cell haemoglobin (HbAS) ranged from 0 to 18.9% in various tribal groups and HbE (16.5%) was observed only among tribals of Assam. Fig. 101 shows the areas from where population samples have been screened and frequencies of G-6-PD deficiency, carriers of sickle-

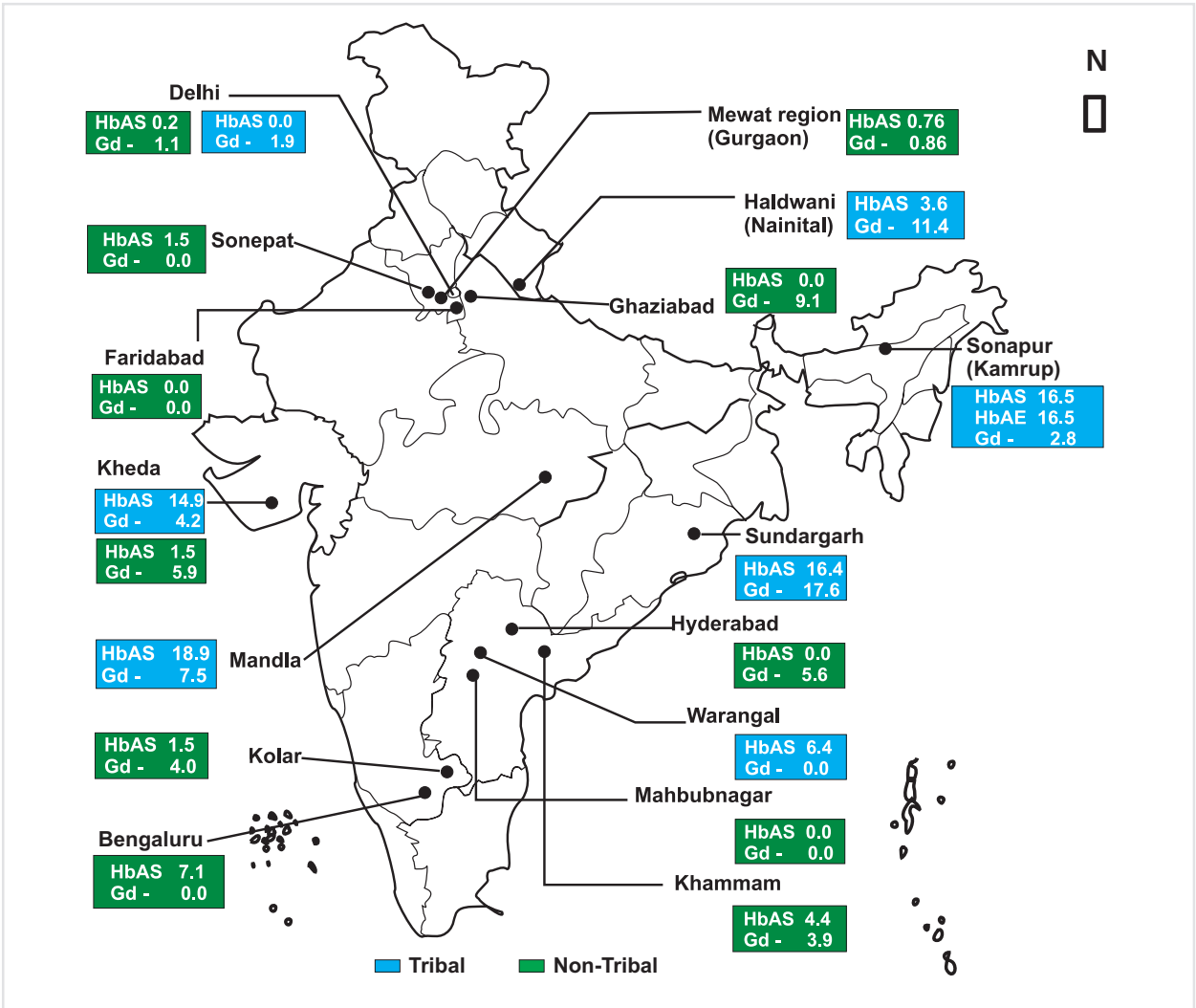


Fig. 101: Surveys of human genetic markers: Frequencies of G-6-PD deficiency (Gd) and haemoglobin (Hb) variants

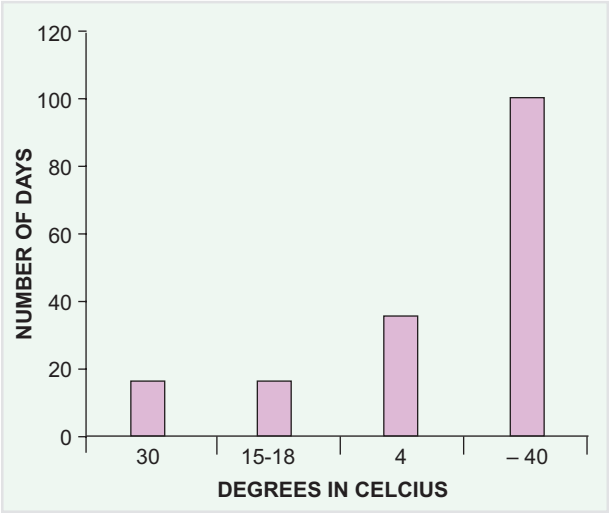


Fig. 102: Stability of the kit stored at different temperatures

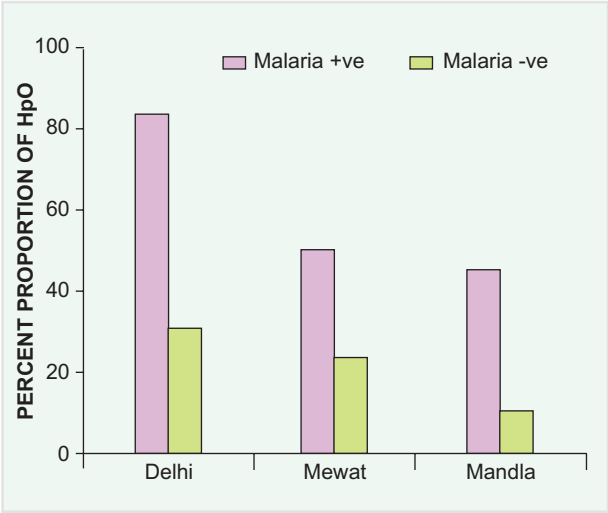


Fig. 103: HpO proportions among malaria positive and malaria negative subjects

cell (HbAS) and haemoglobin E (HbAE) (Joshi *et al* 1985, 1987, 1991, 1998, 1999, 2001). Among non-tribals, G-6-PD deficiency and abnormal haemoglobins occurred in less than 1% of the population with a few exceptions. High incidence of genetic disorders among the tribal groups suggests probable selective role of these genes in the population in highly malarious areas.

A Simple Kit for the Detection of G-6-PD Deficiency

Keeping in view the importance of detecting G-6-PD deficiency in malaria chemotherapy, a simple kit was developed based on the principle of

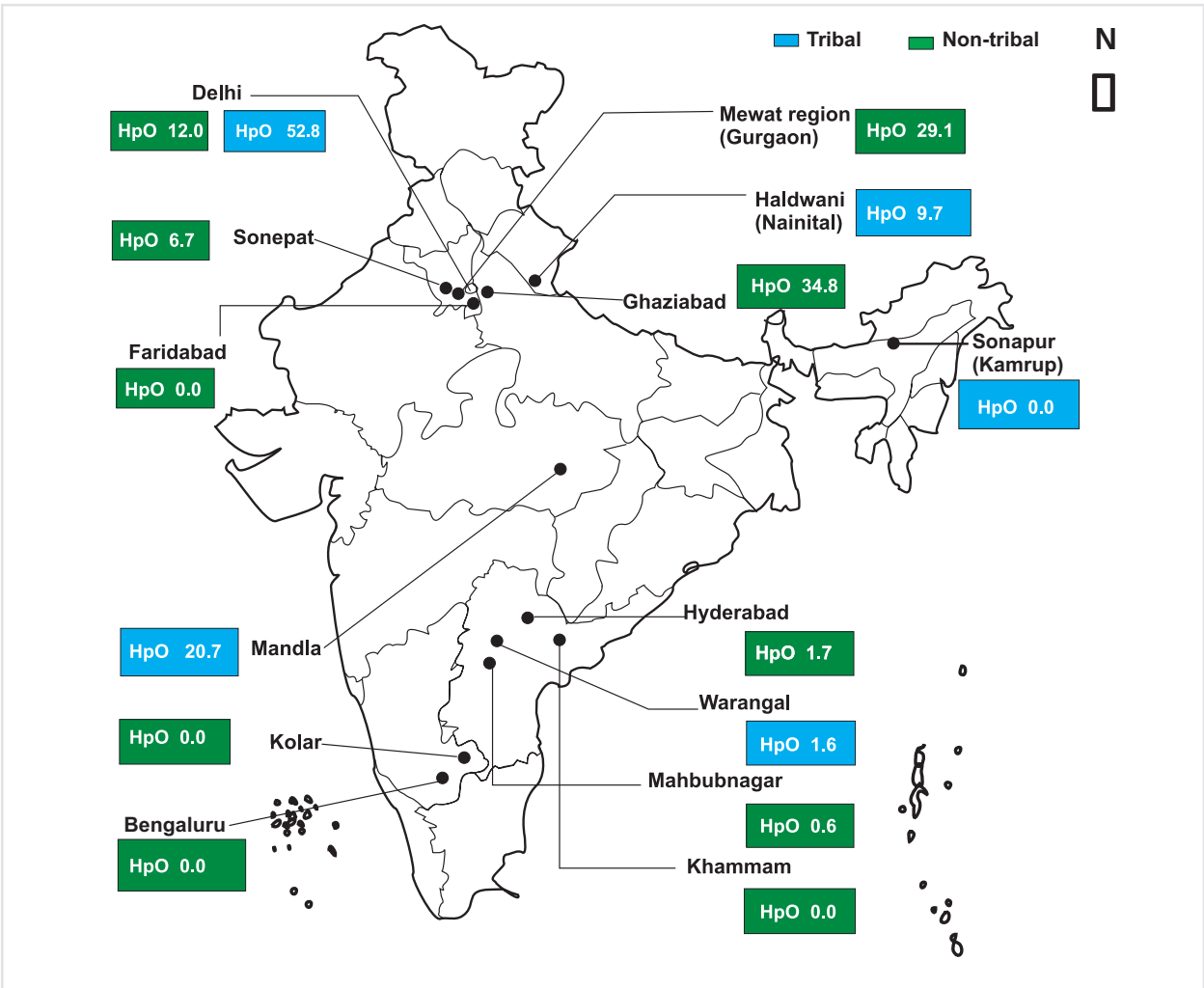


Fig. 104: Surveys of human genetic markers: Frequencies of HpO (ahaptoglobinemia)

fluorescent spot method (Schmidt and Brosions 1978, US Deptt. HEW Pub No. (CDC) 78-8266, p. 77). The kit has been compared with the standard fluorescent spot and electrophoretic method using blood samples collected from Delhi and Sonapur, Assam. This kit has given satisfactory results till 2 weeks (16 days) under field conditions (30°C). Fig. 102 shows the results of evaluation of the kit at different storage conditions. Now the kit is being evaluated at many of the field units of NIMR to test its feasibility under field conditions.

Ahaptoglobinaemia

A high incidence of ahaptoglobinaemia (non-typable haptoglobin–HpO) was observed among malaria patients (Joshi *et al* 1987, 1998) (Fig. 103) and incidence increased with the increase in malaria attacks (Joshi *et al* 1991). Higher incidence of HpO was observed in the population during malaria epidemics (Joshi *et al* 1991, 1999). Antimalarial therapy in ahaptoglobinemic patients has shown normal levels of haptoglobins in about 75% of the subjects within 8–9 days of post-treatment. It is concluded from the study that association of HpO with *P. falciparum* and *P. vivax* malaria is present in Indian population. However, HpO can not be used as an index to study malaria positivity because of its low reliability. Fig. 104 shows the areas from where population samples have been screened and incidence of HpO in the population was surveyed (Joshi *et al* 1985, 1987, 1991, 1998, 1999, 2001). ❑

Health Impact Assessment (HIA) of Development Projects with Reference to Mosquito-borne Diseases

It is recognized that the very development that intends to improve the quality of life of the people, if not managed properly, often leads to conditions hazardous to health of the people and their environment. Poor environmental conditions are the cause of most water-borne, air-borne and vector-borne diseases and contribute to poor health and a poor quality of life. Economically poor communities, children, women, most people in least developed or developing countries and the migrant work force generally constitute the most vulnerable groups. Rio de Janeiro Earth Summit in 1992 stressed that development was about meeting the needs of people, their health, well-being and lives, and a safe environment.

Broadly, the health impact assessment (HIA) is a change in health risk reasonably attributable to a project, programme or policy where a health risk is the likelihood of health hazard affecting a particular community at a particular time. Health impact assessment can make health benefits more explicit

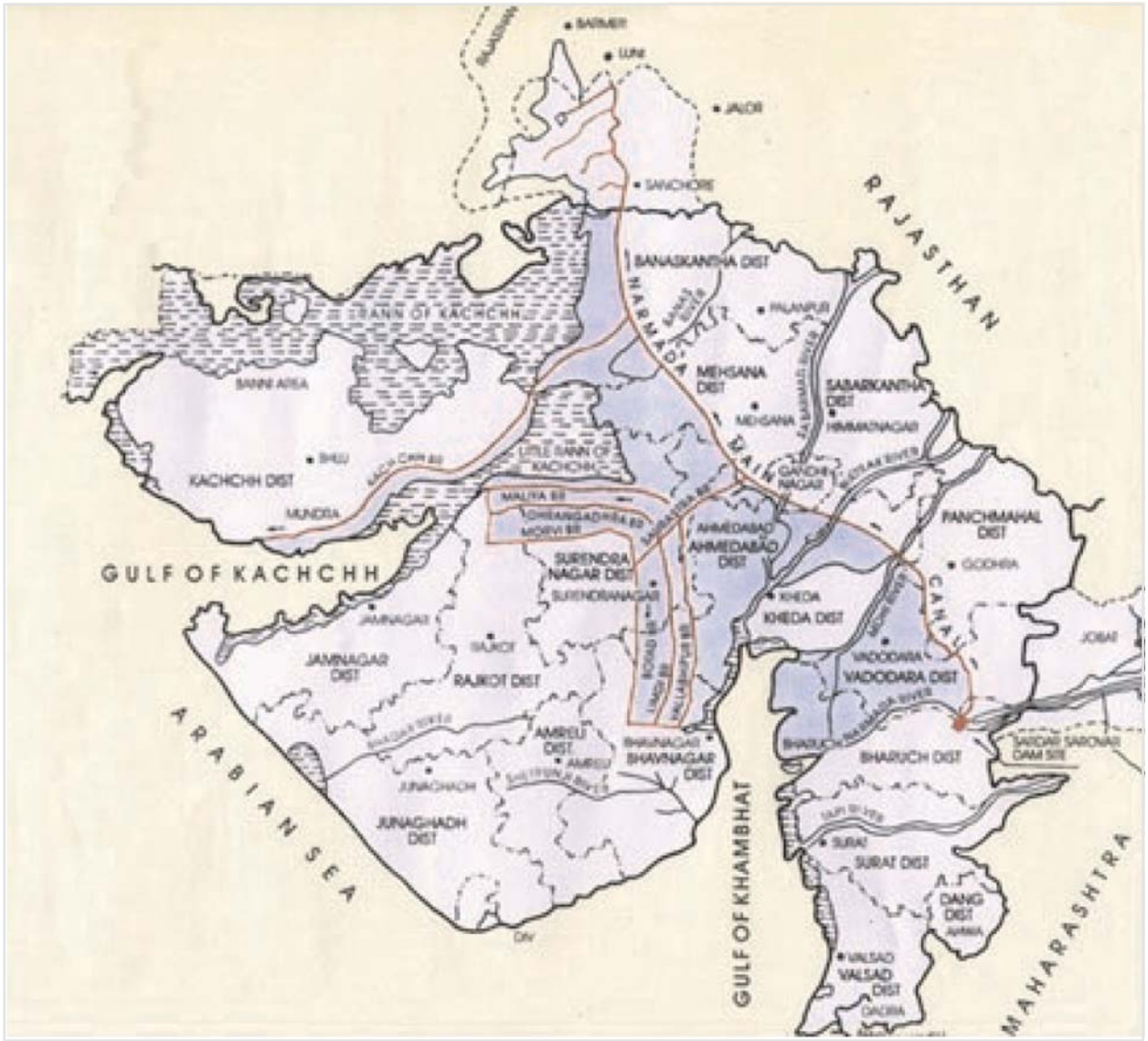


Fig. 105: Command area of the Sardar Sarovar Project in Gujarat

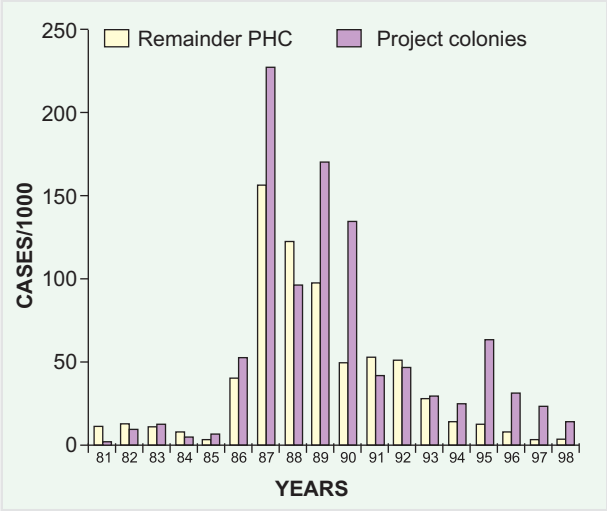


Fig. 106: Secular trend of malaria in project colonies and the surrounding PHC villages (Sardar Sarovar Dam)

Fig. 107: Malaria incidence in the project colonies and the surrounding villages (Sardar Sarovar Dam)

in non-health areas, can look at the unintended health impacts of non-health policies which would not otherwise be explicit and help in either incorporating health safeguards in the project cycle or taking mitigating measures. Whereas positive impacts of development improve overall well-being, the types of health hazard associated with a development project are broadly classified as communicable diseases, non-communicable diseases, nutrition, injuries and psychosomatic disorders.

The problem of vector-borne diseases associated with water resource projects has received a considerable attention for many years. During the pre-DDT era, most major projects in India were being prospectively assessed for adverse health impacts. By providing proper subsoil drainage, the Sarda Canal Barrage in Uttar Pradesh did not create malariogenic conditions. Other noteworthy examples of assessment of impact on malaria of the water resources development projects include Upper Krishna Irrigation Project, Irwin Canal (Karnataka), Thermal Power Plant in Delhi and Vizag Steel Plant, Visakhapatnam.

HIA Studies undertaken by NIMR

Sardar Sarovar Water Resources Development Project (Gujarat)

The Sardar Sarovar is a multipurpose water resources development project created on river Narmada in the Gujarat state (Fig. 105). It will generate electricity, provide irrigation and drinking water to nearly 110 cities and towns in the semi-arid areas of Gujarat and Rajasthan states in western India. Construction of dam started in 1979 and picked up momentum in 1986. Several residential colonies for engineers, workers, labourers and other supporting communities came up in the close vicinity of the dam.

An unusual increase in malaria was recorded at the project site following commencement of the construction of the dam (Fig. 106). An epidemiological investigation undertaken by NIMR during 1994–98 showed that *An. culicifacies* and *An. fluviatilis* were present in the colonies at the project site and in the surrounding villages. *An. culicifacies* was the predominant species. Although the dam has been built in the valley area, creation of dyke ponds and interruption of downstream flow of water caused potential breeding habitats of vector species resulting in a built up of high vector densities. The incidence and prevalence rates of malaria were far higher in the population at the project site as compared to those in the surrounding villages (Fig. 107). The increased transmission of malaria at the project site was attributed to the factor such as the congregation of migrant work force from the malaria endemic areas of the country in close proximity of the mosquito breeding habitats created by the project.

Assessment of the impact of the project on vector-borne diseases was undertaken since July 2002 in Phase I area covering the districts of Narmada, Bharuch, Vadodara and Panchmahals in Central Gujarat. The project was implemented in collaboration with the Department of Health, Govt. of Gujarat, Sardar Sarovar Narmada Nigam Limited, Sardar Sarovar Punarvasvar Agency and Bhaskaracharya Institute of Space Applications and Geometry, Gandhinagar. The main aims were to assess changes in environmental receptivity of the project area, community vulnerability to various diseases and the preparedness of health services to mitigate any adverse effects. Various parameters were monitored regularly at the dam site colonies, in selected villages in the command and non-command areas and rehabilitation and resettlement (RR) sites in the four districts. The activities included geographical reconnaissance (GR) of borrow pits and other mosquito breeding habitats created by

construction of the canal network, studying abundance of mosquito vector population, bionomics of vectors of malaria, filariasis and dengue, evaluating insecticide susceptibility of mosquito vectors, measuring overall vectorial potential, determining parasite prevalence by mass blood/rapid fever surveys, asses malaria risk by application of GIS, and training needs assessment.

The GR of outdoor breeding habitats revealed that *An. culicifacies* was predominant species and preferred irrigation related structures, viz. dyke ponds, canals, siphons, seepage channels and seepage water pools, for breeding. Breeding of JE vector *Cx. vishnui* group was also recorded from some of the structures, particularly during the rainy season. No breeding of *Ae. aegypti* and *Ae. albopictus* was found in any of the peri-domestic breeding sources surveyed. The GR revealed that the development of canal network has considerably increased the environmental receptivity for vector borne diseases in the command area especially near the canal network.

In adult collections by pyrethrum space spraying, *An. culicifacies* was collected in all seasons and accounted for 78–82% of all mosquitoes at the dam site, followed by 61–69% in the non-command area, 32–51% in the command area and 36–44.2% in the RR sites. *An. fluviatilis* and *An. stephensi* were recorded from three districts (except Bharuch) only and their density remained considerably low (<1/room) in all areas. The composition of *Aedes* species in all mosquitoes ranged from 0 to 1.2% and that of all *Culex* spp from 8.2% at the dam site to 23.1% in the non-command area.

An. culicifacies, the main malaria vector in the area, was found to be highly zoophilic (Anthropophilic Index— 0.3%; 7/2660) and its population composed of sibling species B (87.2%; 130/149), C (7.4%), A (4.8%) and B/C (0.7%). *An. fluviatilis* was exclusively zoophilic (0/154) and was represented by species T only (100%; 14/14), which is a non-vector, in this area. During 2003 to 2006, out of 5201 specimens evaluated by ELISA, only three were found to have sporozoites (*Pf* 2; *Pv* 1).

An. culicifacies was found to be resistant to DDT (mortality—51.7%) and malathion (mortality—61.7–86.7%), whereas it was largely susceptible to various synthetic pyrethroids tested (mortality >90%).

To know whether intermediate hosts of Guinea worm disease and Schistosomiasis are present in the project area, a survey of Cyclops and snails was done. Cyclops and some species of Molluscs were found in habitats such as dyke ponds, minor canals, branch canals, siphons of distributory and main canal. Guinea worm disease was last reported in Gujarat in 1994, while Schistosomiasis has never been reported in this area.

Comparison of malaria incidence in the command and non-command area revealed that the disease followed an identical pattern. Annual parasite incidence (API remained low (<1) in the command and non-command areas of Vadodara district during 2000–03 and although it increased during 2004, it remained low (1.9/1000 pop.) in the command than in the non-command (2.7/1000 pop.) areas. Correspondingly, proportion of *P. falciparum* too remained low (18.1%) in the command area than that in the non-command area (26.4%). Malaria indices in both areas in Panchmahals district remained identical. Further, to verify this, two cross-sectional mass blood surveys (MBS) were carried out in the command and non-command area during 2003 (pre-monsoon). Parasite rate was low in both areas (command area—0.19%; 12/6175), non-command area (0.11%; 6/5222). In another MBS in 2005 (post-monsoon), the overall parasite rate was 0.5% (31/6091). GIS-based mapping of malaria incidence in the phase I districts showed low incidence of malaria although malaria showed variations from year to year. In the year 2005 and 2006 too, malaria API was <2 in Vadodara and Panchmahals. In Bharuch, API was >2 in 2005 and declined to <2 in 2006. On the contrary, in Narmada district, there was some increase in malaria in 2006.

The low parasite rate as shown by mass blood surveys, or a low incidence of malaria as shown by low sporozoite rate and routine surveillance data in spite of creation of more mosquitogenic conditions in the project area, were mainly due to an effective anti-malaria programme wherein pyrethroid insecticides were sprayed indoors in all high risk villages or RR sites each year together with strengthening of malaria surveillance and treatment services. The health services at various tiers have largely been strengthened to deal with any real increase in risk of vector borne diseases mainly due to the high priority attached to the project. Yet a few gaps in the form of vacancies and training needs in various cadres of health staff need urgent addressing. Since water release for irrigation has been started in some areas and so far the study covered the pre-irrigation baseline phase, the health impact study will continue both in phase I area as well as well in new areas where canal network is being created.

Bargi Dam Project (Madhya Pradesh)

Bargi Dam has also been created on the River Narmada in Madhya Pradesh during 1974–88. Building of the dam has resulted in the submergence of 162 villages in Jabalpur, Mandla and Seoni districts. A study by NIMR on the impact of the project on malaria following the report of deaths in some submerged villages of District Mandla (Singh *et al* 1997) showed that the villages had high incidence of malaria, gametocyte carriers (13%) and splenomegaly among the children. Submergence caused a several fold increase in the malaria incidence and *P. falciparum* cases. There is

substantial evidence to indicate that *Pf* has invaded almost the whole region and caused immense misery, including many deaths.

Among the malaria vectors—*An. culicifacies* and *An. fluviatilis*, the former was resistant to DDT and HCH. In a newly irrigated area in District Jabalpur, *An. annularis* was the predominant species in the head-end villages and its abundance was directly related to the opening of the canal, whereas *An. culicifacies* was the most abundant species in tail-end villages with scanty irrigation. Malaria infection was due to *P. vivax* and *P. falciparum*. The annual parasite incidence in children and adults was 4-fold higher in head-end villages as compared to that in tail-end villages.

Some of the risk factors identified are: widely distributed clusters of hamlets, increasing operational difficulties in disease surveillance, continuance of the people in some submerged villages, increased groundwater level, fishermen's preference to live close to the reservoir (Fig. 108), migrant fishermen and lack of commensurate increase in medical care and insecticide spraying. Effective prevention and control of malaria in affected villages should include improved disease surveillance, use of effective antimalarial drugs, use of rapid diagnostic kits in epidemic situations, health education, use of effective insecticides, promotion of the use of insecticide impregnated nets. Displaced people should be resettled in healthy areas. Poor engineering design is difficult to correct after construction, and hence early planning is critical. It is already too late for Bargi Dam to prevent some of these consequences. Construction is progressing and action is required now to develop an effective health care programme based on local transmission involving multisectoral action and community participation to prevent the spread of disease in the whole region.

Fig. 108: Photograph showing hutment in Bargi Dam area

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

The project on Health Impact Assessment on Indira Sagar Dam and RR Colonies in SSP Reservoir was submitted to Narmada Valley Development

Fig. 109: Mosquito breeding habitats found near the dam site

Authority, Bhopal in August 2003 and study was initiated in January 2004 with the following objectives: (i) to raise bench mark data on the incidence of vector born diseases (VBD) especially malaria in the rim villages adjacent to estimated high flood level (HFL) on Indira Sagar Dam for comparison during subsequent phases of construction operations; (ii) to assess the adverse health impact of reservoir, in "Drawdown" zone, downstream, canals and command areas on incidence of malaria other vector borne disease; (iii) to assess risk factors related to malaria and other vector borne diseases and water born diseases in Resettlement and Rehabilitation colonies; (iv) to assess the quality of drinking water in terms of toxic minerals in the existing water sources (if any) and microbial contamination in the canal drinking water sources; (v) to make recommendations of mitigation measures for each component in dam, conveyance, command areas and resettlement colonies for control of malaria and other vector borne and water borne diseases.

Till April 2008, 13 surveys in different seasons were carried out in seven districts, viz. Khandwa and Dewas (ISP & OSP), Khargone and Harda (ISP), Badwani, Dhar and Jhabua (SSP). Mosquitogenic conditions created due to dam construction, viz. seepage of the reservoir, pits and pools of down streams, new canals created, curing tanks etc. (Fig. 109) have been identified. Surrounding to these, a total of 32 villages, 18 rehabilitation and resettlement centres and five command area villages and six labour colonies under seven districts have been surveyed for entomological and epidemiological data for all the vector borne diseases, i.e. malaria, dengue, JE and filariasis.

Man hour density/room density of malaria vector *An. culicifacies* and *An. stephensi*, filaria vector *Culex quinquefasciatus,* JE vector *Culex vishnui* and Dengue vector *Aedes aegypti* were calculated in all surveys. Impact of dam construction was observed in nine villages as the vector density was reported high till July–August 2005. To establish the transmission, other entomological parameters, viz. biting habit, parity rate, gonotropic cycle, sporozoite rate, human blood index and presence of sibling species were also carried out. Breeding sites created due to dam construction were surveyed for larval breeding and species-specific breeding sites were identified for all the disease vectors. The susceptibility test for *An. culicifacies* was also carried out in pre-, post-monsoon and winter seasons. *An. culicifacies* was found resistant to DDT and susceptible to synthetic pyrethroids. Report on the vector breeding and malaria positive cases was sent to the state health department and NVDA health authorities.

GIS mapping of all the seven districts had been completed. Digital maps of villages were prepared and attached with attribute and malaria data. Trend analysis of epidemiological data from 2002–05 has been done. The data on various entomological and parasitological parameters which are being collected through periodic surveys are regularly put into GIS based frame work to view the impact of the construction of dams in space and time.

Fig. 110: Meeting with NVDA authorities

Water samples collected from tap, hand pump, tube-well in each survey were analyzed by Public Health Engineering Department and was found safe for drinking. Recently, in April 2008, tap and hand pump water samples from 11 villages of three districts, namely Jhabua, Barwani and Khargone were tested for Coliform and other harmful bacteria. All the tests were negative and water was found safe for drinking.

After completing each survey, meetings was held with Vice-chairman, NVDA and state authorities and survey highlights and actions required for developing situation specific mitigating measures, i.e. engineering, epidemiological and entomological to control the vector borne diseases were suggested (Fig. 110).

From October 2005, the following suggested mitigation measures were implemented in the field by State Health Department, NHDC and NVDA: de-weeding in canals, release of larvivorus fishes in tanks of Narmada Nagar, RR center and ponds and wells of villages, source reduction, spray of pyrethroids in Narmada Nagar and DDT in problematic villages/RR Centers, cleaning and oiling of drains in Narmada Nagar on weekly basis, fogging in power houses, construction of mosquito proof house in Omkareshwar Dam site, IEC activities (Fig. 111) in villages/RR Center and Narmada Nagar and radical treatment to all the *Pf* cases.

Due to this the vector density of malaria, dengue, chikungunya, JE and filariasis were reduced drastically and the impact of this density was also observed on the number of malaria cases which have been reduced from 250 in 2005 to five cases till April 2008. It is important to note that in Sharda Canal area, within four years (1924–28) about 38,000 malaria cases and thousands of deaths were recorded whereas in our study, within three years (2004–07), about 500 malaria cases were recorded with no deaths.

Blood samples were also collected for dengue,

Fig. 111: IEC activities near the dam site

JE and filariasis but none was found positive (Fig. 112). It is also worth to mention that no vector species of kala-azar was recorded from this project area. Schistosomiasis was also not recorded but the detailed study on schistosomiasis and kala-azar will be initiated soon and report will be submitted to NVDA. The health impact study is in progress and will be continued up to December 2010.

In a recent survey (September 2008), we found that drinking water from hand pump is being used in Kakrana, Mahammadpur, Dogargaon, Collector Nagar and Jogakalan. Tube well water stored in tank is supplied in the Borlay village for drinking while in Piplood village drinking water is being supplied from open well. This water was tested for *Salmonella typhimurium*, *S. enteritidis, Citrobactor freundii*, *Vibrio cholerae* and *Vibrio parahaemolyticus* using Hiwater test kit (HiMedia). Presence of *Salmonella typhimurium*, *S. enteritidis, Citrobactor freundii* was recorded in the drinking water of Borlay, Piplood, Mahammadpur, Dongarkalan and Collector Nagar. *Vibrio cholerae* and *Vibrio parahaemolyticus* were also found in the drinking water of Mohammadpur and Dongargaon respectively. None of the bacterial species tested were found in the drinking water of Kakrana and Joga Kalan village.

Fig. 112: Parasitological surveys in the RR villages

Konkan Railway Project

Historical Account

Construction of Railways in malaria endemic tracts was always associated with serious outbreaks of malaria causing enormous morbidity and mortality in the workforce. As a result, these projects were subjected to inordinate delays and cost escalation. In 1900, the survey of line at Liang-Biang (Indo-China) caused 77% mortality amongst Europeans and 80% amongst natives in 8 months. In India, in the hilly tracts between Vizianagram and Raipur, malaria was invincible. Attempts to survey the area failed in 1883, 1897 and 1907. Surveys could only be completed in 1925 when all the staff was duplicated. In the words of Senior-White, the cost in terms of human lives was astounding and the popular phrase to describe the social cost during the project was “A death a sleeper”. Similarly, when the Ambda-Jambda branch of Bengal-Nagpur railway was built through Singhbhum district of Chhota Nagpur in 1923–24, mortality due to malaria amongst engineers and labour was so high that the Frontier Army had to be called in to complete the project amidst large-scale revolt and desertions by the workers. Even after the commissioning of the railway line, malaria transmission continued resulting in heavy economic burden on the railways on account of morbidity and mortality among the railway employees.

On the Western Coast of India, a tentative survey on the Konkan Railway (KR) route had been done about a century ago but owing to a very difficult terrain, railway line could not be built until 1977 when a 65 km stretch was built between Apta and Roha. British had avoided laying a line in this region due to high cost involved in bridging many rivers and valleys and boring of tunnels through several hill ranges. KR project is the biggest new railway line construction undertaken on the Indian sub-continent in the past century. Konkan Railway Route passed through Raigad, Ratnagiri and Sindhudurg districts of Maharashtra, North and South Districts of Goa, Uttara Kannada and Dakshina Kannada Districts of Karnataka State (Fig. 113). KR project, which now connects Roha to Mangalore has been described as a marvel and a rare fete in the civil engineering because an almost impossible 760-km project was executed in a little over 7-years. To have such a railway project along the west coast was the dream

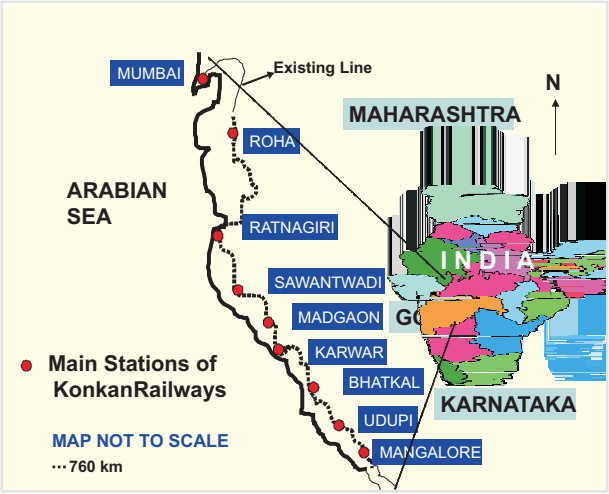


Fig. 113: Konkan Railway line along the west coast of India

of many in Maharashtra, Goa and Karnataka but only a few thought it would ever be realized. The Konkan Railway line was commissioned in phases from 1993 onwards in 11 sectors and finally fully commissioned in 1998 when it was formally declared open by the then Hon'ble Prime Minister of India Shri Atal Bihari Vajpayee.

Environmental Concerns about Konkan Railway Project

In Goa sector, a stretch of 55 km from Mayem to Bali became subject of controversy as it was argued that irreversible damage would result to Goa's ecology and social life. Particularly, it was felt that the coastal silt lands ('Khazan' Lands) which are highly productive would sink and get inundated leading to mosquito breeding and outbreaks of mosquito borne diseases especially malaria and Japanese encephalitis. It was also felt that railway line was passing close to a heritage structure and continuous vibrations would cause damage to the structure of the shrine in Old Goa. The Ministry of Railways appointed a former Railway Board Chairman to give his opinion on the alignment and barring few suggestions concurred with the existing alignment. In June 1992, the Ministry of Environment appointed a 15 member committee headed by Late Mrs. Kamla Chowdhary to examine environmental, heritage and other issues. The committee with majority opinion suggested retaining of the approved alignment. However, 5 dissenting members submitted a separate report. Hence, the Ministry could not take any decision on this report. The agitation continued and chain hunger strikes, rallies, human chains, the burning of contractors' machinery and labour camps were resorted to. To oppose Lathi charge by the police three persons went on fast unto death and this issue and works on Mayem to Bali section was suspended on 25 March 1993. Justice G.J. Oza, a retired Supreme Court judge was appointed to inquire into demand for change of alignment. Justice Oza studied the approved and proposed alignments, held public hearing and submitted a detailed report suggesting remedial measures and extensive additional changes which were accepted by the Government which cost additional Rs. 30 crores.

The Konkan Terrain

The entire coastal belt is characterized by plentiful and regular seasonal rainfall, oppressive weather in the hot months and high humidity throughout the year. There are four distinct seasons, i.e. summer from March to May, the monsoon from June to September, the post monsoon season from October to November and mild winter from December to February. The rainfall increases from North to South and from Coast to Western 'ghats'. It is generally around 2100 mm in the coast while Amboli ghats of Ratnagiri district receive about 7500 mm rainfall annually. Terrain and climatic conditions were highly conducive for malaria transmission in villages, towns and railway colonies all along the track. The scope of water stagnation and consequently mosquito breeding in and around depressions near embankment, cutting sections, pipe and box culverts, drains of washing yards, abandoned masonry and curing tanks, overhead and septic tanks, depressions between two tracks at the stations were enormous to favour mosquito proliferation round the year. The apprehension of enhanced malaria transmission in the entire region was further strengthened by the fact that malaria was already endemic in Mumbai and had also invaded Panjim town in Goa. Accelerated vector breeding and aggregation of tropical labour and movement of carriers (people with malaria infection-reservoir of infection) would establish indigenous transmission of malaria in the entire coastal zone.

KR Project Major Works

- There are 71 tunnels with a total length of 75 km which is 11% of the total length of the project. The Karbude tunnel in Ratnagiri district is 6.5 km long followed by Nathuwadi tunnel (4.4 km), Tike and Bardewadi tunnel (4 km).
- Minor bridges with total span of less than 12 m which included irrigation openings, RCC Pipes, slabs, boxes, arches, were 1599 in number covering about 5.73 km. A total of 171 major and important bridges covering a length of 21.5 km were constructed approximately one in every 4.2 km length (Fig. 114).
- The earthwork constituted major proportion of the line about 627 km (84.8%). The earthwork included cutting of hills or embankment for laying tracks.
- Konkan Railways has in all 56 stations, so designed that they merge with the ambience of the Konkan region.

In the environmental impact assessment (EIA) report prepared by RITES India Ltd., no specific mention was made of any mosquito-borne disease

Fig. 114: To lay railway line long bridges had to be constructed over the broad perennial rivers

potential due to the project activities. This necessitated conduction of an independent HIA especially with regard to malaria. The first step in the risk assessment was the constitution of two teams one for parasitic surveillance to assess the existing load of malaria infection in migrant labour force and the second team to conduct detail vector surveillance and record observation especially on water stagnation points near minor and major construction sites such as pipe culverts, bridges, box culverts, tunnels, stations, staff quarters, yards, sleeper curing tanks, etc.

The Challenge

There were mainly two challenges to ensure prevention and control of malaria along the Konkan Railway: (i) To enumerate the problems arising out of the construction works and suggest remedial measures; and (ii) To supervise field work to ensure that mosquito breeding potential was eliminated on long term basis.

Objective

- Prevention of mosquitogenic conditions and indigenous transmission of malaria and other mosquito borne diseases along the Konkan Railway track.

The Strategy for Prevention of Outbreak of Mosquito Borne Diseases

The following strategy was drafted in consultation with the Konkan Railway management and other stakeholders.

1. Health impact assessment of all works related to Konkan Railway construction.
2. Selection of cost effective and environmentally sustainable interventions
3. Develop team work with the KR, contractors, communities and staff of the NIMR
4. Monitor progress through entomological and parasitological indices.

Cost Sharing

The cost involved in the planning, monitoring and supervision was contributed by the NIMR. Cost of material, equipment, and labour and engineering related inputs/interventions were provided by the Konkan Railway Corporation.

Activities

- Establish linkages and coordination with Konkan Railways
- Provide consultation at planning and design stages
- Blood smear collection of all migrant labour and examination
- Delineation of mosquito breeding potential and ensure interventions
- Monitoring of the mosquito control and malaria treatment works
- Cost estimation of various interventions
- Review, plan and implement decisions taken from time to time
- Make recommendation for maintenance of works

Major findings

Entomological surveys

The entire line of 106 km in Goa sector was covered on foot to conduct a comprehensive survey to detect potential water stagnant areas where breeding of mosquitoes could occur. The NIMR survey team comprised of entomologists, engineers, draftsmen and field workers to assess vector potential at all the sites where water stagnations were possible and to prepare engineering drawings, suggesting corrections to ensure prevention of water stagnations and mosquito breeding. Altogether, 581 sites were inspected including pipe culverts, box culverts, major and minor bridges, tunnels at excavation sites,

Table 24. Mosquito breeding habitat surveys at the project sites and along railway embankment in Goa sector

Type of breeding site	No. checked	No. positive for mosquito breeding (%)
Pipe culverts	147	12 (8.16)
Box culverts	15	3 (20)
Masonry tanks	33	7 (21.2)
Earth depressions	179	23 (12.84)
Drains in the cutting sections	16	5 (31.2)
Drains in the tunnels	40	1 (2.5)
Overhead tanks	7	0 (0)
Fire buckets	20	0 (0)
Water channels	103	17 (16.5)
Sleeper curing tanks	21	12 (57.14)
Total	581	80 (13.76%)

sleeper manufacturing plants, station buildings, staff quarters, etc (Table 24). The mosquitogenic features of each site were recorded and location drawings were prepared by the engineers and draftsman for easy understanding of the problem by railway engineers concerning mosquito potential for rectification.

Mosquitoe Vectors Encountered

An. stephensi
An. culicifacies
An. fluviatilis
Cx. quinquefasciatus
Cx. vishnui group
Ae. aegypti
Ae. albopictus and
Other species —7

Parasitological Surveys

All the 23 project sites under construction such as bridges, tunnels, embankment and sleeper construction plants were visited by the team of NIMR and Medical Officers of Directorate of Health Services, Goa. Konkan Railway site engineers and construction companies ensured availability of all workers and their families for blood screening and clinical examination. Demographically, out of 706 construction workers examined, 665 had migrated from 14 different states of India. Only 36 were natives of Goa and five were natives of Nepal. All 706 construction workers were clinically examined for hepatomegaly and their blood smears were examined for malaria. Of these, six (*P. vivax* 5 and *Pv+Pfr* 1) were suffering from malaria (SPR 0.84%). Presumptive treatment was given to all and positive cases were administered radical treatment within 24 hours as per NVBDCP drug policy. As many as 153 (21.6%) workers had suffered from malaria in the past one year. The spleen rate was 11.7% and average enlargement spleen (AES) was 0.19. Hepatomegaly was observed in 9.91% labourers mostly associated with alcoholism (Table 25). Although, only six cases of malaria were detected in mass surveys, it clearly indicated a threat of outbreak of malaria amongst labour force engaged in the project. Secondly, labour from 14 different states with different parasitic strains and immune status residing in common camps could lead to outbreaks.

Engineering Works

Monitoring of Konkan Railways Project during Operational phase ensured that all interventions were implemented according to the approved designs as illustrated by the following examples.

1. Sloping roof of station and residential quarters (Fig. 115).
2. Installation of mosquito proof over head tanks at station buildings and residential quarters.
3. Construction of septic tanks and soak pits at staff

Table 25. Results of clinical examination for hepatosplenomegaly of Konkan Railway Project workers and malaria diagnosis

Item	Nos.
No. of project sites visited	23
No. of workers examined	706
No. of migrant workers (665 from 14 states of India and 5 from Nepal)	670
Workers native of Goa	36
Positive for malaria (5 *P. vivax* and 1 mix of *Pv* + *Pf*; SPR = 0.84%)	6
No. with history of malaria	153 (21.6%)
Spleen rate	11.7%
Average enlarged spleen	0.19
Hepatomegaly	9.91%

Fig. 115: Konkan Railways Station at Margao and Karmali-Old Goa (Inset). With the timely intervention of NIMR, the design of station building was changed by the Konkan Railway Corporation and sloping roofs were provided to prevent water stagnations on station terrace during heavy monsoons experienced in the Konkan Region

Fig. 116: Pacca drains were provided all along the track in the cutting section for efficient flow of water

Fig. 117: All abandoned masonry tanks were filled with soil to prevent water collection and mosquito breeding

quarters and railway stations.

4. Clearance of drains near the cutting section near the mouth of tunnels and providing concrete drains along the track (Fig. 116).
5. Efficient drainage of water at the railway station and coach washing yards.
6. Filling or demolition of tanks after construction of major and minor bridges tunnels, etc (Fig.117).
7. Clearance of mud/silt from the mouth of the pipe and box culverts to ensure smooth flow of water.
8. Filling up of depressions near major bridges to avoid stagnation of water.
9. All abandoned sleeper curing tanks were either filled and leveled or repaired and converted to larvivorous fish hatcheries (Fig. 118). A system of fish supply to establish hatcheries at the Primary Health Centre's was made operational.
10. Removal/drying up of water stagnations during construction of railway quarters (Fig. 119).
11. Re-usable fibre glass plastic food trays/cups and waste collection system were introduced in the trains.

Konkan Railway Corporation and sloping roofs were constructed to prevent water stagnations on

Fig. 118: Abandoned sleeper curing tanks were stocked with larvivorous fish *Poecilia reticulata* and handed over to the Directorate of Health Services Goa for fish propagation and distribution

Fig. 119: Konkan Railway Engineers and Scientists of NIMR inspecting Railway Quarters site under construction where water stagnation was supporting anopheline breeding

station terrace during heavy monsoons experienced in the Konkan Region.

Cost Profile

In consultation with Konkan Railways financial and technical experts, we computed the cost of anti-malaria surveys along the entire Konkan Railway alignment of 760 km at Rs. 4.566 million (US $ 0.1014 million) (Table 26). While the cost of intervention measures, viz. earth work for bringing soil from hill slopes for creating raised railway embankment,

Recommendation for Routine Maintenance

The recommendations currently under implementation to ensure mosquito free environment in and around the Konkan Railways are listed in Table 27 and few drawings are shown in Figs. 120 and 121.

The Reward

In the entire line no outbreak was reported from anywhere in 760 km line. Even in Goa, where NIMR maintained close watch on the situation, no outbreaks were observed anywhere along 106 km line in labour camps or nearby villages. The inter-sectoral collaboration proved fruitful in averting not only outbreaks of malaria but also of other mosquito-borne diseases.

Table 26. Summary of estimated expenditure for anti-mosquito measures for entire KR project of 760 km from Roha to Mangalore

Item	Description	Cost in millions (Rs)	Cost in million US$*
1	*Cost of survey*		
1.1	Manpower	3.5	0.07
1.2	P.O.L.	0.35	0.007
1.3	Consumables	0.716	0.0159
	Total survey cost	4.566	0.1014
2	*Intervention cost*		
2.1	Earth work	211.5	4.7
2.2	Mosquito proofing of water tanks	0.168	0.0037
2.3	Sloping roofs	2.352	0.0522
2.4	Demolition/filling of masonry tanks	1	0.022
2.5	Laying half round drainage pipes	25.8	0.573
2.6	Minor earth work : filling and levelling of depressions	9.12	0.2
2.7	Repeated clearing of drains in cutting sections, tunnels, coach washing yards	1	0.02
2.8	Cost of re-usable trays	1.46	0.032
Total	HIA and Intervention cost	257.46	5.721
	Intervention cost per km of railway line	0.338	0.0075
	Total cost of KR project	25200	560
	Cost of mosquito intervention in per cent to total Project cost was 1.0%		

*Rs. 45 – 1 US$

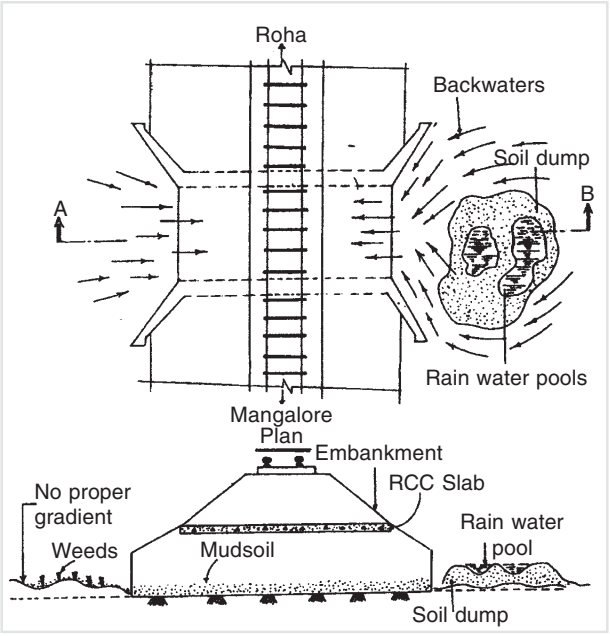


Fig. 120: A line drawing of concrete passage built through the railway embankment submitted to Konkan Railway Corporation highlights water stagnations in the depressions in the soil dump near its mouth in which mosquito breeding was detected

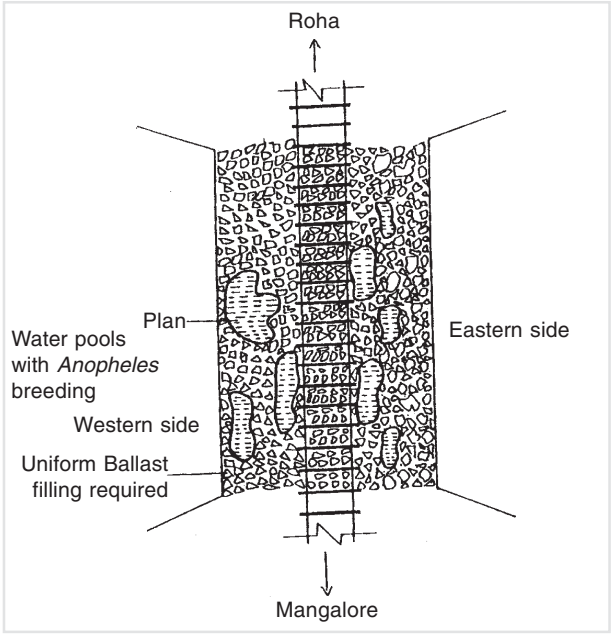


Fig. 121: A line drawing submitted to Konkan Railways highlighting water stagnations in the depressions in ballast on embankment in which *Anopheles* breeding was detected

General Recommendations for Future Railway Projects

Following list for preventive steps in the future projects of Konkan Railway as corporate anti-mosquito policy were suggested.

1. All the future stations, staff quarters and other buildings built by Konkan Railway should have sloping roofs.
2. Only mosquito proof over head tanks and flush tanks should be installed and their over flow pipes will be fitted with mosquito proof arrangement.
3. Large water tanks including over head tanks and

Table 27. NIMR Recommendations for maintenance of Konkan Railway

S.No.	Potential of mosquitoes	Frequency of inspection	Recommended intervention
1.	Overhead tanks	Weekly	Ensure mosquito proofing/repair leakages
2.	Masonry tanks (Abon.)	Recurrent	Demolish and level
3.	Culverts (RCC, Pipe, Box)	Quarterly	Clear silt and debris
4.	Major and Minor bridges	Six monthly	Clear obstructions/filling depressions
5.	Cutting sections	Monthly	De-weeding and clearing drains
6.	Tunnel-side drains	Monthly	Clearing with gradient
7.	Septic tanks	Weekly	Sealing and netting vents
8.	Drains at station/washing	Monthly	De-weeding, de-silting, clearing
9.	Sleeper curing tanks	Annual	Filling of unwanted tanks

ground water sumps should always be fitted with mosquito proof lid assembly as per the specifications given in the enclosed sheet.

4. Septic tanks in the buildings should be kept hermetically sealed and their vents covered with nylon/iron mesh at all times.
5. The masonry tanks should be demolished completely after construction so that no opportunity for water stagnation is provided.
6. Buildings and staff quarters should be provided with mosquito proof wire netting in the doors and windows.
7. No burrow pits shall be created anywhere.
8. Larvivorous fishes should be introduced in water bodies and tanks along the railway alignment.

❑

Situation Analysis of Malaria Control in Five Selected Pilot Areas in the Country for the Implementation of Roll Back Malaria Initiative

Roll Back Malaria (RBM) is a new global initiative against malaria. RBM has been built on the foundations of the accelerated implementation of malaria control in the African region, which was based on the regional and global strategies for malaria control. Its objective was to halve the malaria burden in participating countries within a decade through interventions that are adapted for local needs and by reinforcement of the health sector. The principal mechanism for achieving this is through intensified national action by country-level partnerships working towards common goals within the context of health sector development and using agreed strategies and procedures. As a first step in this endeavor, WHO-SEARO has assigned the task of carrying out situation analysis of malaria control to National Institute of Malaria Research for the implementation of RBM Initiative in India. Five pilot areas were selected for situational analysis. These were Goa state, District Jodhpur (Rajasthan), District Tumkur (Karnataka), District Keonjhar (Orissa) and District Aizawl (Mizoram). The main objective of the study was to provide necessary inputs for the preparation of action plan for implementing the roll back malaria initiative (RBMI).

