Science and Technology Project on
Integrated Disease Vector Control

Annual Report
2015–16

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Contents

An Overview of the Activities v

1. Introduction 1

2. Highlights of the Research Activities 3

3. Detailed Progress of the Work Done 11
   3.1 Bengaluru (Karnataka) 13
   3.2 Chennai (Tamil Nadu) 16
   3.3 Guwahati (Assam) 24
   3.4 Hardwar (Uttarakhand) 27
   3.5 Jabalpur (Madhya Pradesh) 33
   3.6 Nadiad (Gujarat) 41
   3.7 Panaji (Goa) 49
   3.8 Raipur (Chhattisgarh) 55
   3.9 Ranchi (Jharkhand) 61
   3.10 Rourkela (Odisha) 72

4. Research Papers Published (January–December 2015) 80

Annexures

I : Research Advisory Committee of IDVC Project 85
II : Addresses of IDVC Field Units 86
An Overview of the Activities

Continuing the activities of earlier projects, the ten field units of the National Institute of Malaria Research under the Integrated Disease Vector Control (IDVC) Project, initiated several new ventures and research studies during the year 2015–16 on vector control, parasite biology, epidemiology, etc. for prevention and their control, primarily based on applied and operational methods.

Studies on vector biology and bionomics were undertaken in different topographical areas to understand the disease vectors in the changing ecological context. In this effort, introduction of larvivorous fish Gambusia affinis and Guppies Lebistes reticulatus by the Bengaluru Field Unit led to significant reduction in the breeding index of the vector population in JE endemic blocks. Therapeutic efficacy of chloroquine and artemisinin + sulfadoxine-pyrimethamine for Plasmodium vivax and P. falciparum were carried out in Mangalore which showed effective response. A WHOPES supported project on evaluation of the efficacy and duration of effectiveness of a biolarvicide, BactiVec®SC (Bacillus thuringiensis var israelensis SH-14), revealed that the formulation is operationally feasible and effective.

A study for monitoring the antimalarial resistance to P. falciparum and P. vivax was conducted by the Chennai Field Unit in endemic areas of Tamil Nadu. Entomological studies were carried out in the malaria outbreak regions of Kerala to find out the transmission dynamics in affected areas and remedial measures to tackle vector breeding, reduce parasite load in the community and continuation of intervention measures on sustainable basis. A survey was undertaken for evaluation of Sumilarv 2MR as a mosquito larvicide for control of Aedes aegypti in container habitats in Chennai. The study on impact of post-flood scenario on vector breeding potential assessed in extreme flood hit areas in Chennai revealed that the habitat positivity for Anopheles breeding was 18.3% only in the wells, for Aedes it was 8.3% and for Culex species the habitat positivity was found to be 12.2, 12.5, 14.3 and 9.1 for wells, cement tanks, sumps and pools, respectively.

The therapeutic efficacy of arteether + lumefantrine (AL) for treatment of falciparum malaria was monitored by the Guwahati Field Unit in malaria endemic blocks along international borders covering three different locations, viz. Tripura, Mizoram and Meghalaya. The P. falciparum parasite was found as major causative agent in all the three study sites. Entomological investigations were carried out in Mizoram (Indo-Bangladesh border) in order to determine the prevalence of malaria transmitting mosquitoes. To study the malaria treatment practices with special emphasis on private sector in India, a study (Phase-II) was undertaken in Assam. The study concluded that P. falciparum was the predominant infection (75%); all cases had received antimalarial medicines during the infection wherein AL was the most commonly prescribed followed by chloroquine + primaquine and artemesunate + sulfadoxine-pyrimethamine and artemesunate oral/injection.
A survey carried out for identification of risk factors and situation analysis of breeding prevalence of *Aedes* mosquitoes in different areas of Hardwar, Uttarakhand, revealed that desert coolers and containers were the major breeding sites of *Aedes* mosquitoes. A project initiated for the development of botanical insecticide formulation of essential oils from *Lantana camara*, *Valeriana jatamansi* and *Psoralea corylifolia* in large-scale for control of mosquitoes in collaboration with the Defence Research Laboratory, Tezpur showed encouraging results. For stratification of malaria in District Hardwar, a project was initiated to prepare a PHC-wise stratified map of malaria, identify the risk factors of malaria endemicity and undertake intervention measures in one sub-centre so as to demonstrate elimination of malaria.

For assessment of durability of long-lasting insecticidal nets (Phase–III) activities like household surveys, net survivorship and fabric integrity study, insecticide susceptibility test, chemical assays, cone-bioassays (after 12 and 18 month of nets distribution) etc. were performed in different villages of Madhya Pradesh by the Jabalpur Field Unit. Point prevalence studies were performed in Betul and Narsinghpur districts for knowing risk factors associated with malaria re-emergence and dengue transmission, respectively. Entomological surveys were carried out to study the bionomics of malaria vectors and their sibling species, and to establish their role in malaria transmission in Chhattisgarh.

A study initiated for assessing the health impact of Sardar Sarovar project on vector-borne diseases in Gujarat revealed high vector density in command area than non-command area. In command area, house, container and breteau indices were 8.58, 6.98, and 1.47, whereas in non-command area these were 4.35, 2.47 and 0.48, respectively. A comparative study was also carried out for evaluation of efficacy, fabric integrity and community acceptability of PermaNet 3.0 long-lasting insecticidal nets with PermaNet 2.0. The bioassay and chemical assay revealed decrease in deltamethrin and piperonylbutoxide content in nets after one year of use, however, these nets were still effective on mosquitoes. Transmission dynamics and control of malaria was well-studied in tribal areas of Panchmahal district in Gujarat. The peridomestic survey showed that the main breeding habitats are seasonal rivers, river bed pools and wells while species composition revealed that *An. culicifacies* was the dominant species.

For understanding of vector-pathogen interactions, a study was initiated by Goa Field Unit for characterization of salivary gland proteome of dengue, chikungunya and yellow fever vector *Ae. aegypti* Linn. This study will help in identification of proteins playing key role in virus infection process and in devising virus blocking strategies in the salivary glands. Studies on vector infection were conducted under the project on epidemiology of malaria evolution in South Asia, funded by National Institute of Health, USA with the aim of updating knowledge on rapidly evolving vectors necessary for their effective management. The results revealed that *An. stephensi* (which was hitherto considered a non-vector in Goa) and *An. subpictus* contributes significantly in propagation of malaria in urban Goa. In the city of Ponda, Goa, studies were conducted on kinetics of *P. vivax* development in wild and laboratory colonized strains of *An. stephensi*, which revealed wide range of oocyst and sporozoite infection rates (even between individuals of the same batch) and weak correlation between initial parasitemia and infection rates. A national multidistricts study on estimation of malaria burden in India was initiated in different districts including Kolhapur, Dakshin Kannada, Jaipur, Jhabua, Koraput and Chatra to validate recently proposed methodologies.

A study was carried out for monitoring of insecticide resistance against DDT, malathion, alphachempermethrin and deltamethrin by conducting WHO susceptibility tests in different districts of Chhattisgarh. The results showed that *An. culicifacies* has developed resistance to DDT, malathion, deltamethrin and alphachempermethrin in most of the districts. Field
evaluation for determining the efficacy, fabric integrity and acceptability of Olyset +
long-lasting insecticidal nets in comparison to Olyset Net in different villages of Chhattisgarh
state showed that Olyset + long-lasting insecticidal nets were more durable than Olyset
Net and hence more acceptable.

The NIMR, Raipur Field Unit continued its multidisciplinary study on the impact
of insecticide resistance in malaria vectors on effectiveness of combination of indoor residual
spraying (IRS) and long-lasting insecticidal nets (LLINs) in 80 clusters (villages) of Keshkal
block in Kondagaon district in southern Chhattisgarh.

Epidemiological and entomological surveys on malaria in Pakur district, Jharkhand
revealed that mosquito fauna is comprised of >20 species under five genera, i.e. Anopheles,
Aedes, Armigeres, Culex and Mansonia. The P. falciparum was the dominant
species (86.5%) followed by P. vivax (2.8%). Filariaosis surveys carried out in District
Simdega (out of four districts) of Jharkhand state showed that overall, filarial disease and
endemicity rates were 6.07 and 10.45%, respectively. To find out the solutions to control
vector-borne diseases in Jharkhand state, the NIMR, Field Unit at Ranchi is also carrying
out need-based research for the state government (SVBDCP) covering malaria, filariasis,
dengue and kala-azar.

In collaboration with the Govt. of Odisha, the Rourkela Field Unit continued the
MMV funded comprehensive case management programme, in four districts with primary
aim to assess the impact of comprehensive case management system of uncomplicated
malaria on its transmission in different settings. The compliance rates of follow-up in
Bolangir, Dhenkanal, Kandhamal and Angul districts were >90%. A multicentric study
on therapeutic efficacy of artemisinin-based combination therapy (ACT) with the
combination of sulfadoxine-pyrimethamine + artesunate in uncomplicated P. falciparum
was carried out in Angul district of Odisha. Among 75 cases that completed 42 day follow-
up, treatment failures were observed in 3 cases, while the remaining 72 (96%) were
categorized as adequate clinical and parasitological response (ACPR).

All the Field Units provided technical support to the local state health authorities in
terms of investigation of outbreaks of malaria and dengue, training of man power, cross-
checking of slides, and as a societal benefit provided free diagnosis and treatment for
malaria patients through Malaria Clinics.

I, sincerely thank Dr PL Joshi, Chairman, IDVC Research Advisory Committee and its
members for guiding the research activities of the project and Officers Incharge and staff
of the field units for their contribution and providing technical support to the local as well
as national programme. I also wish to thank ICMR and Ministry of Health and Family
Welfare, Govt. of India for providing financial support for IDVC project.

I, take this opportunity to thank all the Officers Incharge and staff of the field units for
their contribution and technical support in implementation of the programmes and
acknowledge the efforts of Dr Ashwani Kumar, Officer Incharge, Field Unit Goa for
compiling the report, Annual Report Committee members, and scientists and staff of
Publication Division, NIMR for editing and bringing out the report.

Neena Valecha
Director
Introduction

Background
Integrated Disease Vector Control (IDVC) project is one of the eight mission mode Science and Technology projects in various fields identified in 1983 by the Scientific Advisor to the then Prime Minister. This project was assigned to the Malaria Research Centre (renamed as National Institute of Malaria Research on 4 November 2005), Delhi (for malaria) and Vector Control Research Centre, Puducherry (for filariasis) in 1985. The IDVC project is being funded jointly by the Indian Council of Medical Research (ICMR) and the Ministry of Health and Family Welfare (MoHFW), Govt. of India. Between 1986 and 1992, Malaria Research Centre (MRC) opened 13 field units at various locations in the country in consultation with the National Anti Malaria Programme, now National Vector Borne Disease Control Programme (NVBDCP). The project was extended up to the eighth plan by the recommendation of the Scientific Advisor to the Prime Minister. Its continuation beyond March 1997 has been done on yearly basis with the approval and financial support from the MoHFW, Govt. of India.

The project successfully demonstrated malaria control in rural, urban, industrial, forest, tribal and coastal areas of the country. In addition to this, the project also successfully demonstrated malaria control through the primary health care system in the country which received appreciation from various agencies. In addition to annual reviews by the Research Advisory Committee (RAC) of IDVC project, Scientific Advisory Committee (SAC) of NIMR, Scientific Advisory Group, Scientific Advisory Board and Governing Body of ICMR, and various committees, namely Nitya Anand (1985), Harcharan (1993), Pattanayak (1995) and Rudrappa (1996) periodically reviewed the IDVC project, and made scientific recommendations in view of the increasing malaria cases in the country and recurrence of epidemics. There is need for continued research to develop and test new technologies for malaria control to be used by NVBDCP. The Scientific Advisory Committee of the MRC which reviewed the annual progress repeatedly also underscored the importance of the regularisation and re-organisation of the IDVC project and made recommendations to effect that from time-to-time. To implement these recommendations, approval of the 32nd Scientific Advisory Board (2000) of the ICMR was taken which endorsed the recommendations made by the Rudrappa Committee on the permanency of long-term extramural projects. Subsequently, the Governing Body of ICMR, New Delhi in 1999 and 2000 recommended that 12 field units located outside Delhi should continue as a permanent activity of MRC and Delhi field unit be closed. And it was further stated that the field units could be shifted as per the needs of the malaria control programme. In continuation, a meeting comprising the representatives from NAMP/MRC/ICMR under the Chairmanship of Dr. S. Pattanayak in the year 2001 discussed the issue of re-organisation of the IDVC project threadbare and recommended the same. The committee also highlighted the areas of applied field research of immediate importance to control malaria in the country.

The Standing Finance Committee of the MoHFW recommended re-organisation of the IDVC project in its meeting on 10 August 2005. Following the re-organisation, 10 field units are functioning under this project in different states of the country. Keeping in view the recommendations of various committees and continued need for research support to malaria programme in the country, NVBDCP and NIRM have jointly identified priority areas to realise the gains in malaria control through research.
Objectives
All the field units have a common research programme of providing support to NVBDCP. However, depending on the local malaria situation, research interest of the field unit and recommendations of the Scientific Advisory Committees of the field unit/NIMR, each field unit has a specific research programme.

The research programmes have been planned up to the end of tenth five year plan. Further planning of research beyond the 10th plan period will be done in consultation with NVBDCP, State Health Departments, Scientific and Research Advisory Committees of NIMR and considering the prevailing malaria situation at a given period.

Common research programme
(i) Basic and applied research on vector biology and transmission dynamics of malaria in different ecosystems in order to provide inputs to malaria control programme.
(ii) Technical support in malaria control activities of the state health programme with reference to the following activities:
- Preparation of annual malaria action plan
- Insecticide resistance monitoring
- Evaluation of insecticide spray quality
- Therapeutic efficacy of antimalarials
- Capacity building in the field of malaria entomology, microscopy and surveillance
- Evaluation of strategies (ITN, LN, larvivorous fish, blister pack, etc.) as and when required by NVBDCP.
(iii) Evaluation of new products (insecticides, drugs, diagnostic, etc.)
(iv) Epidemic investigations for rapid response and management.

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Indian Council of Medical Research (ICMR), V. Ramalingaswami Bhawan, Ansari Nagar, New Delhi-110 029.

Institute
National Institute of Malaria Research (ICMR), Sector 8, Dwarka, New Delhi-110 077.

Collaborating organisation
National Vector Borne Disease Control Programme (NVBDCP), 22 Sham Nath Marg, Delhi-110 054.

Budget
Grant received from ICMR during the financial year 2015–16 was ₹ 192.2 million and expenditure had been shown in Fig. 1.
Highlights of the Research Activities

2.1 Bengaluru (Karnataka)

- Larvivorous fish *Gambusia affinis* (~ 713700) and Guppies *Lebistes reticulatus* (~ 190060) were released in three JE endemic blocks Khorabar, Chagaon and Bhatat for vector control in JE prone areas in Gorakhpur district, eastern Uttar Pradesh. This led to gradual but sharp reduction on breeding index of the vector population.

- A WHOPES-sponsored study ‘Field testing and evaluation of the efficacy and duration of effectiveness of a biolarvicide, BactiVec® SC (Bacillus thuringiensis var israelensis SH-14), was conducted in Bengaluru against immature stages of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* in their main natural habitats following the guidelines of World Health Organization Pesticide Evaluation Scheme.

- Therapeutic efficacy of chloroquine for *Plasmodium vivax* was initiated in Mangalore, Karnataka. A total of 71 patients were successfully followed-up the period of 28 days. All showed good response to chloroquine. Therapeutic efficacy of artemisinin + sulfadoxine- pyrimethamine for *Plasmodium falciparum* was also carried out along with *P. vivax*. There were less number of falciparum cases reported in Mangalore during the study period. A total of 36 patients were successfully followed-up to the period of 42 days. All responded to the ACT.

- In a collaborative project between NIMR and NCBS, Bengaluru on assay development for *P. vivax*-infected hepatocytes in micropatterned co-culture (MPCC) plates, successfully completed with four membrane-fed blood-infected with *P. vivax* to *An. stephensi*. About 55,000 sporozoites per mosquito were generated which is equivalent to other studies, so far. These sporozoites are used to infect in vitro cultured hepatocytes, namely HC-04 cell line.

- In a collaborative project between NCBS, Bengaluru, InStem, Bengaluru and NIMR, Bengaluru Unit to develop a sustainable *P. vivax* liver stage assay using human hepatocytes derived from induced pluripotent stem cells, peripheral monocyte cells from vivax-infected patients were successfully isolated and those undergoing differentiation of hepatocytes. These cells would be utilized for infection from vivax sporozoites.

- A Phase-IIIb, open label trial, to assess the safety, tolerability and efficacy of dihydroartemisinin-piperquine (Eurartesim) in Indian children and adolescent patients with acute uncomplicated *P. falciparum* malaria is underway in Wenlock District Government Hospital, Mangalore. A total of 15 children were recruited and 12 of them were successfully followed-up to the period of 63 days. All showed good response.

2.2 Chennai (Tamil Nadu)

- Given the intervention in force, high transmission of malaria was observed in districts of Mizoram and Tripura sharing international border with Bangladesh. Among two prevalent parasites, *P. falciparum* was the predominant infection (> 80%). The remaining were *P. vivax* cases. Therapeutic efficacy based
on 28-days follow up investigations revealed good success rate (98%) to the given regime of AL (artemether + lumefantrine).

- Entomological investigation in Mizoram revealed that both An. minimus and An. baimaii were prevalent in the study area but An. baimaii was the predominant vector species. In addition, An. maculatus is suspected to play some role in malaria transmission.

- Questionnaire-based study from the medical practitioners revealed that prescriptions and diagnostics practices for malaria treatment were satisfactory. However, monotherapy still continues to be in practice.

- Other activities included providing technical support to the control programme, i.e. health education and capacity building measures, mass propagation and distribution of larvivorous fish (Guppy and Gambusia) in town areas, assessment of mass drug administration in filarial endemic districts and in providing technical expertise on long-lasting insecticide nets for procurement and supplies.

2.3 Guwahati (Assam)
- Monitoring therapeutic efficacy of antimalarials artemether + lumefantrine (AL) in the treatment of P. falciparum malaria in northeastern states was done. In Gandachara, of the total 77 subjects selected for 28 day follow up investigations, 98.7% (76/77) were ACPR. In Chawngte CHC, of the total 83 subjects enrolled 82 (98.8%) showed ACPR (subject to correction by PCR). In Darengeree PHC, as many 32 subjects were selected for follow up investigations all of which all showed ACPR except one which was lost to follow up.

- In order to study prevalence of malaria transmitting mosquitoes in Lawngtlai district of Mizoram, Indo-Bangla border in Northeast India, entomological investigations were undertaken in Chawngte (Lawngtlai district) of Mizoram during July – August 2015. Using different sampling techniques including day-resting mosquito catches in human dwellings, indoors, cattle biting catches in the evenings, overnight CDC trap collections and human landing mosquito catches, 9 anopheline mosquito species were collected including An. aconitus, An. baimaii, An. jameisi, An. kochi, An. maculatus, An. minimus, An. nivipes, An. barbirostris and An. nigerrimus. None of these mosquito species were encountered in day-resting catches in human dwellings indoors.

- A study was undertaken in five different block PHCs of Kamrup district of Assam to ascertain the malaria treatment practices in public and private healthcare providers and use of antimalarial medicines in Assam. Exit interviews were conducted and results have been discussed.

2.4 Hardwar (Uttarakhand)
- During the months July to October, a total of 1525 houses were surveyed, out of them 474 houses were found positive for Aedes breeding. Out of 4185 containers searched, 1034 containers were found positive for Aedes breeding. House index, container index and breteau index were 311, 24.7, and 678 respectively. Four Aedes species, namely Ae. aegypti, Ae. albopictus, Ae. vittatus and Ae. pseudotaienius were identified. Percent species composition of Ae. aegypti in Hardwar proper, Kankhal and BHEL Township was 98.5, 60.4 and 2, respectively. A total of 810 suspected dengue cases were recorded in District Hardwar, out of which 357 cases were confirmed by ELISA test and no death was recorded.

- In order to find out the risk factors of malaria in District Hardwar, four villages in Chanderpuri subcentre of Laksar CHC of population 6651 showing high malaria incidence have been selected and one subcentre (Shivgarh) in Bahadarabad CHC, showing low malaria incidence have been selected for demonstration of elimination of malaria. Prevalence of An. culicifacies was
observed throughout the year and peak density was recorded during the months of July and August in both the areas. During the months of May to March, a total of 665 blood slides were collected in Chanderpuri out of which 78 cases were found positive for P. vivax and 5 cases for P. falciparum, SPR being 12.5, while 656 blood slides were collected from low malaria subcentre, out of which 16 slides were found positive for P. vivax, SPR being 2.4 which coincided with high vector density. Density of An. culicifacies and total anophelines and malaria incidence in Chanderpuri was high as compared to Shivgarh.

- NIMR Field Unit has been working on Industrial malaria control since 1986 and successfully controlled malaria in BHEL, Hardwar. From April 2015 to March, 2016, a total of 2056 blood slides were collected, out of which 71 slides were found positive for P. vivax and 1 for P. falciparum, SPR being 3.5. Out of 72 positive cases, 25 were from Jwalapur, Kankhal and nearby villages and remaining 47 were from the BHEL township.

2.5 Jabalpur (Madhya Pradesh)
- In phase III trial of long-lasting insecticidal nets in Kundam (Madhya Pradesh) revealed that 20% NetProtect nets did not meet the Bioassay test as per WHO criteria. Chemical Assay Reports received from Gambloux showed that after one year of household use, the mean deltamethrin content in LifeNet is 4.42 g/kg corresponding to a loss of 55% of the original dose, 1.11 g/kg corresponding to loss of 38% in NetProtect and 0.92 g/kg corresponding to loss of 32% in PermaNet 2.0. The number of holes in nets were mostly found on lower side.
- Point prevalence study in District Betul where the risk factors associated with re-emergence of malaria showed SPR was 37 and P. falciparum infection 70%.
- Dengue transmission in Narsinghpur district showed container index 42%, breteau index 80% and breeding of Ae. aegypti was 94.5%.
- Bionomics of malaria vectors and their sibling species in Districts Bastar and Koria of Chhattisgarh showed 11 anophelines recorded from both the areas. Anopheles culicifacies C (38%) was dominant followed by species D (23%) and B (22%). Anopheles fluviatilis species T was dominant in the study area. Both the species were P. falciparum positive. Anopheles culicifacies was identified as species C.

2.6 Nadiad (Gujarat)
- A detailed study was initially carried out in Kheda, Surendranagar and Patan districts in Phase-II command area of Sardar Sarovar project. It was further extended to Morbi district of Saurashtra region. Narmada water had reached this region through canal for irrigation as well as for ceramic industries. Entomological activities in these two districts included mosquito collection, peri-domestic and intra-domestic larval surveys, host preference and survivorship of malaria vectors, cross sectional mass blood survey in sentinel villages was done.
- A large-scale (Phase-III) evaluation of efficacy, fabric integrity and community acceptability of PermaNet 3.0 long-lasting insecticidal nets compared with PermaNet 2.0 in India was used by households under field conditions and to assess washing mode and washing habits of LNs by the householders, and to assess the community acceptability of LNs over three years.
- Bioassay, chemical assay and fabric integrity of Cohort nets were done.
- Transmission dynamics and control of malaria in tribal area of Gujarat India was initiated in June 2014 in Panchmahals district of Gujarat state. In total 30% population of this district is tribal. Three PHCs of Gogamba and two PHCs of Jambugoda Taluka were selected for this study. During this year entomological parameters such as adult mosquito density, larval density, parity, human blood index and human landing collection were monitored on bi-monthly basis. Supervision of indoor residual spray (IRS) activity was also done in the study area. During this year, bi-monthly
intra-domestic breeding surveys were carried out. A total of 1026 houses were checked for mosquito breeding and out of these 44 houses were found positive for mosquito breeding (House index = 4.29). Container index (CI) was 3.65 and breteau index (BI) was 2.14.

- Centre for the Study of Complex Malaria in India had been launched in November 2012 in Gujarat with an aim to understand the complexity of malaria, including changing patterns of epidemiology. The objective of the project in Gujarat, is to collect blood samples from the patients with malaria symptoms, then to identify the *Plasmodium* species in samples with three different diagnostic methods, i.e. microscopy, rapid diagnostic kits (RDTs) and polymerase chain reaction (PCR). After sample processing, the DNA samples were sent to NIMR, New Delhi for genomic studies.

- In the year of 2015, 203 suspected cases were enrolled for malaria diagnosis from the clinic of Civil Hospital, Nadiad and Vatva Urban Health Centre (UHC) of Ahmedabad Municipal Corporation, Ahmedabad. Initially these were examined by RDT kit (Zephyr Biomedical) which was followed by microscopy (as per the protocol). The blood samples were collected from the patients after taking informed consent for further molecular diagnosis. The DNA was isolated from the respective blood samples by QIAamp DNA mini kit and processed for the PCR diagnosis. The PCR products were analyzed by electrophoresis technique. Out of 203 cases, 138 were found positive by microscopy (*Pv* = 125, *Pf* = 9, Mixed = 4) as well as by RDT (*Pv* = 125, *Pf* = 10, Mixed = 3). PCR detected 132 cases positive (*Pv* = 119, *Pf* = 13) for malaria.

- Malaria diagnostic support was provided to the Civil Hospital, Nadiad and the data that was generated had been used for sentinel monitoring of malaria situation in the Kheda district of Gujarat. In 2015, 6583 febrile patients have been screened for malaria, of which 93 found positive for malaria infection (*Pv* = 70, *Pf* = 20 and Mixed = 3). The slide positivity rate was 1.4. All the confirmed malaria patients were provided radical treatment by the Medical Officers of Civil Hospital, Nadiad. There is an increase in both *P. falciparum* and *P. vivax* malaria cases as compared to 2014. However, 3 cases of mixed infection have also been reported. The age-wise distribution of cases indicates highest malaria infection among 15 and above age group.

### 2.7 Panoji (Goa)

- Characterization of salivary gland proteome of dengue/DHF, chikungunya and yellow fever vector *Ae. aegypti* I. was done. A large number of proteins were identified and catalogued and their functional analysis was performed.

- Proteomic analysis of urine of malaria patients using high resolution mass spectrometry was performed for identification of candidate biomarkers for *P. falciparum* and *P. vivax* infections. Promising results have been obtained.

- A study on the role of gut microbiota in modulation of longevity, fecundity and fitness of a major malaria vector *An. stephensi* was initiated.

- Larvicidal and pupicidal activity of leaf extracts of *IC_Goa* against *An. stephensi* Liston, *Cx. quinquefasciatus* Say and *Ae. aegypti* was determined and pupicidal activity at low doses showed that promising pupicidal compounds exist in the extract which are further being explored for translational research.

- Vector infection studies under epidemiology of malaria evolution in South Asia project funded by the National Institute of Health, USA were performed. *Anopheles subpictus* was found to play a major role in perennial transmission of malaria in Goa.

- A national multi-distict study entitled, ’Estimation of malaria burden in India was launched and carried out in Kolhapur and Dakshin Kannada district NIMR Field Unit, Goa. Other sample districts in the country were Jaipur, Jhabua, Koraput and Chatra
where similar exercise was undertaken by the NIMR scientists. The project activity included manpower recruitment and training, active surveillance, malaria incidence reporting from private, corporate, municipal and Govt. sectors covering all stakeholders and health providers. Verbal Autopsy of all death cases was carried out on population of 4 lakh population per district.

2.8 Raipur (Chhattisgarh)

- Field evaluation of efficacy, fabric integrity and acceptability of Olyset LNs in 10 villages, Kanker (6) and Balod (4) districts of Chhattisgarh state.

- Monitoring of insecticide resistance in An. culicifacies against various insecticides in all the 27 districts of Chhattisgarh state.

- Monitoring of impact of insecticide resistance in malaria vectors on effectiveness of combination of IRS and LNs in 80 clusters (villages) with population of 75,000 in Keshak block of Kondagaon district, Chhattisgarh.