The objectives were:

(i) to assess the strengths and limitations of the health infrastructure for supporting disease control activities at the state, district and at other administrative levels within the district and of the private and public health care delivery systems for malaria control;

(ii) to identify priority needs of the community for health care delivery with reference to malaria;

(iii) to collect evidence-based data of malaria with special reference to case detection and treatment, disease prevalence, drug sensitivity of *P. falciparum,* prevalence of vector species and their abundance, host preferences, breeding habitats and insecticide resistance status of vector species; and

(iv) to identify potential partners and opportunities for more effective intervention, prevention and treatment of malaria especially at the community level.

A common methodology was drawn for carrying out the task in two phases. Initially, in September/October 2000, an elaborate exercise was made to assess the situation of malaria control in the proposed sites to identify the constraints both technical and administrative, and possible suggestions for the preparation of effective implementation of RBMI. Later, during September/October 2001, surveys were carried out to generate data on various key issues of direct relevance for malaria control and needs for addressing the same in the light of the situational analysis made earlier. The situation analysis also included conducting of workshops at community and district level to inform the concerned partners about the objectives of roll back malaria initiative and the role they could play to achieve the effective malaria control. Data from these evidence-based surveys were analysed and final reports were prepared. Finally, a workshop was conducted in each district in which the officials from all sectors participated. The results of situation analysis were presented and final report was handed over to the District and State Health Officials.

Following is the summary report of each selected area:

Goa State

The Goa state on the western coast of India was selected for the implementation of RBMI. This state is administratively divided into two districts (north Goa and south Goa) and 11 talukas, but for malaria control operations the state is considered as one district (Fig. 122). The district has five community health centres (CHCs), 24 primary health centres (PHCs), four urban health centres (UHCs), 27 rural medical dispensaries (RMDs), one urban medical dispensary (UMD), one Ayurvedic dispensary, two Homoeopathic dispensaries, four National Filaria Control Programme (NFCP) units, 172 sub-centres, 369 villages and 32 malaria clinics, 16 government hospitals and 92 private hospitals with a good net work of private practitioners. There are no drug distribution centres (DDCs) while sub-centres function as fever treatment depots (FTDs). Until 1985, malaria was not a serious problem in Goa. First

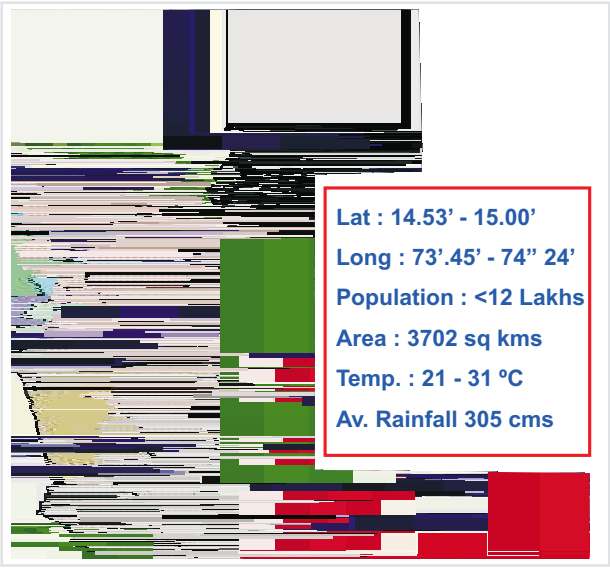


Fig. 122: Map showing location of core malarious UHCs and PHCs of Goa state

outbreak of malaria occurred in 1986 followed by many till 1990s. The outbreaks were around the building construction sites. There was also increased prevalence of *P. falciparum* and deaths were also reported during the years 1996 to 2000.

The major vector responsible for the transmission of malaria is *An. stephensi* and was found profusely breeding in the curing tanks at the construction sites (Fig. 123). *An. culicifacies* was also found in low densities. Though malaria in Goa state is restricted to urban area, urban malaria scheme (UMS) has not yet been established. Transmission was also high among the labour population living in the precincts of the construction sites. The major vector control measure is larviciding of the breeding sites and limited use of larvivorous fishes. Indoor residual spraying against adult mosquitoes is not being undertaken in rural areas due to very low API. *An. stephensi* larvae were susceptible to the two chemical larvicidal insecticides used for antilarval measures, fenthion and temephos. The National Filaria Control Programme units are responsible for vector control but are without requisite technical man power. Municipal bye-laws for implementing legislative measures exist but are not exercised effectively. Focal sprays against adult mosquitoes are undertaken on detection of cases. Adult *An. stephensi* was resistant to DDT but was susceptible to synthetic pyrethroids.

From the results of the studies carried out by RBM team, some constraints were identified that need immediate attention for effective implementation of malaria control. The posts of District Malaria Officers were vacant in both the administrative districts. There were vacancies of other supporting staff as well. To augment the work force for better malaria control, these posts may be filled. Non-existence of sufficient supporting staff is leading to under reporting of cases. Required political support is to be generated to implement legislative measures. Drug susceptibility tests are to be conducted to assess the magnitude of drug failure. Geographical reconnaissance of the breeding habitats in urban areas with special reference to construction sites is to be made to carry out the antilarval measures effectively. Inter-sectoral coordination and

Fig. 123: Curing tanks at construction site in Ward No. 1 and 12 supporting the breeding of *An. stephensi* during the evidence-based situation analysis survey in Panaji, Goa

Information, education and communication (IEC) activities in the community will be of immense help for implementing the initiative. The Government of Goa has in principle accepted to integrate RBMI as a strategy for malaria control into the existing health infrastructure.

District Jodhpur (Rajasthan)

District Jodhpur in the western region of Rajasthan state spread over an area of 22,850 sq km is an arid district. Administratively the district is divided into three sub-divisions, Jodhpur, Pipar City and Phalodi. The health infrastructure has 16 government hospitals, 39 private hospitals, 10 CHCs, 73 PHCs, 512 subcentres and 480 DDCs and 55 malaria clinics. The general health infrastructure is elaborate but ~ 20% key posts of relevance to malaria control are vacant. These posts include Biologists (UMS), Assistant Malaria Officers, laboratory technicians and multipurpose workers.

The slide positivity rate (SPR) and annual parasite incidence (API) of malaria in the district during four years (1997–2000) was >1 with proportion of *P. falciparum* (*Pf %*) in the range of 5–14%. Based on the criterion of the range of API of three years (1997–99), 73 sub-centres (46, API in the range of 2–5; 16 in 5–10 and 11 in API >10) and on the criterion of *Pf*% of >30, 42 sub-centres were identified for high risk of malaria. For treatment of malaria cases National Vector Borne Disease Control Programme (NVBDCP) drug policy is followed. The parasite is susceptible to the first line of drug, chloroquine. Microscopic diagnosis of cases is only done at CHCs and facilities are to be provided at PHCs to avoid delays in administering radical treatment. FTDs are non-existent and the same are to be established at least in the identified high risk areas. Voluntary link workers are to be appointed in high risk areas.

Anopheles stephensi is the main vector responsible for the transmission of the bulk of malaria. In some areas with granite mining, *An. culicifacies* species A was found responsible for transmission of malaria. *An. stephensi* was found to breed and rest preferably in the underground water storage tanks (Tankas) (Fig. 124). Most appropriate strategy for the control of this species would be mosquito-proofing of "Tankas" and this would curtail most of the mosquito breeding. The major vector control measures undertaken in the district are two rounds of IRS of DDT, chemical larvicides, use of larvivorous fishes and limited use of bio-pesticide in breeding places. These measures are more appropriate for the control of malaria in areas where *An. culicifacies* was responsible for the transmission.

Strategies to control *An. stephensi* breeding are to be implemented to control bulk of malaria in the area. The magnitude of malaria is small and is in limited areas and can be controlled with some concerted efforts in the affected areas. Health education camps for sensitising the public and

Water storage tank

Pitcher

Stone quarry

Cement tanks

Fig. 124: Mosquito breeding sites in District Jodhpur

advocacy workshops for private practitioners to follow NAMP drug policy and for improvement of inter-sectoral coordination (both governmental and non-governmental) will be of help to control malaria effectively.

District Tumkur (Karnataka)

This district is situated in Karnataka state. The district has 10 administrative units (Talukas). The health infrastructure in the district comprises of 93 PHCs, 38 PHUs, two general hospitals (400 bed and 100 bed), seven taluka hospitals (50 bed each), 28 Ayurvedic hospitals, 376 sub-centres, 646 DDCs and 4 FTDs.

Malaria in the district is mainly a rural problem. On an average 15,000 malaria cases are reported annually with >25% of *P. falciparum* cases. The main vector of malaria is *An. culicifacies. Anopheles fluviatilis* occur in low densities in some months of the year and based on the prevalence of sibling species, this species was not considered as a vector species in this district. The anti vector measures are

RBM Workshop at Jodhpur

insecticide-based IRS against adult vectors in rural areas and antilarval measures in urban areas. DDT, malathion and synthetic pyrethroids are used for IRS and fenthion and temephos for antilarval treatments.

Analysis of the epidemiological data for the years 1999–2000, revealed that besides a few talukas in District Tumkur a few congruent talukas in Districts Chitradurga, Hassan and Chikmagalur were responsible for 25% of the total cases of malaria and 85% of the total *P. falciparum* cases in Karnataka state (Fig. 125). If major attention is paid for the containment of disease in the regions identified in the above districts most of the *P. falciparum* malaria in the entire state can be controlled. Other administrative and technical constraints were identified during the situation analysis for necessary remedial measures. Generally, it was found that there are vacant of posts and lack of training for the technical staff for collection and correct identification of parasites in blood smears. Delay in administering of radical

Fig. 125: Map showing high risk PHCs Chiknayakanhalli, Arsikere, Hosdurga and Kadur of four districts–Tumkur, Hassan, Chitradurga and Chikmagaluru

treatment to confirmed malaria positive cases was noticed. Good working microscopes, convenient packages of medicine (Blister pack of doses of chloroquine and primaquine may be provided), establishment of DDCs and FTDs would help to control malaria effectively.

Entomological unit of the state in collaboration with NIMR may stratify the areas based on vector species prevalence and some important indicators to understand the transmission dynamics of malaria in the area with special emphasis on the susceptibility status to insecticides in use in public health spray. It has also been emphasised to provide sufficient logistic support and staff for effective implementation of malaria control activities. A good resource of active NGOs and a network of research institutions are available in health sector and can be involved for implementing the strategy. Traditional cultural troupes exist in rural areas and can be utilised for educating the mass community.

District Keonjhar (Orissa)

The Orissa state is endemic for malaria and this state alone contributes over a third of all *P. falciparum* cases and half the deaths due to malaria in India. District Keonjhar is one of the highly malarious districts in the state with >90% prevalence of *P. falciparum* cases. The district health infrastructure comprises of a district headquarter hospital, two sub-divisional hospitals, eight government hospitals, nine CHCs, 13 PHCs, 30 Ayurvedic dispensaries, 48 Homoeopathic dispensaries, 316 sub-centres, 2508 DDCs and 384 FTDs. All the 13 PHCs of the district are at high risk of malaria. The API in the PHCs range from 5 to 124. Two malaria vectors, *An. culicifacies* and *An. fluviatilis* are prevalent in the district. Transmission of malaria is perennial. For vector control, IRS of DDT is being done. In insecticide susceptibility tests, *An. culicifacies* was found resistant to DDT while *An. fluviatilis* was susceptible to DDT and also to malathion and deltamethrin. Based on physiogeographic, sociocultural attributes and vector prevalence, the district was divided into two strata (Fig. 126). Stratum-1 comprises hilly and forested areas of the district inhabited predominantly by tribal population and *An. fluviatilis* is the main vector species which rests indoors and is responsible for transmission of major part of malaria. The API of this stratum ranges from 42 to 120.

Stratum-2 is more or less a plain area inhabited predominantly by non-tribal population with extensive paddy cultivation with irrigation dams and canals. The vector responsible for transmission of malaria is *An. culicifacies* and is resistant to DDT and susceptible to other insecticides. The API in this stratum is in the range of 2.7 to 50. It may be noted that most of the cases of malaria are contributed by stratum-2. A regular spray with DDT following the schedule and good coverage will result in good control of disease. As the parasite is still susceptible to the first line of

Fig. 126: District Keonjhar showing forested (Green) and non-forested (Yellow) areas as Stratum-1 and Stratum-2

treatment, chloroquine if the cases are treated promptly the disease in this district can be controlled. The district has sufficient health infrastructure and if utilised properly the desired control can be achieved. The major constraint for control in the district is remoteness of the villages in the forested areas which delays the reporting and treatment of cases and inadequate supply of insecticides. Some of the indicators which are of direct relevance to the control operation and need immediate attention are information on resistance monitoring of insecticides in the vectors and drug-resistance status in parasites. Other factors that need attention are efforts for inter-sectoral collaboration, health education to community, motivate people to visit government dispensaries for treatment and analysis of the available data for planning control operations.

District Aizawl West (Mizoram)

District Aizawl west of the Mizoram state was selected for the studies. The health infrastructure of the district comprises of two government hospitals, two CHCs, 21 PHCs, 98 sub-centres and 22 DDCs. The general health infrastructure was satisfactory. However, it was noted that the two key posts for malaria control, District Malaria Officer and Assistant Malaria Officer were vacant and the entomology unit was non-functional.

This district is endemic for malaria with API in the range of 1 to 90 in 1999. During the years 1996–2000, the proportion of *P. falciparum* cases was in the range of 72–79%. There are reports of drug-resistant *P. falciparum* (RI–RIII) from different parts of the state. The treatment of confirmed malaria cases is as per the NAMP drug policy but use of quinine (intravenous) and artesunate was prevalent.

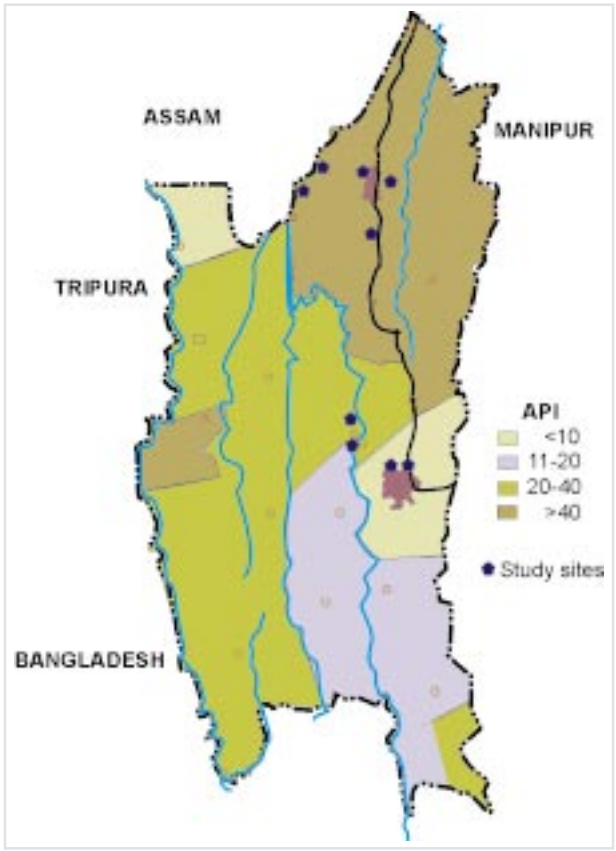


Fig. 127: Map showing API from 1995 to 99 in the study areas

The major vectors of malaria in the region are *An. dirus* and *An. minimus*. Vector control is mainly by two rounds of IRS of DDT. The coverage of the spray was usually >90%. In addition to IRS, insecticide-treated mosquito nets (ITNs) were being distributed since 1996. In the year 1999, one lakh deltamethrin-impregnated nets were distributed and in 2001, 1.5 lakh nets were distributed. During the present entomological surveys very low densities of vector species were observed contrary to the earlier reports of high prevalence. This decreased density in vector species could be due to the extensive use of ITNs resulting in mass-effect on the mosquito population.

Based on the API data of the years 1995–99 the district was stratified into four strata (Fig. 127). The northeastern part of the district was highly malarious with API >40 while the western part had API in the range of 20–40. In southeastern part API was in the range of 11–20. The central part around Aizawl City is least malarious. It was generally observed that over the years the endemicity of malaria in the district is decreasing. To sustain this, key posts be filled up, distribution and use of ITNs in the community be encouraged further, active involvement of result-oriented NGOs present in the region and impart health education to the community will be advantageous for the programme. Health society involving Governmental, military and paramilitary personnel, NGOs and elected leaders be formed. ❑

Malaria Clinics at Headquarters and Field Units

Malaria clinics are being run by NIMR at all its locations. The clinics have been diagnosing and treating patients in the shortest possible time. Hospitals/nursing homes/ private practitioners also refer cases to these clinics. In addition to providing service, these clinics are serving as biological resource for various epidemiological, parasitological, and clinical researches, and for drug trials. A schematic representation of studies carried out/being carried out using clinics' resources are shown in Fig. 128.

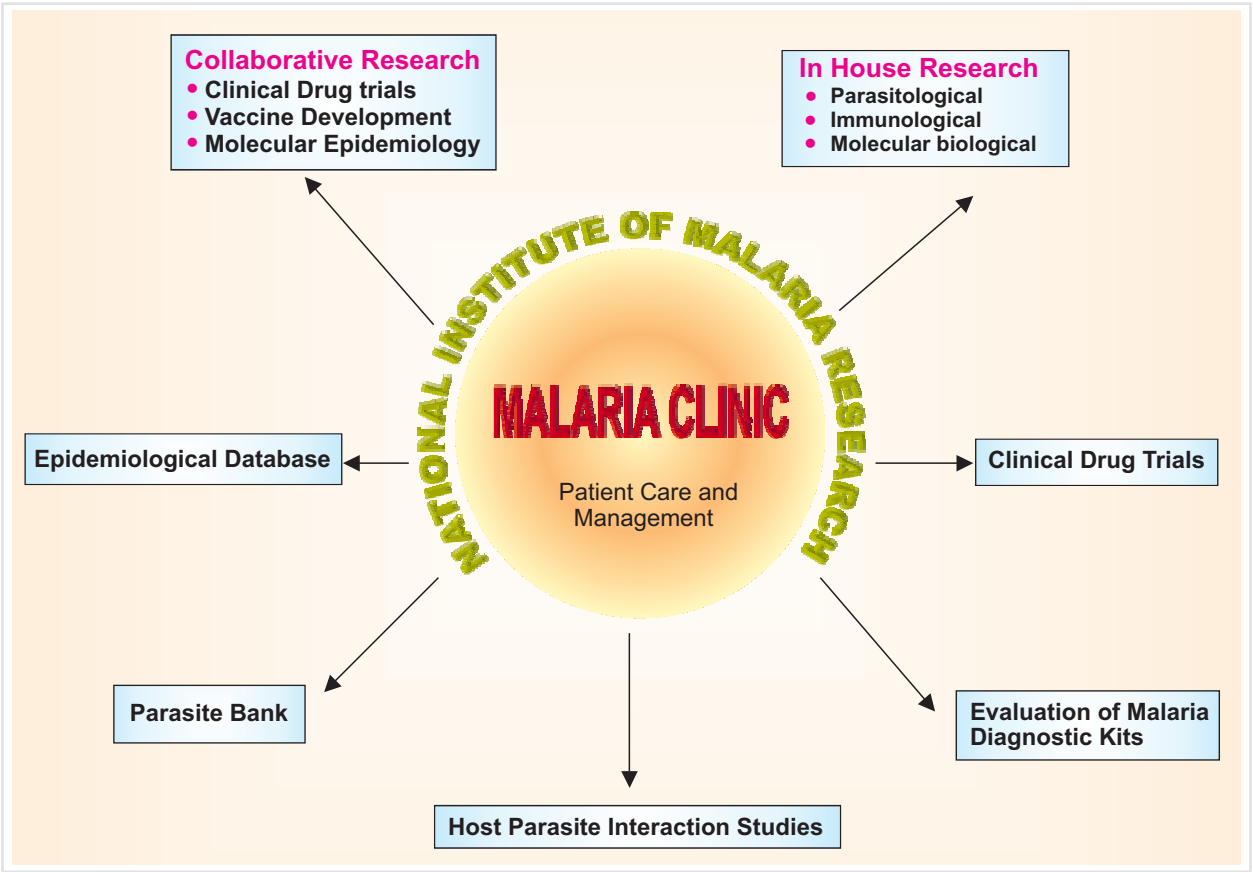


Fig. 128: Malaria clinic as a biological resource

Studies on Other Vector Borne Diseases

Strategy for Integrated Control of Malaria and Dengue Vectors in Northern Gujarat

A research project was undertaken in collaboration with Government of Gujarat with funding support of WHO India office during 2002–04 in six districts Kutchh, Banaskatha, Surendranagar, Ahmedabad and Anand of north-central Gujarat. The objectives of project were to develop an integrated strategy for control of malaria and dengue vectors in combination and synergistically, to suggest a rapid response mechanism to prevent disease outbreaks, to assess training needs of the vector control programme, and to conduct training and inter-sectoral workshops at different levels. Since the early 1990s, dengue has emerged as a serious public health problem throughout Gujarat. Expansion of the provision of piped water supply in the rural areas has led to the progression of *Aedes* mosquitoes from urban areas to the villages. In the absence of drugs and vaccine against dengue, at present vector control alone remains the sole intervention method against the disease. The Vector ecology, bionomics and insecticide susceptibility, dengue situation analysis, health system response to epidemics, staff and training need assessment were studied. Inter-sectoral workshops and trainings of the staff were organized. Evidence-based Integrated Vector Control Strategy comprising of following major components was proposed: (i) assessment of the risk of disease and stratification; (ii) MIS and epidemic containment; (iii) dengue surveillance, diagnosis and treatment; (iv) strengthening of laboratory and diagnostic services; (v) drug supplies and drug resistance monitoring; (vi) dengue surveillance, diagnosis and treatment; (vii) vector management; (viii) biological control; (ix) environmental management; (x) advocacy (IEC); (xi) inter-sectoral cooperation; (xii) legislation; (xiii) capacity strengthening; and (xiv) monitoring and evaluation.

A national workshop was organized by the National Institute of Malaria Research to disseminate outcomes of the studies conducted at three study sites in India, viz. in Gujarat, Madhya Pradesh and Karnataka states.

Dengue Vector Surveys

Vector prevalence surveys and situation analysis were carried out in different states as part of technical support to the National Vector Borne Disease Control Programme (NVBDCP).

Gujarat

On the request of the Joint Director, NVBDCP, Gandhinagar, NIMR teams had carried out dengue vector surveys in the peri-urban areas of Ahmedabad (2004) and Bhavanagar districts (2005) where suspected cases of dengue were reported. High house, container and breteau indices were recorded. Based on the reports intervention measures were strengthened to control the vector population.

Maharashtra

Scientists of the field unit provided technical assistance to the Central Emergency Medical Relief team deployed by the Director General of Health Services (Emergency Medical Relief), New Delhi from 12 to 19 August 2005 in the Leptospirosis and dengue affected areas in Kalyan-Dombivali in Maharashtra. Entomological surveys were carried out in affected localities of Kalyan-Dombivili and Thane Municipal Corporation areas.

Review of Vector Management Programme in West Bengal and Kolkata City for Containment of Dengue

There was a report of wide scale occurrence of dengue cases in Kolkata City and in West Bengal state since mid-August 2005. Till September 12, a total of 2026 cases of dengue were reported in the state by the health department, of which majority (1332) were from Kolkata City alone. Next most affected area was North 24-Parganas district from where 121 cases had been reported. In all, there were 19 deaths of which there were 13 seropositive (IgM) confirmed deaths due to dengue. A rapid assessment of vector management programme in Kolkata City was carried out to provide technical support for containment of dengue from September 9 to 13, 2005. An assessment of vector management programme in Kolkata and West Bengal and breeding potential of dengue vectors was carried out to suggest appropriate remedial measures for effective implementation of various intervention measures to control dengue vectors.

Breeding of *Aedes* mosquitoes was found in almost all the places visited including the premises

of a Borough office and its surroundings and in and around houses in the wards. The breeding was mainly found in discarded tyres, scraps, empty coconut shells, mud/earthen pots, iron/plastic barrels, earthen pots, jerry cans, plastic buckets, bottles, tube-well cap, cement tanks inside/outside houses, flower vases and many other scrap items kept on the roof top. *Aedes aegypti* (59.5%) *Ae. albopictus* (35.2%) and *Ae. vittatus* (5.3%) emerged from the larval samples collected from different areas.

The recommendations based on investigation were given to health authorities to improve anti-larval operations, fogging operations IEC activities and establishment of a Vector Management Programme. Need of Capacity building, Implementation of civic byelaws and Inter-sectoral coordination was emphasized in Kolkata City. In view of the wide prevalence of dengue in West Bengal, measures similar to Kolkata City were recommended to implement in other urban areas of the state.

GIS-based Dengue Information System for Delhi

In India, 21 states have reported dengue cases in 2006. A total of 10,935 cases and 171 deaths were reported from all over the country (provisional). Out of total cases, 31% were reported from Delhi and adjoining areas. Delhi also reported maximum number of deaths among all the states. A GIS-based Dengue Surveillance System was developed for monitoring and control of dengue in Delhi.

Delhi consists of about 139 million population spread over three localities, namely Municipal Corporation of Delhi (MCD), New Delhi Municipal Committee (NDMC) and Cantonment area. In MCD, there are 12 zones and 133 wards. NDMC consists of one zone and nine wards whereas in Cantonment area, there is only one ward in one zone. Digital map up to street level was used to create the GIS database. For all the three areas ward wise number of households, population, literacy rate, scheduled caste population, etc. as per 2001 census were attached. Streetwise reported dengue cases were mapped to identify clusters requiring intense attention for the control of disease (Fig.129). A routine sample survey for breeding sites supporting breeding of dengue vector was carried out by the NIMR. The data was overlaid to identify breeding source contributing more for proliferation of dengue vectors, to undertake situation-specific control measures. Based on GIS mapping, formulation of focused control strategy for dengue is in progress.

Dengue and Chikungunya studies by IDVC Field Unit, Bangalore, Karnataka

NIMR jointly conducted a study on dengue with St. John Medical College in Anugondanahalli, Hoskote, Bangalore rural district from May to July

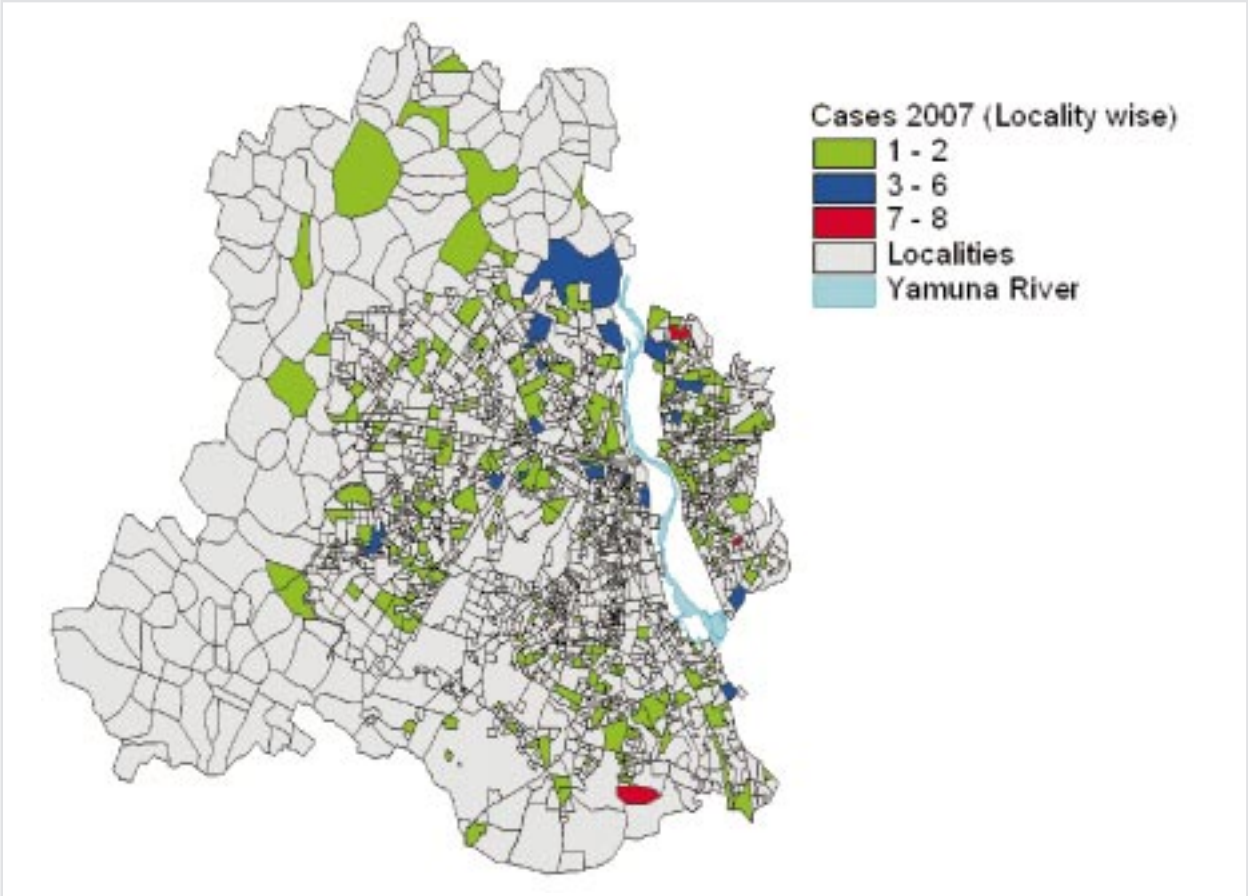


Fig. 129: GIS map showing locality wise dengue cases in Delhi

1991. In this study *Ae. aegypti* was found to breed mainly in indoor cement tanks (91%) whereas *Ae. albopictus* in mud pots (78%). Jointly carried out a dengue outbreak in July 2000 in Bangalore City along with NICD, Community Health Cell, Bangalore and Bangalore Mahanagara Palike. Here *Ae. aegypti* was the main vector mainly breeding in cement tanks. In Bangalore City an action plan has been given to the Bangalore Mahanagara Palike where emphasis has been made for house-to-house survey for control of breeding of all types of mosquitoes including *Aedes* mosquitoes. In Mangalore City, routine mosquito surveys were carried out and the same action plan to the Mangalore City Corporation was suggested. Soon after the recent outbreaks of chikungunya in Karnataka, a study was carried out in Tumkur and Kolar districts. Here indoor cement tanks were the main breeding sources of *Ae. aegypti*. Most of the cement tanks are attached to an oven that maintains temperature at 26 to 28°C and are the most preferred breeding habitats of this mosquito. In all cement tanks checked there was no lid on the tanks and we suggested for use of mosquito proof cement tanks based on experience gained in the field.

A study was undertaken to develop a strategy for Integrated Control of Vectors of Malaria, JE and dengue in Karnataka funded by WHO Country budget. This study was conducted in Mandya district from 2003 to 2004. A total of 769 villages were surveyed in seven talukas. It was found that of the total of 28,584 breeding habitats surveyed 6361 were found dry and in 3928 (13.7%) habitats different mosquitoes were found breeding. Principal malaria vector *An. culicifacies* was breeding mainly in tanks, irrigation wells and seepage water while JE vector *Cx. tritaeniorhynchus* was breeding in tanks, seepage and streams. It is important to note that dengue vector *Ae. aegypti* was exclusively breeding in water storing cement tanks in all the villages surveyed. *Aedes* indices for containers, house, and breteau were 22.8, 18.8 and 20.2% respectively.

Under the Elimination of Lymphatic Filariasis (ELF) programme mass drug administration has been advocated. In this regard, eight northern and coastal districts in Karnataka have been covered under MDA. Two districts Bijapur and Bagalkot were given to NIMR for implementation of the programme.

GIS-based Information System of Decision Support of Kala-azar Control in Bihar

For the first time in India, an attempt has been made to design kala-azar control strategy at national level utilizing GIS platform for Bihar state. There are 38 district in Bihar, where 31 are endemic for kala-azar and eight are severely affected namely, Gopalgunj, Muzaffarpur, Saharsa, Saran, Vaishali, Araria, East Champaran and Madhepura, Despite implementation of various control strategies, the status of morbidity and mortality due to kala-azar in several districts remained the same. For GIS platform geo-referenced digital map of villages/tehsils/districts were used. A three tier database was constructed-districtwise , tehsilwise and village-wise, Attribute data such as village wise population, schedule caste/ schedule tribe population, medical facilities, primary health centres, etc. and data on kala-azar incidence death for six years–from 2001 to 2006, were attached to the village maps and were used for the analysis for decision support in formulation of control strategies.

Overlaying year wise kala-azar cases over Musahar population, a tribe in Bihar, revealed a strong correlation of kala-azar cases with Musahar population. Fig. 130 shows a correlation of Musahar population with kala-azar cases of 2006, the year of high incidence. Villages with Primary Health Centres were mapped and a buffer zone was created at 2 km

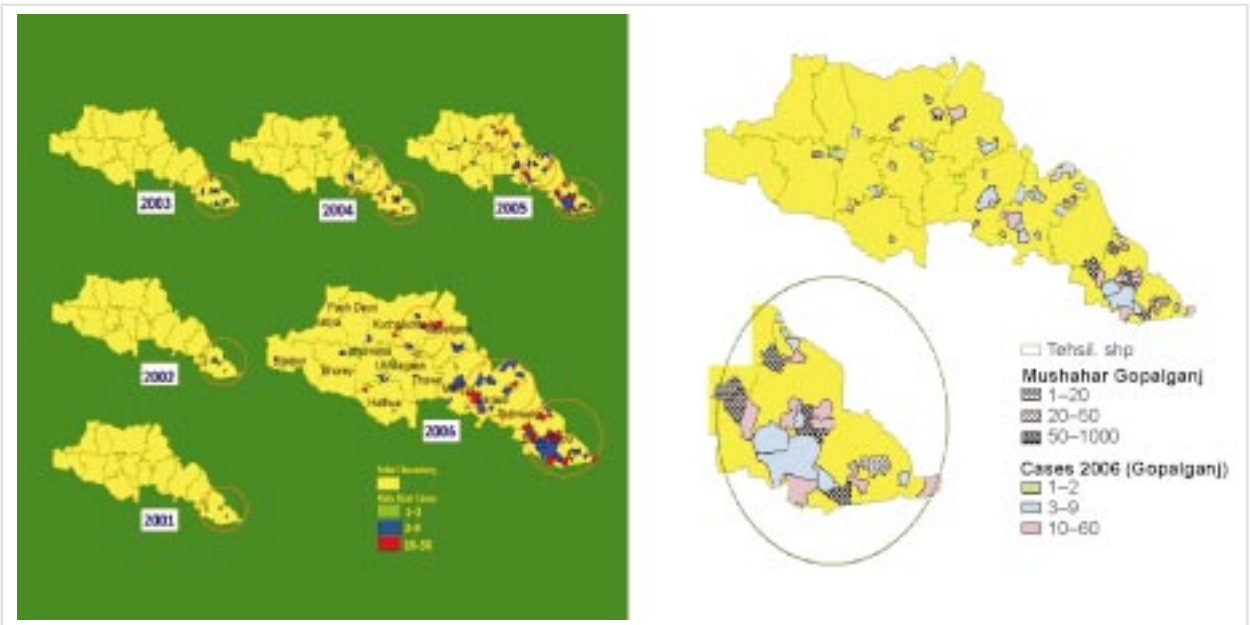


Fig. 130: Kala-azar incidence from 2001 to 2006 and correlation with Musahar population

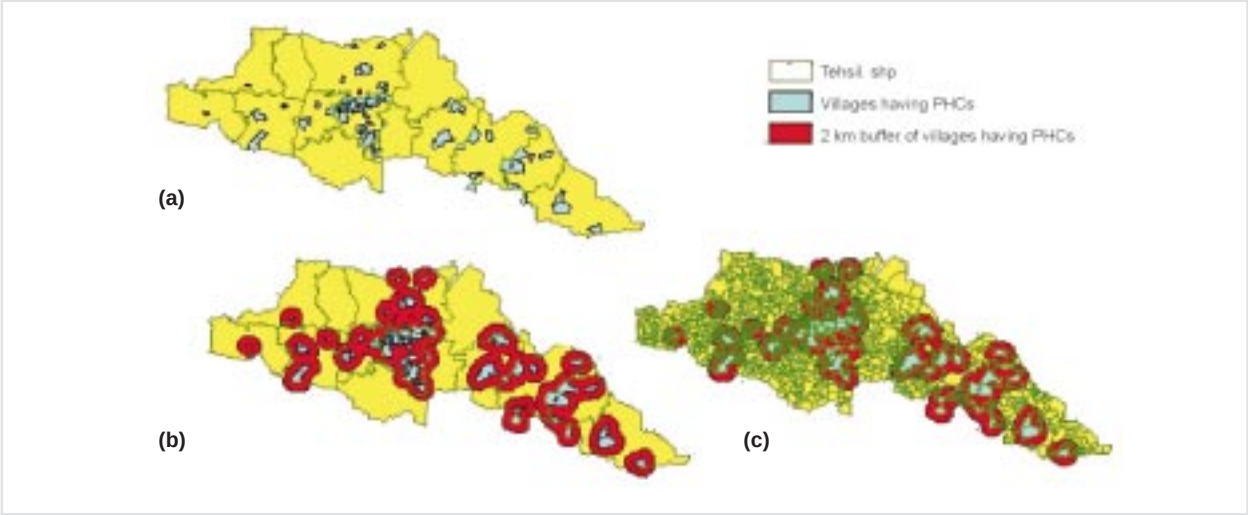


Fig. 131: GIS-based maps for kala-azar endemic pockets in Gopalgunj district

and village boundaries were overlaid to show the accessibility for patients to closest PHCs and the areas where there is need to establish new PHCs. Similar studies have been conducted for other kala-azar affected districts in Bihar and West Bangal. A case study from Gopalgunj is presented here.

Gopalgunj

Gopalgunj has about 8.3 thousand to 2.9 lakh population in its 14 tehsils. In the year 2001 to 2003, the kala-azar cases were 6,19 and 52 respectively, confined to only Baikunthpur tehsil/ PHC of Gopalgunj district. The problem started from east of Gopalgunj and gradually built-up cases in western tehsils with time and in 2006, there was widespread incidence of kala-azar and the disease engulfed 50% of the tehsils and 23 villages having > 10 cases (Fig. 131). Overall eight tehsils of Gopalgunj namely, Baikunthpur, Barauli, Gopalgunj, Hathua, Kuchaikot, Bhorey, Uchkagaon and Manjha have reported kala-azar cases from 2001 to 2006 and kala-azar control may be intensified in these villages.

Chikungunya Outbreak Investigation and Containment in Ahmedabad and Vadodara Cities

In August 2006, a major outbreak of chikungunya transmitted by *Ae. aegypti* was reported in Ahmedabad city. For rapid assessment of the situation and contain the outbreak, NIMR, Nadiad provided technical support. Weekly entomological surveillance was set up in all the 43 municipal wards and newly annexed peripheral municipality areas. *Ae. aegypti* was recorded breeding in all kinds of stored waters in houses, roof tops, zoo/garden tanks, disused tyres, metal scraps, earthen pots with water hung for birds etc. The situation and intervention efforts were monitored by NIMR and municipal authorities on a regular basis.

A household chikungunya morbidity survey was organized with the involvement of Municipal Medical College, Ahmedabad and state health department. NIMR staff participated in entomological surveillance, monitoring of vector borne disease morbidity, review meetings and assisting the govt. and municipal authorities in organizing intensive intervention efforts including training/orientation of newly deployed staff, and IEC at various levels. A number of lectures were given on the need for inter-sectoral coordination for municipal corporators, school teachers and the NGO community. There were > 7000 patients who reported fever with joint pain on 27 August 2006. The breteau index in different wards ranged from 16 to 48 and Container index was >5 in each ward. There was a gradual decline in the prevalence of *Ae. aegypti* (3 per room) with BI <2 together with the patients suffering from fever and joint pain (321) in about 8 weeks of continuous efforts. It was suggested to sustain the intervention by strengthening health system capacity and implementing effective interventions along with monitoring and evaluation of the disease for further containment of chikungunya in the city.

A similar support was given in Vadodara Municipal Corporation. An intradomestic survey was carried out in 10 wards. Altogether, 1000 houses were surveyed in 10 wards (100 houses in each ward) for breeding of *Aedes*. The house, container and breteau indices were 4.8, 2.4 and 5.5%, respectively. Only in Jublinagar ward breteau and house indices were >10%.

Retrospective Study on Chikungunya Outbreak in India

A retrospective study on chikungunya outbreak in India was initiated during 2007 in five states, viz. Delhi, Madhya Pradesh, Orissa, Maharashtra and Kerala (Fig. 132). Seven questionnaires, namely household survey—Q1A; information of all household members—Q1B; knowledge, attitude, belief, practice regarding chikungunya fever prevention and control —Q1C; patient inventory-Q1D; mortality in

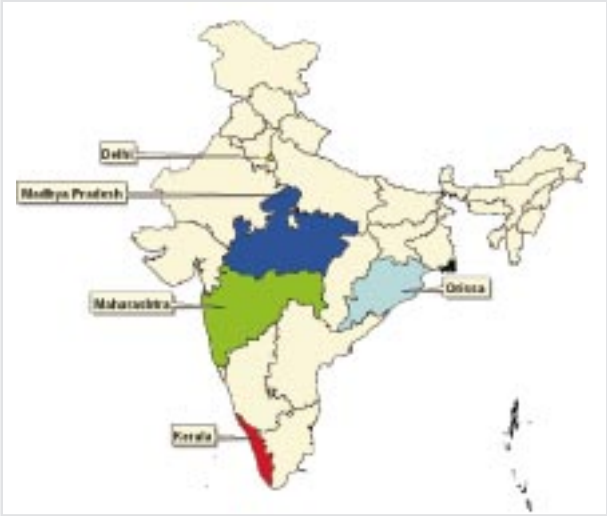


Fig. 132: Study sites selected for the retrospective study on chikungunya outbreak in India

household—Q1E; health facility survey—Q2; and stakeholder interview—Q3 were filled up from urban and rural areas of each state except Delhi from where only urban areas were taken (Fig. 133).

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

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

Maharashtra appeared as the second most

Fig. 133: Filling up of the questionnaire

ignorant state regarding knowledge, attitude, belief and practice for chikungunya fever prevention and control. The average loss of man days/school absenteeism was more than 15 days. Symptoms were mainly recorded as fever and bodyache, and most of the patients took treatment for more than 15 days. In some families of Maharashtra, many members suffered from the disease simultaneously and on an average the expenditure on treatment was high. Chikungunya case management facility was provided by all the health facilities surveyed during this study. Major *Aedes* mosquito breeding sources in Maharashtra are shown in Fig. 134.

In Madhya Pradesh, most of the houses were *Kuchcha* type especially in rural areas. In some high incidence areas, air coolers were found. General sanitary conditions around most of the urban houses of the highest incidence area were found good. Water storage containers mostly used were cement tanks, metal tanks, overhead tanks and buckets (Fig. 135). Regarding other water collection in the houses/ surroundings, mostly water for animals and pet bowls figured out. As far as emptying/drying of all water containers was concerned; some residents said that they did on weekly basis while others said that they did once in a while. Loss of man days/school absenteeism was mostly recorded as 5–10 days. Symptoms were mainly recorded as fever and arthralgia. The duration of treatment recorded was >15 days in most of the cases. Treatment expenditure was mostly ≤Rs. 500 and expenditure on food was ≤Rs. 250. Majority of the patients in Madhya Pradesh didn't receive any information of

Fig. 134: Mosquito breeding sources in Maharashtra

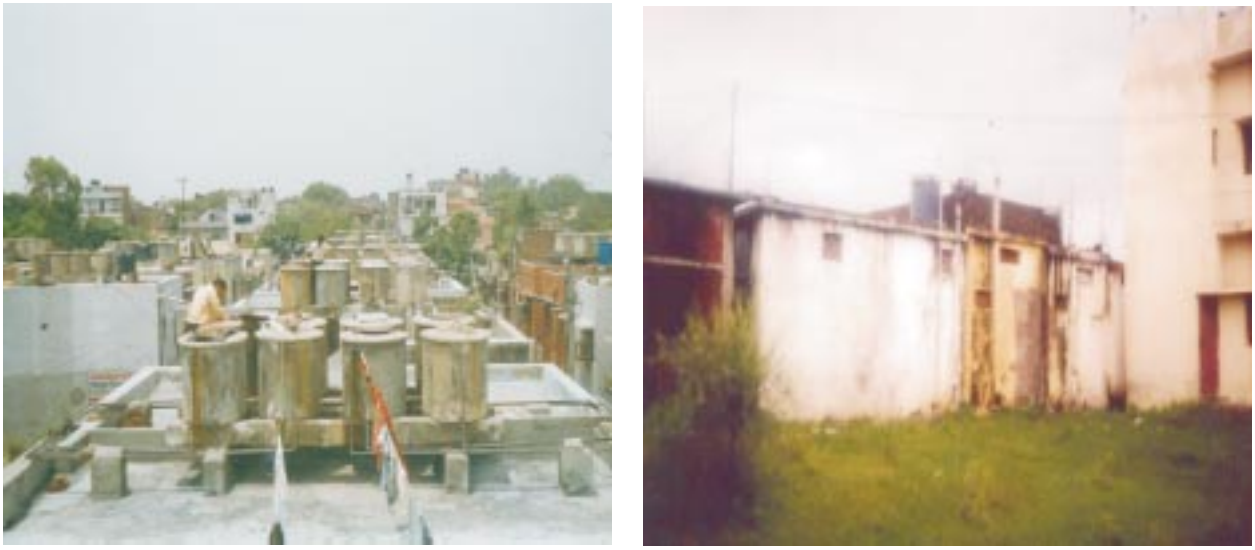


Fig. 135: *Aedes* mosquito breeding sources in Madhya Pradesh

treatment from local hospital and private agencies/ doctors. Chikungunya case management facility was provided by all the health facilities surveyed in Madhya Pradesh.

In Delhi, most of the houses were *Pucca* types and had air coolers. General sanitary conditions around most of the houses were found good. Water storage period in some areas was for 3–6 and >6 days. The key containers in Delhi were overhead tanks, underground tanks, ground level tanks and buckets (Fig. 136). In the highest incidence areas, emptying/drying of water containers was mostly done on weekly basis. Migration has been featured out as a major problem in Delhi. Loss of man days/school absenteeism was mostly recorded as 1–5 days in the highest incidence areas. Symptoms mostly recorded were fever and headache. Duration of treatment in the highest incidence urban area was 1–5 days. Treatment expenditure was mostly ≤Rs. 500 and on food ≤Rs. 250. Chikungunya case management facility was not provided in the MCD Hospital surveyed during the study.

In the urban areas of the highest incidence district of Kerala, more people were found residing in *Kuchcha* houses. In the urban areas of the highest incidence district, very few households had air coolers, but in the low incidence urban areas under the lowest incidence district, all the houses had air coolers. Water storage containers mostly used were: overhead tanks, plastic drums and buckets. Regarding other water collection in the houses/ surroundings were mostly troughs for drinking and coconut shells figured out (Fig. 137). Most of the respondents from Kerala knew answers to the questions related to knowledge, attitude, belief and practice for chikungunya fever prevention and control. Loss of man days/ school absenteeism was mostly recorded as >15 days. Symptoms were mainly recorded as fever and bodyache. Duration of treatment in the highest incidence urban area was >15 days; while in rural areas it varied from 5–10 to 10–15 days. Treatment expenditure was mostly ≤Rs. 500 and on food ≤ Rs. 250. Majority of the patients in Kerala received information for treatment from local hospitals and other sources. Chikungunya case management facility was provided by all the health

Fig. 136: *Aedes* mosquito breeding sources in Delhi


Fig. 137: *Aedes* mosquito breeding sources in Kerala

facilities surveyed in the highest incidence district.

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

The experiment was initiated in Delhi during October 2007 and in Banglore during December 2007. The experiment at Kerala is yet to be started. In Kerala, baseline data has been collected on the basis of which localities have been decided upon. In Delhi about 6500 ovitraps were placed in five localities viz. Mayur Kunj (Trilok Puri), Valmiki Colony (Panchkuiyan Road), Netaji Nagar, R.K. Puram and Railway Colony (Tughlakabad). In Banglore, about 6000 ovitraps were placed in three localities, viz. Ashok Nagar, Kanteerava Nagar and Sanjay Gandhi Nagar + Narayanpura. The experimental ovitraps contained 395 ml water treated with 5 mg C-21, IGR and solvent. Untreated ovitraps contained 400 ml water with solvent only.

Before starting the experiment, a meeting with community was organized at Delhi and Banglore to make them aware about this experiment taking place. Delhi experiment at R.K. Puram location was launched by Mr. Deepak Gupta, IAS, Special Secretary, Ministry of Health & Family Welfare. Training to the newly appointed supervisors and surveillance workers was provided to carry out the experiment and to check the breeding. A surveillance worker was asked to check ovitraps and record the breeding in about 50 houses in a day. Thus a surveillance worker covered about 250 houses in a week. The supervisors monitored the work of the surveillance workers, collected data from the field for processing on computers and provided IEC to the community regarding the experiment being carried out. The study is in progress.

Review of the Progress of Mass Drug Administration in Valsad and Junagarh Districts in Gujarat

National Vector Borne Disease Control Programme, Delhi, had assigned NIMR, Field Unit, Nadiad to assist in the monitoring of the progress of MDA Phase-II for elimination of lymphatic filariasis. In June 2005, the MDA programme in Valsad and Junagarh districts was reviewed by NIMR, Nadiad. District Valsad located in south Gujarat is endemic to lymphatic filarisis. As per the sentinel *mf* survey carried out during June 2005, the average *mf* rate in the district was 0.68% (ranging from 0 to 3.98%), highest rate was in Valsad town (3.98%).

During the verification of MDA activity in district Valsad, two urban areas, viz. Valsad and Vapi towns and two villages of PHC Magod in Valsad taluka, viz. Nanakwada and Bhagdawada were visited. Overall, MDA coverage was 78.2% ranging from a low of 56% to a high of 88.1%. During the field visit, some of the householders were asked the following questions: Whether they knew in advance about the MDA programme? Whether they knew for what purpose the drug is being distributed? Whether they experienced any side effect due to the DEC drug being administered? People informed that announcements were being made over the loud-speakers mounted on auto-rickshaws in the city areas to announce the launch of this programme and to cooperate in this campaign. The MDA teams also carried with them adequate health education materials at the time of the MDA programme to inform the people about various aspects of filariasis. Line-listing of cases with clinical manifestation of cases was also done.

In Junagarh district, the overall MDA coverage was high (89.1%) during 11–13 November 2005. During this period, a major fair was held where the floating population was also administered DEC.

During the visit, discussions were held with the DDO, the CDHO and the District Malaria Officer in Valsad. As mentioned earlier, the District Collector, the DDO and the CDHO took the initiative to achieve the maximum coverage. The DMO was also instructed to implement this programme in all the schools and colleges in the district in consultation with the District Education Officer and heads of various schools and colleges to increase the coverage. ❑

Integrated Vector Management

Bioenvironmental Control in Different Paradigms

An alternative strategy of malaria control based on non-insecticide components was launched by NIMR in mid-1980s. The components of this bioenvironmental control methodology for malaria control were:

Source reduction by

(i) Minor engineering works;
(ii) Environmental manipulation/improvement; and
(iii) Biological control agents.

Treatment by

(i) Early detection; and
(ii) Prompt treatment.

Soliciting

(i) Community participation by creating awareness through health education; and
(ii) Intersectoral coordination.

This approach is naturalistic and holistic. To demonstrate the feasibility of bioenvironmental control strategy in rural areas, Nadiad field unit of NIMR in District Kheda, Gujarat was established in March 1983. Kheda district was selected because of prevailing epidemic situation and other operational as well as technical constraints faced by the antimalaria programme. This methodology was then extended to different eco-epidemiological zones of the country like urban areas, industrial complexes, tribal areas, island ecosystem, north-eastern areas and many other rural areas.

Rural Malaria

The project was launched in seven villages of Nadiad taluka without the use of insecticides and to develop a cost-effective strategy. The main components of the strategy comprised of reduction of mosquito breeding sources, use of EPS beads in unused wells, biological control using mosquito larvivorous fishes, health education, community participation, early case detection and treatment, environmental improvement through social forestry in marsh lands and intersectoral coordination (Sharma *et al* 1985, 1991; Sharma and Sharma 1989). Following initial success, the study area was gradually extended to cover the entire Nadiad and Kapadwanj talukas covering 7,00,000 population by 1987. In order to disseminate the experience gained in the Kheda project, several transfer of technology workshops and training programmes for different categories of health personnel, such as district malaria officers, PHC medical officers, different functionaries of the state health department, municipal corporations, etc. were organised.

Community participation for source reduction of mosquito breeding

This strategy has been accepted in NVBDCP-EMCP World Bank assisted Project. WHO recognises this strategy as sustainable alternative strategy for malaria control

Environmental Improvement by Community Involvement

At many places community came forward to participate in the activities like source reduction, environmental modification and manipulation and use of biological control agents for the control of mosquito breeding. To make the community self-sustainable and motivated, economic incentive schemes like culturing edible fish in large ponds in the area and growing eucalyptus and neem saplings were encouraged.

Economic Incentive Schemes

Success of the Kheda project led NIMR to test the strategy at other rural sites in the country, namely Haldwani in District Nainital, Uttarakhand and Dadraul PHC in District Shahjahanpur and Shankargarh block in District Allahabad, Uttar Pradesh, and PHC Kamasamudram in District Kolar, and PHCs Banavara and Kanakatte in District Hassan, Karnataka. The strategies used were similar to those mentioned above, except in Karnataka where emphasis was given on biological control by larvivorous fish.

In Shahjahanpur, *G. affinis* was extensively used in wells to control vector breeding. Studies carried out in Shahjahanpur revealed that *G. affinis* successfully controlled breeding of mosquitoes and percent positivity of wells reduced from 60–80% to almost nil (Fig. 1).

In Haldwani of Nainital district, *G. affinis* controlled mosquito breeding in ponds very effectively (Fig. 2). These fishes were introduced in ponds during April. By the end of September, fish multiplied many fold and percent positivity of ponds came down from over 80% positivity of ponds to negligible levels.

PHCs where larvivorous fish have been used extensively in Karnataka were PHC Kamasamudram in District Kolar and Banavara and Kanakatte in District Hassan.

In PHC Kamasamudram, in 93 vilages, about 36,000 *Poecilia reticulata* (guppy) were released in wells and tanks during January to April 1994. In this area wells, tanks and streams are the main breeding sites of *An. culicifacies*, the major vector of malaria. Since guppy could not do well in tanks, *Gambusia affinis* was released in tanks during 1996. A significant reduction was observed in all species in all habitats ($p < 0.001$ and < 0.025) (Fig. 3). Significant reduction in *An. culicifacies* densities was observed ($p < 0.001$), while no reduction was observed in *An. fluviatilis* densities ($p > 0.05$) (Fig. 4). Introduction of fish had a major impact in decreasing the malaria incidence in this area as well. In 1993 the API was 41.8, and there was steady decrease in API in the following years. Number of malaria cases reported in the study

Fish culture in ponds (edible fish and prawns were cultured)

Growing of eucalyptus for plantation

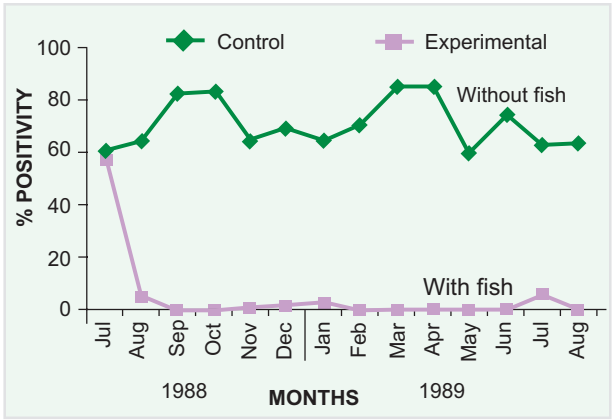


Fig. 1: Impact of *Gambusia* on mosquito breeding in wells in Shahjahanpur

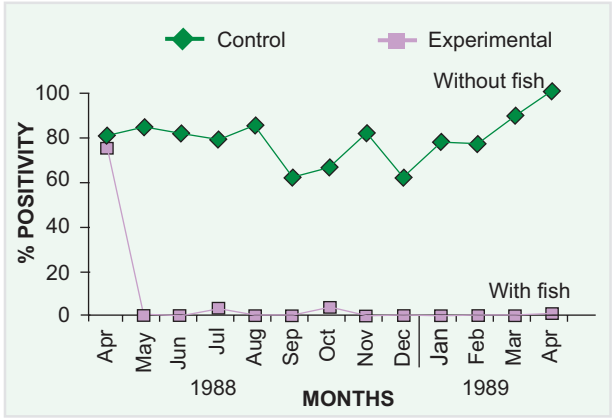


Fig. 2: Impact of *Gambusia* on mosquito breeding in ponds in Haldwani

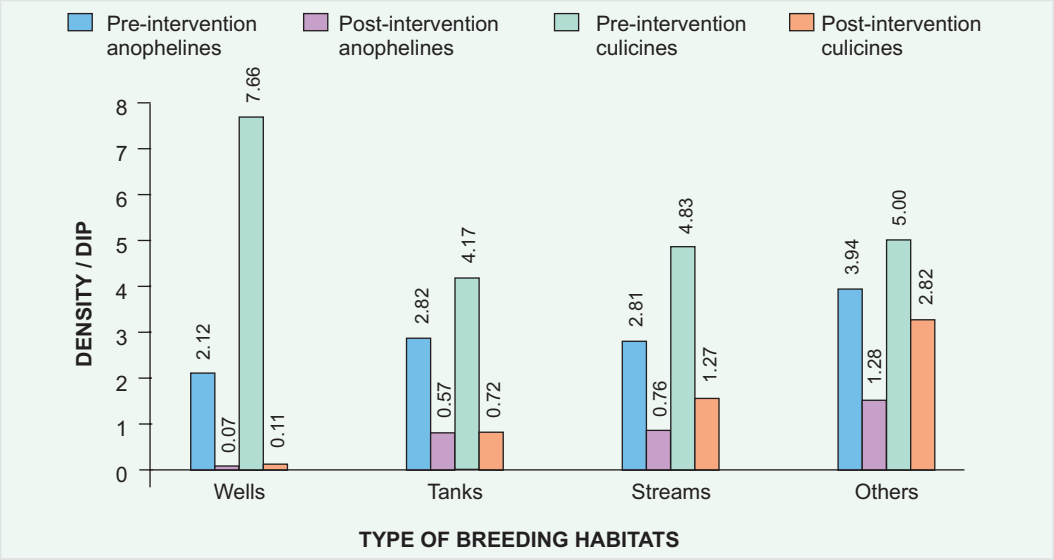


Fig. 3: Changes in larval densities of anophelines and culicines following intervention with larvivorous fish in different breeding habitats in PHC Kamasamudram, District Kolar (Pre-intervention — 1994 to 1996; Post-intervention—1997, 1999 and 2001)

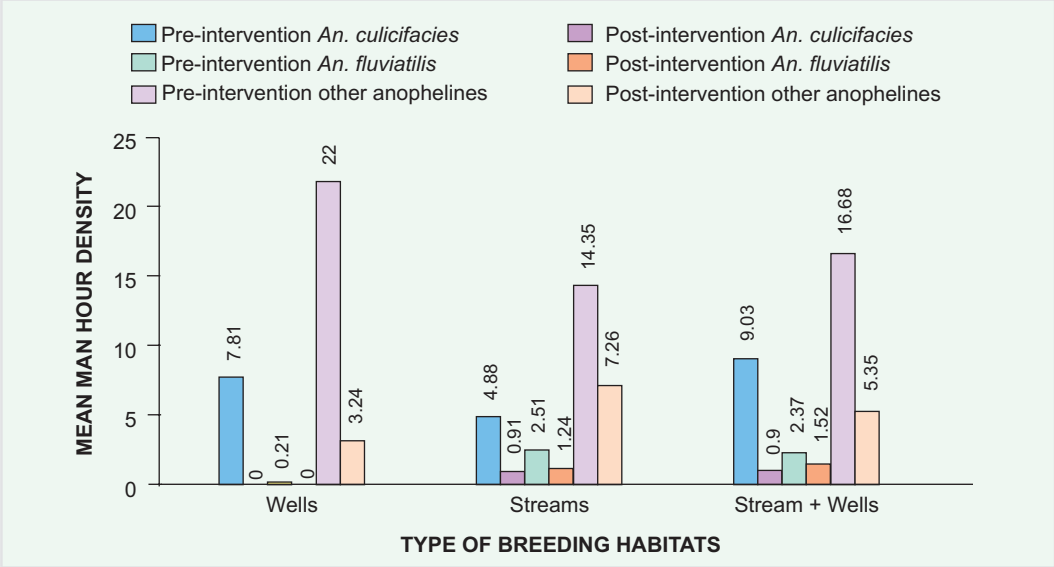


Fig. 4: Changes in adult densities (mean MHD) of anophelines in villages having different types of breeding habitats following intervention with larvivorous fish in PHC Kamasamudram, District Kolar (Pre-intervention—1994 to 1996; Post-intervention—1997, 1999 and 2001)

villages of PHC Kamasamudram from 1993 to 2006 are shown in Fig. 5. There was a drastic decline in number of malaria cases in intervention villages.

A strong focus on malaria contributing 25% of malaria cases of the state involving congruent talukas from four districts, namely Tumkur, Hassan, Chikmagalur and Chitradurga was identified in 2000 by NIMR. As in Kamasamudram of Kolar district and Banvara and Kanakatte of Hassan district, wells and tanks are the main breeding sites and *An. culicifacies* is the major vector. After a detailed geographical reconnaissance fish are being introduced. NIMR had carried out this operation in collaboration with state health personnel. Fish were released in 1766 villages covering a population of 1.2 million in four talukas. There was a considerable decline in malaria vectors

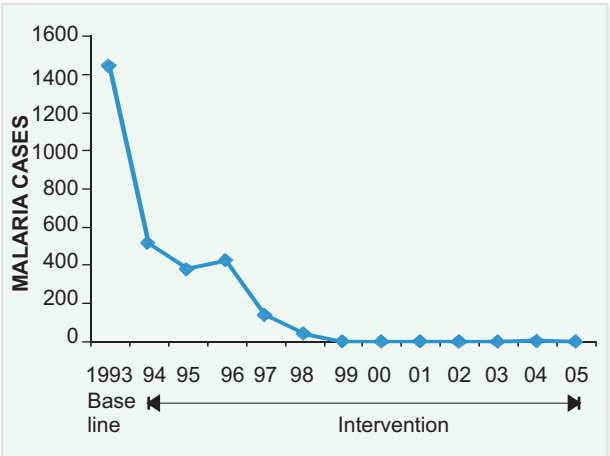


Fig. 5: Impact of larvivorous fish on malaria incidence in villages of PHC Kamasamudram, Karnataka

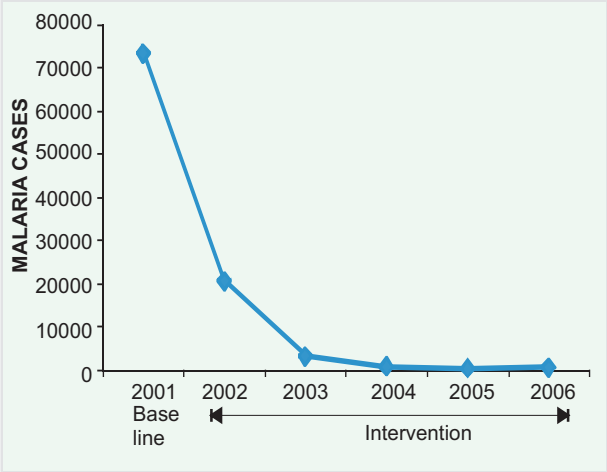


Fig. 6: Impact of larvivorous fish on malaria incidence in 1766 villages in Tumkur, Hassan, Chikmagalur and Chitradurga districts, Karnataka

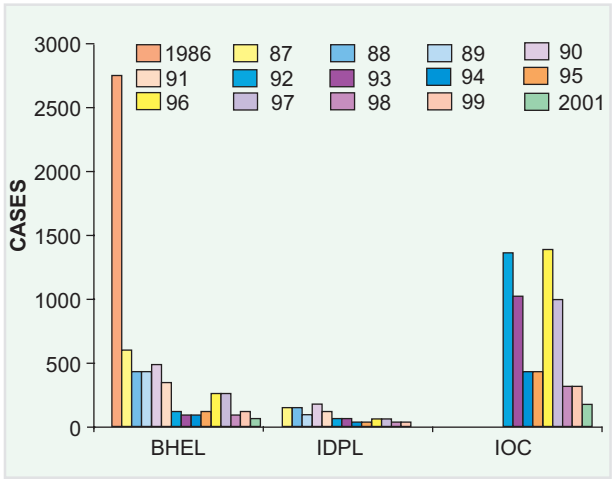


Fig. 7: Incidence of malaria in BHEL, IDPL and IOC industrial complexes

in these villages. Malaria incidence was also decreased significantly in the intervention villages (Fig. 6)

Industrial Malaria Control in BHEL and IDPL Complexes

To find a solution to malaria in industrial areas, the bioenvironmental strategy was tested in the industrial complex of the Bharat Heavy Electricals Limited (BHEL) in Hardwar beginning July 1986 and later on extended to Indian Drugs and Pharmaceuticals Limited (IDPL), Rishikesh (Dua *et al* 1991, 1997, 2000). Surveys were also conducted in areas of IOC, Mathura, NTPC, Shakti Nagar, Rihand Nagar and Unchahar, HEC, Ranchi, Visakhapatnam Steel Plant and Ordnance Factory, Tundla. Mosquito breeding sites in the industrial townships were sluice valve chambers, underground tanks, overhead tanks, ornamental tanks, septic tanks (abandoned), open and blocked drains, stormwater drains, culverts, fire hydrants, open man-holes, used or abandoned wells, pitwells, ponds, oxidation ponds, artificial lakes, coolers and factory scrap like tyres, iron scraps, a large number of borrow pits and low-lying areas.

The intervention strategy used the existing infrastructure of the industries which comprised of filling of borrow pits with fly ash and burnt hard coke ash, construction of sand posts near water taps, soakpits, construction of cemented drains, mosquito-proofing of overhead tanks, application of EPS beads in small underground tanks, sluice valve chambers and choked man-holes, desilting and canalisation, introduction of guppy and *Gambusia* fishes in storm drains and large tanks, periodic cleaning of intradomestic containers, and improvement in case detection and treatment.

There was a marked reduction in malaria cases from 1986 (base year) to 2001 (Fig. 7). Only eight cases were recorded in 2001 as compared to 3049 in 1986. The strategy was found to be cost-effective (Dua *et al* 1997). In IOC, Mathura, malaria cases declined during the period from 1992 to 1995 but increased in 1996 due to flood situation in adjoining areas. It is to point out that the cases declined sharply after 1996.

Overall, the bioenvironmental control of malaria strategy was found feasible, appropriate and cost-effective in industrial complexes. By implementing this strategy, a major reduction in insecticide residues was recorded.

Filling up of breeding sites by fly ash

Urban Malaria Control

Goa

A severe epidemic of malaria in Panaji during 1986–87 was traced due to accelerated developmental activities and importation of labourers. The malaria incidence rose from a mere 10 cases in 1985 to 352 cases in 1986 (API 8.1). It further increased to 4406 cases in 1987 (API 102.4) and 5677 cases in 1988 (API 132). Owing to a public health crisis and threat to economy, a NIMR field unit was set up in 1989 to conduct epidemiological study and evaluate feasibility of bioenvironmental control. Ecology of *An. stephensi* was delineated (Kumar and

Use of guppies in drains to control mosquito larvae

Thavaselvam 1992). Fig. 8 shows contribution of various habitats to breeding of *An. stephensi* in Panaji.

Seasonality of *An. stephensi* was studied. Malaria incidence in construction workers and local community was measured by surveillance. Malaria was strongly related to construction activities (Kumar *et al* 1991). As a part of bioenvironmental strategy, methods applied for vector control were: source reduction (disposal of tyres, domestic containers, barrels, filling of sand in curing tanks, drainage of ornamental tanks and underground masonry tanks with motorised suction pumps, removal of defunct overhead tanks and providing proper cover); introduction of larvivorous fish *Aplocheilus blocki*, *P. reticulata* and *G. affinis*; and use of biolarvicides—*Bacillus sphaericus* H5a5b (B101) and *B. thuringiensis* var *israelensis* H-14 strain 164 in *An. stephensi* larval habitats. Malaria surveillance and case treatment were improved. Health education campaigns were organised to solicit community participation including help of private doctors and the Indian Medical Association. Overall, introduction of bioenvironmental interventions led to malaria transmission control in Panaji and decline in malaria

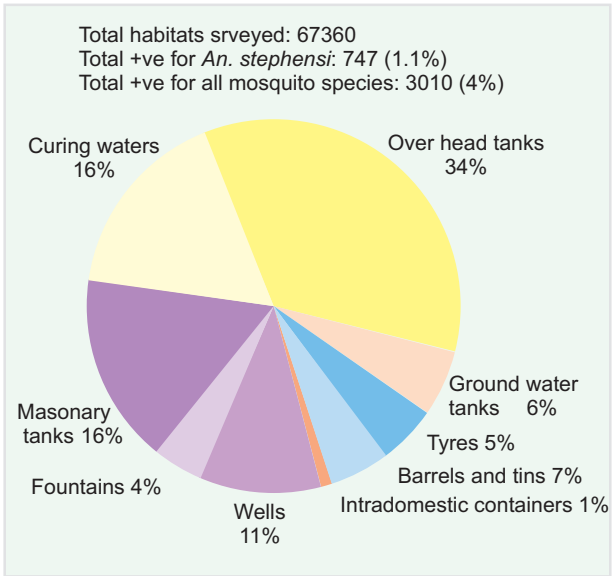


Fig. 8: Contribution of various habitats in breeding of *An. stephensi* in Panaji, Goa

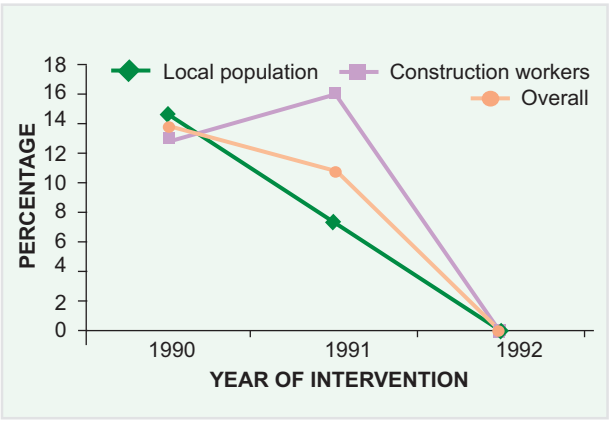


Fig. 9: Impact of bioenvironmental interventions on malaria in Panaji

incidence up to 88.5% by 1992 (Fig. 9).

In conclusion, the bioenvironmental strategy was found to be effective for urban malaria control. However, prior to its implementation, there is a need to study biology and breeding behaviour of vector and then design the strategy suiting to local situation. The focus on vector breeding, source reduction and biological control measures should be greater prior to the vector build-up so that the peak populations are suppressed and active transmission is curtailed. There is also a need to identify population at greater risk so that they are targeted for active parasite surveillance for early detection and prompt treatment. Information, education and communication (IEC) and intersectoral coordination are also beneficial in source reduction and early detection and prompt treatment.

Chennai

A feasibility study on the bioenvironmental control of urban malaria was undertaken in Chennai City during 1987–92. The study was carried out in malaria endemic areas of Chintadripet and Sowcarpet in north Chennai. *An. stephensi* is the vector of malaria in Chennai. Overhead tanks (OHTs), wells and cisterns are the preferred breeding sites. Most of the OHTs and wells are either opened or partially opened. Potential breeding sites were enumerated.

Intervention measures included the use of *G. affinis* fish in tanks and wells, mosquito-proofing of potential breeding sources, application of EPS beads, source reduction and case detection. In OHTs with chlorinated water, fish were not released. Anopheline breeding and presence of fish in OHTs and wells were checked weekly. Many OHTs were permanently mosquito-proofed. Holes were drilled in defunct OHTs to prevent accumulation of water. Fish were introduced in 2657 open OHTs and 2213 open wells and were regularly monitored. No anopheline mosquitoes were observed breeding in OHTs and wells with fish. *Anopheles* breeding was, however, prevalent in breeding habitats without fishes (Fig. 10). Malaria case detection at the Corporation clinics, active case detection and mass blood surveys were

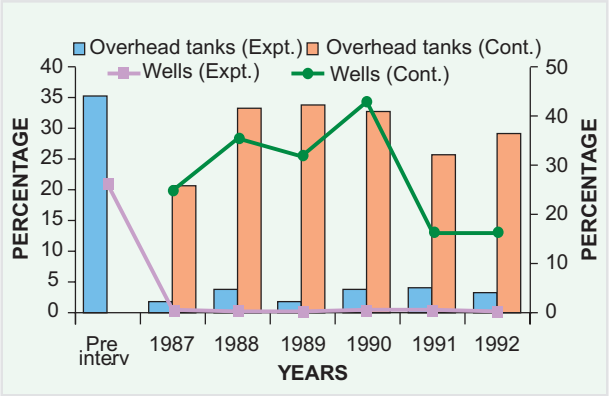


Fig. 10: Breeding of *An. stephensi* in wells and overhead tanks in Chennai, Tamil Nadu

undertaken in slums and at construction sites for early diagnosis and prompt treatment of patients. Health education was given to generate awareness in the community.

These measures resulted in significant control of mosquito breeding and vector densities. There was a steady decrease in slide positivity rate from 0.35 in 1987 to 0.005% in 1990 in Chintadripet area. In Sowcarpet area also, there was a reduction from 2.25 in 1987 to 1.07% in 1989. The study demonstrated the feasibility of bioenvironmental control in an urban area. To provide a sustained solution for malaria control in Chennai, a 7-point action plan for malaria control was developed.

The plan is simple, indigenous, environment-friendly, sustainable and cost-effective, the salient features are: (i) construction and maintenance agencies of government and private buildings were made responsible for taking adequate preventive measures for the control of mosquito breeding; (ii) vector control programme in the city was reorganised with close interaction of City Corporation, Municipality and State Health Department; (iii) Municipal bye-laws must be implemented rigidly; (iv) for any new construction plan clearance from health department was made mandatory; (v) at all construction sites incoming labourers must be screened for malaria infection and given radical treatment; (vi) implementation of malaria control in phased manner; and (vii) constitution of committees for monitoring action plan.

Delhi Action Plan

Mosquito problem in Delhi is mainly due to blocked surface drains, poor sewage disposal, ill-constructed drains, silting of drains, poor maintenance of water supply system, overhead tanks, cisterns, etc. Malaria has also increased in peri-urban areas and at construction sites. In order to organise an effective mosquito control programme and to identify the malariogenic potential, geographical reconnaissance was carried out during 1989–91. An extensive survey was carried out to assess the magnitude of the problem related to actual and potential breeding sites in 12 zones of Delhi, namely Shahdara, Civil Lines, City zone, Sadar Pahar Ganj, Karol Bagh, West zone, South zone, New Delhi, Narela, Nazafgarh, Delhi Cantt. and NDMC zones. All the breeding sites were mapped. Point prevalence survey for malaria incidence was also carried out during 1991–92. In addition, suitable permanent water bodies were also identified for development of hatcheries for larvivorous fishes to be used for urban malaria control programme. Based on the findings of the survey, a detailed Master Action Plan was prepared and handed over to the Government of the National Capital Territory, Delhi for implementation.

Ahmedabad and Surat Cities

Due to the alarming rise in malaria in urban areas, NIMR conducted two studies during 1996–2000 in Ahmedabad and Surat cities with a view to develop comprehensive strategies to control malaria and dengue vectors. The Ahmedabad study had the specific objectives to map mosquito breeding habitats by geographical reconnaissance to plan antilarval measures, study bionomics of vector species, ascertain the knowledge, attitude and practices related to mosquito-borne diseases to implement a health education strategy, estimate the burden of malaria, review malaria surveillance mechanisms and staffing pattern, rationalisation of antilarval measures, test the feasibility of integrated control of malaria and dengue vectors with emphasis on bioenvironmental management of vectors of diseases and develop an antimalaria action plan.

The ongoing Urban Malaria Scheme was reviewed with respect to antilarval and anti-adult measures, malaria case detection and reporting mechanism, entomological monitoring, IEC activities, staffing pattern, laboratory services, training needs and the implementation of public health bye-laws. Following this exercise the rationalising measures undertaken included refresher or reorientation training of antimalaria staff, reorganisation of field staff laying more emphasis on mosquito breeding control in domestic sources, development and use of larvivorous fish resource to reduce reliance on chemical larvicides, strengthening of health education activities especially by involving school children, emphasis on legislative measures to control mosquito breeding and identification of key urban sectors for allocation of responsibilities for taking preventive/ remedial measures.

To evaluate feasibility of the integrated methods of control in Ahmedabad City, four malaria endemic wards with similar ecological conditions were selected randomly on either side of the River Sabarmati. By random allocation, two municipal wards (population 1,65,584) were assigned to integrated strategy comprising larval control using fish, survey and elimination of larval breeding in domestic water storage, community participation in prevention of breeding/larval control, engineering works (e.g.

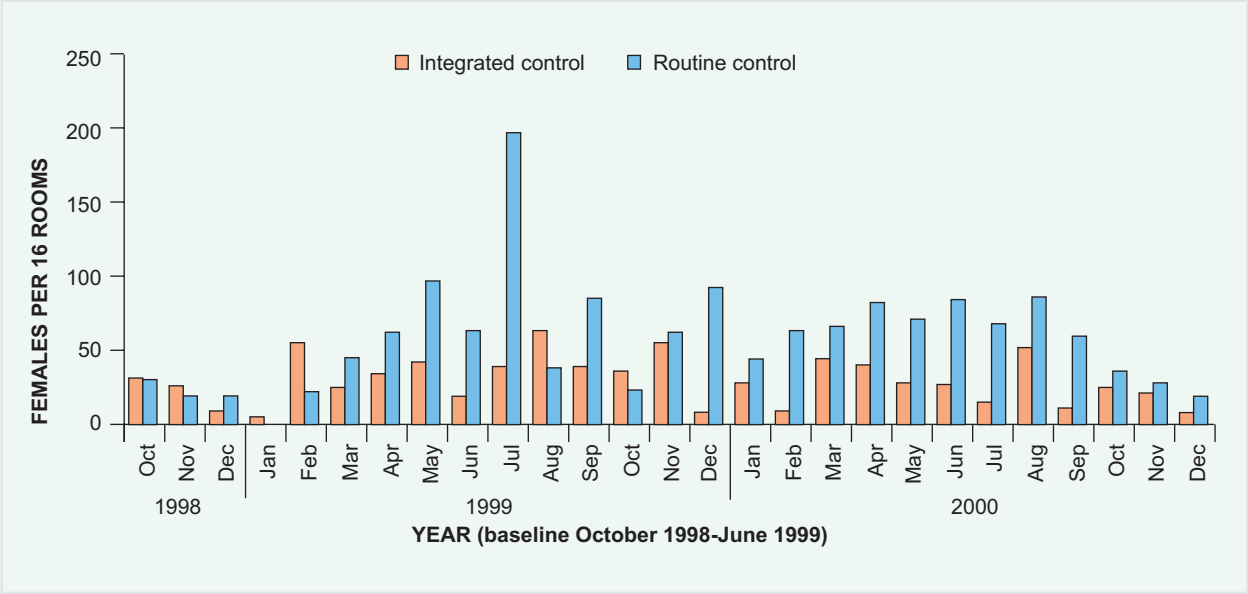


Fig. 11: Impact of integrated control on *An. stephensi* densities in Ahmedabad City

leakage repairs, mosquito-proofing), development of hatcheries and ward-level distribution tanks, removal of tyres/scraps, source reduction and health education. Another two comparison wards (population 1,60,176) continued with the routine control measures of chemical larviciding, fogging and general health education. During the baseline period malaria incidence and vector densities were comparable. During the intervention period beginning July 1999, the integrated strategy reduced malaria incidence and the population densities of *An. stephensi* and *Aedes* species more effectively than the routine control strategy (Figs. 11–12).

The per capita annual operational cost of the integrated strategy (Rs. 8.1; US$ 0.19— Prices of year 2000) was comparable/lesser than the routine control (Rs. 9.3; US$ 0.21). Thus, the integrated control has scope to reduce use of insecticides, improve the urban environment, decrease school absenteeism, generate community awareness, and encourage long-term sustainability. The integrated control strategy implemented in Ahmedabad City is evolving as a model that needs to be carried forward and implemented in other towns of Gujarat and all over India. The benefits of the integrated control strategy may potentially extend to increased community control, reduced insecticide use, improved urban environment, decreased school absenteeism, better community awareness, and long-term sustainability.

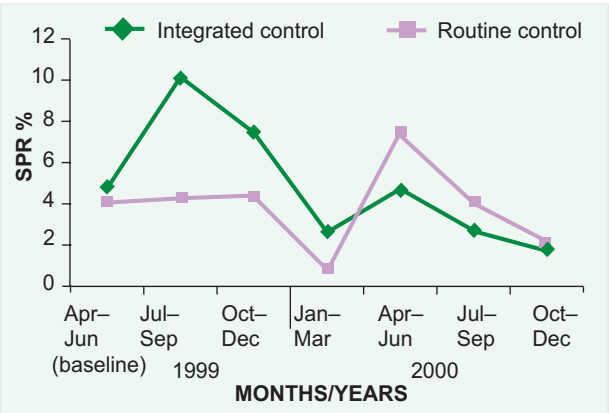


Fig. 12: Malaria incidence in areas under integ-rated control *vs.* routine control in Ahmedabad City

Based on this study a comprehensive action plan for management of malaria and dengue is being prepared and would comprise the elements of: (a) early detection and prompt treatment, and establishment of a malaria information system; (b) integrated vector management; (c) capacity strengthening; (d) IEC and inter-sectoral partnership; (e) implementation of legislative measures; (f) health impact assessment of urban development planning to incorporate health safeguards in urban expansion/ regeneration projects; and (g) operational research on vector-borne diseases to support the control programme.

Surat City is endemic for malaria and filariasis and dengue is an emerging disease. The U.K. Department for International Development supported a study by NIMR in Surat during 1998–2000. Bionomics of malaria, dengue and filariasis vectors and the epidemiology of malaria were studied. A geographical reconnaissance by land-use pattern led to an assessment of the need for environmental and engineering methods of control of malaria, dengue and filariasis. An entomological monitoring unit was set up and all staff trained. Use of malathion indoor spraying in slums was stopped following evaluation of susceptibility of vectors to insecticides. Spraying of pyrethroids indoors has also been stopped for the last two years and reliance on environmental methods involving engineers, architects and builders association, use of fish, and IEC is on the increase. During 2001 and 2002, NIMR staff has participated

in transfer of technology workshops organised by the Surat Corporation to follow-up the work done.

Tribal and Island Malaria Control

The bioenvironmental control startegy was also evaluated in tribal dominated forest areas of Sonapur (Assam), Mandla (Madhya Pradesh) and Rourkela (Orissa) and in Car Nicobar Island by the respective NIMR field units. Overall, because of the presence of innumerable water collections, channels and seepages in inaccessible forested terrains, the environmental management methods were not found practicable and feasible. In view of this, an integrated approach involving insecticide-impregnated bednets along with other methods was tested and found suitable.

Development of Strategy for Integrated Control of Vectors of Malaria and Dengue in Northern Gujarat

The study supported by WHO was undertaken in collaboration with Government of Gujarat from 2002 to 2004 to contribute to the development of rational strategies and policies for control of malaria and dengue. In eight talukas covering six districts, mosquito breeding potential was assessed through survey of domestic water storage practices and geographical reconnaissance of peri-domestic habitats. This determined species specific breeding preference. Sibling species of *An. culicifacies* and susceptibility of vector species were determined. Spatial and temporal trend of malaria in a sentinel area (Rapar Taluka, Kutchh district) during 2000 to 2003 was studied to assess the risk of malaria. Correlation of rainfall and malaria in Kutchh district was studied. Malaria prevalence based on mass blood survey was found to range from 4.4–5.4% during October 2003 to March 2004. A mosaic pattern of use of insecticides indoors in various districts in Gujarat suggested the need for a rationale planning and use of insecticides. Prevalence of dengue was assessed. Results of the study were disseminated in a state level dissemination workshop in Anand and in a national workshop in Bengaluru.

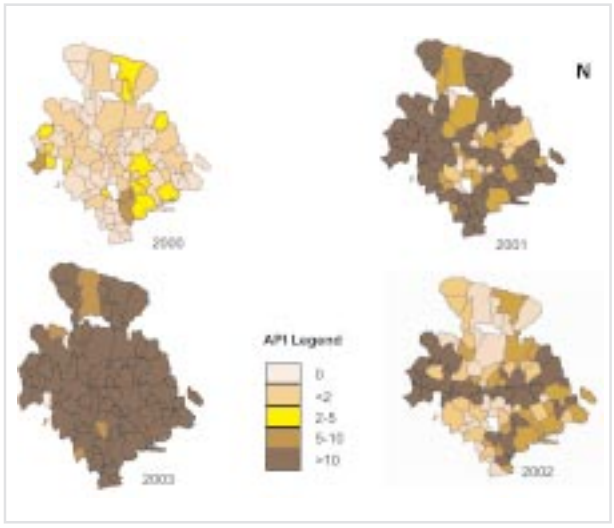


Fig. 13: Spatial and temporal trend of malaria in Rapar taluka, Kutchh district

As an outcome of the study, an evidence-based integrated strategy for control of malaria and dengue vectors in semi-arid zone of Gujarat was developed. The important components of the strategy are: assessment of the risk of disease by stratification (Fig. 13), developing a management information system and epidemic containment, strengthening dengue surveillance, increasing access and coverage to diagnosis and treatment, strengthening laboratory and diagnostic services, drug resistance monitoring, vector management need assessment and implementation, advocacy, inter-sectoral cooperation and legislation. In order to strengthen the proposed strategy operational research, capacity strengthening and monitoring of programme are necessary.

❑

Field Evaluation of Conventional and New Insecticides

Evaluation of new insecticides has been identified as a thrust area in order to guide the NVBDCP for selection of alternative insecticides in areas with double and multiple resistance in vector species and also to suggest ways and means to prolong the useful life of conventional insecticides. In consonance with this policy a large-scale field trial was carried out in District Ghaziabad to compare the relative efficacy of DDT and HCH at single and double doses against *An. culicifacies* which is responsible for about 65 to 70% malaria transmission in northern plain of rural India (Ansari *et al* 1986). Results revealed that DDT spraying @ 1 g/m^2 by increasing the spray coverage from 50 to 90% has interrupted the malaria transmission in predominant area of species A which was found completely susceptible to DDT. There was also no significant difference between 1 and 2 g/m^2 doses of DDT spraying against this species. *An. culicifacies* is mainly a zoophilic species and has inherent tendency to rest in cattlesheds and houses. The study revealed that house spraying alone with >80% room coverage produced the same impact as observed in an area where both houses and cattlesheds were sprayed with similar proportion of coverage.

In addition, several new insecticides were also evaluated to tackle increasing problem of resistance and fulminating outbreaks, particularly in multi-resistant areas. Salient findings of each trial are given below.

Field Evaluation of Deltamethrin against *An. culicifacies* in District Ghaziabad, Uttar Pradesh

An. culicifacies, responsible for bulk of malaria transmission in rural areas, has become resistant to conventional insecticides—DDT, HCH and malathion in most parts of the country. To control double or triple resistant *An. culicifacies,* new insecticides, namely synthetic pyrethroids have been introduced both in the form of indoor residual spray and also for treatment of mosquito nets. The first trial of a synthetic pyrethroid by indoor residual spraying was carried out in some villages of PHC Razapur of District Ghaziabad. Deltamethrin wettable powder formulation (2.5%) was sprayed in three doses—12.5 mg/m^2 (3 rounds), 20 and 25 mg/m^2 (2 rounds each). One section in Dadri PHC located at a distance of 22 km away from this area was taken as control, where three rounds of HCH were sprayed @ 200 mg/m^2. Deltamethrin spraying was carried out for three years. Results revealed that spraying deltamethrin @ 25 mg/m^2 resulted in drastic reduction of DDT and HCH resistant *An. culicifacies* and other anophelines (Fig. 14) and caused interruption of malaria transmission (Fig. 15).

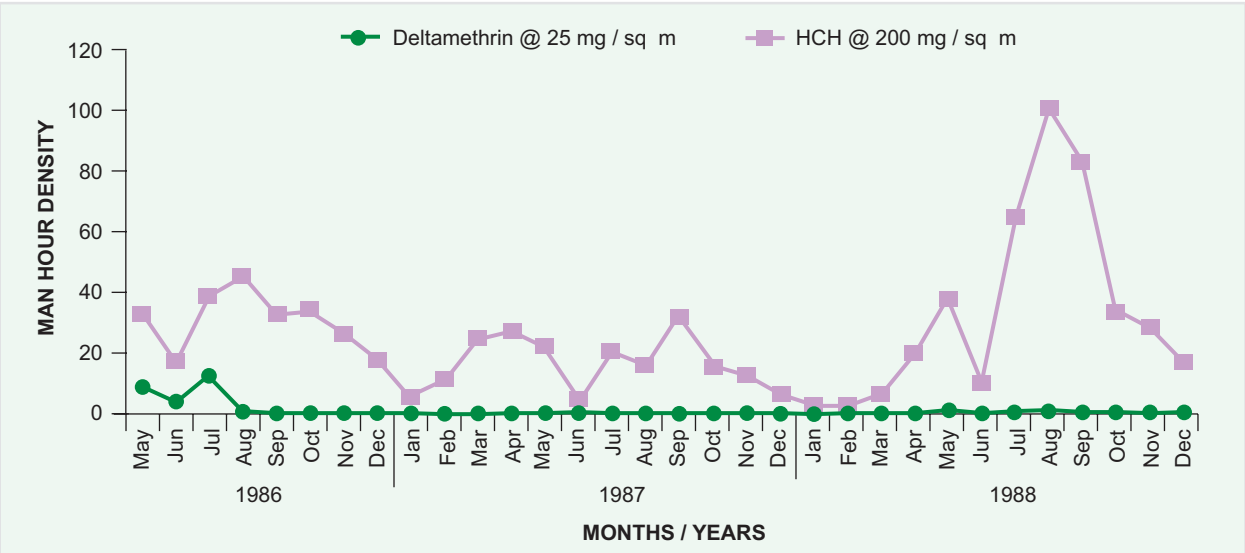


Fig. 14: Impact of deltamethrin spray on indoor resting densities of *An. culicifacies* in District Ghaziabad

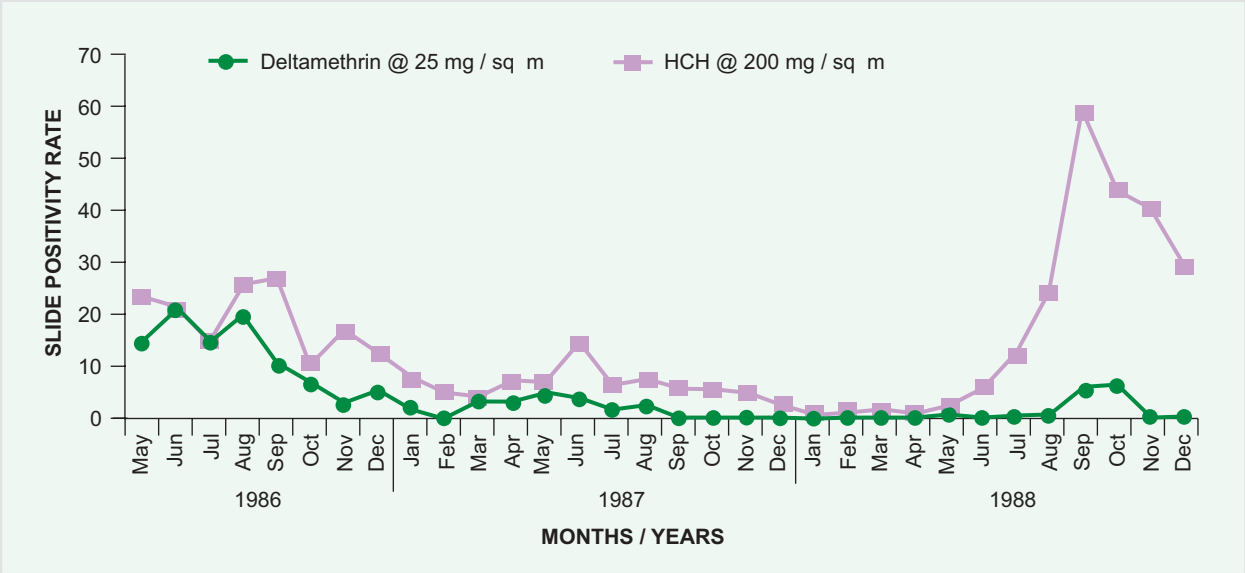


Fig. 15: Impact of deltamethrin spray on slide positivity rate in study areas of District Ghaziabad

Trial of New Insecticides in Collaboration with WHO Pesticide Evaluation Scheme

Phase II Evaluation of Bifenthrin

In an area in Gujarat, where *An. culicifacies,* the main vector of rural malaria has developed triple resistance to DDT, HCH and malathion, a randomised house-scale (phase II) trial of bifenthrin 10%WP was undertaken from July 1999 to March 2000. Baseline tests showed that *An. culicifacies* was 100% susceptible to bifenthrin (0.1% test papers), but only 57% to malathion (5% test papers). Entomological impact of four serial doses of bifenthrin (25, 50, 100 and 200 mg/m^2) sprayed in rooms in five villages was compared with malathion (2 g/m^2), and unsprayed control. In contact bioassays carried out on sprayed surfaces for 24 weeks, bifenthrin 100 and 200 mg doses caused ≥80% mortality in *An. culicifacies* till 24 weeks. The 50 mg dose caused ≥80% mortality on tin, wood and mud surfaces for 24 weeks, and on brick-walls for 16 weeks. Bifenthrin 25 mg dose produced ≥80% mortality for 24 weeks on tin, 20 weeks on mud-walls, 16 weeks on brick-walls, and eight weeks on wood surfaces. Persistence of ≥80% mortality did not differ for 25 and 50 mg doses on any surface except on wood ($p < 0.05$). Malathion sprayed in three rounds of six weeks apart caused ≥80% mortality for 16 weeks on the brick and mud-walls, and for 20 weeks on the tin and wood surfaces. Bifenthrin 25 and 50 mg doses produced a similar impact on the densities of *An. culicifacies* and other mosquitoes but a superior one than malathion or control. Bifenthrin 25 mg dose caused least excito-repellency. Overall, efficacy of bifenthrin was superior to malathion. Considering the duration of the persistence of significant insecticidal action of bifenthrin on the most common surfaces (mud and brick-walls), least excito-repellency and a relative impact on the mosquito densities, the 25 mg dose was found to be most superior among all the four doses evaluated. The trial recommended a further village-scale (phase III) evaluation of bifenthrin 10% WP at 25 mg/m^2 dose (Yadav *et al* 2002).

Phase III Evaluation of Bifenthrin 10% WP and Deltamethrin 25% WG

In the phase III village-scale trial, bifenthrin 10% WP sprayed indoors at 25 mg/m^2 dose was evaluated in Gujarat state during 2000–02 to control *An. culicifacies*. An improved formulation of deltamethrin—Deltamethrin 25% wettable granules, was also evaluated by spraying indoors at 20 mg/m^2 during this period. Both deltamethrin and bifenthrin reduced the elements of vectorial capacity—vector densities (Figs. 16 and 17), survivorship, sporozoite rate and entomological inoculation rate significantly compared to these parameters in unsprayed control villages. A low excito-repellent action of bifenthrin caused a mass killing effect on the indoor resting population of vector mosquitoes. First round of spraying of insecticides was undertaken in mid-July 2001. Bioassays on mud-walls, which are most common surfaces, showed 100% knockdown effect up to next two months which declined markedly by the third month. The indoor resting densities of *An. culicifacies* declined significantly in sprayed villages in the month of August but increased in September though at a level much lower than in the control villages. Considering the built up of indoor resting densities, a second round of spraying was undertaken in October 2001—three months after the first round of spraying. The increasing trend of vector densities with the start of February indicated that the impact of spraying of the second round of bifenthrin lasted for three months.

Based on the detection of sporozoites in *An. culicifacies* collected in early July during the study, main period of the incidence of malaria extends from

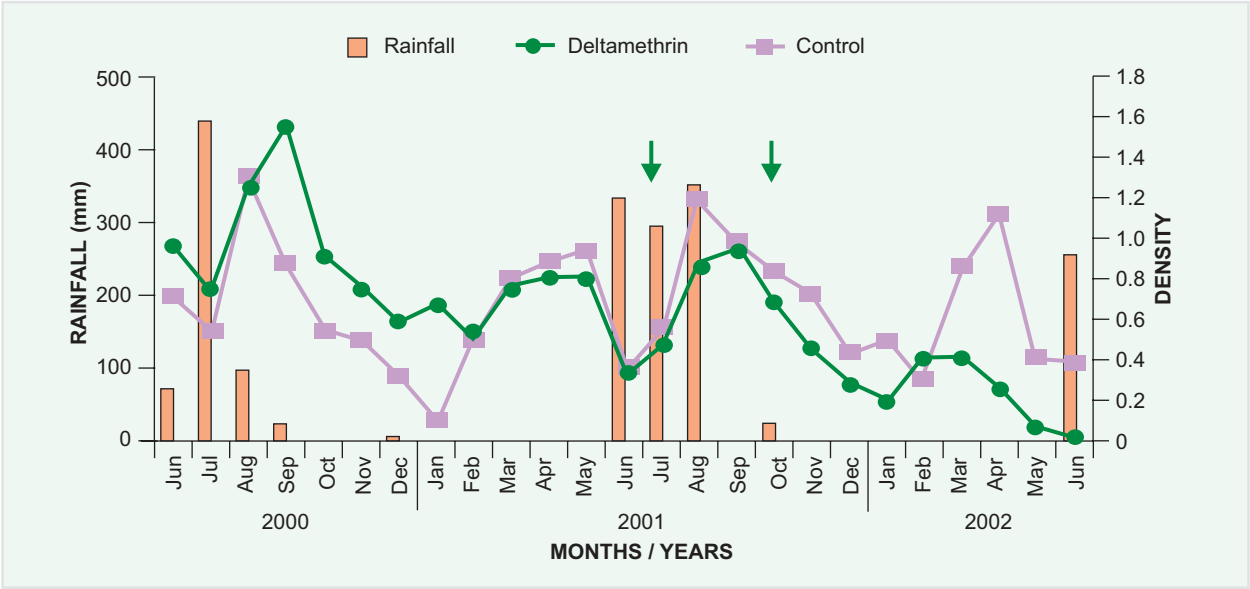


Fig. 16: Geometric mean densities of *An. culicifacies* in intervention and control villages (Gujarat). Arrows indicate first and second rounds of deltamethrin spraying in the intervention villages

July to January. In a riverside village sporozoites of *P. vivax* and *P. falciparum* were also detected in *An. culicifacies* in the month of April in spring season when the vector densities were ascending. Considering the persistence of insecticidal action determined through contact bioassays on sprayed surfaces and the length of malaria transmission in this area, two rounds of spraying with bifenthrin or deltamethrin three months apart would ensure an effective reduction in malaria transmission well over six months. It would be pertinent to undertake spraying in early June and complete the first round as early as possible, preferably by the end of June to interrupt the transmission of malaria. In isolated villages showing high potential of malaria transmission, focal spraying might be required to interrupt the persistent transmission of malaria during the spring. The householders did not report any adverse reaction to these insecticides. Clinical, haematological and urological examinations, and lung function and nerve conduction tests performed on volunteer spray-men showed no adverse effect on short-term relevant exposure during the trial.

Field Evaluation of Lambda-cyhalothrin 10% Capsule Suspension

A village-scale field trial was carried out to evaluate a synthetic pyrethroid insecticide, Lambda-cyhalothrin 10 CS (10% capsule suspension formulation) as an indoor residual spray in District Tumkur in Karnataka state and in Districts

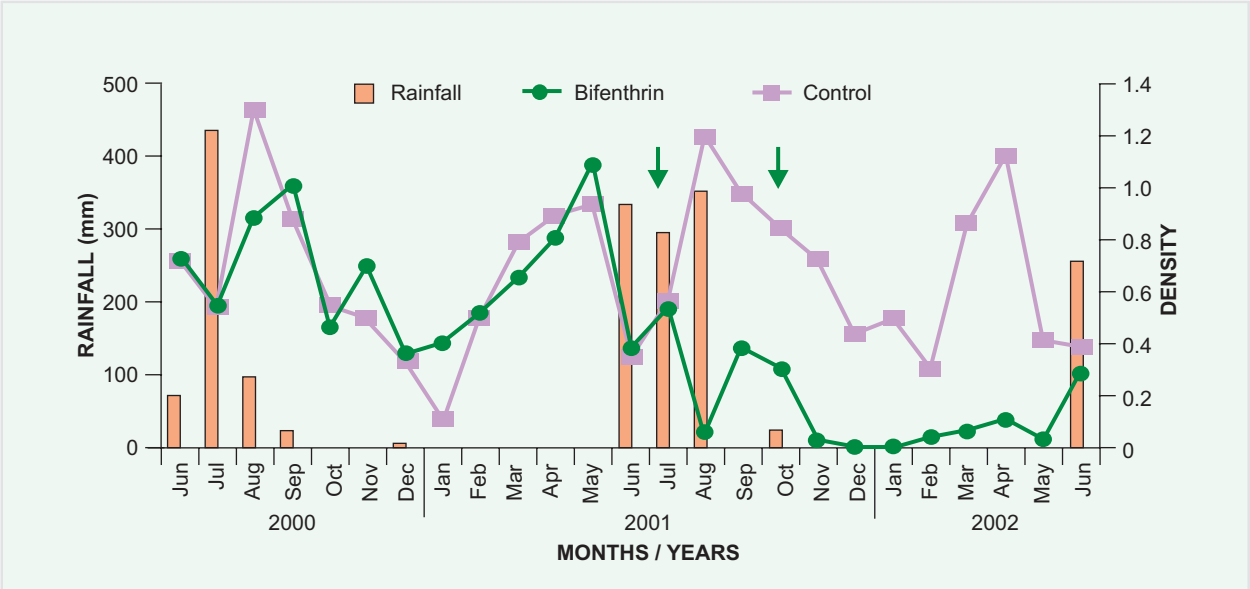


Fig. 17: Geometric mean density of *An. culicifacies* in intervention and control villages (Gujarat). Arrows indicate first and second rounds of bifenthrin spraying in the intervention villages

Dharmapuri and Ramanathapuram in Tamil Nadu during 2005–07. Evaluation was done to assess the entomological and epidemiological impact of the insecticide on malaria transmission. Results were compared with the impact of the routinely used insecticides in indoor residual sprays by the National Vector Borne Disease Control Programme (NVBDCP). The evaluation was carried out essentially following the 'Protocols for uniform evaluation of insecticides for use in vector control' jointly published by National Institute of Malaria Research, Delhi and Vector Control Research Centre, Puducherry. The district-wise observations are as follows:

District Tumkur (Karnataka)

In this district villages of PHC Taverkere were selected for the studies. Lambda-cyhalothrin 10 CS was sprayed in four villages (experimental) @ 25 mg/m^2 comprising of a population of 3098 while malathion was sprayed in three villages (control) comprising of 2820 population. The villages had similar ecotype and *An. culicifacies* was the principal vector of malaria in this region. In insecticide susceptibility tests *An. culicifacies* registered 82 and 97% mortality to malathion in experimental and control areas while against lambda-cyhalothrin 82 and 93% respectively. Insecticide spray was done following the insecticide spray schedule followed by NVBDCP. Two rounds of lambda-cyhalothrin 10 CS was sprayed in experimental villages and the room coverage in first round (October 2005) and second round (February 2006) was 67.4 and 69%, respectively. Two rounds of malathion (first round September 2005 and second round February 2006) were sprayed in control villages with respective room coverage of 59 and 55.7%.The pre-spray mortalities in cone bioassays in experimental and control villages in *An. culicifacies* on wall surfaces were 29 and 33% respectively. After first round spray the species registered 100% mortality to lambda-cyhalothrin 10 CS sprayed surfaces in experimental areas while in control villages on malathion sprayed surfaces it was 86%. The mortality in cone bioassays on lambda-cyhalothrin sprayed surfaces was >80% up to three months after spray while in the control area on malathion sprayed surfaces it was >80% only in the first month and decreased by second month (the residual efficacy of malathion is 6–8 weeks). The man hour densities (MHD) of *An. culicifacies* in the pre-spray period in experimental and control areas was 16.4 and 24.3 respectively. The MHD reduced to 0.7 (range 0.14–1.7) after first round in the experimental area while it was 8.0 (range 1.4–40) in the control area. In post-spray period of second round the MHD in experimental area was in the range of 0–0.5 while in the control area it was 1.0–2.5. Lambda-cyhalothrin 10 CS has shown relatively higher efficacy. Pre-spray densities in total catch collection in the experimental area in cattlesheds and human dwellings were 6 and 18 respectively while in the control area it was 51 and 14 respectively. In the immediate post-spray periods the densities decreased in both the experimental and control areas. However, the impact of lambda-cyhalothrin 10 CS IRS was better than malathion IRS. The post-spray densities in experimental area in human dwellings was in the range of 1–4 (post-spray first round—1–4 and post-spray second round it was >1). The densities in post-spray period in control areas were in the range of 2–22 (post-spray first round—10–22 and post-spray second round it was >3).

The parity rates during pre-spray period were 69 and 75 respectively in the experimental and control areas and decreased to 39 and 43 respectively after the spray. In both the experimental and control areas malaria incidence reduced after spray in comparison to pre-spray. Human safety of lambda-cyhalothrin 10 CS was evaluated in human volunteers (spraymen and inhabitants) in experimental area on general health, six biochemical parameters and routine and biochemical analysis of urine and differential counts of blood. No significant changes were observed in profiles in the pre- and post-spray periods. Inhabitants have expressed satisfaction about the benefits of spray in their villages and did not report any adverse effect during or after the spray operation.

District Dharmapuri, Tamil Nadu

In District Dharampuri, two villages Hoggenakal and Ootamalai PHC Nagadasampatti, comprising of a population of 2810, were selected for evaluation of lambda-cyhalothrin 10 CS (experimental area). Five villages, namely Balrampatti, Seriampatti, Chinnappankottai, Pooniyan Kottai and Seklinatham were selected as control villages where deltamethrin 2.5% WP was sprayed as a comparative insecticide. *An. culicifacies* is the vector of malaria in this region. First round of spray in experimental villages with lambda-cyhalothrin 10 CS was carried out in February 2006 with house coverage of 66.3%. Second round spray in experimental villages was carried out in July 2006 with 72% house coverage. In control villages deltamethrin 2.5 WP was sprayed in first round (March 2006) with 51.9% house coverage which in second round (August 2006) increased to 59.7%. Pre-spray MHD of vectors in experimental villages was 12 and that of in control villages it was 8. In the post-lambda-cyhalothrin spray period in experimental villages the density was >1. While in control villages sprayed with deltamethrin, the MHD was >1 in the post-spray period after first round and was in the range of 2–4 in post-spray period after second round. The impact of lambda-cyhalothrin 10 CS was found superior than deltamethrin. Per-structure density in total catch collection of *An. culicifacies* was respectively 9 and 8 in experimental and control villages during pre-spray. After the first round spray the per-structure density in the experimental area was 0–2 and 4–7 in

the control area. After the second round spray it ranged from 4–6 in the experimental villages while it ranged from 2–13 in the control villages. Similar impact was found on total anophelines and *Culex* species populations. The impact was significant ($p<0.05$) indicating superior efficacy of lambda-cyhalothrin 10 CS being a slow release formulation. Persistence studies indicated similar efficacy of the two insecticides in comparison. The residual activity (80% mortality rate of mosquitoes) remained for three months after the first and second rounds of spray in the experimental area and up to three months and two months respectively in control area.