- Questionnaire-based surveys were undertaken to ascertain the presence of distributed nets, their physical integrity in 7 districts of Chhattisgarh state where LNs were distributed by the State/District Health Department through Public Distribution System (PDS).

- Undertook study with the objective to assess usability of PermaNet 3.0 in Kanker, Dhamtari and Janjigar-Champa districts of Chhattisgarh state with malaria vectors having different levels of deltamethrin resistance.

- Monitoring of therapeutic efficacy of ACT against uncomplicated P. falciparum in Balod and Kanker districts, Chhattisgarh and Gadhiroli district, Maharashtra.

- Provided technical support to the programme by cross-checking of malaria blood slides and by checking of filaria blood slides received from various districts of Chhattisgarh state.

- Imparted training in malaria and its control to M.B.B.S. students from Govt. Medical College, Raipur and B.H.M.S. students of Maharana Pratap Homoeopathic Medical College and Hospital, Raipur.

- Refresher training course in malaria microscopy and training in examination of blood slides for microfilaria to Laboratory Technicians of 10 and 7 districts, respectively of Chhattisgarh state.

- Participated in monthly review meeting of Chief Medical Officers/District Malaria Officers organised by the State Health Secretary at Raipur.

2.9 Ranchi (Jharkhand)

- Mosquito fauna survey was undertaken with reference to anophelines at Noamundi area of West Singhbhum district. Four recognised malaria vectors An. culicifacies, An. annularis, An. fluviatilis and An. minimus were recorded. Low density of An. minimus (MHD) 2–4 was recorded from Noamundi (Badajamda, Bajihar and Kadajamda villages) where high density (20–25) of An. minimus was recorded. Breeding was observed in hill tops slow moving streams, and pools. The feeding of An. minimus was observed in indoors and in outdoors (less numbers). Mosquito blood meal (MBM) analysis of An. minimus revealed high anthropophilic index 60% positive for human blood index.

- Susceptibility test of An. minimus using DDT (4%), malathion (5%) and deltamethrin (0.05%) was carried out in the villages of Noamundi (Barajamda, Bajihar and Kadajamda). An. minimus showed 95% mortality to DDT (4%) and 100% mortality to malathion (5%) and deltamethrin (0.05%). Four An. minimus were sequenced. Sequencing of 28r DNA confirmed that specimen identified morphologically as An. minimus s.l. were actually An. minimus sensu stricto.

- On the request of Air Force HQ, New Delhi an epidemiological and entomological investigation on malaria was carried out in and around Air Force unit, Singarsi of Pakur district, Jharkhand. In the present surveillance,
mass blood survey smear examination result was presented. The result revealed slide positive rate (SPR) 37.9%. The highest SPR was observed in Singhars (45.7%) and the lowest was observed in Madgama (19.0%); however, in the clinic at AF unit the SPR was 72.2% from Santhal and Pahadi tribes. Plasmodium falciparum was the dominating species recorded 86.5%. The Plasmodium vivax was 2.8 and 2% Pf cases showed gametocyte in the peripheral blood smear. The highest percentage of asymptomatic carrier of Pf was detected from the population that has no symptoms during a mass blood survey in all the villagers. Therefore, they act as a reservoir for transmitting the cases to the healthy persons through the vectors available in the local area. It was observed that the tribes were not using any protective measures for the control of malaria. Thus becoming vulnerable for malaria contact visa-à-vis transmitting malaria. The entomological survey revealed the mosquito comprise over 20 species. A total of 791 anopheles mosquito was collected. Major malaria vectors were An. culicifacies (39.9%) followed by An. annularis (7.2%) and An. fluviatilis (1.5%).

- Insecticide susceptibility test reported that An. culicifacies, and An. annularis were resistant to DDT (4.0%) and their susceptibility to malathion (5%) and deltamethrin (0.05%). However, it was observed that An. fluviatilis was susceptibility to DDT (4%).

- Suggested remedial measures were provided to the Air Force base. LLINS (long-lasting insecticide treated bednets) must be distributed and used covering all the population of Air Force, MES and civilians residing in AF units. All the three malaria vectors are susceptible to insecticides (malathion and deltamethrin). Therefore, it was suggested to overcome the entire problem, it is necessary to undertake proper surveillance, indoor spraying (IRS) of SP (synthetic pyrethrroids) in the human dwelling and cattleshed at AF units. Active surveillance should be carried out the entire nearby village with the radius of 6 km weekly once and treatment must be provided to block the malaria transmission. Insecticide-treated nets (ITMNs) may be provided to all the nearby villages. Introduction of ACT in the AF unit and village level for more effective treatment.

- Monitoring of the therapeutic efficacy of ACT (artesunate + pyrimethamine and sulfadoxine) against uncomplicated P. falciparum malaria was carried out at Mahuadand CHC of Latehar district, Jharkhand state. All the malaria positive cases were susceptible to ACT.

- Filariasis survey was carried out in the four districts of Jharkhand state (Simdega, Dhanbad, Palamu and Lohardaga). The district is dominated by Santhal, Munda, Oraons, Ho, Kharia, Karmali, Asur and Birhor tribes. The microfilaria rate was 6.07% in Bano PHC of Simdega district, 4.06% in Baliapur PHC of Dhanbad district, 4% in Mediningar PHC of Palamu district and 3.03% in Bhandra PHC of Lohardaga district. All the districts are in hotspot area. No MDA was carried out in Simdega and Palamu district and 9 and 10 rounds of MDA was carried out in Dhanbad and Lohardaga districts. The study highlights the problem of filariasis in the Jharkhand state.

- To facilitate early diagnosis and prompt treatment, a Malaria Clinic function at NIMR, Field Unit, Itki, Ranchi. All the cases from Itki PHC and TB Sanatorium Hospital were diagnosed. A total of 248 patients attended the malaria clinic during the year 2015–16, out of which 14 cases were found to be positive for malaria of these 2 cases were positive for P. vivax and 12 cases were positive for P. falciparum. Overall percentage was—SPR 5.64, SR 4.83 and Pf 85.71. One P. falciparum positive patients showed gametocyte in the peripheral blood.

- A filarial clinic is functioning at IDVC Field Unit, Itki, Ranchi. A total of 46 patients of filariasis attended the clinic during the year. Most of the cases were of old cases of filariasis. These cases were with acute manifestation of filariasis starting from hydrocele to
elephantiasis. Two cases of epididymo-orchitis was observed. Five patients had multiple manifestations (10.86%).

- Support provided to NVBDCP and State Health Department: MDA evaluation, capacity building in the field of malaria entomology, microscopy and surveillance, insecticide resistance monitoring, evaluation of RDT kits, epidemic investigation for rapid response and management, quality control of laboratory services (diagnosis of malaria and filariasis) and training for transmission assessment survey (TAS).

2.10 Rourkela (Odisha)

- MMV funded comprehensive case management programme launched in 2013 in four districts of Odisha with the primary objective to assess the impact of comprehensive case management system of uncomplicated malaria on its transmission in different transmission settings continued in Bolangir, Dhenkanal, Angul and Kandhamal districts. The study was undertaken in collaboration with the Government of Odisha after completion of recruitment and training of project staffs as well as orientation training of the Medical Officers and other existing staffs of the Community Health Centres. The results were discussed.

- Monitoring the therapeutic efficacy of antimalarial medicines in India. A multicentric study on therapeutic efficacy of artemisinin-based combination therapy (ACT) with the combination sulfadoxine-pyrimethamine + artesunate in uncomplicated *P. falciparum* malaria was carried out in Thakurgarh New PHC under Madhapur CHC in Angul district of Odisha. A total of 77 subjects who fulfilled all the inclusion criteria were enrolled in the study comprising of 35 (45.5%) females and 42 (54.5%) males.

- Eco-epidemiology and transmission of complex malaria in India (under NIH Sponsored CSCMi project) was carried out in 11 villages located in forest, plain and riverine areas under Bisra, Kuarmunda and Birkera CHCs of Sundergarh district, Odisha. In this study health seeking behaviour, diagnostic methods used, proportion of asymptomatic cases, mosquito fauna and composition of vector sibling species, host blood meal preferences and various environmental factors supporting growth of vector immature and adult survival were studied.
Detailed Progress of the Work Done

- Bengaluru (Karnataka)
- Chennai (Tamil Nadu)
- Guwahati (Assam)
- Hardwar (Uttarakhand)
- Jabalpur (Madhya Pradesh)
- Nadiad (Gujarat)
- Panaji (Goa)
- Raipur (Chhattisgarh)
- Ranchi (Jharkhand)
- Rourkela (Odisha)
Larvivorous fish in vector control in JE prone areas in Gorakhpur district, eastern Uttar Pradesh

This project has been undertaken to address the long problem of JE-related AES cases and related deaths mostly among the children. In July 2012 about 15000 Gambusia were brought to Gorakhpur from Bengaluru. The fish multiplied and established in a pond at Gorakshnath Temple. Following this a team was formed at the block level for fish release work. Three JE endemic blocks Khorabar, Chagaon and Bhatat were selected for the programme. In total, in Khorabar block fish was released in 316 tolas in 58 villages, population 146,827. A total of 51,930 guppies in 547 wells and 109,200 Gambusia in 201 ponds were released. Similarly, in Chagaon block fish was released in 195 tolas in 50 villages, population 159,432. A total of 70,630 guppies in 386 wells and 130,000 Gambusia in 115 ponds were released. In Bhatat block fish was released in 136 tolas, in 52 villages population 133,800. A total of 67,500 guppies in 436 wells and 474,500 Gambusia in 350 ponds were released. The work was carried out with the active participation of the local administration.

The entomological data revealed that the vector mosquito Culex tritaeniorhynchus and Cx. pseudovishnui were breeding in wells, rice-fields, ponds and burrow pits. This area is represented by extreme weather conditions. In the monsoon season rice-fields provide additional breeding grounds for the vectors. Following winter, the rice-fields are replaced with wheat cultivation and in the summer almost all the places get dry. Here ponds and wells are the main grounds for the vectors and these two habitats were targeted with fish which was found to be very effective antilarval measure. There was a sharp and gradual reduction on breeding index of the vector population. In general, about 82% reduction on vector breeding index was observed at the end of two-year fish intervention.

Field testing and evaluation of the efficacy and duration of effectiveness of a biolarvicide, BactiVec® SC (Bacillus thuringiensis var israelensis SH-14), in Bengaluru, India

This was a WHOPEES sponsored project conducted in Bengaluru City of Karnataka state from June to December 2015. Extensive surveys were carried out in 15 localities of Bengaluru north and east and two study localities have been identified based on the prevalence of Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus. BactiVec SC (Bti) has been tested against three species in natural breeding habitats. Different formulations of Bacillus thuringiensis var israelensis have been tested against different mosquito vectors and other insects for its residual activity. In the present study we evaluated efficacy and residual activity of a new formulation of Bti against immature stages of An. stephensi, Ae. aegypti and Cx. quinquefasciatus in natural habitats in small and large-scale in Bengaluru City, India.

Preferential breeding habitats of above mentioned species of mosquitoes were selected and four dosages each were tested in small-scale trial. Two most effective dosages were selected for large-scale trial. Evaluation was carried out essentially following the guidelines of World Health Organization Pesticide Evaluation Scheme. Pre- and post-treatment densities were recorded at regular intervals and > 80% reduction in pupae was taken as effective duration of the test product. BactiVec SC-treated at the dosage of 1 ml/50 L could produce 10–17 days efficacy (> 80% reduction in pupae) in clean water habitats tested, whereas 0.5 ml/50 L dosage showed residual
activity from 7–14 days against *Aedes aegypti* and *An. stephensi*. In polluted water habitats, 4–7 days efficacy could be recorded against *Cx. quinquefasciatus*. No noticeable impact due to application of BactiVec SC could be seen in other non-target organisms such as crabs, snails, dragonfly naiads, water boatman, etc.

The BactiVec SC formulation is operationally feasible and easy to handle. For the control of Anophelines and *Aedes* mosquitoes in fresh water habitats, 0.5 and 0.1 ml/50 l dosages are found effective, whereas in polluted water habitats against *Cx. quinquefasciatus* 5 ml/m² was found effective.

**Therapeutic efficacy of antimalarial drugs in India—Mangalore site**

*Plasmodium vivax:* Determination therapeutic efficacy of chloroquine for *P. vivax* has been initiated in Mangalore, Karnataka. A total of 71 patients have been completed successfully in a follow-up period of 28 days. All showed good response to chloroquine.

*Plasmodium falciparum:* Determination therapeutic efficacy of artemisinin + sulfadoxine-pyrimethamine for *P. falciparum* was carried out along with *P. vivax*. There were less number of falciparum cases reported in Mangalore during the study period. A total of 36 patients have been successfully completed for a follow-up period of 42 days. All responded to the ACT.

**Assay development for Plasmodium vivax-infected hepatocytes in micropatterned co-culture (MPCC) plates**

This is a collaborative project between NIMR and NCBS, Bengaluru. We have successfully completed four membrane-fed blood-infected with *P. vivax* to *An. stephensi*. We were able to generate 55,000 sporozoites per mosquito which is equivalent to other studies, so far. These sporozoites are used to infect in vitro cultured hepatocytes, namely HC-04 cell line.

**Development of a sustainable Plasmodium vivax liver stage assay using human hepatocytes derived from induced pluripotent stem cells**

This is a collaborative project between NCBS, Bengaluru, InStem, Bengaluru and NIMR, Bengaluru Unit. Peripheral monocyte cells from vivax-infected patients were isolated and undergoing differentiation of hepatocytes. These cells will be utilized for infection of vivax sporozoites.

A Phase-IIIb, open label trial, to assess the safety, tolerability and efficacy of dihydroartemisinin-piperquine (Eurartesim) in Indian children and adolescent patients with acute uncomplicated *Plasmodium falciparum malaria*

This project is undergoing at the Wenlock District Government Hospital, Mangalore. A total of 15 children have been recruited and 12 of them have been successfully followed-up to the period of 63 days. All showed good response.

**Technical support to the programme**

**Investigation of Japanese encephalitis cases reported in Bengaluru City**

On the request of the State Health Department, Govt. of Karnataka, detailed entomological investigations were undertaken to find out the mosquito vector potential and other aspects of local transmission of the JE cases in Bengaluru City from 18 September to 1 October 2015. Altogether, 10 cases have been assigned to NIMR for investigation. Out of the 10 cases, seven cases could be investigated and other three cases could not be traced. In the preliminary investigation, the team found that Yerappanahalli area, Kumarswamy layout area and Titalara Palya, Peenya 2nd stage area showed presence of conducive atmosphere for incidence of JE, like presence of free moving, un-housed country pig population, presence of herons, eagles, and other migratory birds, suitable habitats for breeding and proliferation of JE vectors.

Of the seven cases investigated, four could be probably JE cases as per the case history; symptoms reported that the place of residence supporting the conditions for JE incidence. However, virus isolation should be undertaken in hosts as well as mosquitoes prevailing in the affected areas to confirm the indigenous transmission in the affected localities.

Detailed entomological investigations are proposed to study the establishment of JE foci in the Bengaluru urban area. The report was submitted to the State Health Department for further action.

**Training to Medical Officers**

Training on mosquito identification, handling of insecticides, compression sprayer, knap-sack
sprayer, spraying techniques and other aspects related to vector control, demonstration of larvivorous fishes, maintenance, release, etc. was imparted to 75 newly joined Medical Officers of Karnataka state in three batches.

Training was imparted to four batches of PG Medical graduates of MS Ramaiah Medical College, Bengaluru, Institute of Medical Sciences and Research, NIMHANS.

**Dengue investigation in Bengaluru**

In Bengaluru upsurge of dengue was reported, and related investigation was carried out in four places of the city. Investigation revealed that the vector **Ae. aegypti** breeds in water storing cement tanks, and small containers mostly in congested colonies.

**Parasite panel preparation**

NIMR has undertaken the responsibility for validation of rapid diagnostic kit in the country, for which it needed to have measured parasite labelled parasite panel. For the same, parasite panel were prepared for both **P. vivax** and **P. falciparum**. The clinical samples were collected from the Wencolk District Government Hospital, Mangalore from June to September 2015.
Chennai (Tamil Nadu)

Monitoring of antimalarial resistance to *Plasmodium vivax* and *Plasmodium falciparum* malaria in endemic areas of Tamil Nadu, India

The study was designed to find out the therapeutic efficacy of chloroquine to vivax malaria in Rameswaram Island based on the request from Directorate of Public Health & Preventive Medicine, Govt. of Tamil Nadu. The study has been carried out in Thangachimadam and Pamban PHCs of Rameswaram Island, Ramanathapuram district, Tamil Nadu. A total of 91, 70 and 53 blood smears were taken from patients attending the malaria clinic at Thangachimadam, Pamban and Rameswaram Clinics, respectively. Out of the blood smears examined, one each from Thangachimadam, Pamban and Rameswaram were found to be positive for *Plasmodium vivax* malaria. Altogether 20 *P. vivax* patients were followed up for the last two years from Thangachimadam (7) and Pamban (13) PHCs. The haemoglobin level of patients at the time of enrolment ranged from 7.5 to 12.9 g/dl and the minimum and maximum parasitaemia was 740 and 14,240 parasites/μl, respectively. All the patients were followed up on Days 1, 2, 3, 7, 14, 21 and 28 and on any other day they reported for fever. Results indicated adequate clinical and parasitological response (ACPR). Further enrolment and follow up is planned to enrol more patients when the malaria incidence peaks.

Center for Study of Complex Malaria in India (CSCMI)

The community and clinic study, the two major arms of eco-epidemiology project was initiated in Besant Nagar, Chennai from December 2012. Enrolment for community (quarterly) and clinic study (weekly) was carried out and all the positive cases were followed up on Days 2, 7, 14, 21, 28 and 42 in clinic study.

A total of 116 fever patients presented at the clinic were screened to find out the prevalence of symptomatic malaria and 5 (4.3%) were positive by microscopy and 7 (6%) were positive by PCR. Species prevalence indicated 5 *Pv* (100%) on microscopic examination. However, 6 (85.7%) *Pv* and 1 (14.3%) *Pf* were detected by PCR. All the five malaria positive cases were males and one enrolled subject was followed on Days 2, 7, 14, 21, 28 and 42. A total of 45 individuals were enrolled for reactive case detection (RCD) surveillance from households (4 individuals were enrolled from 2 index households) in response to positive case reported at malaria clinic involving screening of all the household members of the index case. Also, individuals living in close proximity (<50 m radius; proximal) to passively detected case and households within 100 m radius (distal) were surveyed for the presence of fever within 2 weeks and screened for presence of malaria parasites by microscopy, RDT and PCR. The prevalence of malaria (including index cases) and RCD surveillance was 2 *Pv* (4.4%) by microscopy and PCR (Figs. 1 and 2).

Adult *An. stephensi* collections were carried out in cattlesheds (dawn) besides, weekly pyrethrum
spray sheet collections (PSC) in human dwelling, to check the vector density and seasonal fluctuations. The average man hour density (MHD) ranged from 23.8 (December 2014) to 68.6 (August 2015) (Fig. 3). CS-ELISA was performed for 2199 (2139 cattlesheds and 60 huts) mosquito samples collected from February 2013 to August 2015 during the reporting period and nine mosquito samples were observed to harbour *Plasmodium* sporozoites (7 *Pv* 210 and 2 *Pf* 210) and the percentage positivity was 0.41. One mixed and 2 *Pf* infections were detected out of 394 *An. subpictus* collected from 2013 to 2015. Besides, blood meal analysis performed on 342 *An. stephensi* collected from cattlesheds and human dwellings indicated 319 (93.3%) to prefer bovine blood meal, 3 (0.9%) human blood meal and the remaining 20 (5.8%) negative for both human and bovine blood. Out of 3 samples positive for human blood meal, 2 were collected from human dwellings and one from cattlesheds. Blood meal of 10 samples from cattlesheds and 2 samples from human dwellings were cross-checked with PCR. The two samples (human dwelling) which were negative in counter current immuno-electrophoresis, turned out to be positive for bovine blood in PCR. Susceptibility of larvicide (Temephos) carried out with three gene pools on the pre-adults (late III and early IV instars of F1 generation) of *An. stephensi* from the study sites (Thiruvanmiyur, Adyar and Besant Nagar), and also other sites such as Kotturpuram (malarious) and Virugambakkam (3 gene pools), Trilpican, Padikkuppam (non-malarious) areas, were found to be susceptible to WHO diagnostic dose of 0.25 ppm. Further, studies on the operational field dosage is in progress.

Studies on water temperature (using water immersible HOBOs) of the major breeding habitats at the study site have been initiated in May 2015. HOBOs were placed in 2 OHTs (cement and sintex) and 2 wells (shaded and non-shaded) and the readings are downloaded every week. Infectivity studies (10 experiments) were undertaken which involved membrane feeding of *Pv*-infected blood collected from the study site. Dissections were carried out from Day 3 and it was found that oocysts started developing on Day 7 and sporozoites after Day 12 when the fed mosquitoes were exposed at room temperature. Incubator-based studies on the immature stages of *An. stephensi* and its emergence rate at varied temperatures were initiated based on month-wise mean temperatures obtained from longitudinal study done previously with HOBOs. Average temperature of wells and OHTs for the month of February was considered and accordingly set in two incubators. Five replicates of 50 eggs each were exposed to 30°C (mean OHT temp, DTR 14.21°C and RH 88%) and 27.4°C (mean well temp, DTR 1.19°C and RH 77%). Hatching rate of the eggs was found to be 94.4% at 27.4°C and 96% at 30°C and 72% of the hatched out eggs became pupae and from pupae 82.9% emerged into adults (27.4°C). However, 89.6% of the eggs became pupae and 98.1% emerged into adults at 30°C. Hatching/development rate was observed to be faster at higher temperature (30°C). In all 141 (56.4%) adults emerged on Day 14 out of 250 eggs at 27.4°C and 211 (84.4%) adults emerged at 30°C. Emergence rate was observed to be high at 30°C when compared to mean temperature of wells, which seems to support our longitudinal larval study that OHTs are the potential breeding habitats of *An. stephensi*. Further studies are in progress (Fig. 4).
Assessment of malaria gametocythaemia with duration of symptoms: A potential programme monitoring tool for delay in seeking treatment

The study has been planned to determine the validity of the proportion of patients with gametocytes as a monitoring tool for delayed access to health care and treatment. During the period, a total of 25 malaria patients (23 \( P. falciparum \) and 2 \( P. vivax \) cases) were enrolled in the malaria clinic. All malaria patients were 15 years or above the number of males and females was 21 (84%) and 4 (6%), respectively. Previous history of malaria and treatment seeking period of patients were recorded and 24 (96%) of the patients informed that they had received treatment elsewhere, but it could not be confirmed whether the treatment was for malaria. All these patients visited NIMR clinic for further diagnosis and treatment. When taking into consideration both treated and untreated patients, 21 (84%) of the patients visited the clinic for treatment within a week and the remaining 4 (6%) patients between 1st and 2nd week after the onset of fever. In patients with vivax malaria, gametocytes were observed in all the patients. The minimum and maximum gametocytaemia was 160 and 4800/\( \mu \)l, respectively. The proportion of gametocytes to asexual stages was proportionally high in 7 out of the 23 \( P. falciparum \) patients enrolled. Further study to know the health/treatment seeking behaviour in an endemic, rural area is in progress.

A comparative study on the susceptibility of Anopheles stephensi from geographically diverse ecotypes in Tamil Nadu to Plasmodium species

Anopheles stephensi mosquitoes were collected in different areas of Chennai and Coimbatore for carrying out infectivity studies. Adult mosquito collections were carried out in cattlesheds in Virugambakkam area in Chennai; and Gounderpallyam, Vellandipallyam, Edarapallyam, Veera Keralam, Vadavalli and Linganoor areas in Coimbatore City. A total of 45 (MHD: 22.5) and 54 (MHD: 1.5) mosquitoes were collected, respectively and brought to the laboratory for rearing. Immature collections were undertaken in Ananda Nagar, Ramasamy Layout, Peelamedu, Ganapathy Nagar and other in Coimbatore. Eighteen over head tanks (OHTs) (Habitat positivity: 16.7%), 10 underground tanks (10%), 15 outside tanks (13.3%) and 33 tap pits (24.2%) were checked for Anopheles breeding and the immature collected for rearing.

A total of six trials to study differences in infectivity in Chennai and Coimbatore strains have been carried out during the period. Artificial membrane feeding technique was followed. The mean, minimum and maximum gametocytaemia of malaria infected patients blood were 2807, 1040 and 3880/\( \mu \)l. Except in one trial, development of parasites was observed in all other trials. Based on the availability of infected blood fed mosquitoes, dissections were carried out from Day 3 to check development of parasites. A total of 117 and 123 Chennai and Coimbatore strains of \( A. \) stephensi mosquitoes were dissected out of which, 36 and 26 mosquitoes were found to contain oocyst. Oocyst was first observed on Day 5 (one exp.) and 6 (3 exp.) in Chennai strain; Day 5 (one exp), Day 6 (one exp) and on Day 7 (2 exp) in Coimbatore strain. The oocyst positivity rate from the dissections made from Day 5 onwards was 32.7 and 23.4%, respectively (Fig. 5). Sporozoites were not observed in the trials due to lack of survivability of infected mosquitoes. Study is in progress.
Battle assay to monitor Insecticide resistance in vector mosquitoes

The studies were conducted in the laboratory following standard operating procedures at room temperature. The result was assessed based on knockdown of exposed vectors in one hour time period. Adult An. stephensi mosquitoes were collected from cattle sheds in Thiruvaniyur (southeast; malarious) and Virugambakkam (southwest; non-malarious) areas of Chennai. The F₁ progeny obtained from these mosquitoes was sugar-fed and used for the experiments. In the case of Aedes aegypti the experiments were conducted in adults that emerged from field collected larvae/pupae from Ayavanaram area. A total of three trials each (gene pools) in An. stephensi from Thiruvaniyur, Virugambakkam areas; and in Ae. aegypti from Ayavanaram area have been carried out. The KD₅₀ value was 100% in all the trials indicating that the vector mosquitoes are susceptible to dosage of deltamethrin. Further studies with other malaria vectors in the state have been planned.

Situation analysis of the malaria outbreak in Vizhinjam CHC, Thiruvananthapuram district, Kerala, India

PHC Mukkola (Vizhinjam CHC) of Thiruvananthapuram district recorded 23 malaria cases (22 P. vivax and one P. falciparum) from 21 to 31 July 2015. The cases were reported mainly from Pallithura, Kottapuram and Township colony, all coastal hamlets adjacent to Vizhinjam harbour area. Evidence-based situational analysis besides, entomological investigations were carried out by the field unit to find out the transmission dynamics in the affected areas.

Out of 23 malaria positive blood smears/slides 20 were provided by the Directorate of Health Services, Govt. of Kerala and re-examined and confirmed. All the 20 blood smears were correctly identified and there was no discrepancy in identification of malaria parasite species. The parasitaemia for P. vivax and P. falciparum ranged from 160 to 1,1360 and 360 to 12,800 parasites/µl, respectively. On enquiry with the patients, it was found that none of them had any recent travel history prior to the infection which indicated indigenous nature. The cases (23) were also plotted with global positioning system (GPS) for accurate distribution to demarcate the hotspot areas. The team could collect follow up blood smears from malaria patients (19 nos) declared positive/admitted at CHC Vizhinjam, besides samples for RDT, filter paper and microvete samples for molecular diagnosis of the parasites to find out any sub-microscopic forms and mixed parasite populations. Clinical profile including auxiliary temperature and haemoglobin was recorded using Haemocue equipment. Contact smears (43 nos.) were taken from attenders/immediate family members who had stayed with the patient prior and during the present episode. Furthermore, blood smears were also collected from clinically febrile patients (14 nos.) of Pallithura and Township colony/areas (Fig. 6). RDT tested were found to be negative for malaria parasites. However, the microslides were examined for screening of malaria parasites and blood samples for molecular diagnosis of parasite species. It was observed that the admitted malaria patients were provided mosquito nets to prevent the transmission of infection to other patients and 14 days Primaquine regimen was administered to P. vivax
cases by directly observed treatment, revealed by the case sheet at Vizhinjam CHC.

Immature sampling of mosquitoes was undertaken in Pallithura, Kottapuram and Township colony/areas. The domestic and peri-domestic breeding habitats inspected includes OHTs, wells, outside tanks, inside tanks, cisterns (sintex), besides boats docked on the sea shore (Fig. 7). Out of 113 sources (with water) inspected habitat positivity for mosquito breeding was 14 (12.4%). However, anopheline positivity in the above breeding habitats (wells and cisterns) was only 2 (1.8%). In total 14 (28.6%) house premises were found with mosquito breeding but anopheline breeding was only in 2 (4.1%) households. It was observed that almost all the breeding habitats were treated with Temephos (Abate) and even twice a week in most of the habitats in the affected areas ever since the outbreak was reported. Anopheline breeding could be observed in a slow moving fresh water stream in Kottapuram area and on adult emergence it was found to be An. culicifacies. Nevertheless, adult emergence was not observed from other habitats with breeding due to intensified larvicide application.

Adult collections (dawn and dusk) were also undertaken in Pallithura, Kottapuram and Township areas, where cases were reported. A total of 43 anophelines (3 dawn; 40 dusk) could be collected from cattle sheds (3 nos.) in Kottapuram and Township colony/areas. Anopheles culicifacies (12 nos.) and An. stephensi (16 nos.) were the vector mosquitoes collected, both from dawn and dusk collections. The anopheline/vector density was found to be more in dusk collections. The other anopheline mosquitoes collected and identified were An. jameisi, An. vagus and An. culiciformis. The vector mosquitoes were screened for vector incrimination by CS-ELISA and blood meal for host preference by counter current immunoelectrophoresis method. Houses visited in the affected areas had the inscriptions of IRS carried out recently. The team discussed with the programme managers/officials on the remedial

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**Fig. 7:** Immature vector survey in outbreak areas of Thiruvananthapuram district—(a) Well; (b) Stream; (c) Roof/Terrace; and (d) Boats.
solutions to tackle vector breeding, reduce parasite load in the community and continuation of intervention measures on sustainable basis and report submitted to DHS, Govt. of Kerala.

Ecology and distribution of *Aedes albopictus* and *Aedes aegypti* with special reference to *albopictus* subgroup species of the subgenus *Stegomyia* in Kerala, India

Discussions were made by the investigators of both the institutes and plans have been chalked out to make arrangements for advertisement and recruitment of the staff besides, preliminary visit to initiate the study in selected districts. Accordingly, a survey was undertaken in two study districts, namely Pathanamthitta and Idukki in Kerala during February 2016 to select sites/habitats for collection of target mosquito species, *Ae. albopictus* and *Ae. aegypti*. Vandanpathal forest, Kombukuthi tribal village, Murinjapuzha forest, Koruthodu, Chittar and forest areas in Erumeli, Manimala, Kodumudi, Maniyar and Nilackal besides, rubber plantations in the adjoining areas of the forests in Pathanamthitta district were surveyed. In Idukki district, forest areas and rubber plantations in Malankara, Nadukani, Kulamavu and Cheruthony were surveyed. Collections undertaken in the forest areas were not productive with only a few numbers of *Ae. albopictus* owing to the dry nature of the habitats. Nevertheless, collections made in the rubber plantation areas obtained 122 *Ae. albopictus* mosquitoes. All of them have been preserved for detection of virus, if any. Further, plans have been chalked out for carrying out immature and adult collections after the onset of monsoon rains.

Monitoring of existing intervention tools/methods in the programme for scaling-down malaria in Rameswaram island, Tamil Nadu, India

As suggested by the IDVC/RAC, situation analysis of the existing tools was carried out in Rameswaram island in 2015 after obtaining necessary clearance from Directorate of Public Health & Preventive Medicine (DPH & PM), Govt. of Tamil Nadu. Discussions were held with DDHS, Ramanad district and also with the Medical Officers of Thangachimadam and Pamban PHCs (Fig. 8). It was observed that 35.6 and 68.1% reduction in malaria was observed in Pamban and Thangachimadam PHCs from 2013–14. Further, 63.2 and 45.9% reduction in malaria was observed on analysis of data from 2014 to September 2015 in the respective PHCs. The parasite and vector control methods existing and at present functional in the programme were discussed. It was observed that most of the people in the locality approach private practitioners for diagnosis and treatment. The RDT was the most common method of diagnosis by the private practitioners and artesunate plus lumefantrine combination was administered for *Pl* treatment. Alphacypermethrin 5% WP was

Evaluation of SumiLarv 2MR as a mosquito larvicide for control of *Aedes aegypti* in container habitats in Chennai, India

A survey was undertaken in Ayanavaram Railway Colony and Panathoppe area in Ward No. 69, Zone VI of Corporation of Chennai to identify the breeding potential *Ae. aegypti* for the proposed study. A total of 984 houses were visited and breeding sources present inspected. Out of these houses checked, 294 (30%) of houses were found with water storage containers positive for *Aedes* breeding. The water storage containers prevalent in the area were barrels (temporary storage facilities) and cement tanks (permanent storage facilities). The cement tanks were found to be located both indoors and outdoors. The number of barrels, indoor and outdoor cement tanks checked were 1045, 941 and 175 and *Aedes* breeding was observed in 91 (8.70%), 280 (29.75%) and 24 (13.71%) of the habitats, respectively. Immatures were collected and brought to the laboratory for emergence and identification. A total of 1038 *Aedes* mosquitoes were emerged and all were found to be *Ae. aegypti*.
the insecticide used for IRS to control vector mosquitoes besides, bacticides and to certain extent larvivorous fish, *Poeclia reticulata*. Further, study is in progress in close collaboration with local health officials.

**Dynamics of malaria in endemic areas of Chennai (Thiruvottiyur), Tamil Nadu, India**

Discussions were held with the Health Department of Corporation of Chennai and Directorate of Public Health and Preventive Medicine for necessary concurrence to initiate the study, logistic assistance and details of the study site. Health Department of Corporation of Chennai has given the concurrence to initiate the study. Background information of the study site has been obtained from the concerned zone and efforts have been initiated to carry out the longitudinal/seasonal studies. The research findings would be provided to the programme managers for realistic planning and sustainable vector control measures to scale-down the malaria prevalence.

**Post-flood scenario on vector mosquito breeding potential in Chennai, India**

Chennai witnessed heavy rains during November to December 2015. The entire city including water bodies, water storage facilities and wells in low-lying areas were flooded/affected. Many wells were submerged in flood waters and in some areas water level was up to the rim. As a result, the well water and the other domestic storage waters were polluted. Effective steps were taken up by the Corporation of Chennai immediately after the recession of flood to treat the wells. As advised by the Secretary, DHR & DG, ICMR, to assess the impact of floods on the breeding of mosquitoes, a survey was undertaken in extreme flood hit areas of Chennai, namely Manali New Town, Tondairpet, Madhavaram, Mel Aynambakkam, Maduravoyal, West Mambalam, Manuel, Virugambakkam, Valasaravakkam, Adayar and Mudichur. Houses were selected on a random basis and the breeding habitats present were inspected. Immatures present were collected and brought to the laboratory for rearing and identification of species.

A total of 222 houses were surveyed. The mosquito breeding habitats inspected were open domestic wells (164), sumps (7), outside cement tanks (24), barrels (61), tap pits (12), puddles (4), cesspools (22) and other sources (boats 14). Immatures of anophelines were present only in wells (30) and the habitat positivity for *Anopheles* breeding was 18.3%. All emerged 613 *Anopheles* mosquitoes were found to be *An. stephensi*. Likewise, *Aedes* mosquitoes were found to be breeding only in outside cement tanks (2); the habitat positivity was 8.3% and all the 64 Ae. immatures emerged were found to be *Aedes aegypti*. *Culex* species. were found breeding in wells (20), outside cement tanks (3), sumps (1) and cess pools (2) and the habitat positivity was 12.2, 12.5, 14.3 and 9.1, respectively. Among the culicines, emergence results showed 826 *Cx. quinquefasciatus*, 38 *Cx. vishnui* and 15 *Cx. pseudovishnui* to be breeding in wells and 103 *Cx. gelidus* and 81 *Cx. tritaeniorhynchus* in cess pools. Mosquito breeding was not observed in tap pits, barrels and puddles.

**Technical and training support to the programme**

**Diagnosis and treatment of malaria patients**

The malaria clinic continued to function at the office premise. The patients from nearby areas, namely Ayapakkam, ICF colony, Mogappair, Nolambur, Padi and Anna Nagar used to attend the clinic for prompt diagnosis and treatment. During the period, 146 blood smears were taken out of which four were found positive for malaria. The number of *Pv* and *Pf* cases was three and one, respectively. The SPR, SIR and PI% were 2.7, 2.05 and 25, respectively. All the malaria patients were treated as per the NVBDPC drug schedule. Clinic data on malaria cases were handed over to the Health Department of Corporation of Chennai and DPH & PM, Govt. of Tamil Nadu for follow-up and control measures.

**Health education/Training programmes/Awareness on malaria**

During the period, two health education/training/summer internship programmes were conducted and 9 students (2 from the Zoology Department of Madras Christian College, Tambaram, Chennai and seven students pursuing MD course in Sri Ramachandra Medical College and Research Institute, Porur, Chennai) participated. The students were trained on blood smear preparation, staining techniques, identification of malaria parasites and different diagnostic methods for malaria. Lectures on epidemiology and control of malaria was delivered to them (Fig. 9).

Awards/Honours received:

Dr Alex Eapen
Selected as ‘Chartered Biologist (CBiol)’ by the Royal Society of Biology, London, UK.

Ph.D. students under guidance

Dr Alex Eapen (as a Co-guide)

Ms Shalu Thomas CSIR JRF
Ph.D. topic (General): Micro-environmental factors influencing immature and adult densities of Anopheles stephensi (Liston, 1901), urban malaria vector and its role in malaria transmission in Chennai, India. Department of Zoology, Madras Christian College, University of Madras, Chennai, India.
Status: Thesis compilation and documentation

Ms G Sri Lakshmi Priya SRF
Ph.D. topic (General): Genetic epidemiology of Plasmodium vivax and P. falciparum in Chennai, India. Department of Zoology, Madras Christian College, University of Madras, Chennai, India.

Ms R Sangamithra SRF
Ph.D. topic (General): Vector mosquitoes and persistent malaria transmission in Chennai, India. Department of Zoology, Madras Christian College, University of Madras, Chennai, India.
Monitoring therapeutic efficacy of antimalarials in India

Artemether + lumefantrine (AL) was introduced in 2013 (replacing AS + SP therapy) for treatment of *P. falciparum* malaria in northeastern states. The broad objectives of the study were to monitor the current therapeutic efficacy of AL in malaria endemic blocks along international borders. The study was undertaken in three different locations including Tripura (Gandachara CHC, Dhalai district), Mizoram (Chawngte CHC, Lawngtlai district) and Meghalaya (Darengere PHC, Tura) during June to October 2015 in collaboration with the respective state health authorities (Fig. 1). In Gandachara, of the total 736 blood-smears screened for malaria parasite, 212 (28.8%) were positive for malaria parasite of which *P. falciparum* was the majority infection (94%). Of the total 77 subjects selected for 28-day follow up investigations, 98.7% (76/77) were ACPR. In Chawngte CHC, of the 1058 blood-smears examined, 408 (38.6%) were malaria positive of which 83% were *P. falciparum* cases. Of total 83 subjects enrolled 82 (98.8%) showed ACPR (subject to correction by PCR). In Darengere PHC, as many 32 subjects were selected for follow up investigations, all of which showed ACPR except one which was lost to follow up (Table 1).

Prevalence of malaria transmitting mosquitoes in Lawngtlai district of Mizoram, Indo-Bangla border, northeast India

Entomological investigations were undertaken in Chawngte (Lawngtlai district) of Mizoram during July–August 2015 to ascertain the relative abundance of mosquito vectors in the locality (Fig. 2). Different sampling techniques were applied; these included day-resting mosquito catches in human dwellings, indoors, cattle biting catches in the evenings, overnight CDC trap collections and human landing mosquito catches. Nine different anopheline mosquito species were collected; these included *Anopheles aconitus*, *An. baimai*, *An. jamesii*, *An. kochi*, *An. maculatus*, *An. minimus*, *An. nivipes*, *An. barbirostris* and *An. nigerimus*. Of these *An. kochi*, *An. nivipes* and

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**Table 1** Prevalence of malaria and success rate of artemether + lumefantrine (AL) for the treatment of *P. falciparum* malaria in northeastern states of India

<table>
<thead>
<tr>
<th>Study location/ District/ State</th>
<th>Study period</th>
<th>No. of fever cases</th>
<th>Malaria (+)ve cases (%)</th>
<th><em>P. falciparum</em> cases (%)</th>
<th>No. of cases enrolled</th>
<th>% success rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chawngte/ Lawngtlai/ Mizoram</td>
<td>Jun–Aug</td>
<td>1058</td>
<td>408 (38.6)</td>
<td>338 (83)</td>
<td>83</td>
<td>(98.8)</td>
</tr>
<tr>
<td>Gandachara/ Dhalai/ Tripura</td>
<td>Jun–Aug</td>
<td>736</td>
<td>212 (28.8)</td>
<td>212 (94.3)</td>
<td>77</td>
<td>(98.7)</td>
</tr>
<tr>
<td>Darengere/ West Garo Hills/ Meghalaya</td>
<td>Aug–Sep</td>
<td>864</td>
<td>65 (7.5)</td>
<td>64 (98)</td>
<td>32</td>
<td>(100)</td>
</tr>
</tbody>
</table>
An. *nigerimus* were most abundant in cattle biting collections; others included *An. barbirostris* and *An. maculatus* in order of their relative abundance. *Anopheles maculatus* and *An. baimaii* were the most predominant collections in CDC-trap collections as well as human landing catches in human dwellings (Table 2). None of these mosquito species were encountered in day-resting catches in human dwellings indoors.

**Malaria treatment practices with special emphasis on the private sector in India – Phase-II**

The study was undertaken in five different block PHCs of Kamrup district of Assam to ascertain the malaria treatment practices in public and private health care providers and use of antimalarial medicines in Assam. The cases were detected based on passive surveillance at malaria clinic in Sonapur, Kamrup district (Table 3). Of the total 167 exit interviews, all reported fever and had blood test for malaria positivity. Among these, 44% (74/167) had malaria test done by both microscopy as well as RDK kit. Only 24% (40/167) had blood test exclusively by microscopy whereas 32% (53/167) by RDKs. Among test results, *P. falciparum* was the predominant infection (75%, 126/167). All had received antimalarial medicines during illness. Among prescribed drugs, AL was the most commonly prescribed (56%, 93/167) followed by CQ+PQ (25%, 41/167), AS+SP (12%, 20/167) and Artesunate oral/injection (8%, 13/167). Among attending physicians interviewed, all were either were MBBS (51%, 15/29) or had PG degree (31%, 9/29), and 17% (5/29) were non-allopathic. Among 42 chemists interviewed most by reported sale of E-mal, larinate and artesunate. The study is concluded.

**Technical support to the control programme**

1. Stocks of both guppy and Gambusia are being maintained in Guwahati metropolis. These larvivorous fishes are being provided to other northeastern states, various districts of Assam and other establishments/cantonment areas on the request of the Vector Borne Disease Control Programme, NHM (Assam).
Workshops/Seminars/Conferences/Training courses/Important meetings attended

Dr Vas Dev

1. Served as member committee for LLIN technical bid for the Government of Assam in the meeting chaired by the Director Health Services on 22 December 2014 and then on 27 January 2015 for procurement.

2. Served as Resource person for Regional review meeting of northeast states held at Kohima, Nagaland April from 23–24, 2015 taken by the NVBDCP and deliberated on “Vector bionomics and control” specific to NE region.

3. Serving as member on board of the Institutional Academic and Research Committee (SSNIA & RC) of Sri Sankaradeva Nethralaya, Guwahati for preparing research documentation and building proposals for suitable funding.

4. Coordinated the ICMR JRF Entrance Examination held at Guwahati for providing local logistics support as per directives of the DG, ICMR on 19 July 2015.

5. Reviewed manuscripts for ACTA TROPICA, Indian Journal of Medical Research (IJMR) and Open Entomology Journal, Transactions of the Royal Society of Tropical Medicine & Hygiene and for Journal of Vector Borne Diseases (Subject Editor).

6. Served as “External Member” of the DRDC board for assessment of the Technical Cadre employees of the DRDO held at Tezpur on 10 July 2015.

7. Developed institutional linkage with the Department of Zoology, University of Science and Technologyj, Meghalaya for serving as resource institute for PG research.

8. Served as guest speaker at the University of Science and Technology, Meghalaya on 28 August 2015 and delivered lecture on, “Mosquito vectors of malaria and their bionomics in northeast India” for the benefit of PG students at the inaugural session 2015–16 (by invitation).

9. Coordinated the establishment of Brahmaputra Gallery in the Assam Water Research & Management Institute (AWRMI) in Guwahati under the aegis of the National Academy of Sciences, Allahabad.

10. Served as reviewer of short-term studentship (STS) fellowship project proposal for ICMR on 17 December 2014 and 6 February 2015.

11. Observed World Mosquito Day on 20th April 2015 in collaboration with the Regional Science Centre, Guwahati (National Council of Science Museums), and delivered a popular lecture on “Mosquito borne diseases in Assam” hosted by the NCSM.

Other Scientists

1. Dr Vas Dev and HP Gupta participated in the Orientation and GCP meeting on study project, “A multicentre open labelled randomized trial to assess efficacy of triple ACTs (TACT) for treatment of P. falciparum malaria held at Agartala, Tripura from 25–27 July 2015.

2. Dr HP Gupta participated in inter-country meeting on cross-border collaboration to eliminate malaria in south asia held at WHO Regional Office for Southeast Asia, New Delhi, India from 12–13 February 2016.

3. Dr Vas Dev and Sh GG Tewari attended the meeting on “India Innovation Growth Programme” organized by the FICCI held at Guwahati on 16 January 2015.
Situation analysis and identification of risk factors of dengue in District Hardwar

Focal outbreak of dengue was reported in Hardwar district of Uttarakhand state during 2013. A total of 1412 suspected dengue cases were recorded in District Hardwar, out of which 468 cases were confirmed by ELISA test and 7 deaths were recorded. Survey of breeding prevalence of Aedes in selected areas of District Hardwar, viz. Kankhal (urban area) and BHEL township (Bahadarabad CHO) was undertaken during 2015. House index (HI), container index (CI) and breteau index (BI) of Aedes and their percent species composition is given in Table 1. During the months from July to October, a total of 1525 houses were surveyed, out of which 474 houses were found positive for Aedes breeding. Out of 4185 containers searched, 1034 containers were found positive for Aedes breeding. House, container and breteau indices were 31.1, 24.7, and 67.8, respectively.

Breeding prevalence Aedes in different breeding sites are given in Table 2. It was observed that desert coolers and containers were the major breeding sites in the area which supported heavy breeding of Aedes.

Four Aedes species namely, Aedes aegypti, Ae. albopictus Ae. vittatus, and Ae. pseudosetaenius were identified. Percent species composition of Aedes aegypti in Hardwar City, Kankhal and BHEL Township was 98.6, 60.4 and 1.5, respectively. Percent species composition of Aedes aegypti larvae was more than 60% in urban areas of Hardwar and Kankhal, whereas its percent composition was very low in BHEL Township (Table 3). Insecticide

<table>
<thead>
<tr>
<th>Area</th>
<th>Houses searched</th>
<th>Houses positive</th>
<th>Containers searched</th>
<th>Containers positive</th>
<th>Positive for pupae</th>
<th>HI</th>
<th>CI</th>
<th>BI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kankhal</td>
<td>236</td>
<td>84</td>
<td>638</td>
<td>164</td>
<td>49</td>
<td>35.6</td>
<td>25.7</td>
<td>69.5</td>
</tr>
<tr>
<td>Hardwar</td>
<td>102</td>
<td>41</td>
<td>365</td>
<td>72</td>
<td>32</td>
<td>40.2</td>
<td>19.7</td>
<td>70.6</td>
</tr>
<tr>
<td>BHEL</td>
<td>1187</td>
<td>349</td>
<td>3182</td>
<td>798</td>
<td>281</td>
<td>29.4</td>
<td>25</td>
<td>67.2</td>
</tr>
<tr>
<td>Total</td>
<td>1525</td>
<td>474</td>
<td>4185</td>
<td>1034</td>
<td>362</td>
<td>31.1</td>
<td>24.7</td>
<td>67.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Breeding sites</th>
<th>Hardwar city</th>
<th>Kankhal</th>
<th>BHEL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. surveyed</td>
<td>% positivity</td>
<td>No. surveyed</td>
</tr>
<tr>
<td>Desert coolers</td>
<td>71</td>
<td>50.7</td>
<td>205</td>
</tr>
<tr>
<td>Tanks</td>
<td>11</td>
<td>18.2</td>
<td>5</td>
</tr>
<tr>
<td>Containers</td>
<td>29</td>
<td>41.4</td>
<td>156</td>
</tr>
<tr>
<td>drums</td>
<td>5</td>
<td>40</td>
<td>11</td>
</tr>
<tr>
<td>Refrigerators</td>
<td>5</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>Mud pots</td>
<td>0</td>
<td>0</td>
<td>57</td>
</tr>
<tr>
<td>Tyres</td>
<td>0</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Pits</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Flowers pots</td>
<td>244</td>
<td>8.2</td>
<td>155</td>
</tr>
<tr>
<td>Tree holes</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>365</td>
<td>19.7</td>
<td>638</td>
</tr>
</tbody>
</table>
Table 3. Species composition of immature *Aedes* collected from field

<table>
<thead>
<tr>
<th>Area</th>
<th>No. of immature identified</th>
<th>Percent species composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ae. aegypti</td>
</tr>
<tr>
<td>Kanial</td>
<td>386</td>
<td>60.4</td>
</tr>
<tr>
<td>Hardwar</td>
<td>145</td>
<td>98.6</td>
</tr>
<tr>
<td>BHIL</td>
<td>615</td>
<td>1.5</td>
</tr>
<tr>
<td>Total</td>
<td>1,146</td>
<td>33.6</td>
</tr>
</tbody>
</table>

susceptibility test to different groups of insecticides revealed that *Ae. aegypti* was resistance to DDT and susceptible to malathion and deltamethrin. Block-wise dengue cases of District Hardwar are given in Table 4. A total of 810 suspected dengue cases were recorded, out of which 357 cases were confirmed by ELISA test and no death was recorded. More than 70% cases were recorded from Hardwar urban area and Bahadarabad block.

Table 4. Block-wise dengue cases in District Hardwar

<table>
<thead>
<tr>
<th>Block</th>
<th>Suspected cases</th>
<th>ELISA +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bahadarabad</td>
<td>644</td>
<td>275</td>
</tr>
<tr>
<td>Bhagwanpur</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Laksar</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Narson</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Roorktee</td>
<td>88</td>
<td>49</td>
</tr>
<tr>
<td>Migrated</td>
<td>65</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>810</td>
<td>357</td>
</tr>
</tbody>
</table>

Stratification of malaria in District Hardwar: A demonstration of elimination in one subcentre

The project has been initiated in May 2015, for a period of three years. The objectives of the project are to prepare a PHC-wise stratified map of malaria, identify the risk factors of malaria endemicity and undertake intervention measures in one subcentre so as to demonstrate elimination of malaria. Based on PHC-wise epidemiological data of malaria in District Hardwar one high and one low malaria incidence CHC has been selected for the study. Subcentre wise data of District Hardwar of last three years has been collected and a stratified map of the area has been prepared (Fig. 1). Four high malaria incidence villages in Chanderpuri subcentre of Laksar CHC (Khanpur) and one low malaria incidence village in Shiv Garh subcentre of Bahadarabad CHC have been selected for the study. Socio-demographic profile of the area has been compiled in Tables 5 and 6. Entomological and parasitological investigations were carried out.

Table 5. Socio-demographics of four villages of Chanderpuri subcentre of Laksar CHC

<table>
<thead>
<tr>
<th>Villages</th>
<th>Population</th>
<th>No. of houses</th>
<th>Type of houses</th>
<th>Economic status of families</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Type</td>
<td>APL</td>
</tr>
<tr>
<td>Chanderpuri Khurd</td>
<td>1149</td>
<td>929</td>
<td>383</td>
<td>380</td>
</tr>
<tr>
<td>Chanderpuri Kala</td>
<td>838</td>
<td>791</td>
<td>297</td>
<td>278</td>
</tr>
<tr>
<td>Madha Bela</td>
<td>665</td>
<td>608</td>
<td>197</td>
<td>171</td>
</tr>
<tr>
<td>Sherpur Bela</td>
<td>614</td>
<td>521</td>
<td>350</td>
<td>250</td>
</tr>
<tr>
<td>Total</td>
<td>3266</td>
<td>2849</td>
<td>1227</td>
<td>1079</td>
</tr>
</tbody>
</table>
in both the areas. Man hour density of *An. culicifacies, An. fluviatilis* and total anophelines are given in Figs. 2 and 3. Prevalence of *An. culicifacies* was observed throughout the year and peak density was recorded during the months of July–August. *An. fluviatilis* was not recorded in the Chanderpuri subcentre. Prevalence of *An. culicifacies* and *An. fluviatilis* were observed in Shiv Garh subcentre and peak density of *An. culicifacies* was recorded in months of July–August.

![Graph](image)

Prevalence of *An. fluviatilis* was observed from September to December in low density. Density of *An. culicifacies* was very low from November to March in both the areas. During the months of May to March, a total of 656 blood slides were collected in Chanderpuri subcentre, out of which 78 cases were found positive for *P. vivax* and 5 cases for *P. falciparum**, SPR being 12.5. In Shiv Garh a total of 656 blood slides were collected, out of which 16 slides were found positive for *P. vivax*, SPR being 2.4. The results coincide with high vector density (Figs. 4 and 5) in the area. Overall man hour density

![Graph](image)

![Graph](image)

![Graph](image)

![Graph](image)
of *An. culicifacies* and total anophelines and malaria prevalence in Chanderpuri subcentre was high as compared to Shiv Garh subcentre.

**Synthesis, pKa determination and in vivo toxicity of new promising antimalarial 6-methoxy-5, 8-dii-(4-amino-1-methylbutyl-amino)-quino line**

The project was initiated in July 2013 for a period of two years in collaboration with Jamia Hamdard University, Delhi to synthesis, pKa determination and *in vivo* toxicity of new promising antimalarial 6-methoxy-5, 8-dii-(4-amino-1-methylbutyl-amino)-quino line. The work was mainly divided into two sections, viz. synthesis and *in vivo* toxicity study. Primary target was to design synthetic scheme for the promising antimalarial N4, N4-(6-methoxyquinoline-5, 8-diy) bis (pentaene-1, 4-diamine). Various synthetic strategies were proposed and finally, 7 step synthetic scheme was adopted. Out of 7 synthetic steps, 6 have been successfully optimized. The synthesis for the side chain has been obtained. The trace quantity of the 5th step product was confirmed via mass spectrometry, but yet reaction needs to be optimized to get good yield. Acute oral toxicity test was performed as per OECD-423 guidelines for primaquine as a standard drug until we get the target molecule. No significant differences were noticed in the body and organ weights and physical and behaviour parameter between the control and 100, 200 mg dose primaquine treated groups while 500 mg/kg primaquine treatment group showed some change in physical behaviour pattern like lethargy and tremors. In biochemical parameter, increase level of lipid MDA, SGOT, SGPT and AKT in blood serum were recorded but no significant change in level of glucose, total cholesterol, triglyceride in blood serum and glucose 6-phosphate dehydrogenase in blood was found.