Parity rates of *An. culicifacies* decreased from 76 in pre-spray period to 44 in post-spray period in the experimental area and from 68 to 42 in the control area indicating equal effectiveness of the insecticides ($p>0.05$). Active and passive surveillance studies indicated prevalence of both *Pv* and *Pf* with increased prevalence of *Pv* in the experiental area. In post-monsoon months 18 *Pf* cases were reported in the control area as against two cases in experimental areas indicating continued transmission in the control area. Though the number of malaria cases reported in the experimental village was slightly higher than the control village, statistically no significant difference was observed between the two villages ($\chi^2 = 1.56$; $p>0.05$). Similarly, in mass blood surveys *Pf* cases were found prevalent in control villages. It may be stated that the villages in both areas were receiving deltamethrin spray and being an effective insecticide kept the transmission under control. However, lambda-cyhalothrin CS has shown better effect in curtailing the malaria transmission. No adverse effects were reported by inhabitants or spraymen during the spray and overwhelming response was received from the inhabitants for IRS.

District Ramanathapuram (Tamil Nadu)

In District Ramanathapuram, villages of PHC Pamban in Rameshwaram Island were selected for the studies. Five villages, namely Mundal, Thoppukadu, Chinnapalam, Kundakal and Tharavai Thopu comprising a population of 2827 were selected as experimental villages and village Akkalamaddam and hamlets comprising of a population of 2626 were selected as control villages. *An. culicifacies* is the vector of malaria in these villages. Spray was carried out as per the NVBDCP schedule of indoor residual spray. Both experimental and control areas received different formulations, CS and WP of lambda-cyhalothrin. Three rounds of lambda-cyhalothrin 10 CS spray (July 2006, November 2006 and March 2007) was carried out in experimental villages with respective per cent house spray coverage of 98.6, 98.2 and 98.6%. In control villages, three rounds of lambda-cyhalothrin 10 WP IRS was carried out simultaneously with 95.5, 90.8 and 81.4% coverage in first, second and third rounds respectively.

The MHD of *An. culicifacies* remained low in experimental areas throughout the study period (0–2) while in the control area it reached up to 4.5. Total catch assessment in structures in post-spray period indicated per structure density of *An. culicifacies* as 0–4 except in March 2007, and of anophelines remained in the range of 1–5 except in November 2006. While in control area it was below 12 except in October and November 2006. Similar impact was seen on *Culex* species density. This data on indoor densities indicated better impact of lambda-cyhalothrin 10 CS probably owing to the slow release characteristic of the CS formulation over the WP formulation. A significant decrease in parity rates ($p<0.05$) was observed. Pre-spray parity rate of the vector in the experimental area was 62 and decreased to 22 in post-spray period. Similarly, in the control area it decreased from 58 to 24. The malaria incidence in the experimental and control villages was very low throughout the study period. The positives in experimental area was 31 (20 *Pv* + 11 *Pf*) while in control area it was 61 (45 *Pv* + 16 *Pf*) indicating a better interruption of transmission in the experimental villages. Mass blood surveys did not show any difference between the control and experimental villages indicating equal effectiveness of insecticides. Inhabitants and spraymen did not report any adverse effects due to spray operation. There was a overwhelming response from the inhabitants for the spray.

Multicentric field trials at three sites has established that lambda-cyhalothrin 10 CS formulation was found relatively more effective than malathion 25% WP, deltamethrin 2.5 WP and lambda-cyhalothrin 10 WP in some important evaluation parameters like indoor resting mosquitoes, parity rates of vector mosquitoes, increased persistence, etc.

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

Phase 1 evaluation of Chlorfenapyr, of pyrrole group of insecticides, was carried out following WHO guidelines and common protocols published jointly by NIMR, Delhi and VCRC, Puducherry. Chlorfenapyr is a pro-insecticide which is converted by oxidases in animals to an active form. This insecticide is a mitochondrial electron transport inhibitor (METI) whose mode of action is to disrupt the conversion of ADP to ATP (Oxidative phosphorylation) in mitochondrion. Because chlorfenapyr's mode of action is novel, it is unlikely to show cross resistance to neurotoxic insecticides. It has low mammalian toxicity (acute oral rat—2582 mg/kg BW) and classified as WHO toxicological class III and placed in the category 'slightly hazardous' to humans. The insecticide has shown good efficacy against three species of mosquitoes, *An. culicifacies, An. stephensi* and *Cx. quinquefasciatus*. The efficacy against *Ae. aegypti* was erratic and hence was not included in

the study. Adult bioassays with impregnated paper were carried out using different concentrations and exposure times. The results showed that exposure to impregnated papers for 2 h and 48 h holding period was suitable for susceptibility tests. Susceptibility was determined by exposing the mosquitoes to impregnated papers in a range of concentrations from 0.25 to 5% for 2 h exposure and holding period of 48 h. The dose-mortality response data were subjected to log-probit regression analysis and LD_{50} and LD_{99} value were calculated. LD_{50} values for *An. culicifacies* species A was 0.411%, species C was 0.676%, *Cx. quinquefasciatus was* 0.370% and *An. stephensi* was 0.437%, while LD_{99} were respectively 2, 2.39, 2.23 and 2.13%. Thus, LD_{99} was in the range of 2 to 2.4% for different species. As per the standard criteria double the LD_{99} was chosen as the diagnostic dose for assessment of susceptibility in the field mosquitoes. Accordingly, 5% impregnated paper with 2 h exposure and 48 h holding is suggested for assessing the susceptibility of mosquitoes in field. Persistence studies were carried out on five different fabricated surfaces, namely mud, mud + lime, cement, cement + distemper and wood. These surfaces were sprayed with Chlorfenapyr 10% SC was sprayed in a range of 12.5 to 800 mg/m^2 following NVBDCP norms. Efficacy of insecticide on the sprayed surfaces was assessed by cone bioassays with 30 min exposure and 24 h holding period and the results indicated a dose of 400 mg/m^2 to be appropriate dose for effectiveness. Chlorfenapyr @ 400 mg/m^2 was found to be effective up to 28 weeks against *An. culicifacies* and up to 34 weeks with *An. stephensi* . While against *Cx. quinquefasciatus* two surfaces namely mud + lime and wood have shown consistent results up to 34 weeks and with other surfaces persistence of effectiveness was variable. Studies to asses cross resistance with 5% chlorfenapyr impregnated papers for 120 min with laboratory selected permethrin resistant *Cx. quinquefasciatus* (74%) has shown 100% mortality at the end of 48 h while lambdacyhalothrin resistant *Cx. quiquefasciatus* (78%) has shown 100% mortality within 24 h. In exposures with 2% chlorfenapyr for 60 min in *An. culicifacies* field strain (Surat) which was double resistant to DDT (80%) and malathion (43%) registered 100% mortality within 24 h. No side- effects such as eye irritation, skin irritation, etc. were reported during and after spray by the applicators and investigating staff.

Multicentric Study on the Susceptibility of *Culex quinquefasciatus* Larvae to Fenthion in Urban Areas

Application of Fenthion (100% EC) @ 5 cc in 10 litres in breeding habitats of *Cx. quinquefasciatus* @ 20 cc/m^2 was carried out in urban areas of Delhi, Bengaluru and Nadiad from September to November 2006, to evaluate the efficacy in field conditions.

The results revealed that the spraying of Fenthion produced a very low impact on larval densities in general. The effect lasted only up to three days in Delhi and Nadiad and till seven days of post-spray in Bengaluru with 71.98% reduction after first round and no reduction after third round of spray. The gradual rounds of spray resulted in further reduction in larval densities. On Day 21 of post-spray, the larval density was more than Day 0, indicating no impact of larvicide in Delhi, Nadiad and Bengaluru.

The results of the larval susceptibility tests on *Cx. quinquefasciatus* indicated that the corrected percent mortality in larvae at the dose of 0.05 ppm was 18.7, 53.4 and 30.5 in Nadiad, Delhi and Bengaluru respectively which revealed that *Cx. quinquefasciatus* has developed resistance to the insecticide in all the study areas.

Trials of Insecticide-treated Mosquito Nets and Curtains

Nets or curtains treated with various formulations of pyrethroids such as deltamethrin, cyfluthrin, lambda-cyhalothrin and bifenthrin have been evaluated in laboratory and through field trials.The results are given specifically in this publication.

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Studies on the Reliance of DDT, HCH and Malathion in Vector Control Programme

Studies were carried out in field to assess the efficacy of DDT and HCH in vector control in early eighties. These studies were actually conducted amidst the conflicting reports on the usage of DDT for vector control in indoor residual sprays owing to the reports of wide-spread resistance in major vectors to DDT and HCH.

In the year 1981, a study was conducted in villages of District Faridabad (Haryana) which were under regular spray of HCH (Sharma *et al* 1982). The main vector species in this region was *An. culicifacies* and was reported 89% resistant to DDT. Comparative entomological and epidemiological evaluation was made in villages sprayed with DDT @1 and 2 g/m^2 with unsprayed villages as control. The first round of DDT was sprayed in June and the second in August. Entomological evaluation indicated no difference in the impact of two doses of DDT spray (Fig. 18) while parasitological evaluation also did not indicate any relative advantage in the two areas of DDT spray but the slide positivity rate decreased to half in subsequent months (Fig. 19). This study indicated that the usual dose of DDT spray @ 1 g/m^2 with good coverage resulted in desired epidemiological impact.

With this background a more elaborate study was carried out in 1984 in the villages of Loni primary health centre, in District Ghaziabad (Sharma *et al* 1986). The villages were under regular spray of HCH @ 0.2 g/m^2. Spraying was carried out in 4-dose regimen in four zones. Three zones were sprayed under the supervision of NIMR) staff—HCH @ 0.2 g/m^2 (normal recommended dose) and 0.5 g/m^2 (enhanced dose) and DDT @ 1 g/m^2 (normal recommended dose) while the fourth zone was sprayed @ 0.2 g/m^2 with HCH under the supervision of state health personnel and this zone served as control for the above three zones. Three rounds of HCH were sprayed in the months of May–June, June–August and August–October respectively while DDT was sprayed in the months of May–July and August–September. Enhanced dose of HCH @ 0.5 g/m^2 was contemplated to kill the heterozygotes and some of the homozygous resistant genotypes. *An. culicifacies*, the major vector of malaria

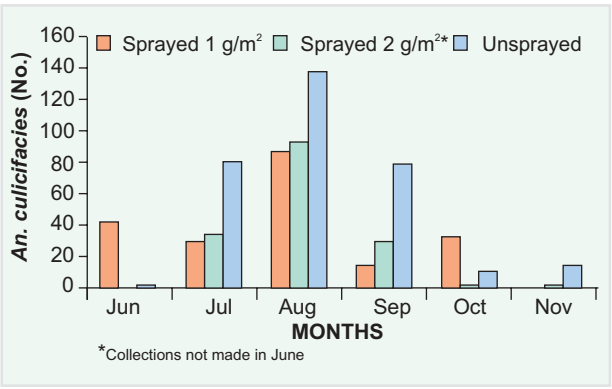

Fig. 18: Observed number of *An. culicifacies* in total catch in different months in DDT sprayed and unsprayed villages in District Faridabad, Haryana

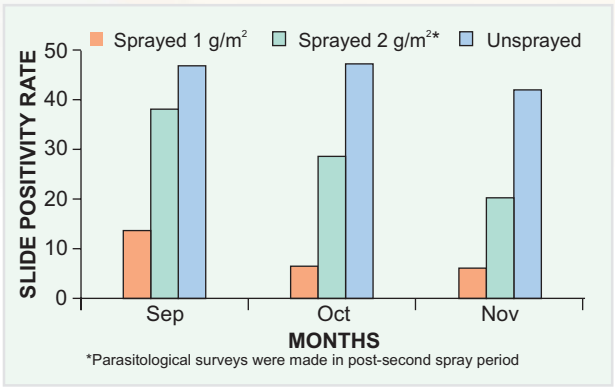

Fig.19: Parasitological data during the intervention periods in villages with 2 dose regimens of DDT and unsprayed villages in District Faridabad, Haryana

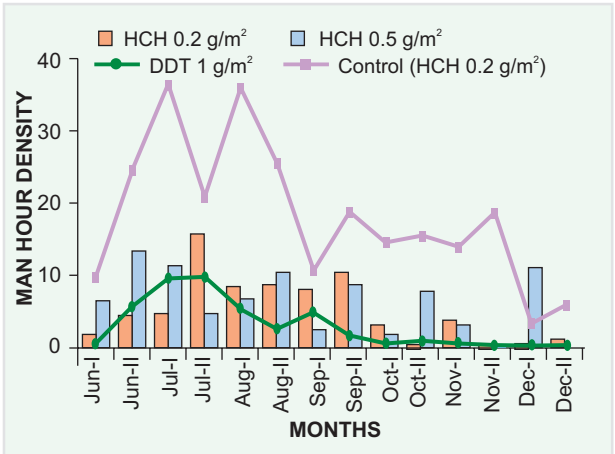

Fig.20: Man hour density of *An. culicifacies* in villages with different dose regimens of HCH and DDT in villages of PHC Loni, District Ghaziabad

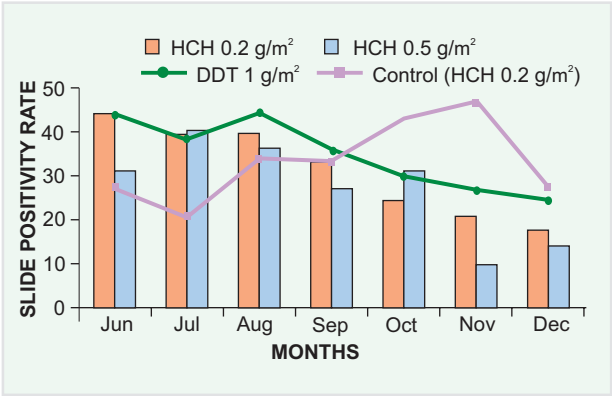

Fig. 21: Slide positivity rates in different months during the intervention in villages with spray under different dose regimens of HCH as compared to DDT

in this region was >60% resistant to DDT and >90% resistant to dieldrin. Entomological evaluation revealed no significant difference in the impact of two doses of HCH (Fig. 20). Usual dose of DDT @ 1 g/m^2 with good coverage indicated desirable impact even on the parasitological indices (Fig. 21).

In continuation of these studies, another study was carried out during 1985–86 to assess the comparative advantage of DDT spraying in cattlesheds alone and human dwellings and cattlesheds with two doses of DDT spray @ 1 and 2 g/m^2 and HCH @ 0.2 g/m^2 (Ansari *et al* 1988). The study was aimed at studying the impact of this different regimens of sprays on the main malaria vector in the area, *An. culicifacies* which is primarily zoophagic. Entomological and epidemiological studies revealed no comparative advantage of increased DDT dose (2 g/m^2) as was observed in earlier studies and spraying. These studies have again brought out that improved coverage of human dwellings with the usual dose of 1 g/m^2 could provide the desired impact on parasitological indices (Fig. 22).

Due to continuous usage of DDT, HCH and other insecticides in vector control programme, *An. culicifacies* has developed resistance to all the insecticides used in public health. Presently from the available database, this species has become resistant to DDT in 286 districts, DDT and HCH in 233 districts, DDT, HCH and malathion in 71 districts and synthetic pyrethroids in two districts. It may be mentioned here that this species is responsible for ~60–70% of new cases of malaria each year.

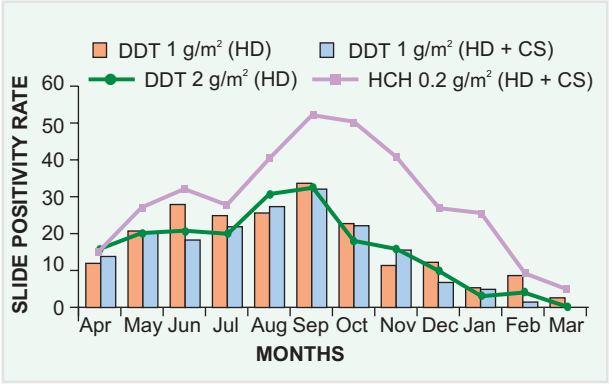

Fig. 22: Slide positivity rates in villages with different dose regimens and spray pattern of DDT anf HCH

In spite of multiple insecticide-resistance in *An. culicifacies*, DDT is continued to be used in indoor residual spray in rural areas to control especially *An. culicifacies*. DDT is still a cost-effective insecticide. Recently, DDT has been designated as an exempted insecticide from the persistent organic pollutant chemicals in Stockholm Convention which suggested that this insecticide could be continued for use till a cost-effective sustainable alternative strategy is found.

Meanwhile, The Mandate Committee of the Government of India for the use of DDT formed under the Chairmanship of the Secretary, Health & Family Welfare, decided to evaluate the efficacy of DDT and malathion in malaria and kala-azar control programmes. Accordingly, Indian Council of Medical Research was requested to conduct a multicentric study in different parts of the country and the Director, NIMR, Delhi, coordinated the project. The NIMR, Delhi and the Vector Control Research Centre, Pondicherry carried out the studies on malaria control and the Rajendra Memorial Research Institute of Medical Sciences, Patna, on kala-azar control. Multicentric studies were undertaken in 10 districts of eight states (Fig. 23). Both entomological and epidemiological evaluations were done. Studies were carried out during pre- and post-spraying periods of the respective rounds of spray. Evaluation was done in areas under the influence of the three major vectors of malaria, namely *An. culicifacies, An. fluviatilis* and *An.minimus*; and Kala-azar vector—*Phlebotomus argentipes.*

This multicentric study revealed that *An. culicifacies* was resistant to DDT in three districts, namely Chhindwara (Madhya Pradesh), Mandya (Karnataka) and Bareilly (Uttar Pradesh). In general, indoor spraying of DDT has indicated that DDT was ineffective in reducing the vector densities to a desired level. The excito-repellent effect lasted for the initial 2–3 weeks. Though the studies were done on limited scale, the data available on susceptibility status of *An. culicifacies* showed that DDT is not the insecticide of choice of spray in areas with *An. culicifacies* prevalence—rural plains and peri-urban areas of India. However, the use of DDT can be continued in areas under the influence of *An. fluviatilis* and *An. minimus*.

Malathion was evaluated in two districts, namely Hardwar (Uttarakhand) and Kheda (Gujarat) and was found effective in reducing *An. culicifacies* population. In other districts in Gujarat variable level of malathion susceptibility was observed. This suggested a further need to assess the susceptibility to malathion and its use as per the need.

Kala-azar vector—*Phlebotomus argentipes*

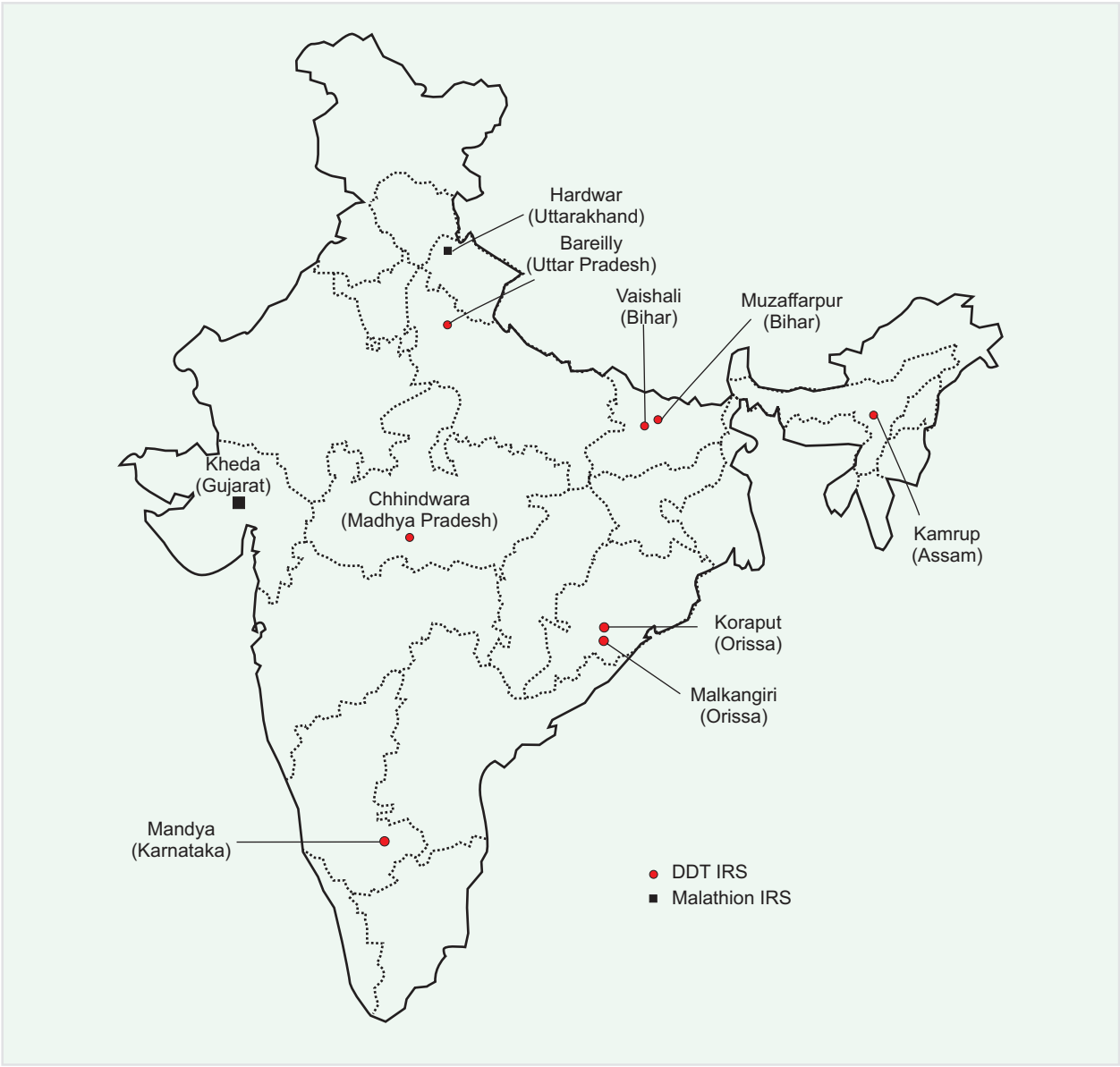

Fig. 23: Map showing districts selected for evaluation of DDT and malathion indoor residual sprays

showed variable resistance to DDT in Vaishali (Bihar) and a few other districts. This calls for regular monitoring of resistance in this vector, as DDT is going to be used extensively in the kala-azar control programme. Also more studies have to be conducted to see the efficacy of DDT in these areas.

Present Status of DDT Use in India

The Stockholm Convention on persistent organic pollutants (POPs) in 2001 identified DDT as one of the 12 POPs. But DDT ban had certain restrictions applicable to countries that have notfied to the secretariat for its continued use and India is one of it. The restriction permits indoor residual sprays (IRS) of DDT in malaria control as per the WHO specifications for its production and following safety precautions for its safe use and disposal. Phasing out of DDT is delayed till an effective, affordable and safe alternative is available. In India, the use of DDT in agriculture was banned in 1989 with a mandate to use a maximum of 10,000 tons of DDT per annum for the control of malaria and kala-azar and this policy is strictly adhered to till date.

There are reports that *An. culicifacies*, a major malaria vector is highly resistant to DDT since 1960s, now shows increased susceptibility in Gujarat and other parts of the country where DDT was withdrawn since 1969. Meanwhile, WHO has issued a clean bill to use DDT supported by scientific and programmatic evidences (http://www.who.int/mediacentre/news/releases/2006/pr50/en/print.html)

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Insecticide-treated Nets and Curtains

One of the major innovations in the field of malariology during the past two decades is the development of the technology of insecticide-treated mosquito nets (ITNs). Mosquito nets or curtains have been in use since long in many civilizations including in India. The Italian traveler Marco Polo had mentioned in his travelogue that nobles in southern India used the mosquito curtains made of light cane work in the 13th century AD. Although deliberate treatments on nettings with repellents and even DDT were tried to provide additional protection to individuals against blood sucking insects like mosquitoes. Development of stable pyrethroids during the 1980s led to evaluation of pyrethroid-treated netting and clothing materials against mosquito vectors. Until 1990, several small-scale trials in various countries demonstrated the effectiveness of nets treated with pyrethroids in reducing malaria and vector populations. In China, nets treated with deltamethrin were used on a large-

Steps involved in impregnation of net: (1) measuring the surface of net; (2) measuring the volume of insecticide for impregnation; (3) Impregnation of net; and (4) drying of impregnated net on a plastic sheet

scale during this period. In many endemic countries, laboratory and field trials with untreated and treated mosquito nets were carried out including India.

ITN Trials in Assam

To evaluate effectiveness of mosquito nets in malaria control, the first field trial was carried out successfully in Sonapur PHC, Assam during 1987 to 1991 in areas where *An. minimus* was the main malaria vector (Jana-Kara *et al* 1995 and Sharma *et al* 1996). Cotton nets treated with deltamethrin WP at 25 mg/m^2 were found effective in reducing *An. minimus* population and malaria incidence (Fig. 24). In villages with untreated cotton nets malaria situation remained unchanged, whereas in villages without nets malaria incidence rose significantly during the trial period. General acceptance of nets by the communities was found to be satisfactory.

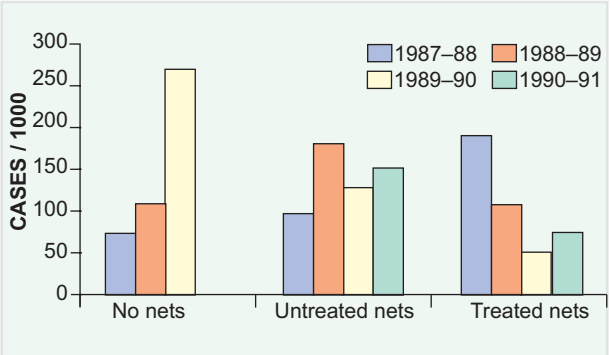


Fig. 24: Impact of untreated nets and deltamethrin-treated nets on malaria in Sonapur PHC, Assam

Another trial comparing efficiency of nylon nets treated with deltamethrin (25 mg/m^2), lambda-cyhalothrin (25 mg/m^2), untreated nets and no nets in villlages in Sonapur, Assam, also showed a superior protection of treated nets over untreated nets or no nets (Fig. 25).

ITN Trials in Orissa

Most of the rural malaria in India is transmitted by *An. culicifacies* in plain areas and in Orissa hill forests by *An. fluviatilis*. A trial was carried out from 1989 to 1994 in areas of north Orissa where above

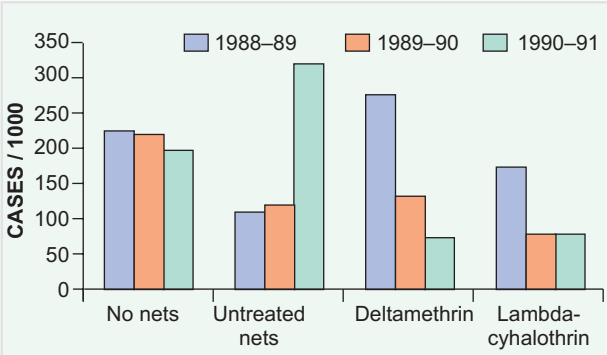


Fig. 25: Impact of untreated nets and nets treated with lambda-cyhalothrin and deltamethrin in another trial in Assam

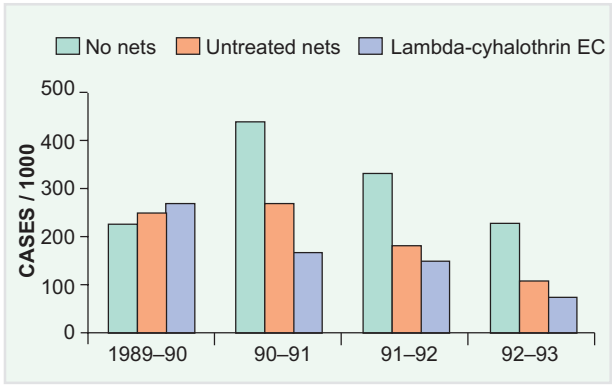


Fig. 26: Impact of lambda-cyhalothrin-treated nets on malaria in villages of Sundargarh district

mentioned two species were the main malaria vectors. In tribal villages of Sundargarh district, nylon nets treated with deltamethrin SC or lambda-cyhalothrin EC at 25 mg/m^2 reduced indoor densities of malaria vectors, *An. culicifacies* and *An. fluviatilis*, their biting rate and the malaria incidence significantly (Figs. 26 and 27) (Sampath *et al* 1998 and Yadav *et al* 1998, 2001).

In the mining areas, where malaria caused tremendous economic loss due to loss in man days, use of cyfluthrin-treated nets resulted in considerable reduction in *An. fluviatilis,* and incidence of malaria (Fig. 28), as well as anaemia and spleen rates in protected children (Sharma and Yadav 1995).

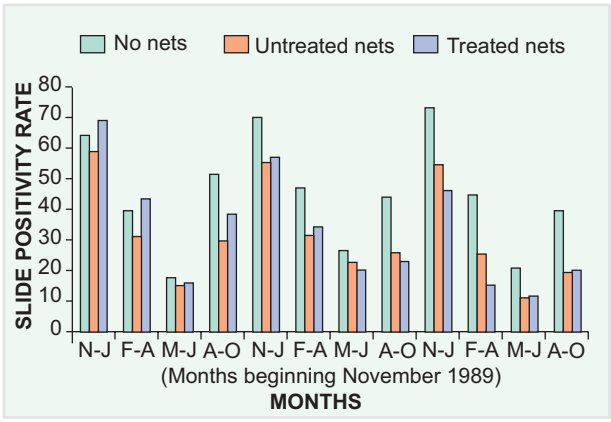


Fig. 27: Impact of deltamethrin-treated nets on malaria in villages of Sundargarh district, Orissa

Hospital occupancy due to malaria in two mining hospitals decreased. Minor communities accepted the treated nets very well and perceived that such nets even reduced other household insect pests besides mosquitoes. Human toxicity studies reported that the treated nets were safe to impregnators and users (Yadav *et al* 1996 and Satpathy *et al* 1997).

Comparative Field Trial with different Pyrethroids and Formulations

A study on the efficacy of impregnated bednets in controlling malaria was conducted from May 1990 to April 1993, covering a population of 6100

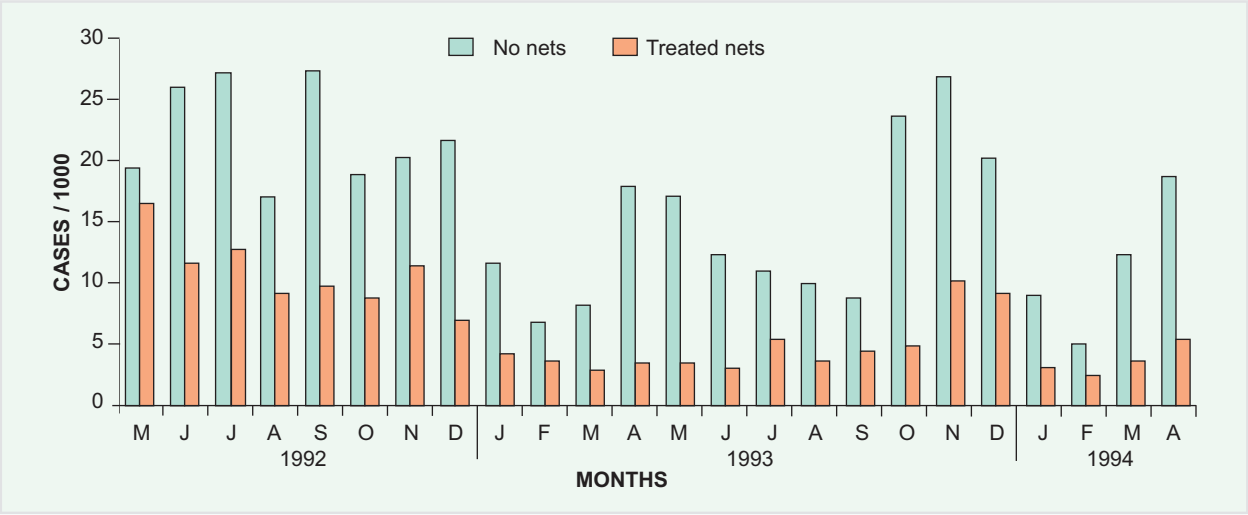


Fig. 28: Efficacy of cyfluthrin-treated nets on malaria in mining settlements, Orissa

in 15 villages of Kuarmunda PHC. The study area was divided into five groups of comparable endemicity. Bednets treated with lambda-cyhalothrin 5% EC (ICON), deltamethrin in 2.5% WP (DM, WP) and deltamethrin 2.5% flow (DM, FLOW) (@ 25 mg/ m^2) were provided to three experimented groups with population of 1220, 1850 and 1100 respectively. The fourth group with a population of 1403 was provided with untreated nets while the fifth group with a population of 814 did not receive nets.

Mosquito densities were monitored fortnightly by hand collection. After first impregnation in May 1990 the density of *An. culicifacies* in ICON treated net area, declined significantly as compared to no net area. The observable impact lasted for nine months. Second impregnation in June 1991 further checked vector density. Based on the results of residual toxicity bioassay six monthly impregnation cycle was scheduled. The impact of DM WP and DM FLOW treated nets on the density of *An. culicifacies* were also similar to that of ICON. However, DM WP treated nets resulted in the itching of skin and the last re-impregnation in the area was carried out with DM FLOW in June1992 (Fig. 29).

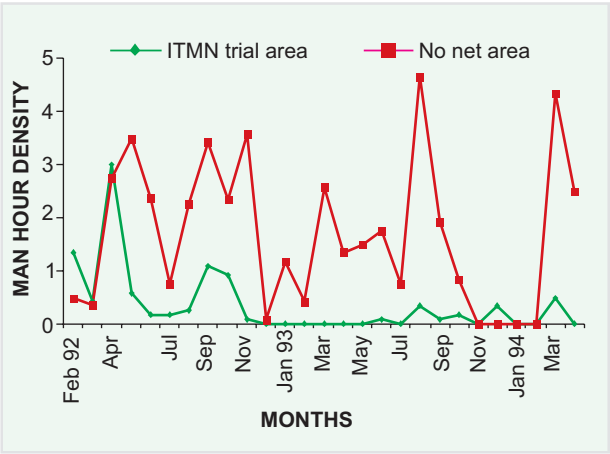


Fig. 29: Impact of insecticide-treated mosquito nets on the density of *An. culicifacies* in the mining area of Sundargarh district, Orissa

During night bite catches (1 night per month for 12 months) using four human baits the total number of vectors (*An. culicifacies* and *An. fluviatilis)* collected were in order of 63 in no net; 19 in untreated net; 12 in DM WP treated net; 11 in DM FLOW treated net; and two in ICON treated net.

In no net area malaria incidence increased from API of 223.6 in the base line year 1992 to 227.3 in the 3rd year of intervention with a considerable increase during the intervening period. The API of the area with untreated nets decreased by 58.5%— from 252.5 to 104.8. The API in the area where ICON treated nets were distributed decreased by 72.5% —from 270.7 to 74.6. DM WP treated nets resulted in a decrease of API from 208.5 to 121.6 (41.8%) after three years of intervention. Nets treated with DM FLOW were provided in November 1990, which resulted in a decrease in API by 59%— from 327.6 to 133.6 after two years of trial. After the completion of the study it was found that haemoglobin levels (g/dl) in children below 10 years of age sleeping under treated nets were higher in children using untreated nets or without nets (DM FLOW 14.9 g/dl > ICON 14.5 g/dl > DM WP 14.3 g/dl > untreated net 11.6 g/dl > no net 11 g/dl). There was also a marked improvement in morbidity of children as the spleen rate dropped by 81% in ICON, 86% in DM FLOW and 90% in DM WP areas, whereas it increased by 35.5% in no net areas.

Bednet Trial in Mining Areas

The mining area of Sundargarh district is hyperendemic for malaria, which is responsible for high morbidity and mortality as well as economic loss. On the request of the authorities of the Rourkela Steel Plant, a bednet trial was conducted in three mining settlements from May 1992 to April 1994. The area is located at an elevation of 700–800 m above the MSL and received annual rainfall of about

2000 mm. The temperature remains favourable for mosquito breeding throughout the year (range 17 to 32°C).

Impregnated nets were provided to a population of 11,762 in three settlements—Tensa, Barsuan and Kalta whereas another three settlements, viz. Toda, Khandadhar and Sasikala with a population of 2920 were kept as control area without net. The nets were treated with cyfluthrin @ 50 mg/m^2 at half yearly intervals starting with April/May 1992. Endemicity of two areas were comparable at the beginning of the study (Parasite incidence—Experimental (E) 8.2, Control (C) 9.7; SPR—E 37.8%, C 21.9%; spleen rate—E 59.3%, C 56.1% and Hb—E 9.9 g/dl, C 11.4 g/dl).

Treated nets caused a considerable reduction in the densities of major malaria vectors, *An. fluviatilis* and *An. culicifacies* as well as the densities of total anophelines and total mosquitoes. Before the distribution of nets, the MHD of *An. fluviatilis* was fluctuating around 5 which was drastically reduced to 0 immediately after the intervention and never exceeded 0.5, that too was occasionally throughout the period of study, whereas in the control area it fluctuated in between 2 and 20. The MHD of *An. culicifacies* also reduced drastically from 4.5 at the beginning of the study to 1 after the distribution of net which was further reduced to 0.2 in the subsequent period. After the second impregnation in November 1992 *An. culicifacies* was totally absent in the hand catches for about six months and found in a very low density in the subsequent period. On the other hand the MHD of *An. culicifacies* remained above 2 in the control area during most part of the study. Besides, treated nets provided total protection from the biting of *An. fluviatilis*.

Epidemiological impact was studied on the basis of fortnightly surveillance. In Tensa and Barsuan areas annual incidence of malaria declined by 68.5 and 69.6% during the I and II year of study respectively, while SPR declined by 25.5 and 27.5% in comparison with the control area (Fig. 30). Similarly, in Kalta area the incidence of malaria was reduced by 11.6% during the first year and 48.7% during the second year compared with the control area. SPR also reduced by 6.2 and 36% in the I and II year respectively.

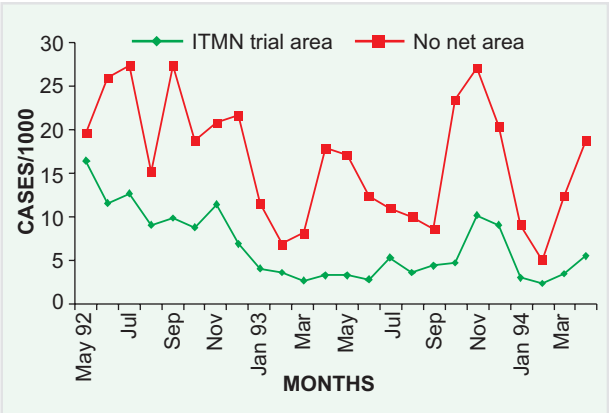


Fig. 30: Impact of ITMNs on malaria incidence in the mining area of Sundargarh district, Orissa

There was a significant reduction in the indoor admissions, due to sever/complicated malaria in the hospitals of Tensa and Kalta. The number of admission in the base line year was 656 which was reduced to 328 in the first year and 170 in the second year of intervention, thereby registering a total decline of 74.1%. During study, no possible toxic effects of cyfluthrin treated bednets were recorded. The compliance of bednet usage was generally good and the trial was well received by the people.

Community Acceptance of Insecticide Treated Mosquito Net (ITN) Trial in a Tribal Population of Keonjhar district, Orissa

A collaborative field trial project under operational research studies was conducted in a tribal area of Jhangira sector in Keonjhar district. The project was financed by Orissa Health Projects, Office of the British Council Division with ODA funds with the objectives to explore whether the communities with poor socio-economic status and living in malaria endemic areas can finance and sustain mosquito net usage and to assess epidemiological impact of the ITN usage.

The duration of the project was from June 1994 to March 1997. The generation of demand for mosquito nets and their subsequent distribution through social marketing at a subsidised price of Rs. 50/- per net was undertaken by CARE-India while NIMR took the responsibility of providing technical support and monitoring the epidemiological impact of the intervention. The distribution of Deltamethrin treated nets (dosage : 25 mg/m^2) commenced from May 1995 in all the 55 villages (population 29000) falling under 7 sections of Janghira sector and a total population of 7799 nets were sold covering approximately 81% of the total population. The nets were re-impregnated at six month interval under the supervision of NIMR.

For epidemiological evaluation, regular entomological and parasitological monitoring was carried out and for comparison a control area of about 22,000 population in the adjoining Jirang sector was selected. The impact was measured on vector densities (Fig. 31), human biting rate, malaria incidence (Fig. 32) and infant mortality rate. The study indicated that treated mosquito nets provide full protection from the bites of mosquitoes and there was 50% reduction in malaria incidence and 34% reduction in infant mortality in experimental area. Surveys conducted to ascertain compliance rate of bednet usage revealed that usage rate ranged from 45 to 60% in different seasons. The trial was well accepted by the people and there was more awareness among the communities to fight against malaria.

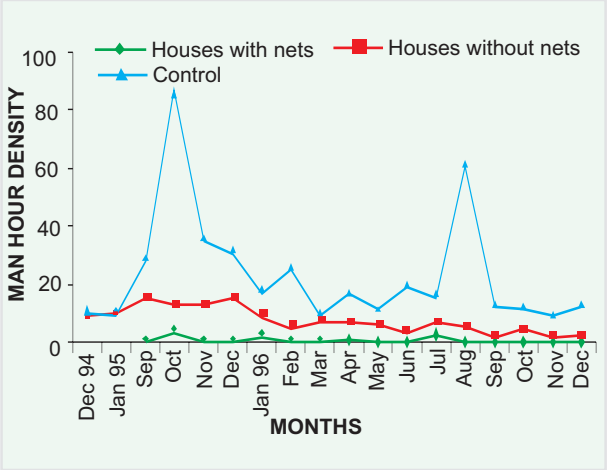


Fig. 31: Impact of insecticide-treated mosquito nets (ITN) on the vector density in Keonjhar district, Orissa

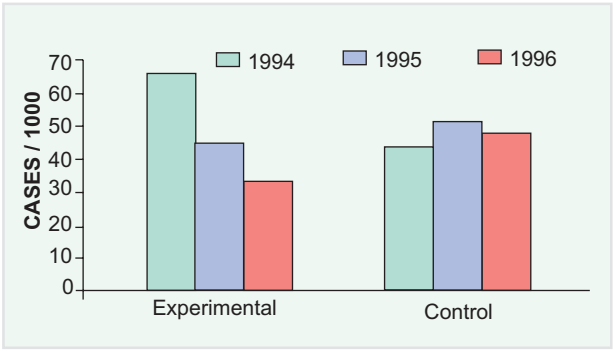


Fig. 32: Malaria incidence in the experimental and control areas of ITN trial in Keonjhar district, Orissa

Evaluation of Mosquito Nets Treated with Deltamethrin Tablet Formulation against Malaria Vectors *An. fluviatilis* and *An. culicifacies* and its Impact on Malaria Transmission

The studies were conducted during 2002–03 in three villages under Bisra PHC of Sundargarh district. These three villages were Birkera (Pop. 506) which was the trial village and village San Pokhari (Pop. 367) and Dudarta (Pop. 271) were control villages. Based on entomological and parasitological parameters, the impact of mosquito nets treated with tablet formulation of deltamethrin was assessed. The bio-efficacy studies showed 100% mortality up to six months in *An. fluviatilis* and 80–100% mortality in *An. culicifacies* up to six months. Therefore, the bio-availability of the insecticide persists on mosquito nets up to six months. The man hour density (MHD) of malaria vectors *An. culicifacies* and *An. fluviatilis* in houses with treated nets was significantly low as compared to houses with untreated nets and no nets. There was overall reduction in entry rates of mosquitoes into the houses with treated nets compared to other two control villages with untreated nets and no nets. There was 39.6% immediate mortality in mosquitoes coming in contact with treated nets and 43.3% of mosquitoes succumb to lethal dose within 24 hours. The low feeding success rate of mosquitoes in the trial village in comparison to villages with untreated net and no net also confirm efficacy of tablet formulation of deltamethrin. The insecticide formulation was found to have low excito-repellency rate for both the vector species, which is likely to produce a better mass killing effect on the mosquito population. There was significant reduction in malaria incidence in the trial village in comparison to control villages with plain nets and no nets. Cross-sectional malaria prevalence data collected during pre-intervention and intervention period also showed that there was 65.2% reduction in malaria parasite rate (Fig. 33) in the population using treated nets, which is significantly higher in comparison to the population using untreated nets (35.4%) and no net (7.5%).

Data on splenomegaly and parasite prevalence in 2–9 years children are given in Table 1. Baseline data collected during December 2002 showed no significant difference in spleen rates in children from all three villages ($\chi^2 = 0.2$, Df-2, $p > 0.05$). At the end of one year of intervention in December 2003, the spleen rate in children using treated net showed significant reduction (χ^2=15.4, Df-1, $p<0.001$). The reduction in spleen rate in children using untreated net was also significant (χ^2=9, Df-1, $p<0.005$), whereas the spleen rate was found higher in children using no net, in comparison to baseline data although the increase in spleen rate was insignificant (χ^2=0.6, Df-1, $p>0.05$). Parasite rate in children 2–9 years showed no significant change between baseline and after one year data in the population using no nets (χ^2=0.8, Df-1, $p >0.05$) but significant reductions were observed in population using untreated net (χ^2=5.2, Df-1, $p <0.025$) and treated nets (χ^2=12.2, Df-1, $p <0.001$). The study showed that in areas with persistent malaria throughout the year such as Sundargarh district, two treatments of mosquito nets at an interval of six months would provide effective protection against malaria.

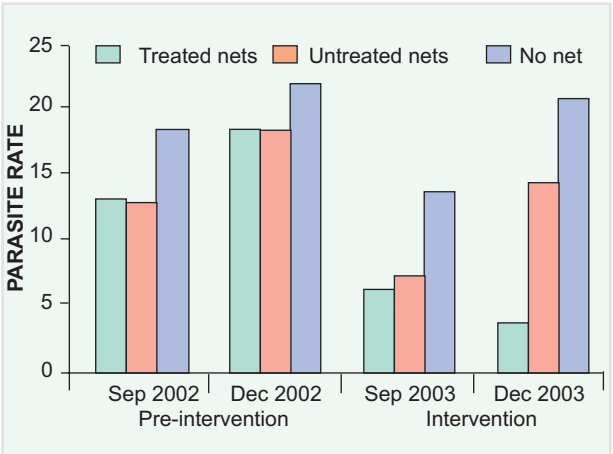


Fig. 33: Malaria prevalence in the study villages with K-O TAB treated nets, untreated nets and no net as recorded through cross-sectional point prevalence surveys conducted in September and December during pre-intervention and intervention phases

Table 1. Impact of use of treated mosquito nets on splenomegaly and child parasite rate (CPR) in children aged 2–9 years, as recorded through cross-sectional surveys conducted in pre-intervention (December 2002) and post-intervention (December 2003)

Population using	Year	n	Spleen rate (%)	χ^2	AES	CPR (%)	χ^2
Treated net	2002	41	63.4	1.54	36.6		
	2003	47	12.7	15.4 ($p<0.001$)	1.12	10.6	2.2 ($p<0.001$)
Untreated net	2002	35	69.6	1.9	42.8		
	2003	42	20.7	9.0 ($p<0.005$)	1.0	19.0	5.2 ($p<0.025$)
No net	2002	37	59.5	1.64	37.8		
	2003	39	74.3	0.6 NS	1.43	28.2	0.8 NS

NS: Not significant; AES: Average enlarged spleen; *Chi-square test was applied to test the significance of difference between data collected at baseline and after one year of intervention.

Phase III Evaluation of High Density Polyethylene (HDPE) Mosquito Nets Treated with Insecticide

A field trial was completed on the efficacy of High Density Polyethylene (HDPE) bednets indigenously manufactured by small scale industries in Tamil Nadu. The nets were treated with deltamethrin flow at a dosage of 25 mg/m^2 and were evaluated against malaria vectors, *An. culicifacies* and *An. fluviatilis* as well as its impact on malaria transmission in one of the highly endemic areas of Orissa. The study was sponsored by NVBDCP. The trial was conducted in Kuarmunda and Bisra blocks of Sundargarh district. The study area comprised 13 villages which were randomized into three clusters and designated as treated net cluster (4 villages 2069 pop.), untreated net cluster (4 villages 1859 pop.) and no net cluster (5 villages 1863 pop.). Baseline pre-intervention data were collected from December 2006 to February 2007 followed by three months of intervention phase from March to May 2007. Baseline studies showed that both the vector species *An. culicifacies* and *An. fluviatilis* were 100% susceptible to deltamethrin. Bioassays conducted on treated HDPE nets after 4th washing resulted in reduced mortality of 35 and 57.5% in *An. culicifacies* and *An. fluviatilis* respectively. The median knock-down time for these species during the first month of intervention (March, 2007) was 4.30 and 4.25 min respectively and after 3 months of use, the median knock down time for these vector species was 4.55 and 4.30 respectively. In treated HDPE net area, there was a significant reduction of 83 and 89% in the entry rate of *An. culicifacies,* and *An. fluviatilis* with an over all reduction of 51.0% in total mosquitoes in comparison to pre-intervention phase ($p<0.05$). Floor sheet collections in houses with treated net indicated 29% immediate mortality in total mosquitoes. The overall feeding success rate of mosquitoes in the trial village was only 17.7% in comparison to 35.7 and 76% in villages with untreated nets and no nets respectively. A significant reduction of 87 and 100% was recorded in parity rate and human blood index of vector species in deltamethrin treated HDPE net area respectively. During intervention phase, there was a reduction of 63 and 50% in malaria incidence in treated net and untreated net areas respectively. However, no significant reduction was recorded in control villages without nets ($p>0.05$). The community acceptance of HDPE nets was high as there was 85–99% compliance rate of net usage in the study population during different months. The treated HDPE nets provided relief not only from mosquitoes but also from other household pests. The HDPE nets were found to be safe for human and no adverse events were reported either by the users or net impregnators.

ITN Trials in Madhya Pradesh

Simultaneously during the years 1989–91 another trial conducted in some tribal villages of Mandla district in Madhya Pradesh, where *An. culicifacies* and *An. fluviatilis* are malaria vectors, showed a low impact of treated nets. The reasons for low impact were poor compliance by inhabitants due to their varied socioeconomic status and means of livelihood. Later, another trial in the villages of Jabalpur district, however, showed a marginally better efficacy of cyfluthrin-treated nets over routine or supervised spraying of DDT indoors.

Comparative Efficacy of ITN and IRS in Karnataka

A study was carried out in PHC Kanakatte, District Hassan, Karnataka during the years 1995–2001. During the year 1995, baseline data were collected. Cyfluthrin impregnated mosquito nets @30 mg/m^2 were distributed to inhabitants in seven villages in 1996. Compliance for the usage of bednets was ensured by imparting health education.

First impregnation was made by villagers under NIMR supervision in March 1996 and subsequent re-impregnations were made in November 1996,

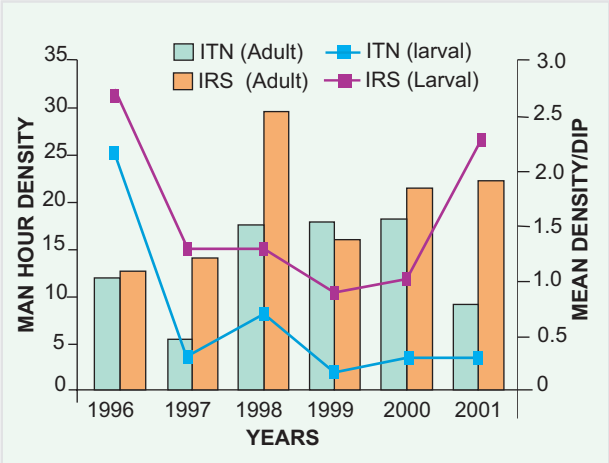


Fig. 34: Comparative efficacy of ITN and IRS with cyfluthrin on adult and larval densities

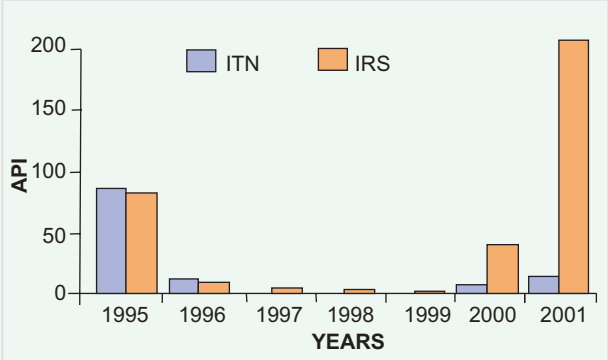


Fig. 35: Comparative efficacy of ITN and IRS with cyfluthrin on malaria incidence

May 1997 and December 1997 respectively. Entomological evaluations were done by NIMR at regular intervals during the intervention period. Malaria surveillance was carried out by the State Health Department. PHC, Belagur, District Chitradurga, Karnataka was under IRS with cyfluthrin. Comparative efficacy of ITN and IRS areas was studied for these two areas.