**Development of botanical insecticide formulation of essential oils extracted from Lantana camara and Valeriana jatamansi and Psoralea corylifolia for the control of mosquitoes**

The project has been initiated in October 2013 for a period of 3 years in collaboration with Defence Research Laboratory, Tezpur. The objective is to isolate essential oils from *Lantana camara*, *Valeriana jatamansi* and *Psoralea corylifolia* in large-scale and to develop easy to use, relatively safe botanical insecticide formulation(s) of essential oils extracted from proposed plants for the control of mosquitoes. To investigate toxicity of the developed formulation against human cell lines, 100 ml oil was extracted from the leaves of *Lantana camara*, and from roots of *Valeriana jatamansi*. LD<sub>50</sub> and LD<sub>90</sub> values of *L. camara* were 0.06 mg/cm<sup>2</sup> and 0.10 while, LD<sub>50</sub> and LD<sub>90</sub> values of *V. jatamansi* were 0.14 mg/cm<sup>2</sup> and 0.24 mg/cm<sup>2</sup>. KDT<sub>50</sub> and KDT<sub>90</sub> values of essential oil of *L. camara* with 0.208 mg/cm<sup>2</sup> (1.5% w/v) impregnated papers were 14 min and 25 min, while KDT<sub>50</sub> and KDT<sub>90</sub> values of essential oil of *V. jatamansi* were 17 and 30 min with 0.28 mg/cm<sup>2</sup> (2.016% w/v) impregnated papers. Attempts were made to find out the impact of DEPA (N,N-diethyl phenyl acetamide) a synthetic mosquito repellent developed by DRDO on the KD<sub>90</sub> values on the oils *L. camara* and *V. jatamansi* against *An. stephensi*. Results revealed that the combination of DEPA in Lantana oil reduces the KDT<sub>90</sub> from 25 min to 20 min. Similarly, a combination of (2.0%) *V. jatamansi* oil with 2% DEPA reduces the KDT<sub>90</sub> from 30 min to 25 min against *An. stephensi*. Experiments against *Ae. albopictus* and *Culex quinquefasciatus* also revealed that addition of DEPA in oils of Lantana and *V. jatamansi* resulted in the reduction of KD values thus enhanced the insecticidal activity. It is to point out that DEPA did not show any adulticidal activity against mosquitoes. Results of formulations in combination of 1.5 per cent LEO with 4% DEPA showed significant reduction in KDT<sub>100</sub> as compared to 1.5% LEO alone. Combination of 1.5% LEO with 4% DEET also showed significant reduction in KDT<sub>100</sub> as compared to 1.5% LEO alone. KDT<sub>100</sub> values are much lower if the combinations were prepared in isopropyl alcohol as compared to acetone. Combination of LEO with DEET is more effective as compared to LEO with DEPA. Application of LEO + DEET on wall surface showed > 80% reduction in mosquito mortality up to 21 days while with LEO + DEPA showed > 80% reduction in mosquito mortality up to 13 days.

**Industrial malaria control**

This NIMR Field Unit is working on industrial malaria control since 1986 and successfully controlled malaria in BHEL, Hardwar. From April 2015 to March 2016, a total of 2056 blood slides were collected of which 71 slides were found positive for *P. vivax* and one for *P. falciparum*, SPR
being 3.5 (Fig. 6). Out of 72 positive cases, 25 were from Jwalapur, Kankhal and nearby villages and remaining 47 were from the BHEL Township. During the months of August–September average man hour density (MHD) of *An. culicifacies* was 25. Three anopheline species ie. *An. culicifacies*, *An. splendidus*, and *An. annularis* were collected from the township area. Insecticide susceptibility test of *An. culicifacies* was carried out against 4% DDT, 5% malathion, 0.05% deltamethrin and 0.05% lambdacyhalothrin. *An. culicifacies* was found resistant to DDT and susceptible to malathion, deltamethrin and lambdacyhalothrin.

**Technical support provided to the vector control programme**

- Cross-checking of blood slides of district Hardwar for malaria parasites.
- Entomological surveillance of Dengue vector in District Hardwar, Uttarakhand.
- IEC activities were carried out extensively in dengue affected areas of District Hardwar and discussions were held with the state health authorities on various control measures taken by the administration.
- Two Larvivorous fish hatcheries have been established in BHEL Township for their propagation and distribution in the district.
- Treatment of malaria positive cases were carried out in collaboration with State Health authorities to curtail malaria transmission in two subcentres of Laksar and Bahadarabad CHC’s.

**Other activities**

- A meeting was held on 10 July 2015 with the Director General, UCOST at Dehradun regarding funding of scientific projects. Dr Ashish Gupta Dr AC Pandey and Mr HC Pandey attended the meeting.
- Dr Ashish Gupta Dr AC Pandey and Mr HC Pandey met Mr Rajan Kapoor, District NGO Head on 31 July 2015 at Ambuja Cement, Bhagwanpur in connection with training programmes of ASHA.
- Dr NC Gupta discussed about treatment of malaria positive cases in Laksar CHC with the District Malaria Officer on 28 July 2015.
- Dr Ashish Gupta and Mr HC Pandey attended the 20th half yearly meeting of Town official Language implementation committee meeting (Narakas) on 6 August 2015 at Hardwar.
- Dr NC Gupta and Mr HC Pandey met the Principals of Delhi Public School, Kendriya Vidhyalaya and Educational Management Board Schools of BHEL from 3–4 August 2015 to create awareness of dengue control activities amongst school children.
- Dr Ashish Gupta and Dr AC Pandey held discussions with Chief Medical Officer, BHEL, Hardwar on 25 August 2015 regarding dengue control measures in the township and factory area.
- A meeting was held with Dr Jangpangi, Ardra Kumbha Mela Incharge (Health), Hardwar on 8 September 2015, regarding control of house flies and mosquitoes. Dr Ashish Gupta, Dr AC Pandey and Mr HC Pandey attended the meeting.
- Dr Ashish Gupta, Dr AC Pandey and Mr HC Pandey attended a meeting at Mela Control Bhawan on 29 September 2015 regarding control of dengue in District Hardwar. The Chief Development Officer, Chief Medical officer and other officials of different state departments participated in the meeting.
- A review of work progress of the field unit was carried out by Dr Shivalkal, Chairman, NIMR, and Dr PL Joshi, Chairman, on 8 June 2015.
- Dr RC Dhiman visited the field unit on 30 October 2015, to identify suitable villages for the ongoing projects.
- Dr BN Nagpal, Dr T Adak and Dr CP Batra along with a training team of 20 Biologist from Ujjain, Madhya Pradesh visited Hardwar field unit from 14–15 January 2016 for epidemiological and entomological field
training. Dr Jangpangi, Ardha Kumbha Mela Incharge (Health), Hardwar delivered a lecture to the training team.

- Dr Ashish Gupta and Dr AC Pandey attended the Town official Language implementation committee meeting (Narakas) on 28 January 2016 at THDC, Rishikesh.
Assessment of durability of LifeNet® long-lasting insecticidal nets in a Phase-III study in Madhya Pradesh, India

The study is continuing in 13 villages of Kundam, Jabalpur (Fig. 1) covering 1530 households and population 6701. A total of 3568 LLINs (1202 LifeNet®, 1192 NetProtect and 1174 PermaNet 2.0) were distributed from March to May 2014. The baseline HH survey, community sensitization, coding of nets, net master list and distribution, base line susceptibility tests, adverse effect surveys, base line chemical assay, cone bioassays base line and 6 month and net survivorship and fabric integrity after 6 month of net distribution all were conducted during last 2 years. Following activities were conducted during the period from April 2015 to March 2016:

Chemical assay

For insecticidal activity, 30 each of 3 types of nets (LifeNet®, NetProtect and PermaNet 2.0) were sampled and sent to Gambloux, Belgium for chemical analysis after 12 months of household use of nets in field. Five netting samples (30 cm × 30 cm) from each of 30 randomly selected LNs cut from position 1 to 5 were used for this. Reports received from Gambloux showed that after 1 year of household use, the mean deltamethrin content in LifeNet is 4.42 g/kg corresponding to a loss of 55% of the original dose, 1.11 g/kg corresponding to a loss of 38% in NetProtect and 0.92 g/kg corresponding to a loss of 32% in PermaNet 2.0.

Cone-bioassays (after 12 and 18 month of distribution)

Cone bioassays were performed under ambient conditions using 100% deltamethrin susceptible laboratory reared, 2–5 day-old, and sugar-fed females of Anopheles culicifacies. Five netting samples (25 × 25 cm) from each of 30 randomly selected LNs cut from position 1 to 5 were used for the bioassays. On each netting sample, standard WHO cone was placed and held in place using a plastic manifold. Five mosquitoes were introduced into each cone and exposed for 3 minutes. This was repeated twice on each of the five netting samples cut from a net.

Thus a total of 50 mosquitoes were exposed on each net. After the exposure, mosquitoes were removed gently from the cones and kept in plastic cups provided with cotton-wool moistened with 10% glucose solution. Knockdown was recorded after 60 min and mortality after 24 h. Mosquitoes exposed to untreated nets were used as controls.

The bioassay results for the netting pieces from each sampled LN were pooled to determine if the net met with the WHO efficacy criteria, i.e. knock-down of ≥95% after 60 min post-exposure and or mortality of ≥80% after 24 h holding. The results revealed that In 12 months, all 30 LifeNet, 27 NetProtect and 29 PermaNet passed (met WHO criteria). In 18 months, 29 LifeNet, 25 NetProtect and 30 PermaNet met criteria of WHO.

Household surveys

The questionnaire-based surveys after 12 and 18
month of net distribution were undertaken to get information regarding net survivorship and fabric integrity, usage pattern, storage and washing behaviour etc.

**Net survivorship and fabric integrity**

To measure net survivorship for cohort study, 250 IDs from the net master list for each of LifeNet®, PermaNet 2.0 and NetProtect LNs were selected for durability and fabric integrity to be done after 6, 12, 24 and 36 month of net distribution. These nets are shown as cohort nets. Apart from this, all 30 nets of each type that were withdrawn for bioassays were also checked for durability and fabric integrity at every 6-month sampling period. These nets are shown as bioassay nets.

**Usage and washing pattern**

**12 month survey**

The survey for cohort study revealed that >81.1% LNs given as part of the study were found in place with the households and out of those >97% of available nets were found to be in use (Table 1). Cohort nets were reported to be used year round by 66, 62 and 68% LifeNet®, NetProtect and PermaNet householder. Most of the participating households (>58%) reported use of nets over cut bamboo. These users mainly tuck the net in at night (>82%). Attrition rate of cohort nets after 12 month was 16, 19 and 10%, respectively. Regarding washing of cohort nets, survey revealed that >76% LNs were washed with locally available detergent powder. These nets (>57%) after washing were dried mainly outside in the shade.

The survey for bioassay nets revealed that 100% LNs given as part of the study were found in place with the households and all were found to be in use. These nets were reported to be used year round by 43, 43 and 37% LifeNet®, NetProtect and PermaNet householders. Most of the participating households (>53%) reported use of nets over cut bamboo. Only about 53% users tuck the net at night. Attrition rate of bioassay nets was zero in these three types of nets. Regarding washing of bioassay nets survey revealed that >73% LNs were washed with locally available detergent powder (>72%). These nets (>52%) after washing were dried mainly outside in the shade.

**18 month surveys**

The survey for bioassay nets revealed that 100% LNs given as part of the study were found in place with the households and all were found to be in use (Table 2). These nets were reported to be used year round by 76, 57 and 60% LifeNet®, NetProtect and PermaNet householder. Most of the participating households using LifeNet (>59%) reported use of nets over wooden bed frame sticks whereas NetProtect and PermaNet users used these nets over cut bamboo (>60%). More than 63% users tuck the net in at night. Attrition rate of bioassay nets was zero in these three types of nets. Regarding washing of bioassay nets survey revealed that >73% LNs were washed with locally available detergent powder.
Table 2. Net survivorship and pattern of usage of bioassay nets by the households at 12 and 18 month survey

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LifeNet</th>
<th>NetProtect</th>
<th>PermaNet 2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 month</td>
<td>18 month</td>
<td>12 month</td>
</tr>
<tr>
<td>(A) Survivalship</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Net used for sleeping</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Year round</td>
<td>43.33</td>
<td>75.86</td>
<td>43.33</td>
</tr>
<tr>
<td>Occasionaly</td>
<td>56.66</td>
<td>24.13</td>
<td>56.66</td>
</tr>
<tr>
<td>Used last night</td>
<td>36.7</td>
<td>89.66</td>
<td>36.7</td>
</tr>
<tr>
<td>Used past week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All 7 night</td>
<td>20</td>
<td>58.62</td>
<td>33.3</td>
</tr>
<tr>
<td>5–6 night</td>
<td>43.33</td>
<td>24.14</td>
<td>20</td>
</tr>
<tr>
<td>1–4 night</td>
<td>36.7</td>
<td>13.79</td>
<td>46.7</td>
</tr>
<tr>
<td>0–night</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(C) Sleeping places</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wooden bed frame (sticks)</td>
<td>20</td>
<td>58.62</td>
<td>36.7</td>
</tr>
<tr>
<td>Cut bamboo</td>
<td>53.3</td>
<td>41.38</td>
<td>53.3</td>
</tr>
<tr>
<td>Other</td>
<td>26.7</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>(D) Tuck the net in</td>
<td>53.3</td>
<td>89.66</td>
<td>53.3</td>
</tr>
<tr>
<td>(E) Storage of nets</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hanging lose over sleeping places</td>
<td>50</td>
<td>51.72</td>
<td>40</td>
</tr>
<tr>
<td>Hanging tied in knot</td>
<td>10</td>
<td>24.14</td>
<td>30</td>
</tr>
<tr>
<td>Hanging folded</td>
<td>16.67</td>
<td>3.45</td>
<td>10</td>
</tr>
<tr>
<td>Visible but not hung up</td>
<td>10</td>
<td>6.9</td>
<td>6.67</td>
</tr>
<tr>
<td>Stored away</td>
<td>13.33</td>
<td>13.79</td>
<td>13.33</td>
</tr>
<tr>
<td>(F) Washing of nets</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent washes</td>
<td>83.33</td>
<td>79.31</td>
<td>73.33</td>
</tr>
<tr>
<td>Time of washing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 week ago</td>
<td>4</td>
<td>8.7</td>
<td>0</td>
</tr>
<tr>
<td>1 week to 1 month ago</td>
<td>12</td>
<td>8.7</td>
<td>18.18</td>
</tr>
<tr>
<td>1–3 month ago</td>
<td>28</td>
<td>30.43</td>
<td>22.73</td>
</tr>
<tr>
<td>3–6 month ago</td>
<td>44</td>
<td>43.48</td>
<td>18.18</td>
</tr>
<tr>
<td>&gt; 6 month ago</td>
<td>12</td>
<td>8.7</td>
<td>40.91</td>
</tr>
<tr>
<td>Type of soap</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detergent powder</td>
<td>72</td>
<td>30.43</td>
<td>77.27</td>
</tr>
<tr>
<td>Local bar soap</td>
<td>0</td>
<td>0</td>
<td>4.55</td>
</tr>
<tr>
<td>Bar and detergent</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>28</td>
<td>69.57</td>
<td>18.18</td>
</tr>
<tr>
<td>Net scrubbed hard surface</td>
<td>20</td>
<td>13.04</td>
<td>13.6</td>
</tr>
<tr>
<td>Where dried</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Out side in sun</td>
<td>48</td>
<td>13.04</td>
<td>40.91</td>
</tr>
<tr>
<td>Outside in shade</td>
<td>52</td>
<td>86.96</td>
<td>59.09</td>
</tr>
<tr>
<td>Inside</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3. Insecticide susceptibility status of adult Anopheles culicifacies in Kundam and Bargi CHC village

<table>
<thead>
<tr>
<th>Area</th>
<th>Insecticide</th>
<th>No. of replicates</th>
<th>No. exposed</th>
<th>Knocked down in 1 h</th>
<th>No. dead in 24 h</th>
<th>% mortality</th>
<th>Corrected % mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net area (1 year after net distribution)</td>
<td>Deltamethrin 0.05%</td>
<td>34</td>
<td>510</td>
<td>492</td>
<td>502</td>
<td>98.4</td>
<td>97.7</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>10</td>
<td>150</td>
<td>8</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Without net area</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deltamethrin 0.05%</td>
<td>24</td>
<td>360</td>
<td>352</td>
<td>357</td>
<td>99.2</td>
<td>99.2</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>8</td>
<td>120</td>
<td>0</td>
<td>1</td>
<td>0.8</td>
<td></td>
</tr>
</tbody>
</table>

powder (> 58%) for NetProtect and PermaNet nets, however, LifeNet users (69%) did not use detergent or any bar. These nets (> 70%) after washing were dried mainly outside in the shade.

**Insecticide susceptibility test**

After one year, insecticide susceptibility tests were carried out to assess the susceptibility of targeted disease vector An. culicifacies of study area against deltamethrin 0.05%, using the WHO kit and method. Results revealed 98.4% mortality of An. culicifacies in 24 h with knockdown 96.4% (Table 3). It shows the susceptible status of An. culicifacies in this area. However, in without bednet area the mortality was almost similar to bednet area (99.2% 24 h with 98% KD)
12 month surveys
During cohort, a total of 22% LifeNet®, 30% NetProtect and 25% PermaNet LNs were found with number of holes (Table 4). Majority of holes were found in PermaNet (214) followed by 197 in NetProtect and 129 in LifeNet®. The highest number of holes observed per net were of size 1 (0.5–2 cm) and 2 (2–10 cm) in all 3 types of nets. Based on holes of different sizes, mean hole index was found highest 73 (95% CI 72.4–73.6) in NetProtect followed by 58.7 (95% CI 58–59.4) in PermaNet 2.0 and 28.8 (95% CI 28.2–29.5) in LifeNet. These holes were observed mainly on the lower side of all types of LNs (Table 5). The number of holes per net on the lower side was 1.5, 2.2 and 2.7 in LifeNet®, Netprotect and PermaNet LNs respectively. Quite a few LNs were found with repairs in the form of stitches and knots.

During survey for bioassay nets a total of 40% LifeNet®and NetProtect and 50% PermaNet LNs, were found with number of holes (Table 6). Majority of holes were found in NetProtect (51) followed by 33 in LifeNet®and 28 in PermaNet. The highest number of holes observed per net were of size 1 (0.5–2 cm) and 2 (2–10 cm) in all three types of nets. The mean hole index was highest 50.5 (95% CI 48.7–52.3) in PermaNet 2.0 followed by 47.4 (95% CI 45.6–49.2) in NetProtect and 30.8 (95% CI 29.2–32.5) in LifeNet®. These holes were observed mainly on the lower side of all types of LNs (Table 7). The number of holes per net on the lower side was 2.1, 3.0 and 1.2 in LifeNet®, NetProtect and PermaNet LNs, respectively. Very few bioassay nets were found with repairs.

### Table 4. Physical aspects of cohort nets—Hole size (12 month)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LifeNet</th>
<th>NetProtect</th>
<th>PermaNet 2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of net</td>
<td>22.82</td>
<td>30.5</td>
<td>25.64</td>
</tr>
<tr>
<td>with any holes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of holes/net</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size 1 (0.5–2 cm)</td>
<td>1.40</td>
<td>1.33</td>
<td>1.96</td>
</tr>
<tr>
<td>Size 2 (2–10 cm)</td>
<td>0.94</td>
<td>1.07</td>
<td>1.58</td>
</tr>
<tr>
<td>Size 3 (10–25 cm)</td>
<td>0.34</td>
<td>0.70</td>
<td>0.62</td>
</tr>
<tr>
<td>Size 4 (&gt; 25 cm)</td>
<td>0.06</td>
<td>0.13</td>
<td>0.12</td>
</tr>
<tr>
<td>Mean hole index</td>
<td>28.84</td>
<td>73.06</td>
<td>58.70</td>
</tr>
<tr>
<td>95% CI</td>
<td>(28.23–29.46)</td>
<td>(72.44–73.67)</td>
<td>(58.0–59.39)</td>
</tr>
</tbody>
</table>

### Table 5. Physical aspects of cohort nets—Holes position (12 month)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LifeNet</th>
<th>NetProtect</th>
<th>PermaNet 2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position of Holes/Net</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roof side</td>
<td>0.47</td>
<td>0.57</td>
<td>0.94</td>
</tr>
<tr>
<td>Upper side</td>
<td>0.66</td>
<td>0.36</td>
<td>0.6</td>
</tr>
<tr>
<td>Lower side</td>
<td>1.49</td>
<td>2.16</td>
<td>2.72</td>
</tr>
<tr>
<td>Seam side</td>
<td>0.13</td>
<td>0.13</td>
<td>0.02</td>
</tr>
<tr>
<td>Repair holes/net</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stitched</td>
<td>0.4</td>
<td>0.11</td>
<td>0.38</td>
</tr>
<tr>
<td>Knotted</td>
<td>0.09</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>Patched</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 6. Physical aspects of bioassay nets (Hole size)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LifeNet®</th>
<th>NetProtect</th>
<th>PermaNet 2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of net with any holes</td>
<td>40</td>
<td>20.69</td>
<td>40</td>
</tr>
<tr>
<td>No. of holes/net</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size 1 (0.5–2 cm)</td>
<td>1.33</td>
<td>0.67</td>
<td>2.33</td>
</tr>
<tr>
<td>Size 2 (2–10 cm)</td>
<td>1.17</td>
<td>2.67</td>
<td>1.5</td>
</tr>
<tr>
<td>Size 3 (10–25 cm)</td>
<td>0.25</td>
<td>0.5</td>
<td>0.42</td>
</tr>
<tr>
<td>Size 4 (&gt; 25 cm)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mean hole index</td>
<td>30.87</td>
<td>33.1</td>
<td>47.4</td>
</tr>
<tr>
<td>95% CI</td>
<td>(29.24–32.55)</td>
<td>(30.35–33.69)</td>
<td>(45.62–49.19)</td>
</tr>
</tbody>
</table>

### Table 7. Physical aspects of bioassay nets (Hole position)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LifeNet</th>
<th>NetProtect</th>
<th>PermaNet 2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position of holes/Net</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roof side</td>
<td>0.5</td>
<td>0.67</td>
<td>0.33</td>
</tr>
<tr>
<td>Upper side</td>
<td>0.17</td>
<td>1</td>
<td>0.92</td>
</tr>
<tr>
<td>Lower side</td>
<td>2.08</td>
<td>2.17</td>
<td>3</td>
</tr>
<tr>
<td>Seam side</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Repair holes/net</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stitched</td>
<td>0</td>
<td>0.17</td>
<td>0.08</td>
</tr>
<tr>
<td>Knotted</td>
<td>0</td>
<td>0.17</td>
<td>0</td>
</tr>
<tr>
<td>Patched</td>
<td>0</td>
<td>0</td>
<td>0.22</td>
</tr>
</tbody>
</table>
18 month surveys
During survey for bioassay nets a total of 20.7% 
LifeNet®, 30% NetProtect and 43.3% PermaNet LNs 
were found with number of holes. Majority of holes 
were found in PermaNet (68) followed by 37 in a 
NetProtect and 23 in LifeNet®. The highest number 
of holes observed per net were of size 1 (0.5–2 
cm) and 2 (2–10 cm) in all three types of nets. The 
mean hole index was highest 91.8 in PermaNet 
2.0 followed by 86.5 in NetProtect and 33.1 in 
LifeNet®. These holes were observed mainly on the 
lower side of all types of LNs. The number of holes 
per net on the lower side was 2.2, 1.8 and 2.8 in 
LifeNet®, NetProtect and PermaNet LNs, 
respectively. Very few bioassay nets were found 
with repairs (Tables 6 and 7).

Point prevalence study in District Betul of Madhya 
Pradesh for the risk factors associated with re-
emergence of malaria in the area
A study was conducted in 4 villages of CHC, 
Shahapur and 4 villages of Bhimpur CHC in 6000 
population from Oct to Dec 2015. During active 
surveillance (Table 8), 217 fever cases screened 
out of which 81 were found with malaria positive 
infection (Pf 57, Pv 22, mixed 2). The SPR, SFR and 
Pf% were 37.3, 26.3 and 70.3, respectively.

Entomological survey also recorded per man 
hour density (PMH) total Anopheles was 13.6 of 
which 73% were An. culicifacies and An. fluvialis 
was 0.42. Per room density of Anopheles 10.8 of 
which 80% were An. culicifacies and per trap per 
night of Anopheles mosquito was 14.7 of which 
An. culicifacies and An. fluvialis were 9.3 and 
0.3, respectively.

Questionnaire-based survey also conducted 
among the population which revealed that sleeping 
mainly indoors (78%) in winter and rains and 
about 95% in outdoors in summer. For mosquito 
prevention, households use smoke (57%) only 14% 
use bednets; 95% households know about malaria 
disease in which only 46% know that malaria is 
spread through mosquitoes; and 82% households 
know that diagnosis of malaria through blood test 
and treatment is done (52%) mainly in Government 
hospitals.

Point prevalence study to know the risk factors 
associated with dengue transmission in Narsinghpur 
district
In the district, there were dengue outbreaks in 
rural, semi-urban areas from 2011 to 2015. During 
the period 792 samples were tested from which 
354 (44.7%) were positive for dengue with serotype 
DENV-1 and DENV-3. Entomological and 
sociocultural questionnaire-based study conducted 
in November 2015 to January 2016. Entomological 
survey showed container index was 42.1 and 
breteau index 80. Emergence of mosquitoes from 
the breeding place recorded 74% Aedes and 36% 
Culex species. Further, among Aedes species was 
Ae. aegypti 94.5%, Ae. albopictus 3.6% and Ae. 
vittatus 1.8%, respectively.

Household survey also revealed that they store 
water in metal/mud pots and cement tanks, which 
are almost remain open. In urban area 65% house-
holds have coolers and only 50% of them empty 
out water in 7 days interval. Among the population 
95% know about the dengue disease but only 4% 
of them know that disease spread through Aedes 
mosquito. However, 45% of households know that 
a mosquito breeds in stagnant water which bites in 
day-time. For prevention of mosquito bite they 
mostly use bednets and smoke.

Bionomics of malaria vectors and their sibling species, 
and to establish their role in malaria transmission in 
Chhattisgarh India (NIRTH and NIMR collaboration)

The study was carried out in two malarious 
districts, i.e. Bastar and Korea of Chhattisgarh (Fig. 
2). Two CHCs in the district and 4 villages in each 
CHC were selected for this study. Entomological 
surveys were carried during the period from July 
2014 to Aug 2015. Indoor resting mosquitoes (per 
man-hour) were collected at monthly intervals from 
the 16 selected villages. Pyrethrum spray sheet 
collections (PSC) were made once in a month from 
human dwellings (HD) randomly selected other 
than those selected for indoor resting collection. 
Collected mosquitoes were identified in the field. 
Monthly species specific breeding site survey was 
carried out at each study site. Anopheles culicifacies 
and An. fluvialis were assayed for the presence 
of malaria parasite by employing PCR-based on 18s

Table 8. Results of month-wise surveillance of fever cases

<table>
<thead>
<tr>
<th>Month (2015)</th>
<th>BSC</th>
<th>Positive</th>
<th>Pf</th>
<th>Mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>149</td>
<td>67</td>
<td>22</td>
<td>45</td>
</tr>
<tr>
<td>November</td>
<td>45</td>
<td>9</td>
<td>–</td>
<td>7</td>
</tr>
<tr>
<td>December</td>
<td>23</td>
<td>5</td>
<td>–</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>217</td>
<td>81</td>
<td>22</td>
<td>57</td>
</tr>
</tbody>
</table>
rRNA target gene. DNA was isolated and sibling species identification was carried out using DNA sequencing of ITS2 and D3 region.

The average per man hour density of Anopheline mosquito was 18.17 (ranging from 8.09 to 46.6) of which 44.3% were An. culicifacies and 4.6% were An. fluvialitis in indoor resting collection. The average per man hour density of An. culicifacies in Bastar and Korea districts was 6.6 (95% CI 5.02–8.2) and 9.6 (95% CI 7.5–11.8), respectively. The average PMH density of An. fluvialitis in Bastar and Korea districts was 0.39 (95% CI 0.02–0.8) and 4.34 (95% CI 1.82–8.84), respectively. The average PMH density of An. fluvialitis was significantly higher in Korea as compared to Bastar (p < 0.001), while An. culicifacies has shown no significant difference in Bastar and Korea in indoor resting collection.

Ecotype-wise analysis revealed that the relative abundance of An. culicifacies was high in each ecotype while An. fluvialitis was dominant in both forest and foothill ecotype as compared to the plain (Figs. 3–4). Season-wise data (Figs. 5–6) showed the highest density of Anopheles during spring season in both the districts. Anopheles culicifacies was almost equal in each season in Bastar while in Korea it was comparatively higher in spring and winter seasons. In the Pyrethrum spray sheet collection, data revealed that An. culicifacies was
most abundant (43%) species during study period.

The anopheline fauna of study villages consisted of 11 species in Korea and 10 species in Bastar district of which *An. culicifacies* species caught throughout the year in indoor resting collections from both the study sites. *Anopheles fluvatilis* was caught from September to April in Korea while in Bastar only from October to February month. *Anopheles culicifacies* was found breeding in all the places such as rocky pit, rocky stream, running stream and seepage water, while *An. fluvatilis* breeding was found in seepage water, rocky pit and running stream, whereas, other anophelines were mostly found breeding in running stream (Tables 9 and 10). A total 123 mosquitoes tested for blood meal preference 5 (4%) were found having Human blood (anthropophilic) and 118 (96%) were having cattle blood (zoophilic). A total 358 *An. culicifacies* samples and 153 *An. fluvatilis* were analyzed by DNA sequencing. *Anopheles culicifacies* C (38%) was dominant followed by species D (23%) and B (22%). *Anopheles fluvatilis* species T was dominant in the study area (Tables 11 and 12). Both *P. falciparum* positive *An. culicifacies* was identified as species C.
### Table 9. Number of anopheline larval emergence from different breeding sources (Bastar)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Species</th>
<th>Breeding places</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ditches</td>
<td>Pits</td>
</tr>
<tr>
<td>1.</td>
<td><em>An. culicifacies</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td><em>An. fluviatilis</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3.</td>
<td><em>An. jeyropensiis</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.</td>
<td><em>An. subpictus</em></td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>5.</td>
<td><em>An. vagus</em></td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>6.</td>
<td><em>An. annularis</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7.</td>
<td><em>An. barbirostris</em></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>8.</td>
<td><em>An. nigerimus</em></td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>9.</td>
<td><em>An. tessellatus</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>218</td>
<td></td>
</tr>
</tbody>
</table>

### Table 10. Number of anopheline larval emergence from different breeding sources (Korea)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Species</th>
<th>Breeding places</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Marshall pools</td>
<td>Rock pools</td>
</tr>
<tr>
<td>1.</td>
<td><em>An. culicifacies</em></td>
<td>2</td>
<td>54</td>
</tr>
<tr>
<td>2.</td>
<td><em>An. fluviatilis</em></td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>3.</td>
<td><em>An. jeyropensiis</em></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>4.</td>
<td><em>An. subpictus</em></td>
<td>37</td>
<td>2</td>
</tr>
<tr>
<td>5.</td>
<td><em>An. vagus</em></td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>6.</td>
<td><em>An. annularis</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7.</td>
<td><em>An. barbirostris</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8.</td>
<td><em>An. nigerimus</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9.</td>
<td><em>An. pallidus</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10.</td>
<td><em>An. splendidus</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11.</td>
<td><em>An. theobaldi</em></td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 11. Site-wise distribution of sibling species of *An. culicifacies*

<table>
<thead>
<tr>
<th>Site</th>
<th>No. of tested mosquito</th>
<th>Species A</th>
<th>Species B</th>
<th>Species C</th>
<th>Species D</th>
<th>Species E</th>
<th>Unidentified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bastar</td>
<td>174</td>
<td>7 (4)</td>
<td>42 (24)</td>
<td>61 (35)</td>
<td>47 (27)</td>
<td>2 (1)</td>
<td>15 (9)</td>
</tr>
<tr>
<td>Korea</td>
<td>184</td>
<td>9 (5)</td>
<td>38 (21)</td>
<td>74 (40)</td>
<td>37 (20)</td>
<td>5 (3)</td>
<td>21 (11)</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate percentages.

### Table 12. Site-wise distribution of sibling species of *An. culicifacies*

<table>
<thead>
<tr>
<th>Site</th>
<th>No. of tested mosquito</th>
<th>Species T</th>
<th>Species S</th>
<th>Species U</th>
<th>Unidentified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bastar</td>
<td>38</td>
<td>24 (63)</td>
<td>3 (8)</td>
<td>7 (18)</td>
<td>4 (11)</td>
</tr>
<tr>
<td>Korea</td>
<td>115</td>
<td>91 (79)</td>
<td>5 (4.5)</td>
<td>14 (12)</td>
<td>5 (4.5)</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate percentages.

### State workshop conducted

A three-days workshop for medical officers from different district, viz. Chhindwara, Shahdol, Jhabua, Shivpuri, Betul, Dhar and Hoshangabad. Balaghat and Mandla of Madhya Pradesh on malaria and other vector-borne disease were organized during the reported year. The workshop was organized jointly by NIRTH, NIMR FU Jabalpur and Directorate of Health Services, Bhopal. Professor of Medical College Jabalpur, Scientists of NIRM, NIRTH and State Health Officers imparted training on various aspects of vector-borne diseases.

### Participation in Meetings/Conferences/Workshops/Trainings, etc.

- Supervision of Indoor residual spray in 8 districts of Madhya Pradesh by the Scientist of NIMR on the request of Govt. of Madhya Pradesh.
- Delivered two lectures to ANM, MPW training workshop at training centre of Jabalpur.
Health impact assessment of development projects: Impact of Sardar Sarovar project on vector-borne diseases in Gujarat

This study was initially carried out in Kheda, Surendranagar and Patan districts in Phase-II command area of Sardar Sarovar project. It was further extended to Morbi district of Saurashtra region. Narmada water had reached this region through canal for irrigation as well as for ceramic industries. Entomological activities in these two districts included mosquito collection, peri-domestic and intra-domestic larval surveys, host preference and survivorship of malaria vector, cross-sectional mass blood survey in sentinel villages was done.

Bi-monthly monitoring of adult mosquito density was carried out during this year. Results of vector density/room (GMD) in command and non-command area are shown in Figs. 1 and 2. It shows that the vector density was comparatively high in command area as compared to non-command area. Anopheles culicifacies female adults were dissected for determination of parity from command and non-command area. Results of parous rate are shown in Fig. 3. Results show that it was high in both command and non-command area in the month of February, August and October. However, in non-command area it was very low in April and June months.

The geographical reconnaissance of mosquito breeding habitats showed that 72.5 and 62.5% habitats were positive for anopheline and culicine mosquitoes in command and non-command area, respectively. Mosquito larval density was higher (17 larvae/dip) in peri-domestic habitats of command area than the non-command area (8.5 larvae/dip). Results of mosquito species composition are presented in Table 1. During this
year, Anopheles and two Culex species were found in peri-domestic breeding habitats in command area whereas in non-command area only 4 Anopheles and 2 Culex species were found. In both arm, An. stephensi is dominant species among anophelines. Results of mosquito breeding in domestic water containers are presented in Table 2. In command area, house index, container index and breteau index were 8.58, 6.98, and 1.47, whereas in non-command area 4.35, 2.74 and 0.48, respectively.

One mass blood survey was carried in the month of June 2015 in sentinel villages of command and non-command area. A total of 580 and 150 blood smears were prepared from command and non-command areas, respectively. Results of mass blood survey are presented in Tables 3 and 4. Slide positivity rate in command area was higher (1.38) than the non-command area (0.7).

One more fish hatchery for Aphanius dispar was established during this year at Kheda district. A total of five hatcheries of Aphanius dispar are being supervised regularly. These hatcheries are being maintained by state health department and fishes are being used as an antilarval measure by state public health department (Table 5). Collection of Aphanius dispar from salt pan area of Kheda district and fish hatchery are shown in Figs. 4 and 5.

Table 1. Larval density and species composition in peri-domestic habitats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Command</th>
<th>Non-command</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habits checked</td>
<td>80</td>
<td>24</td>
</tr>
<tr>
<td>Habitat positive</td>
<td>58</td>
<td>15</td>
</tr>
<tr>
<td>% Positive habitats</td>
<td>72.5</td>
<td>62.5</td>
</tr>
<tr>
<td>Density (Larvae/dip)</td>
<td>17.15</td>
<td>8.15</td>
</tr>
<tr>
<td>Species composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>An. culicifacies</td>
<td>5.6</td>
<td>3.16</td>
</tr>
<tr>
<td>An. stephensi</td>
<td>17.15</td>
<td>27.37</td>
</tr>
<tr>
<td>An. anturalis</td>
<td>14.75</td>
<td>10.53</td>
</tr>
<tr>
<td>An. flaviscutelus</td>
<td>8.13</td>
<td>0</td>
</tr>
<tr>
<td>An. subpictus</td>
<td>8.96</td>
<td>5.26</td>
</tr>
<tr>
<td>Cx. quinquefasciatus</td>
<td>38.96</td>
<td>47.37</td>
</tr>
<tr>
<td>Cx. vishnui</td>
<td>4.38</td>
<td>3.16</td>
</tr>
</tbody>
</table>

Table 4. Age-wise distribution of malaria cases in mass blood surveys of Morbi district

<table>
<thead>
<tr>
<th>Age group (yr)</th>
<th>Command</th>
<th>Non-command</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BSE</td>
<td>Pv</td>
</tr>
<tr>
<td>&lt; 1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 to 4</td>
<td>27</td>
<td>1</td>
</tr>
<tr>
<td>5 to 8</td>
<td>29</td>
<td>1</td>
</tr>
<tr>
<td>9 to 14</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>≥15</td>
<td>495</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>580</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 5. List of Aphanius dispar hatcheries established in command area

<table>
<thead>
<tr>
<th>District</th>
<th>Place</th>
<th>Number of fishes introduced</th>
<th>Size of hatchery (Cubic foot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kheda</td>
<td>Palana</td>
<td>28000</td>
<td>240</td>
</tr>
<tr>
<td></td>
<td>Chakalsi</td>
<td>22000</td>
<td>240</td>
</tr>
<tr>
<td></td>
<td>Alina (2015)</td>
<td>10000</td>
<td>150</td>
</tr>
<tr>
<td>Vadodara</td>
<td>Dabhoi</td>
<td>25000</td>
<td>490</td>
</tr>
<tr>
<td>Anand</td>
<td>Kheda</td>
<td>11300</td>
<td>350</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>96300</td>
<td>1470</td>
</tr>
</tbody>
</table>

Fig. 4: Aphanius dispar fishes are being collected from salt pan.

Fig. 5: Fish hatchery of Aphanius dispar.
Large-scale (Phase-III) evaluation of efficacy, fabric integrity and community acceptability of PermaNet 3.0 long-lasting insecticidal nets compared with PermaNet 2.0 in India

The main objectives of this study was to determine and compare the insecticidal activity and fabric integrity of PermaNet 3.0 LNs with PermaNet 2.0 over three years of use by households under field conditions and to assess washing mode and washing habits of LNs by the householders, and to assess the community acceptability of LNs over three years.

Bioassay and chemical assay

A total of 30 nets of each arm (PermaNet 3.0 and PermaNet 2.0) were withdrawn from the study area after six month and one year use. Cone bioassay tests were performed on each net withdrawal on laboratory reared *An. culicifacies* and *An. stephensi* mosquitoes. All nets were passed in cone bioassay tests as per the WHOPES guidelines. Results are shown in Tables 6 and 7. One set of net samples (30 each) from both arms withdrawn after one year were sent for chemical assay to Wallon Agriculture Research Centre (CRAW), Pesticide Research Department, Rie de Borda, Belgium. Baseline nets samples were also sent for chemical assay tests. Results of chemical analysis of both types of nets (baseline and 12 month withdrawal) are presented in Table 8. It shows that there is decrease in deltamethrin and piperonylbutoxide contents after one year of use. However, the bioassay shows LNs (PermaNet 3.0 and PermaNet 2.0) are still effective on mosquitoes.

Fabric integrity (cohort nets)

To evaluate fabric integrity of nets of both arms, 350 nets of each arm were physically inspected in presence of net users in the study area in six and 12 months after use. In six month cohort survey, 291 nets were actually inspected among 350 PermaNet 3.0 nets. The rest of nets (59) could not be inspected because these nets were carried by the net users to their working places or lost somewhere. Similarly, 298 PermaNet 2.0 nets were actually inspected and 62.2% (181/291) PermaNet 3.0 nets were found clean, whereas it was 84.23% (251/298) for PermaNet 2.0 nets. It is also observed that 40.55% (118/291) PermaNet 3.0 nets were washed during six months use. However, it was 25.17% (75/298) in PermaNet 2.0 nets and 9.28% (27/291) nets were found having at least one hole

<table>
<thead>
<tr>
<th>Parameters</th>
<th>No. of mosquito exposed/net</th>
<th>PermaNet 3.0 (n=30)</th>
<th>PermaNet 2.0 (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Knockdown 3 min</td>
<td>% Knockdown 1 h</td>
<td>% Mortality 24 h</td>
</tr>
<tr>
<td>Mean</td>
<td>97.40</td>
<td>97.30</td>
<td>100</td>
</tr>
<tr>
<td>SD</td>
<td>4.96</td>
<td>0.87</td>
<td>0</td>
</tr>
<tr>
<td>Range</td>
<td>96–100</td>
<td>96–100</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameters</th>
<th>No. exposed/net</th>
<th>PermaNet (3.0) (n=30)</th>
<th>PermaNet (2.0) (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Knockdown</td>
<td>% Mortality</td>
<td>% Knockdown</td>
</tr>
<tr>
<td>Mean</td>
<td>96.67</td>
<td>97.83</td>
<td>91.50</td>
</tr>
<tr>
<td>SD</td>
<td>4.75</td>
<td>3.27</td>
<td>8.03</td>
</tr>
<tr>
<td>Range</td>
<td>85–100</td>
<td>92.5–100</td>
<td>72.5–100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of net</th>
<th>Parts</th>
<th>Baseline net samples</th>
<th>Nets withdrawal after one year of use</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Deltamethrin contents (g/kg)</td>
<td>Piperonylbutoxide contents (g/kg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>PermaNet 3.0</td>
<td>Lower side</td>
<td>2.86</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>Upper side</td>
<td>2.69</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>Roof</td>
<td>4.32</td>
<td>0.13</td>
</tr>
<tr>
<td>PermaNet 2.0</td>
<td>Whole</td>
<td>1.32</td>
<td>0.14</td>
</tr>
</tbody>
</table>
in PermaNet 3.0 nets, whereas it was 3.02% (9/298) in Permanet 2.0 nets. Similarly, in 12 months cohort survey, 307 nets were actually inspected among 346 PermaNet 3.0 nets. A total of 327 PermaNet 2.0 nets were actually inspected among 347 nets and 49.19% (151/307) PermaNet 3.0 nets were found clean, whereas it was 61.16% (200/327) for PermaNet 2.0 nets. It is also observed that 80.46% (247/307) PermaNet 3.0 nets were washed during 12 months period; however, it was 72.28% (238/327) in ParmaNet 2.0 nets. During cohort survey after 12 months period, 39.41% (121/307) nets were found having at least one hole in PermaNet 3.0 nets, whereas it was 37% (121/327) in PermaNet 2.0 nets. Figures 6 to 8 show the inspection of cohort nets of both arms.

Transmission dynamics and control of malaria in tribal area of Gujarat, India

This study was initiated in June 2014 in Panchmahals district of Gujarat state. A total of 30% population of this district is tribal. Three PHCs of Gogamba and two PHCs of Jambugoda taluka were selected for this study. The main goal of this study was to contribute to the reduction of the burden of malaria in tribal ecosystems with the specific objectives to understand the ecosystem dynamics of malaria including epidemiology of malaria parasites, to develop/evaluate situation-specific, appropriate interventions and to contribute to capacity strengthening for effective control of malaria and other vector-borne diseases.

During this year entomological parameters such as adult mosquito density, larval density, parity, human blood index and human landing collection were monitored on bi-monthly basis. Supervision of indoor residual spray (IRS) activity was also done in the study area. Technical support on IRS was given to spray team at village level and district level authorities during spraying of insecticide. At present alphacypermethrin is being used for indoor residual spraying. Under epidemiological parameters, supervision of surveillance mechanism, laboratory services and mass blood surveys were also conducted.

Mosquito density was measured by pyrethrum space collection method. Bi-monthly monitoring was done during this year. Results of adult mosquito density are shown in Table 9. Results showed that density of An. culicifacies were remained high in pre-monsoon and post-monsoon season. It varied from 8.17 to 42.50 per room. An. fluvialis density varied from 0.0 to 1.67 per room. Figure 9, shows the geometrical mean mosquito density per room of vector population. Mosquito density was also collected by light-trap collection in all sentinel villages of study area. Figure 10, shows that An. culicifacies was dominant vector species in all seasons. During this period four human landing collections were also conducted and the mean density of An. culicifacies was 0.88/bait/night. The parous rate of An. culicifacies was remained high.
Table 9. Mean mosquito density/room

<table>
<thead>
<tr>
<th>Month</th>
<th>An. culicifacies</th>
<th>An. stephensi</th>
<th>An. annularis</th>
<th>An. subpictus</th>
<th>An. fluviatilis</th>
<th>An. splendidus</th>
<th>Total Culex</th>
<th>Total Aedes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan</td>
<td>29.17</td>
<td>0</td>
<td>0.33</td>
<td>8.33</td>
<td>1.67</td>
<td>0</td>
<td>22.33</td>
<td>0</td>
</tr>
<tr>
<td>Mar</td>
<td>40.50</td>
<td>0.17</td>
<td>1</td>
<td>37.67</td>
<td>1.17</td>
<td>0.17</td>
<td>31.67</td>
<td>0</td>
</tr>
<tr>
<td>May</td>
<td>8.17</td>
<td>0</td>
<td>0.17</td>
<td>17.17</td>
<td>0</td>
<td>0</td>
<td>13.33</td>
<td>0.17</td>
</tr>
<tr>
<td>Jul</td>
<td>29.67</td>
<td>0</td>
<td>1.50</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>26</td>
<td>0.17</td>
</tr>
<tr>
<td>Sep</td>
<td>42.50</td>
<td>0</td>
<td>1.50</td>
<td>52</td>
<td>0.67</td>
<td>0</td>
<td>29.17</td>
<td>1.33</td>
</tr>
<tr>
<td>Dec</td>
<td>23</td>
<td>0</td>
<td>11.67</td>
<td>19.67</td>
<td>0</td>
<td>0</td>
<td>13.17</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 9: Geometrical mean mosquito density (per room).

Fig. 10: Mean vector densities (light-trap collection).

throughout the year. It ranged from 41.52 to 51.79%. Month-wise distribution of parous rate in An. culicifacies is shown in Fig. 11.

During this year, bi-monthly intra-domestic breeding surveys were carried out. A total of 1026 houses were checked for mosquito breeding. Out of this 44 houses were found positive for mosquito breeding (House Index = 4.29). Container Index (CI) was 3.65 and Breteau Index (BI) was 2.14. The peri-domestic survey showed that the main breeding habitats for mosquitoes breeding are seasonal rivers, river bed pools and wells. Species composition of mosquito breeding is given in Table 10. Anopheles culicifacies was dominant species among all mosquito species being 36.9%. Another vector species in this area was An. fluviatilis which contributed 4.35%. During the entomological investigation, 559 mosquito blood meals were prepared and analyzed for the purpose of host preference. Blood meal samples were analyzed by gel diffusion technique (precipitin test). Seven blood meals were found positive for human blood (HBI = 1.25%) and 56.89% mosquitoes prefer bovine blood whereas, 48.48% samples did not show any reaction. 1717 ELISA samples of An. culicifacies and 59 An. fluviatilis samples were also prepared for vector incrimination. These samples will be analyzed by ELISA technique.

Two mass blood surveys were conducted in sentinel villages of the study area in different

Table 10. Percent mosquito species composition in peri-domestic habitats

<table>
<thead>
<tr>
<th>Mosquito species</th>
<th>Percent composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>An. culicifacies</td>
<td>36.90</td>
</tr>
<tr>
<td>An. stephensi</td>
<td>2.01</td>
</tr>
<tr>
<td>An. annularis</td>
<td>4.68</td>
</tr>
<tr>
<td>An. fluviatilis</td>
<td>4.35</td>
</tr>
<tr>
<td>An. subpictus</td>
<td>13.79</td>
</tr>
<tr>
<td>An. barbirostris</td>
<td>2.68</td>
</tr>
<tr>
<td>An. nigerrimus</td>
<td>2.19</td>
</tr>
<tr>
<td>An. splendidus</td>
<td>0.45</td>
</tr>
<tr>
<td>Cx. quinquefasciatus</td>
<td>20.55</td>
</tr>
<tr>
<td>Cx. vishnui</td>
<td>10.36</td>
</tr>
</tbody>
</table>
seasons. Pre-monsoon mass blood survey was carried in April 2015 and post-monsoon survey was done in December 2015. In pre-monsoon survey, the slide positivity rate (SPR) was 0.14 and slide falciparum rate (SfR) was 0.05, whereas in post-monsoon survey, SPR and SfR were 0.32 and 0.32, respectively (Table 11). Age-wise distribution of malaria positive cases in both mass blood surveys are presented in Table 12.

Centre for the Study of Complex Malaria in India

This project had been launched in November 2012 in Gujarat with an aim to understand the complexity of malaria, including changing patterns of epidemiology. The objective of the project in Gujarat, is to collect blood samples from the patients with malaria symptoms, then to identify the *Plasmodium* species in samples with three different diagnostic methods, i.e. microscopy, rapid diagnostic kits (RDTs) and polymerase chain reaction (PCR). After sample processing, the DNA samples were sent to NIMR, New Delhi for genomics studies.

In the year of 2015, 203 suspected cases were enrolled for malaria diagnosis from the Clinic of Civil Hospital, Nadiad and Vatva Urban Health Centre (UHC) of Ahmedabad Municipal Corporation, Ahmedabad. Initially, they were examined by RDT kit (Zephyr Biomedical) which was followed by microscopy (as per the protocol). The blood samples were collected from the patients after taking informed consent for further molecular diagnosis. The DNA was isolated from the respective blood samples by QIamp DNA Mini kit and processed for the PCR diagnosis. The PCR products were analyzed by electrophoresis technique. Out of 203 cases, 138 were positive by microscopy ($P_v = 125$, $P_f = 9$, Mixed = 4) as well as by RDT ($P_v = 125$, $P_f = 10$, Mixed = 3). PCR detected 132 cases positive ($P_v = 119$, $P_f = 13$) for malaria (Table 13). Clinical presentation of the cases are shown in Table 14. The average hemoglobin level in these patients was 12.57 g/dl and only one patient had hemoglobin level <7.

A total of 127 cases enrolled from Vatva Urban SFR Health Centre (UHC) of Ahmedabad Municipal Corporation, Ahmedabad were also processed at this field unit. Result of microscopy, RDT and PCR examination are presented in Table 15. Attempts

<p>| Table 11. Mass blood surveys in Panchmahals district |
| --- | --- | --- | --- | --- | --- |</p>
<table>
<thead>
<tr>
<th>Season</th>
<th>Population covered</th>
<th>BSC Positive</th>
<th>$P_v$</th>
<th>$P_f$</th>
<th>SPR</th>
<th>SfR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-monsoon</td>
<td>12804</td>
<td>2079</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0.14</td>
</tr>
<tr>
<td>Post-monsoon</td>
<td>12781</td>
<td>1559</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0.32</td>
</tr>
</tbody>
</table>

| Table 12. Age-wise distribution of malaria cases in mass blood surveys in Panchmahals district |
| --- | --- | --- | --- | --- | --- |
| Age group (yr) | BSE | Pre-monsoon season | Post-monsoon season |
| --- | --- | --- | --- | --- | --- |
| <1 | 20 | 0 | 0 | 0 | 0 |
| 1 to 4 | 161 | 0 | 0 | 0 | 0 |
| 5 to 8 | 169 | 0 | 0 | 0 | 0 |
| 9 to 14 | 348 | 1 | 1 | 0 | 0 |
| ≥15 | 1381 | 1 | 0 | 0 | 0 |
| Total | 2079 | 2 | 1 | 0 | 0 |

<p>| Table 13. Cases enrolled in clinical study diagnosed by different methods |
| --- | --- | --- | --- | --- | --- |</p>
<table>
<thead>
<tr>
<th>BSE</th>
<th>Enrolled</th>
<th>Microscopy</th>
<th>RDT</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_v$</td>
<td>$P_f$</td>
<td>Mixed</td>
<td>$P_v$</td>
<td>$P_f$</td>
</tr>
<tr>
<td>4567</td>
<td>203</td>
<td>125</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>138</td>
<td>138</td>
<td>132</td>
<td></td>
</tr>
</tbody>
</table>

<p>| Table 14. Clinical presentation of cases (n=203) enrolled under clinical study |
| --- | --- | --- | --- |</p>
<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Gender</th>
<th>Mean Temp. ($^\circ$F)</th>
<th>Average Hb* g/dl (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>Male</td>
<td>176 (62.06)</td>
<td>99.49</td>
</tr>
<tr>
<td>2–14</td>
<td>Female</td>
<td>77 (37.93)</td>
<td>(96.3–110.8)</td>
</tr>
<tr>
<td>&gt;15</td>
<td>*Average Hb: 12.57; Anaemic: 0.49% Figures in parentheses indicate percentages.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<p>| Table 15. Clinical study—Ahmedabad (Vatva – UHC) |
| --- | --- | --- | --- | --- | --- |</p>
<table>
<thead>
<tr>
<th>Duration</th>
<th>Enrolled</th>
<th>Microscopy</th>
<th>RDT</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_v$</td>
<td>$P_f$</td>
<td>Mixed</td>
<td>$P_v$</td>
<td>$P_f$</td>
</tr>
<tr>
<td>Jan–Dec 2015</td>
<td>127</td>
<td>117</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>127</td>
<td>127</td>
<td>120</td>
<td></td>
</tr>
</tbody>
</table>
were made to follow all positive patients. However, only 39 cases could be followed completely as per protocol (Table 16).

### Technical support to state health programme

#### Malaria Clinic

Malaria diagnostic support was provided to the Civil Hospital, Nadiad and the data so generated have been used for sentinel monitoring of malaria situation in the Kheda district of Gujarat. In 2015, 6583 febrile patients have been screened for malaria, of which 93 found positive for malaria infection \((Pv = 70, Pf = 20\) and Mixed = 3). The slide positivity rate was 1.4. All the confirmed malaria patients were provided radical treatment by Medical Officers of Civil Hospital, Nadiad. There is an increase in both *P. falciparum* and *P. vivax* malaria cases as compared to 2014 (Fig. 12). However, 3 cases of mixed infection have also been found. The age-wise distribution of cases indicates highest malaria infection among 15 and above age group.

### Trainings/Workshops/Seminars/Conferences organized, participated

1. Re-orientation training on entomology to insect collectors from NVBDCP, Gandhinagar has been conducted at NIMR, Nadiad in the month of March 2015.
2. Entomological training to DMOs, Biologists, Sanitary Inspectors, Deputy Assistant Entomologists and Malaria Inspectors from various districts of Gujarat state, had been held at NIMR, Nadiad on March 2015. Dr CS Pant delivered a lecture on Integrated Vector Management (IVM) to 20 participants.
3. Training of Urban Malaria Scheme (UMS) staffs from different districts had been held at NIMR in the month May 2015. Dr CS Pant had participated as faculty and delivered lecture on Entomological monitoring.
4. Dr Ranvir Singh attended NVBDCP sub-committee in the State Health Society meeting held at Gandhinagar, Gujarat on 16 July 2015.
6. Re-orientation training in Malaria microscopy for laboratory technicians of PHCs/CHCs/UHCS were organized at this Field Unit. Six batches (one batch each month) from July to December 2015. A total of 127 laboratory technicians were trained.
7. Dr CS Pant attended Review meeting of NVBDCP at Gandhinagar on 30 September 2015.
8. On the request of the Director, NVBDCP, Delhi, Dr Ranvir Singh and Dr CS Pant organized meeting with Dr Chandana Day, Senior Regional Director, NVBDCP, Government of India, Ahmedabad on 24 August 2015 in relation to proactive action for malaria control in the flood-affected districts of Gujarat.
Epidemiological investigation

On the request of NVBDCP, Gandhinagar, epidemiological investigation was carried out in three districts, namely Morbi, Dahod and Panchmahals of Gujarat state. Under this investigation, supervision of surveillance mechanism, laboratory services, indoor residual spraying (IRS), LLIN distribution and cross-checking of positive and negative blood smears were done. Entomological parameters had also been monitored in some villages. Results of field observations and recommendations had been sent to the Joint Director, NVBDCP, Gandhinagar for necessary action and corrective measures.