It was observed that adult and larval densities of *An. culicifacies* (vector mosquito) were low in areas with intervention of ITN (Fig. 34). The results of the five year study indicated substantial decrease in malaria incidence in ITN area as compared to area with IRS (Fig. 35). During the years 1996–98, in both bednet and IRS areas there was decrease in malaria incidence compared to 1995. It was observed that during the years 1999–2001, the malaria incidence in IRS areas in the absence of indoor spray increased substantially, while in ITN areas the incidence remained low even with the use of bednets without further re-impregnation after 1998.

ITN Trials in Ghaziabad

The relative efficacy of insecticide-treated mosquito nets was evaluated under field conditions in Dehra village of Dhaulana PHC, District Ghaziabad (Uttar Pradesh), India during 1996. Nylon nets were impregnated with deltamethrin, cyfluthrin, lambda-cyhalothrin and etofenprox at 25 mg/m^2 by standard methods. Repellency and excito-repellency, killing and airborne actions were monitored from dusk-to-dawn by hourly collection of mosquitoes that entered and rested in rooms and also females that landed on treated and untreated mosquito nets. Results revealed 15.3–22.9% repellent action, 98.3–99.3% excito-repellency action and 100% mortality of females that landed on treated fabrics (Ansari and Razdan 2000). No significant differences were observed in the efficacy of different synthetic pyrethroids against anophelines. However, against *Cx. quinquefasciatus* Say there was a significant difference between deltamethrin and etofenprox. Control of anophelines was more pronounced than *Cx. quinquefasciatus*. There was no pronounced airborne action with any insecticide tested. Synthetic pyrethroids with strong airborne action may be more

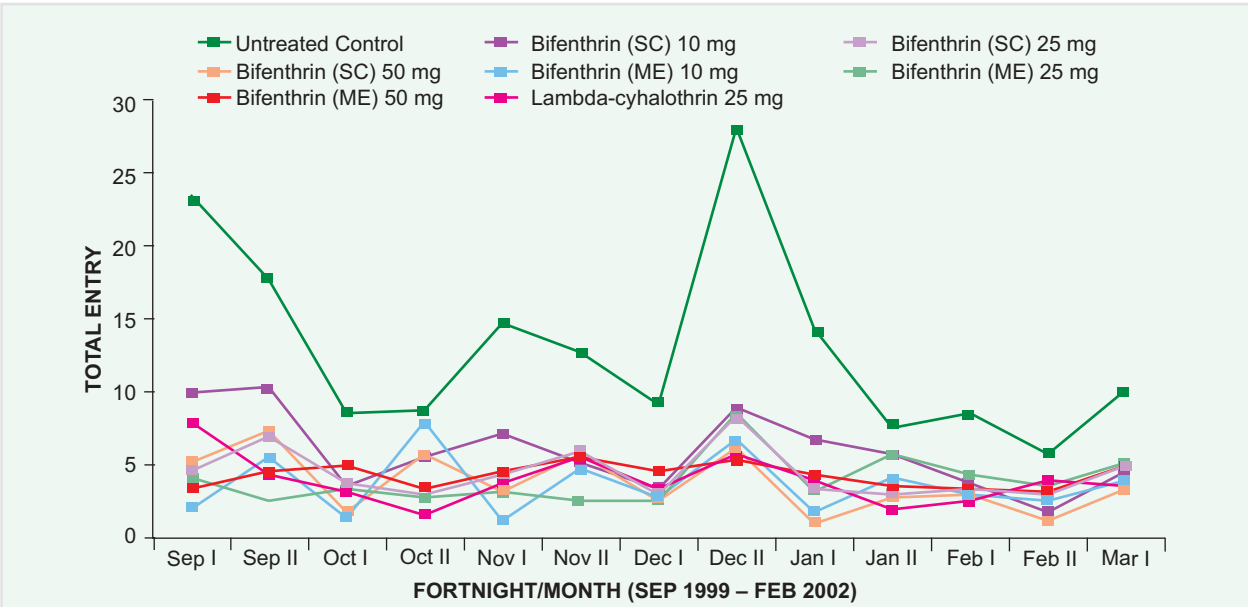


Fig. 36: Total entry of mosquitoes into the habitats in different months during the intervention period

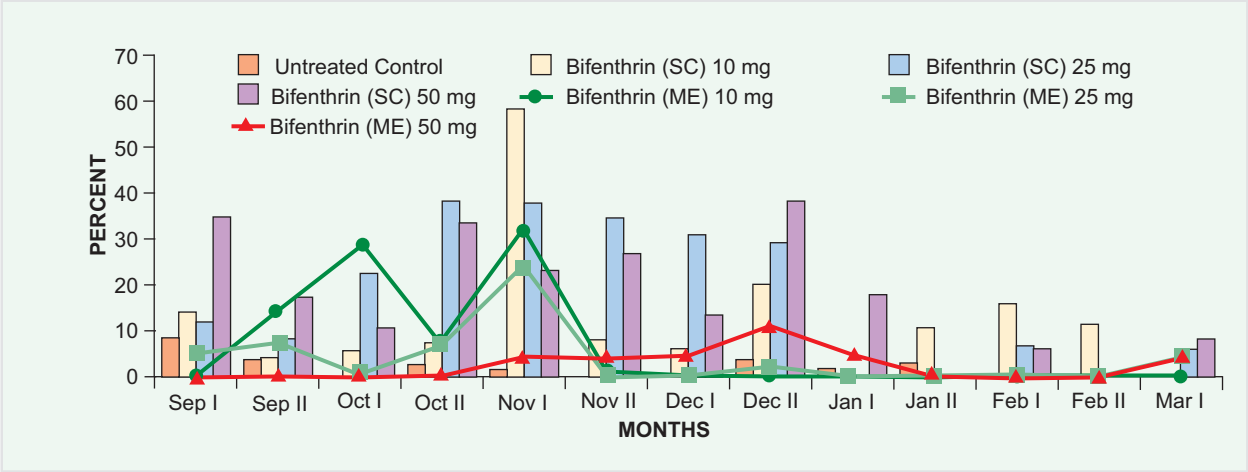


Fig. 37: Excito-repellency rates in the mosquitoes with different doses and formulations of bifenthrin

appropriate for impregnation of mosquito nets.

ITN Trials in Haryana

A field trial was carried out in the year 2000 in villages of District Sonepat, Haryana to assess the efficacy of bifenthrin as an impregnant on mosquito nets. Phase II trial was conducted to assess the comparative efficacy between two formulations, namely suspension concentrate and micro-emulsion. Efficacy was studied for both the formulations at three doses of impregnation, 10, 25 and 50 mg/m^2. Net bioassays to assess the persistence indicated bioavailability up to 11 fortnights (~ 5 months) at all the doses of the two formulations. There was an estimated 50% reduction in the entry of the mosquitoes into the houses with intervention of treated nets compared to the houses without treated nets indicating the impact of the insecticide (Fig. 36). The excito-repellency rates in the mosquito were low in houses with nets impregnated with ME formulations and was marginally higher than the houses with non-impregnated nets. These results indicated increased human-mosquito contact in intervention with ME formulations (Fig. 37). Uniform bio-availability of the insecticide on nets was observed with all the doses of the two formulations including the ME formulation and in view of the increased excito-repellency rates with this formulation, Bifenthrin ME formulation was suggested for impregnation @ 10 mg/m^2. for future use.

Efficacy of Water Dispersible Deltamethrin Tablets for Treatment of Individual Nets of Different Fabrics

Deltamethrin water dispersible tablets (K-O TAB®) offer operational advantages and ease in use over the liquid formulations of pyrethroids. K-O Tab were evaluated for bio-efficacy and persistence on different fabrics, namely cotton, nylon and polyester nets against malaria vectors *An. culicifacies* and *An. stephensi* in Kheda, Gujarat during 2001–02. Mosquito nets treated @ 25 mg/m^2 were distributed in three villages, during June and July 2001. Both treated and untreated nets were distributed in three villages. A set of 4 nets (2 each treated and untreated) of each fabric was kept in the laboratory for evaluation of shelf life of treated nets. Cone bioassays were carried out at monthly interval using laboratory reared *An. culicifacies* and *An. stephensi* by exposing to the treated surface for 3 minutes. Impact of washing frequency on the efficacy and persistence of insecticide on different types of fabrics, at first wash at one-month and second wash at three months interval was studied.

In cone bioassays on cotton and polyester nets, distributed for field use indicated more tolerance in *An. stephensi* as compared to *An. culicifacies*. Bioassays on unwashed field used cotton, nylon and polyester nets registered 100% mortality in *An. culicifacies* up to four months after the treatment (Fig. 38) and in *An. stephensi* mortality ranged from 90–100% till that period (Fig. 39). Bioassays on unwashed cotton, nylon and polyester nets, five months after the treatment, produced 75, 91.7 and 83.3% mortality in *An. stephensi* and 83.9, 83.3 and 100% mortality in *An. culicifacies,* respectively.

Bioassays of *An. stephensi* on cotton nets washed once and twice produced 40 and 55% mortality respectively as against 75% mortality on unwashed nets after five months of treatment. Similarly, mortalities on washed polyester nets were

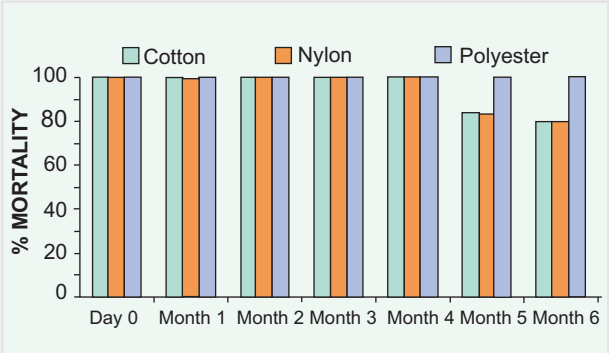


Fig. 38: Bioassay of K-O TAB treated nets on *An. culicifacies*

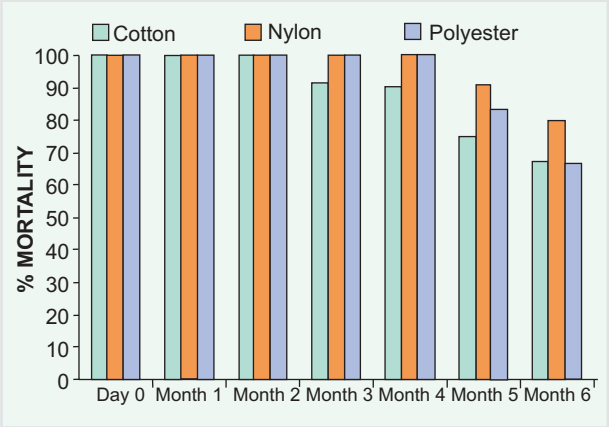


Fig. 39: Bioassays of K-O TAB treated nets on *An. stephensi*

70% (1 wash) and 66.7% (2 washes) as against 83.3% on unwashed nets. Contrary to this, bioassays on nylon nets washed once and twice produced less mortality in *An. stephensi* (93.3 and 86.7% respectively) than in *An. culicifacies* (73.3 and 76.7% respectively). Washing had no significant impact on efficacy against *An. culicifacies* exposed to cotton nets; washed once (81.7%) and twice (80%), and to polyester nets washed once (100%) and twice (75%). Thus K-O Tab would help ensure a better community acceptability and sustainability.

Evaluation of Operational Programme of ITNs

In 2003, NVBDCP initiated a pilot project on insecticidal treatment of community owned nets in 15 malaria endemic districts in 11 States. The project used public-private partnership where insecticide manufacturers would make available commercial packs of insecticide and community would pay for the cost of insecticide. Certain NGOs were involved for social mobilization towards promotion of use of bed nets and their treatment with insecticide. IEC activities were carried out in all the areas to promote the use and acceptance of ITNs. A survey in these 15 districts indicated presence of large number of mosquito nets owned by community, of which a great majority were treated under the pilot project. The project has been relatively more successful in the north-eastern states where communities use nets traditionally. In Darrang 37% of the available nets, in Karbi-anglong 45% nets and in West Aizwal 46% of the targeted number of nets could be treated. In Anand district, Gujarat state, 25% of 55,000 nets in 1.9 million population could be treated. The manufacturer had made the deltamethrin flow available at the district level. Communities were found willing to procure insecticide from the health authorities. Training of community volunteers and health workers has helped in capacity building for treatment of bed nets on an ongoing basis. Household surveys conducted by NIMR in districts of Anand in Gujarat, Betul in Madhya Pradesh, Ranchi and East Singhbhum in Jharkhand, Kanker in Chhattisgarh and Keonjhar in Orissa reported that the ITN programme was largely successful in areas where proper IEC and training motivated the local communities before initiating it (Yadav and Bhatt 2004; Batra *et al* 2004a,b; Bhatt and Gupta 2004). Where communities were not well informed, the programme couldn't take off, such as in Betul. In Anand, nearly 40% children under 5 years had access to a net/ITN (Yadav and Bhatt 2004).

Sustainability of ITNs

The efficacy of ITNs against malaria transmitted by local mosquito vectors in many areas has been proved in field by NIMR, but the question of sustainability needs to be addressed (Yadav and Sharma 1997). A field project in collaboration with British Council Division (U.K.) and M/s. CARE India was undertaken in Keonjhar, Orissa in 1994 (Yadav 1997). The project showed that it was possible to promote purchase and use of treated nets by community financing (sale of nets). The National Anti Malaria Programme adopted the ITN-technology and distributed ITNs in north-eastern states. These are now being promoted through the state antimalaria programmes of different states and this constituted a major advancement in vector and malaria control. Also, in National Anti Malaria Programme's Enhanced Malaria Control Project being implemented in 100 districts in eight states, sustainability of ITNs was evaluated.

In considering sustainability, distinction needs to be made between programme sustainability from the managers' point of view, which is restricted to the programme cycle and overall sustainability of ITNs as a component of the antimalaria strategy. While sustainability is not easy to define or measure, three main indicators that could be used are: proportion of population using or willing to use ITNs; proportion of ITNs actually retreated; and decreased reliance on single-source external intervention and shift towards a greater community ownership/participation. Thus, sustainability of an ITN programme will depend upon various factors such as the acceptability, feasibility, affordability, technical support requirements, production, sale and distribution systems of nets.

Relative Efficacy of Insecticide-treated Curtains (ITCs) against Mosquitoes under Laboratory Conditions

The relative efficacy of pyrethroid-impregnated fabrics was evaluated against *An. stephensi*, *Aedes aegypti* and *Cx. quinquefasciatus* under laboratory conditions (Ansari *et al* 1998). Results revealed that deltamethrin (D) was significantly superior in comparison to lambda-cyhalothrin (L) and cyfluthrin (C). Results of bioassay tests and relative toxicity index (RTI) revealed that deltamethrin was 1.5 and 1.9 times more effective than lambda-cyhalothrin and cyfluthrin, respectively, against *An. stephensi* exposed to cotton fabric treated at 100 g/m^2. Similarly, deltamethrin was 3.9 and 4.6 times more effective

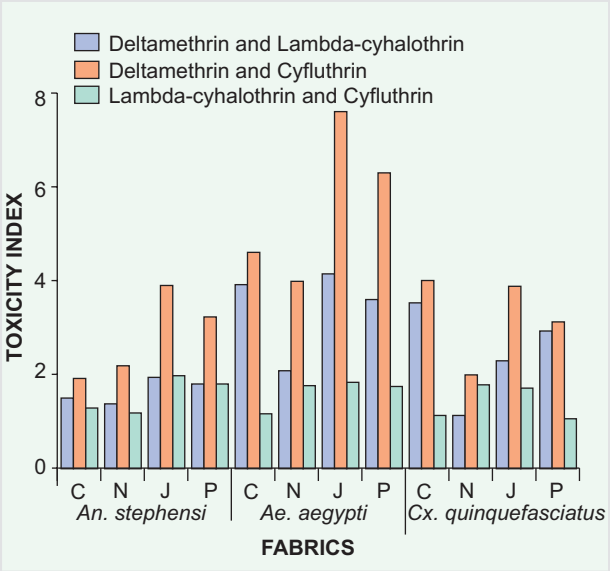


Fig. 40: Relative toxicity indices on different fabrics (C: Cotton, N: Nylon; J: Jute; and P: Polyethylene)

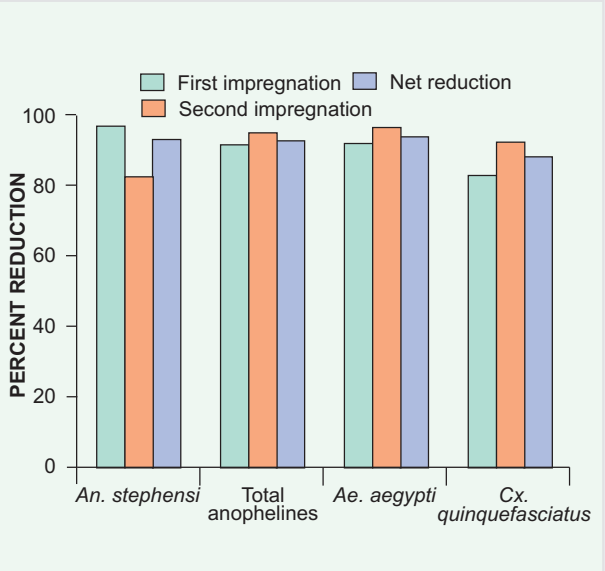


Fig. 41: Mean reduction of adult densities of mosquitoes in deltamethrin-treated curtain area, Moti Bagh, New Delhi

than lambda-cyhalothrin and cyfluthrin respectively against *Ae. aegypti* and 3.5 and 4.0 times more effective against *Cx. quinquefasciatus* respectively. Of the four fabrics—cotton, nylon, polyethylene and jute, cotton was the best on the basis of median lethal dose (LD_{50}) and 90% lethal dose (LD_{90}) values and persistence of insecticide (Fig. 40).

ITC Trials in Delhi

A field trial in an area in New Delhi Municipal Committee to demonstrate composite control of *An. stephensi* and *Ae. aegypti* by spraying deltamethrin at 100 mg/m^2 on window and door curtains of habitations showed 88–93% reduction of the vector species in the experimental area (Fig. 41) (Ansari and Razdan 2001). The impact of deltamethrin-treated curtains was also evident against non-target species (67.9–85.7%; $p<0.05$). Treated curtains provided 100% kill of *Anopheles stephensi* and *Ae. aegypti* for 3–4 months, followed by a gradual decline in successive months. Use of deltamethrin-treated curtains resulted in 92% reduction in SPR and 95.4% reduction in malaria cases/1000 population. The cost of deltamethrin treatment was less than per house per annum. Insecticide-treated mosquito window and door curtains, along with legislative measures, may provide cost-effective concurrent control of mosquitoes and other domestic pests.

ITC Trials in District Ghaziabad (U.P.)

In selected villages of District Ghaziabad, operational feasibility and efficacy of hessian curtains impregnated with deltamethrin @ 100 mg/m^2 was evaluated in comparison to indoor residual spraying of HCH @ 0.2 g/m^2. The impregnation was carried out before the onset of transmission and observations were continued up to two transmission periods. District Health Authorities sprayed HCH indoors. There was 87% reduction of *An. culicifacies* up to six months and 61.6% reduction in total mosquitoes in comparison to HCH indoor residual spraying (Ansari and Razdan 2000). Follow-up studies revealed that the impact of deltamethrin impregnated curtains declined after 6–7 months. The results of bioassay tests revealed 100% mortality up to 6–7 months. Epidemiological evaluation revealed 81.9% reduction in total malaria cases as against 88.5% in *P. falciparum* cases. Similar reduction was also observed when slide positivity rate (SPR), slide falciparum rate (SFR), cases/1000 and *Pf*/1000 were compared to corresponding village. Pilot studies have indicated to evaluate the efficacy of impregnated curtains on a large-scale as these are relatively cheaper than the conventional vector control method—insecticide residual spraying.

❑

Long-lasting Insecticidal Nets and Materials

Insecticide treated nets (ITNs) have been proved efficacious in several parts of the world in reducing the mosquito densities and also reduction in malaria incidence. However, periodic re-impregnation, erratic dose of the insecticide, and other factors diluted the efficacy of ITNs in long-term. To overcome these problems, long-lasting insecticidal nets (LN) have been developed and are being promoted in many malaria endemic areas.

Long-lasting insecticidal net is *'A net treated at factory level with insecticide either incorporated into or coated around fibers resisting to multiple washes and whose biological activity last as long as the net itself (3–4 years for polyester nets, 4–5 years for polyethylene ones)'.*

The LN is the most technologically advanced form of treated net available today. LNs, which maintain efficacy without re-treatment for 4–5 years, represent an important innovation that could facilitate sustainable scale-up of malaria prevention. They are constructed of special synthetic fibers (polyester and polyethylene) that have been compounded with an insecticide. The net both kills and repels mosquitoes and it provides a physical barrier to them. Tests have proven that the presence of a LN or long-lasting net material hung as curtain, also discourages mosquitoes from remaining in the surroundings. A few brands of LNs are already in use in some countries. World Health Organization (WHO) has given full recommendation to Olyset® Net and interim recommendation to PermaNet® 2.0, Duranet®, Net Protect® and Interceptor®. K-O Tab 1-2-3® and ICON® MAXX (long-lasting insecticide treatment kits were also approved by WHO.

LN Trials carried out by NIMR

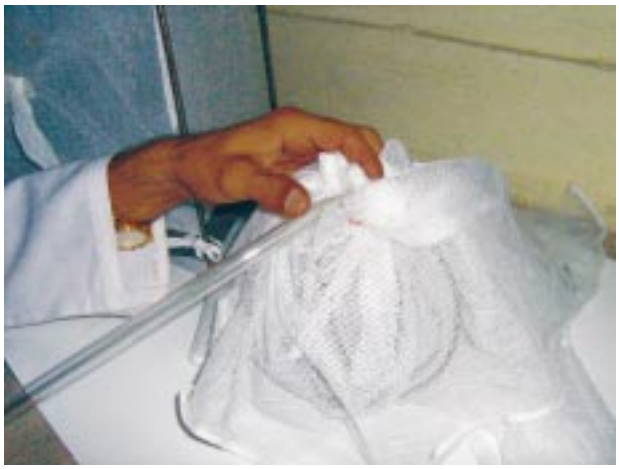
NIMR has carried out Phase II and Phase III trials of three long lasting insecticidal nets, namely Olyset net, PermaNet, Interceptor and K-O Tab1-2-3. Trials on few LNs are underway. The results of laboratory and field trials on the above mentioned LNs are presented below.

Olyset® Nets

Bio-efficacy and Wash Resistance

Results of cone bioassay tests for determining discriminatory exposure time and bio-efficacy revealed that Olyset Net produced 100% mortality in *An. culicifacies*, *An. subpictus*, *An. stephensi* within three minutes of exposure and after 24 hours holding. In *Ae. aegypti,* 100% mortality was observed at 5 min exposure, while in *Cx. quinquefasciatus* at 30 min exposure time and 24 hours holding. From the results 3 min was established as discriminatory exposure time for *An. culicifacies*, *An. stephensi* and *An. subpictus;* 5 min for *Ae. aegypti* and 30 min for *Cx. quinquefasciatus.* Cone bioassay tests on washed Olyset Nets at an interval of 24 hours up to 20 washes showed that washing did not dilute the efficacy of Olyset net as evidenced by the fact that 100% mortality was observed in *An. culicifacies* even

Cone bioassay on LN



Ring net bioassay on LN

after 20 washes, whereas in *Cx. quinquefasciatus* 100% mortality was observed only up to 12 washes. The results clearly shows that Olyset® Net is highly effective in killing anopheline mosquitoes even after repeated washings, which did not dilute the efficacy (Ansari *et al* 2006).

Bio-efficacy and wash resistance studies in Orissa on *An. culicifacies* and *An. fluviatilis* showed 100% mortality in *An. culicifacies* up to 11 washings, whereas 100% mortality was observed in *An. fluviatilis* even after 20 washings. The median knock down time for these species ranged between 4.55 – 6.00 min and 4.45 and 5.45 min respectively against *An. culicifacies* and *An. fluviatilis*during one year of intervention (Sharma *et al* 2007). Bio-efficacy and wash resistance studies in Assam indicated that the bio-availability of insecticide on Olyset net fibre was consistent (100% kill effect) up to 10 months of monitoring, and nets were observed to be wash-resistant even after 20th wash at fortnightly intervals.

Phase II Evaluation of Olyset Nets

Beel Akbarpur village located in the PHC Dadri, Distt. Ghaziabad, Uttar Pradesh, India was selected for the field trial. Indoor resting density of of *An. culicifacies* mosquitoes (MHD) in Olyset Net, untreated net and without net structures was 53.75 ± 11.2, 59 ± 20.6 and 55.5 ±23.1, respectively (Fig. 42). However, the density of *An. culicifacies* was reduced drastically in Olyset Net used structures. The percent reductions in *An. culicifacies* density in the Olyset Net used structures when compared with structures, where nets were not used during pre- and post-experiment were 94 and 95.2% based on density in untreated net and without net structures respectively. In case of *Cx. quinquefasciatus* and total mosquitoes the percent reduction observed over untreated net and without net structures were 47.2 and 54.1, and 50 and 57.7 respectively.

It was observed that landing rate on Olyset Net was drastically reduced. However, 100% mortality was observed in those mosquitoes which landed on the Olyset Net. Mortality was also observed in indoor resting collected mosquitoes within 24 hours, which were exposed to the net for few seconds and settled to rest on walls. The average mortality was 50.8% in *An. culicifacies*, 41.5% in total anophelines, 33.6% in *Cx. quinquefasciatus* and 44.8% in total mosquitoes. This clearly emphasizes that the Olyset® Net not only kills the landed mosquitoes but also kills the mosquitoes which enters in to the room having Olyset Net and are accidentally exposed to the net.

The results also revealed that Olyset net produced strong repellent action. Repellent action of Olyset Net was more pronounced in *An. culicifacies* as compared to total anophelines and *Cx. quinquefasciatus*. The repellent action of the Olyset Net was 55.2% in *An. culicifacies* as against 38.6% in *Cx. quinquefasciatus*. Results also revealed that excito repellency action (ERA) was almost 100% against all the mosquito species over a period of six months (Ansari *et al* 2006)

Phase III Evaluation of Olyset Nets

Large-scale field evaluation of Olyset nets to study the entomological and epidemiological impact was undertaken in Uttar Pradesh, Orissa and Assam states. The results of these trials are furnished below.

Uttar Pradesh

A village-scale trial was undertaken from August 2003 to August 2007 in Khandera, Beel Akbarpur and Anandpur villages in Dadri PHC, Distt. Gautam Budh Nagar, Uttar Pradesh, India. Olyset nets were distributed in Khandera village, untreated nets in Beel Akbarpur and Anandpur village was kept as control, where nets were not used. Results of entomological evaluation revealed a marked difference in the indoor

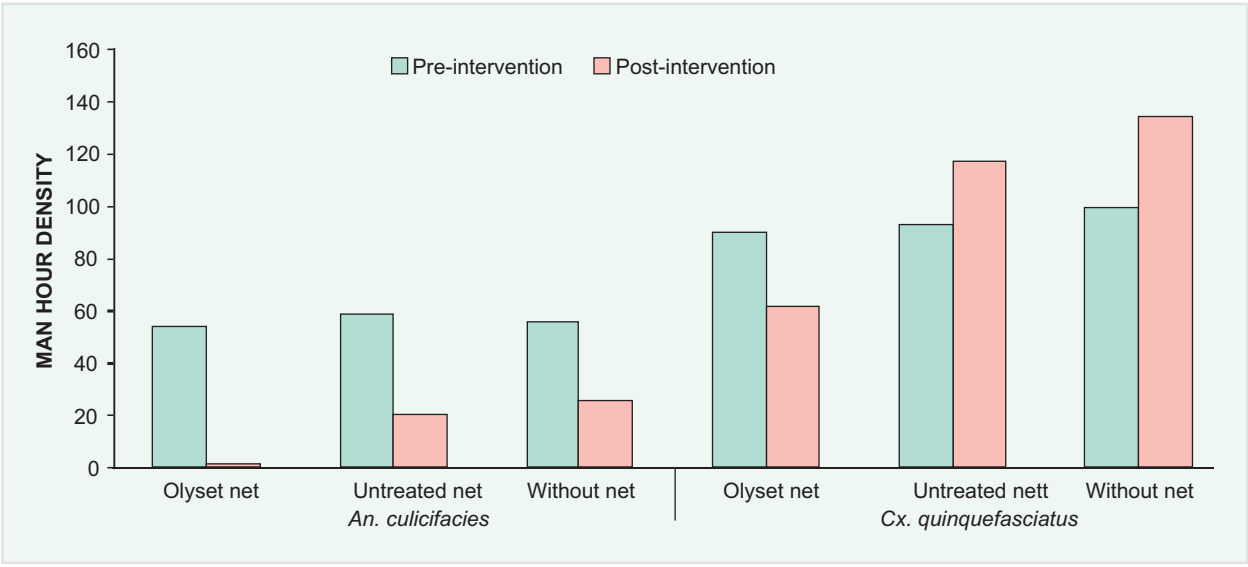


Fig. 42: Mean man hour density of *An. culicifacies* and *Cx. quinquefasciatus* in structures with Olyset nets, untreated nets and without nets

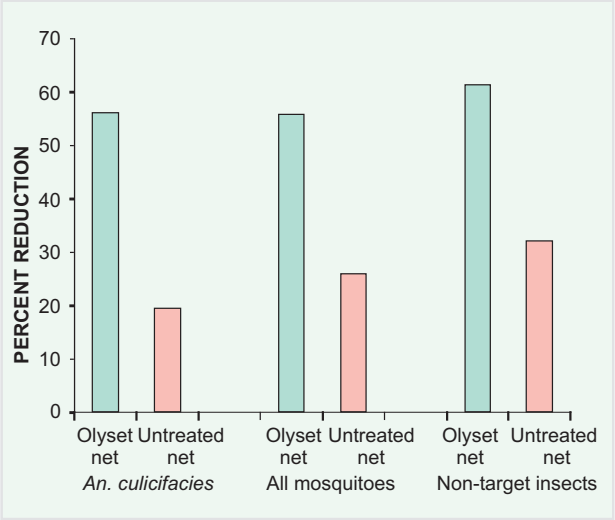


Fig. 43: Percent reduction in the density of *An. culicifacies,* all other mosquitoes and non-target insects in Olyset or untreated net villages over no net village (from August 2004 to July 2006)

resting density of the major malaria vector *An. culicifacies* in the Olyset-net village, when compared to the untreated net and without net villages (Fig. 43). The use of Olyset nets not only reduced the indoor resting density of *An. culicifacies* but also reduced mosquito entry into the Olyset net used structures.

There was no landing on the Olyset net resulting complete protection of users. Results of epidemiological evaluation in the three villages revealed that parasite incidence (Cases per thousand population) in village with Olyset net during 2003–04 (pre intervention year) was 39.5,which declined to 0.12, 0 and 2.5 during 2004–05, 2005–06 and 2006–07 in the post intervention years respectively. In the untreated net and without net villages, the cases/1000 during 2003–04 (pre-intervention) was 44 and 19 and as compared to 6.1, 3.8 and 8.8 during 2004–05, 2005–06 and 2006–07 in the untreated net village and 19.5,11.5 and 10 in without-net village (Fig. 44). Similar trend in Pf/1000 in the three villages was also observed. Random checking for the usage pattern of Olyset net during the peak transmission season revealed that more than 80% people are still using the Olyset nets even after three years of distribution of these nets. Results of the present study revealed that Olyset nets are highly effective in reducing the indoor resting density of *An. culicifacies*, man-vector contact and malaria incidence even after three years of use in field. Inhabitants expressed overwhelming response to Olyset nets and they did not report any adverse effects due to the use of Olyset nets. Follow-up trials on Olyset nets are still in progress.

Rourkela ,Orissa

A village scale-trial was conducted in Bisra block of Sundargarh district. The study area comprised 22 villages which were randomized into three clusters and designated as Olyset net cluster (8 villages, 1953 pop.), untreated net cluster (9 villages, 2019 pop.) and no net cluster (5 villages, 1863 pop.). Baseline pre-intervention data were collected from November 2005 to January 2006 followed by one year of intervention phase from February 2006 to January 2007. In the Olyset net study area, there was a significant reduction of 80.6, 94.1 and 76.7% in the entry rate of *An. culicifacies, An. fluviatilis,* other anopheline species respectively with an over all reduction of 63.5% in total mosquitoes. Floor sheet collections in houses with Olyset net indicated 39% immediate mortality in total mosquitoes. The person-hour density of *An. culicifacies* and *An. fluviatilis* in houses with Olyset net, untreated net and no net are shown in Figs. 45 and 46 respectively.

The overall feeding success rate of mosquitoes in the trial village was only 18% in comparison to 44.2 and 79.1% in villages with untreated nets and no nets respectively. A significant reduction was also recorded in parity rate and human blood index of vector species in Olyset net area. There was a significant reduction of 65–70% in malaria incidence and 46% in malaria prevalence (parasite rate) in Olyset net area. The attack rate of *P. falciparum* in different age groups during intervention phase also showed significant reduction in Olyset net area as

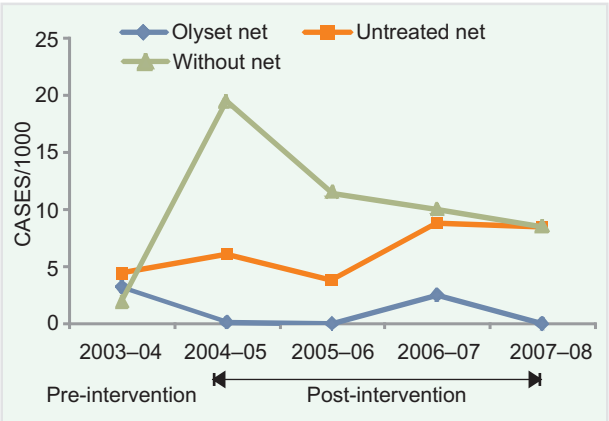


Fig. 44: Malaria cases/1000 population in Olyset, untreated and without net villages before and after intervention periods

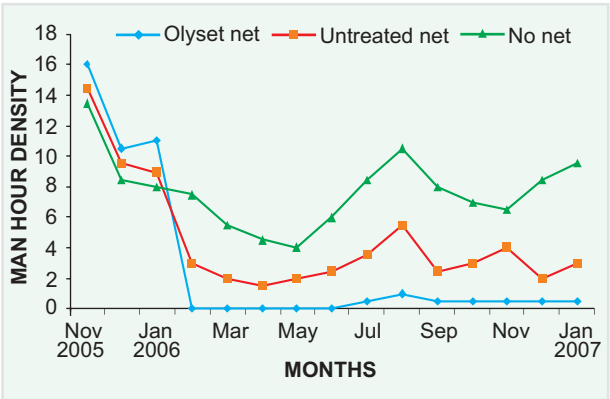


Fig. 45: Density of *An. culicifacies* in houses with Olyset net, untreated net and without net during pre-intervention (Nov 2005 to Jan 2006) and post-intervention (Feb 2006 to Jan 2007) in Orissa

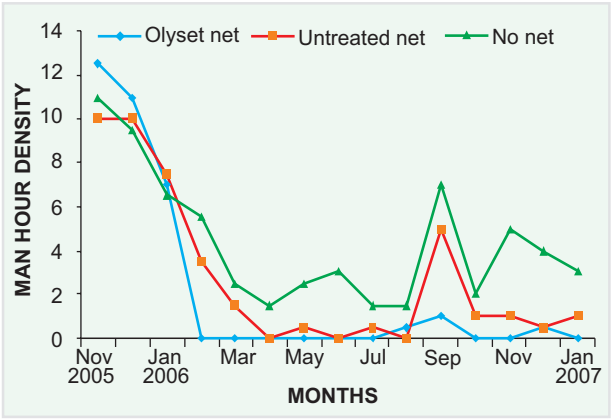


Fig. 46: Density of *An. fluviatilis* in houses with Olyset net, untreated net and without net during pre-intervention (Nov 2005 to Jan 2006) and post-intervention (Feb 2006 to Jan 2007) periods in Orissa

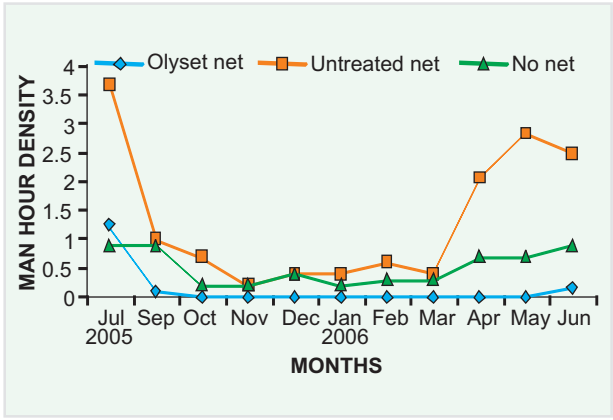


Fig. 48: Relative abundance of *An. minimus* in experimental villages of Sonapur PHC, Assam, India subject to introduction of Olyset® nets in August 2005

compared to untreated net and no net areas (Fig. 47).

The community acceptance of Olyset nets was high as there was 80–98% compliance rate of net usage in the study population during different months. The use rate of untreated nets in control villages was between 70 and 90% in different months.

Assam

A village-scale trial was conducted in Kamrup district of Assam from July 2005 – June 2006. It was observed that there was virtual disappearance of *An. minimus*, the mosquito vector species (Fig. 48) corroborated by data on mosquito-landing rates in Olyset net villages. A consistent decline in malaria incidence was observed in Olyset net villages, and overall impact on malaria transmission was highly pronounced and significant when compared to untreated net intervention and no net villages for the corresponding study periods (Fig. 49). The Olyset nets were safe to use for which community compliance and acceptance was high, and are operationally feasible community-based intervention for sustainable management of disease vectors.

PermaNets

PermaNet® 2.0 is manufactured by M/s. Vestergaard Frandsen (India) Pvt. Ltd. The net is coated with Deltamethrin @ 55 mg a.i./m^2 on a polyester fabric with100 denier fiber with Warp-Knetheo weave type and 156 het/sq inch mesh size.

Wash Resistance and Bio-efficacy

Results of cone bio-assay tests revealed that PermaNets produced 100% mortality in *An. culicifacies*, *An. fluviatilis*, *An. stephensi*, *An. minimus*, *An. philippinesis* and *Ae. aegypti* within three min of exposure and after 24 hours recovery period. In *Cx. quinquefasciatus,* 100% mortality was observed at 30 min exposure. The results clearly show that PermaNets are highly effective in producing 100% mortality in *Anopheles* mosquitoes in a short time of exposure.

Results of ringnet bioassays on unwashed and washed PermaNets showed median knock-down time (KT_{50}) of *An. culicifacies* (Uttar Pradesh) on unwashed PermaNet was 6.5 min and it progressively increased with the number washes up to 7 min after

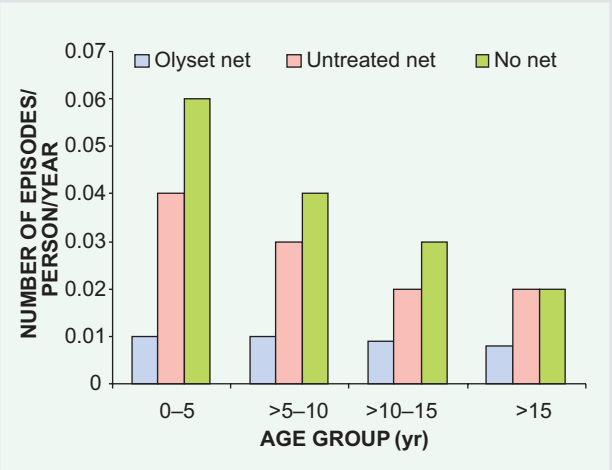


Fig. 47: Attack rate of *P. falciparum* in different age groups using Olyset net, untreated net and no net

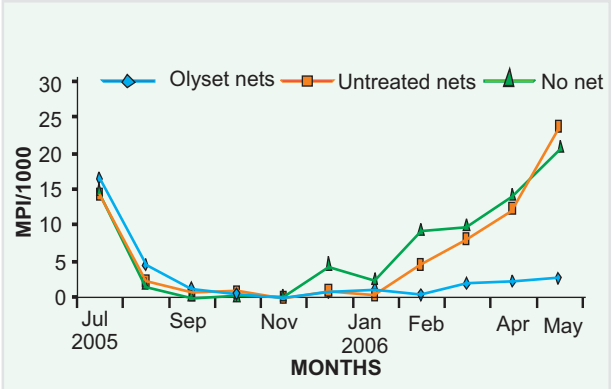
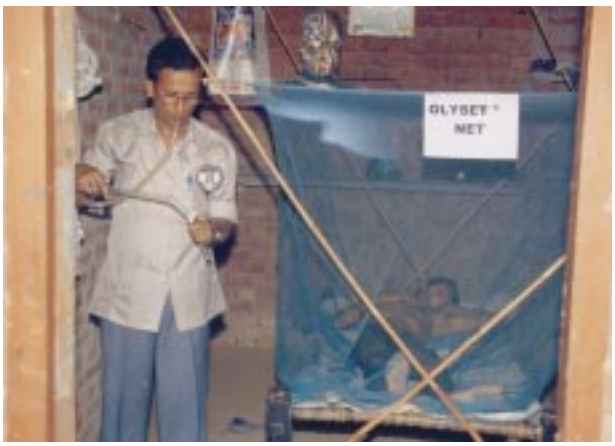


Fig. 49: Malaria transmission trends in experimental villages of Sonapur PHC, Kamrup district, Assam, India during pre-intervention (July 2005), and post-intervention months beginning September 2005 (Olyset nets were introduced in August 2005)



Hand catch collections in LN used houses

20 washes. The KT_{50} of *An. culicifacies* (Orissa) was 11 minutes on unwashed PermaNet. Similarly, KT_{50} of *An. stephensi* was 8 min on unwashed PermaNet and after 20 washes, it was 16 min. The KT_{50} of *An. fluviatilis* was 8 min on unwashed PermaNet. The results showed high efficacy of PermaNet against both the species as evidenced by the fact that even after 20 washes the mortality in both the species remained >80%. Further, there was no significant difference between the mortalities of the two species ($p>0.05$). In ring net bioassays the baseline median knock-down time against *An. culicifacies* is 393 sec. However, after progressive washings the median time for knockdown (MTKD) also increased. The MTKD of *An. culicifacies* was less than that of *An. stephensi* and the difference was statistically significant ($p<0.05$) (Sreehari *et al* 2007).

Uttar Pradesh

Phase II Evaluation

A small-scale field trail was conducted from April 2005 to November 2005 to study the efficacy of PermaNets. Three villages, namely Nawada, Harampur and Durgawali in Loni PHC, District Ghaziabad, Uttar Pradesh, India. Depending on the prevalence of vector species PermaNets were distributed in Nawada village, untreated nets in Durgawali village and Harampur village was kept as control, where nets were not used. Nets were distributed to all the inhabitants of the two villages on 1 July 2005. The results revealed that the MHD of *An. culicifacies* and *An. stephensi* the major malaria vectors in the structures selected for PermaNet, untreated net and without net during pre distribution period was 27 and 53, 22 and 32 and, 20 and 22 respectively. After the distribution of nets the densities reduced gradually in PermaNet village. Though there was also a reduction in the village using untreated nets, the impact was less pronounced than that observed in the PermaNet village (Fig. 50).

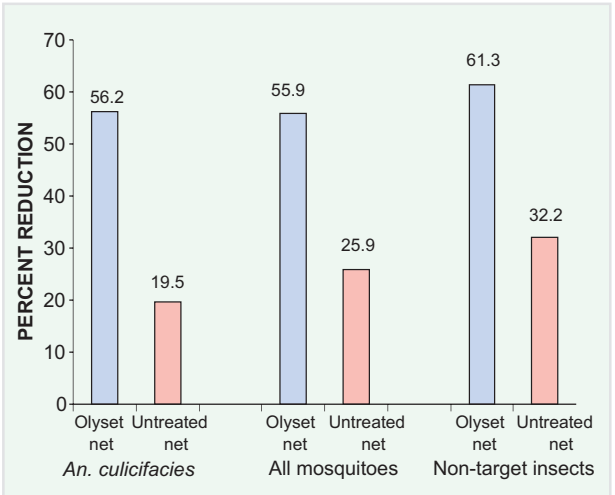


Fig. 50: Percent reduction in per man hour density of *An. culicifacies, An. stephensi* and all other mosquitoes (collected by hand catch) in PermaNet or untreated net villages over no net village (from July to November 2005)

Repellent action and excito repellent action of PermaNet against different mosquito species were calculated based on untreated nets. Results revealed that PermaNet showed high excito repellent activity (82–97%) against *An. culicifacies*, 77–97% against *An. stephensi*. Killing action of PermaNet calculated over untreated net revealed that PermaNet has high killing activities as evidenced by the fact that all the landed mosquitoes on the net died after 24 hours of observation. About 20 to 40% mortality was observed in mosquitoes entered the room and accidentally exposed to the net and rested on the walls. The results clearly demonstrate that PermaNet showed high efficacy in producing mortality in the mosquitoes, which are exposed to the net.

Phase III Evaluation of PermaNet

Village-scale trials were conducted with Permanets in Uttar Pradesh and Orissa states to study the efficacy of these nets on prevailing disease vectors in respective states and also on malaria incidence. Results of these trials are furnished below separately in different states.

Uttar Pradesh

Phase III field evaluation of Permanet 2.0 was carried out in District Gautam Budh Nagar, in western Uttar Pradesh in April 2007.Three villages with similar malaria endemicity, topography and mosquito prevalence in District Gautam Budh Nagar in Uttar Pradesh, where malaria is transmitted mainly by *An. culicifacies* and *An. stephensi.* Entomological evaluations revealed that the man hour density (MHD) of *An. culicifacies* during pre-intervention period in PermaNet, untreated net and no net villages was in the range of 10–13, 24–27 and 8–12 respectively. With the commencement of intervention, there was sharp decline in density of *An. culicifacies* in June 2007, whereas the density in the untreated and no-net villages did not decline in June 2007. However, there was an increase in the resting density of *An. culicifacies* in all the villages during the

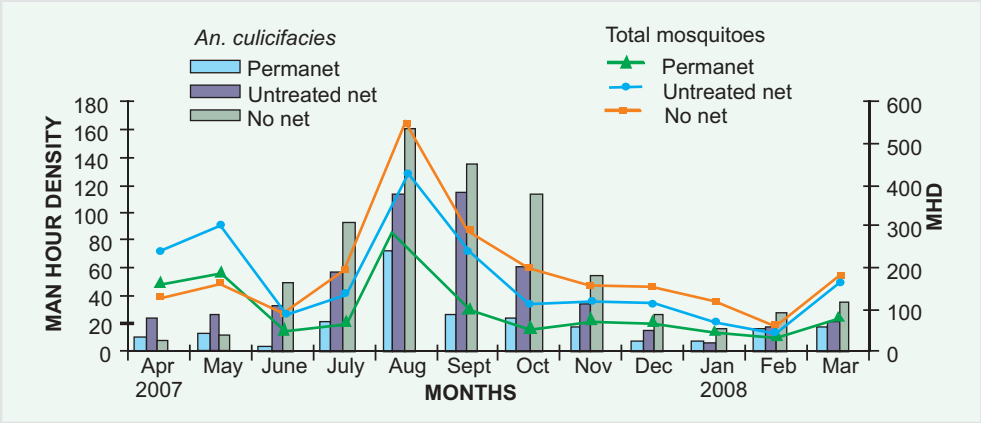


Fig. 51: Impact of PermaNet on mosquito density in study villages in Distt. Gautam Budh Nagar, Uttar Pradesh during April 2007–March 2008

monsoon and post-monsoon period of August to November 2007, but the build-up of *An. culicifacies* density was much higher in the control villages as compared to PermaNet village (Fig. 51). The parity rate of *An. culicifacies* was low in PermaNet village as compared to untreated net and no net villages. The parity rate of *An. culicifacies* in June 2007 in the first month during post-intervention period in PermaNet untreated net and no net villages was 20, 66.6 and 60% respectively.

Comparison of malaria incidence data showed that during pre-intervention period of April-May, 2007, the parasite index (PI) or number of cases per 1000 population in the treated net villages was 2.5 and in the control villages with untreated nets and no nets was 1.7 and 2.9 respectively. There was no significant difference ($p > 0.05$) in the malaria endemicity in the trial and control villages (Fig. 52). During intervention phase, the malaria incidence in the treated net villages was much less than the untreated net and without net villages (0.84, 5.19 and 13.46 respectively). There was 85–99% compliance rate of net usage in the study population during different months.

Mosquito landing catches of the LN

Orissa

Field evaluation of PermaNets was initiated during August 2007 in malaria endemic block of Gurundia in Sundargarh district. Density of malaria vector species *An. culicifacies* and *An. fluviatilis* was monitored in houses having PermaNets, untreated nets, and no nets in trial and two control villages respectively during pre-intervention period (August to October 2007) and intervention period (November

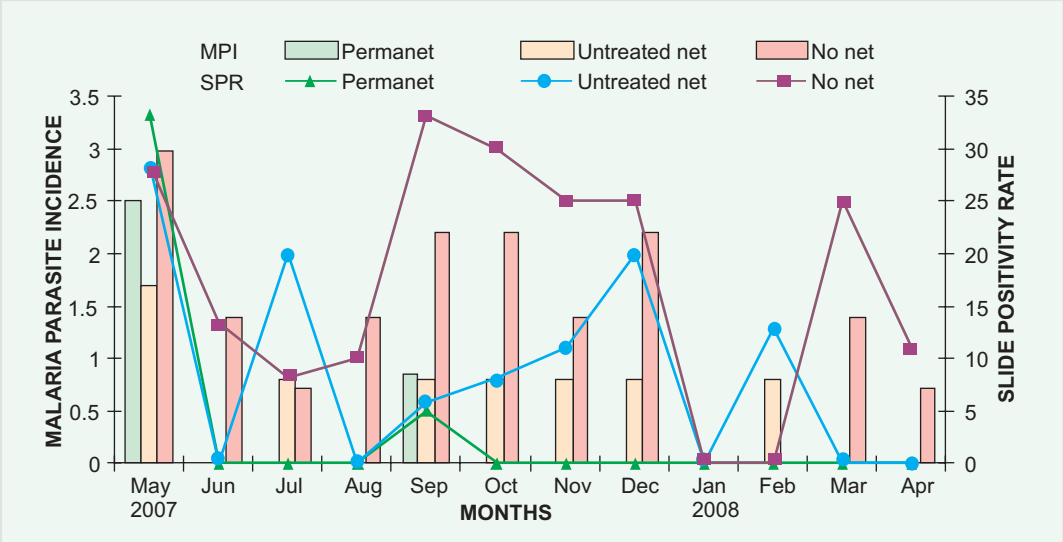


Fig. 52: Malaria incidence in PermaNet, untreated net ant no net villages during May 2007–April 2008 in Uttar Pradesh

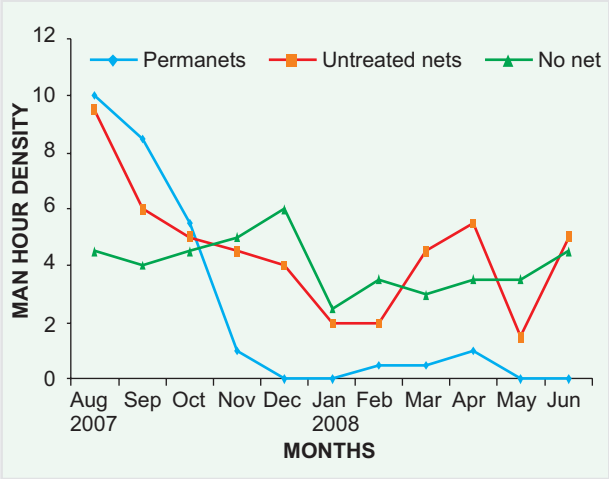


Fig. 53: Density of *An. culicifacies* in houses with PermaNets, untreated nets and without nets during pre-intervention (Aug to Oct 2007) and post-intervention (Nov 2007 to Jun 2008 phases in Orissa

2006 to June 2008). The MHD of *An. culicifacies* during pre-intervention period in trial villages, untreated net villages and no net area ranged from 8.5–10, 5–9.5 and 4–4.5 respectively. However, with the commencement of intervention during November, 2007, there was sharp decline in the density of *An. culicifacies* (Fig. 53) in PermaNets area (range: 0–1) as compared to areas with untreated nets (range: 1.5–5.5) and without net (range: 2.5–6). Similarly, density of *An. fluviatilis* during pre-intervention period in PermaNets, untreated net and no net area was ranging between 1–2, 0.5–2, and 2.0–11.5 respectively (Fig. 54). The impact of introduction of PermaNets in the trial villages on *An. fluviatilis* was so remarkable that the man hour density of this species came down to zero and remained so during most part of the intervention phase, whereas in control villages with untreated nets and no nets the density was in the range of 0.5–1.5 and 2–9 respectively during intervention period. The density of *An. culicifacies* in PermaNets area showed a significant reduction of 89% as compared to untreated net and no net area respectively. Similarly, a reduction of 100% was recorded in the density of *An. fluviatilis* in houses with PermaNets in comparison to houses with untreated nets and no net respectively.

The parasite rate or percent of individuals positive for malaria parasite in PermaNets, untreated net and no net population during pre-intervention was 6.8, 5.8 and 7 respectively and no significant difference was observed between all the thee clusters ($p > 0.05$). During intervention phase the parasite rate in PermaNets users had come down to 2.9, whereas in untreated net and no net users, the parasite rate was 3.4 and 15.1 respectively (Fig. 55). Comparison of parasite rate in PermaNets net villages during intervention with that of pre-intervention period showed that there was 57% reduction in malaria prevalence, which was statistically significant ($p<0.01$). Malaria prevalence in untreated net area also showed a significant reduction of 41.4% in comparison to pre-intervention phase, whereas there was an increase of 115.7% in no net villages.