Exhibition

Participated as National Institute of Malaria Research (NIMR) representative at ‘Global Meet and Vibrant Gujarat 2015’ event under the umbrella of Indian Council of Medical Research, New Delhi held at Gandhinagar from 7 to 13 January 2015. An exhibition on life cycle of mosquitoes and malaria parasites along with live demonstration of three larvivorous fishes (Cambusia affinis, Poecilia reticulata and Aphanius dispar) and different stages of mosquito larvae and adults of An. stephensi, Ae. aegypti and Cx. quinquefasciatus were displayed at exhibition ground during the event. New tools of mosquito control were also displayed at the exhibition. Visitors from India and foreign countries took keen interest in our demonstration (Fig. 13).

Visitors

The students from the following Colleges/School visited NIMR, FU, Nadiad.
1. Three PG Students from BJ Medical College, Ahmedabad, Gujarat.
2. Forty-seven III Year students from Ayurvedic College Nadiad.
3. Three PG students from SBKSMI and RC Pipariya, Vadodara.
4. Twenty students from General Nursing School, Nadiad.
Characterization of salivary gland proteome of dengue/DHF, chikungunya and yellow fever vector *Aedes aegypti* Linn.

*Aedes aegypti* Linn. (Diptera: Culicidae) is a principal vector for dengue and chikungunya in India and many parts of the world. The complete genome sequence of *Ae. aegypti* was released in the year 2007. There are 15,988 genes which contain ~1376 million base pairs and 17,402 proteins. However, large majority of predicted protein coding genes remained hypothetical. Proteogenomic analysis using high resolution Fourier transform mass spectrometry, will not only validate most of predicted protein coding genes but also allow discovery of novel proteins and corrected gene models. The ultimate aim is to identify those proteins which play role in virus infection process.

In this study, proteins from the salivary glands of female *Ae. aegypti* were extracted using ultrasonication. Proteins were first resolved on the SDS-PAGE and entire gel was cut into 18 fragments followed by digestion with trypsin. Trypsin digested peptides were further analyzed on high resolution LTQ-OrbitrapVelos mass spectrometer. The MS/MS data was searched against a protein database comprised of known and predicted proteins reported from *Ae. aegypti* using proteome discoverer software. The bioinformatics analysis of protein data was done using VectorBase resources for assigning Gene Ontology (GO) terms. KEGG pathway portals were used to assess involvement of proteins in different metabolic pathways.

LC-MS/MS analysis of 18 fractions of *Ae. aegypti* salivary gland proteins processed on high-resolution mass spectrometer resulted in acquisition of over 80000 MS/MS spectra. These spectra when searched against protein database of *Ae. aegypti*, led to identification of more than 5000 unique peptides. These peptides belonged to a total of 1208 salivary gland proteins. To assign molecular functions and biological processes to these proteins, VectorBase resource and assigned Gene Ontology (GO) terms were used. The most prominent biological processes represented in our data were found to be translation, metabolism, oxidation-reduction and cellular organization (Fig. 1). Several proteins were found to be involved in one or more metabolic pathways as described for *Ae. aegypti* in KEGG Pathways portal. As expected, majority of the proteins were found to be involved in housekeeping pathways including glycolysis, TCA cycle, fatty acid metabolism, amino acid metabolism, biosynthesis of antibiotics, glyoxylate and dicarboxylate metabolism and carbon metabolism. Apart from these, proteins from WNT signaling pathway, Hedgehog, TGF-beta, Hippo, FoxO, MTOR and peroxisome signaling pathways were also found to be enriched in our data, signifying their functional presence in salivary glands. A number of proteins involved in immunity related pathways in salivary glands were also identified. A subset of these proteins, are known to interact with disease viruses. This is the largest catalog of proteins identified in salivary gland of *Ae. aegypti* thus far. A complete proteome of salivary gland of *Ae. aegypti* female when available, will aid in understanding of vector-pathogen interactions prior to virus transmission. These findings will enable in devising, at molecular level, virus blocking strategies in the salivary glands.

**Proteomic analysis of urine of malaria patients using high resolution mass spectrometry for identification of candidate biomarkers for *Plasmodium falciparum* and *P. vivax* infections**

In India, malaria is a major public health problem with reported ~1 million cases and ~500 confirmed deaths annually. Two main human
parasites *P. falciparum* and *P. vivax* have extensive global distribution as well as in India. Apart from preventive vector control measures, early diagnosis and complete treatment are the two important modalities that have been adopted to contain the disease. To achieve this objective, active case detection at the community level and passive collection of blood smears at facility level are performed under the national programme. Besides the time tested microscopy for diagnosis, many rapid tests have become commercially available in last two decades. Apart from certain advantages, there are several limitations of these tests, viz. false negativity at very low parasitaemia (<100/µl), persistent *Pf* HRP-II antigenemia post successful treatment, deletion of *HRP-II* gene, lower than acceptable sensitivity of LDH-based *P. vivax* rapid tests, antibody stability issues at high temperatures in the field conditions, costs and need for blood draw to perform the test.

Thus, there is need for developing a new diagnostic method with improved sensitivity and specificity to overcome drawbacks/limitations which persist with the diagnostic kits available in the market. It is desired that such a test should be simple, convenient to perform in the field, robust and cost-effective. This project envisages identification of novel antigens of *P. falciparum* and *P. vivax* excreted in the urine of the patients that would form basis for the development of urine based non-invasive diagnostic kits for both *P. falciparum* and *P. vivax* infections.

Urine samples were collected from *P. vivax* patients and control individuals. Samples
were selected based on age and sex matches and further processed for protein concentration. Individual protein samples from urine were fractionated and analyzed on Orbitrap Fusion mass spectrometer. The peptide profile of urine obtained is being studied. Peptides will be further validated by employing multiple reaction monitoring (MRM) assays. Finally, suitable assays of candidate antigens will be developed to screen malaria and their sensitivity and specificity across large cohorts of urine samples will be determined.

A study on the role of gut microbiota in modulation of longevity, fecundity and fitness of Anopheles stephensi as a malaria vector

It is well-known that vector gut microbiota plays a vital role in biological processes including longevity, fecundity and Plasmodium infectivity in vectors. Since, the midgut flora of An. stephensi is poorly understood, the aim of this study is to characterise the microbial diversity and their abundance in the midgut of An. stephensi. The larvae actively feed on micro-organisms from their aquatic environment.

The larvae and pupae of An. stephensi were collected from the breeding habitats in Goa. The immature were brought to the laboratory for processing. After emergence adult mosquitoes were morphologically identified using standard keys. Isolation of the midgut was done at each stage (i.e. larva, pupa and adult) of An. stephensi. All precautions were taken to dissect and grow bacteria from the gut in different conditions. These plates were incubated in suitable conditions for defined time frame. After incubation, total viable count (TVC) was recorded and the isolates were re-streaked onto fresh medium to obtain the pure culture. The purified colonies were maintained at 4°C.

The TVC of bacteria was obtained from the midgut of larvae, pupae, adult males and females. Each life stage showed variation in total viable count on different media used and overall the midgut of male mosquito showed minimum viable count as compared to female. It was found that in general there is variation in the diversity of microbiota within a gut and also their preferences to grow on different media. It is important to identify the microbial diversity to understand their role in the life processes of a mosquito vector.

A study on isolation, characterization and efficacy of naturally occurring mosquito pathogenic bacilli in Goa, India

Bacillus-based bio-toxins are gaining importance in the control of insecticide resistant populations of insect pests and vectors of human diseases. Bacillus thuringiensis sub sp. israelensis (Bti) and B. sphaericus (Bsp) are entomopathogenic bacteria that produce a parasporal crystal, and are being used widely as larvicidal bacteria for mosquito control. Nature store a formidable pool of natural enemies of mosquitoes and their bioactive compounds form a strategic source for new and successful mosquitocidal products.

We collected soil samples from harsh environment in Goa and screened them for potential mosquitocidal strains by conducting bioassays against mosquito vectors of local importance (Culex quinquefasciatus, An. stephensi, Ae. aegypti and Ae. albopictus). Dose finding bioassays with isolate in order to obtain LC_{50} and LC_{90} values were performed following which biochemical and physiological characteristics of the potential mosquitocidal isolates were also studied according to Bergey’s Manual of Systematic Bacteriology. Biochemical tests, namely catalase, starch hydrolysis, gelatin liquefaction, tween hydrolysis, casein hydrolysis, indole test, methyl red test, vogue Proskauer test, sugar fermentation test, nitrate reduction test, oxidase test, and citrate utilization test were performed.

Healthy III instar larvae (n=25) were taken in 250 ml plastic bowls containing 100 ml of sterilized distilled water. For each dose three replicates were maintained along with control. The percentage mortality was recorded post 24 and 48 h of exposure. The culture supernatant of some active isolates, obtained by centrifugation was tested against the immature of the three test vector species (Cx. quinquefasciatus, An. stephensi and Ae. aegypti) and the percentage mortality was recorded.

Microscopic examination of the eight isolates following gram staining showed all eight isolates to be gram positive rods and endospore producers. The biochemical tests were performed with the eight isolates, a gram negative control and un-inoculated control and results obtained were recorded.

One isolate showed high larvicidal activity against laboratory reared healthy III instar Ae. albopictus larvae. The percentage mortality increased with increase in the dose administered. The highest dose administered showed 98.66%
mortality on 24 h of exposure and 100% mortality on 48 h of exposure. Further detailed studies are in progress.

Larvicidal and pupicidal activity of leaf extracts of IC_Goa against Anopheles stephensi Liston, Culex quinquefasciatus Say and Aedes aegypti Linn.

Plant based insecticides have been used in many parts of the world against different mosquito species, as they are environment friendly and easily biodegradable. Plants are considered rich source of bioactive chemicals and also source of mosquito control agents. More than 2000 plant species have been reported to possess chemicals which are helpful in the control of pests due to their insecticidal properties. The test plant IC_Goa is widely distributed in India and known to possess medicinal properties. We tested leaves for their pupicidal activity against III instar larvae and freshly emerged pupae of An. stephensi, Cx. quinquefasciatus and Ae. aegypti, the three well-known vectors of malaria, Bancroftian filariasis and dengue/DHF. The mortality in larvae was observed at 24 and 48 h of exposure and LC₅₀ and LC₉₀ were calculated for each time interval. Methanolic extract showed greatest pupicidal activity during bioassays against An. stephensi with LC₅₀ = 0.67 ppm and LC₉₀ = 1.4 ppm; followed by Cx. quinquefasciatus with LC₅₀ = 20.1 ppm and LC₉₀ = 115.0 ppm; and Ae. aegypti with LC₅₀ = 24.7 ppm and LC₉₀ = 185.7 ppm at 24 h. In conclusion, methanolic extract was found to be more effective than the chloroform extract against all the tested mosquito species. Further, crude extract will be subjected to fractionation on column chromatography under gradient elution of non-polar to polar ratio and different fractions will be collected. Different fractions as well as purified active compounds will be assayed against pupae of three mosquito species, viz. An. stephensi, Cx. quinquefasciatus and Ae. aegypti for pupicidal activity by conducting standard bio-assays using WHO guidelines. Characterization and structure elucidation of the compounds will be carried out by IR, MS, NMR and X-ray diffraction techniques.

Vector infection studies under epidemiology of malaria evolution in south Asia project funded by National Institute of Health, USA

India contributes about 1.5–2 million annual confirmed malaria cases and 46,970 (14,757–94,945) estimated deaths per annum. Since, both parasites and vectors are evolving rapidly, updated information on parasite prevalence in mosquitoes is important in vector management and disease control, in India and broader regions of Asia.

Using 1036 CDC traps placed at four major malaria focal points in Goa, India from May 2013 to April 2015, 1375 anopheline specimens comprising of 10 species were caught. The mosquito species were identified using morphological keys. Mosquito DNA was extracted using Qiagen DNA kits and analysed for human blood as well as for P. falciparum and P. vivax infections using nested PCR.

Human host feeding was confirmed in An. stephensi (30%), An. subpictus (27%), An. jamesii (22%), An. annularis (26%), and An. nigrimus (16%). None of the An. vugus, An. barbirostris, An. tessellarus, An. umbrosus nor An. karvari specimens contained human blood. Importantly, An. subpictus, which was hitherto considered a non-vector in Goa was found to be a dominant vector in terms of its numbers and Plasmodium carriage. Plasmodium infections were detected in 14 An. subpictus (2.8%) and seven An. stephensi (2.1%). In An. subpictus, nested PCR demonstrated three Plasmodium infections in gland; a P. vivax, and two mixed infection of P. falciparum and P. vivax. In addition to 10 gut infections (1 P. vivax, 6 P. falciparum and 3 mix infections) were seen in this new competent vector. In contrast, the seven An. stephensi infections included three P. vivax, two P. falciparum and two mix infection of these two. Longitudinal mosquito collections pointed to a bimodal annual appearance of An. subpictus with peaks flanking the months showing a single peak for An. stephensi. Together, parasite positive mosquitoes were seen year round.

In addition to An. stephensi, An. subpictus contributes significantly to the propagation of malaria in urban Goa. It is a dominant vector species, and appears to work in concert with An. stephensi to maintain perennial malaria transmission cycle of both P. vivax and P. falciparum in Goa. The study has been published in Malaria Journal in 2016 and its results have been shared with NVBDCP, Goa and Delhi.

Studies on kinetics of Plasmodium vivax development in Anopheles stephensi

Human Plasmodia exhibit a complex life cycle which involves two hosts a vertebrate host (human) and an insect vector (mosquito). The sexual phase of malaria parasite is completed inside the mosquito.
host, a process known as sporogony which is a highly complex and involves transformation of malarial parasites into six different forms. An. stephensi is one of the major vectors of malaria in India and neighbouring countries. In India, P. vivax contributes about 45% of total malaria cases. In this study the kinetics of P. vivax development were determined in wild and laboratory colonized strains of An. stephensi.

Anopheles stephensi larvae and pupae were collected from different curing water (breeding habitats) in construction sites in the city of Ponda, Goa, India. The larvae and pupae were reared at NIMR insectary until they emerged into adult mosquitoes. Emerged adult mosquitoes were identified using standard keys. The colonized An. stephensi pure line used has been generated from a single mother is currently in its 76th generation. Adult mosquitoes from colonized and wild An. stephensi were used for mosquito infection assays and comparison.

In this study, we have observed wide range of oocyst and sporozoite infection rates even between individuals of the same batch. We found weak correlation between initial parasitemia and infection rates. In some cases we have also observed melanized oocysts. The infection and infectivity rates were compared between wild and laboratory strain. Seven successful infections were obtained during the period of report.

Estimation of malaria burden in India to validate recently proposed methodologies: A national study

A national multi-districts study entitled, ‘Estimation of malaria burden in India’ was launched and carried out in Kolhapur and Dakshin Kannada district under NIMR FU, Goa. Other sample districts in the country were Jaipur, Jhabua, Koraput and Chatra where similar exercise was undertaken by NIMR. The project activity included manpower recruitment and training, active surveillance, malaria incidence reporting from private, corporate, municipal and Govt. sectors covering all stakeholders and health providers. Verbal autopsy of all death cases was performed in a population of 4 lakh per district which included two arms, surveillance and death arms covering population of 2 lakh each. In each study district, active surveillance was conducted in 2 lakh population with the help of voluntary surveillance monitors. Blood smears and rapid tests were performed on all fever cases. Supervisory staff of field workers ensured supply chain of material, examination of blood smears and treatment of malaria patients. Field workers performed verbal autopsies of all death cases after 15 days of death and interviewed the nearest kin of the deceased and collected all medical records if available. The proforma were based on RGI prescribed formats and in local language. Each filled up VA form was gone through by two qualified medical attendants and independently catageorized as per four prescribed death categories. Morbidity estimates were done by collating all cases detected from the study population by all health providers. Care was taken not to miss cases and avoid duplicate entries in data bases. Further analysis of data is in progress.

Ph.D. Students at NIMR Field Unit

Dr Ashwani Kumar

(Guide for S.No. 1–4 and Co-guide for S.No. 5–10)

1. Dr Ajeet Kumar Mohanty
   Topic: Midgut proteome analysis of female An. stephensi Liston, a vector for human malaria
   University: Goa University
   Current status: Degree awarded

2. Col (Dr) Rakhi Dhawan
   Topic: Proteogenomic analysis of midgut and salivary gland and identification of barriers to transmission of Plasmodium falciparum infection in Aedes aegypti Linn.
   University: Goa University
   Current status: Progressing

3. Minisha Pereira
   Topic: A study on the role of gut microbiota in modulation of longevity, fecundity and fitness of Anopheles stephensi
   University: Goa University
   Current status: Progressing

4. Joleen Almeida
   Topic: A study on isolation, characterization and efficacy of naturally occurring mosquito pathogenic bacilli in Goa, India.
   University: Goa University
   Current status: Progressing

5. Dr Narayani Prasad Kar
   Topic: Study of malaria transmission dynamics in two different ecosystems in district Deogarh, Orissa
   University: Goa University
   Current status: Degree awarded
6. **Sh. Manish Kumar**  
*Topic*: Proteomic analysis of fat body of *Anopheles stephensi* Liston using high-resolution mass spectrometry  
*University*: Manipal University  
*Current status*: Progressing

7. **Sh. Gourav Dey**  
*Topic*: Mass spectrometry-driven genome annotation of *Anopheles stephensi* Liston, a major malaria vector in India  
*University*: Manipal University  
*Current status*: Progressing

8. **Ms KS Sreelakshmi**  
*Topic*: Integrated transcriptomic and proteomic analysis of the Indian malaria vector—*Anopheles stephensi*  
*University*: Manipal University  
*Current status*: Progressing

9. **Sh. Kamlesh Kaitholia**  
*Topic*: Effect of residual antimalarials in malaria patients enrolled for therapeutic efficacy studies and its effect on spread of drug resistant parasites in high malaria endemic districts in India  
*University*: Goa University  
*Current status*: Progressing

10. **Ms Ruchi Gupta**  
*Topic*: Studying artemisinin resistance in selected malaria endemic sites of India  
*University*: Goa University  
*Current status*: Viva voce held.

**Trainings**

1. Sixteen students and 2 faculty member (Dr Md Khalid Saifullah and Dr Seemab Zehr) M.Sc., Zoology (Parasitology & Fisheries) from Aligarh Muslim University, Aligarh visited the Field Unit on 1 March 2016 as a part of their academic tour. They were lectured on proteogenomics and Bacilli-based vector control to control malaria and other vector-borne diseases.

2. Ms Ayesha Shetkar, B.Sc. Final year student of Government College of Arts, Sciences and Commerce Sanquelim, Goa University received guidance from January to March 2016 who carried out her dissertation work on ‘Biocontrol of mosquito larvae using herbal extracts’ in partial fulfilment of her degree.


**Visits**

1. Dr Ashwani Kumar presented work on Vector collections and *Plasmodium* infection studies in India at 5th ICEMR Annual Workshop at Cali, Colombia from 17–18 August 2015.

2. Dr Ashwani Kumar presented plenary lecture “Insecticide resistance, an impediment to malaria elimination: Challenges and alternatives” at 4th Symposium “Perspectives on malaria elimination” at Cali, Colombia from 19–21 August 2015.

3. Exploring new vectors that might carry human *Plasmodium* in urban areas of India at conference organized by Association of Entomology at Punjabi University, Patiala from 29 to 30 October 2015.

4. MESA malERA Refresh tools for elimination meeting as Expert Panel Member from 12–13 October 2015 at Washington, USA.

5. VCAG: Vector Control Advisory Group meeting held at Geneva, WHO (HQs) from 16–18 November 2015.

6. VCAG emergency response consultation on new vector control tools for control of Zika virus at WHO (HQs), from Geneva 14–15 March 2016.
Large-scale (Phase-III) evaluation of efficacy, fabric integrity and community acceptability of Olyset Plus long-lasting insecticidal nets compared with Olyset Net in India

A household randomized trial was launched in June 2014 with the objectives to: evaluate and compare the insecticidal activity and fabric integrity of Olyset Plus with Olyset Net over three years of use by the households under field conditions; assess community washing methods and washing habits for Olyset Plus with Olyset Net and assess and compare the community acceptability of Olyset Plus with Olyset Net. After population census, assessment of net requirement and obtaining informed written consent from the households to participate in the trial, Olyset Plus (1103) and Olyset Nets (1135) were distributed during August–September 2014. Net were distributed among 928 households (pop. 4098) in 10 villages of Kanker (6) and Balod (4) districts. Anopheles culicifacies is the main malaria vector and was found resistant to deltamethrin (74.1% mortality), permethrin (90.6%) and bendiocarb (85.6%).

Olyset Plus LLIN is made of 150 denier high-density mono-filament polyethylene yarn containing 2% permethrin (w/w) corresponding to 20 g AI/kg ± 25% and piperonylbutoxide (PBO) 1% (w/w), as synergist, corresponding to 10 g PBO/kg (about 400 mg of PBO/m²) which is incorporated in all the fibres on all sides and also on the roof. The rate at which permethrin and PBO migrate to the surface of the net has been adjusted to provide rapid regeneration, making the net active again within 1–2 days after washing. Olyset Net is WHO approved LLIN made of a 100% high density polyethylene, 150 denier net, blended with permethrin 2% (w/w) as active ingredient, corresponding to 20 g/kg ± 25%.

The mean permethrin content in 30 samples of Olyset Plus at baseline was 19 g/kg, corresponding to 736.9 mg/m², complying with the target dose of 20 g/kg ± 25%. In 30 nets withdrawn after one year of household use, the mean permethrin content was 14.2 g/kg corresponding to a loss of 25% of the original dose. The mean PBO content in Olyset Plus at baseline was 10.1 g/kg, corresponding to 392.2 mg/m² (target dose of 10 g/kg ± 25%). After one year the mean PBO content was 3.9 g/kg corresponding to a loss of 62% of the original dose. The mean permethrin content in 30 samples of Olyset Nets at baseline was 19.8 g/kg, which complied with the target dose of 20 g/kg ± 25%. After one year of household use, the mean permethrin content was 17.1 g/kg corresponding to a loss of 14% of the original dose.

Thirty each of Olyset Plus and Olyset Nets sampled at the baseline and those withdrawn after six and 12 months of distribution fulfilled the WHO criteria of ≥95% knockdown or ≥80% mortality in cone bioassays (Fig. 1) when tested against susceptible An. culicifacies. Out of 30 Olyset Plus nets withdrawn after 18 months, eight failed in cone bioassays whereas all 30 Olyset Nets passed. All failed nets when subjected to tunnel tests, met the

Fig. 1: Cone bioassays being performed on LLIN withdrawn at Raipur Field Unit.
eﬃcacy criteria of ≥80% mortality and ≥90% blood-feeding inhibition. In control tunnel tests with untreated netting piece, the blood-feeding rate was > 50% while mortality was < 10%. Nets withdrawn from villages for eﬃcacy monitoring at an interval of every six months were replaced with new net of the same brand.

Surveys of cohort nets for fabric integrity after six and 12 months of distribution revealed that the proportion of Olyset Plus with holes was 13.8 (368) and 27.9% (368) and that of Olyset Net was 26.4 (387) and 39.3% (387), respectively. Number and position of holes of diﬀerent sizes on all cohort nets as well as those withdrawn for bioassays (Fig. 2) were counted and recorded to calculate the hole index. Attrition rate (100 minus survivorship) of Olyset Plus was 5.6 (358) and 5.9% (338) and that of Olyset Net was 7 (384) and 4.2% (357), respectively after six and 12 months of distribution.

Questionnaire-based surveys were carried every six months in randomly selected 30 households in each study arm and in households with cohort nets after six and 12 months of net distribution to also collect information on net usage rate and washing behaviour.

![Fig 2: An used LLIN withdrawn from a household has been draped over a cube-shaped frame for counting of holes.](image)

**Impact of insecticide resistance in malaria vectors on the eﬀectiveness of combination of indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) in India: A multidisciplinary approach**

In an observational and clustered trial in 80 clusters (villages) selected in Keshkal block of Kondagaon district in southern Chhattisgarh, universal distribution of long-lasting insecticidal nets was accomplished in a population of 75,000 during November–December 2014 after obtaining informed written consent from every household participating in the trial. A total of 40,000 Perma Net 2.0 were supplied by the State Health Department out of which 30,468 nets were distributed in study clusters. In addition to this, the project staﬀ helped the Keshkal Community Health Centre (CHC) in distributing the nets in non-study villages (21), tribal residential schools (21) and paramilitary camps (5). Average LLIN distribution/ HH was 1.99 (95% CI: 1.67–2.31) and per capita distribution as per old norms was 2.42 (national average 2.2). LLIN compliance survey of ~3000 households carried out in the post-monsoon season in 2015 covering all clusters, last-night usage of LLIN was 81.1% among children of < 5 yr of age. However, overall LLIN usage by the community on the previous night was 94.8%.

In the baseline blood survey of 6582 cohort children of < 12 yr of age enrolled in the study in 80 villages after obtaining written informed consent, 488 children were having malaria infection (SPR 7.4%). Parasite rate (PR) was highest in villages of Dhanora PHC (16.4%) followed by Keshkal PHC (7.4%). PR among cohorts in rest of the two PHCs, Bahigaon and Singanpur was 1.95 and 0.5%, respectively. In a follow-up survey carried out in August 2015, out of 5862 cohort children screened for malaria infections, 82 (PR 1.4%) were found positive for malaria parasites showing a reduction of 81%, as a result of LLIN intervention and high compliance rate. Fortnight fever surveillance of cohort children by Mitans (124) and non-cohort population by the Malaria Surveillance Workers (30) is continuing in all clusters and the data are being uploaded regularly to an online data management programme developed by National Informatics Centre, Raipur which can be accessed at [http://nirm.cg.nic.in](http://nirm.cg.nic.in).

Susceptibility tests carried out in 2015 by exposing wild-caught *An. culicifacies* females collected from all clusters to deltamethrin 0.05% indicated the vector was resistant in 51 clusters, indication of possible resistance in 18 clusters and was susceptible in 11 clusters with average mortality of 83.8% (range 53–100%). This indicated a loss of 13% susceptibility when compared to results of 2014 in which average mortality of 97% was recorded. Tests against bendiocarb 0.1% showed that *An. culicifacies* was susceptible in 76 clusters while in four the results indicated possible resistance with an average mortality of 99.7% (95 to 100%).

Synergistic bioassays were carried out using PBO
and TPP on wild-caught An. culicifacies to identify the biochemical mechanism of resistance in the vector. Mosquito collections were made in sentinel villages to monitor the vector density. Anopheles culicifacies females were dissected to determine parous rate. Blood meal and sporozoite ELISA were carried out to determine the blood feeding preference (HBI = 0.04; n = 655) and sporozoite rate (SR = 0, n = 457). Cytotaxonomic identification of sibling species complex of An. culicifacies (n = 560) revealed that species B was dominant and accounted for 90% while species C accounted for 10%. It is planned to introduce an IRS intervention arm with bendiocarb during the 2016 transmission season by randomizing the study clusters into high and low levels of deltamethrin resistance based on fresh susceptibility test data.

**Monitoring of insecticide resistance in malaria vector Anopheles culicifacies in different districts of Chhattisgarh**

WHO susceptibility tests were carried out by exposing wild caught An. culicifacies against DDT, malathion, alphacypermethrin and deltamethrin treated papers in different districts of Chhattisgarh. In all tests were carried out in 16 districts (DDT 4%—6 districts, malathion 5%—14, alpha-cypermethrin 0.1%—6, deltamethrin 0.05% and bendiocarb 0.1%—16 districts each) out of 27 districts in the state (Fig. 3).