Interceptor® Net

Wash Resistance and Bioefficacy

Interceptor nets have shown wash resistance up to observed 20 washes. Bioavailability of insecticide on net fibers was found effective against malaria vectors, *An. culicifacies* and *An. fluviatilis* in Orissa; *An. culicifacies* in Chhattisgarh; and *An. minimus* in Assam. Exposure of mosquitoes in Sundargarh district and Kamrup district registered 100% mortality in cone bioassays, while in Chhattisgarh, 100% mortality was registered in first 2 washes and in subsequent washes up to 20 times, the mortality

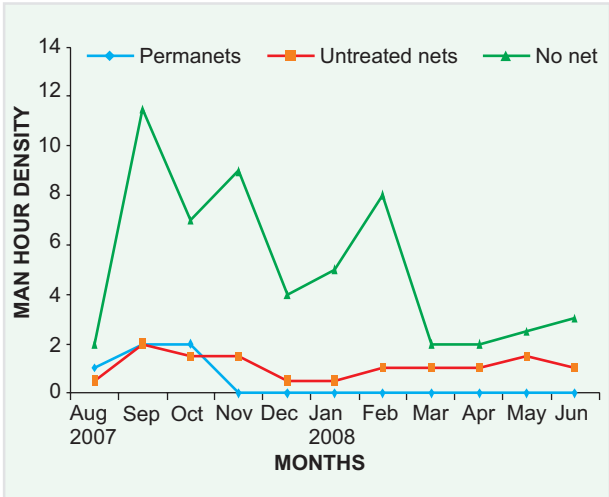


Fig. 54: Density of *An. fluviatilis* in houses with PermaNets, untreated nets and without nets during pre-intervention (Aug to Oct 2007) and post-intervention (Nov 2007 to Jun 2008 phases

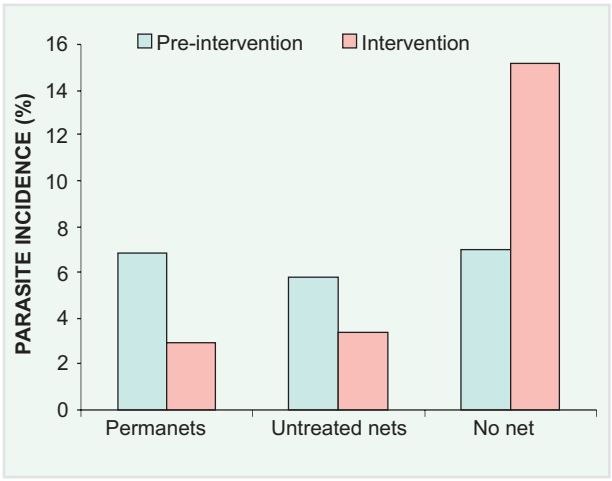


Fig. 55: Malaria prevalence in PermaNet, untreated net and without net villages during pre-intervention (Aug to Oct 2007) and post-intervention (Nov 2007 to Jun 2008) phases recorded through cross-sectional surveys in Orissa

Delivering instructions on the use of LNs to the users

remained >85%. The studies on wash resistance indicated good wash resistance of interceptor nets and also bioefficacy against important malaria vectors (WHO criterion: >80% mortality in 3 min cone bioassays).

Field Evaluation

Field evaluation of Interceptor®, alpha-cypermethrin-treated long-lasting insecticidal nets (LNs), was undertaken to assess entomological and epidemiological impact in malaria endemic villages of Sundargarh district (Orissa), Kamrup district (Assam) and Kanker district (Chhattisgarh). Three clusters of villages in each study area were selected. In one cluster of villages Interceptor nets were distributed to all the inhabitants, in the second cluster, untreated nets were distributed and the third cluster was kept as control where nets were not distributed.

The interceptor nets were found to be effective in controlling the indoor resting mosquitoes. There was a decline in the overall entry rate of mosquitoes in houses with interceptor nets at all the three study sites coupled with reduction in feeding success rate. In Sundargarh district, a significant reduction up to 87.5% was registered in entry rate of mosquitoes during intervention compared to pre-intervention ($p<0.01$). There was virtual disappearance of *An. minimus* in villages with Interceptor nets, but were found in untreated net and without net villages in Assam. In Kanker district, Chhattisgarh, the entry rate of mosquitoes (40.3) in interceptor used houses was comparatively lower than the untreated net (151) and without net (136) houses. The results suggested good performance of Interceptor nets in reducing the mosquito density and also preventing entry of mosquitoes into the houses with interceptor nets.

The impact of Interceptor nets on malaria incidence in Sundargarh district indicated 57–76% reduction compared to untreated net ($p<0.01$) and without net villages ($p<0.001$). Mass blood surveys in the study villages in Sundargarh district indicated similar results as that of the active surveillance. In Sundargarh, the parasites rates in the interceptor net

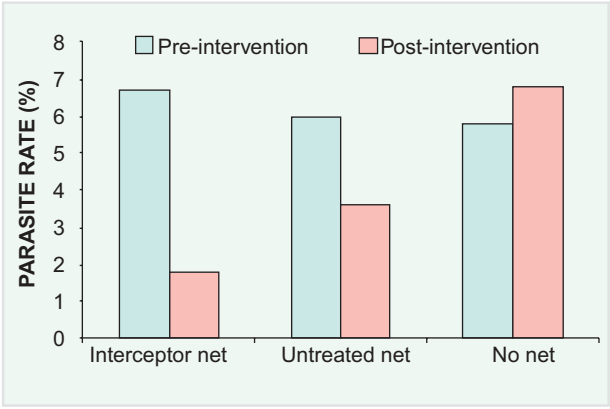


Fig. 56: Malaria prevalence in Interceptor net, untreated net and without net villages during pre-intervention and post-intervention phases recorded through cross-sectional surveys in Sundargarh district, Orissa

used villages reduced (73%) after the intervention over the pre intervention period, in the untreated net villages also 40% reduction was observed, but in the without net villages the parasite rates increased during the intervention phase than the pre-intervention period (Fig. 56).

In Assam, villages with interceptor nets registered drastic reduction in *P. falciparum* cases during intervention period. The total cases observed in Interceptor net used villages during the intervention period (October 2006 to April 2007) were 10, while in untreated net villages 82 cases and without net villages 233 cases were reported during the intervention period (Fig. 57).

In Kanker district, Chhattisgarh the slide positivity rate (SPR) during pre-intervention period was 8.4, 6.9, and 3.2 in Interceptor net, untreated net and without net villages respectively, whereas after intervention the SPR was 3.7, 6.5 and 11 respectively. The results indicated the impact of Interceptor nets in reducing the malaria transmission. Similarly, malaria incidence (Cases/000) reduced from 36.3 during pre-intervention to 14.7 post intervention in

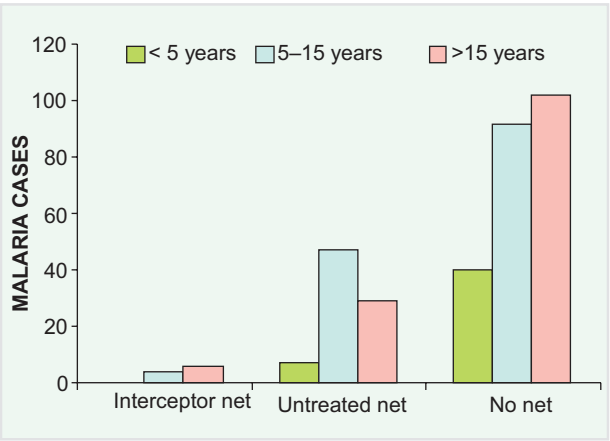


Fig. 57: Malaria prevalence in different age groups in Interceptor net, untreated net and without net villages in Kamrup district, Assam

Recording of perceptions on the use of LNs from the inhabitants

Interceptor net villages, while it increased from 15 in pre-intervention to 23.1 in post-intervention in untreated net villages and from 34.2 to 42.9 in without net villages in respective periods (Fig. 58)

The interceptor nets were found to be safe for human. The community compliance and acceptance was high . No adverse health events were reported by the users. Surveys carried out in Orissa indicated 98.2% compliance, in Assam it was 100% and in Chhattisgarh it was 81.7%. All the users in the three study areas informed that there is reduction in mosquito bites (74–90%) with effect on other non-target domestic insects like cockroaches, house flies, spiders, etc. Studies carried out to assess the human safety didn't indicate any contraindications owing to net use and the nets were found to be safe for use by inhabitants of all age groups.

The results of multicentric field evaluation in three malaria endemic states in India revealed good performance of Interceptor nets in reducing the mosquito densities and malaria incidence.

K-O- Tab 1-2-3

This is a deltamethrin formulation developed by M/s. Bayer Environmental Science. This is specifically meant for converting untreated nets into long-lasting insecticidal nets. The formulation consists of deltamethrin tablets and a binder; upon impregnation it converts the net into a long-lasting net.

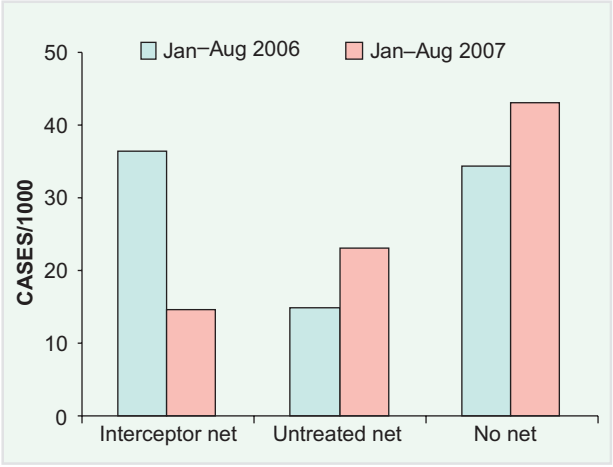


Fig. 58: Malaria prevalence in Interceptor net, untreated net and without net villages in Kanker district, Chhattisgarh

A project on the evaluation of bioefficacy and wash resistance of K-O Tab 1-2-3 tablets supplied by M/s. Bayer India Ltd. was undertaken. Nylon mosquito nets were impregnated with the water dispersible tablet formulation of deltamethrin

(K-O Tab 1-2-3) @ 25 mg/m^2 dose + binder and washed as per the study protocol. Bio-efficacy of the insecticide treated mosquito nets was evaluated against *An. fluviatilis*, *Ae. aegypti* and *An. stephensi* using WHO cone bioassay test. The bioefficacy studies showed 100% knockdown of *An. fluviatilis* up to 30 washes. K-O Tab 1-2-3 nets were 100% effective against *An. fluviatilis* even after 15 washes. Thereafter, percent mortality recorded was 92, 92, 84.6 at 20, 25 and 30 washes, respectively.

Ring net bioassay test was also carried out to determine the median knockdown time (KDT_{50}), 90% knockdown time (KDT_{90}) and percent mortality. There was 100% mortality in wild caught full-fed *An. fluviatilis* in 1 hour exposure and 24 hour recovery period up to 30 washes. The KDT_{50} and KDT_{90} were 11, 15; 12, 18; 14, 23; 14, 25; 18, 25; 18, 26; and 20, 28 min at 0, 5, 10, 15, 20, 25, and 30 washes respectively. KDT_{50} and KDT_{90} value of *An. fluviatilis* against 5% deltamethrin impregnated paper were 9 and 14 min respectively.

Further studies on the bioefficacy of K-O Tab 1-2-3 were conducted to examine wash resistance against *Ae. aegypti*. There was 100% knockdown in laboratory reared *Ae. aegypti* up to 15 washes at 1 hour observation and thereafter between 85 and 92% knockdown was recorded in subsequent 20 to 30 washings. Percentage mean mortality of mosquitoes was 31% (27.2–33%) up to 15 washes at 24 hour recovery period. Thereafter, percent mean mortality was 13.8% (13.6–14.3) up to 30 washes.

Long-lasting Insecticide Incorporated Plastic Sheets

Spraying of canvas tents with residual pyrethroid insecticide is an established method of vector control in refugee camps. In recent times plastic sheeting (polythene tarpaulins) has replaced canvas as the utilitarian shelter material for displaced populations in complex emergencies. Advances in technology enable blankets to be impregnated with pyrethroid during the process of manufacture. The efficacy of such fabrics against mosquitoes when used for protection in different ecological conditions needs evaluation. Presently the anti-malaria campaigns include insecticide based intervention measures both against larvae and adult mosquitoes. Prevention of malaria is a major technical and operational problem in displaced and mobile populations. This has necessitated a continued need by the programme managers to evaluate new efficacious strategies to control vectors in complex emergencies. Insecticide incorporated blankets is a new technology to control

mosquitoes in emergency shelter places, temporary habitations in different locations, slum settlers, tribal population, etc. The blankets can be used for covering the body for protection against mosquitoes and other insects. The results of the trial will definitely provide the usefulness of the blankets in controlling the mosquito nuisance and also disease prevalence. In India no such trial was undertaken on insecticide incorporated blankets. To tackle complex emergencies it is desirable to evaluate insecticide incorporated blanket in different ecological conditions in India to provide relief to distress people affected by natural calamities or living in remote and unhygienic environment.

ZeroFly

ZeroFly is an insecticide incorporated plastic sheet with deltamethrin @ 265 mg ai/ m^2 manufactured by Vestergaard Frandsen India Pvt. Ltd. This was field evaluated in Delhi and Orissa states for its efficacy. The results of the trial are furnished below.

Delhi

This study was initiated in the month of Aug 2006 in Labour camps in Delhi and Noida. In both the localities ZeroFly plastic sheets were fixed at a distance of at least one km from the control localities where plastic sheets without insecticide (untreated) were fixed. In addition to the two localities the study was also carried out in RAC police camp in Delhi. Bioassay tests on ZeroFly sheets with 3-min exposure period resulted in 100% mortalities in *An. culicifacies* and *An. stephensi.* The effect of ZeroFly sheets persisted at 100% mortality against *An. culicifacies* even after one year of use under field conditions. Fortnightly monitoring of entomological parameters showed almost complete reduction in the indoor resting density of vector and non-vector insects in the labour camps provided with ZeroFly plastic sheeting as compared with the camp provided with untreated plastic sheetings. Entomological and epidemiological parameters were monitored at fortnightly interval. Fortnightly monitoring of entomological and epidemiological parameters have shown considerable reduction in the indoor resting density of vector and non-vector insects in the labour camps provided with ZeroFly plastic sheeting, as compared with the untreated plastic sheeting. In labour camp at a construction site in Noida, man hour density (MHD) of malaria vectors *An. culicifacies* and *An. stephensi* during post intervention period was in the range of 0–05 in the experimental area as compared to 0–12 and 0–15.5 in the control area. Similarly in JJ cluster of agricultural labor in the Jamuna belt area in Delhi, man hour density of malaria vectors *An. culicifacies* and *An.stephensi* were in the range of 0–05 in the experimental area as compared to 0–13 and 0–15 in the control area. Parasite incidence (cases per thousand population)

Tent line with ZeroFly plastic sheeting

in the construction site labour camp in Noida was 37 in the experimental area as compared to 73 in the control area. Similarly, in the JJ cluster in Delhi the PI in experimental and control areas was 42 and 62.7 (Fig. 59). In RAC police camp, intervention with Zerofly sheeting revealed in the reduction of culicine density in the experimental tents as compared to the control tents. Survey about the perception of the users about side effects versus and benefits revealed a highly positive response in favour of the benefits of Zerofly sheeting.

Orissa

A field trial was conducted on the efficacy of ZeroFly® plastic sheeting against malaria vectors *An. culicifacies* and *An. fluviatilis* and its impact on malaria transmission in one of the highly endemic areas of Orissa—Birkera block of Sundargarh district. The study area comprised three villages which were randomized into three clusters and designated as ZeroFly plastic sheet (330 pop.), untreated plastic sheet (372 pop.) and no plastic sheet area (382 pop.). Baseline pre-intervention data were collected from July to September 2006 followed by nine months of

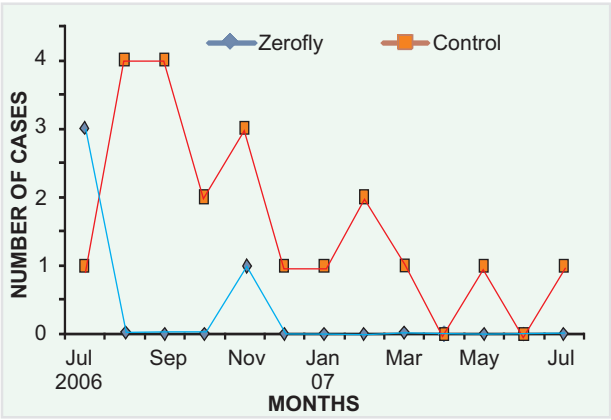


Fig. 59: Number of malaria cases reported in ZeroFly and untreated plastic sheets used localities in Noida, Uttar Pradesh during pre-(Jul 2006) and post-(Aug 2007–Jul 2008) intervention periods

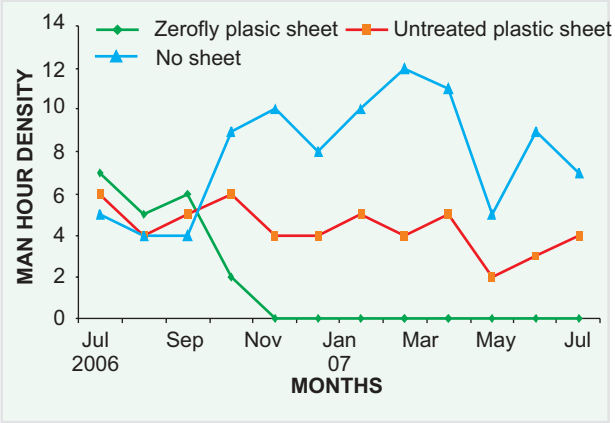


Fig. 60: Density of *An. culicifacies* in houses with ZeroFly plastic sheets, untreated plastic sheets and without plastic sheets during pre-intervention (Jul- Sep 2006) and post-intervention (Oct 2006 to Jun 2007) periods in Sundargarh district, Orissa

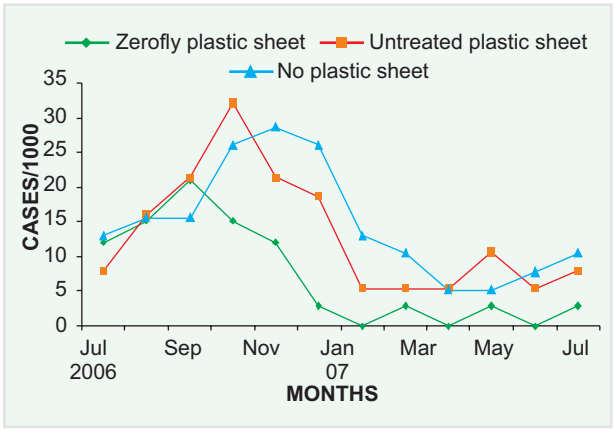


Fig. 61: Month-wise malaria cases/1000 in study villages during pre-intervention (Jul–Sep 2006) and post-intervention (Oct 2006 to Jun 2007) periods in Sundargarh district, Orissa

Temporary shelter camp made with ZeroFly plastic sheets

intervention phase from October 2006 to June 2007. Baseline studies showed that both the vector species *An. culicifacies* and *An. fluviatilis* were 100% susceptible to deltamethrin. Cone bioassay tests were performed on *An.culicifacies* and house flies every month during intervention period on the ZeroFly plastic sheets while in use in the field conditions and these species were exposed for 3 and 30 minutes respectively. The results showed a knock-down effect of 95–100% and 90–100% on these species respectively during different months of intervention. 100% mortality was recorded after 24 h of recovery period in both the species. In ZeroFly plastic sheeting area, there was a significant reduction of 86.7, 100 and 84% in the entry rate of *An. culicifacies, An. fluviatilis* and other anopheline species respectively with an over all reduction of 84.7% in the entry rate of total mosquitoes in comparison to pre-intervention phase. The density of malaria vector *An. culicifacies* in houses with ZeroFly plastic sheeting, untreated plastic sheeting and no sheet is given in Fig. 60.

Floor sheet collections in houses with ZeroFly plastic sheet indicated 56.2% immediate mortality in total mosquitoes. The overall feeding success rate of mosquitoes in the trial village was only 12.5% in comparison to 49.7% and 51.1% in villages with untreated plastic sheet and no sheet respectively.

The excito-repellent rate of all the mosquitoes in houses with ZeroFly plastic sheets and untreated plastic sheets was 40.6% and 3.1% respectively. The month-wise parasite index in the trial and control areas is shown in Fig. 61. There was a significant reduction of 65% and 70.5% in malaria incidence in ZeroFly plastic sheeting area as compared to untreated plastic sheet and no sheet area respectively. The ZeroFly plastic sheeting was found to be safe for human and no adverse health events were reported. The community acceptance was high as 91% of study population was sleeping in the rooms fixed with ZeroFly plastic sheeting. The ZeroFly plastic sheeting provided relief not only from mosquitoes but also from other household pests, such as bed bugs, cockroaches, ants and houseflies as revealed by the study population. The study showed that ZeroFly plastic sheeting may be used as an effective intervention strategy that is operationally feasible to control malaria, particularly in remote high risk areas and also during complex emergency situations. ❑

Larvivorous Fish in Mosquito Control

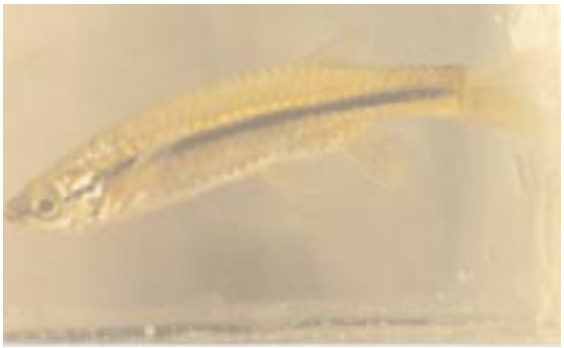
Use of fish in mosquito control has been well-known for more than 100 years. In India, as far back in 1904 larvivorous fishes were used in Mumbai City for the control of malaria vector *An. stephensi*. Larvivorous fishes *Poecilia reticulata* (Guppy), a native of South America and *Gambusia affinis* (Gambusia), a native of Texas were imported in India in 1908 and 1928, respectively for the control of malaria vectors. Soon after that use of larvivorous fish became a common practice in India, e.g. in Bengaluru and Kolkata cities and during the construction of Sharda Canal in Uttarakhand. During mid-1980s National Institute of Malaria Research demonstrated the use of larvivorous fish as part of an integrated vector control strategy. Though use of larvivorous fish is an important component of vector control in the urban malaria schemes in India, use of larvivorous fish in control of rural malaria was shown for the first time in India.

Fish fauna surveys have been undertaken at different NIMR field units which revealed that there are several indigenous fish like *Danio rerio, Esomus danricus, Badis badis, Chanda nama, Puntius ticto, Rasbora daniconius, Colisa fasciata,* etc. which are commonly found in Indian fresh waters. These fish were found to have significant larvivorous potential in various conditions. However, they either can not be mass produced or are not hard enough to withstand transportation, variation of water quality, turbidity and temperature. Also these fish produce smaller broods than the exotic fish.

During laboratory trials at Rourkela, Orissa, *Danio rerio* and *Oryzias melastigma* showed a high predatory efficacy against the mosquito larvae. A single tiny *Danio* fish (2.7–3.0 cm) consumed on an average 52 IV instar anopheline larvae per day, whereas *Oryzias* sp (2.5 cm) consumed 98 larvae per day. The results obtained during the trials in rice-field quadrates showed that both the fish are highly effective in reducing the density of mosquito immatures in the rice-fields. The reduction in density of III and IV instars and pupae became evident right from the beginning. On Day 6 *Danio* and *Oryzias* lowered the densities by 86.8 and 76.2% respectively.

Recently, in north Gujarat (Kutchh district) an indigenous fish *Aphanius dispar* was found in many water bodies such as in the rivulets, seepage waters of dams/check dams and ponds. As no previous study was done in India to evaluate its effectiveness in vector/malaria control, a randomised controlled trial

Danio rerio



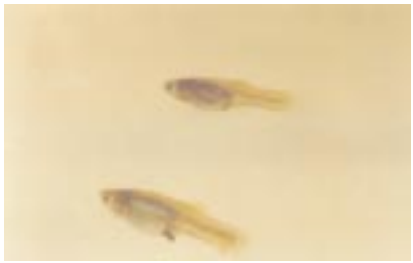
Rasbora daniconius

Esomus danricus

Puntius sp.

Colisa fasciata

Poecilla reticulata (guppy)



Gambusia affinis

Mass production of Fish

was undertaken in Rapar taluka during 2005–07 with the objectives to assess the impact of *A. dispar* on malaria vector population, malaria prevalence, operational feasibility, social acceptance and sustainability of *A. dispar* under the vector-borne disease control programme. Results of the trial indicate that the fish is highly effective in malaria vector control and propagates very well in brackish water as well freshwater in the earthen and concrete irrigation tanks, wells where malaria vector *An. culicifacies* and *An. stephensi* breed profusely. Monitoring of of the incidence of malaria by routine surveillance system and cross-sectional surveys indicated that the fish use was as effective as IRS. The SPR in the fish area was 1.4% compared to 1.6% in the IRS area (Fig. 62). The acceptance of fish by the native farming community was high.

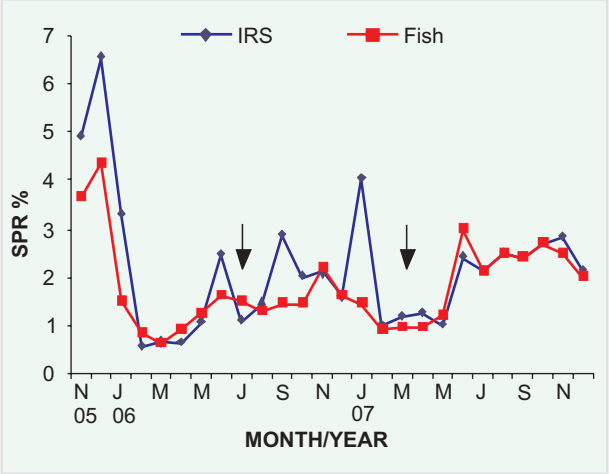


Fig. 62: Slide positive rate in *A. dispar* used and IRS villages in Rapar Taluka, Gujarat. Arrows indicate malathion spray in the control areas

Mass Production of Larvivorous Fish

Mass production of *P. reticulata* and *G. affinis* was undertaken for mosquito control programme as part of the bioenvironmental control of malaria at many places in India. Some innovative methods have been developed to reduce the cost of mass production and distribution of fish. A number of hatcheries for mass production were established and fish were transported to the villages where they were stocked and introduced in the mosquito breeding places from time-to-time.

Mass production of *G. affinis* was undertaken in District Nainital in Uttarakhand and Districts Shahjahanpur and Allahabad in Uttar Pradesh. Big ponds were converted into fish hatcheries as well as new hatcheries were made. *G. affinis* were introduced into these ponds for multiplication. *G. affinis* breeds thrice in a year and within a year all the field units had good stocks. Similarly, large stocks of *P. reticulata* (Guppy) were established at Nadiad, Gujarat and BHEL, Hardwar. The fish from these stocks were used for mosquito control in various breeding sites like drains and underground tanks. Regular introduction and monitoring was undertaken.

Composite Fish Culture

Improvement of village economy by promoting local raw materials and natural resources was an important component of the programme. Carps are grown by the farmers all over and the adjoining areas of Haldwani, District Nainital, Uttarakhand. As a result of health education and personal discussions with fish farmers, it was possible to culture *Gambusia* along with carps. The tendency of *Gambusia* to remain near margins convinced farmers that this fish does not compete with edible fish for space and food. It was amply clear to the farmers that it eats mosquito larvae at the margins while carp fish is mainly herbivorous. Experiments on composite culture of the larvivorous fish along with food fish were carried out, which showed that guppies and *Gambusia* can be cultured without causing an adverse effect on the edible fish production, as well as generate income to the rural communities. Culture of *G. affinis* with food fish and *P. reticulata* was carried out at Nadiad and Shahjahanpur field units involving local fish farmers and village *Panchayats*.

In Nadiad, >50% village ponds were infested with water hyacinth. Water hyacinth was manually removed from these ponds and these important resources were used for fish production. *P. reticulata* (Guppy) along with the major carps was used in mixed cultures in four experimental ponds in Kheda district. An average annual yield of 847 kg/ha of carps in such ponds was comparable with 839 kg/ha of four other experimental ponds without guppies and there was no adverse effect on the population of food fish due to rearing of guppies with carps.

Culture of *Gambusia* along with carps in fish culture ponds in Shahjahanpur district showed very little change in the fish productivity—1539 kg/ha in

ponds with *Gambusia* and 1572 kg/ha in ponds without them. The growth and survival of *Gambusia* were found normal in most of the composite fish culture ponds. Using this technique, large-scale production of larvivorous fish for vector control could be achieved which helped the community to generate resources for the developmental activities in experimental villages. These studies showed that composite fish culture can yield double benefits of food fish production as well as the suppression of mosquitoes. Culture of larvivorous fish *G. affinis* and *P. reticulata* at village-level is very vital for the successful implementation of bioenvironmental control of vector borne diseases. This may be done with the active community participation under the technical guidance of malaria control agencies.

Larvivorous fish hatchery

Operational Use of Larvivorous Fish

Suitable fish species and rate of application in different breeding habitats for the control of different mosquito vector species are shown in Table 2. Use of larvivorous fish for vector control is a simple, inexpensive, effective and local measure. Larvivorous fish has been accepted as a selective vector control method in the Enhanced Malaria Control Projects launched with World Bank financing in eight states of India since 1997. An independent assessment of the performance of the larvivorous fish programme as an integrated control measure was carried out in Ahmdabad City and in rural areas of Nagpur (Maharashtra) and Chhindwara (Madhya Pradesh) districts during 2003–04. The assessment included the status of development of fish resources, introduction of larvivorous fish and maintenance of hatcheries, methodologies adopted for producing fish stocks, transportation and distribution/release, available infrastructure and manpower, training needs, social acceptance and community perception. The impact of the larvivorous fish on the mosquito borne diseases and the sustainability of the larvivorous fish programme was also assessed. It was observed that the use of larvivorous fish in malaria

Training on collection of fish

Table 2. Suitable fish species and rate of application in different mosquito breeding habitats

Aquatic habitats	Main vector mosquito	Fish species	Number of fish to be released
Ponds/rain water pools	*An. culicifacies*	*Gambusia* and *Guppy*	10–20 fish/m^2
Water storage tanks, ornamental tanks, fountains, swimming pool and cisterns	*An. stephensi, Aedes* sp.	*Guppy* and *Gambusia*	5–10 fish/m^2
Wells	*An. stephensi*	*Guppy* and *Gambusia*	50–250 (For rapid control)
Paddy-fields	*An. culicifacies*	*Gambusia, Danio, Aplocheilus, Oryzias* and *Aphanius*	5000/acre
Farm ponds and check dams	*An. culicifacies*	*Gambusia, Aphanius* and *Aplocheilus*	25–50/m^2
Mine pits	*An. culicifacies*	*Gambusia* and *Guppy*	2500/acre (For immediate control)
Swamps	*Cx. quinquefasciatus*	*Guppy, Aplocheilus, Colisa,*	10,000/acre
Drains	*Cx. quinquefasciatus*	*Guppy* and *Colisa*	

control programme has been taken up on operational scale with variable performance levels.

Currently, the use of larvivorous fish is in full swing to cover the entire states of Gujarat, Maharashtra and Karnataka. In Maharashtra, the bioenvironmental methods have been spread to cover the entire state through the primary health care system. In Gujarat, hundreds of fish hatcheries have been established for use in the entire state and training programmes are being organised in association with the National Institute of Malaria Research.

Cost-effectiveness

Although no specific studies have been conducted to evaluate comparative per capita cost of fish and insecticides under programmatic conditions. Some of the examples of integrated vector control using larvivorous fish were found very effective in controlling the breeding of malaria and dengue vectors in a variety of habitats (Fig. 63) in an urban setting. The per capita annual operational cost of the integrated strategy was Rs. 8.1 (US$ 0.19; 2000 prices) compared as Rs. 9.3 (US$ 0.21) with chemical control. The strategy was found feasible, appropriate, cost-effective and resulted in a major reduction in antimalarials and insecticide consumption in industrial complexes.

Future Plan

At present in India or elsewhere large-scale operational use of larvivorous fish for vector control

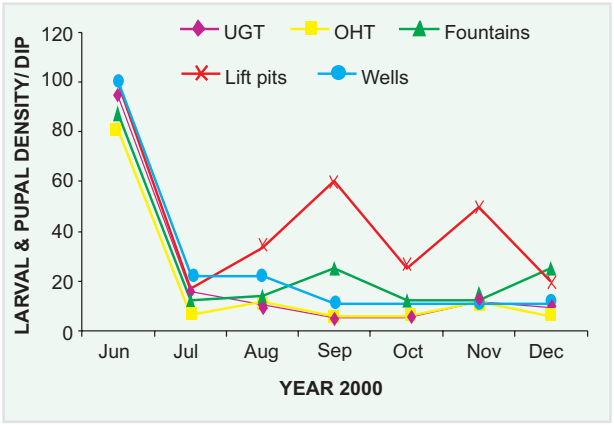


Fig. 63: Impact of the use of fish on mosquito breeding in selected larval habitats of an urban setting. UGT–Underground tanks; OHT–Overhead tanks

remains under exploited in situations where there is technical feasibility of use of fish as part of an integrated vector management approach. There is an urgent need to strengthen facility to impart operational training on operational aspects on larvivorous fish. Keeping in view of the increasing role of fish in vector borne disease control and training requirements of the personnel involved in antimalaria programme, NIMR has been in the process of supporting capacity strengthening of the National Vector Borne Disease Control Programme such as in Gujarat state to scale-up use of larvivorous fish including indigenous fish *A. dispar* in suitable ecosystems.

❑

Evaluation of Biolarvicides

Biolarvicides

Over the last few decades, there has been growing realisation that alternative methods to chemical control of vector mosquitoes should be developed. One such method is the use of *Bacillus sphaericus* and *B. thuringiensis* var *israelensis (Bti)* Commercial formulations of *Bti* are available and can be used in large-scale mosquito control operations. The major advantages of biolarvicides are—reduced application costs, safety to environment, human beings, animals and other non-target organisms. Various formulations of *B. sphaericus* and *B. thuringiensis* var *israelensis* have been evaluated during the last two decades.

Laboratory and Field Evaluations

Biocide-S

Biocide-S (*B. sphaericus* 1593M) developed by Madurai Kamraj University was the first biocide tested at NIMR in 1983, later three experimental formulations of Biocide-S–HIL-8, 9 and 10 wettable powder and dust formulations were developed and tested in laboratory and small-scale field trials (Mittal *et al* 1985). These formulations were found to be effective against both *Culex* and *Anopheles* larvae in laboratory and small pits in field. However, further development of various aqueous formulations, MKU-1, 2 AU, etc. based on Biocide-S were not much effective against *Anopheles* sp, particularly *An. culicifacies*. In addition to Biocide-S, various other formulations of *B. sphaericus* obtained through WHO and various other laboratories in India and abroad were tested both in laboratory and small-scale field trials (Ansari *et al* 1989, 1995). All these formulations were, however, effective only against culicines and some anopheline species, particularly against *An. stephensi* and *An. subpictus* and not against *An. culicifacies*.

Solvay, Spherimos and Vectolex

Solvay, a liquid and Abotts granular formulations of *B. sphaericus* 2362 obtained through WHO, were tested against *An. stephensi*, *An. culicifacies* and *Cx. quinquefasciatus* both under laboratory and field conditions (Ansari *et al* 1989). Later two more formulations of *B. sphaericus* 2362 namely Spherimos and Vectolex 2.5 AS obtained through WHO were tested (Ansari *et al* 1995). It was found that *Cx. quinquefasciatus* was more susceptible to all these formulations followed by *An. stephensi* and *An. culicifacies.* In the field condition, absolute mortality in *Culex* larvae was obtained with Spherimos @ 2 ml/m^2 for one week in pools and 99% reduction

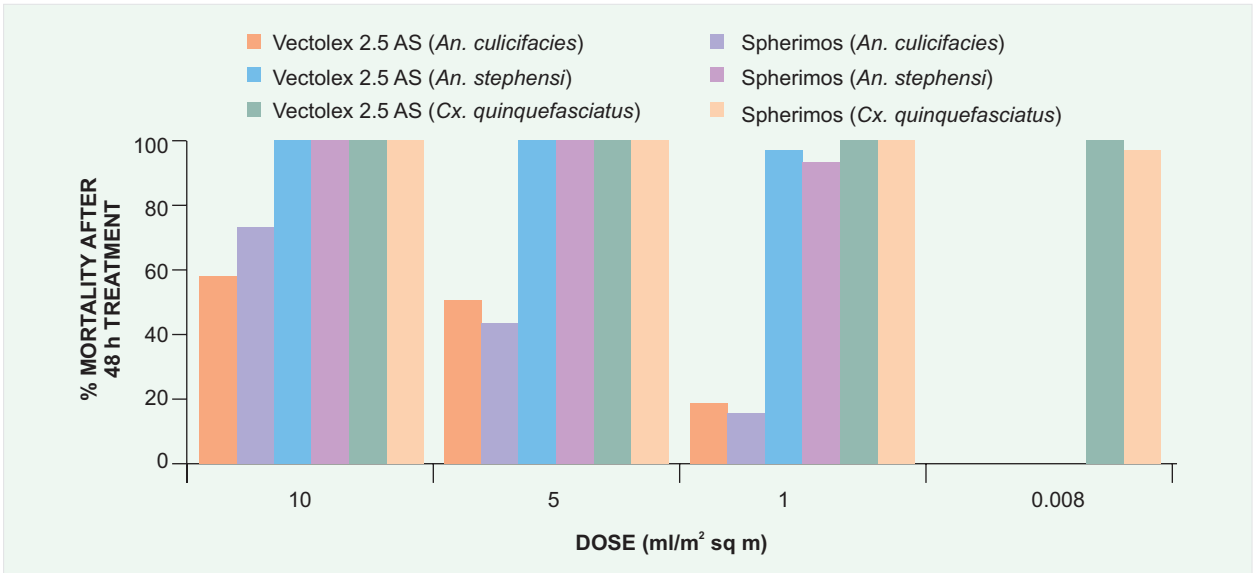


Fig. 64: Laboratory evaluation of *B. sphaericus* 2362 flowable formulations against larvae

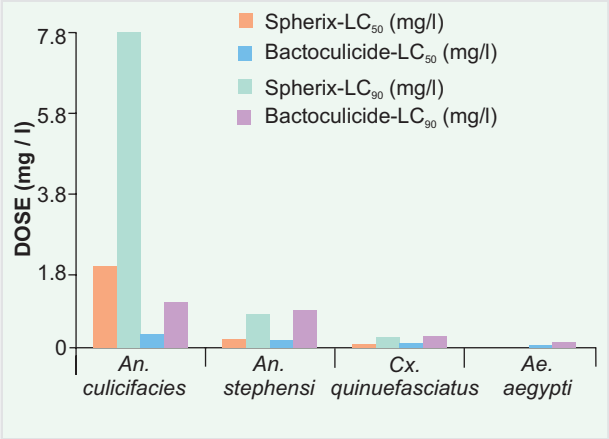


Fig. 65: Comparative toxicities of spherix and bactoculicide against III instar mosquito larvae

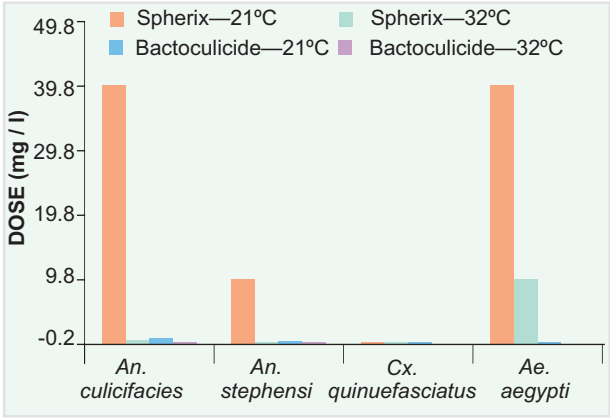


Fig. 66: Effect of temperature on larvicidal activity (LC_{50}) of spherix and bactoculicide against different vector species

for three weeks in the wells. The activity was enhanced for three weeks in pools when the dose was increased to 10 ml/m^2. On the other hand, against anophelines the absolute impact was seen for one week @ 2 ml/m^2 and up to 90.8% for two weeks in the pools. In some pools, the impact was moderate. In the wells, the impact was seen for one week @ 2 ml/m^2, whereas @ 10 ml/m^2 it was prolonged for two weeks. A good impact of Vectolex was observed on *Culex* sp in the field which lasted for four weeks in the pools @ 2–5 ml/m^2 and for six weeks in unused wells @ 5–10 ml/m^2 (Fig. 64).

Spherix or Bactoculicide

In the year 1992, National Malaria Eradication Programme (now NVBDCP) procured two Russian formulations of biolarvicides—Spherix (*B. sphaericus*, serotype H 5a5b, strain B-101) and Bactoculicide (*B. thuringiensis* var *israelensis,* serotype H-14, strain 164). NVBDCP provided 40 MT of Spherix and 10 MT Bactoculicide to NIMR for scientific evaluation by conducting multicentric field trials in different eco-climatic situations in India.

Prior to this, the laboratory bioassays with these two formulations were conducted in Delhi from July to October 1991. It was observed that both Spherix and Bactoculicide were effective against *An. culicifacies*, *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* larvae (LC_{50} values of Spherix ranged from 0.19 to >40 mg/l and Bactoculicide from 0.034 to 0.16 mg/l) (Fig. 65). It was found that Spherix was most effective against *Cx. quinquefasciatus* and the least against *Ae. aegypti,* whereas Bactoculicide was the most effective against *Ae. aegypti* and the least against *An. culicifacies.* Among the two malaria vectors tested, *An. stephensi* was much more susceptible than *An. culicifacies* to both formulations, this observation was also corroborated later by the findings of the field trials (Mittal *et al* 1993).

The safety profile of these formulations to non-target organisms (NTOs) such as *Gambusia affinis*, *Poecilia reticulata*, frog tadpoles, notonectid bugs (*Enithares indica* and *Anisops sardae*) and Cyclops (Copepods) was also established prior to large-scale field trials in the country. The LC_{50} values for above NTOs ranged from 50 to >2000 mg/l, which were many fold higher than the recommended field dose for larval control.

Simultaneously, studies on effect of temperature showed that a 10°C rise in temperature from 21 to 31°C increased efficacy of Spherix and Bactoculicide by 2–100 fold against different vector species (Fig. 66) (Mittal *et al* 1993). This indicated that biolarvicides would lose efficacy in cold climatic conditions and prove very useful in warmer months or tropical climatic conditions which exist in India for most of the year (Fig. 67).

Similarly, it was observed that beyond pH 9.5 the efficacy of both Spherix and Bactoculicide was reduced drastically (Mittal *et al* 1995). There was, however, no significant difference observed in the activity in the pH range 3.5 to 9.5.

Multicentric trials were carried out with Spherix in different NIMR field units, namely at Farrukhabad, Shahjahanpur, Hardwar, Mathura, Ghaziabad, Delhi,

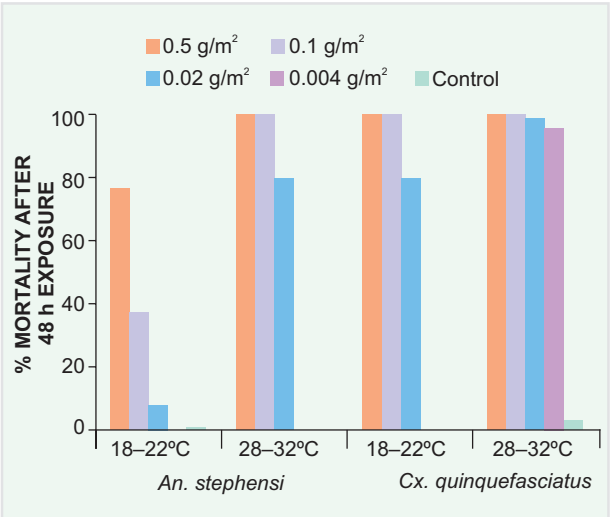


Fig. 67: Studies on the effect of temperature on the efficacy of spherix on *An. stephensi* and *Cx. quinquefasciatus*

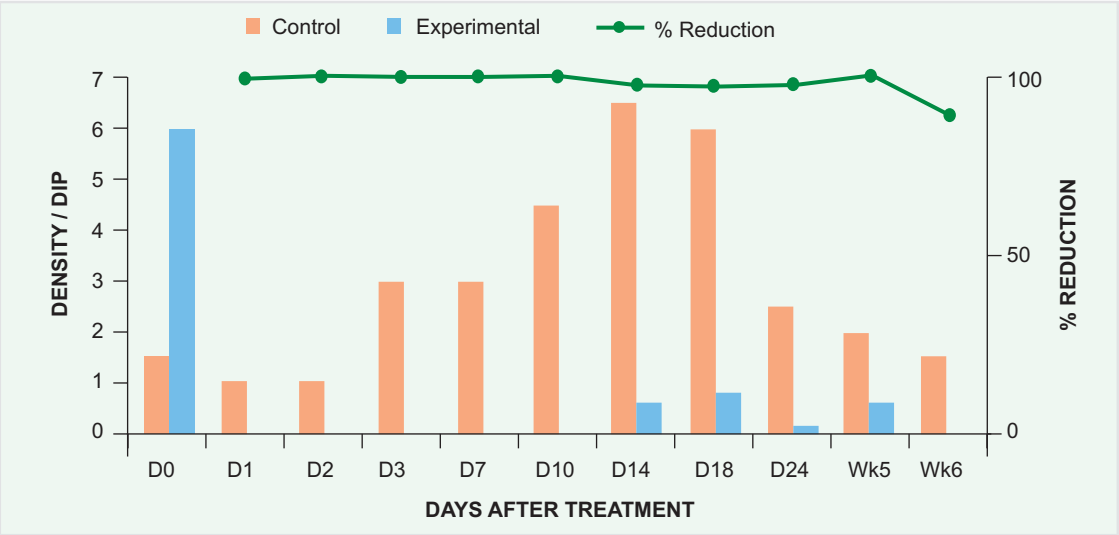


Fig. 68: Efficacy of bactoculicide against mosquito larvae in industrial scraps at BHEL, Hardwar, Uttarakhand

Panaji and Vasco da Gama, Goa, Chennai, Mandla, Nadiad, Shankargarh, Rourkela and Car Nicobar Islands. From the results of these multicentric studies it became evident that both the Russian formulations—Spherix and Bactoculicide were effective against anophelines and culicines including disease vectors in different ecological conditions (Fig. 68). The residual activity of biocides depended upon vegetation, algae and organic pollution. The larval control was longer when breeding habitats were less polluted had less or no vegetation as effect could be seen for up to four weeks in them, whereas the impact lasted for 3–7 days in others (Figs. 69 and 70). Further studies in field and also in laboratory showed the development of resistance to *B. sphaericus*-based biocides but no cross-resistance to *Bti* was observed (Adak *et al* 1995, Mittal *et al* 1998) Later, extensive field trials were also conducted by NIMR to assess the efficacy of the above formulations. Later

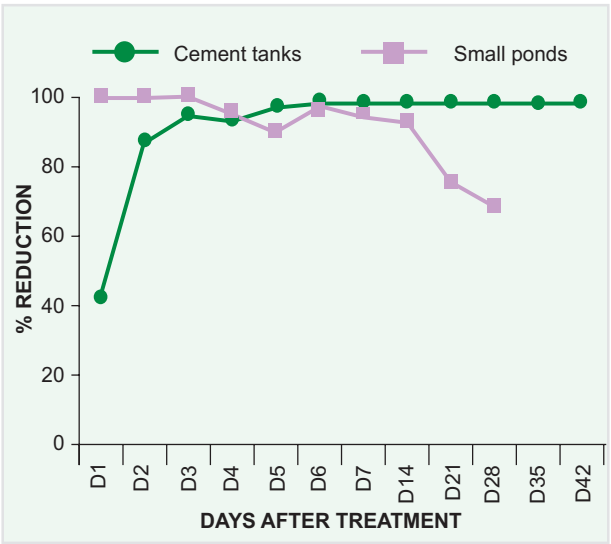


Fig. 69: Larvicidal efficacy of spherix against anopheline larvae in cemented tanks and small ponds after a single application in Bhabhar area, District Nainital, Uttarakhand

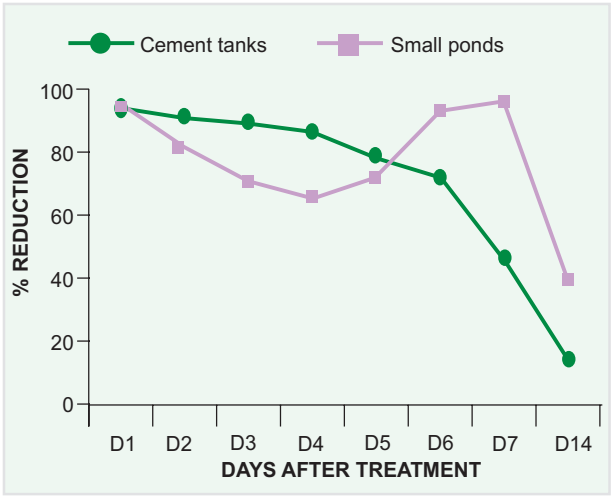


Fig. 70: Larvicidal efficacy of bactoculicide against anopheline larvae in cemented tanks and small ponds

various other formulations of *Bti* tablet, granule and wettable powder against different disease vector species and in urban and rural areas were tested (Batra *et al* 2000; Dua *et al* 1993; Kar *et al* 1997; Kumar *et al* 1994, 1995, 1996, 1998; Mittal *et al* 2000; Shukla *et al* 1997, Yadav *et al* 1997). A commercial formulation (Wockhardt *Bti*) of *B. thuringiensis* var *israelensis* H-14 50% WP was evaluated in field at three NIMR field units at Goa, Haldwani (Uttarakhand) and Shahjahanpur (Uttar Pradesh). Field efficacy was tested in different breeding habitats, namely paddy-fields, river-bed pools, pokhars, pits and drains, septic tanks, ornamental fountains and cement tanks. The formulation was tested at 2 doses, 0.5 and 1 g/m^2. Over 90% reduction in larval density was observed in most of the breeding habitats at a dose of 0.5 g/m^2 except in septic tanks. In septic tanks 1 g/m^2, was effective. Residual effect lasted for one week in most of the breeding sites except in ornamental fountains and cement tanks. In paddy-fields, the efficacy was observed for only two days (Fig. 71).

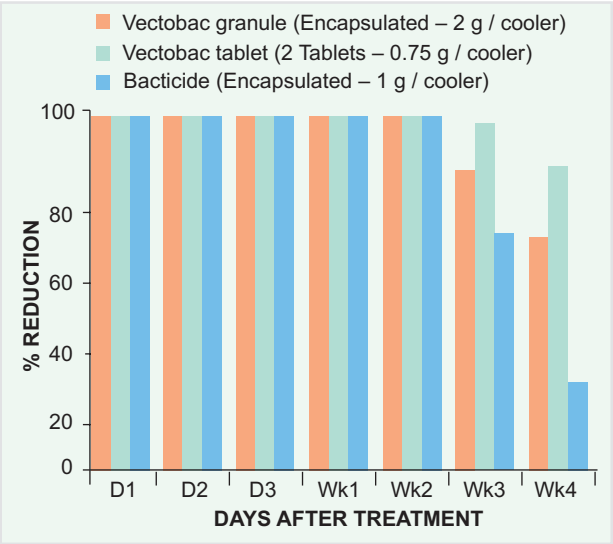


Fig. 71: Efficacy of different formulations in containing *Ae. aegypti* breeding in treated desert coolers

Tacbio

Trial of an Indian strain of *B. thuringiensis* var *israelensis* H14 (*Bti* AS, VCRC B17) against immatures of *An. culicifacies, An. stephensi, Cx. quinquefasciatus* and *Aedes* sp showed 100% mortality up to 100 µl dose and 70–90% mortality at lowest dosages of 10 to 50 µl of biolarvicide. The lowest field application dose of 0.5 ml/m^2 remains effective for about 10–12 days in clean unpolluted water bodies, which support anopheline breeding. However, in stagnant polluted waters such as drains and cesspools supporting culicine breeding, the same dose persists for 5–6 days only. Based on the field observations, an operational dose of 0.5 ml/m^2 at fortnightly intervals is required for clean water sources supporting anopheline breeding. However, to control breeding of culicine mosquitoes in stagnant and polluted waters, an operational dose of 1 ml/m^2 at fortnightly intervals was suggested.

Biolarvicides (*Bti* AS, VCRC B17 strains) were evaluated for their efficacy against immatures of *Anopheles* and *Culex* species larvae in natural habitats and their persistence was assessed. Studies were carried out in Rourkela Municipal area with a control urban area 10 km away from the experimental area. Field application was done with standard equipment following the NVBDCP guidelines for spray. Percent reduction was estimated on subsequent days to determine the efficacy. Impact on adult densities was also determined. Application was done @ 0.5 ml/m^2 in clean waters and @ 1 ml/m^2 in polluted waters. Study was carried out for one year.

The product was found effective for 10–12 days at an application dose of 0.5 ml/m^2 (5 litres per hectare) in clean water and 1 ml/m^2 (10 litres per hectare) in polluted waters with impact on adult densities.These results have shown that this new strain of bacterial larvicide is effective for use in larval control.

One year trial was completed on an Indian strain of *B. thuringiensis* (*Bti* AS, VCRC B17) being manufactured by Tuticorin Alkali Chemicals and Fertilizers and marketed by Godrej Hi-Care under the brand name of Tacbio. The objectives of the trial were: to evaluate the efficacy against mosquito larvae in different natural habitats, impact on adult mosquito density, persistence of biolarvicide in different breeding habitats and to assess the operational dose and its frequency of use. Field application with Tacbio showed that it is effective against immatures of anopheline and culicine mosquito species in different breeding habitats commonly encountered in urban areas. The application of Tacbio resulted in significant reduction in the larval density of III and IV instars ranging from 73–100% in different breeding habitats. (Figs. 72 and 73).