Mean An. culicifacies mortality against DDT was 5.9% (95% CI: 1.8–10), in tests against malathion it was 63.1% (57.2–69), against alphacypermethrin mean mortality was 56.9% (36.8–77), while in tests against deltamethrin it was 63.6% (55–72.1). Mean mortality in tests against bendiocarb was 93.3% (89.2–97.5). An. culicifacies was susceptible to bendiocarb in six districts ≥98% mortality), in another six districts the mortality ranged from 90–97% (verification required) and in four districts the mortality was <90%, indicating resistance. Bendiocarb, a carbamate compound has never been used in malaria control programme in India. Thus, resistance to a carbamate insecticide in four districts may be due to use of another molecule of this group in agriculture conferring cross-resistance to bendiocarb. The results clearly indicate that An. culicifacies has developed resistance to DDT, malathion, deltamethrin and alphacypermethrin in most of the districts while its susceptibility to bendiocarb in four districts showing <90%
mortality needs more confirmatory tests. It is planned to undertake tests against bendiocarb, deltamethrin and alphacypermethrin 0.05% in few districts to generate more data.

**To evaluate the efficacy of PermaNet® 3.0 long-lasting insecticidal nets in areas with variable deltamethrin-resistant malaria vectors in India**

Study was undertaken with the objective to assess usability of PermaNet 3.0 in areas with malaria vectors having different levels of deltamethrin resistance mediated by CYT P450s to determine the level of resistance that can be synergized.

PermaNet 3.0 (PN3) is a combination LLIN made of polyethylene roof and polyester side panels. The roof of PermaNet 3.0 is incorporated with deltamethrin and a synergist piperonylbutoxide (PBO) while deltamethrin alone is coated onto the polyester fibers. The net is suggested for malaria vector control with metabolic resistance mechanism mediated by Cytochrome P450s. Studies so far undertaken in India have not revealed any superior effect of this net on pyrethroid resistant vector probably due to low level of resistance to be enabled for synergism.

Based on previous deltamethrin susceptibility test data of various districts generated by the Field Unit, three districts, viz. Kanker, Dhamtari and Janjigir-Champa were selected for the study. Susceptibility tests against deltamethrin 0.05% were again carried out by exposing wild-caught fresh fed An. culicifacies in all three districts. In all tests, more than 100 mosquitoes were exposed. Knockdown after one hour was recorded as 77.5, 57 and 30% while mortality of 85.8, 72 and 64% was recorded in Kanker, Dhamtari and Janjigir-Champa districts, respectively indicating resistance to deltamethrin. Synergistic assays were carried out subsequently by first exposing An. culicifacies to PBO 5% impregnated papers for one hour followed by exposure to deltamethrin 0.05%. Synergistic bioassays resulted in 100% mortality in An. culicifacies in Kanker and Dhamtari while it was 96.2% in Janjigir-Champa. There was no mortality in mosquitoes when exposed to PBO impregnated papers alone.

WHO cone bioassays were performed by exposing wild caught blood fed An. culicifacies on two PN3. On each net 5 cones were fixed on five sides (1 roof + 4 side panels). In all 50 mosquitoes were exposed on each net (10 females/cone in 2 replicates of 5 each) for 3 minutes. Simultaneously 25 mosquitoes (5 females/cone) were exposed on one untreated net to serve as control. Knockdown after one hour and mortality after 24 h were recorded. In Kanker and Dhamtari knockdown of 100% was recorded while in Janjigir-Champa it was 99%. Overall mortality after 24 h was 100% in Kanker and Dhamtari and 99% in Janjigir-Champa. Exposure on roof produced 100% mortality in all three districts while mortality on side panels was 100% in Kanker and Dhamtari and 98.7% in Janjigir-Champa. Further studies are in progress.

**A longitudinal study on survivorship and physical integrity of field distributed long-lasting insecticidal nets for malaria control in Chhattisgarh state**

Questionnaire-based surveys were undertaken in sample of households in at least two villages each in different districts to ascertain the presence of distributed nets, their physical integrity and usage pattern by the community. In all net surveys were carried out in seven districts, five in south and two in the north. On an average 26 houses were surveyed in each district representing at least two villages where long-lasting insecticidal nets (PermaNet 2.0) were distributed > 2 years ago by the State/District Health Department through Public Distribution System (PDS). For almost all villages no information was available on the total number of households and number of nets distributed either at the district/block/CHC/PHC levels. The present survey was carried out in 184 households representing a population of 799 with an average family size of 4.34/HH. The households possessed 249 nets (1.3/HH) of which 211 (84.7%) were LLINs (1.1/HH) while there were on an average 2.2 sleeping spaces per house. Of the 211 LLNs recalled by HH to have received through the PDS, 143 (68%) were physically present thus there was an attrition of 32% after about 2 years of net distribution. About 93% of the available nets were still being used by the households while 37% nets had holes of different sizes.

Considering very few LLINs have been left with households, it is pertinent to strengthen this intervention further by undertaking universal distribution of new LLINs as per the revised norms as only this intervention if effectively used can make any dent in malaria transmission due to high level of resistance in malaria vector An. culicifacies to
DDT and alphacypermethrin being used in indoor residual spraying for the last many years.

**Monitoring the therapeutic efficacy of antimalarial medicines in India**

Therapeutic efficacy of ACT was monitored in Balod and Kanker districts, Chhattisgarh and Gadchiroli district, Maharashtra where it is being used as a first line of treatment for uncomplicated *P. falciparum* malaria positive cases.

**Chhattisgarh:** In the present study, 23 cases at Chikhalda Kasa PHC, District Balod and 38 cases at Antagarh CHC, District Kanker fulfilling the inclusion criteria were enrolled in the study. In all 61 patients with uncomplicated *P. falciparum* mono-infection were given ACT under medical supervision over 3 days as per National Drug Policy. All the patients enrolled in the study, administered with drug were followed-up to 42 days from Day 0 (day of enrolment) for parasitological and clinical evaluation. Haemoglobin was checked on Day 0 and Day 42. In all, 54 patients completed 42-days follow up. Two patients were lost in follow up and consent was withdrawn by five patients. The 42-days cure rate with ACT (AS+SP) was 100% and no clinical or parasitological failure was recorded. All the patients tolerated the drug very well and no adverse event was observed. The study indicates that response of 3-dose regimen of ACT (AS+SP) is effective in clearing the asexual parasitaemia in 100% of patients within three days. Therefore, ACT (AS+SP) should be continued as first line of treatment for uncomplicated *P. falciparum* malaria in chloroquine resistant high risk areas.

**Maharashtra:** A total of 62 cases fulfilling the inclusion criteria were enrolled in the study at Malewada PHC, District Gadchiroli and were successfully followed up to 42 days. The cure rate with ACT (AS+SP) was 100% and no clinical or parasitological failure was recorded. All the patients tolerated the drug very well and no adverse event was observed.

**Technical support to the programme**

**Cross-checking of malaria blood slides**

Cross-checking facility for malaria slides was provided by the Field Unit. During the reporting period, 9644 malaria slides received from 15 districts of Chhattisgarh through State Programme Officer (Malaria), Raipur were cross-checked at the Field Unit. Discrepancy of 0.8% was observed in positive slides and discrepancy of 0.2% was observed in negative slides. Results were communicated to the concerned agency.

**Examination of filarial slides**

In all, 257 slides received from District Janjgir-Champa were checked for presence of microfilaria. Out of these, five slides were positive for microfilaria. Mf rate was 1.9.

**Malaria Clinic**

A total of 25 persons with fever attended the Malaria Clinic at the Field Unit. None of the blood smear was found to be malaria positive.

**Training support**

**Orientation training to MBBS and BHMS students**

III year MBBS 119 students from the Govt. Medical College, Raipur and 14 students of 4th year BHMS from Maharana Pratap Homoeopathic Medical College and Hospital, Raipur were imparted 1-day orientation training on various aspects of vector-borne diseases and their control.

**Refresher training to laboratory technicians**

Refresher training in malaria microscopy was imparted to 39 laboratory technicians from 10 districts, posted in 16 CHCs, 18 PHCs, and 5 Hospitals in three batches from 11-15, 18-22 May 2015 and 14-18 March 2016.

**Training to laboratory technicians in filaria slide examination**

Two-days training for examination of blood slides for microfilaria was imparted to 35 laboratory technicians of seven districts in two batches from 30 November to 1 December 2015.

**Meetings/Workshops attended**

1. Dr RM Bhatt and Dr GDP Dutta attended a meeting on Preparatory activities for vector-borne and water-borne diseases in monsoon season which was chaired by the Principal Secretary, Health, Govt. of Chhattisgarh held at Raipur on 13 May 2015.
2. Dr RM Bhatt and Dr GDP Dutta attended a meeting on Control of vector-borne diseases held at Raipur on 18 May 2015.
3. Dr GDP Dutta attended a meeting on Technical evaluation of rapid diagnostic kit for malaria held at Raipur on 11 August 2015.

4. Dr RM Bhatt attended a meeting on Control of vector-borne diseases in 11 states organized by NVBDCP held at Raipur from 18–19 August 2015, and made a presentation on Insecticide resistance status of An. culicifacies to various insecticides in different Districts of Chhattisgarh.

5. Dr RM Bhatt attended a Brain storming workshop on Integrated Vector Management organized by WHO SEARO at NCDC, Delhi on 2 November 2015.

6. Dr RM Bhatt participated as resource person in NVBDCP-WHO National Training workshop on Indoor residual spray under IVM held at NCDC, Delhi from 3–7 November 2015.

7. Dr RM Bhatt participated as resource person in the State level Training of Trainers Course for indoor residual spraying for control of Leishmaniasis held at Patna from 30 November–4 December 2015.

8. Dr RM Bhatt participated as resource person in NIMR-WHO Joint workshop on Malaria vector insecticide resistance monitoring and management in India held at New Delhi from 7–11 December 2015.

9. Dr RM Bhatt attended 19th WHOPES working group meeting at WHO (HQs), Geneva as Temporary Adviser from 5–8 February 2016.
Ranchi
(Jharkhand)

The Jharkhand state is highly endemic for malaria contributing about 7% of the total malaria cases of the country. On the basis of epidemiological information out of 24 district, 14 districts, namely West Singhbhum, Latehar, Simdega, Khunti, Palamu, Godda, Giridih, Hazaribagh, Chatra, Garhwa, Gumla, Lohardaga, Koderma and Saraikela have been identified as most vulnerable for malaria (Fig. 1).

The state has relatively stable malaria transmission with yearly average slide positivity rate (determined from both passive and active surveillance) of 15% over the last three years. *Plasmodium falciparum* accounts for 44% of the cases while *P. vivax* accounts for 56%. The forest, hilly terrain, favourable climate, inaccessible area, tribal culture, migration and social unrest have aggravated the malaria situation. After the introduction of new epidemiological tools, bivalent RDT kits for diagnosis, ACT for the treatment of *Pf* malaria and long-lasting insecticide net (LLINs), the API of Jharkhand state was 2.9% in the year 2015. The other vector-borne diseases prevalent in the state are filariasis, dengue, chikungunya, Japanese encephalitis and kala-azar. The health seeking behaviour of the tribal population is very poor. In addition to this there is very scanty information on the transmission dynamics of vector-borne diseases like malaria, filaria, kala-azar and dengue. To find out the solution to control vector-borne diseases in Jharkhand state, the National Institute of Malaria Research, Field Unit at Ranchi is carrying out need

Fig. 1: District-wise annual parasite incidence (API) of malaria in Jharkhand state (Source: SVBDCP)
based research for the State Government (SVBDCP) covering malaria, filariasis, dengue and kala-azar.

Mosquito fauna survey and susceptibility status of Anopheles minimus

Altogether 14 anopheline species were recorded from Noamundi area out of with four recognized malaria vectors An. culicifacies, An. annularis, An. flaviatilis and An. minimus. Low density of An. minimus (MHD: 2–4) was recorded from Noamundi, Badajamda, Balijharan and Kadajamda villages where high density (MHD: 20–25) of An. minimus was recorded. Breeding was observed in hill tops slow moving streams, and pools. The feeding of An. minimus was observed in indoors and in outdoors (less numbers). The resting of An. minimus was observed under the table and sleeping beds occasionally on wall hangings. Mosquito blood meal (MBM) analysis of An. minimus revealed high anthropophilic index 60% positive for human blood index.

Susceptibility test was done as per standard WHO susceptibility test using DDT (4%), malathion (5%) and deltamethrin (0.05%) in the villages of Noamundi, Badajamda, Balijharan and Kadajamda (Table 1). Anopheles minimus showed 95% mortality to DDT (4%) and 100% mortality to malathion (5%) and deltamethrin (0.05%) (Table 1). Four An. minimus were sequenced. Sequencing of 28r DNA confirmed that specimen identified morphologically as An. minimus s.l. were actually An. minimus sensu stricto.

Epidemiological and entomological studies on malaria in and around Air Force unit, Singharsi of Pakur district, Jharkhand

On the request of Air Force HQs, New Delhi an epidemiological and entomological investigation was carried out in eight villages in and around Singharsi Air Force unit in the dense forest hilly areas of east Jharkhand. The villages were dominated by primitive Pahari tribes (Fig. 2).

Table 1. Susceptibility status of An. minimus to adulticides at Noamundi area

<table>
<thead>
<tr>
<th>Adulticides</th>
<th>Adulticides (% Conc.)</th>
<th>Exposure time (min)</th>
<th>No. exposed (24 h)</th>
<th>No. of mosquito dead</th>
<th>% Mortality</th>
<th>Susceptibility status</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDT</td>
<td>4</td>
<td>60</td>
<td>20</td>
<td>19</td>
<td>95</td>
<td>$</td>
</tr>
<tr>
<td>Malathion</td>
<td>5</td>
<td>60</td>
<td>20</td>
<td>20</td>
<td>100</td>
<td>$</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>0.05</td>
<td>60</td>
<td>20</td>
<td>20</td>
<td>100</td>
<td>$</td>
</tr>
</tbody>
</table>
Mass blood survey (MBS) was carried out in eight villages in and around of 507 SU, AF unit, Singharsi of Pakur district, Jharkhand. Malaria cases were identified at village level by rapid diagnostic test (RDT) and the treatment was provided immediately. Blood smears (thick and thin) were prepared and stained with Giemsa and examined under Primo microscope. Epidemiological parameters like different species of parasites, i.e. SPR, SfR, Pf% among different age groups and sex-wise malaria cases were analyzed.

The adult mosquito collection was carried out in all the eight villages and the campus of the AF station from human dwellings (HD) and cattlesheds (CS) were collected by suction tube method used from dusk to dawn. The immature samples were collected from ponds, river beds, streams and unused wells and reared in the field laboratory until emergence. All the mosquitoes were identified in the laboratory of NIMR, Field Unit, Ranchi using the catalogues of Night and Stone; Nagpal et al; and Wattal and Kalra.

CDC miniature light-traps (CDC, Atlanta, USA) were used from dusk to dawn for collection of adult mosquitoes. Susceptibility status of malaria vectors, An. culicifacies, An. annularis and An. fluviatilis was carried out against DDT (4%), malathion (5%) and deltamethrin (0.05%).

In the present surveillance, mass blood survey smear examination results were presented (Table 2). The results revealed slide positive rate (SPR) 37.9%. The highest SPR was observed in Singharsi (45.7%) and the lowest was observed in Madgama (19%); however, in the clinic at AF unit the SPR was 72.2% from Santhul and Pahadi tribes. The P. falciparum was the dominant species recorded (86.5%) (Fig. 3). The P. vivax was 2.8 and 2% Pf cases showed gametocyte in the peripheral blood smear. The highest percentage of asymptomatic carrier of Pf was detected from the population that

---

**Table 2. Area-wise malaria prevalence in and around AF unit, Singharsi of Pakur district, Jharkhand**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Area</th>
<th>BSCE</th>
<th>RDT positive</th>
<th>Microscopy positive</th>
<th>SPR</th>
<th>SfR</th>
<th>Pf%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pv  Pf Mix  Total</td>
<td>Pv  Pf Mix Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Rakha</td>
<td>47</td>
<td>0  24 1 25</td>
<td>0 18 1 19 40.4 38.3 94.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Paktadi</td>
<td>94</td>
<td>5  41 6 52</td>
<td>3 31 4 38 40.4 33 81.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Balmi</td>
<td>21</td>
<td>1  6 1 8</td>
<td>1 5 1 7 33.3 23.8 71.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Madgama</td>
<td>21</td>
<td>0  4 1 5</td>
<td>0 3 1 4 19 14.3 75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Mamamod</td>
<td>42</td>
<td>5  21 2 25</td>
<td>1 16 2 19 45.2 38.1 84.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Singhari</td>
<td>70</td>
<td>3  38 1 42</td>
<td>2 29 1 32 45.7 41.4 90.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Damdam</td>
<td>66</td>
<td>3  19 1 23</td>
<td>1 14 1 16 24.2 21.2 87.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Chabi</td>
<td>51</td>
<td>3  16 0 19</td>
<td>2 13 0 15 29.4 25.5 86.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Clinic</td>
<td>18</td>
<td>0  16 2 18</td>
<td>0 12 1 13 72.2 66.7 92.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>430</td>
<td>17 185 15 217</td>
<td>10 141 12 163 37.9 32.8 86.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BSCE—Blood slide collected/examined; SPR—Slide positivity rate; SfR—Slide falciparum rate.
Table 4. Distribution of adult anopheline species and man hour density given village-wise in and around AF unit, Singharsli of Pakur district, Jharkhand

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>An. culicifacies</td>
<td>TMC</td>
<td>MHD</td>
<td>TMC</td>
<td>TMC</td>
<td>TMC</td>
<td>TMC</td>
<td>TMC</td>
<td>TMC</td>
<td>TMC</td>
<td>TMC</td>
<td>TMC</td>
<td>TMC</td>
</tr>
<tr>
<td>104</td>
<td>6.2</td>
<td></td>
<td>49</td>
<td>2.9</td>
<td>2</td>
<td>0.1</td>
<td>11</td>
<td>0.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>72</td>
<td>4.3</td>
<td></td>
<td>31</td>
<td>1.9</td>
<td>0</td>
<td>0.1</td>
<td>1</td>
<td>1.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>98</td>
<td>5.9</td>
<td></td>
<td>31</td>
<td>1.9</td>
<td>0</td>
<td>0.1</td>
<td>11</td>
<td>0.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>29</td>
<td>1.7</td>
<td></td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>0.5</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>92</td>
<td>5.5</td>
<td></td>
<td>23</td>
<td>1.4</td>
<td>0</td>
<td>0.2</td>
<td>3</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>92</td>
<td>5.5</td>
<td></td>
<td>23</td>
<td>1.4</td>
<td>0</td>
<td>0.2</td>
<td>3</td>
<td>0.2</td>
<td>0</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>69</td>
<td>4.1</td>
<td></td>
<td>0</td>
<td>0</td>
<td>22</td>
<td>1.3</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>308</td>
<td>18.4</td>
<td>163</td>
<td>5</td>
<td>0.3</td>
<td>27</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>31</td>
<td>1.9</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>803</td>
<td>48.1</td>
<td>12</td>
<td>0.7</td>
<td>58</td>
<td>3.5</td>
<td>11</td>
<td>0.7</td>
<td>6</td>
<td>0.4</td>
<td>3</td>
<td>0.2</td>
</tr>
<tr>
<td>Mean</td>
<td>100.4</td>
<td>6.0</td>
<td>12</td>
<td>0.7</td>
<td>58</td>
<td>3.5</td>
<td>11</td>
<td>0.7</td>
<td>6</td>
<td>0.4</td>
<td>3</td>
<td>0.2</td>
</tr>
</tbody>
</table>

TMC—Total number of adult mosquito species collected; MHD—Man hour density.
mosquitoes was collected. Major malaria vectors encountered were *An. culicifacies* (39.9%) followed by *An. annularis* (7.2%) and *An. fluviatilis* (1.5%). *Anopheles culicifacies*, *An. annularis* and *An. fluviatilis* were incriminated as malaria vectors by the National Institute of Malaria Research, Ranchi (unpublished reports).

*Anopheles culicifacies* and *An. annularis* were resistant to DDT (4%) and susceptible to malathion (5%) and deltamethrin (0.05%). However, it is observed that *An. fluviatilis* was susceptibility to DDT (4%) (Table 7). This indicates that malathion/deltamethrin will be useful/helpful for insecticide residual spray (IRS) in controlling malaria vectors vis-à-vis malaria in AF unit and villagers of Singharsi area.

### Suggested remedial measures

- LLINS (Long-lasting insecticide-treated bed-nets) must be distributed and used covering all the population of Air Force, MES and civilians residing in AF units.
- All the three malaria vectors are susceptible to insecticides (Malathion and deltamethrin). Therefore, it was suggested to overcome the entire problem, it is necessary to undertake proper surveillance, indoor spraying (IRS) of SP (Synthetic pyrethroids) in the human dwelling and cattlesheds at AF units.
- Active surveillance should be carried out the entire nearby village with the radius of 6 km weekly once and treatment must be provided to block the malaria transmission.
- Insecticide treated nets (ITMNs) may be provided to all the nearby villages.
- Distribution of Odomos cream, coils, spray (repellants) to the AF units personnel and particularly those who engage in the night duty.
- Introduction of ACT in the AF unit and village level for more effective treatment.
- To impart health education to the villages by

### Table 6. The identification of positive breeding habitats of anopheline species in and around AF unit, Singharsi of Pakur district, Jharkhand

<table>
<thead>
<tr>
<th>Breeding habitat</th>
<th>Type of habitat</th>
<th>Types of mosquito species emerge from larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ponds</td>
<td>Natural BH</td>
<td><em>An. culicifacies</em>, <em>An. annularis</em>, <em>An. subpictus</em>, <em>An. vagus</em>, <em>An. splendidus</em> and <em>An. palidus</em></td>
</tr>
<tr>
<td>Streams</td>
<td>Natural BH</td>
<td><em>An. culicifacies</em> and <em>An. fluviatilis</em></td>
</tr>
<tr>
<td>Riverbeds</td>
<td>Natural BH</td>
<td><em>An. culicifacies</em>, <em>An. annularis</em>, <em>An. splendidus</em> and <em>An. theobaldi</em></td>
</tr>
<tr>
<td>Tree holes</td>
<td>Natural BH</td>
<td><em>An. culicifacies</em></td>
</tr>
<tr>
<td>Unused well</td>
<td>Artificial BH</td>
<td><em>An. culicifacies</em>, <em>An. annularis</em>, <em>An. subpictus</em>, <em>An. vagus</em>, <em>An. fluviatilis</em> and <em>An. barbirostri</em></td>
</tr>
<tr>
<td>Others</td>
<td>Discards BH</td>
<td><em>An. culicifacies</em>, <em>An. annularis</em>, <em>An. subpictus</em>, <em>An. vagus</em>, <em>An. fluviatilis</em> and <em>An. barbirostri</em></td>
</tr>
</tbody>
</table>

BH—Breeding habitats.
audiovisuals and to promote the use of insecticide treated bednets among the AF unit and villagers.

- Antimalarial drugs and RDT kits should be adequately stocked in AF unit. Early detection and prompt treatment of malaria cases carried out.
- The ASHA (locally called as Sahiya) in the village should be properly trained on the use of RDT kits and proper use of ACT drugs.
- The ban of monotherapy should be highlighted in the villages carried by local quacks.
- All the breeding habitats must be surveyed weekly once and antilarval measures should be implemented.
- Chemoprophylaxis should be continued.
- The sun sets means everybody must use a full sleeve which is not followed properly.

Molecular identification of the vectors, vector incrimination by ELISA, MBM analysis by ELISA and sibling species identification is in progress.

The survey revealed slide positivity rate 37.9% ranging between 19% (Madga) to 45.7% (Singhars). *Plasmodium falciparum* was the dominant parasite which accounts for 86.5% and *P. vivax* was 2.8%. Results of mass blood survey indicated the presence of high percentage of asymptomatic carriers of malaria parasites in local population. In an entomological survey 20 species of mosquitoes 5 genera was collected by CDC miniature light-trap and hand catch method. Identification of entire mosquitoes revealed the genus *Anopheles* consists of 10 species. A total of 791 Anopheline mosquitoes were collected. Major malaria vectors encountered were *An. culicifacies* (39.9), *An. fluvialitis* (1.5) and *An. annularis* (7.2%). Immature mosquito larvae were collected from static tanks and slow moving streams of the Air Force unit. One *Cambusia affinis* hatchery was noticed during the survey.

![Fig. 5: Map showing study area of Mehuadarr CHC, Latehar district, Jharkhand.](image-url)
Monitoring of therapeutic efficacy of ACT (artesunate + pyrimethamine and sulfadoxine) against uncomplicated *Plasmodium falciparum* malaria at Mahuadann CHC, Latehar district, Jharkhand

Therapeutic efficacy of ACT (AS + SP) was investigated at Mahuadann CHC, Latehar district (Fig. 5). The site initiation meeting was carried out at Mahuadann CHC on 23rd November 2015 (Fig. 6). About 35 participants participated in the meeting including Doctors, Technicians and other staffs. Majority of the patients were enrolled in the study through rapid fever survey in remote CHC villages. This area is tribal dominated and endemic for malaria (Fig. 7).

Malaria outbreak and social unrest is also prevailing in this area. A total of 59 patients of uncomplicated *P. falciparum* fulfilling the inclusion criteria (as per WHO protocol) were enrolled in the study after taking the written informed consent.

The parasitic density varied from 1030 to 69033/µl. *Plasmodium falciparum* patients were given treatment for three days under the supervision of medical doctors as per the National Drug Policy and were followed up to 42 days. The drug used in the study was collected from malaria control programme of SVBDCP.

Out of 59 patients 52 patients completed 42 days follow up and seven patients were lost to follow up. All the 52 patients showed adequate clinical and parasitological response (ACPR). The age group of the patients varies from 1.5 to 62 years. The parasite clearance was 48 h after the ACT treatment in all the cases. It was observed that all the patients tolerated the drug very well and no adverse effect was observed. The study indicates that the response of three dose regimen of ACT (AS+SP) combination therapy is highly effective in clearing the asexual parasitaemia within two days in Jharkhand state. Therapeutic efficacy study provided guidance to the antimalarial drug policy of the programme.

**Filarisis survey**

Filarisis survey was carried out in four districts (one PHC of each district) of Jharkhand state out of 24 districts, during the year 2015 (Fig. 8). The districts surveyed were Simdega (Bano PHC), Palamu (Medininagar), Dhanbad (Baliapur), and Lohardaga (Bhandra).
Simdega district (Bano)

Filariasis survey was carried out in Simdega district (Bano PHC) of Jharkhand state during the month of August 2015 (Fig. 9). The district is dominated by Oraons, Kharia, Mundas, Asur, Birhor.

A total of 2190 persons were surveyed for filariasis, out of which microfilariae were detected in 133 individuals with an overall prevalence of 6.07% (Table 8). The prevalence of microfilaria among males (6.81%) was significantly different ($z = 1.9539, p < 0.05$) with prevalence among females (5.37%), (Table 9). The youngest microfilaria carrier detected was a five-year old boy and the oldest microfilaria carrier was a 70-year old male.

A total of five microfilariae carriers were observed in the blood smears of 133 positive persons (Fig. 10). The mean microfilaria (mf) density per positive person was 5.02 per 20 μl of blood.

The mean mf density in males and females was 5.07 and 2.83 and the microfilaria ranged from 1–43 in males and 1–63 in females, respectively. The maximum number of parasites found in a single slide was 27 in a 12-year old girl. The microfilaria identified was Wuchereria bancrofti. The highest microfilaria was detected in Soye village (15.21%) and the lowest microfilaria rate in Sahubera (1.16%).

A total of 96 patients had manifestations of filariasis out of which 12 (12.5%) being positive for microfilaria (Fig. 11 a & b). The most common manifestation was hydrocele. In males, hydrocele was the predominant chronic manifestations accounting for 50.54% followed by lymphoedema 32.96%. The overall filarial disease rate and filarial endemicity rate was 10.45 and 4.38%, respectively. The manifestation rate 5.68% among males and 3.17% among females. The filarial endemicity rate among males (12.5%) was significantly higher.
(z = 1.8225, p < 0.05) with filarial endemicity rate among females (8.54%). The presence of microfilaria among person with clinical disease was lower than in person without clinical disease. It was observed that the mf rate increased with the increase in age.

Binax now (antigen detection card) showed 23.33% (730) positive among 2–5 years children indicating high transmission in this area. All the 133 microfilaria positive cases were treated with standard dose of DEC (diethylcarbamazine) 6 mg/kg/day for 12 days.

Fig. 10: Age-wise distribution of microfilaria data of Bano PHC (numbers in red indicate mf positive samples).

Fig. 11: (a) Elephantiasis of leg, foot, the toe web space having hand and feet, upper limbs; and (b) Lymphoedema of lungs infection, multiple skin nodules due to lymphoedema infection an uncommon finding.
During the entomological survey, a total of 150 female *Culex quinquefasciatus* were collected from human dwellings. The man hour density (MHD) of *Cx. quinquefasciatus* was 37.5. A total of 120 mosquitoes were dissected for the presence of different stages of filarial larvae. Out of which 12 were found to be positive for L1 and L2 stage showing 10% of infection rate, eight were positive for L3 larvae and showing 6.67% of infectivity rate.

**Malaria Clinic**

Malaria clinic at the Field Unit provides free diagnosis facility to the patients attending the clinic. A total of 248 patients attended the clinic from April 2015 to March 2016 of which 14 cases were positive for malaria. Of these two cases were positive for *P. vivax* and 12 cases for *P. falciparum*. Overall SPR was 5.64%, SIR 4.83% and PIR was 85.71. One *P. falciparum* positive patient showed gametocyte in the peripheral blood (Table 10).

**Table 10. Malaria Clinic data of IDVC FU, Itki, Ranchi (2015-2016)**

<table>
<thead>
<tr>
<th>Month</th>
<th>BSC Total (+)ve</th>
<th>Pv</th>
<th>Pf</th>
<th>SPR</th>
<th>SFR</th>
</tr>
</thead>
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<tr>
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<td>21</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>May</td>
<td>9</td>
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<td>0</td>
<td>11.12</td>
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<tr>
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<td>0</td>
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<td>23.52</td>
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<td>Mar</td>
<td>25</td>
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<td>0</td>
</tr>
<tr>
<td>Total</td>
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<td>14</td>
<td>2</td>
<td>12</td>
<td>5.64</td>
</tr>
</tbody>
</table>

**Filaria Clinic**

Filaria clinic is functioning at IDVC Field Unit, Itki, Ranchi. A total of 46 patients of filariasis attended the clinic during the year. Most of the cases were the old cases of filariasis. These cases were with acute manifestation of filariasis starting from hydrocele to elephantiasis. Two cases of epididymo-orchitis were observed and five patients had multiple manifestations (10.86%) (Table 11).

**Future programme**

1. To study the prevalence of G-6-PD deficiency and haemoglobinopathies in tribal population of Jharkhand state (collaboration with the RIMS).
2. Integrated approach to control filariasis (*Wuchereria bancrofti* and *Brugia malayi*) in Santhal Pargana of Jharkhand state.
3. A pilot study to monitor the quality of antimalarial drugs in Jharkhand and Odisha states of India and to distinguish counterfeit and substandard drugs in formal and informal health facilities.
4. Entomological studies of Phlebotomine sand-flies (Diptera: Psychodidae), a vector of visceral leishmaniasis and its control in four endemic district of Jharkhand state, India.

**Support provided to NVBDCP and the state health programme with reference to the following activities**

Therapeutic efficacy of antimalaria drug ACT:
1. MDA evaluation.
2. Capacity building in the field of malaria entomology, microscopy and surveillance.
3. Insecticide resistance monitoring.
5. Epidemic investigation for rapid response and management.
6. Quality control of laboratory services (Diagnosis of malaria and filariasis).

**Health education and IEC activities**

Ten health education camps were organized in villages (Balmi, Chabi, Dhadam, Madama and Rakha) of Singharsi of Pakur district, Jharkhand state. The inhabitants of the villages are primitive Pahari tribes. The role of early detection of malaria cases and prompt treatment was discussed among the tribes. The use of LLINs for protection against mosquito and malaria was discussed among the tribal people. Role of RDT and ACT combination therapy was also discussed. Lectures and demonstrations regarding malaria and use of LLINs were delivered to the tribal people.
In addition to this, eight health education camps were organized in PHC Bano (Simdega district), Baliapur (Dhanbad district), Medininagar (Palamu district) and Bhandra PHC of Lohardaga district for filariasis. Role of MDA for control of filariasis and role of LLINs were discussed among the tribal people.

**Trainings provided**

**Dr MK Das**

1. Provided training on Transmission assessment survey at Namkum IPH Conference Hall to DVBDs/DVBD Consultant/Malaria Inspector/Laboratory Technician (on the request of SPO) regarding evaluation of filarial kits on 30 March 2016.

2. Training provided to Doctors of Ranchi district and VBD consultant regarding Elimination of lymphatic filariasis on 30 April 2015.

3. MDA and filariasis control training provided to Doctors of PHCs/CHCs at CS Office, Ranchi on the request of DMO, Ranchi on 5 May 2015.

4. Provided one-day training on MDA and filariasis control to officers, health workers and NGOs of Jharkhand state on 15 October 2015.

5. Training on MDA and filaria elimination provided to the Doctors of PHCs/CHCs of Ranchi district on the request of DMO, Ranchi on 30 November 2015.

6. VBD re-orientation training provided at District VBD, Hazaribagh, Koderma, Chatra, Ramgarh, Bokaro and Dhanbad to MTS and MI by NIMR scientists from 26–29 August 2015.
Rourkela (Odisha)

Comprehensive case management programme in Odisha

The MMV funded Comprehensive case management programme was launched, in July 2013, in four districts of Odisha with the primary objective to assess the impact of comprehensive case management system of uncomplicated malaria on its transmission in different transmission settings. Puintala, Athamallick, Hindol and Nuagaon are intervention blocks, whereas Saintala, Bhuban, Chhendipada and Khajuripada have been taken as control blocks in Bolangir, Dhenkanal, Anugul and Kandhamal districts, respectively (Fig. 1). The study is being undertaken in collaboration with the Government of Odisha after completion of recruitment and training of project staffs as well as orientation training of the Medical Officers and

Fig. 1: Map showing the intervention and control blocks in four districts of Odisha under CCM project.
other existing staffs of the Community Health Centres.

In the low endemic Bolangir district the ABER, TPR and API were 17.5, 0.9 and 1.6 in the intervention block, whereas the corresponding indicators in the control block were 21.5, 2.6 and 5.6 during the year 2015–16 (Fig. 2). In the intervention block of meso-endemic Dhenkanal district the ABER, TPR and API were 18.5, 5.3 and 9.8, whereas in the control block the corresponding indicators were 17.4, 1 and 1.8 during the year of reporting (Fig. 3). In the high endemic Kandhamal district the ABER, TPR and API were 36.9, 7.3 and 27 in the intervention block, whereas the corresponding indicators in the control block were 32.4, 14.7 and 47.8 (Fig. 4). In the hyper-endemic Angul district the ABER, TPR and API were 26.2, 15.4 and 40.3 in the intervention block whereas the corresponding indicators in the control block were 23.1, 6.8 and 15.74 during the year of reporting (Fig. 5). The malaria indicators were lower in the intervention blocks and higher in control blocks during the current year in comparison to those of previous years in all the four districts.

In the intervention blocks of all the four districts P. falciparum and P. vivax cases were followed-up on Day-5 and Day-14, respectively for drug compliance and adverse events. The compliance rates of follow-up in Bolangir, Dhenkanal, Kandhamal and Angul districts were above 90%. Various works under CCM project were closely monitored.

**Monitoring the therapeutic efficacy of antimalarial medicines in India**

A multicentric study on therapeutic efficacy of artemisinin-based combination therapy (ACT) with the combination sulfadoxine-pyrimethamine + artesunate in uncomplicated P. falciparum malaria was carried out in Thakurgarh New PHC under Madhapur CHC of Angul district in Odisha. Angul district was selected as one of the 13 sites in the country where therapeutic efficacy of the antimalarial medicine, being operational in the area, was carried out in collaboration with the National Vector Borne Disease Control Programme. Informed consent of all adult subjects and assent of parents in the cases of all minor subjects were obtained before enrolment in the study.

A total of 77 subjects who fulfilled all the inclusion criteria were enrolled in the study comprising of 35 (45.5%) females and 42 (54.5%)
males. Children belonging to age groups 0 to 4, 5 to 8 and 9 to 14 yr comprised of 6.5, 27.3 and 14.3% of the subjects, whereas adult comprised of 51.9% of the subjects, the range of age being 3 to 60 yr. The mean height, mean weight and mean mid-arm circumference of the subjects were 136.5 ± 22.5 cm, 33.1 ± 14.55 kg and 20.1 ± 4.19 cm, respectively. Haemoglobin level of the subjects was measured on Day 0 and on the last day of follow-up. Frequency distribution of all the subjects in terms of haemoglobin level is shown in Fig. 6. It is observed that most of the subjects were having haemoglobin level in the rage of 9.3 to 11.7 gm/dl. Majority of the subjects were having higher haemoglobin level on the last day of follow-up in comparison to Day 0 (Fig. 7).

The mean parasite density of enrolled subjects on Day 0 was 22,564 per µl blood while the range was 1038 to 95,238 µl blood. All the subjects became negative for asexual stages of malaria parasite by Day 2. Out of the 77 subjects enrolled for the study 75 subjects completed 42-day follow-up while two subjects withdrew from the study.

### Table 1. Results of the therapeutic efficacy of ACT (Sulfadoxine-pyrimethamine + artesunate) for the treatment of uncomplicated falciparum malaria

<table>
<thead>
<tr>
<th>Results of therapeutic efficacy study</th>
<th>(n = 77)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects enrolled</td>
<td>77</td>
</tr>
<tr>
<td>Number of subjects who completed 42 day follow-up</td>
<td>75</td>
</tr>
<tr>
<td>Number of subjects withdrawn from the study</td>
<td>2</td>
</tr>
<tr>
<td>Number of treatment failures—late clinical failures (LCF)</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>Number of adequate clinical and parasitological response (ACPR)</td>
<td>72 (96%)</td>
</tr>
<tr>
<td>Number of subjects encountered with adverse events</td>
<td>0</td>
</tr>
</tbody>
</table>

Among two withdrawn cases one withdrew on Day 1 for seeking alternative treatment and another withdrew on Day 30 due to the detection of *Plasmodium vivax* in the blood smear. Among 75 cases who completed 42-day follow-up, 3 treatment failures—all of them late clinical failures (LCF) were observed while the remaining 72 (96%) were categorized as adequate clinical and parasitological response (ACPR). Summarized results of the therapeutic study are given in Table 1.

### Ecoepidemiology and transmission of complex malaria in India (under CSCMI project)

#### Epidemiological studies

The multicentric study is being carried out with an objective to understand the basic ecoepidemiology of malaria; to determine the genetic diversity and population structure of *P. falciparum* and *P. vivax*; to quantify the role of environmental conditions in determining transmission intensity and to evaluate the evolutionary response of key Indian vectors to the adoption of insecticide-based intervention. The study area comprised of 11 villages located in forest, plain and riverine areas under Bisra, Kuarmunda and Birkera CHCs of Sundergarh district, Odisha.

### Enrolments under clinical, cross-sectional and longitudinal studies

A total of 1859 subjects were enrolled in the clinic study, whereas 1539 and 258 subjects were enrolled in cross-sectional and longitudinal studies, respectively. In the clinic study the enrolment of female subjects (44.4%) were less in comparison to male subjects (55.6%), whereas in both cross-sectional and longitudinal studies the enrolment of female subjects (55 and 57.8%) were more than that of male subjects (45 and 42.2%). In all the three
Table 2. Study specific enrolment summary
(Cumulative 2013 to 2015)

<table>
<thead>
<tr>
<th>Sex and age distribution</th>
<th>Passive case detection (Clinical study)</th>
<th>Active case detection</th>
<th>Cross-sectional study</th>
<th>Longitudinal study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consented subjects</td>
<td>1859</td>
<td>1539</td>
<td>258</td>
<td></td>
</tr>
<tr>
<td>Sex distribution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>825 (44.4)</td>
<td>847 (55)</td>
<td>149 (57.8)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1034 (55.6)</td>
<td>692 (45)</td>
<td>109 (42.2)</td>
<td></td>
</tr>
<tr>
<td>Age distribution (yr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>186 (10)</td>
<td>157 (10.2)</td>
<td>21 (8.1)</td>
<td></td>
</tr>
<tr>
<td>5–9</td>
<td>192 (10.3)</td>
<td>181 (11.8)</td>
<td>31 (12)</td>
<td></td>
</tr>
<tr>
<td>10–17</td>
<td>275 (14.8)</td>
<td>186 (12.1)</td>
<td>52 (20.2)</td>
<td></td>
</tr>
<tr>
<td>&gt;17</td>
<td>1206 (64.9)</td>
<td>1015 (66)</td>
<td>154 (59.7)</td>
<td></td>
</tr>
</tbody>
</table>

Figures in parentheses indicate percentages.

studies adults accounted for the majority of the enrolments (59.7 to 66%). The enrolment percentage of children belonging to the age groups <5, 5–9 and 10–17 yr ranged from 8.1 to 20.2% (Table 2).

Malaria cases and species composition by different diagnostic methods

The blood smears/samples of all subjects enrolled under cross-sectional and longitudinal studies were examined/analyzed by microscopy, RDT and PCR. However, diagnosis through RDT was not done in all cases in the clinic study. In the clinic study microscopic examination was done in all the 1859 subjects in which 64 (SPR 3.4%) cases were found positive for malaria. Rapid diagnostic tests and PCR analysis were done in 1188 and 1488 cases which detected 44 (TPR 3.7%) and 46 (TPR 3.1%) malaria positive cases, respectively. In the cross-sectional study microscopic examination and PCR analysis were done in all the 1539 cases, whereas RDT was done in 1503 cases. The microscopic examination revealed 130 (SPR 8.4%) malaria cases, whereas RDT and PCR analysis revealed 99 (TPR 6.6%) and 123 (TPR 8.0%) malaria cases, respectively (Table 3).

Asymptomatic malaria cases

In cross-sectional and longitudinal studies data on history of self-reported fever within the 48 h were recorded apart from taking the body temperatures at the time of clinical examination. In cross-sectional study 52.3, 44.4 and 51.2% of the malaria cases diagnosed by microscopy, RDT and PCR respectively were found to be asymptomatic. Similarly, during initial enrolment in the longitudinal study 40% of the malaria cases diagnosed through microscopy were found to be asymptomatic whereas only one positive cases each were diagnosed by RDT and PCR which were found to be symptomatic (Table 4).

Table 4. Proportion of asymptomatic malaria cases by different diagnostic methods

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cross-sectional study</th>
<th>Longitudinal study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N  n (%)</td>
<td>N  n (%)</td>
</tr>
<tr>
<td>Microscopy</td>
<td>130 68 (52.3)</td>
<td>5 2 (40)</td>
</tr>
<tr>
<td>RDT</td>
<td>99 44 (44.4)</td>
<td>1 0 (0)</td>
</tr>
<tr>
<td>PCR</td>
<td>123 63 (51.2)</td>
<td>1 0 (0)</td>
</tr>
</tbody>
</table>

N—Number examined/tested/analyzed; n (%)—Number (percent) positive.

Entomological studies

Mosquito species composition

Indoor resting mosquitoes were collected in the study villages located in plain, riverine and forest areas monthly between 0600 to 0800 hrs from four human dwellings and four cattlesheds. A total of 5504 female anopheline mosquitoes representing 16 Anopheles species were collected from the study villages. Anopheles culicifacies (32.23%) was the dominant species followed by An. annularis (26.10%) and An. palidus (18.1%). The percentage of An. fluviatilis was 3.07%.

Sibling species composition and blood meal analysis

Females of An. culicifacies and An. fluviatilis identified morphologically were used for identification of sibling species and blood meal source. Genomic DNA samples of the mosquitoes were subjected to allele specific PCR assay using

Table 3. Proportion of malaria cases by different diagnostic methods

<table>
<thead>
<tr>
<th>Diagnostic methods</th>
<th>Passive case detection (Clinical study)</th>
<th>Active case detection</th>
<th>Cross-sectional study</th>
<th>Longitudinal study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N  n (%)</td>
<td>N  n (%)</td>
<td>N  n (%)</td>
<td></td>
</tr>
<tr>
<td>Microscopy</td>
<td>1859 64 (3.4)</td>
<td>1539 130 (8.4)</td>
<td>258 5 (1.9)</td>
<td></td>
</tr>
<tr>
<td>RDT*</td>
<td>1188* 44 (3.7)</td>
<td>1503 99 (6.6)</td>
<td>258 1 (0.4)</td>
<td></td>
</tr>
<tr>
<td>PCR**</td>
<td>1488** 46 (3.1)</td>
<td>1539 123 (8.0)</td>
<td>247 1 (0.4)</td>
<td></td>
</tr>
</tbody>
</table>

N—Number examined/tested/analyzed; n (%)—Number (percent) positive; *RDT of all samples not done in the clinic in cross-sectional study; **PCR of all the samples have not been completed.
species specific assay. Midgut blood smears of An. \textit{fluvitatis} and \textit{An. culicifacies} specimens, identified to sibling species, were subjected to blood meal source identification by PCR using an established protocol.

\textbf{Sibling species composition}

The sibling species composition revealed by PCR is given in Table 5. Out of the total \textit{An. culicifacies} samples, 99.8\% were type B, C and E, whereas A and D-types were found very infrequently (0.2\% of the species total). \textit{Anopheles fluvitatis} species complex comprised of type S and T only while U and V-types were not found at all. However, T-type was numerically dominant in the study villages comprising of 86.4\% while S-type comprised of 13.6\%.

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|}
\hline
Species & Sibling/type & Percentage \\
\hline
\textit{An. culicifacies} & B, C & E & 99.8 \\
 & A & D & 0.2 \\
\textit{An. fluvitatis} & T & 86.4 \\
 & S & 13.6 \\
\hline
\end{tabular}
\caption{Sibling species composition of \textit{Anopheles culicifacies} and \textit{An. fluvitatis}}
\end{table}

\textbf{Host blood meal analysis}

Source of blood meal was determined by PCR in individual \textit{An. culicifacies} identified to sibling species type. All the A & D samples (0.22\% of the total \textit{An. culicifacies} analyzed) were positive for mixed meals of bovine and human blood. The B, C and E-types showed no clear feeding preferences, irrespective of whether mosquitoes were collected from human dwellings or cattle-sheds (Table 6).

Blood meal analysis of \textit{An. fluvitatis} provided limited evidence for exclusive human blood feeding as human blood was found only in 0.6 to 5.3\% of those comprising of both S and T-types caught from cattle-sheds and human dwellings. Higher percentage of bovine blood feeding and mixed bovine + human blood feeding was observed in both types of \textit{An. fluvitatis} collected, irrespective of whether mosquitoes were sampled from cattle-sheds or human dwellings (Table 7).

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|c|}
\hline
 & Bovine & Human & Bovine + human & Unfed & Others \\
\hline
\textit{S. fluvitatis} & 0 & 0.6 & 0.6 & 0 & 0 \\
\textit{S. CS} & 4.1 & 5.3 & 1.2 & 1.2 & 0 \\
\textit{T. HD} & 3 & 5.3 & 0 & 1.2 & 0 \\
\textit{T. CS} & 17.1 & 4.7 & 49.1 & 3.5 & 2.3 \\
\hline
\end{tabular}
\caption{Percent host blood meal analysis of \textit{Anopheles fluvitatis}}
\end{table}

\textbf{Effects of temperature and larval diet on development and survival of \textit{Anopheles culicifacies}}

\textit{F}_1 generation of field collected \textit{An. culicifacies} were reared under standard insectary conditions. Larval development rate and survival were quantified in artificial containers over gradients of temperature and food concentration (mg/ml/day). Performance across different conditions was evaluated by recording mortality rates during development (dead individuals/cup), time to pupation (days since hatching), and time to emergence (days since hatching). From the data it is observed that good larval survival (70–80\%) at peak transmission temperature. Survival rate was slightly better at 30°C than 27°C. Lower food concentration reduced survival rate (Table 8).

\textbf{Effects of temperature and density on the development rate and survival of \textit{Anopheles culicifacies}}

Larvae were reared at five different temperatures (20, 24, 27, 30 and 33°C) and three densities (20, 40 and 80). Density and temperature strongly interacted to determine the development and survival of the mosquitoes. Lowest mortality rate was observed at the intermediate temperature of 27°C while mortality rates increased in both the higher and lower temperatures (Fig. 8). Mortality rate also increased with the increase in the density of larvae (Fig. 9). Time of pupation decreased with the increase in temperature from 20 to 33°C and it increased with the increase in the density of larvae (Figs. 10 and 11). Highest percentage of adult emergence was observed in 27°C and it decreased in both higher as well as lower temperatures (Fig. 12). The percentage of adult emergence was higher in lower density and it decreased with the increase in density (Fig. 13).
Table 8. *Anopheles culicifacies* larval to adult survival at two temperatures representative of peak transmission season and with high and low food

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Food concentration (mg)</th>
<th>Replicate no.</th>
<th>No.of 1st instar larvae</th>
<th>% pupation</th>
<th>% adult emergence</th>
<th>% mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>0.6</td>
<td>1</td>
<td>50</td>
<td>76</td>
<td>94.80</td>
<td>28</td>
</tr>
<tr>
<td></td>
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<td>50</td>
<td>86</td>
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<td>73.60</td>
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<td>38</td>
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<td></td>
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<td>50</td>
<td>66</td>
<td>95.50</td>
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<td>5</td>
<td>50</td>
<td>44</td>
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</tr>
<tr>
<td>Total</td>
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<td></td>
<td></td>
<td>61.60</td>
<td>92.90</td>
<td>42.8</td>
</tr>
<tr>
<td>30</td>
<td>0.6</td>
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<td>80</td>
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<td>76</td>
<td>100</td>
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<td></td>
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<td>80.80</td>
<td>99</td>
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<td>70</td>
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</tr>
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<td>56</td>
<td>95.50</td>
<td>95.90</td>
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</tr>
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<td>48</td>
<td>95.90</td>
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<td></td>
<td>4</td>
<td>50</td>
<td>72</td>
<td>100</td>
<td>100</td>
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<tr>
<td></td>
<td>5</td>
<td>50</td>
<td>60</td>
<td></td>
<td></td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>250</td>
<td></td>
<td></td>
<td>62</td>
<td>98.70</td>
<td>38.80</td>
</tr>
</tbody>
</table>

Fig. 8: Effect of temperature on the mortality rate of *An. culicifacies* larvae in different temperatures.

Fig. 10: Effect of temperature on the time of pupation of *An. culicifacies* larvae.

Fig. 9: Effect of density on mortality rate of *An. culicifacies* larvae.

Fig. 11: Effect of larval density on the time of pupation in *An. culicifacies* larvae.
out of which 112 were found positive for malaria comprising of 40 *P. falciparum*, 68 *P. vivax* and 3 with mixed infection. The SPR, SFR and PF% were 2.7, 10 and 38.4, respectively (Table 9). All the malaria positive cases were given free treatment for malaria as per the National Drug Policy.

**Technical support to national programme and local health authorities**

1. The OIC, NIRM FU, Rourkela is a permanent invitee to the State Task Force-cum-review meeting on malaria control programme held periodically under the Chairmanship of Commissioner-cum-Secretary, H & FW Department, Government of Odisha.

2. The OIC is a member of the District Task Force headed by the District Magistrate and Collector for the control of dengue in Sundergarh district, Odisha.

3. During the current year more than 4000 febrile patients reported to the Malaria Clinic run by the Field Unit which caters to the need of the people living in and around Rourkela. All the malaria positive cases were given free treatment for malaria as per the National Drug Policy.

4. Cross-checked the blood slides of Sentinel site of NVBDCP, Odisha being operational in the Ispat General Hospital, Rourkela on regular basis.

5. NIRM, Field Unit, Rourkela actively participated in “Malaria Samadhan Shivir” organized by the State Health Department in different Block-CHC HQ in order to generate awareness among people for the prevention and control of malaria.

6. Organized Orientation training on 9 November 2015 on “Monitoring therapeutic efficacy of antimalarial drugs in India which was attended by the Medical Officers, Block Level Manager, Laboratory Technicians and Health Workers of Madhapur CHC under Angul district in Odisha.

7. Provided training to the students of Nursing Training Institute, Ispat General Hospital, Rourkela Steel Plant, SAIL, Rourkela on the Control of malaria and other vector-borne diseases.

8. Carried out Aedes breeding survey in the Rourkela Steel Township, as per the request of Public Health Authorities, Rourkela Steel

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**Malaria Clinic**

During the year under reporting from April 2015 to March 2016 a total of 4106 patients with fever reported at the clinic run by the NIRM Field Unit

**Table 9. Parasitological data of Malaria Clinic at NIRM Field Unit, Rourkela from April 2015 to March 2016**

<table>
<thead>
<tr>
<th>Month</th>
<th>BSE</th>
<th>Total Pf</th>
<th>Pf</th>
<th>Pv</th>
<th>Mixed</th>
<th>SPR</th>
<th>SFR</th>
<th>PF%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apr 2015</td>
<td>205</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>2.9</td>
<td>2.0</td>
<td>66.7</td>
</tr>
<tr>
<td>May</td>
<td>142</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>6.3</td>
<td>3.5</td>
<td>55.6</td>
</tr>
<tr>
<td>Jun</td>
<td>188</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Jul</td>
<td>385</td>
<td>23</td>
<td>5</td>
<td>15</td>
<td>2</td>
<td>6.0</td>
<td>1.8</td>
<td>30.4</td>
</tr>
<tr>
<td>Aug</td>
<td>551</td>
<td>12</td>
<td>3</td>
<td>9</td>
<td>0</td>
<td>2.2</td>
<td>0.5</td>
<td>25.0</td>
</tr>
<tr>
<td>Sep</td>
<td>650</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>1.4</td>
<td>0.9</td>
<td>66.7</td>
</tr>
<tr>
<td>Oct</td>
<td>466</td>
<td>13</td>
<td>6</td>
<td>7</td>
<td>0</td>
<td>2.8</td>
<td>1.3</td>
<td>46.2</td>
</tr>
<tr>
<td>Nov</td>
<td>346</td>
<td>16</td>
<td>7</td>
<td>9</td>
<td>0</td>
<td>4.6</td>
<td>2.0</td>
<td>43.8</td>
</tr>
<tr>
<td>Dec</td>
<td>294</td>
<td>14</td>
<td>0</td>
<td>13</td>
<td>1</td>
<td>4.8</td>
<td>0.3</td>
<td>7.1</td>
</tr>
<tr>
<td>Jan 2016</td>
<td>257</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.8</td>
<td>0.4</td>
<td>50.0</td>
</tr>
<tr>
<td>Feb</td>
<td>372</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0.8</td>
<td>0.5</td>
<td>66.7</td>
</tr>
<tr>
<td>Mar</td>
<td>250</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1.2</td>
<td>0.4</td>
<td>33.3</td>
</tr>
<tr>
<td>Total</td>
<td>4106</td>
<td>112</td>
<td>40</td>
<td>68</td>
<td>3</td>
<td>2.7</td>
<td>1.0</td>
<td>38.4</td>
</tr>
</tbody>
</table>
Plant and provided technical support to them on the control of dengue.

**Swachh Bharat Mission**

Employees of Rourkela Field Unit participated voluntarily in cleaning the office premises and its surroundings in order to make it clean and to propagate the message of "Swachh Bharat Mission".


11. Kushwah RB, Mallick PK, Ravikumar H, Dev V, Kapoor N, Adak TP, Singh OP. Status of DDT and pyrethroid resistance in Indian Aedes albopictus and absence of knockdown


26. Uragayala S, Kamaraju R, Tiwari S, Ghosh SK, Valecha N. Small-scale evaluation of the efficacy and residual activity of alphacypermethrin WG (250 g a.i./kg) for indoor
spraying in comparison with alpha-cypermethrin WP (50 g a.i./kg) in India. *Malar J* 2015; 14: 223.


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