The persistence of Tacbio varies in different breeding habitats. The lowest field application dose of 0.5 ml/m^2 remains effective for about 10–12 days in clean unpolluted water bodies, which support anopheline breeding. However, in stagnant polluted waters such as drains and cesspools supporting

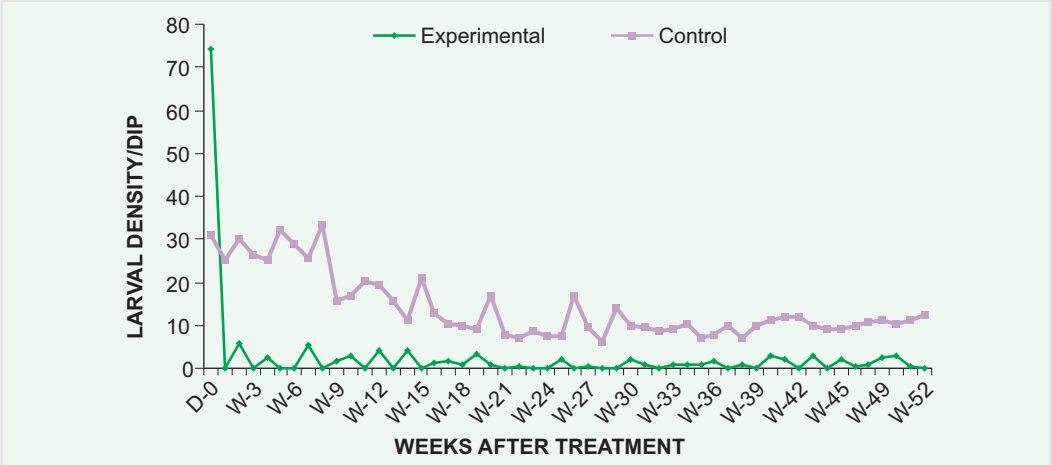


Fig. 72: Impact of Tacbio on the anopheline larval density in cement tanks applied @ 0.5 ml/m^2

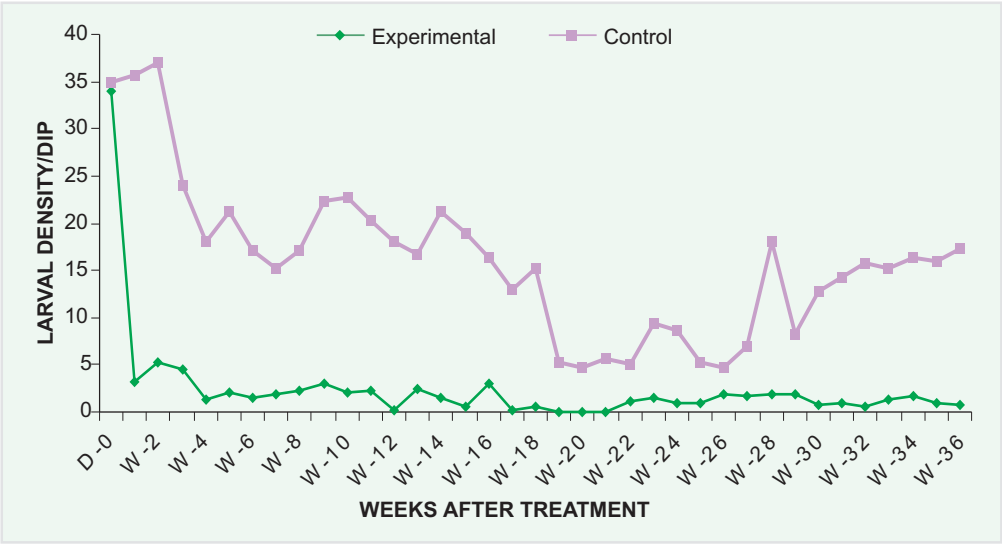


Fig. 73: Impact of Tacbio on the *Cx. quinquefasciatus* larval density in stagnant drains

culicine breeding, the same dose persists for 5–6 days only. A dose of 1 ml/m^2 in stagnant polluted waters was found to be effective for about 10–12 days in controlling culicine breeding. During intervention phase, the overall breeding positivity rate had come down to 15% as compared to 76% in the control area. There was a reduction of 69–86% in the adult mosquito density in comparison to control area.

VectoBac

VectoBac 12AS is a liquid formulation of *Bti* and was also found effective in the control of *An. stephensi, Ae. aegypti, Cx. quinquefasciatus* mosquitoes in a field trial. It was found operationally very feasible to apply in the field. Two more formulations of *Bti*, namely VectoBac WDG and VectoBac tablets, produced by M/s. Valent Bioscience U.S.A and supplied by M/s. Sumitomo Chemicals have been tested in the natural breeding habitats of different mosquito species in different areas. VectoBac WDG formulation was found to be effective against different mosquitoes @ 0.1 to 0.5 g/m^2 for one to three weeks in different habitats. Large scale trials have shown its efficacy @ 0.1 to 0.2 g/m^2 in clear and polluted water habitats when applied at fortnightly intervals.

Most recently a phase III trial of VectoBac WDG was conducted in Ahmedabad City to evaluate its efficacy and persistence against *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* in intradomestic and peridomestic mosquito breeding habitats. It was applied at the dose of 1.5 kg/ha in clear water (equivalent to 150 mg/m^2 area or 150 mg/100 litre water volume of containers) and 3 kg/ha in polluted water or peridomestic habitats (equivalent to 300 mg/m^2 area). Each application of VectoBac WDG was found to give effective control of larvae/pupae (70–100% mortality) up to three weeks in intradomestic containers and up to two weeks in peridomestic habitats (mortality, 80–100%). After two weeks of VectoBac WDG application, gradual declination in ovitrap positivity in treated area indicates reduction in prevalence of *Ae. aegypti*. For operational reasons, therefore, blanket spraying of VectoBac WDG every two weeks was found to produce effective control of mosquitoes in all kinds of habitats in urban and peri-urban settings.

Bacticide DT

A multicentric trial of Bacticide DT formulation was carried out in urban areas to see persistence in domestic and peridomestic breeding containers, such as desert coolers, containers, water tanks which are potential mosquito breeding habitats of *Ae. aegypti* and *An. stephensi* and also against *Cx. quinquefasciatus.* The study was carried out in urban/periurban areas of Raipur (Chhattisgarh), Hardwar (Uttarakhand) and Sonepat (Haryana) areas. The methodology was based on the common protocol developed by NIMR for evaluation of biolarvicide. The Bacticide DT (400 mg) was evaluated in natural breeding habitats of *Anopheles, Culex* and *Aedes* species. Application of one dispersible tablet (400

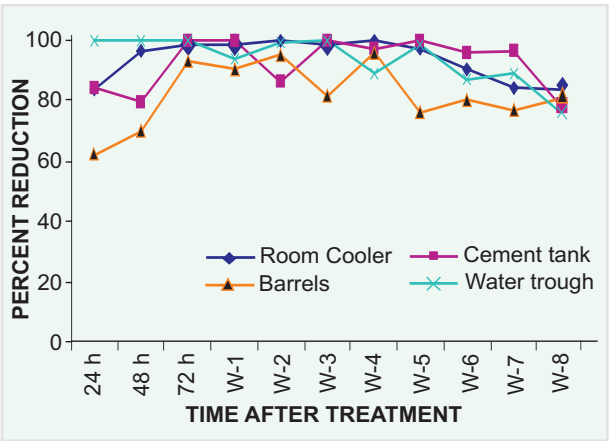


Fig. 74: Effect of Bacticide DT 400 mg on late instars of *Ae. aegypti* in different breeding habitats in Raipur

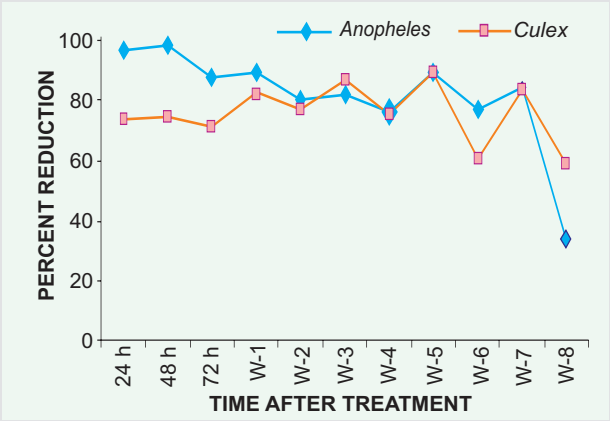


Fig. 75: Effect of Bacticide DT 400 mg on late instars of *An. stephensi* and *Cx. quinquefasciatus* in different breeding habitats in Sonepat

mg)/m² of water surface or one tablet per container with <50 litre of water produced >80% reduction of late instars up to two weeks . In container habitats of *Anopheles* and *Aedes*, where water was >20 litre, the effect was up to four weeks (>80% reduction) (Figs. 74–76). Bacticide DT formulation was more effective against *Aedes* compared to *Anopheles* and *Culex* species.

Bacticide WP

This study was carried out to evaluate the effectiveness of Bacticide WP formulation for control *of An. culicifacies, An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* in a locality as a multicentric trial. Bacticide WP was evaluated at the dose of 200 mg/m² in natural breeding habitats against *An. stephensi*, *An. subpictus*, *Cx. quinquefasciatus* at Raipur and Sonepat. It was also evaluated against *Ae. aegypti* in Raipur. In Mathura, it was evaluated against *An. culicifacies* and also against *Cx. quinquefasciatus*.

Results showed within a week maximum of 100 percent reduction of late instar larvae of target species *An. stephensi* in coolers, cemented tanks, *An.*

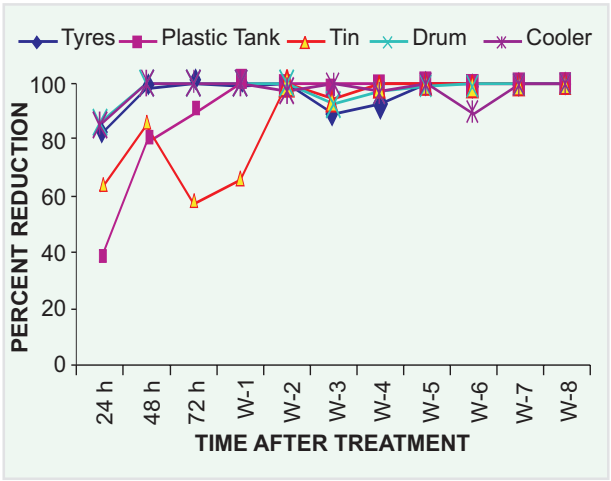


Fig. 76: Effect of Bacticide DT 400 mg on late instars of *Ae. aegypti* in different breeding habitats in Hardwar

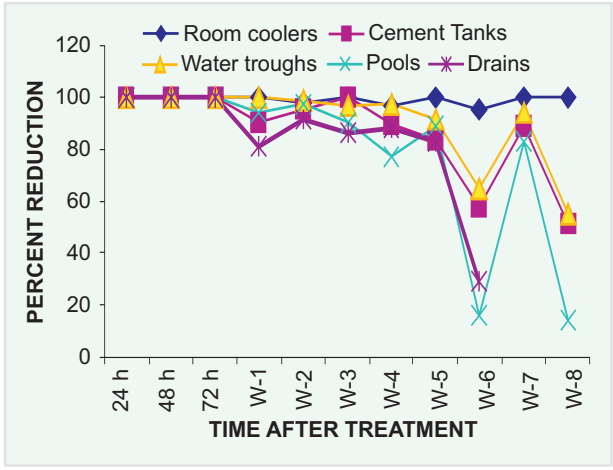


Fig. 77: Effect of Bacticide WP @ 200 mg/m² on late instars of anophelines in Raipur

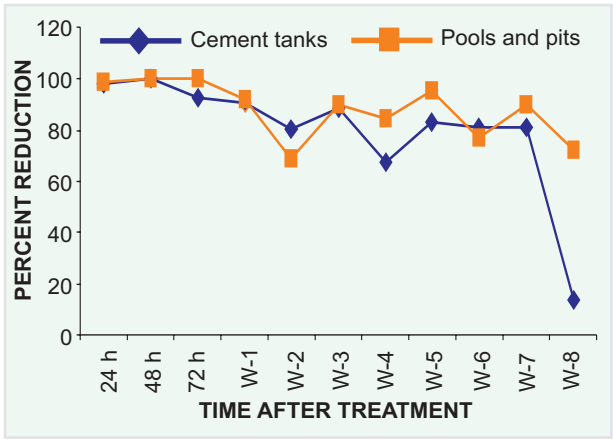


Fig. 78: Effect of Bacticide WP @ 200 mg/m² on late instars of anophelines in Sonepat

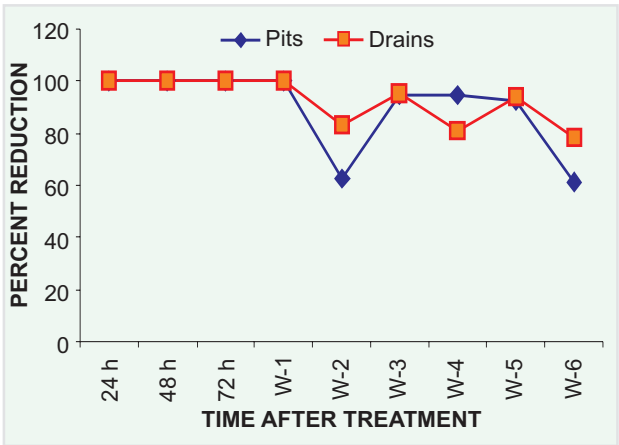


Fig. 79: Effect of Bacticide WP @ 200 mg/m² on late instars of anophelines in Mathura

subpictus in pits and pools, cemented tanks, clean water drains and *Ae. aegypti* in coolers during post-treatment period. Bacticide WP was effective (>80% reduction) in clean water small habitats for two weeks. (Figs. 77–79). Against *Cx. quinquefasciatus* in surface drains with organic matter, the reduction was >80% for seven days and in small containers such as coolers, tanks the effect was (>80%) for two weeks. In general, the formulation was more effective against *Aedes* compared to *Anopheles* and *Culex*.

❑

Evaluation of IGR Compounds

Insect growth regulators (IGRs) are chemical compounds that are highly toxic to insect larvae or pupae, interfering with their development into adults. IGR compounds can be categorised as (a) Juvenile hormone analogues that prevent the development of larvae into pupae or of pupae into adults and (b) Chitin synthesis inhibitors which interfere with moulting process and kill the larvae when they moult. IGRs have a very low toxicity to mammals, birds, fish and adult insects, but are highly toxic to crustaceans and immature stages of aquatic insects. They break down rapidly in the environment but may last for several weeks/months when applied ad granules, microcapsules or briquettes.

Hilmilin

Hilmilin is an insect growth regulator, highly effective against immature stages of mosquitoes and does not produce harmful effects on non-target organisms. Insecticidal activity of diflubenzuron, the active ingredient of Hilmilin is based on interference with the formation of chitin in insect cuticle, thus inhibiting the moulting.

Laboratory Evaluation

Hilmilin WP 25 and 22 SL formulations were dissolved in distilled water and laboratory reared larvae of *An. culicifacies, An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* were exposed to the Hilmilin and observations were made at 24 hours intervals till the emergence of adults and control was run concurrently. Four replicates of each concentration were tested. Different stages—larvae, pupae mosaics and incomplete emergence of adult was taken as dead pupae. LC_{50} and LC_{90} values were calculated as per standard procedure. Percent inhibition was calculated on the basis of untreated control run concurrently. Results of laboratory evaluation revealed that the Hilmilin formulations were highly effective against larvae of all the species tested. However, anopheline immatures were more susceptible than culicines. LC_{50} and LC_{90} values were 0.000869 and 0.0004775 (WP 25), respectively.

Field Evaluation

Tests were carried out in pools and unused wells against immatures of *An. culicifacies, An. stephensi* and *Cx. quinquefasciatus*. Hilmilin WP 25 and Hilmilin 22 SL @ 0.003 and 0.005 ppm, respectively were applied. The WP formulation was broadcast manually over the water surface, while 22 SL formulation was sprayed with the help of stirrup pumps. Observations were made till the emergence of adult. Field evaluation revealed that Hilmilin formulations showed varying degree of inhibition against mosquitoes. Cent percent inhibition of adult emergence was observed in *An. culicifacies* up to one week as against 95 and 93% in *An. stephensi* with two formulations. The persistence of the compound was also variable. The average percentage inhibition obtained @ 0.005 ppm WP 25 was 84.5, 83.16 and 75.16 up to 56 weeks against *An. culicifacies, An. stephensi* and *Cx. quinquefasciatus* as against 94.3, 92.0 and 82.5, respectively with 22 SL.

Diflubenzuron and Triflumuron

The IGR compounds Triflumuron and Diflubenzuron produced delayed impact (such as lesser pupal production and inhibition of adult emergence) at very low concentrations. Of the two products, Triflumuron displayed slightly higher toxicity against two mosquito species at EC_{50} level but at EC_{90} level, Diflubenzuron was slightly more toxic (EC_{90}= 0.0005 ppm) than Triflumuron (EC_{90}= 0.0024 ppm) against *An. stephensi*, while reverse was observed against *Ae. aegypti* (Table 3). At present IGR compounds are well in use as they do not pose any hazard to mankind and other wild life. These are also non-toxic against fish and can be used for prolonged antilarval effect.

Field evauation

Both the formulations supplied by M/s. Crompton Uniroyal Chemicals Asia Pacific Pvt. Ltd., Mumbai, were tested for bioefficacy against immatures of *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* vectors of urban and rural malaria, dengue and dengue haemorrhagic fever (DHF) and filariasis respectively in small field condition in the respective habitats of different species in and around Delhi.

Dilmilin TB-2% was applied in contained water habitats such as tanks and moderately polluted small pools on volume basis and Dimilin GR-2 formulation was applied on the surface area basis. Water sample from treated habitats was brought to the laboratory

Table 3. Toxicity of insect growth regulators formulation against *An. stephensi* and *Ae. aegypti*

IGR formulation	Concentration in ppm (mg/l)		
	LC/EC values	*An. stephensi*	*Ae. aegypti*
Diflubenzuron (25% WP)	EC_{50}	0.0002	0.0004
	EC_{90}	0.0005	0.0033
Triflumuron (48% SC)	EC_{50}	0.0001	0.0002
	EC_{90}	0.0024	0.0026

on the same day and after three days and later at an interval of one week. Laboratory colonised late III instar larvae were introduced in subsequent weeks till the complete pupation or adult emergence under controlled conditions. Also late instar larvae collected from the treated habitats were observed till the complete pupation or adult emergence under controlled conditions. Percent inhibition was calculated on the basis of untreated control run concurrently.

Dilmilin TB-2%, applied at half tablet and 1 tablet per 40 litre water (equivalent to 0.5 and 1 ppm) produced complete inhibition of the development of pupae and emergence of adult mosquitoes of *An. stephensi, Ae. aegypti* and also *Cx. quinquefasciatus* up to four weeks of observation in tanks with clear water, and *Cx. quinquefasciatus* in moderately polluted small pools.

The efficacy of Dimilin GR-2 formulation in cement tanks when treated @1.5 kg/ha also showed 100% inhibition of *An. stephensi*; *Ae. aegypti* and *Cx. quinquefasciatus* up to four weeks. However, at lower dose, i.e. 1 kg/ha, 100% inhibition was observed against *Cx. quinquefasciatus* up to three days only, whereas against *An. stephensi* and *Ae. aegypti* only 97 and 96% inhibition was observed

Field evaluation of Dimilin GR-2 against *Cx. quinquefasciatus* larvae in pools produced 100% inhibition of adult emergence up to two weeks @ 1.5 kg/ha but at lower doses only 86 and 89% inhibition was observed on Day 3 itself which declined to 73 and 81% after four weeks.

The study revealed that the tablet and granule formulations of Dimilin were effective in the prevention of adult emergence of mosquitoes even at very low dosages. Of the three species tested, *An. stephensi* was most susceptible followed by *Ae. aegypti* and *Cx. quinquefasciatus*. ❑

Evaluation of Repellents, Herbal and Fungal Products

Insecticidal and repellent properties of some plants to mosquitoes are well-known. Phytochemicals obtained from huge diversity of plant species are an important source of safe and biodegradable chemicals, which could be screened for mosquito repellent and insecticidal activities.

Neem Oil as Mosquito Repellent

Azadirachta indica, commonly known as neem in India, has been used in various ways since ancient times. Dried neem leaves are commonly used in villages for protection against infestation of stored grains and other products by insects. Smoke produced by burning of neem leaves is used for the protection against mosquitoes. Neem oil has also been used in various insecticidal and medicinal preparations, but its mosquito repellent activity is not known. Therefore, systematic studies were undertaken at NIMR to see the mosquito repellency of neem oil.

Topical Applications

Repellent action of neem oil was evaluated against different vector species of malaria—*An.culicifacies, An. stephensi, An. minimus, An. fluviatilis and An. sundaicus* in the villages of Mandla district (Madhya Pradesh), Ghaziabad district (Uttar Pradesh) and Hardwar district (Uttarakhand), Kheda

Table 4. Repellent activity of various herbal products against mosquitoes and sandflies

Plant species	Plant product	Species tested	% Protection	References
Azadirachta indica	5–40% Neem oil-mixed with coconut/ mustard oil as topical application	*An. culicifacies*	80–100	Sharma *et al* 1993a
		Cx. quinquefasciatus	61–100	Mishra *et al* 1995
		Ae. aegypti	85	Sharma *et al* 1995
				Rajnikant and Bhatt 1994
		Phlebotomus papatasi	97.6	Dhiman and Sharma 1994
		Phlebotomus argentipes	100	Sharma and Dhiman 1993
	5% Neem oil in a cream-base topical application	*Ae. aegypti*	84	Dua *et al* 1995
		Ae. albopictus	78	Singh *et al* 1996
		Anopheles spp	93–100	
		Culex spp	89–94	Nagpal *et al* 2001
	5-10% Neem oil-impregnated on mats (Vapours)	*Anopheles* spp	98	Sharma *et al* 1993
		Cx. quinquefasciatus	63	
	1% Neem oil in Kerosene oil (Smoke)	*An. culicifacies*	99–100	Sharma and Ansari 1994
		Culex spp	79–81	Valecha *et al* 1996
Cymbopogan spp	Oil as topical application	*An. culicifacies*	99–100 for 10 h	Ansari and Razdan 1995
		Cx. quinquefasciatus	95–97 for 6 h	
Lantana camara	Methanol + Coconut oil extract	*Ae. albopictus*	94 for 2 h	Dua *et al* 1996
		Ae. aegypti	50 for 4 h	
Mentha piperita essential oil	Steam distilled	*An. annularis*	100	Ansari *et al* 1999
		An. culicifacies	92	
		Cx. quinquefasciatus	85	

Use of Expanded Polystyrene Beads for Mosquito Larval Control

To find a new technology for control of immature stages of mosquitoes, the expanded polystyrene (EPS) beads were evaluated in the laboratory for mosquito control (Sharma 1994). The EPS beads are the expanded form of polystyrene granules which are produced indigenously and are available commercially as hard translucent glass-like beads with diameter ranging from 0.6 to 2.5 mm. The unexpanded beads contain an expanding agent. When exposed to super heated steam, they expand about 35 to 40 times of their original volume and are thus named as expanded polystyrene (EPS) beads. EPS beads are used to produce thermocol sheets for insulation, packaging materials, ice boxes, etc. The raw material for EPS beads is easily available. The size of expanded beads generally suitable for application in mosquito breeding habitats is of 2 to 4 mm in diameter. As the volume of expanded beads is enormous (1 kg of expanded beads equals to approximately 57–60 litres of water-volume), their transportation becomes a problem. The raw material is 35 to 50 times less voluminous than the expanded beads. To overcome the difficulty of transportation of large volumes of expanded beads, NIMR has designed and fabricated a machine mounted on a truck for on-site expanding of raw granules into EPS beads.

The EPS beads are light in weight, inert, non-toxic, non-wettable and resistant (do not interact) to sea water, salt solutions, soap and wetting agents. Direct exposure to intense sunlight can turn them yellowish and brittle due to ultra-violet radiation. These are not acted upon by any micro-organisms, are non-biodegradable and remain for years on the surface of water on a single application. Being light in weight, the EPS beads when applied @ 500 g to 1 kg/m^2 in different habitats, float on water surface in several layers. Since mosquitoes lay eggs only on the water surface, the physical barrier formed by the floating blanket of EPS beads prevents them in doing so. Further, the immature stages of mosquito trapped under the layer of the beads die of suffocation and mosquitoes do not emerge from the treated habitats.

Application of EPS beads in unused wells

Field Evaluations of Expanded Polystyrene (EPS) Beads

The EPS beads were extensively evaluated at Nadiad (Gujarat), Shahjahanpur (Uttar Pradesh), Hardwar (Uttarakhand) and Chennai (Tamil Nadu) field units of the National Institute of Malaria Research for control of mosquito breeding in habitats such as wells, overhead tanks, underground tanks, sluice-valve chambers, chocked manholes and tanks of biogas plants (Sharma *et al* 1985; Chandrahas and

Sharma 1987). These studies showed that the EPS beads provide control of mosquito breeding on long-term basis.

The trials conducted at different places showed that the mosquito breeding habitats suitable for application of EPS beads are confined and stagnant permanent/semi-permanent water bodies, water collections that can not be drained off, water surfaces not subjected to wind currents (as slight breeze can drift them away exposing the water surface for mosquito oviposition), habitats not interfered by humans or animals, deep quarry pits not exposed to wind, permanent underground water collections, temporary rainwater pools, cisterns and unused wells.

The technology of EPS beads has not been used on an operational scale in the country so far. There is a good scope of using this approach in a variety of situations in urban, industrial and rural areas as part of an integrated vector management strategy for control of mosquito vectors, breeding in a wide variety of man-made, large-size containers/habitats which are not usually amenable for cleaning regularly such as large industrial tanks, domestic cisterns, wells, septic tanks, etc. ❑

EPS beads making machine mounted on a truck

Information, Education and Communication

Health Education

NIMR has been involved in health education activities for creating awareness among various communities and school children. Messages regard-ing malaria and its control are conveyed to the communities through various means like print media and electronic media.

Some of the major activities which have proved successful are: health education camps, exhibitions in many languages, live demonstrations, door-to-door campaign, distribution of pamphlets and folders, slide shows, video films, popular lectures, radio talks, telecast on TV, debates and painting competitions by students on malaria related topics. Health education messages are also spread through various activities during celebration of National Science Day and observance of Anti Malaria Month. Several brochures, flyers and posters were designed and developed for health education and awareness activities.

Audio-Visual Programme Unit

Audio-Visual Programme Unit was established at the Institute for producing video films on health education, teaching programmes, documentaries, scientific discussions and mosquito control activities. The Unit has made several films on various aspects of malaria as listed. The video films are in great demand and several copies of these films have been sold to educational institutions, government health departments, NGOs etc.

Video Films Produced

Documentaries

1. Fighting Malaria (English) (2000) Duration – 18'
2. Malaria Control in Shahjahanpur (English) (6003) 14' 47"
3. Malaria Control in Shahjahanpur (Hindi) (6001) 14' 47"
4. Defeating the Invincible—Hardwar (English) (6004) 22'
5. A Seven Point Action Programme for Malaria Control in Madras (English) (2010) 17' 27"
6. A Seven Point Action Programme for Malaria Control in Madras (Tamil) (2208) 17' 6"
7. Tackling Malaria in Orissa (English) (2011) 16' 17"
8. Insecticide Impregnated Bednets for Malaria Control (Assamese) (2008) 19' 39"
9. Insecticide Impregnated Bednets for Malaria Control (English) (2006) 19' 39"
10. Insecticide Impregnated Bednets for Malaria Control (Hindi) (2061) 19' 39"
11. Man-made Malaria (English) (2002) 12' 20"
12. *Sirf Ek Muskan* (Hindi) (2018) 17' 12"
13. *Ek Anootha Prayog* (Hindi) (2003) 19' 51"
14. Malaria Control in Madras (English) (2153) 11' 37"
15. Man, Mines and Malaria (English) (2018) 7' 37"
16. Mosquito Menace (English) (6049) 30' 29"
17. Mosquito and the Neem (English) (3015) 15' 49"
18. Konkan Railways – No Ticket for Mosquitoes (English) (3096) 14"

Teaching Programmes

1. Life Cycle of Malaria Parasite (English) (2247) 11'
2. The Microscope (English) (2240) 14' 49"
3. How to Treat Uncomplicated Malaria (English) (6045) 25' 58"
4. Cerebral Malaria (English) (2200) 8' 57"
5. Malaria in Pregnancy (English) (6060) 23'
6. Laboratory Diagnosis of Malaria (English) (6060) 29' 53"
7. Morphology of Mosquitoes (English) (2474) 16'
8. Breeding Sites of Mosquitoes (English) (2533) 10'

Health Education

1. Malaria—Bednets a TV Spot (Hindi) (2013) 56''
2. Malaria—Bednets a TV Spot (English) (2072) 1' 2"
3. Malaria—Spread the Knowledge (English) (2071) 7' 23"
4. *Malaria—Mukti Pavoo* (Hindi) (2236) 5' 9"
5. Malaria—Arivay Parappivoo (Tamil) (2214) 7' 10"
6. Malaria—Gnanava Haradona (Kannada) (2261) 9' 15"
7. Malaria—Overhead Tanks and Malaria Control–A TV Spot (Tamil) (2282) 1' 18"

Scientific Discussions

1. Synthetic Malaria Vaccine: A Hope for Future (English) (2121) 15' 6"
2. Malaria Vaccine: A Perspective (English) (2204) 18' 57"
3. Malaria Vaccine: A State of Art (English) (2122)

17' 38''

4. Malaria Vaccine: Status and Future Prospect (English) (2211) 19' 26''
5. Dengue Fever (Hindi) (2421) 7' 52''
6. *Dengue Bukhar* (2422) 3' 32''
7. M-10: A New Environment Friendly Insecticide for Disease Vector Control (English) (2212) 16' 46''
8. Global Malaria Control—An Approach Plan (English) (2275) 21' 28''
9. Chelating Agent in Severe Malaria (2140) 17' 45''

Health Education through Folk-theatre (Kalajatha)

Kalajatha is a popular, traditional art form of folk-theatre which depicts various life processes of a local socio-cultural setting. This effective medium of mass communication was carried out in malaria endemic villages in Karnataka in 2001. Thirty local artists, NGOs, health staff, NIMR, members from Panchayat participated in this programme. Messages conveyed through Kalajatha were very effective in imparting community mobilization for fish-based malaria control programme. ❑

Publications

Periodicals

Journal of Vector Borne Diseases (*Formerly *Indian Journal of Malariology)

The Institute has been publishing a quarterly journal on vector borne diseases. This journal superceded the *Indian Journal of Malariology* in 2003. The *Indian Journal of Malariology* was started in 1947 by the Malaria Institute of India with an aim to disseminate scientific information to ongoing malaria control programmes and research. The journal was discontinued in 1963 due to inadequate interest and poor response from the scientific community. However, in the wake of widespread resurgence of malaria, research on malaria was started at many centres in the country. There was, therefore, a felt need of a journal exclusively on malariology. The *Indian Journal of Malariology* was revived by the Malaria Research Centre in 1981 after 17 years of its discontinuation with the following objectives: to disseminate the scientific information on malaria to provide support to the ongoing malaria control programme and research; to promote the interest in malaria research; and to meet the growing demand for publication of journal exclusively devoted to basic, applied and operational research in the field of malariology. The journal is devoted to original research communications on all aspects in the field of vector borne diseases such as malaria, filaria, Japanese encephalitis, dengue, dengue haemorrhagic fever, leishmaniasis, trypanosomiasis and other vector borne diseases and their control. The journal also publishes book reviews and letters to the editor, inviting frank scientific opinion on the papers published in the journal. The *Journal of Vector Borne Diseases* is indexed by PubMed, BIOSIS, Drugs and Pharmaceuticals Current Indian Titles, R & D Highlights, Industry Highlights, EMBASE/ Excerpta Medica, Indian Science Abstracts, Protozoological Abstracts, Quarterly Bibliography of Major Tropical Diseases, etc.

The *Journal of Vector Borne Diseases* (JVBD) stood at 10th rank in country-wise ranking of Biomedical Journals in India as per the latest rank ings provided by SCImago Journal Rank (SJR) in 2006. The SJR of JVBD is 0.081.The articles publish-ed in JVBD can be openly accessed through www.mrcindia.org/journal. This journal has been included in Directory of Open Access Journals (DOAJ).

***Malaria Patrika* (Hindi)**

The results of scientific findings are usually published in English which do not reach to common man due to illiteracy and also lack of awareness and command on English language among the majority of the population in India. It has been realized that malaria control is everybody's concern. In order to disseminate the basic knowledge about the components and conditions, spreading of malaria and recent scientific developments taking place in the field of malaria control, it was thought prudent by the Institute to publish *Malaria Patrika* in Hindi because Hindi is the National language of India. Therefore, *Malaria Patrika* was launched in 1993 with 4 issues in a year—March, June, September and December in order to disseminate the scientific information in the form of popular, non-technical with common vocabulary of language, so that common man can understand the intricacies of science of malaria. If necessary, technical words in English are used in Devnagiri script. In addition to scientific articles, national and international news on malaria and Institute's activities are also included to create awareness among the community. Publication of the patrika has been applauded by the people from different walks of life—students, village leaders, NGOs, teachers, government departments, etc.

***Plasmodium* (English & Hindi)**

NIMR has also been bringing out a biannual Newsletter since January 2006 in both English and Hindi. The newsletter is informative on the research activities of the Institute, publications, latest ongoing research on malaria, other activities of the Institute, etc.

Books

Published by NIMR

1. *The Anophelines of India* by T. Ramachandra Rao (Revised edition), published by Malaria Research Centre (ICMR), Delhi (1984)

2. Seroepidemiology of Human Malaria: A Multicentric Study (1989)
3. Indigenous Larvivorous Fishes of India by Prof. A.G.K. Menon (1991)
4. Community Participation in Malaria Control edited by Dr. V.P. Sharma (1993)
5. A Profile of Malaria Research Centre (2002)
6. Malaria Parasite Bank: A National Repository (2004)
7. Protocols for Uniform Evaluation of Insecticides for use in Vector Control (2005)
8. Integrated Disease Vector Control Project: A Profile (2007)

Authored by NIMR Scientists

1. Indian Anophelines by B.N. Nagpal and V.P. Sharma, published by Oxford & IBH Publishing Co., New Delhi (1995)
2. Anopheline Species Complexes of Southeast Asia by Sarala K. Subbarao, published by World Health Organization, Regional Office for Southeast Asia Region, New Delhi in 1998 (WHO Technical Publication, SEARO No. 18)

Proceedings

1. Indo-UK Workshop on Malaria: Proceedings of the Workshop held at ICMR, New Delhi, 14–19 November 1983 (1984)
2. Forest Malaria in Southeast Asia: Proceedings of an Informal Consultative Meeting, WHO/MRC (1991)
3. Community Participation for Disease Vector Control: Proceedings of the ICMR/WHO Workshop to Review Research Results, 3–9 February 1986 (1986)
4. Larvivorous Fishes of Inland Ecosystem: Proceedings of the MRC-CICFRI Workshop, New Delhi, 27–28 September 1989. Editors V.P. Sharma and Apurba Ghosh (1994)
5. Proceedings of Drug Policy Workshop (2006)

Reprints

1. Malaria Incidental to Engineering Construction by Lt. Col. Jaswant Singh and I.M. Puri (1989)
2. Malaria in Bombay 1928 (1990)

Manuals/Souvenirs

1. Manual for Community Health Workers on Diagnosis and Treatment of Pesticide Poisoning (1991). UNDP/WHO-SEARO Project on Safety and Control of Toxic Chemicals and Pollutants in collaboration with MRC
2. Manual for Trainers of Community Health Workers on Diagnosis and Treatment of Pesticide Poisoning (1991). UNDP/WHO-SEARO Project on Safety and Control of Toxic Chemicals and Pollutants in collaboration with MRC
3. Guide for Village-level Malaria Workers (1992)
4. *Grameen Stariya Malaria Karyakartaon ke Liye Margdarshika* (1992)
5. Souvenir for International Symposium on Challenges in Malaria and Prospects for Research (2002)
6. Pictorial Identification Key for Indian Anophelines (2005)
7. IX International Conference on Vectors and Vector Borne Diseases (2008)

Folders

1. Bioenvironmental Control of Industrial Malaria: A Systems Approach (1987)
2. Malaria and Tribal Health: An Integrated Approach (1987)
3. Bioenvironmental Control of Malaria: A Holistic Approach (1989)
4. *Malaria Ki Rokthaam* (*Jaiv-Paryavaran Paddatti*)*: Ek Anootha Prayog* (1989)
5. Bioenvironmental Control of Malaria [Regional Language] Assamese (1989)
6. Bioenvironmental Control of Malaria [Regional Language] Gujarati (1989)
7. Bioenvironmental Control of Malaria [Regional Language] Oriya (1989)
8. Bioenvironmental Control of Malaria [Regional Language] Tamil (1989)
9. The Kheda Experiment : An Innovative Approach (1989)
10. Malaria Control in Kumaon Foothills (1990).
11. Shankargarh: Malaria Control by Bioenvironmental Methods (1990)
12. *Shankargarh: Jaiv Paryavaran Paddatti dwara Malaria ki Rokthaam* (1990)
13. Bioenvironmental Control of Malaria: A Holistic Approach [Revised] (1992)
14. *Jaiv-Paryavaran Paddatti dwara Malaria Niyantaran: Ek Anootha Prayog* (Revised) (1992)
15. Industrial Malaria Control– Bioenvironmental Approach (1992)
16. Malaria Education Campaign for School Children (1993)
17. Insecticide Spray Strategy for the Control of *An. culicifacies*: The Rural Malaria Vector (1996)
18. Expanded Polystyrene (EPS) Beads to Control Mosquito Breeding (1996)
19. Neem Oil Mosquito Repellent and Larvicide (1996)
20. Major Vectors of Malaria in India (1996)
21. Quick Identification Key for Indian Anophelines (1996)
22. Biological Charts Folder (1996)
23. Biological Control of Mosquitoes for Prevention of Malaria (1996)
24. Malaria Research Centre: Mandate, Achievements and Opportunities (2003)
25. *Malaria Anusandhan Kendra: Uddeshya, Upalabdhiyan aur Avsar* (2003)
26. Expanded Polystyrene Beads for Mosquito Control (2009)

Brochures

1. Information on *Indian Journal of Malariology* (1986)
2. Announcing Quarterely Publication of IJM (1988)
3. Insecticide Impregnated Bednets—Illustrated (1992)
4. 7-Point Action Plan for Malaria Control in Madras City (1992)
5. National Malaria Control Strategy (1994)
6. National Malaria Control Strategy (Revised) (1995)
7. Larvivorous Fishes in Mosquito Control—Illustrated (1995)
8. Insecticide Impregnated Bednets (Revised) (1996)
9. 7-Point Action Plan for Malaria Control in Urban Areas (1996)
10. Pyrethroid Impregnated Mosquito Nets : Protection from Mosquitoes and Malaria (1996)
11. Biolarvicides: *Bacillus thuringiensis* var. *israelensis* Serotype H-14 (1996)
12. Malaria: Diagnosis and Treatment Illustrated (1996)
13. Cummulative Index 1981–95 (1996)
14. Roll Back Malaria: Asian Concept (1998)
15. *Rajbhasha Karyanvayan Sambandhi Sandarbha Pustika* (2005)
16. Journal of Vector Borne Diseases: Information Brochure (2006)
17. Larvivoroush Fish for Mosquito Control (2009)
18. Insecticide Treated Nets,kong-kasting nets and Materials for Malaria Control (2009)
19. *Anopheles culicifacies* and *An. fluviatilis* Complexes and their Control (2009)
20. Biolarvicides for Mosquito Control (2009)

Flyers

1. *Malaria Ki Rokthaam: Aassan Tarike* (Hindi) (1991)
2. Save Yourself from Malaria (2001)
3. *Malaria Se Bachiye* (2001)

Coloured Biological Charts

1. Life-Cycle of *Plasmodium falciparum*
2. Life-Cycle of *Plasmodium vivax*
3. Life-Cycle of *Plasmodium malariae*
4. Fever Periodicity in Malaria Infection
5. Differences between various Developmental Stages of *Anopheles, Aedes* and *Culex* Mosquitoes

Reports

1. Malaria Research Centre—Annual Reports (1977–2008)
2. Annual Progress Report of Science and Technology Project on Integrated Disease Vector Control of Malaria, Filaria and other Vecor-borne Diseases (1986–2008)
3. In-depth Evaluation of the Community-based Integrated Vector Control of Malaria Project in Kheda, Gujarat (October 1987)
4. Action Plan for Delhi Metropolis (1989)
5. 7-point Action Plan to Control Malaria in Madras (1989)
6. Progress Report of Biolarvicides in Vector Control: *Bacillus sphaericus/thuringiensis* (1990)
7. Progress Report of Biolarvicides in Vector Control (1993)
8. Mosquito Control in Bangalore City: Geographical Reconnaissance (GR) Report of a Pilot Study Area, MRC, Bangalore March (2001)
9. Final Report of Situational Analysis of Malaria in District Aizawl West, Mizoram under Roll Back Malaria Initiative (2001)
10. Final Report of Situational Analysis of Malaria in District Jodhpur, Rajasthan under Roll Back Malaria Initiative (2001)
11. Final Report of Situational Analysis of Malaria in District Tumkur, Karnataka under Roll Back Malaria Initiative (2001)
12. Final Report of Situational Analysis of Malaria in District Keonjhar, Orissa under Roll Back Malaria Initiative (2001)
13. Final Report of Situational Analysis of Malaria in Goa state under Roll Back Malaria Initiative (2001)
14. Evaluation of the Impact of DDT and Malathion Indoor Residual Spraying being used in Malaria and Kala-azar Programmes on the Disease Prevalence, MRC, Delhi June (2002)
15. Larvivorous Fishes for Mosquito Control [Gujarati] (2002)
16. Action Plan for Management of Malaria and Dengue in Ahmedabad City (2002).
17. In-depth Review on Malaria for National Vector Borne Disease Control Programme 2006–07 (2007).

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Trainings Organized

Short-term Trainings

1. Training in Malaria Control to the Community Workers of Mapusa Municipality at NIMR Field Unit, Goa on 24 November 1992.
2. Training in Malaria Control to Jr. Red Cross Counsellors at NIMR Field Unit, Goa on 5 December 1992.
3. Orientation Training in Remote Sensing of Leishmaniasis was imparted to two Scientists of RMRI (ICMR), Patna from 19–30 June 1995.
4. Malaria Microscopy Training to the Laboratory Technicians/Assistant Malaria Officers of Gujarat at Nadiad from 8–12 January 1996.
5. Capsule Training Course on Malaria Entomology for the staff of Surat Malaria Control and Research Project, organized by NVBDCP and British Council Division (U.K.) at Surat, Gujarat from 6–7 March 1996.
6. Trainings in Mosquito Control to the Construction Supervisors and Workers at NIMR Field Unit, Goa in March and May 1996.
7. Training Course on Field Application of Biolarvicides held at NIMR, Delhi from 10–12 June 1996.
8. Training in Malaria Control to Jr. Red Cross Counsellors at NIMR Field Unit, Goa from 28–29 November 1996.
9. Training in Malaria Microscopy to the staff of PHC Banavara, Hassan, Karnataka in 1996.
10. Laboratory and Field Training to the Volunteers of Rushi Smruty Vidya Kendra, Goa in 1997.
11. Training in Malaria Parasitology to the Medical Laboratory Technicians at NIMR Field Unit, Nadiad, Gujarat in March 1997.
12. Training Programmes on Dengue for the Medical Officers of Bangalore City Corporation, held at Bengaluru in April 1997.
13. Trainings in Malaria Entomology to the Staff of Surat Malaria Control and Research Project at Surat from 21–30 April 1997 and at Nadiad from 5–10 May 1997.
14. Training in Malaria Entomology to the Staff of MCRP, Surat at Nadiad from 9–10 May 1997.
15. Training in Malaria Microscopy to the Technicians from different PHCs of Surat and Surat Municipal Corporation from 12–20 May 1997.
16. Training in Bionomics of *Aedes* Mosquitoes and Control of Dengue for MCD Officials at Delhi from 26 May to 6 June 1997.
17. A Practical Training on Malaria Microscopy to the Laboratory Technicians of N.F. Railways at NIMR Field Unit, Goa from 16–20 June 1997.
18. Training in Malaria Control to Jr. Red Cross Counsellors at NIMR Field Unit, Goa from 19–21 June 1997.
19. One day Training Programme on Vector Biology and Mosquito Control for the Students of M.Sc., Deptt. of Zoology, Loyola College, Chennai on 25 June 1997.
20. Training/Refresher Course on Malaria Diagnosis for Laboratory Technicians on 28 June 1997 in collaboration with ARMA Medical Foundation, Regional Office for Health and Family Welfare, Chennai and the Directorate of Public Health and Preventive Medicine, Govt. of Tamil Nadu.
21. Training in Malaria and its Control to State Officials at NIMR Field Unit, Goa on 19 September 1997.
22. The Orientation Course for the students of M.Sc. (Medical Entomology) and PG Diploma, VCRC, Puducherry at Delhi from 5–14 April 1998.
23. Training in Insecticide Bioassay to the staff of Surat Malaria Control and Research Project at Surat from 30 November to 6 December 1998.
24. Malaria Microscopy Training for the Laboratory Technicians of Gujarat at Nadiad, Gujarat from 8–12 March 1999.
25. Govt. of Gujarat sponsored Training on Field Application of Biocides to the District Medical Officers at Nadiad, Gujarat on 27 July 1999.
26. Training in Malaria Microscopy to the Laboratory Technicians at Nadiad, Gujarat from 20–24 December 1999.
27. Training Course on Vector Control for the PG Students of Public Health Entomology, Institute of Vector Control and Zoonosis, Hossur, at Delhi from 19–23 February 2000.
28. One day Training Course on Malaria Diagnosis and Treatment Aspects at Guwahati on 11 February 2003.
29. Training on Vector Control in Advance Level Training Programme for Laboratory Technicians on 1 October 2004
30. A Training on Indoor Residual Spraying for Block Health Officers of Kheda and Anand districts at Field Unit, Nadiad on 15 July 2005.

31. Three Training Courses for Entomologists/ Biologists under the World Bank supported Enhanced Malaria Control Project from 31 January to 18 March 2005.
32. Two Capsule Training Courses in Malaria Microscopy for newly recruited Laboratory Technicians at NIMR Field Unit, Nadiad from 12–16 and 18–22 July 2005.
33. Two Trainings on Malaria Treatment for the MBBS students of PS Medical College at NIMR Field Unit, Nadiad in July 2006.
34. A Health Education-cum-Training Programme for the Orissa State Armed Police for Identification of Breeding Habitats of *Aedes* sp in the Township of Orissa at Rourkela on 30 August 2006.
35. A 3-day Training in Malaria Surveillance and Treatment at Nadiad in February 2006.
36. Two Training Courses for Technicians of Municipal Corporation from 8–12 and 15–19 April 2006 at NIMR, Delhi.
37. Five Training Courses on Quality Assurance of RDT and Malaria Microscopy at NIMR, Delhi during January to February 2007.
38. A Training Programme on Malaria by NIMR Field Unit, Goa from 16–26 April 2007.
39. A Training Programme for 12 Volunteers of a Local NGO on Vector-borne Diseases on 9 October 2007.
40. Two Training Courses on Malaria and other Vector-borne Diseases at NIMR Field Unit, Jabalpur in January and February 2008.
41. Six reorientation Training Courses organized for Laboratory Technicians on Malaria Microsocopy in collaboration with Commissionerate of Health, Govt. of Gujarat at NIMR Field Unit, Nadiad in February and March 2008.

Long-term Trainings

1. MRC-NAMP Training Course in Malariology for the District Public Health Officers from Nepal, at Delhi from 12 April to 11 June 1993.
2. WHO sponsored Training for Bangladesh Health Officers at Delhi with field visit to Patna from 21 November to 17 December 1994**.**
3. Training in various aspects of Bioenvironmental Control of Malaria to the Assistant Entomologists, NVBDCP, Mysore Zone at Chennai from 2–23 May 1996.
4. Training Course in Entomology to the District Malaria Officers/Assistant Malaria Officers at Alwar and Haldwani from 12 January to 20 February 1998.
5. WHO Sponsored Training Course in Vector Borne Diseases for Health Officials from Bangladesh at Delhi from 29 June to 28 July 1998.
6. Training Course in Malaria Entomology for the Entomologists/Biologists of the country at Delhi from 17 August to 26 September 1998.
7. Training Course in Malaria Entomology for the Entomologists/Biologists of the Country at Delhi from 10 January to 18 February 2000.
8. NAMP/World Bank Sponsored Training Course on Comprehensive Vector Control (CVC) for Biologists and Entomologists from 13 February to 27 March 2001.
9. A 15-days Entomological Training on the request of the Directorate of Health Services and Deputy Director, Malaria, NVBDCP, Port Blair in 2002.
10. Seven Training Courses (each for three days) for PHC Medical Officers by National Institute of Malaria Research (NIMR) and National Vector Borne Disease Control Programme (NVBDCP) under the Enhanced Malaria Control Project from 27–29 January; 3–5, 9–11 and 23–25 February; 9–12 and 24–26 March 2004.
11. Seven Courses for Medical Officers of MCD, NDMC, Railways and Armed Forces under EMCP, jointly organized by NIMR and NVBDCP at NIMR, Delhi from January to March 2004
12. Three Training for Entomologists/Biologists jointly organized by NIMR and NVBDCP during January to March 2005.
13. A 5-day Training on Preparation of Blood Smear for Diagnosis, Life Cycle of Malaria Parasites and Mosquitoes for Student of General Nursing School, Nadiad.
14. Training Course on Malaria in Pregnancy in India: Research Coordination Meeting organized by NIMR at Pusa, New Delhi from 10–11 April 2006.
15. Two Training Courses for Laboratory Technicians of MCD organized by NIMR at NIMR, Delhi form 8–12 and 15–19 May 2006.
16. Five Training Courses on Quality Assurance of RDT and Malaria Microscopy for Laboratory Technicians organized by NIMR during 2–6, 15–19, 22–27 January; and 5–10 and 12–16 February 2007.
17. Training Course for Entomologists/Biologists organized by NIMR and NVBDCP at NIMR Delhi from 19 February to 16 March 2007.
18. Reorientation Training Programme for Insect Collectors of Antimalaria Operations—MCD in 2008. ❑

Conferences and Workshops Held

1. Workshop for Engineers and Architects, held at Delhi from 19–24 September 1988.
2. Workshop for Engineers and Architects, held at Delhi, Hardwar and Haldwani from 4–29 October 1988.
3. Workshop for Engineers and Architects, held at Delhi on 27 January 1989.
4. Workshop for Engineers and Architects, held at Goa and Mumbai from 13–17 February 1989.
5. Workshop for Engineers, held at Chennai from 18–20 February and 15–17 May 1989.
6. Workshop for Engineers, held at Chennai from 25–28 July 1989.
7. Workshop for Engineers, held at Chandigarh from 1–4 August 1989.
8. MRC/CICFRI Workshop on Larvivorous Fishes of Inland Ecosystem, held at New Delhi from 27–28 September 1989.
9. Workshop on Cytotaxonomic Techniques in Mosquitoes, held at New Delhi from 1–15 October 1994.
10. WHO sponsored Workshop on Engineering and Environmental Management Methods of Malaria Control, held at Delhi from 28–30 November 1994.
11. Intersectoral coordination Workshop on Integrated Disease Vector Control of Malaria for Public Health Experts, held at Nadiad from 22–24 February 1996.
12. WHO sponsored Workshop on Bioenvironmental Control Methods of Malaria for Health and Non-health Sectors, held at Port Blair in collaboration with NVBDCP, DHS, Port Blair from 21–23 May 1996.
13. For Medical Practitioners conducted in collaboration with ARMA Medical Foundation, Directorate of Public Health and Preventive Medicine, Govt. of Tamil Nadu on 13 June 1997.
14. WHO Inter-Country Workshop on Identification and Confirmation of Sibling Species of Malaria Vector, held at Chennai from 15–27 September 1997.
15. Workshop on Larvivorous Fish for Malaria Control for the District Malaria Officers of Gujarat held at Nadiad from 12–13 November 1998.
16. A Workshop for Health Workers on various aspects of Bioenvironmental Control of Malaria at NIMR Field Unit Nadiad in 1998.
17. Ten NAMP-EMCP sponsored Workshops on Intersectoral Coordination for Malaria Control, held at Ahmedabad, Panaji, Jodhpur, Kolkata, Chennai, Mumbai, Hyderabad, Bharuch, Bhopal and Dindigul during March 1999.
18. Workshop to develop Common Working Protocol for Situation Analysis of Malaria in five Pilot Districts under RBM initiative at NIMR, Delhi from 23–25 May 2001.
19. Training on Identification of Sandflies and Mosquitoes to Officials of Defence Research Development Organization (DRDO), Gwalior at NIMR, Delhi from 4–22 June 2001.
20. Workshop on Roll Back Malaria—Partnership and Linkages at Tumkur, Karnataka for Health, Non-health and NGO Officials on 3 September 2001.
21. Workshop on Roll Back Malaria—Community's Role, at Taluka C.N. Hally, District Tumkur, Karnataka for Village Heads, Voluntary Organizations, PHC Officials and Community Leaders on 7 September 2001.
22. Workshop on Roll Back Malaria—Community's Role, at Cansarvarnem PHC, Goa for Village and Community Leaders, Voluntary Organizations, Block and PHC Officials on 8 September 2001.
23. Workshop on Roll Back Malaria—Partnership and Linkages, at Jodhpur Rajasthan for Health, Non-health and NGO Officials on 11 September 2001.
24. Workshop on Roll Back Malaria—Partnership and Linkages, at Aizawl, Mizoram for District-level Health, Non-health and NGO Officials on 13 September 2001.
25. Workshop on Roll Back Malaria—Community's Role, at CSC, Banar, District Jodhpur, Rajasthan, for Community Leaders, Village Heads, Voluntary Organizations, Block and PHC Officials on 14 September 2001.
26. Workshop on Roll Back Malaria—Partnership and Linkages, at Panaji, Goa for Health, Non-health and NGO Officials on 15 September 2001.
27. Workshop on Roll Back Malaria—Community's Role, at Kolasib PHC, Aizawl, Mizoram for Community Leaders, Village Heads, Voluntary Organizations, PHC and Block Officials and Villagers on 20 September 2001.
28. Workshop on Roll Back Malaria—Partnership and Linkages, at Keonjhar, Orissa for Health, Non-health and NGO Officials on 21 September 2001.

29. Workshop on Roll Back Malaria—Community's role, at Banspal PHC, Distt. Keonjhar, Orissa for Block and PHC Officials, Community Leaders, Village Heads, Voluntary Organizations and Villagers on 24 September 2001.
30. Workshop on Therapeutic Efficacy of Antimalarials for Scientists of NIMR HQs and its Field Units on 8 November 2001.
31. Workshop on Insecticide Resistance—Methods and Modalities for Scientists of NIMR on 8 November 2001.
32. Workshop on Prevention and Control of Malaria for Divine United Organization Health Workers (over 50) in December 2001.
33. International Conference on Challenges in Malaria and Prospects for Research held at New Delhi from 29–31 October 2002.
34. Workshop on Treatment of Severe and Complicated Malaria at IGH on 23 February 2005 at NIMR Field Unit, Rourkela.
35. Workshop on Clinical Management of Severe and Complicated Falciparum Malaria at RMRC, Bhubaneswar, jointly organized by NIMR and NVBDCP from 21–23 March 2005.
36. Workshop on Treatment of Severe and Complicated Malaria at Ispat General Hospital on 23 February 2005 at NIMR, Field Unit, Rourkela.
37. Workshop on Treatment of Severe and Complicated Malaria at RMRC, Bhubaneswar from 21–23 March 2005.
38. Workshop on Identification and *in vitro* Cultivation of Malaria Parasites, Screening of Antimalarials, Establishment of Malaria Parasite Bank in Sudan from 28 May to 12 June 2005.
39. Workshop on Malariology held at NIMR Field Unit, Jabalpur from 4–8 July 2005.
40. Workshop on Monitoring of Drug Resistance in *P. falciparum* Malaria Cases at Keonjhar on 20 August 2005.
41. Workshop on Drug Policy at Rourkela on 30 August 2005.
42. Workshop on Drug Policy for Malaria at NIMR, Delhi from 26–29 October 2005.
43. International Conference on Malaria at New Delhi from 4–6 November 2005.
44. Workshop on Assessment of Malaria Treatment Practices in Public and Private Health Sectors at NIMR, Delhi on 27 December 2005.
45. Five Workshops for Medical Officers of 12 districts of Madhya Pradesh organized by NIMR Field Unit, Jabalpur from 19 to 21, 23 to 25 January; 6 to 8, 13 to 15 and 20 to 22 February 2006.
46. Seminar on Malaria Vector Control for the Urban Malaria Staff of Nadiad at NIMR Field Unit, Nadiad on 23 February 2006.
47. Workshop on Assessment of Malaria Treatment Practices in Public and Private Health Sectors at NIMR, Delhi on 22 March 2006.
48. Workshop on Assessment of Therapeutic Efficacy of Antimalarial Drugs against Uncomplicated *P. falciparum* Malaria at Ranchi, Jharkhand from 23–25 March 2006.
49. Workshop on Malaria Burden in India at National Agriculture Science Complex, New Delhi from 29–30 March 2006.
50. Seminar on Vector Borne Diseases organized in different parts of Gujarat and the Union Territory of Diu by the Nadiad Field Unit from 24–29 April 2006.
51. Workshop on Urban Malaria at Ajmer, Rajasthan on 24 January 2006.
52. Workshop on Malaria Vector Control at Nadiad on 23 February 2006.
53. Five Workshops on Urban Malaria under USAID Project at NIMR during February and March 2006.
54. Training Workshop on Vector Borne Diseases in Gujarat from 24–29 April 2006.
55. Workshop on Malaria Epidemiology and Drug Policy at PS Medical College, Nadiad on 27 July 2006.
56. Two USAID-sponsored Workshops on Rapid Assessment of Malaria in Pregnancy in Madhya Pradesh at Satna on 5 September and at Bhopal on 15 December 2006 .
57. Two Seminars on National Malaria Drug Policy organized by NIMR Field Unit, Nadiad held at Ahmedabad in October and November 2006.
58. Workshop on Malaria Drug Policy and Treatment Practices at NHL Medical College, Ahmedabad on 30 October 2006.
59. NIMR Field Unit, Jabalpur organized a Malariology Training Workshop from 23–25 November 2006.
60. Two Training Workshops on Malariology organized by NIMR Field Unit, Jabalpur from 15–17 January; and 8–10 March 2007.
61. Organised Malaria Symposium and field visit at Bhubaneswar from 13–14 February 2007.
62. Workshop on Development of Scientific Communication Skills organized by NIMR from 15–16 February 2007.
63. Workshop on Environmental Management of Vector Borne Diseases organsied by NIMR Field Unit, Rourkela on 10 August 2007.
64. Workshop on Molecular Epidemiology and Immunology of Malaria and other Vector Borne Diseases organized by NIMR Field Unit, Jabalpur from 16–19 October 2007.
65. An International Workshop on Insecticide Resistance held at Puri on 14 February 2008.
66. IX International Symposium on Vectors and Vector Borne Diseases at Puri, Orissa from 15–17 February 2008.
67. Workshop on Basic Malaria Parasitology and Entomology organized by NIMR from 28 April to 2 May 2008.

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Other Information

List of Extramural, Collaborative and Sponsored Research Projects Undertaken

1. "A pilot study to estimate malaria incidence using sampling methods" in collaboration with NAMP/ IRMS and State Governments (1980–83) funded by ICMR.
2. "A laboratory and field study of malathion resistance in *An. culicifacies"* in collaboration with NAMP (1980–85) funded by WHO/TDR.
3. "To study the impact of residual insecticidal spray operations in areas with varying degrees of resistance and its relationship to the malaria transmission" in collaboration with NAMP and State Governments (1981–83) funded by ICMR.
4. "A pilot study to assess the impact of antimalaria operations on the morbidity (duration of febrile illness)" in collaboration with NAMP/State Governments (1982–83) funded by ICMR.
5. "Studies on cell biology, ultrastructural and electron cytochemistry of the human malaria parasite *Plasmodium vivax* and *Plasmodium falciparum"* in collaboration with Delhi University, Delhi (1982–85) funded by DBT.
6. "Seroepidemiology of human malaria—multicentric trials" (1983–85) funded by ICMR.
7. "Incrimination of *An. culicifacies* using immunoradiometric assay" (1984–86) funded by International Agency for Funding under Gandhi-Regan Science and Technology Initiative in collaboration with New York University School of Medicine, U.S.A.
8. "Impact of Deltamethrin (K-othrine) spraying in areas with DDT and HCH resistant *An. culicifacies"* in collaboration with NAMP (1985) funded by ICMR/NAMP.
9. "Evaluation of *Bacillus sphaericus* in laboratory and field" (1986–87) funded by WHO/TDR.
10. "Field trials of Deltamethrin (K-othrine) in Razapur PHC, District Ghaziabad" (1986–88) funded by NAMP.
11. "Science and Technology Project on Integrated Vector Control of Malaria, Filaria and other Vector-borne Diseases" (1986 onwards) funded by ICMR and Deptt. of Health, Ministry of Health and Family Welfare, Govt. of India.
12. "Applied field research on malaria" (1987–89) funded by SIDA.
13. "Transmission dynamics of *Plasmodium falciparum*" (1987–90) funded by WHO/TDR.
14. "Tribal health" (1988) funded by UNDP/WHO.
15. "Establishment of the culture system for exo-erythrocytic stage malarial parasites" (1992–94) funded by Indo-French Centre, Delhi.
16. "Malaria Parasite Bank" (1992 onwards) funded by DBT (1992–97) and ICMR (1997 onwards).
17. "Multicentric field trials with *Bacillus sphaericus* and *Bacillus thuringiensis* var. *israelensis* (1993–94) funded by ICMR/NAMP.
18. "Field evaluation of an antigen dipstick test for falciparum malaria" (1994) funded by WHO-SEARO.
19. "A comparative field study on malaria transmission by the use of insecticide-impregnated bednets vs DDT residual spraying" (1995) funded by WHO/TDR.
20. "Ecological adaptations of malaria vectors in different climatic zones and their epidemiological implications" (1996–97) funded by WHO through NAMP.
21. "Community acceptance of insecticide-treated mosquito nets (ITMNs) trial in tribal area of Keonjhar district" (1996–98) funded by British Council Division, U.K.
22. "Malaria prevalence among pregnant women and children in malaria endemic tribal area of Orissa" (1997–98) funded by CARE-India.
23. "The information modelling of malaria distribution process in India under ILTP of cooperation in Science and Technology between India and Russia" (1998–2000) funded by DST.
24. "Molecular analysis of cytoadherence phenotype and invasion pathways of Indian isolates of *P. falciparum*" in collaboration with ICGEB, New Delhi (1998–2001) funded by ICMR.
25. "Molecular markers for the identification of members of *Anopheles culicifacies* complex", Indo-Sri Lanka under bilateral collaboration in Science and Technology (1998–2001) funded by DBT.
26. "Evaluation of ICT Malaria *Pf/Pv* test kit for diagnosis of malaria" (1999–2000) funded by Becton Dickinson, U.S.A.
27. "Phase-II evaluation of Bifenthrin 10% WP and Fipronil 80% WDG indoor residual spraying for malaria vector control in India" (1999–2000) funded by WHOPES, WHO, Geneva.
28. "Phase-II evaluation of mosquito nets treated with Bifenthrin SC and ME formulations vis-à-

vis Lambda-cyhalothrin CS formulation for control of *Anopheles culicifacies*, a major vector of malaria in District Sonepat, Haryana state, India" (1999–2000) funded by WHOPES, WHO, Geneva.

29. "A randomized, double blind comparative study of Azithromycin vs Chloroquine as treatment of uncomplicated *P. vivax* and *P. falciparum* malaria at Delhi, Jabalpur and Sonapur" (1999–2001) sponsored by Pfizer.
30. "Study on the vector bionomics and evaluation of the sensitivity of malaria parasites to antimalarials in Surat City" (1999–2001) funded by DFID, U.K.
31. "Phase-II evaluation of Bifenthrin 10% WP and Deltamethrin 80% WDG indoor residual spraying for malaria vector control in India" (1999–2001) funded by WHOPES, WHO, Geneva.
32. "Impact of residual spraying of Reldan 40% EC against DDT and HCH resistant malaria vector *An. culicifacies* in malaria endemic villages of District Ghaziabad (U.P.), India" (1999–2002) sponsored by Denocil.
33. "Impact of residual spraying of Bendiocarb 80% WP (Carbamate) against DDT and HCH resistant malaria vector *An. culicifacies* in malaria endemic villages of District Ghaziabad (U.P.), India" (1999–2002) sponsored by Hoechst Schering Agro Evo Ltd.
34. "Laboratory and field evaluation of Hilmilin against mosquitoes" (1999–2002) sponsored by Hindustan Insecticides Ltd.
35. "Bioefficacy and operational feasibility of Alphacypermethrin (Fendona) impregnated mosquito nets/curtains to control rural and urban malaria" (1999–2002) sponsored by CYNAMID Agro Ltd.
36. "Population genetic analysis of *Anopheles culicifacies* species A" (1999–2003) funded by WHO/TDR (Molecular Entomology), Geneva.
37. "Field research on situation analysis in some selected districts and towns in the context of Roll Back Malaria Initiative" (2000–01) funded by WHO-SEARO, New Delhi.
38. "Phase-III evaluation of Bifenthrin 10% WP and Deltamethrin 25% WG indoor residual spraying for malaria vector control in India" (2000–02) funded by WHOPES, WHO, Geneva.
39. "Molecular characterization of pyrethroid resistance in *An. culicifacies*" (2000–02) funded by Wellcome Trust, U.K.
40. "Bioenvironmental control of mosquitoes in Mormugao Port Trust: technology transfer phase" (2000–02) funded by Mormugao Port Trust.
41. "Application of remote sensing in identifying and mapping sandfly distribution in endemic and non-endemic Kala-azar foci in Bihar (RMRI, Patna)" (2000–03) funded by ICMR Task Force.
42. "Application of RS and GIS for decision support in malaria control" (2000–03) funded by ICMR Task Force.
43. "Delineation of breeding habitats and landscape features suitable for *An. culicifacies* abundance using satellite remote sensing data" (2000–03) funded by ICMR Task Force.
44. "Evaluation of Rapid diagnostic technique, Parasight for diagnosis of malaria" (2000–05) funded by Becton Dickinson.
45. "Multicentric field trials with *Bacillus thuringiensis* var *israelensis* serotype H-14 against mosquitoes in Haldwani (Uttaranchal), Shahjahanpur (U.P.) and Vasco-da-Gama (Goa)" (2001) sponsored by Wockhardt.
46. "Efficacy of Azithromycin + Chloroquine combination in the treatment of uncomplicated *P. falciparum* malaria cases at Jabalpur and Sonapur" (2001) sponsored by Pfizer.
47. "Primary screening of herbal products for antimalarial activity" (2001) sponsored by DRDO, Tezpur, Assam.
48. "Operational evaluation of the stability of Iodine in double fortified salt—a multicentric study" (2001–02) funded by ICMR.
49. "Operational evaluation of the stability of Iodine in double fortified salt—a multicentric study (ICMR– NIN–MRC–RMRC, Dibrugarh–RMRC, Bhubaneswar–TRC-IRR)" (2001–02) funded by ICMR.
50. "Evaluation of the impact of DDT and malathion indoor residual spraying being used in malaria and Kala-azar control programmes on the disease prevalence—a multicentric study" (2001–02) funded by ICMR Task Force.
51. "Evaluation of Temeguard 50% EC at Chennai" (2001–02) funded by Gharda Chemicals.
52. "Phase III evaluation of Bifenthrin 10% WP and Deltamethrin 25% WG indoor residual spraying for malaria vector control in India" (2001–02) funded by FMC Bengaluru.
53. "Comparative evaluation of bioefficacy and persistence of mosquito nets treated with Deltamethrin tablet formulation (K-O Tab® against malaria vectors *Anopheles culicifacies* and *An. stephensi"* (2001–02) funded by Aventis/ Bayer Crop Sciences, Mumbai.
54. "Bioenvironmental control of mosquitoes in Mormugao Port Trust, Vasco-da-Gamma, Goa" (2001–02) funded by Mormugao Port Trust, Goa.
55. "Testing of Rapid Diagnostic test kits manufactured by M/s. Orchid Biochemicals" (2001–03) funded by M/s. Orchid Biochemicals System, Goa.
56. "Ecological adaptation of malaria vectors in three different climatic zones of country" (2001–03) funded by World Bank.
57. "Laboratory and field evaluation of Teknar HP-D (*Bacillus thuringiensis* var *israelensis – Bti*)" (2001–03) funded by Margo Bio Control Pvt Ltd.
58. "Field evaluation of Triflumuron (IGR) against

larvae of mosquito vectors" (2001–03) sponsored by Bayer.

59. "Evaluation of Azithromycin as treatment against *P. falciparum* and *P. vivax* using chloroquine as comparison drug" (2001–03) funded by Pfizer Ltd.
60. "Delineation of breeding habitats and landscape features suitable for *An. culicifacies* abundance using satellite data" (2001–03) funded by ICMR Task Force.
61. "Use of molecular and cytotaxonomic techniques to study *Anopheles culicifacies* complex and its relationship in malaria transmission (Indo-Sri Lanka)" (2001–03) funded by DBT.
62. "Population genetic analysis of *Anopheles culicifacies* species A" (2001–03) funded by WHO-TDR.
63. "To develop strategy for integrated control of vectors of malaria, JE and dengue in Karnataka" (2001–03) funded by WHO.
64. "Primary screening of the medicinal plants for north-east states of India for its antimalarial activity" (2001–04) funded by DRDO.
65. "Process of development for production of recombinant malaria vaccine based on *P. vivax* duffy binding protein" in collaboration with ICGEB-NIH (2001–04) funded by DBT.
66. "To develop strategy for integrated control of vectors of malaria and dengue" (2001–04) funded by WHO.
67. "Prospecting for botanical pesticides – All India coordinated research project" (2001–05) funded by DBT.
68. "Field evaluation of mosquito larvicide and pupicide Agnique MMF in different urban habitat against malaria vector *An. stephensi*" (2002) funded by WHOPES, WHO, Geneva.
69. "Laboratory (Phase-I) evaluation of Phenthoate against urban mosquito vectors *Anopheles stephensi* and *Culex quinquefasciatus*" (2002–03) funded by EID Perry, India Ltd.
70. "Operational feasibility of use of larvivorous fish for control of malaria in a high areas of Karnataka state" (2002–03) funded by WHO.
71. Therapeutic efficacy of chloroquine in *P. vivax* malaria" (2002–03) funded by WHO-SEARO.
72. "Evaluation of Binax: a rapid *Pf* diagnostic kit" (2002–03).
73. "Evaluation of Deltamethrin 25% WG indoor residual spraying for malaria control in India" (2002–04) funded by Aventis/Bayer Crop Science.
74. "Strategy for integrated control of vectors of malaria and dengue in northern Gujarat" (2002–04) funded by WHO country budget.
75. "Impact of climate change on malaria in India" (2002–04) funded by Ministry of Environment and Forest.
76. "ICT *Pf* kit programme" (2002–04) funded by DSS Imagetech.
77. "Comparative evaluation of bioefficacy and persistence of mosquito nets treated with Deltamethrin tablet formulation against malaria vectors *An. fluviatilis* and *An. culicifacies* and its impact on malaria transmission in malaria endemic tribal area of Sundargarh district (2002–04) funded by Aventis Crop Science Ltd.
78. "Genetic diversity of human malaria parasites *Plasmodium falciparum* and *P. vivax:* development of microsatellite markers" (2002–05) funded by ICMR.
79. "Genetic polymorphism of T-helper cell epitopic regions of *Plasmodium falciparum* isolates from India: relevance for vaccine development" (2002–05) funded by CSIR.
80. "Development of field site for malaria vaccine trial" (2002–07) funded by DBT.
81. "Studies on estimation of disease burden of identified infectious diseases—collection and review of literature for estimating malaria burden in India using DALYs as a summary measure" (2003–04) funded by WHO.
82. "Evaluation of Primiphos-methyl 50% EC against the immatures of *Anopheles stephensi/An. culicifacies* (malaria vectors) *and Culex quinquefasciatus* vector bancroftian filarial" (2003–04) funded by Syngenta Chemicals Ltd.
83. "WHO collaborative study for the establishment of diagnostic concentrations for bifenthrin and alpha-cypermethrin for resistance monitoring in malaria and dengue vectors" (2003–04) funded by WHO.
84. "Multicentric study on evaluation of VectoBac WDG**,** a formulation of *Bacillus thuringiensis israelensis* (*Bti*) against larvae of mosquito vectors" (2003–04) funded by Sumitomo Chemicals.
85. "Antimalarial drug against uncomplicated falciparum malaria in West Bengal as part of Indo-Nepal cross border activity" (2003–04) funded by USAID.
86. "Entomological evaluation of Cyphenothrin 5% EC as space spray against mosquitoes" (2003–05) funded by Sumitomo Chemicals.
87. "Cerebral malaria associated neurological disorders in central India" (2003–05) funded by Medical College, Jabalpur, More House School of Medicine and Centre for Disease Control and Prevention, Atlanta, U.S.A.
88. "Mosquito control in Bangalore City" (2003–05) funded by Bengaluru Mahanagar Palike.
89. "Molecular characterization of *Anopheles fluviatilis* complex: development of species specific diagnostic markers and microsatellite markers" (2003–05) funded by ICMR Genomics.
90. "Enhanced Malaria Control Programme-sponsored by NAMP" (2003–05) funded by EMCP/NAMP.
91. "Accumulation of persistent organochlorine compounds in sub-Himalayan region of north

India" (2003–06) funded by G.B. Pant Institute of Himalayan Environment.

92. "Videography of Olyset Net" (2004) funded by Sumitomo Chemicals.
93. "Monitoring of NVBDCP programme implementation in high risk districts" (2004–05) funded by NVBDCP.
94. "Operational activity for the assessment of therapeutic efficacy of Chloroquine and Sulpha-Pyrimethamine combination for uncomplicated falciparum malaria in Assam, Orissa and Tripura" (2004–05) funded by WHO.
95. "Pictorial identification key for Indian anophelines" (2004–05) funded by DRL, Tezpur.
96. "Evaluation of Diflubenzuron GR-2 and TB-2 for control of mosquito larvae" (2004–05) funded by Bayer India Ltd.
97. "Entomological evaluation of Etofenprox 20 WP against malaria vectors" (2004–05) funded by Sumitomo Chemicals.
98. "Entomological evaluation of Olyset nets impregnated with Permethrin 2% w/w at three sites" (2004–05) funded by Sumitomo Chemicals.
99. "Evaluation of Pyriproxifen 0.5 G against larvae of mosquito vectors" (2004–05) funded by Sumitomo Chemicals.
100. "Assessment of state implementation capacities for IMCP" (2004–06) funded by NVBDCP.
101. "Health impact assessment of Indira Sagar Dam and resettlement and rehabilitation colonies in SSP reservoir impoundment areas in Narmada Valley in Madhya Pradesh" (2004–06) funded by NVDA, Bhopal.
102. "Assessment of therapeutic efficacy of antimalarial drugs against uncomplicated *P. falciparum* malaria" (2004–06) funded by USAID/WHO.
103. "Assessment of malaria treatment practices in public and private health sectors" (2004–06) funded by USAID/WHO.
104. "Transmission dynamics of malaria in tribal areas" (2004–07) funded by RMRC, Jabalpur under Tribal Sub-plan of ICMR.
105. "Phase-II evaluation of VectoBac tablets (*Bti* H-14 formulation) against larvae of mosquito vectors" (2005) funded by Sumitomo Chemicals India Pvt Ltd.
106. "Bionomics and role of *Anopheles stephensi* in transmission of malaria in rural areas of Gujarat" (2005) funded by Govt. of Gujarat and IDVC.
107. "Phase-III evaluation of Olyset nets for malaria control in India" (2005) funded by Sumitomo Chemicals.
108. "Assessment of the state implementation capacities for IMCP" (2005–06) funded by NVBDCP.
109. "Monitoring of NVBDCP programme implementation in high risk districts" (2005–06) funded by NVBDCP.
110. "Studies on the epidemiology of urban malaria in mega, medium and small cities of India" (2005–06) funded by USAID.
111. "Discovery of antimalarials from marine organisms" (2005–06) funded by DBT.
112. "Screening of chloroquine sensitivity status of *P. falciparum* parasites from western border areas of India" (2005–06) funded by DRDO.
113. "Phase-III evaluation of Olyset nets (LLINs) against *An. minimus* transmitted malaria in Assam" (2005–06) funded by Sumitomo Chemicals.
114. "Entomological evaluation of PermaNets against malaria vector" (2005–06) funded by Vestergaard.
115. "Situation analysis of malaria in Car Nicobar after Tsunami attack" (2005–06) funded by DBT.
116. "Monitoring of Post-Tsunami malariogenic conditions in Car Nicobar Island using Satellite Remote Sensing" (2005–06) funded by ISRO.
117. "Environmental assessment of use of insecticides and insecticide treated material under NVBDCP" (2005–06) funded by NVBDCP.
118. NIH funded project "Promotion of *Plasmodium* research and training in India" (2005–06) funded by New York University School of Medicine NY.
119. "Effect of formulation and encapsulation on the efficacy of microbes against mosquito larvae" (2005–06) funded by CSIR.
120. "Bioefficacy of advanced odomos cream against *An. stephensi* a malaria vector and *Aedes aegypti* vector of dengue and dengue haemorrhagic fever" (2005–06) funded by Balsara Home Products, Mumbai.
121. "Study to assess the repellency of a natural oil formulation (DRP-1) on disease mosquito vectors" (2005–06) funded by Dabur Research Foundation.
122. "Laboratory evaluation of wash resistance of bioefficacy and insecticidal persistence of K-O Tab 12% treated LLN against *An. culicifacies* and *An. stephensi"* (2005–06) funded by Bayer Crop Sciences India Ltd, Mumbai.
123. "Randomized control trial of an indigenous fish, *Aphanius dispar* for malaria control in Gujarat" (2005–06) funded by Govt. of Gujarat and IDVC.
124. "Phase-III village scale trial of Lamda-cyhalothrin CS for indoor residual spraying against malaria vectors" (2005–06) funded by WHOPES, WHO.
125. "Evaluation of Biolarvicide (*Bti* B-17) in different larval habitats" (2005–06) funded by Godrej Hi Care India Ltd.
126. "Engineering of Indian malaria vector *Anopheles culicifacies* mosquito genetically using transposable element (IMVAC Project)" (2005–08) funded by DBT.
127. "Estimation of malaria disease burden in Jharkhand" (2005–08) funded by USAID.
128. "Evaluation of Primiphos-methyl 50% against the immatures of *Anopheles stephensi, An. culici-*

facies (MV) and *Culex quinquefasciatus*, vector of bancroftian filariasis" (Jul–Sep 2006) funded by Syngenta Crop Protection Pvt Ltd.

129. "Bioefficacy laboratory studies on Interceptor long-lasting insecticide treated nets (LLINs) impregnated with Alpha-cypermethrin" (July to December 2006) funded by OBASE India Ltd.
130. "A phase-III comparative, open level, randomised multicentric, clinical study to assess the safety and efficacy of fixed dose formulation (Paramex *Pv*)" (August to September 2006) funded by Medicine for Malaria Venture, Switzerland.
131. "Laboratory evaluation of the wash resistance of bioefficacy and insecticidal persistence of K-O Tab 123 treated long-lasting insecticidal nets against *An. culicifacies* and *An. stephensi* K-O Tab (Hardwar)" (2006) funded by Bayer Crop Sciences India Ltd.
132. "Laboratory evaluation of the wash resistance of bioefficacy and insecticidal persistence of K-O Tab 123 treated long-lasting insecticidal nets against *An. culicifacies* and *An. stephensi* K-O Tab (Nadiad)" (2006) funded by Bayer Crop Sciences India Ltd.
133. "Phase-II and Phase-III field evaluation of a neem based larvicide 0.15% Azaec by BMR and Compune" (2006) funded by BMR Agency.
134. "A phase-III comparative, open level, randomised multicentric, clinical study to assess the safety and efficacy of fixed dose formulation (Paramex *Pf*)" (July to August 2006) funded by Medicine for Malaria Venture, Switzerland.
135. "Meeting expenditure for village wise digital map of nine districts for GIS mapping of kala-azar in India and mosahar population in villages of Bihar under the direction of NVBDCP" (2006–07) funded by NVBDCP.
136. "Micro-level mapping of high malaria risk district in India for decision support of malaria control" (2006–07) funded by NVBDCP.
137. "Use of Artemisinin and Curcumin combination in treatment of uncomplicated *P. falciparum* malaria" (September 2006 to March 2007) funded by DST.
138. "Phase-III randomized, non-inferiority trial to assess the efficacy and safety of Dihydro-artemisinin and Piperaquine (DHA+PPO, Artekin) in comparison with Artesunate + Mefloquine (AS+MQ) in patients affected by acute uncomplicated *Plasmodium falciparum* malaria—Goa, Bangalore and Sonapur" (October 2006 to January 2007) funded by MDS-Life Sciences, Singapore.
139. "Follow-up studies on the efficacy of Olyset net in malaria vector and incidence of malaria in a village of District Gautam Budh Nagar, U.P" (2006–07) funded by Sumitomo Chemicals Pvt Ltd.
140. "Malaria in pregnancy in M.P. Agreement for performance of work (APW) under allotment and SE-IND MAL 002 x W06 re. Rapid assessment of burden malaria in pregnancy in Satna, Katni and Jabalpur" (2006–07) funded by WHO.
141. "WHOPES field testing and evaluation of bio-flash GR (*Bti*, Nature Bio Tek, Iran) for mosquito larviciding" (2006–07) funded by WHO.
142. "Phase-I laboratory studies to assess the larvicidal efficacy of (SSCL-30 mixture of essential) on mosquito vector species" funded by AccuDx Consultants, New Delhi.
143. "Laboratory studies to assess the bioefficacy of herbal based repellent spin cream (AD 101-mixture of essential oils) against the different mosquito vector species on human volunteers" (2-months) funded by AccDIGM Bio Med Trading Pvt Ltd, Delhi.
144. "Community randomized evaluation of the effectiveness of insecticide treated nets for malaria control in construction work in the urban slums in Goa" (2006–08) funded by NVBDCP.
145. "Identification of malaria risk factor in different ecosystem of Assam using remote sensing" (2006–09) funded by DRL.
146. "Antimalarial properties of some plants from Garhwal region of northwest Himalaya" (2006–09) funded by DST.
147. "Characterization of monoclonal antibodies raised against erythrocytic stages of India *Plasmodium vivax* isolates (2006–09) funded by CSIR.
148. "Identification of malaria risk factors in different ecosystems of Assam using remote sensing" (2006–09)) funded by DRDO.
149. "Field evaluation of Bacticide DT (Dispensable tablets) a formulation of *Bacillus thuringiensis* var *israelensis* H-14, strain 164 against larvae of mosquito vector" (July to December 2007) funded by Bio Tek International Ltd Delhi.
150. *"In vitro* sensitivity of Indian *Plasmodium falciparum* strains to antimalarial agents" (2007) funded by DNDI.
151. "Field evaluation (Phase-III) Bacticide WP, a formulation of *Bacillus thuringiensis* var *israelensis* H-14, strain 164 against larvae of mosquito vector" (July to December 2007) funded by Bio Tek International Ltd.
152. "Studies to assess the comparative bioefficacy of two different samples of Olyset nets (LLINs) 2% Permethrin incorporated (HD PE) bednets" (2007) funded by Sumitomo Chemicals India Pvt Ltd, Mumbai.
153. "Molecular characterization of different gl-chromosomal forms of *Anopheles fluviatilis"* (2007).
154. "External audit of DDT use in NVBDCP regarding" (2007) funded by NVBDCP.
155. "Field evaluation of BIODART-M, a formulation of *Bacillus thuringiensis* var *israelensis* (5% WP) against larvae of mosquito vector" (November

2007 to October 2008) funded by Ajai Bio Teck India Ltd.

156. "Multicentric, open-label, randomised clinical trial of efficacy and tolerability of the fixed-dose Artesunate/Amodiaquine (AS/AQ) combination therapy and Amodiaquine (AQ) monotherapy for treatment of uncomplicated falciparum malaria in India" (2007–08) funded by DNDI, Geneva.
157. "Evaluation of malaria (AG)/(AB-rapid test diagnostic kit)" funded by AccuDx Technologies.
158. "Monitoring on malaria programme implementation activities under World Bank" funded by NVBDCP.
159. "Monitoring on malaria programme implementation of programme activities in high risk district" funded by NVBDCP.
160. "Assessment of the state implementation capacity for global fund supported IMCP" funded by NVBDCP.
161. "RBX-combo study" funded by Ranbaxy.
162. "Multicentric study on efficacy trial with enhance dose of Fenthion 82.5% EC against mosquitoes in polluted water" (2007–08) funded by Bayer Crop Sciences.
163. "Assessment of efficacy safety and population pharmacokinetics of the fixed dose combination of Artesunate + Mefloquine in the treatment of acute uncomplicated *P. falciparum* malaria in India" (2007–09) funded by DNDI, Geneva, Switzerland.
164. "Phase-III evaluation of high density polythene (HDPE) nets" (3-months) funded by NVBDCP.
165. "Follow-up study of Olyset net in Sonapur and Rourkela" (2007–09) funded by Sumitomo Chemicals India Pvt Ltd.
166. "Developing a frame work for predicting malaria outbreak in rural and urban Gujarat, India" (2007–09) funded by NVBDCP/University of Michigan.
167. "A health monitoring study among inmates of households using bednet impregnated with Interceptor" (2007–09) funded by BASF India Ltd.
168. "Efficacy of a chloroquine and sulphadoxine-pyrimethamine (SP) for treatment of uncomplicated *P. falciparum* malaria with special reference to bio availability of chloroquine and sulphadoxine in Chhattisgarh (2008) funded by NVBDCP.
169. "Significance of asymptomatic carriers in malaria transmission vis-à-vis development of surveillance strategy in Orissa" (2008) funded by NVBDCP.
170. "Assessing the burden of malaria in pregnancy in east India, Jharkhand" (2 years) funded by WHO.
171. "Evaluation of Icon life nets having 0.2% Deltamethrin incorporated into the fibre with polyethylene material" (2008) funded by Syngenta Crop Protection Pvt Ltd.
172. "Environmental assessment under the proposed World Bank assisted Vector Borne Disease Control Project" (2008) funded by NVBDCP.
173. "Operational research on surveillance and intervention strategies in Chad Chiroli district of Maharashtra" (2008) funded by NVBDCP.
174. "Multicentric Phase-III evaluation of effectiveness of Diflubenzuron 25% WP (DI-LAEB) an insect growth regulator for mosquito vector control in urban settings" (2008) funded by Chemtura Chemicals India Pvt Ltd.
175. "Assessment of the impact of climate change on malaria and dengue at national scales and adaption strategies for short medium to long term scale (Nat.Com)" (2008–09) funded by Unirock International India.
176. "HRP-II/P-LDH based diagnostic kit for differential detection malaria parasite Phase-I" (2008–10) funded by Deptt. of Biotechnology, Govt. of India.
177. "Studies on the transmission dynamics of acute encephalitis syndrome in relation to vector and virus in the District Saharanpur of Uttar Pradesh in action plan for the prevention and control" (2008–10) funded by NVBDCP.
178. "Therapeutic efficacy of antimalarials" funded by World Bank (2008–09).
179. "Pharmacovigilance of antimalarials in India" funded by World Bank (2008–09).
180. "Quality assurance of laboratory diagnosis of malaria" funded by World Bank (2008–09).
181. "Monitoring of insecticide resistance of mosquito vectors in India" funded by World Bank (2008–09).

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Awards/Honours/Prizes Received by NIMR Scientists

Dr. T. Adak

Dr. M.O.T. Iyengar Memorial Award for Malaria of the Indian Council of Medical Research—1996.
Best Scientist Award related to Environmental Aspects of Vector Borne Diseases, National Academy of Vector Borne Diseases—2002.
Fellow of the Indian Society for Malaria and other Communicable Diseases, Delhi.
Fellow of the National Academy of Sciences, India, Allahabad.

Late Dr. M.A. Ansari

Best Scientist Award from National Environmental Science Academy—1994.
Fellow of the Indian Society for Malaria and other Communicable Diseases, Delhi.
Fellow of the National Environmental Science Academy.
Biotech International life time achievement award for contributions in vector borne diseases (posthumously in 2008).

Dr. C.P. Batra

Fellow of the Indian Society for Malaria and other Communicable Diseases, Delhi—1990.
Best poster presentation award at the National Congress of Parasitology—1994.

Dr. Aparup Das

Young Scientist Award from the Department of Science and Technology, Govt. of India—1993.
Post-doctoral fellowship award by the Deutsche Forschungsgemeinschaft (German Science Foundation)—2000.
Overseas associateship award from the Department of Biotechnology, Govt. of India—2007.

Prof. A.P. Dash

Dr. T.R. Rao Award by the ICMR for the year 1991 for outstanding contributions in Medical Entomology in India—1991.
Rajiv Gandhi Award for outstanding contributions to Science in India by the Rajiv Gandhi Foundation—2001.
Oration Award of the Indian Society of Epidemiologists and Indian Society for Malaria and other Communicable Diseases for best contributions in Malaria Research in India—2002.
Rajiv Gandhi Sadbhawna Award by Rajiv Gandhi Forum, Orissa as best personality in Health Services—2008.
Fellow of the National Academy of Sciences.
Fellow of the Indian Society for Malaria and other Communicable Diseases.
Fellow of the Zoological Society of India.
Fellow of the Environmental Science Academy.

Dr. Vas Dev

Bio-medical Research Award of the ICMR—1995.
Best poster presentation award at the Indian Society for Parasitology—1995.
Fellow of the Indian Society for Malaria and other Communicable Diseases, Delhi.
Fellow of the National Academy of Sciences, India, Allahabad.

Dr. Virender K. Dua

Dr. M.O.T. Iyengar Memorial Award for Malaria of the Indian Council of Medical Research—1997.
Best Scientist Award related to Environmental Aspects of Vector Borne Diseases, NAVBD, Bhubaneswar, India—1998.
Wellcome Trust (U.K.) Award to work in U.K. for six months—Oct 1999 to Apr 2000.
Dr. M.O.T. Iyengar Memorial Award for Malaria of the Indian Council of Medical Research—1999.

Dr. Hema Joshi

Fellow of the Indian Society for Malaria and other Communicable Diseases, Delhi—1987.
Awarded British Council (ODA) fellowship, U.K. for Post-doctoral for six months—1996.
Best poster presentation award at the National Congress of Parasitologists—2000.
Wellcome Trust (U.K.) Award to work in U.K. for two months—2003.
Best Scientist Award related to Molecular Biology of Vector Borne Diseases, NAVBD—2008.

Dr. Ashwani Kumar

Red Cross Award for outstanding services to the Society—1994.
Outstanding Young Personality Award from the Cuncolim Junior Chamber of Goa—1997.
Outstanding Young Indian Award under Toyi Programme in Technology and Scientific Development

Category by Indian Junior Chamber during their National Conference, Secunderabad—1997.

Outstanding Young Personality Award from the Zone XI of the Indian Junior Chamber at Zoncon—1998.

Outstanding Young Person of the World Award in Technology and Scientific Development Category by International Junior Chamber presented at the World Congress of JCI at Manila—1998.

Bharat Gaurav Award by Society for International Friendship and Understanding, New Delhi—2002.

Dr. P.K. Mittal

Fellow of the Indian Society for Malaria and other Communicable Diseases, Delhi—1993.

Best poster presentation award in Vector Control at the Indian Society for Parasitology—1994.

Dr. B.N. Nagpal

Silver Medal received for best paper in Hindi presentation in "Man and Environment" at National Environment Science Academy—1995.

T. Ramachandra Rao Award for young scientist of the Indian Council of Medical Research—1996.

Dr. Nutan Nanda

Awarded Ist prize for the best presentation in the poster session at the All India Cell Biology Conference—1984.

Awarded Ist prize for the best presentation in the poster session at the Asian Congress on Parasitology—1986.

Fellow of the Indian Society for Malaria and Other Communicable Diseases—1993.

Awarded certificate of merit in the "Micrograph Contest", Electron Microscope Society of India—1994.

Dr. S.K. Sharma

WHO fellowship for training in 'Management of Tropical Diseases' at London School of Hygiene and Tropical Medicine, London—1994.

Certificate of Honour by Rotary Club of Rourkela (Central) for outstanding efforts on Rotary against Malaria (RAM)—2001.

Best Poster Award during III Global Meet on Parasitology held at Bengaluru—2004.

Best Scientist Award related to Environmental Aspects of Vector Borne Diseases, NAVBD, Bhubaneswar, India—2008.

Fellow of the Academy of Zoology.

Dr. V.P. Sharma

Dr. B.K. Srivastava Foundation Award of Medical College, Udaipur—1977.

Om Prakash Bhasin Award in Health and Medical Sciences of the Bhasin Foundation—1985.

Dr. R.V. Rajam Oration Award of the National Academy of Medical Sciences—1987.

L.N. Gupta Trust Award of the L.N. Gupta Memorial Charitable Trust, Hyderabad—1989.

M.O.T. Iyengar Award for Malaria of the Indian Council of Medical Research—1989.

B.N. Singh Oration Award—Gold Medal of the Indian Society for Parasitology—1990.

Ranbaxy Award in Applied Medical Sciences of the Ranbaxy Foundation—1990.

Padma Shree by the Honb'le President of India Shri R. Venkatraman—1992.

Best Scientist Award of the National Environment Science Academy— 1995.

Federation of Indian Chambers of Commerce and Industry (FICCI) cash award in Biomedical Sciences—1998.

Fellow of the Royal Asiatic Society of London and Ireland.

Fellow of the Indian National Science Academy, New Delhi.

Fellow of the National Academy of Sciences, India.

Fellow of the National Academy of Medical Sciences, New Delhi.

Fellow of the Academy of Sciences, Bengaluru.

Fellow of the Entomology Society of India, New Delhi.

Fellow of the National Environmental Science Academy, Patna.

Fellow of the Indian Society for Parasitology, Lucknow, U.P.

Fellow of the Indian Society for Malaria and other Communicable Diseases, Delhi.

Dr. Neeru Singh

Indian Council of Medical Research award for outstanding work in the field of Biomedical Research in under developed areas—1996.

Best Scientist Award related to Environmental Aspects of Vector Borne Diseases, National Academy of Vector Borne Diseases—1999.

Dr. Aruna Srivastava

Gold Medal for best poster presentation in "Man and Environment", National Environment Science Academy—1995.

Best analysis award in ESRI/ERDAS conference—1996.

Dr. S.K. Subbarao

Dr. M.O.T. Iyengar Memorial Award for Malaria of the Indian Council of Medical Research—1991.

Best Scientist Award related to Environmental Aspects of Vector Borne Diseases, National Academy of Vector Borne Diseases—1996.

Fellow of the National Academy of Sciences, India.

Fellow of the Indian Society for Malaria and other Communicable Diseases, Delhi.

Fellow of the Indian National Science Academy, New Delhi.

Dr. Neena Valecha

Fellow of the Indian Society for Malaria and other Communicable Diseases, Delhi.

Dr. R.S. Yadav

Certificate of Honour by the Indian Medical Association, Vadodara—1997.

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Human Resource Development

Ph.D Awardees worked in NIMR

Name	Title of the Thesis	University	Year
S.K. Ghosh	Morphological variations of *Plasmodium vivax* and *Plasmodium falciparum* in the human hosts and in the vectors during post-eradication resurgence of malaria	University of Delhi, Delhi	1985
B.N. Nagpal	A contribution to the knowledge of family Culicidae (Diptera) from Orissa	Berhampur University, Berhampur	1986
Nutan Nanda	Studies on ultrastructure and development of human malaria parasite *Plasmodium vivax* (Grassi & Feletti, 1890)	University of Delhi, Delhi	1989
T. Adak	Studies on gene enzyme system of two important vectors of malaria in India, *Anopheles stephensi* and *Anopheles culicifacies*	University of Delhi, Delhi	1989
Babita R. Jana	Laboratory and field evaluation of pyrethroid impregnated netting in India	University of London, U.K.	1991
R.K. Chandrahas	Host parasite interaction in rice field and feasibility of mosquito control in urban area	Berhampur University, Berhampur	1991
Dhrubaa Ghosh	Characterization of cultured *P. falciparum* isolates with respect to antimalarials, ultrastructure and genomic organization	University of Delhi, Delhi	1992
Narain Sharma	Investigation on resurgence of malaria in urban areas of Delhi and the study on current bionomics of *An. stephensi* and *An. culicifacies* responsible for resurgence	CCS University, Meerut	1992
Rajni Kant	Studies on the ecological succession of mosquito breeding in rice fields with reference to their possible control	Kanpur University, Kanpur	1993
Tanusri Chakraworti	Present status of resurgence of malaria in and around Delhi after failure of eradication of programme	Visva Bharti University, West Bengal	1993
K. Raghavendra	Studies on malathion-resistance in different sympatric sibling species of *Anopheles culicifacies* Giles	University of Delhi, Delhi	1994

Name	Title of the Thesis	University	Year
Bharati Atrie	Cytogenetic aspects of *An. annularis* and *An. subpictus*	University of Delhi, Delhi	1995
B. Bhaskar Rao	Field evaluation of impregnated bednets for malaria control	Dibrugarh University, Assam	1995
C.P. Batra	Malariogenic stratification in Delhi	CCS University, Meerut	1995
Neera Kapoor	Evaluation of insecticide-impregnated curtains as personal protection measure against mosquitoes	University of Delhi, Delhi	1995
T.S. Satyanarayan	Population dynamics of *An. culicifacies* sibling species	University of Delhi, Delhi	1996
C.S. Pant	Occurence of organochlorine pesticides in different components of the ecosystems	HNB Garhwal University, Srinagar, Uttarakhand	1996
Reema Sarin	Chromatographic studies on some antimalarials	Utkal University, Bhubaneshwar (Orissa)	1997
Roop Kumari	Studies on bioconcentrations of organo-chlorine insecticides in environment due to their use in malaria control in India with special reference of terai region of Uttar Pradesh	HNB Garhwal University Srinagar, Uttarakhand	1997
Suman Lata	Isoenzyme analysis of *An. culicifacies* species complex	University of Kalyani, (West Bengal)	1998
S.N. Sinha	Isolation and characterization of oxidation products of primaquine and test their antimalarial activity	Bihar University, Muzaffarpur	1998
Sarbjit Kaur	Susceptibility of the *An. culicifacies* species complex to *Plasmodium* species	Himachal University, Shimla	1999
R.M. Bhatt	Effect of extracts on insect pests: a critical study	Sardar Vallabbhai Patel University, Gujarat	1999
Vasanti	Biology of *An. stephensi* in Tamil Nadu	University of Madras, Chennai	2000
T.R.R. Sampath	Evaluation of pyrethroid impregnated bednets for control of malaria in a tribal area of Orissa	Sambalpur University, Orissa	2001
M.A. Haque	Malaria and its control through personal protection measures in the mining areas of Orissa	Sambalpur University, Orissa	2001
John Ravindran	Studies on mosquito breeding in riceland agroecosystem near Chennai	University of Madras, Chennai	2001

Name	Title of the Thesis	University	Year
Kulvir Sra e Dhindsa	Isolation, characterization culture and efficacy studies on bacilli pathogenic to mosquitoes in Goa, India	Goa University, Goa	2001
N.C. Gutpa	High performance liquid chromatographic studies on 4-aminoquinolines and their application for the concentration determination during the treatment of malaria cases	HNB Garhwal University, Srinagar, Uttarakhand	2005
A.C. Pandey	Repellency of *Lantana camara* Linn. (Family: Verbenanceae) against mosquitoes	Gorakhpur University, Uttar Pradesh	2005
Geeta Goswami	Development of molecular technique(s) for the differentiation of members of *Anopheles culicifacies* Complex	MD University, Rohtak	2005
V.P. Ojha	Studies on isolation and antimalarial activity of different compounds from *Andrographis paniculata,* family: Acanthaceae	CCS University, Meerut	2006
Firoz Alam	Insecticidal properties of some plants from Garhwal region of the North-West Himalaya against mosquitoes	Gurukula Kangri University, Hardwar	2006
Deepak Tomar	Development of immuno-reagents for the detection of *Plasmodium falciparum* HRP-2 and LDH antigen using antigen capture assay	AIIMS, New Delhi	2006
G.N. Kiwanuka	Characterization of *P. falciparum* parasites of normal as well as sickle-cell children among Uganda population using molecular markers	Mbarara University, Uganda	2007
Anil Sharma	Immune response of *Anopheles culicifacies* sibling species	MD University, Rohtak	2007
Alex Eapen	Systematics and larvivorous potential of Indian fishes of the genus *Aplocheilus* McClelland (Pisces: Cyprinodontiformes) with special reference to *Aplocheilus parvus* Raj	University of Madras, Chennai	2007
U. Sreehari	Efficacy of long-lasting insecticide treated net technology against *Anopheles culicifacies*, a principal malaria vector in India	Jamia Millia Islamia, New Delhi	2008
Sharmila Pahwa	Environmental epidemiology of malaria in three states of India	CCS University, Meerut	2008

Ph.D candidates currently Working in NIMR

Name	Title of Research Topic	University
Rosy Gurwara	Development and validation of analytical methodology for synthetic pyrethroid insecticides in contaminated air due to the use of mosquito repellents and their impact on human health	Gurukul Kangri Vishwavidhyalaya, Hardwar
Anil Sharma	Immune response of *Anopheles culicifacies* sibling species	MD University, Rohtak
O.P. Singh	Molecular characterization of different chromosomal forms of *Anopheles fluviatilis*	Jiwaji University, Gwalior
Nandini Korgaonkar	An epidemiological study on risk factors responsible for enhanced receptivity and vulnerability to malaria in Goa	Goa University, Goa
Swapnil Roy	Accumulation of organochlorine residues in sub-Himalayan region of north India	Gurukul Kangri Viswavidhyalaya, Hardwar
Praveen Kumar Bharti	Study of nature and external of polymorphism in vaccine candidate antigen (MSP-1, MSP-2 and MSP-3) and drug resistance gene (*Pfcrt*) of *Plasmodium falciparum* in central India	Rani Durgavati University, Jabalpur
Surendra K. Prajapati	Molecular studies on house keeping genes of *Plasmodium vivax*	Jamia Millia Islamia, New Delhi
Prashant K. Mallick	Studies on drug resistance	University of Delhi, Delhi
Ajaz A. Bhat	Developing epitope based immunogen using different stages of *Plasmodium vivax* with in-built immuno-adjuvants and delivery in microspheres	AIIMS, New Delhi
Mayank Madhukar	Complement receptor 1 (CR1) and its gene polymorphisms in relation to the pathophysiology and susceptibility to severe malaria	AIIMS, New Delhi
Sanghamitra Verma	Studies on sequence variation and immunogenicity of recombinant fusion proteins of T-helper cell epitopes of circumsporozoite protein of *Plasmodium falciparum* isolates from India: Relevance for vaccine development	Jiwaji University, Gwalior
Jai Prakash N. Singh	Studies on genetic polymorphism and immunogenicity of synthetic peptides of T-helper cell epitopic regions of circum-sporozoite protein of *Plasmodium falci-parum* isolates from India: Relevance for vaccine development	Jiwaji University, Gwalior

Name	Title of Research Topic	University
Suresh Yadav	Study of acute and sub-acute toxicity of some plant extracts against malaria vector *Anopheles stephensi*	Dr BR Ambedkar University, Agra
A.K. Upadhyay	Studies on the mosquito fauna and bio-ecology of malaria vectors in the malaria endemic tribal area of northern Orissa	Jiwaji University, Gwalior
Gaurav Verma	Antimalarial properties of some plants from Garhwal region of northwest Himalaya	Jiwaji University, Gwalior
Prerna Sethi	Determination of some new antimalarials by using high performance liquid chromatography and their application to malaria cases	Jiwaji University, Gwalior
Mahesh B. Kaliwal	Studies on bio-ecology of filarial vector *Culex quinquefasciatus* in Goa	Goa University, Goa
Deeparani Prabhu	Studies on mode of action and bioefficacy of fungi pathogenic to larvae of *Anopheles stephensi (*Liston), *Culex quinquefasciatus (*Say*)* and *Aedes aegypti (*Linnaeus*)*	Goa University, Goa
Ratanesh K. Seth	Isolation and characterization of monoclonal antibodies against erythrocytic stages of Indian *Plasmodium vivax* isolates	Jiwaji University, Gwalior
A.S. Pradeep	Development of more specific and sensitive Histidine rich protein 2 (HRP2) based diagnostic system for *Plasmodium falciparum* malaria	Jiwaji University, Gwalior
Gauri Awasthi	Genetic diversity of the 7th chromosomal genes in Indian *Plasmodium falciparum*	Jiwaji University, Gwalior
Jyotsana Dixit	Population genetic studies of malaria vector *Anopheles minimus* in northwestern parts of India using bioinformatic and evolutionary approaches	Jiwaji University, Gwalior
Hemlata Srivastava	The effect of natural selection on immune response genes of *Anopheles minimus* species A	Jiwaji University, Gwalior
Bhavna Gupta	Population genetic studies of Indian *Plasmodium vivax*	Jiwaji University, Gwalior
Anita C.	Population genetic and evolutionary studies of duffy gene in Indian humans	Jiwaji University, Gwalior
Sonam Vijay	Characterisation of nitric oxide synthase (NOS) in *Anopheles culicifacies:* Implication for an innate immune mechanism of refractoriness	Jiwaji University, Gwalior

Name	Title of Research Topic	University
Manmeet Rawat	Molecular characterisation of aspartic protease gene from *Plasmodium vivax*	Jiwaji University, Gwalior
Sneh Shalini	Molecular characterisation of Plasmep sin gene in *Plasmodium vivax* and their comparative study with primates malaria parasites	Jiwaji University, Gwalior
B. Prasad Rao	Biochemical and molecular characterization of insecticidal resistance in *Anopheles culicifacies*	Jiwaji University, Gwalior
Vaishali Verma	Studies on insecticide resistance and its management: Biochemical and molecular approaches for characterisation	Jiwaji University, Gwalior
B.P. Niranjan Reddy	Characterisation of insecticide resistance mechanisms in Indian malaria vectors	Jiwaji University, Gwalior
Mahesh B. Kaliwal	Bioecology of *Culex quinquefasciatus*, the principal vector of Lymphatic filariasis in Goa	Goa University, Goa
Ajeet Kumar Mohanty	Salivary gland proteome analysis of *Anopheles culicifacies*, the principal vector of human malaria in rural India	Goa University, Goa
Sompal Singh	Low dose radiation induced molecular changes in human blood cells	CCS University, Meerut
Bijayalaxmi Sahu	Molecular epidemiology of drug resistance in *Plasmodium falciparum* in Orissa, India	Jiwaji University, Gwalior
Geeta Sharma	Target antigens of immunity to *Plasmodium vivax:* characterization in areas of north-eastern India	Jiwaji University, Gwalior